Response of plant-pollinator interactions to landscape transformations in the Greater Cape Floristic Region (GCFR) biodiversity hotspot

by

Opeyemi Adebayo Adedoja

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Supervisor: Prof MJ Samways
Co-supervisor: Dr TO Kehinde

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Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated) that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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Abstract

Landscape transformation is one of the leading causes of global biodiversity decline. This decline is seen in terms of loss of species of ecological importance, and the collapse of important ecological interactions in terrestrial ecosystems. Ecological interactions are highly sensitive to environmental changes, as they are more vulnerable to disruptions than the species involved. Understanding the stability of these interactions in the face of growing environmental changes is key to identifying suitable conservation strategies for ameliorating species loss in transformed landscapes. This is of major conservation concern for the Greater Cape Floristic Region (GCFR), a globally important biodiversity hotspot and rich floral kingdom, which is home to many endemic species.

I assessed here the response of plant-pollinator interaction networks to landscape transformation in the GCFR. I examined the influence of changing abiotic and biotic conditions across elevation zones, and I further investigated the influence of fire and invasive alien trees as drivers of environmental change on plant-pollinator interactions. I used a multi-taxon approach to highlight the effects of these drivers on these interactions. I sampled insects and flowering plants, as well as their interactions in areas impacted differentially by fire, invaded areas, and in areas with stratified elevation zones.

My results showed a significant response of bees and beetles to environmental factors influencing species distribution across elevation gradients. Ecotones were an area of high conservation interest, as they were the most diverse in terms of species abundance and richness, although there was a mismatch between bees and flowering plant abundance peaks. Furthermore, species restricted to the highest elevation, peak zone are most at risk of local extinction, especially for the insect pollinators, as shown by interaction networks here having the lowest Shannon diversity index, generality, and interaction evenness.

Fire influences plant-pollinator interactions and species dispersion patterns through its direct effect on flower abundance and nest provision. Bees were the only group associated with flower abundance. Results showed the importance of flower-rich fire refuges for the persistence of insect pollinators, especially the specialized species during fire events. Finally, increases in alien pine tree age and density were associated with a decline in plant-pollinator interactions, species abundance, and richness. However, dense, tall pine tree patches supported unique interactions involving large-sized pollinators.

Overall, this study highlights the important response of plant-pollinator interaction networks to different drivers of environmental change. Habitat physical structure that sustains plant-pollinator interactions, especially those involving specialized species, holds important solutions for conservation action in this region. Controlled burning of overgrown areas should be encouraged for the proliferation of flowering plants. In addition, careful consideration should be given to trade-offs when instigating important conservation actions, such as restoration, especially when these actions can lead to local loss of some endemic species.

Opsomming

Landskapstransformasie is een van die belangrikste oorsake van afname in globale biodiversiteit. Hierdie afname is as gevolg van die verlies van ekologiese belangrike spesies sowel as die ineenstorting van belangrike ekologiese interaksies in terrestriële ekosisteme. Hierdie ekologiese interaksies is hoogs sensitief vir omgewingsveranderinge, aangesien hulle meer vatbaar is vir veranderinge as die betrokke spesies. In die aangesien van toenemende omgewingsveranderinge, is dit belanrik om die stabiliteit van hierdie interaksies te verstaan, want hulle is kritiek tot die identifisering van geskikte bewarings-strategieë vir die voorkoming van spesieverlies in getransformeerde landskappe. Hierdie is ‘n groot bewarings-bekommernis vir die breër Kaapse Floristiese Streek (KFS), ‘n globale belangrike biodiversiteit-hotspot en ’n weelderige blommeryk wat die tuiste van verskeie endemiese spesies is.

Ek het hier die reaksie van plant-bestuiwer interaksie-netwerke op landskapstransformasie in die KFS geëvalueer. Ek het die invloed van veranderde abiotiese- en biotiese toestande op die hoogte bo seespieël-sones ondersoek, en het verder ondersoek ingestel na die invloed van brande en indringerplante as drywers van omgewingsveranderinge op plant-bestuiwer interaksies. Om die effek van dié drywers op hierdie interaksies te beklemtoon, het ek ‘n multi-takson metode gebruik. Ek het monsters van insekte en blomplante geneem, asook hul interaksies ondersoek in gebiede wat deur verskillende tipes brande beïnvloed word, gebiede wat binnegedring is deur indringerplante, en in gebiede met verskillende hoogte bo seespieël-sones.

My resultate het getoon dat bye en kewers model bestuiwer-takson is, vir die evaluering van omgewingsveranderinge, veral dié met betrekking tot ruimtelike of hoogte bo seespieël gradiënte. Die grens ekotone was areas van hoë bewaringsbelang, aangesien hulle die mees divers was in terme van die aantal individue en spesies-rykheid, alhoewel daar was ‘n wanverhouding tussen die hoeveelheid bye en blomplante. Verder, spesies wat beperk is tot die hoogste areas bo seespieël, die piek-sone, is die kwesbaarste vir plaaslike uitwissing, veral die insekbestuiwers, soos bewys deur die interaksie-netwerke met die laagste Shannon diversiteits-indeks, algemeenheid, en interaksie-gelykheid.

Brande beïnvloed plant-bestuiwer interaksies en spesies-verspreidingspatrone deur sy direkte effek op blom-hoeveelheid en nes-bepaling. Bye was die enigste groep wat deur blom-hoeveelheid gedryf word. Die resultate het ook die belangrikheid van blom-ryke brand-skuiltes vir die deursettingsvermoë van insekbestuiwers aangetoon, veral vir die gespesialiseerde spesies gedurende brand gevalle. Ten slotte, ‘n styging in die ouderdom en digtheid van indringer dennebome het ‘n afname in plant-bestuiwer interaksies, aantal individue, en spesies-rykheid veroorsaak. Nietemin, digte, hoë denneboom plate het unieke interaksies ondersteun waarin groot bestuiwers betrokke is.

Dus, in opsomming, hierdie studie beklemtoon die belangrike reaksie van plant-bestuiwer interaksie-netwerke tenoor die verskillende drywers van omgewingsveranderinge. Die fisiese strukture van habitatte wat plant-bestuiwer interaksies ondersteun, veral dié waarby gespesialiseerde spesies betrokke is, bevat belangrike oplossings vir bewaringsaksie in hierdie gebied. Beheerde verbranding van toegegroeide areas moet aangemoedig word vir die verspreiding van blomplante. Versigtige oorwegings ten opsigte van potensiële voor- en nadele moet ook geneem word wanneer belangrike bewaringsaksies van stapel gestuur word, soos byvoorbeeld restorasie, veral wanneer hierdie aksies na ‘n plaaslike verlies van sekere endemise spesies kan ly.

Biographical Sketch

My name is Opeyemi Adebayo Adedoja; I am Nigerian with a BSc-degree in Zoology from Obafemi Awolowu University, Nigeria in 2012. I also received my Masters degree from the same institution in 2014. My research has been in pollination ecology in context of landscape transformation. With the completion of my PhD research program at Stellenbosch University, I am prepared for a postdoctoral research with a long-term career goal of lectureship in an University.

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Preface

This dissertation is presented as a compilation of seven chapters. Each chapter is introduced separately and written according to the style of the journal Ecology and Evolution to which Chapter 5 was published.

Chapter 1 General Introduction, Literature review and project aims

Chapter 2 Research results

Insect-flower interaction networks vary among endemic pollinator taxa over an elevation gradient

Chapter 3 Research results

Asynchrony among insect pollinator groups and flowering plants with elevation: significance for global climate change studies

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Chapter 1

General Introduction

Biodiversity crisis

The variety of life forms and all their interactions determine the well-being and resilience of ecosystems globally. However, biodiversity is declining at an alarming rate (Sala et al., 2000) with further decline predicted in the future. One of the most understood mass extinction events was the loss of dinosaurs that occurred about 65 MYA (Cretaceous-Palaeocene). Nevertheless, extinction in this present age is 1 000 times the former background rate (Pimm et al., 2014; de Vos et al., 2014). For example, 10, 533 species were classified as threatened by IUCN in 1996, and this has grown by approximately 100% in 20 years, with a total of 24, 307 threatened species in 2016 (IUCN Red list, 2018). Consequences of biodiversity loss include the failing delivery of ecosystems services (Myers, 1996), decreased productivity (Hector et al., 1999), altered nutrient cycles (Hooper and Vitousek, 1998), and changed trophic interactions (Hooper et al., 2005), among other functions. Thriving biodiversity provides functional ecosystem processes (Balvanera et al., 2006), with species functions established in food-webs at different trophic levels (Cardinale et al., 2006).

One of the most important tools for effective conservation of species is the availability of ecological information such as life history, distribution etc. According to Pimm and Joppa, (2015), several species have already gone extinct before they could be identified and studied. Insects constitute the highest diversity among life forms on earth. However, due to their small size and limited niche range, most species are still undiscovered or understudied. Usually, loss of important species yields loss of essential interactions and ecosystem services on which other species depend for nutritional requirement, reproduction or shelter (Koh et al., 2004; Colwell et al., 2012). Loss of such species may result in co-extinction of different species in the ecosystem. This is highly pronounced in insect-flower interactions, where the flowering plants are highly dependent on the visitation rate of insect pollinators for reproduction (Ollerton et al., 2011). Disruption of interaction due to decline of pollinators usually results in reduced productivity in both natural- and agro-ecosystems (Anderson et al., 2011; Garibaldi et al., 2013).

Pollinator crisis and causes

Pollinators are responsible for 75% of food consumed by humans (Dirzo et al., 2014), with insects alone contributing approximately a 35% increase to agricultural yield through pollination (Klein et al., 2006). Pollination services from insects are crucial in African terrestrial ecosystems, as they are elsewhere in the world, for maintenance of natural biodiversity on the one hand, and production of insect-pollinated food plants on the other. The economy of some countries is partly dependent on pollination services provided by insects. For example, pollination by insects yields about £430 million of agricultural output in the UK (Smith et al., 2011) and about $361 billion globally (Lautenbach et al., 2012). Also in South Africa, honeybees are responsible for the pollination of about 27% of herbs, 44% of shrubs and 28% of trees in the Cape Floristic Region (Hepburn and Radloff, 2013). Pollinators are essential component of natural biodiversity, and threats to this group have cascading effects on various components of biodiversity. Viera et al. (2013) found a decline in phylogenetic diversity of flowering plants in response to co-extinction simulation of pollinators. Furthermore, community composition of flowering plants may also be determined by their pollinators. Overall, ecosystem service delivery of pollination is compromised in situations of pollinator decline and this affects the integrity of many ecosystems (Chapin et al., 2000; Hopper and Gioia, 2004).

Honeybees, which are the most important pollinators, are declining rapidly in different parts of the world, with highest documentation in America and Europe (vanEngelsdorp et al., 2008; Potts et al., 2010a). *Varroa destructor*, an invasive parasitic mite is the major cause of colony collapse disorder (Yang and Cox-Foster, 2007; Berthoud et al., 2010; Dainat et al., 2012), a major threat to beehives causing major declines in bee populations. Different attempts have been made to ameliorate this decline, largely by increasing the number of beehives globally. Although beehives have been increased by 45% since 1961, the increase in human population and demand on agriculture, has increased by about 300%, and has been recorded on a number of crops that depend on bees and other insects for pollination (Aizen and Harder, 2009). This increasing demand for agricultural production has also been predicted to increase by 70% by 2050 (FAO, 2010). The constant dependence on bee pollination services and increased demand on agricultural products have masked the effort of conservationists and beekeepers in different regions of the world to conserve important insect pollinators.

Another major driver of pollinator decline is the application of harmful pesticides, especially in agricultural ecosystems (Brittain et al. 2010; Mullin et al. 2010; Henry et al. 2012; Whitehorn et al. 2012). Most of these chemicals were not designed to target pollinators. However, through direct exposure, pollinators, most especially bees, have shown various responses (Thompson, 2002; Romeis et al., 2008). Neonicotinoids are some of the leading harmful substances causing a decline in bee health and population levels (Henry et al., 2012; Whitehorn et al., 2012). While some of these pesticides can extinguish an entire generation of bees in a colony, some influence the foraging activities of pollinators by altering brain function (Desneux et al., 2007; Henry et al., 2012). Brittain et al., (2010) showed how wild bees are highly vulnerable to the application of pesticides, leading to a major decline in bee populations within a short period of the use of pesticide. Also, according to Park et al., (2015), pollinator abundance declines with increased application of pesticides. However, this may be ameliorated by the availability of natural patches where pollinators may seek refuge away from treated areas. In the era of increased dependence on agriculture, it is essential to apply more sustainable management practices that are pollinator friendly.

In the face of growing ecological threats to natural and agricultural ecosystems, great transformation in various terrestrial ecosystems is highly evident. These transformation events are often accompanied by habitat destruction and loss of nutrient requirements for important insect pollinators (Biesmeijer et al., 2006; Potts et al., 2010b; Winfree et al., 2011). Over time, pollinator species are displaced from transformed ecosystems, creating a temporal loss until suitable conditions are restored (Kormann et al., 2016). Kaiser-Bunbury et al. (2017) showed how vegetation restoration could restore high visitation rate and pollinator activity to previously degraded habitat. Patches of natural habitat are of great importance for the persistence of bee species in urban areas (Bates et al., 2011). Winfree et al., (2009) illustrated the correlation of bees and habitat loss, where decline in quality habitat yields a strong decline in the population of bees. Several factors such as global warming, land use type and invasive alien species are responsible as drivers of global change and influence ecological process in natural ecosystems and the relative success of pollination activities (Memmott et al., 2007; Didham et al., 2007; Tylianakis et al., 2008; Grass et al., 2013). Some of these drivers also act in synergy, usually increasing their impact on the ecosystem, as well as their effect on natural biodiversity (Schweigr et al., 2010; Grass et al., 2013; Rafferty, 2017). It is important to assess the extent and intensity of the declines associated with important drivers in Africa’s region of high plant diversity: the Greater Cape

Floristic Region (GCFR), with its high concentration of rare and threatened plants. A total of 1 805 plant species are on the IUCN Red List as threatened taxa (South African National Biodiversity Institute (SANBI) 2014, Cowling and Hilton-Taylor 1994).

Greater Cape Floristic Region Biodiversity Hotspot

A biodiversity hotspot is a biogeographical region that is both home to biodiversity of global significance and also under severe threat for possible destruction (Myers 1990; Ginsberg, 1999). Overall, 36 biodiversity hotspots have been identified globally, and the Greater Cape Floristic Region (GCFR) is one of the richest in terms of plant diversity. The GCFR, although the smallest of the six recognised floral kingdoms, is also the richest floral kingdom in the world. In addition, the GCFR is a point where the pollinator diversity meets plant diversity (Kuhlmann et al., 2012). This region supports over 9 000 vascular plant species, of which 69% are endemic to the region (Odendaal et al., 2008). The fynbos biome is one of the unique vegetation classes of the region, and this has contributed greatly to the economy of the country, with about R77 million yield from harvested fynbos (Odendaal et al., 2008). The GCFR is also where the biodiversity hotspot overlaps with UNESCO biosphere reserves. Five main biosphere reserves have been identified in this region, with Kogelberg Biosphere Reserve being one of the most diverse biosphere reserves in the world, with about 1 300 plant species per 10 000 sq km (Pool-Stanvliet et al., 2018).

The central zone of the GCFR is confined to the Cape Fold Mountains and adjacent montane valleys and coastal plains. However, combining this region with other diverse areas in the south-western tip of South Africa constitute a greater biodiversity of higher ecological significance. According to Born et al., (2007), the central zone of the GCFR, known as the Cape Floristic Region, is the most diverse in this region. However, combining the vegetation here with the unique endemism of Hantam-Tanqua-Roggeveld Region and the Namaqualand Region constitutes the Greater Cape Floristic Region (GCFR).

This region of great endemism is under threat from different types of transformation through direct human-mediated activities such as alien species invasion, altered fire regimes, and increased land use. Overall, these events alter the local distribution of biodiversity in this region, and are of great ecological significance. This has attracted recent intensive research to fill knowledge gaps, and to make recommendations on how to conserve what is left of the region's biodiversity. I now discuss various considerations and transformation events that are significant for making conservation recommendations with regards to pollinators.

Topographic elevation

Several plant lineages are known to radiate from different heterogeneous topographic landscapes, for example *Gentiana, Globularia* and *Soldanella* are known to radiate from the European Alps (Kadereit et al., 2004), *Lupinus* from the Andes (Hughes and Eastwood, 2006), *Rhododendron* from the Himalayas (Milne et al., 2010) and *Macowania* originating from the Draskensberge (Bentley et al., 2014). The GCFR is known for the unique complexity of its toposcape, consisting of the small hills and undulations (microtopography), the large hills (mesotopography) and the mountains (macrotopography). These complex topographic patterns of the GCFR are known to be ten times older than the European Alps, and six times older than the Himalayas and Andes. A great diversity of plants has been observed in the Andes, Himalayas and the Cape Fold Mountains (Mittermeier et al., 2004; Mutke and Barthlott, 2005; Kier et al., 2009). Complex topography is an important driver of the diverse fynbos vegetation in this region, which supports a wide range of nectar sources for bird and insect pollinators, and made up of three plant families: the Proteaceae, Restionaceae and Ericaceae (Johnson et al., 2006). With no glaciations for tens of millions of years, and a complex topography with complex orographic patterns and soil types, there has been considerable speciation (Ellis et al., 2013), leading to very high levels of GCFR endemism among both the plants and the fauna.

While the topography has led to high levels of endemism, it is still unclear how biodiversity at different elevations responds to changing climate conditions associated with increased elevation. On a latitudinal scale, species richness has been shown to increase towards the equator (Willig et al., 2003; Armbruster, 2006). Similarly, for elevation gradients, species richness usually increases to a certain level in the middle elevation and then decreases with further increases in elevation. However, this pattern of response is different among different taxa and functional traits (Rahbek, 2005; Kessler et al., 2011; Guo et al., 2009; Sundqvist et al., 2013). Air temperature has been established as a major factor driving species distribution in this era of increased in global warming (Barry, 2008). At high elevations, weather conditions can become extreme and relatively unstable, which limits the species that can survive at high elevations (Guo et al., 2013). Lefebvre et al. (2018) showed that bee and beetle populations decrease rapidly above 1 500 m elevation on the Alps, which limits pollination activities. Warren et al., (1988) found a similar pattern in the distribution of anthophilous insects across various mountain ranges, with hymenopterans being the dominant flower visitors at lower elevations, but flies dominant at higher elevations, and a great decline in all insects at the peak.

Several studies have shown how changes in air temperature across elevation gradients influence biodiversity response to elevation (Kessler 2000; Yu et al., 2013; Kearns et al. 2017). While temperature should yield a monotonic decline in species richness and abundance, the pattern of abundance and richness is often humped-shape across elevation gradients. This suggests other factors apart from simply temperature are also playing a role, such as soil moisture, sunshine exposure, season length, among others (Körner, 2000). Indeed, topographic attributes alone could play a major role in determining the distribution of biodiversity across elevation gradients. Bertuzzo et al., (2016), found that connectedness of topographic features was the main factor determining the number of species that can occupy certain elevations on a topographic gradient, while most features of topography, such as peaks and valleys, are isolated, yielding few dominant species. In contrast, the mid-zone elevation has more connected patches forming a larger land area. This characteristics feature of mid-elevations is the main factor driving highest species richness at this zone compared to other elevations across the topographic gradient. In a sensitive ecosystem like the GCFR, where the complex topography is a key driver for species radiation and distribution, understanding the pattern of species assemblages may require a multi-directional approach, where many environmental factors associated with elevation are considered.

Land use change

Land use change, which results from the conversion of natural areas for other human uses, is one of the leading causes of pollinator decline globally (Kremen et al., 2002; Cairns et al., 2005; Potts et al., 2010b). With continuous increase in the global human population, the rate of habitat modification and destruction from landscape fragmentation, as well as habitat loss, is continually increasing. A total of 40% of ice-free land surface area was being used by humans in the year 2000, and an additional 37% was bordered by anthropogenic agricultural activities and modified areas (Eliss et al., 2010). These figures have been predicted to increase further as time passes (Tilman et al., 2001). Bellard et al., (2014) suggested non-natural habitats might increase by 8% in the next decade, and important biodiversity hotspots globally, including the Succulent Karoo, will lose about 20% of their herbaceous cover.

The current trend in global decline of natural areas will incur co-extinction of flowering plants and pollinators, and lead to loss of important ecosystem interactions. Weiner et al., (2014) showed the dependence of specialist insect pollinators on floral hosts, where loss of the flowering plant yielded a decline in pollinator diversity. Species sensitivity to loss of partners in mutualisms usually differs among taxonomic groups, based on how easily species adapt to transformation by choosing from other options available for their basic requirements. This is mostly influenced by ecological traits such as tongue length, dietary requirement, range size etc. (Murray et al., 2010; Roulston and Goodell, 2011). Species with limited dispersal ability relative to niche, time, interactions etc. are more vulnerable to displacement (Kassen, 2002). For example, sensitive specialist pollinators with a limited range of floral requirement are most vulnerable to increase land use intensity (Weiner et al., 2014). De Palma et al., (2015) showed how bee species respond to different degrees of land use, based on their traits, which include foraging range, niche breadth, reproductive strategy and phenology. Thus, understanding the species basic requirements based on functional traits in an ecosystem with diverse land use intensity may help develop conservation strategies for important species.

In the GCFR, common land use types include the conversion of natural areas for agricultural purposes (Heydenrych et al., 1999). One-third of the region has been transformed by agricultural practices (Rouget et al., 2003), and vineyards in the region constitute about 95% of the total vineyards in South Africa. This has contributed to the loss of important flora and fauna in this area of great ecological significance (Fairbanks & McGwire, 2004). Predictive land use modelling showed a total of 14 849 ha of threatened habitat is necessary for a viable vineyard, thus predicting 89.3% irreplaceable loss of fynbos/renosterveld mosaic in this region (Dean et al., 2004). Restoring degraded habitat may yield undesirable outcomes, as it may eventually be impossible to restore the food web and species composition to its initial state (Whitmore and Sayer, 1992; Novacek and Cleland, 2001). In a system where conservation plans are weighed against human needs through agriculture, it may be important to apply management strategies that optimise both requirements. For example, Kehinde and Samways (2014) found higher diversity of flower-visiting insects in organic vineyards that are bordered by natural land compared to the highly disturbed conventional vineyards in the CFR. Moderately managed agricultural lands, with patches of natural areas that act as refuges for pollinators may be the key factor in conserving pollinator diversity in this era of increased demand on agriculture (Tucker and Rehan, 2017).

Invasive alien species

Invasive alien plant species often compete with the indigenous species for resources, and can cause a decline in abundance and diversity of native plant species. Invasive alien species are often regarded as the second greatest cause of biodiversity decline globally (Mooney and Hobbs, 2000). Sometimes, alien species are characterized by floral traits that attract a variety of pollinators away from native species, resulting in more pollinator visits to alien than to indigenous plants (Aizen et al., 2008), resulting in a decline in visitation of pollinators to native species (Dietzsch et al., 2011; Montero-Castano and Vila, 2012). Impact of invasive alien plants is a major threat to biodiversity in South Africa, as many alien plants have invaded ecosystems and continue to spread (Kotze et al., 2010). Alien plants usually flower earlier than native species (Lake and Leishman, 2004), and some species of alien plants that flower after the native species are usually more abundant and colonize the habitat faster than native ones (Lloret et al., 2005). This may increase the competition rate for pollinators, and result in a decrease in pollinator visitation rate to the native plants, reduced seed set, and pollen deposition for native species (Brown and Mitchell, 2001). Competition for flowering plants among pollinators may also alter species composition of pollinators in invaded areas; in this case, pollinator species that are attracted to alien plants dominate the pollinator composition of the habitat and yield fewer visitations to native species (Muñoz and Cavieres, 2008). However, some studies have shown positive influence of alien species on the visitation rate of pollinators to native plants (Moragues and Traveset, 2005; Bartomeus et al., 2008). Both negative and positive impacts of alien plants result in alteration of composition and evolutionary trends of native species and pollinators in an ecosystem (Schlueter et al., 2009).

Sometimes, invasive alien trees are highly successful in colonised environments, and they can replace the native flowering plants in a short period (Harding, 2001). The long-term effect of flowering alien plants on pollinators and native plants has received little attention, and is poorly understood, especially in the CFR. Invasive alien trees have the greatest significant influence on biodiversity community composition and distribution of water in the CFR (Le Maitre et al., 2000; Gaertner et al., 2012). Pine (*Pinus* spp.) trees are significant group of alien trees in this region with features that enable species of this genus to invade new areas. These trees often form a closed canopy when fully grown, and they reduce the amount of sunlight reaching the understorey, thereby causing a loss in flowering plants and shrubs in invaded areas (Leege and Murphy, 2010; Franzese et al., 2017). Pine trees also have a higher water consumption rate compared to native fynbos shrubs, and they are highly significant in spreading wild fires quickly, especially in areas with high invasion intensity (van Wilgen et al., 2008; Wilson et al., 2018).

The GCFR has the highest proportion of tree invasion in the whole of South Africa (Henderson, 2007). The invasive trees (*Pinus*, *Eucalyptus*, *Acacia*, etc.) in this region compete with other native flowering plants for nutritional requirements, water and land area. Overall, the shrub-like native vegetation of the region is being displaced, leading to a decline in flower resources for insect pollinators. While more attention has focused on native flowering plants facing competition from flowering invasive plants for pollinators (Gibson et al., 2012; Gibson et al., 2013), little is known on the proportion of the native shrubs that are being lost to shade from invasive trees in the GCFR. With increasing transformation of landscapes in the GCFR through invasion and tree plantation, assessing how endemic native flowering shrubs respond to shading effect of the alien trees is important to quantify, not just in terms of the loss of native plants, but also the effect on stability and diversity of plant-pollinator interaction networks in the GCFR.

Fire and plants in the Greater Cape Floristic Region

Fire is a disturbance that has a major influence on landscapes, both in terms of their physical and biotic components (Bond et al., 2005), and has a major effect on terrestrial ecosystems globally (Keeley, 2012; Keeley and Brennan, 2012). While fire has always been affected by local climatic conditions, it is of particular concern in this era of rapid, anthropogenic climate change (Wilson et al., 2010; Trouet et al., 2010; Moritz et al., 2012). Recent global climate change patterns have a major effect on fire intensity and frequency (Westerling et al., 2006; Wilson et al., 2010; Aldersley et al., 2011). In many regions of the world, especially Mediterranean-type ecosystems (MTEs), warmer and drier weather conditions are predicted, resulting in an increase in frequency and intensity of fire in MTEs (Gitay et al., 2001).

The frequency of fire, its intensity, and its severity are important factors of fire regimes (Keeley et al., 2011) that impact various components of the ecosystems differently. The species assemblages of an ecosystem may be altered following increased exposure to increasing fire intensity (Schaffhauser et al., 2008; Vilà-Cabrera et al., 2008). Also, an increase in phylogenetic clustering (Ojeda et al*.,* 2010) and hierarchy of well adapted species may be influenced by differential fire intensity among localities. This is particularly evident in the GCFR, where fire is a major factor driving plant diversity (Kraaij and van Wilgen, 2014). Most of the plants in this region have traits that encourage the spread of fire, and the smoke from fire is essential for seed germination of many fynbos plant species (Brown et al., 2004).

While fire and smoke greatly influence the composition of plant communities, plant-pollinator interaction networks are prone to disruption from fire events. The flowering of plants following a fire event may precede the recovery of pollinators in the habitat, and this may affect the pollination process (Geerts et al., 2012) causing a mismatch. This is because the recovery of pollinators may be affected by the intensity and extent of the fire, and also availability of patches of unburned areas within the habitat (Watson et al., 2012). The pollinator assemblage may also differ after the fire event, and this may impact the effective pollination of the plants. Pryke and Samways (2009) found differences in composition of butterflies assemblages between recently burned and unburned areas in the GCFR. Although there is paucity of information on how pollinating insects respond to fire, there are reports of changes in butterfly composition after fire (Vogel et al., 2007), with fire listed as the major threat to three South African butterfly species that are on the IUCN Red List (Henning et al., 2009).

Response of biodiversity to fire differs among species and taxonomic groups. While fire can result in population decline of some group, other species may thrive better during and post fire. Species life cycle process and physical features can mediate both immediate and post-fire response in terms of survival, reproduction, and recolonization in fire prone landscape. For example, some plants are fire tolerant as they resprout after fire Most of these resprouters are adapted to multiple fire cycles and are mostly perennials (Pausas and Keeley, 2014). Most animal groups with positive immediate survival response to fire are vertebrates, with a few reptiles and amphibians showing population increases soon after fire in burned areas (Greenberg et al., 2008). For many insect species, the immediate response is emigration from fire area, or they perish (Silveira et al., 2010). However, some insects do exhibit positive long-term recovery with higher diversity post-fire in burned landscape. For example, Galbraith et al., (2019) found a higher diversity of bees in areas with highest fire severity in a burned landscape. However, the study also showed that some cavity nesting bees hid in insect-mined holes and among flower patches to enhance their survival during fire. Thus, for insects, especially the less mobile groups, in addition to their life cycle traits which differ among species and taxonomic groups, other environmental factors which include habitat quality, nesting, and niche selection may influence insect survival and recolonization of burned areas during fire.

Protecting these interacting insect species during periods of fire requires a feature of the ecosystem that can withstand the impact of the fire and other adverse environmental factors (such as loss of nectar resources, and increased exposure to the elements). This feature is usually specific land areas that escape fire, known as refuges (Lindenmayer et al., 2009; Brennan et al., 2011). In fire prone areas like the GCFR, refuges are important in retaining the original species composition and characteristics of the landscape, despite the changes brought on by fire in the surrounding area. The effectiveness of refuges to do this however, depends on the intensity, re-occurrence, and size of the fire (Nimmo et al., 2013). Most refuges, such as patches of unburned forest (Perera et al., 2007), have sufficient resources to sustain species of flora and fauna, and their interactions, after the fire. The size and number of these refuges depends on the severity of the fire and its dynamic behaviour (e.g. in response to wind intensity and direction), and this in turn, determines the percentage of species that can survive in the refuges.

The presence of refuges may influence ecosystem stability, and the occurence of these refuges in ecosystems may be related to post-fire successional trends (Banks et al., 2015). With increasing anthropogenic disturbances to ecosystems, the effect of fire is in synergy with other direct human impacts such as land degradation. This has led to indications that the higher impact of fire in recent times affects the components of ecosystems to a greater extent than it did prior to the Anthropocene (Brook et al., 2008). This implies a greater need for more effective refuges that can help preserve biodiversity during fire events.

The recovery and reassembly of insect pollinators and plants in interactions after fire events depends on the nature of the fire in terms of size, frequency, and intensity (Lindenmayer et al., 2013). There is limited information on composition of a long-term post fire succession, but this will vary between pollinator and plant taxa, as the functional traits of species can define the rate of persistence despite fire events, or because of them. Also, recolonization of pollinators depends on the spatial isolation of the refuge (Driscoll et al., 2012; Robinson et al., 2013) and this may also be in synergy with some traits of the pollinators, such as mobility of the species. Often, after a fire, there is rapid expansion of populations from within the refuge, and this expansion spreads to the burned areas when conditions are suitable and habitable for the pollinators (Watson et al., 2012). The duration and success of the recolonization of the burned area however, depends on the severity of the fire, and also on the pollinator assemblages that have persisted in the refuge areas. Importantly, the persistence of pollinators in refuges depends on how well the refuge is able to provide sufficient resources needed by the pollinators to sustain them until the burned area has recovered sufficiently for them to recolonize it (Watson et al., 2012).

Aims of this study

Knowledge of species' responses to factors driving community distribution in a transformed landscape is essential for the conservation of sensitive ecosystems. In the GCFR, topography, invasive alien trees, and fire are among the leading factors influencing species distribution across the CFR biodiversity hotspot (Brown et al., 2017; Verboom et al., 2015; Wilson et al., 2018). While response of flowering plants and other species of ecological significance to these drivers has been studied (Wilson et al., 2015; Kemp and Ellis, 2017), little is known about how mutualistic interactions, especially plant-pollinator interactions, respond to this pattern.

In the GCFR, the high rate of ecological speciation and radiation of the fynbos vegetation in the Cape Fold Mountains has been well documented (Goldblatt, 1997; Goldblatt and Manning, 2002; Linder et al., 2003; van der Niet and Johnson, 2009; Verboom et al., 2015). However, no published study has shown how the complex topography and its features influence the diversity of different pollinator taxa in interactions with flowering plants in this region. Similarly, while competition for insect pollinators between flowering invasive and indigenous plant species in this region has been documented (Gibson et al., 2012; Gibson et al., 2013), the shading effect of invasive alien trees (which have occupied many parts of the GCFR) on native flowering shrubs and on important interactions, has received less attention. In the case of fire, the fynbos community depends largely on fire at moderate intervals for reproduction (Vlok and Yeaton, 2000). However, how pollinators and plant-pollinator interactions persist during fire, and are recruited post-fire, has also received little attention in this region. These are important knowledge gaps in this region of great endemism, and where bee diversity matches plant diversity (Kuhlmann, 2005).

This study aims to elucidate how important native flowering plant and endemic pollinator taxa respond to changes across GCFR landscapes. It also aims to determine the effect of these changes on plant-pollinator interaction networks, which have only recently received more attention in this region (Kehinde and Samways, 2012; Gibson et al., 2012, Gibson et al., 2013; Brown et al., 2017; Benadi and Pauw, 2018). The influence of drivers of global change on interaction networks may be observable from the network topology beyond what can be assessed through changes in abundance and species richness (Memmott et al., 2007; Hegland et al., 2009). For example, some studies have shown the importance of conservation action through making inference from interaction network properties, even when the species richness is unaffected (Memmott et al., 2007; Aizen et al., 2008). A decline in flowering plant species richness may have a direct consequence on loss of mutualistic interactions such as pollination (Biesmeijer et al., 2006). Generally, there is an increase in the awareness of incorporating ecological interactions between species in understanding biodiversity response to changes across landscape (Tylianakis et al., 2007).

In this study, the impact of these drivers (topography, invasive alien trees and fire) will be assessed on four pollinator taxa (bees, beetles, flies and wasps), and on native flowering plant species diversity and abundance. Also, the response of plant-pollinator interaction network metrics that explain network stability, diversity and partitioning, based on floral requirements, will be assessed. In view of these aims, I ask the following research questions:

- Insect-flower interaction networks vary among endemic pollinator taxa over an elevation gradient:
    - How does species composition of pollinators vary across an elevation gradient?
    - Does change in pollinator composition track flowering plant communities across the elevation gradient?
    - What are the most important factors predicting pollinator distribution across the elevation gradient?
    - What is the pattern of change in network topology across the elevation gradient?

- Asynchrony among insect pollinator groups and flowering plants with elevation: significance for global climate change studies
    - How does abundance peak vary among pollinator taxonomic group and elevation zones?
    - How do flower and pollinator abundance and species richness vary among taxonomic group and elevation zones?

- Time since fire strongly influences pollinator composition in a historically fire-prone landscape:
    - How do species composition of pollinator and flowering plants differ according to time since last fire?
    - Does the dispersion pattern of pollinators track that of flowering plants across habitats with different post-fire age?

- Refuges from fire maintain plant-pollinator interaction networks:
    - How do fire refuges encourage the persistence of plant-pollinator interaction networks in fire-prone landscape?
    - How does elevation influence the effectiveness of fire refuges for the conservation of pollinators during fire?
    - How do fire refuges affect plant-pollinator interaction networks and species specialization in fire-prone landscapes?

- Trade-offs between retaining stands of alien trees and the conservation of indigenous flowering plants, pollinators, and rare vertebrates in a biodiversity hotspot:
    - What is the response of flower abundance to the proportion of shade from invasive alien trees in areas with different invasion density?
    - How does pine tree density influence plant-pollinator interaction network metrics and species diversity in invaded areas?

Limitation of study design

Like most ecological studies, the inference here may be limited by other site-specific conditions other than fire, elevation, and invasive alien trees. For instance, variation in other abiotic features such as soil structure, microclimate, etc. may influence observations here. Observation recorded from the clustering of sites at each elevation zone in chapters 2 and 3 may be driven by other spatial factors not measured here. Also, while the distribution of medium-term and short-term burned sites reduces variation in other confounding spatial structures, the block design of the long-term burned area may be influenced by different spatial factors not associated with medium-term and short-term burned areas in chapters 4 and 5. However, both small and tall alien trees sites were distributed randomly across the invaded landscape with limited influence of other cofounding variables on the observed pattern in chapter 6, and also showing site independence.

To reduce the effect of other spatial environmental variables, sites in all fire classes and across elevation should be distributed evenly across the landscape. This may be possible in a controlled burning experiments, or where interactions were observed across a continuous elevation gradient rather than topographic block zones. However, the situation here reflects how topographic zonation influences species interactions in chapters 2 and 3, and also how natural fire creates uneven patches of vegetation distribution across burned area in chapters 4 and 5. In block design of spatial distribution experiments such as natural fire and topographic zonation, caution is needed when drawing conclusion about the effect of measured environmental variables on pollinators and flowering plants across landscapes.

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Chapter 2

Insect-flower interaction networks vary among endemic pollinator taxa over an elevation gradient

Abstract

Interaction networks are sensitive to elevation gradients through changes in local distribution of interacting partners. Here, I use plant-pollinator interaction network metrics to assess the effect of elevation on flowers and flower-visiting insect assemblages on a sentinel mountain used for monitoring climate change in the flower- and insect-rich Greater Cape Floristic Region. I also use these interaction metrics to explain the effect of environmental factors on the interaction networks. I did this over four vegetation zones <1 640 m a.s.l., as determined by former botanical studies. Overall, bees were the dominant flower visitors, followed by monkey beetles (Hoplinii), and far behind were wasps and flies. The middle elevation zone (650-744 m a.s.l), which is also an ecotone between two distinct botanical zones, had the highest species richness and abundance of interacting plants and insects. Interaction frequency and size of network were also greatest in the middle zone, as were network diversity, generality, and linkage density, while lowest in the summit zone. In sum, there was distinct elevation zoning of flower-visiting insects. The greatest zonal change was between species at the middle compared with summit zone. Large monkey beetles, bees and flies characterized the unique assemblage in the summit zone (1 576-1 640 m a.s.l.). The insect zonation tracked that of plant assemblages, with air temperature (lapse rate) having a high influence on bee distribution, with lowest levels in the summit zone. In contrast, beetle distribution was mostly associated with flower assemblages as well as air temperature. In turn, wasp and fly interaction networks were not affected by any of the measured environmental variables. I conclude that increased elevation stress from reduced temperatures, changing abiotic weather conditions (e.g. strong winds at high elevations), and decline in flowering plant abundance and species richness across elevation zones cause breakdown of interaction networks involving bees and beetles but not that of flies and wasps.

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Introduction

Plant-pollinator interaction networks are valuable for assessing biodiversity change and landscape quality in response to stressors (Heink and Kowarik, 2010). Changes in these networks also lead to changes in the interaction metrics, most of which are defined by interaction frequency. However, careful analysis and interpretation of these metrics are important for identifying particular stressors on communities (Soares et al., 2017). As there are several network metrics used to interpret stressors, it is necessary to identify the ones that best explain specific patterns of change in interaction networks.

Natural ecosystems change across environmental gradients, as well as from turnover of mutualistic relationships among species (Devoto et al., 2005; Carstensen et al., 2014). Changes in mutualistic interactions, such as plant-pollinator interactions across latitudinal gradients, lead to interactions in the tropics being more specialised through high species diversity compared to that in temperate regions (Schleuning et al., 2012). Community composition and mutualistic interactions also respond to changes across elevation gradients, which illustrate how environmental stress influences biotic communities (Hodkinson, 2005; Beck et al., 2010). Responses of bee-plant interactions across elevation gradients have been explored (Miller-Struttmann and Galen, 2014; Widhiono et al., 2017). However, little information is available on bees compared to other pollinator taxonomic groups such as beetles, wasps and flies as regards their response to elevation gradients, especially in Africa.

Elevation gradients are an important component of many natural landscapes, and can greatly affect environmental variables, even over short range (Körner, 2007). These gradients provide opportunities for studying biotic responses to changes in air temperature (the lapse rate), precipitation, solar radiation, soil properties, reduced land area, and other abiotic features of montane ecosystems (Körner, 2000; Hodkinson, 2005; Brown and Vellend, 2014). With every 100 m increase in elevation, there is a drop of 1^0C in air temperature (Rolland, 2003), resulting in delayed growth and flowering of plants. Decrease in growth and density of flowering plants especially at peak elevations may reduce the attraction of pollinators to these plants and thereby yield a negative effect on pollination (Hadley and Betts, 2012).

Plant-pollinator interactions are sensitive to abiotic conditions that affect the interacting partners. Distribution of plant and insect pollinators across an elevation gradient determines pollination success, especially as there is often an increase in frequency and intensity of adverse weather with increasing elevation. With the exception of some plant species (e.g *Homogyne alpine, Primula farinose*) which show higher flower longevity in response to harsh conditions at peak elevations especially in the Alps (Trunschke and Stöcklin, 2017), the warmer conditions at low elevations allow longer flowering times, as well as affecting the local distribution of various insect species (Koti et al., 2005; Radmacher and Strohm, 2011). This 'low-elevation effect' positively affects mass flowering of some plant species (Schauber et al., 2002), with experimental warming in the arctic increasing reproductive success of flowering plants through an increase in number of flowers (Arft et al., 1999; Inouye et al., 2003).

There are few studies on how insect pollinator taxa are differentially affected across elevation gradients with their differing environmental conditions. However, there is some information on the differential effects of weather on various insect groups. For example, cold and wet weather positively influences the distribution of flies, while bees respond better to warm and dry conditions (Lázaro et al., 2008; González et al., 2009; Kovac and Stabentheiner, 2011; Nicolson et al., 2013). The response of different groups also depends on life history traits (Petanidou et al., 1995; Hodkinson, 2005; Purcell, 2011), including sociality, nesting behaviour, body size, reproduction pattern, diet requirements etc. Higher insect sociality is usually an attribute of warmer low elevations (Purcell, 2011), with voltinism depending on length of season and time of appearance of flowers, both of which decline or change with decreasing temperatures associated with increasing elevation (Chown and Gaston, 2010).

Availability of interacting partners at various elevations is an important determinant of the types of mutualistic interactions present. The local presence or absence of interacting partners is determined by temperature across the elevation gradient, as well as the respective, innate ability of the flowers to produce floral resources and the insects to pollinate at the various temperatures, as well as under particular weather conditions. Increase in temperature beyond the thermal tolerance level of a biotic community results in an upward shift along the elevation gradient to a cooler region (Deutsch et al., 2008; Hegland et al., 2009). Declines in abundance of ants and plants at high elevations result in fewer interacting partners high up, due to reduced richness and abundance of plant and ant species. At high elevations, there are fewer interactions, encouraging more connected networks, where they are less specialized (Plowman et al., 2017).

The bee fauna of the Cape Floristic Region (CFR) is among the most vulnerable to increased global warming due to the high level of endemism of most species, as well as the small size of this region in the southwestern tip of Africa (Kuhlmann et al., 2012). Bees, like most other pollinator taxa, are dependent on the environmental temperature for their activities. This may be a critical factor associated with foraging activities, body size at maturity, and the insect's life span (Radmacher and Strohm, 2011). Large-bodied bees, such as megachilids and apids, are capable of generating internal heat to optimize foraging activities, even when the environmental temperature is low (Oyen et al., 2016). However, smaller bees such as *Lasioglossum* spp. have to hibernate to avoid colder habitats during a temperature drop below thermal tolerance (Bishop and Armbruster, 1999). In Wyoming, USA, large bumblebees have high tolerance for low temperatures of about 1°C at a high elevation of 3 290 m asl. Conversely, bumblebee species at lower elevations are smaller, and have reduced tolerance to extreme temperatures (Oyen et al., 2016).

Elevation has been used to assess the effect of climate change on pollinators (Morris et al., 2015). However, little is known about how elevation influences plant-pollinator interaction networks. Most studies have been in the northern hemisphere with its history of glaciation events, while there are no studies yet in southern Africa which has had no glaciation for >200 myr. This is an important knowledge gap for a biodiversity hotspot like the CFR, where it is predicted that there will be a change in plant communities through a rise of 1.8°C by 2050 (Midgley et al., 2003).

I aim here to determine how different groups of insect pollinators and their interactions respond to changes in abiotic conditions with elevation. I hypothesize that: (i) species composition of flower-visiting insects will vary across the elevation gradient due to changing environmental conditions, (ii) change in flower-visiting insects species composition will track changes in flowering plant communities, (iii) flowering plant diversity and area of floral display will be the most important factors predicting changes in insect species composition, and (iv) since flower resource composition and diversity changes with elevation in my study area (Agenbag et al, 2008), network properties will also change across elevation zone

Material and methods

The study was conducted on Jonaskop Mountain (33°58'10.67"S, 19°30'21.96"E), Western Cape Province, South Africa, in the Greater Cape Floristic Region biodiversity hotspot with a research permit from CapeNature. The bee diversity of the GCFR is exceptionally high, coinciding with that of plants (Kuhlmann, 2005). The focal study area is 1 640 m high, and supports many localized sclerophyllous fynbos plant species. The mountain has distinct vegetation zones, and is a sentinel mountain for recording climate change (Camill and Clark, 2000).

My study sites with increasing elevation on the mountain were based on previous vegetation profiling (Agenbag et al., 2008). Lower elevations (< 550 m asl, 33°55'03.8"S , 19°30'46.1"E, 'Base zone') are characterized by succulent Karoo. Elevations 650-744 m asl (33°55'28.2"S, 19°30'59.4"E ,'Middle zone') are ecotone areas between the lower elevations, and the third zone (33°57'06.5"S, 19°31'02.0"E'High zone'), characterized by Mid-elevation Sandstone Fynbos at 953-1303 m asl. The summit elevation (>1576 m asl, 33°58'09.0"S, 19°29'45.3"E, 'Summit zone') is classified as High-elevation Sandstone Dwarf Fynbos (Mucina and Rutherford, 2005).

Plant-pollinator interactions were recorded at 18 sites within each of the four zones of the mountain at the peak flowering season (August – October, 2017). Each site was a 50 m^2 plot, and plots within any one zone were 100-500 m apart. Groups of these sites, representing the four zones, were 0.8-2 km apart. I observed interactions fortnightly at each zone, with plant-pollinator interactions conducted during five visits to each zone, except the summit, where the short flowering period permitted only three visits. I conducted all observations on days with no rainfall and minimal wind (usually < 6 km/s). I computed Moran I index (Moran, 1950) to assess spatial autocorrelation for interaction frequency among study sites established in each elevation zone. Moran I index (0.284 ± 0.104, P = 0.009) showed a weak site clustering of interactions at each elevation zone across the mountain.

Five replicate of 2 m^2 subplot were established in each 50 m^2 plot of a site. Timed observation of insect activity was standardized to 10 min/2 m^2 plot to avoid over-emphasizing the specialization of flowering plants (Gibson et al., 2011). During this time, an interaction was noted when an insect visited the stamen of a floral unit of a plant. Flower-visiting insects were identified in the field, or caught for later identification. Five replicates per 2 m^2 sampling unit, made a total of 50 min observation time per site for each visit. Insects were identified to morphospecies level with reference to collections from this region and using appropriate taxonomic guide (The bee genera and sub-genera of sub-saharan Africa (Abc Taxa, Eardly et al., 2010), Wasps and Bees in Southern Africa (Gess and Gess, 2014) and Insects of Southern Africa (Scholtz and Holm, 1985)). Rare/new species (mostly singletons) were identified as morphospecis by expert taxonomist in different insect taxon at the Department of Conservation Ecology and Entomology, Stellenbosch University. This enabled the effective comparison of network metrics and species composition in this study.

Flower abundance of each plant species was estimated in each 2 m^2 plot where insect activities were observed. A flower unit was defined here as the unit from which a honeybee-sized insect will fly to the next unit rather than walk (Dicks et al., 2002). Area of floral display was determined for each open flowering plant species by measuring the diameter of 1-10 flowers per plant species. Areas of flowers with circular outline was estimated using πr^2 and L x B for rectangular surface outline flowers. A flower with visible depth, such as that of *Protea repens*, was estimated using $2\pi r^2 d + \pi r^2$. The mean flower area for a plant species, together with the total abundance of flowers, was used to estimate the plant flower area per site (Vrdoljak et al., 2016).

Ambient air temperature was measured at each sampling period at the height of the flowers, and facing away from the direct radiation of sunlight in each 2 m^2 subplot using hand held thermometer (Testo 410-1). Temperature reading was computed twice per sampling period, I made temperature reading between 09:00 and 11:00, and also between 13:00 and 14:30 across sampling sites in each elevation per sampling period. I then computed the mean temperature per sampling period for each site visit. Plant indices included flowering plant species richness, estimated by counting the number of flowering plant species per 2 m^2 subplot and extrapolating this to the 50 m^2 plot, plant species common to two or more subplots were regarded as the same entry for species richness per study site. The Shannon diversity index, which takes into account the flower abundance and richness, was used to estimate flowering plant diversity.

Statistical analyses

Web structure for plant-pollinator interaction networks was computed for each site visit. Eighteen interaction web structures and metrics were computed using the plotweb, network level and species level function in the bipartite package (Dormann et al., 2008) in R (R Core Team, 2017). Network qualitative properties, such as species richness and abundance of flower and insects, were also computed.

I compared number of interactions, network size, and flower-visiting insect species richness across elevation zones using generalized linear mixed effect models. I applied the “glmer” function and specified “poisson” family for my data. To account for overlap between sampling of each zone, I included the sites as a random variable in my model. I computed the square root for flowering plant species richness and I included this in a general linear model (glm) using the function ‘glm’ in R to compute differences in plant richness across zones. Network metrics, such as connectance, network nestedness, linkage density, network specialization (H_2’), network generality, and interaction strength asymmetry were computed using the network level command in the bipartite package.

Definitions used here are as follows: 1) Connectance: the proportion of realized interactions out of all possible interactions in a network (Blüthgen et al., 2008), 2) Generality: explains the number of plant species available for an insect in the interaction network (Bersier et al., 2002), 3) Nestedness: describes the ability of specialist species in the network to interact with the species that also receive interactions from most generalised species in the network (Spiesman and Inouye, 2013), and ranges from 1 to 100, and usually confers stability to interaction networks where the higher the nestedness value, the more stable and resilient the network is to disruption (Tylianakis, et al., 2013), 4) Network specialization (H_2’):estimates the selection and constancy of interaction between partners in a network by calculating the deviation of observed interaction from the expected null frequencies of interactions (Blüthgen et al., 2006), and ranges from 0 (generalized network) to 1 (perfectly specialized network), 5) Species level of specialization (d’): describes the deviation of observed visits to expected visits based on interaction of a focal species of insect in a network, and is determined by the availability of floral resources (Benadi et al., 2014), 6) Linkage density: describes the degree of distribution of interacting partners in a network, and takes into account species richness and evenness of the distribution. Linkage density may be a better descriptor of network stability compared to nestedness of a network, but this is only the case for large networks (Dormann et al., 2008), and 7) Interaction strength asymmetry (ISA): the strength and degree of interaction between partners is not usually the same in a network, which means that the effect of an interaction between an insect and a flowering plant is not the same as the effect of interaction that the plant has with the insect. This metric helps to understand the mismatch in the effect that a species has on interacting partner, and is reciprocal in an interaction network (Vázquez et al., 2007).

Network indices were log transformed to fit into normal distribution following Shapiro-Wilk’s test, and data compared among zones using simple Analysis of Variance (ANOVA). Tukey HSD post-hoc test was computed to observe pairwise comparison among groups. I computed linear model to assess how interactions of insect taxonomic groups are associated with flower diversity and area of floral display using log-transformed data. Difference in composition of flower-visiting insects and flowering plant species among zones was estimated using Bray Curtis distance between zones in Primer v6. Analysis of Similarity (ANOSIM) was then computed to determine the degree of separation or similarity of interacting species between zones of elevation. Principal Component Ordination (PCO) was also computed to visualize the separation of insect activities across elevations.

The effect of area of floral display, flower richness, flower diversity, flower abundance, and temperature on frequency of interaction were computed using the Distance based linear model (Distlm) in Primer V6. Stepwise selection regression, together with Alkaike information criterion (AIC), were then used to assess the most important predicting variables that determine the frequency of interaction for individual taxonomic groups of flower-visiting insects. To see how changes in interaction, made by flower-visiting insects, tracks flowering plants, the RELATE function in Primer was used to compare the resemblance matrix of flower-visiting insects' interaction to the resemblance matrix of flowering plants. This function is important for comparing similarity of two sets of multivariate data matrices by calculating the rank correlation coefficient of the element of the two matrices (Clarke and Warwick, 2001).

Results

A total of 1 344 interactions were observed between 71 (Appendix 2a) flower-visiting insect morphospecies and 32 (Appendix 2b) flowering plant species. For all zones combined, interactions consisted of bees (53.5%), beetles (28.5%), wasps (9.1%), and flies (8.9%). This pattern was mostly consistent at each zone separately, with bees making up half of all interactions, except at the summit zone where bee interactions dropped to 36% and beetle interactions increased to 34%.

There were significant differences in both flower-visiting insect species and plant species richness across all zones combined. The highest species richness of flower-visiting insects ($z = 3.141$, $p = 0.008$, $df = 13$, Figure 2.1) and plants ($z = 3.532$, $p = 0.003$, $df = 14$, Figure 2.2) was recorded at the middle zone, and the lowest at the summit zone.

There was also a significant difference in frequency of interaction and network size across zones. Highest number of interactions ($z = 7.049$, $P<0.0001$, $df = 13$, Figure 2.3) and largest number of networks (z = 4.322, p<0.0001, df = 13, Figure 2.4) were in the middle zone, which differed significantly from the small-sized and few interactions in the summit zone. Most of the network metrics showed no significant differences across zones. However, network linkage density ($F_{3,14}$ = 4.145, p=0.027, Table 2.1), network generality ($F_{3, 14}$ = 5.528, p= 0.0101, Table 2.1), and network Shannon diversity ($F_{3,14}$ = 18.11, p=0.00004, Table 1) showed significant differences across zones. At the species level, flower visitors were moderately specialized (bee=0.43±0.03, beetle=0.45±0.06, fly= 0.32±0.04, wasp=0.33±0.05). However, mean specialization (d') index did not differ significantly across zones ($F_{3,129}$ = 0.795, p = 0.499), or among taxonomic groups ($F_{3,129}$ = 1.506, p = 0.216).

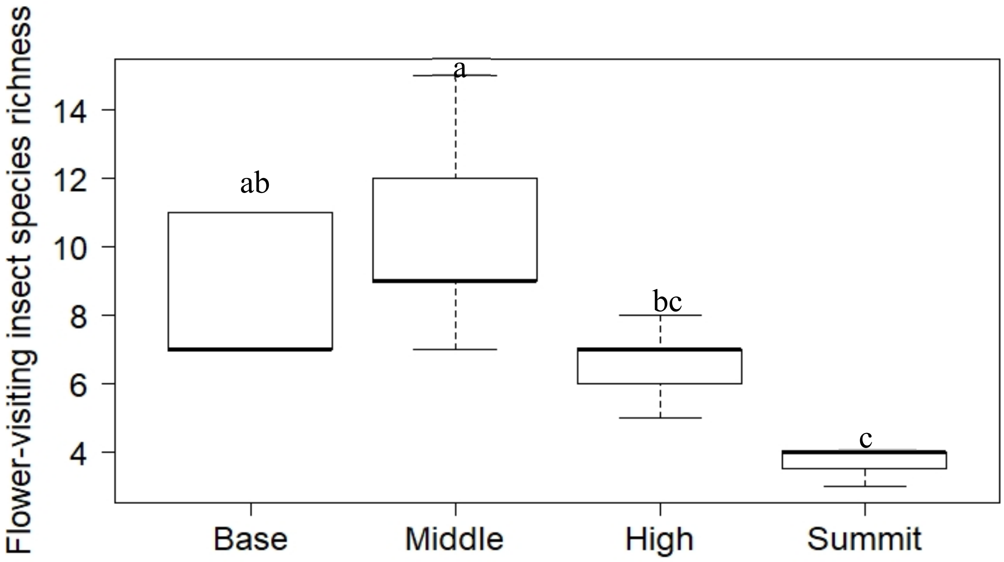

Figure 2.1. Species richness of flower-visiting insects across elevation zones. Zones with common letters are not significantly different at P > 0.05.

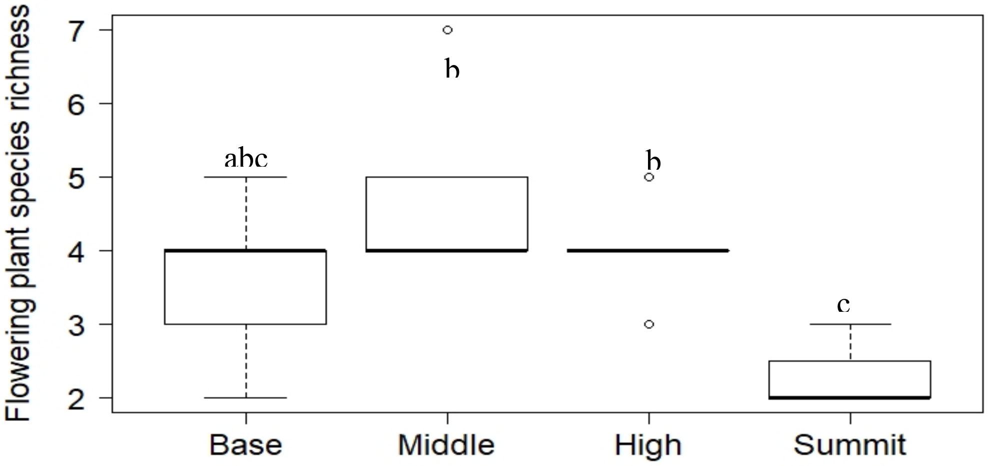

Figure 2.2. Species richness of flowering plants across elevation zones. Zones with common alphabets are not significantly different at P>0.05.

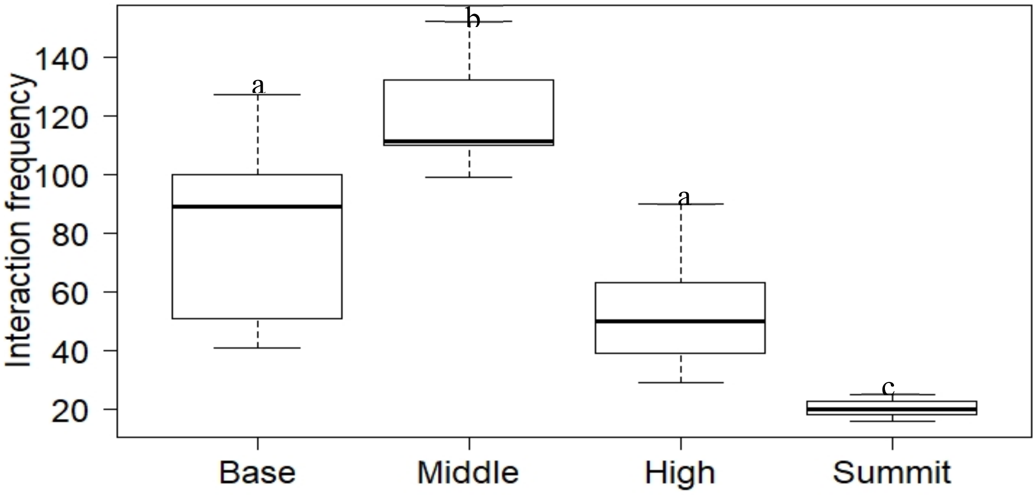

Figure 2.3. Interaction frequency across elevation zones. Zones with common alphabets are not significantly different at P>0.05.

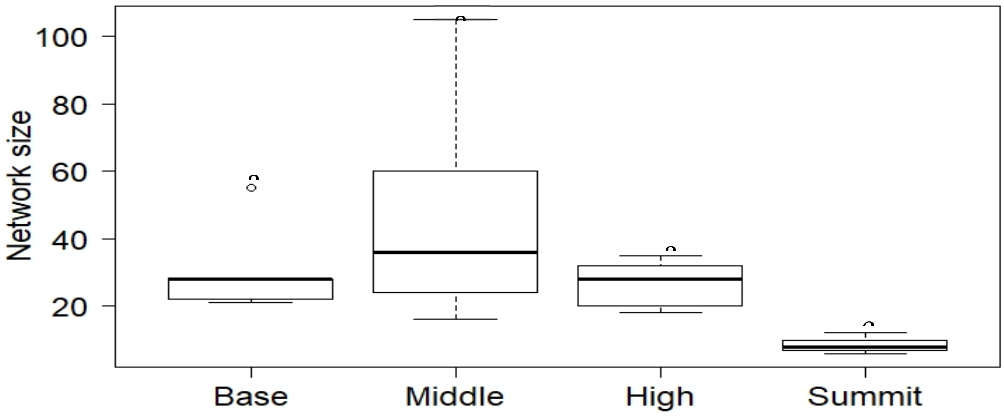

Figure 2.4. Interaction network size across elevation zones. Zones with common alphabets are not significantly different at P>0.05.

Overall, insect taxa activity increased significantly with flower diversity ($F_{1,\,51}$ = 9.085, R = 0.389, p = 0.004). However, this varied among taxonomic groups. Only bees showed significant association with flower diversity ($F_{1,\,16}$ = 6.782, R = 0.546, p = 0.019). There was no significant association between flower diversity and other taxa.

There was also a significant positive relationship between flower-visiting insect activity of all taxa and flower area ($F_{1,\,51}$ = 10.7, R = 0.417, p = 0.002), although the strength and direction of the relationship varied among insect groups. Similarly, only bees showed significant association with flower area ($F_{1,\,16}$ = 10.59, R = 0.631, p = 0.005). There was no significant association between flower area and other taxa.

Species separation across zones

The ANOSIM indicated a significant separation in species of flower-visiting insects across zones (R = 0.516, p = 0.001, Table 2.2). The greatest separation was between insect species at the middle zone and the summit zone (R = 0.968, p = 0.018, Table 2.2). The degree of separation differed significantly among taxonomic groups. Bees showed the greatest separation (R = 0.454, p = 0.001, Table 2.2) across zones, followed by beetles (R = 0.25, p = 0.005, Table 2.2), then wasps (R = 0.115, p = 0.008, Table 2.2). However, none of the pairwise comparisons was significant. There was no significant separation in fly species across zones (R = 0.034, p = 0.204, Table 2.2).

The PCO gives a visual representation of the separation among zones, as well as the direction of the environmental variables. Insect activities in the summit zone strongly separated out from those of all other zones (Figure 2.5). Temperature increased towards the base zone, which was similar to the direction of flow for flower richness, flower diversity, and flower abundance, while flower area increased towards the high zone (Figure 2.5)

Table 2.1. Mean (±SE) of network metrics in the four elevation categories.

	Base zone	Middle zone	High zone	Summit zone	F value	P value
Connectance	0.4±0.04	0.37±0.05	0.34±0.04	0.60±0.08	3.31	0.05
Nestedness	42.88±5.07	39.80±4.39	30.21±4.56	32.21±4.10	1.68	0.22
ISA	0.41±0.09	0.25±0.06	0.22±0.14	0.21±0.11	0.78	0.53
Shannon diversity	2.17±0.13	2.53±0.12	1.83±0.07	1.33±0.10	18.11	0.0004
Generality	1.42±0.11	1.91±0.15	1.40±0.10	1.24±0.07	5.53	0.01
Linkage density	2.52±0.39	2.83±0.20	1.92±0.20	1.53±0.06	4.15	0.03
Specialisation (H_2')	0.73±0.06	0.57±0.08	0.80±0.11	0.89±0.03	2.41	0.11

ISA =interaction strength asymmetry.

Table 2.2. Analysis of Similarity showing pairwise comparison of interaction frequency of insect taxa for elevation categories.

Elevation zones		R	P
All insects		**0.516**	**0.001**
Base	Middle	0.478	0.008
Base	High	0.68	0.008
Base	Summit	0.41	0.036
Middle	High	0.236	0.040
Middle	Summit	0.969	0.018
High	Summit	0.559	0.018
Beetles		**0.25**	**0.005**
Base	Middle	0.078	0.222
Base	High	-0.002	0.444
Base	Summit	0.333	0.054

Table 2.2 (cont.)

Middle	High	0.014	0.373
Middle	Summit	0.846	0.018
High	Summit	0.462	0.036
Wasps		**0.115**	**0.008**
Base	Middle	0.105	0.206
Base	High	0.118	0.111
Base	Summit	0.1	0.25
Middle	High	0.125	0.206
Middle	Summit	0.111	0.464
High	Summit	0.143	0.464
Bees		**0.454**	**0.001**
Base	Middle	0.568	0.008
Base	High	0.65	0.008
Base	Summit	0.462	0.036
Middle	High	0.22	0.063
Middle	Summit	0.744	0.018
High	Summit	0.251	0.107
Flies		**0.034**	**0.204**
Base	Middle	-0.034	0.762
Base	High	0.154	0.167
Base	Summit	-0.039	0.607
Middle	High	0.077	0.286
Middle	Summit	-0.1	0.786
High	Summit	0.143	0.036

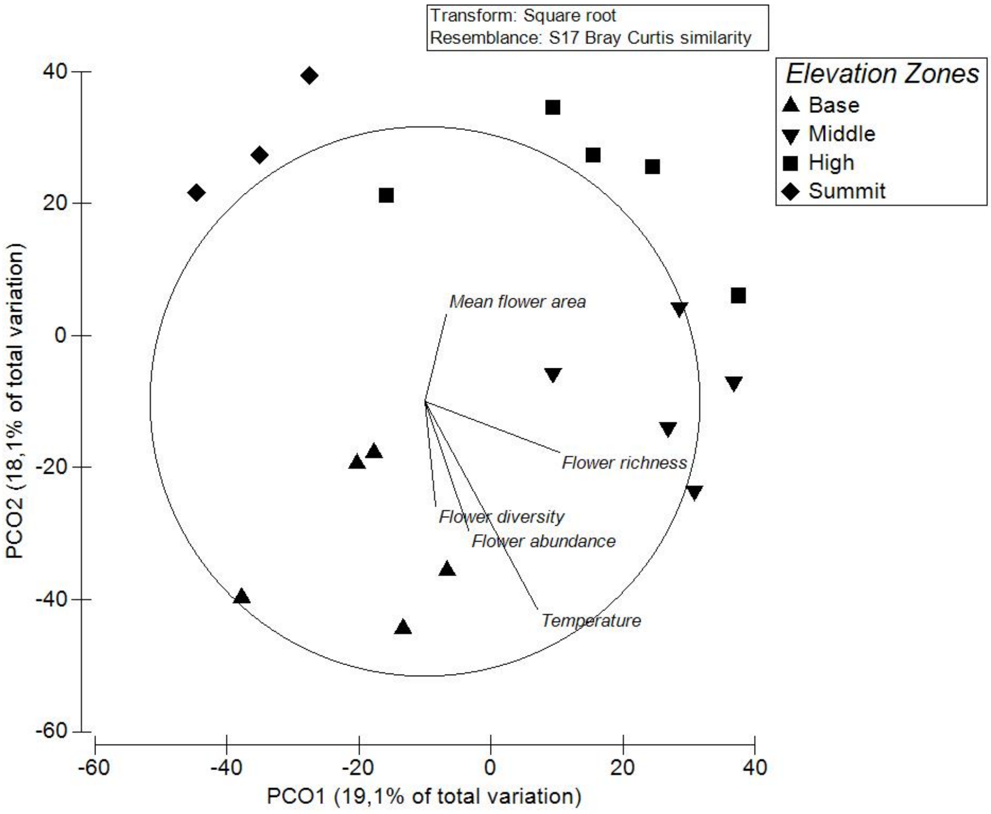

Figure 2.5. PCO showing separation of insect activities at different elevation zones, and the direction of environmental variables.

Effect of environmental variables on flower-visiting insects activities

Of all the explanatory environmental variables in my model, air temperature was the only significant factor driving the pattern of interaction of flower-visiting insects across the elevation gradient with a prediction of 14.4% of total insect activity (Table 2.3).

Similarly, when I assessed effect of these variables on interaction of different taxonomic groups, air temperature was the only significant factor explaining the variation of bee interactions across the elevation gradient, with a prediction of 15.6% (Table 2.3). In turn, variation in interactions involving beetles was more strongly influenced by flower abundance and flower area, with estimated predictions of 37.8% and 27.6% respectively, and a moderate prediction of 16.9% in the case of temperature (Table 2.3). None of the environmental variables in my model showed significant predictive ability for pattern of interaction observed in wasps and flies.

Relationship between plant composition and insect activities

The RELATE comparison showed a significant relationship between the resemblance matrix of plant composition and activities of flower-visiting insects across all zones (rho=0.418, p=0.001).

Table 2.3. Distlm model showing important predictors of insect visitation frequency among taxonomic group.

	Variable	AIC	SS(trace)	Pseudo-F	P	Prop.	Cumul.	Res.df
All insects	Temperature	149.28	9711.3	2.697	0.001	0.144	0.144	16
Beetles	Temperature	149.25	1170.6	3.257	0.002	0.169	0.169	16
	Flower area	148.78	7364.9	2.203	0.031	0.106	0.276	15
	Flower abundance	148.06	7051.9	2.291	0.023	0.102	0.377	14
Bees	Temperature	148.73	10319	2.955	0.003	0.156	0.156	16

AIC =Akaike information criterion, SS = Sum of square, Prop.= Percentage variation explained by variable in model, Cumul. = Cumulative percentage variation explained by model, Res.df = Residual degrees of freedom

Discussion

Bees are important pollinators, with most species being actively dependent on floral resources. The CFR is the only biodiversity hotspot where high plant and bee diversity coincide (Kuhlmann, 2005); this explains the dominance of bees in interactions with flowering plants, as seen here. The flower-visiting insect groups here showed a strong relationship with flower area and diversity, however, bees and beetles showed stronger association with flower area than with flower diversity in my result. Mass flowering, an attribute of several flowering plant species in the GCFR has a strong influence in attracting flower-visiting insect with wide area of display of compact inflorescence (Vrdoljak et al., 2016, Lázaro et al., 2009). In my study, mass flowering *Lobostemon, Helichrysum* and *Senecio* were diverse with wide open areas at the base, middle and high zones. This may explain the association especially between beetles and flowering plants here.

There was a significant difference in species richness of flower-visiting insects and flowering plants across the different elevation zones. Insects are usually highly sensitive to fluctuations in environmental factors, including those across different elevations. One of the major factors associated with elevation change is temperature, which usually influences foraging activities of flower visiting insects (Kilkenny and Galloway, 2008), and also the productivity of flowering plants (Zhao et al., 2012). Thus, flower visiting insects composition tracks flowering plant as I found here, and as reported by Winfree et al. (2009). This implies that the presence of different species of flowering plants at different zones of elevation in my study supports the distribution of different species of flower-visiting insects across the elevation gradient.

Although quantity of floral resource available in an ecosystem is one of the most important factors driving flower visitation by insects (Carnicer et al., 2009), here I show that the species of plant available is also of great importance, especially in a system like this one, with its distinct zonation of flowering plants. This largely supports my second hypothesis. Also, for a mountain like in this study, with varying level of environmental stress and a unique distribution of flowering plants, loss of important flower species at any zone may have a direct effect on the displacement of flower-visiting insects across elevations.

Interaction network properties

Here I show significant changes in some network quantitative indices across elevation zones. There was a significant difference in interaction linkage density across zones. Linkage density, which is the average number of feeding links for a species, is dependent on availability of interaction partners. On my mountain, this decreased at the summit elevation, as also observed on Mt. Wilhelm, Papua New Guinea (Plowman et al., 2017). The decline in interacting partners at high elevation also drove the pattern of network generality in my study. Highest network generality recorded in the middle zone is indicative of more floral resources, even though flower abundance was not significantly different across zones. Presence of highly rewarding flowering plants like *Lobostemon glaber* and *L. trichotomus* in high abundance at the base, middle and high zones may have led to higher generality at these elevations compared to the summit zone. Flowers of this genus are attractive to bees (Goldblatt et al., 2002; Gess and Gess, 2006) and received high visitation rate in my study. Highest network generality at the middle zone, which is also an ecotone, may be important for the stability of these networks and their resilience to environmental stress. As a link between zones, ecotones are evidence of rapid climatic transitions along gradients, and are important conservation areas in ecosystems (Kark, 2013). Furthermore, plant species richness was also highest in this zone. More flower resources may encourage selectiveness of flower-visitors, especially for more specialized insect species, and overall will confer stability and robustness to interaction networks (Bascompte et al., 2003; Olesen et al., 2007; Thébault and Fontaine, 2010).

Network diversity accounts for the distribution of interaction frequency among interacting species (Montoya and Yvon-Durocher, 2007; Hass et al., 2018). Communities rich in interacting partners are usually more stable than communities made up of few individual species (Cebrian, 1999; Dunne, 2006). In addition, network generality and linkage density were also highest at lower elevations, these also support more roboust networks at lower elevations compared to higher zones. Peak elevations are usually associated with extreme abiotic factors, and sometimes, these are associated with low species diversity in this zone. Although network diversity is low at the summit elevation zone here, nevertheless, some species are unique to this region and were not observed elsewhere on this mountain. This shows the resilience of these species to the harsh environmental conditions. While a few plants here are adapted to wind pollination, little is known about adaptive measures influencing the persistence of the unique insects at this zone. Understanding the ecology of these species at the summit zone may influence scientific knowledge of their distribution and preferred environmental conditions.

Separation across elevation and effect of temperature

Agenbag et al. (2008) showed a distinct separation in flowering plant species communities on this mountain. For the elevation-sensitive species here on this mountain such as *P. repens*, which prefers high rather than low zones, this plant species may be vulnerable to climate change as warmer and drier conditions begin to prevail in this Mediterranean-type ecosystem (Westerling et al., 2006).

Flower-visiting insect species at the middle zone were very different from those in the summit zone. Bees were the most sensitive to change in elevation, with significantly different species composition at each elevation zone. This suggests huge turnover in interactions among bees, determined mostly by temperature rather than flower indices, as explained by my model selection of environmental variables. In the case of ants, temperature was the major predicting factor for their distribution across a nearby elevation gradient (Longino and Colwell, 2011). Body size is important for how bee species tolerate adverse climatic conditions with elevation (Oyen et al., 2016). Here, I observed *Xylocopa* spp. and moderate-sized Megachilidae spp. across all elevations. However, *Lasioglossum* spp. and other small Halictidae differed in species composition at different elevations. Big Bombyliidae and the beetle *Clania glenlyonensis* were only recorded at the summit zone, with small monkey beetles only at the lower elevations.

The greatest difference in species interactions was between middle and summit zones. However, there was also a significant difference between the base and middle zones. The middle, ecotonal zone had the highest number of bee species interactions, as well as highest species richness, indicating the presence of most suitable abiotic conditions necessary for a rich interaction between bees and flowers. It appears that such ecotonal zones in general are rich in species and interactions (Loehle, 2000; Evans and Brown, 2017).The summit zone here had the fewest interactions involving bees, indicating that it is the least climatically suitable zone for supporting bee-flower interactions. Although temperature was the major predicting factor for local bee elevation distribution, the decline in flower diversity at the summit elevation may also have acted in synergy with temperature to reduce bee diversity (Scaven and Rafferty, 2013).

Beetles showed a weaker zonal difference than bees, although there was a highly significant difference in species composition between the lowest two zones and the summit, determined, as with bees, by temperature and flower composition. CFR plant diversity may explain the diversity of insect assemblages better than abiotic factors (Kemp and Ellis, 2017), with monkey beetles being one of the most important pollinator groups for most flowers here (Colville et al., 2002). Although bees have highest flower visitation, monkey beetles carry higher pollen loads, at least of Asteraceae and Aizoaceae species (Mayer et al., 2006). Decline in flower richness and diversity, especially in the summit zone, may be the most important factor, rather than temperature, influencing reducing beetle diversity over the elevation gradient. Nevertheless, plant diversity may have been driven in part by temperature in addition to the underlying factors of soil types, low nutrients, and orographic patterns (Agenbag et al., 2008), which in turn, may influence beetle diversity.

I found that flies and wasps were not significantly influenced by temperature or flower indices, and unlike bees, these groups are not obligate nectar feeders, and are less affected by flower abundance and composition (Lázaro et al., 2016). I show here that flies and wasps are less sensitive to changes in environmental factors, and so may be less suitable for monitoring of changes in flower indices and the abiotic effects of elevation.

Conclusion

As elsewhere, elevation stress here influenced interactions between plants and flower-visiting insects, but with the various insect groups being influenced differentially, by the direct effect of temperature and the indirect effect of flowering plant diversity and area. With an anticipated overall temperature increase in the area of about 1.8°C by 2050 (Midgley et al., 2003), my results suggest that the current insect-flower interactions, especially those involving bees, are vulnerable to temperature changes where interactions decline with reduced temperature at summit elevation. This may not necessarily be negative in view of the richer and more robust interactions at the lower elevations where temperature is higher, and the presence of monkey beetles, wasps and flies. However, there is likely to be loss of certain local species that are currently only at the summit elevation such as *Clania glenlyonensis* and *Bombyliidae* sp2. Also, with flowering plants driving insect composition across the elevation gradient, loss of flowering plant species unique to any of the zones, especially the summit zone where very few flowering plant species are available, may eventually result in a local displacement of visiting insects on this mountain. Finally, I provide here some data against which interaction networks could be compared in the future.

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APPENDIX

Appendix 2a. List of flower-visiting insect species

Taxa	Family	Genus	Species/Morphospecies
Bee	Halictidae	*Allodapula*	*melanopus*
Bee	Halictidae	*Halictus*	sp.1
Bee	Halictidae	*Halictus*	sp.2
Bee	Halictidae	*Halictus*	sp.3
Bee	Halictidae	*Halictus*	sp.4
Bee	Halictidae	*Halictus*	sp.5
Bee	Halictidae	*Halictus*	sp.6
Bee	Halictidae	*Halictus*	sp.7
Bee	Halictidae	*Lasioglossum*	sp.1
Bee	Halictidae	*Lasioglossum*	sp.2
Bee	Colletidae	*Colletes*	sp.1
Bee	Colletidae	*Colletes*	sp.2
Bee	Colletidae	*Colletes*	sp.3
Bee	Colletidae	*Colletes*	sp.4
Bee	Colletidae	Colletidae	sp.
Bee	Megachilidae	*Megachile*	sp.1
Bee	Megachilidae	*Megachile*	sp.2
Bee	Megachilidae	*Megachile*	sp.3
Bee	Apidae	*Amegilla*	sp.
Bee	Apidae	*Anthophora*	sp.1
Bee	Apidae	*Anthophora*	sp.2
Bee	Apidae	*Anthophora*	sp.3
Bee	Apidae	*Anthophora*	sp.4
Bee	Apidae	*Apis*	*mellifera*
Bee	Apidae	Apidae	sp.1
Bee	Apidae	Apidae	sp.2
Bee	Apidae	*Xylocopa*	*olivacea*
Bee	Apidae	*Xylocopa*	sp.1
Bee	Apidae	*Xylocopa*	sp.2
Bee	Apidae	*Xylocopa*	sp.3
Bee	Apidae	*Xylocopa*	sp.5
Bee	Apidae	*Xylocopa*	sp.6
Bee	Apidae	Apidae	sp.3
Bee	Apidae	*Tetralonia*	sp.1
Bee	Apidae	*Tetralonia*	sp.2
Bee	Apidae	Apidae	sp.
Beetle	Cerambycidae	Cerambycidae	sp.1
Beetle	Cerambycidae	Cerambycidae	sp.2
Beetle	Cerambycidae	Cerambycidae	sp.3
Beetle	Cerambycidae	Cerambycidae	sp.4
Beetle	Cerambycidae	*Typocerus*	sp.
Beetle	Scarabaeidae	*Heterochelus*	sp.
Beetle	Scarabaeidae	*Hedybius*	sp.
Beetle	Scarabaeidae	*Peritrichia*	sp.1
Beetle	Scarabaeidae	*Peritrichia*	sp.2
Beetle	Scarabaeidae	*Peritrichia*	sp.3
Beetle	Scarabaeidae	*Clania*	*glenlyonensis*
Beetle	Scarabaeidae	*Anisonyx*	*ursus*
Beetle	Scarabaeidae	*Anisonyx*	sp.
Fly	Muscidae	Muscidae	sp.1
Fly	Muscidae	Muscidae	sp.2
Fly	Syrphidae	Syrphidae	sp.1
Fly	Syrphidae	Syrphidae	sp.2
Fly	Syrphidae	Syrphidae	sp.3
Fly	Bombyliidae	Bombyliidae	sp.1
Fly	Bombyliidae	Bombyliidae	sp.2

Fly	Bombyliidae	Bombyliidae	sp.3
Fly	Bombyliidae	Bombyliidae	sp.4
Fly	Calliphoridae	Calliphoridae	sp.
Fly	Culicidae	Culicidae	sp.
Wasp	Sphecidae	Sphecidae	sp.1
Wasp	Sphecidae	Sphecidae	sp.2
Wasp	Sphecidae	Sphecidae	sp.3
Wasp	Sphecidae	Sphecidae	sp.4
Wasp	Vespidae	Vespidae	sp.1
Wasp	Vespidae	Vespidae	sp.2
Wasp	Eumenidae	Eumenidae	sp.1
Wasp	Eumenidae	Eumenidae	sp.2
Wasp	Pompilidae	Pompilidae	sp.1
Wasp	Pompilidae	Pompilidae	sp.2
Wasp	Icheumonidae	Ichneumonidae	sp.

Appendix 2b. List of plant species

Family	Genus	Species
Aizoceae	*Ruschia*	*tecta*
Asteraceae	*Arctotis*	*gousblom*
Asteraceae	*Oedera*	*squarrosa*
Asteraceae	*Helichrysum*	*dasyanthum*
Asteraceae	*Oncosiphon*	*grandiflorum*
Asteraceae	*Cullumia*	sp.1
Asteraceae	*Cullumia*	sp.2
Asteraceae	*Senecio*	sp.1
Asteraceae	*Senecio*	sp.2
Asteraceae	*Senecio*	sp.3
Asteraceae	*Heterolepis*	*aliena*
Asteraceae	*Dimorphotheca*	*nudicaulis*
Asteraceae	*Othonna*	*bulbosa*
Asteraceae	*Metalasia*	*muricata*
Boraginaceae	*Lobostemum*	*glaber*
Boraginaceae	*Lobostemum*	*dorotheae*
Boraginaceae	*Lobostemum*	*trichotomus*
Geraniacea	*Pelagonium*	sp.
Malvaceae	*Hermannia*	*alnifolia*
Montiniaceae	*Montinia*	sp.
Montiniaceae	*Montinia*	sp.2
Polygalaceae	*Polygala*	*fruticosa*
Polygalaceae	*Muraltia*	sp.
Proteaceae	*Protea*	*repens*
Proteaceae	*Protea*	*laurifolia*
Proteaceae	*Leucadendron*	*laureolum*
Proteaceae	*Leucadendron*	*salignum*
Ericaceae	*Erica*	sp.1
Ericaceae	*Erica*	sp.2
Ericaceae	*Erica*	sp.3
Rhamnaceae	*Phylica*	*ericoides*
Fabaceae	*Acacia*	sp.

Chapter 3

Asynchrony among insect pollinator groups and flowering plants with elevation: significance for global climate change studies

Abstract

Mountains influence species distribution through differing climate variables associated with increasing elevation. These factors determine species niche ranges and phenology. Although the distribution patterns of some insect groups relative to elevation have been established, how differing environmental conditions across elevation zones differentially influence the phenology of various insect groups is largely unknown. This is important in this era of rapid climate change. I assess here how species composition and seasonal peaks in abundance among different insect pollinator groups and flowering plants differ across four floristically distinct elevation zones up a sentinel mountain subject to strong weather events. I sampled insect pollinators in four major groups (bees, wasps, beetles and flies) over two spring seasons. Pollinator species composition across all elevation zones track flowering plant species composition. In terms of abundance, beetles were the dominant group across the three lower zones, but declined greatly in the summit zone, where flies and bees were more abundant. Bee abundance peaked earlier than the other groups across all four elevation zones, where there were significant peaks in abundance. Bee abundance peaked earlier than flowering plants at the middle zone and slightly later than flowering plants at the base zone, suggesting a mismatch. I conclude that, while elevation shapes species distribution, it also influences species phenology, and can lead to a mismatch as seen here. This may be of great significance in long-term assessment of species distribution in sensitive mountain ecosystems.

Introduction

Mountains are highly significant drivers of species diversity and dispersion patterns. They occur in about half of earth’s biodiversity hotspots (Spehn et al., 2010) and they are often rich in the diversity of endemic species. Different elevation zones have different degrees of exposure to weather and climate (Van Beusekom et al., 2015). Besides the standard lapse rate in temperature, there is great variation in wind, humidity, precipitation, and orographic patterns, differentially affecting the biota at various elevations (Körner, 2000), especially species abundance and distribution patterns across the elevation gradient (Hodkinson, 2005).

There is not always a monotonic response in species diversity across elevation gradients. Sometimes, species richness and abundance increase and peak at mid-elevations (Kessler 2000), then decrease with increasing elevation. This response pattern is however, dependent on taxonomic group. For example, the abundance of beetles show an arched-relationship with elevation (Yu et al., 2013), as do bumble bees (Kearns et al. 2017) at mid elevations. However, army ants show a linear decline in abundance with increasing elevation (Kumar and O'Donnell, 2009). Rahbek (2004) found that 50% of the studies done on species response to elevation gradients show arch-shaped species richness patterns, while species richness with linear patterns made up only 25%.

The determining factors of differential response among taxa to elevation gradients are not well understood, although bees track flowering plant diversity (Steffan-Dwenter and Schiele, 2008; Winfree et al., 2009), which in addition to other abiotic factors, influence bee distribution in terms of species richness and abundance across elevation gradients. Flies on the other hand, are better associated with cold temperatures, and often dominate at high elevations beyond the limits of other taxa (Kearns, 1992; Zoller et al., 2002; Lefebvre et al., 2018).

In addition, elevation shapes abundance peaks and species composition (Benadi et al., 2014). The adaptation of different taxa to differing air temperatures associated with elevation may influence species abundance and composition. For example, flies are well adapted to cool and wet areas, while bees are mostly in dry and warm habitats (Totland, 1993; Lázaro et al., 2008). In the case of flowering plants, Fitter and Fitter, (2002) showed how temperature influences first flowering day among plant functional groups, first flowering day of insect-pollinated plants were more prone to temperature fluctuation compared to wind pollinated plants. Furthermore, some flowering plant species have low flower abundance associated with increasing spring temperature (Inouye et al., 2003) especially at low elevations. However, for some mass flowering species, high spring temperature drives high abundance (Schauber et al., 2002). In a sensitive ecosystem like the Greater Cape Floristic Region (GCFR) of South Africa, where bee diversity matches plant diversity (Kuhlmann, 2005), high species seed set may ensue when mass flowering is associated with high bee abundance in warm elevation zones. Studies assessing influence of air temperature change on bee composition and diversity are few, yet there may be differences in species response influencing productivity across elevation zones.

Factors driving species distribution patterns across elevation gradients also influence their phenology over space and time (Hegland et al., 2009; Hodgson et al., 2011). Phenology usually describes species natural seasonal pattern, which may be associated with seasonal appearance (Fitter and Fitter, 2002; Menzel et al., 2006), abundance peaks (Waser, 1976; Lefebvre et al., 2018), flowering duration (Menzel and Fabian, 1999; Miller-Rushing and Primack, 2008), insect flight duration (Roy and Sparks, 2000; Sparks and Collinson, 2007), among other life activities. Shifts in phenology of important flower-visiting insect taxa provide evidence of global change (Roy and Sparks, 2000; Stefanescu et al., 2003). For example, butterflies are a model taxon for understanding climate change through shifts in phenology (Stefanescu et al., 2003; Dell et al., 2005; Menzel et al., 2006).

Using temporal patterns of species phenology to understand environmental change is well established (Roy and Sparks, 2000; Forister and Shapiro, 2003; Hegland et al., 2009). However, studies assessing spatio-temporal response of phenology are few (Doi and Takahashi, 2008; Hodgson et al., 2011). Response of species phenology to spatial patterns may differ from year to year (influenced by time) with varying climate (Thompson and Clark, 2006; Doi et al., 2008; Doi and Takahashi, 2008). Although global warming can bring about early onset in appearance of flowering plant and bee species (Hegland et al., 2009; Mohandass et al., 2015), little information is available on how elevation mediates this pattern.

While most of the spatio-temporal studies addressing insect and plant diversity have been across latitudinal gradients (Doi and Takahashi, 2008), studies across elevation gradients have only recently been conducted (Benadi et al., 2014; Lefebvre et al., 2018). I investigate this in the CFR, a Mediterranean-type ecosystem with a rich and complex topography across most of its expanse. The mountains are ancient (about 600 my at base to 300 my at summits), and support extensive species radiation of angiosperms, characterized by the sclerophyllous fynbos vegetation. Specifically, I focus on how elevation zones influence species composition and peaks in richness and abundance of different insect pollinator taxa in the CFR. I hypothesize that 1) since species sensitivity to abiotic conditions differ across elevation zones (Lefebvre et al., 2018), there will be differences in abundance and richness among taxa across elevation zones, 2) there will be differences in species composition of flowering plants and insect pollinators across elevation zones, since species niche range and flora requirement may differ across elevation zones, 3) since flower visiting insects especially pollinators track floral resources (Winfree et al., 2009), changes in species composition of insect pollinators will track composition of flowering plants across the elevation zones, 4) since flowering peak period may differ among plant species, seasonal abundance peaks will also vary among insect pollinator taxa tracking flower distribution across elevation zones

Material and methods

The study was undertaken on Jonaskop Mountain (33°58'10.67"S, 19°30'21.96"E), Western Cape Province, South Africa, in the Cape Floristic Region biodiversity hotspot. The bee diversity of the CFR is exceptionally high, coinciding with that of plants (Kuhlmann, 2005). Jonaskop Mountain, my focal study area, reaches 1 640 m a.s.l., and supports many localized sclerophyllous fynbos plant species. The mountain is highly exposed to extreme weather events (cold, wet and windy in winter, and hot and dry in summer), has distinct vegetation zones (Agenbach et al. 2008), and is a sentinel mountain for monitoring climate change.

Our study sites, with increasing elevation on the mountain, are those used previously in vegetation profiling (Agenbag et al., 2008). Low elevations (< 550 m asl, 33°55'03.8"S , 19°30'46.1"E, ‘Base zone’) are characterized by succulent karoo. Elevations 650-744 m a.s.l. (33°55'28.2"S, 19°30'59.4"E ,‘Middle zone’) are an ecotone between the lower elevations, and the third zone (33°57'06.5"S, 19°31'02.0"E ‘High zone’) and is characterized by Mid-elevation Sandstone Fynbos at 953-1303 m a.s.l. The highest elevation (>1576 m asl, 33°58'09.0"S, 19°29'45.3"E, ‘Summit zone’) is classified as High-elevation Sandstone Dwarf Fynbos (Mucina and Rutherford, 2006).

Flower visiting insects in the area belong mostly to four major taxa: bees and wasps (Hymenoptera), beetles (Coleoptera: mostly Scarabaeidae, and also some beetle families whose roles as flower visitors have been identified from literature), and flies (Diptera: mostly Syrphidae, Bombyliidae and Muscidae, including other families whose role in flower-visitation has been recorded). These taxa were sampled across 18 study sites (as in Chapter 2) August-November 2017 and 2018, the flowering time of most plants in the area. Five study sites each were established in the first three zones with only three study sites established at the summit zone due to low flower distribution. Sites were selected with increasing altitude from 385 m – 550 m asl (Base zone), 712 – 740 m asl (Middle zone), 990 m – 1 250m asl (High zone) and >1570 m asl (Summit Zone). Sites were selected along increasing elevation at each zone based on their abundant flower cover to account for areas with highest composition of flowering plants at each elevation zone.

Each site was a 50 m^2 plot. Plots within any one zone were 100-500 m apart. Groups of these sites, representing the four zones, were 0.8-2 km apart. I made nine visits to each of the first 3 zones, each site was visited twice (once in each year) with the exception of one site in each of the middle, high and base zone which were partly or completely burned in the second year. I only made six visits to the summit zone with every site visited in the second year. These were the only areas with flowers at the summit elevation with zero flowers recorded elsewhere at this zone. Study sites were visited fortnightly in each sampling year, pooled insect and plant abundance data in each site were used to assess differences in species abundance across elevation zones. However, repeated collections in different sampling days in the same sites were used in assessing species phenology across sampling days and elevation zones.

Insects were sampled with yellow pan traps at all elevation zones. I also included white and blue pan traps during a pilot study. However, these collected very few insects and were omitted from the study. At each collection time, 10 yellow bowls (2 000 ml) were half-filled with water, and raised to vegetation height at each elevation zones for a period of 24 hours. Insects caught in each bowl were collected, stored in 70% ethanol, and then sorted. Insects were identified based on morphospecies with reference to collections from this region and using appropriate taxonomic guild (The bee genera and sub-genera of sub-saharan Africa, Abc Taxa, (Eardly et al., 2010); Wasps and Bees in Southern Africa (Gess and Gess, 2014) and Insects of Southern Africa (Scholtz and Holm, 1985)). Rare/new species (mostly singletons) were identified as a new species/morphospecis by expert taxonomist in different insect taxon at the Department of Conservation Ecology and Entomology, Stellenbosch University. Morphospecies count was used as abundance measure in my statistical analysis. I estimated flower abundance at each study site in five replicates of 2 m^2 plots. A flower unit was defined here as the unit from which a honeybee-sized insect will fly to the next unit rather than walk (Dicks et al., 2002). I counted the flower units of identified plant species in each plot, and I pooled these data over the entire 50 m^2 plot. I also estimated flower species richness at each study plot across all elevation zones at each visit.

Statistical analyses

To asses sampling adequacy, I estimated species rarefaction curves for flowering plants and anthophile species richness across study sites using the ‘rarefy’ function in ‘vegan’ R package (Oksanen et al., 2006).

I used Analysis of variance (ANOVA) to test for difference in overall insect abundance and species richness across elevation zones and insect taxonomic groups. Pooled data for abundance and species richness of insects and flowering plants across site visits were normally distributed according to Shapiro-Wilk’s normality test. Tukey HSD post-hoc test was computed to observe pairwise comparison among groups. To assess how insect abundance and species richness differ among taxonomic group at each elevation, I used a generalised linear model (GLM) in R version 3.4.1 (R core team, 2017) and specifying Poisson distribution. Here I specified abundance and species richness as dependent variables in different models, insect taxa at each elevation was specified as the fixed factor. To investigate how species composition varied across elevation gradient, I used a Bray Curtis dissimilarity matrix, and compared this among elevation zones using PERMANOVA in Primer 6 software. I also repeated the same procedure for species composition among taxonomic group. The PERMANOVA was performed in both cases using the Bray-Curtis similarity index obtained from the square-root transformation of abundance data and selecting Type III SS sum of square to account for unbalanced design (Anderson et al., 2008). Data were permuted 999 times for the analysis.

I further tested for pairwise comparisons where significant differences were observed. Similarly, I used the same pattern to assess the difference in the species composition of flowering plants across elevation zones using square-root transformation of abundance data. To understand whether flower composition tracks insect composition, I compared the resemblance matrix of flower-visiting insects with that of flowering plants across elevation zones using the RELATE function in Primer 6 software as in Chapter 2.

To determine the period of peak abundance during the sampling season and differences among taxonomic groups across sampling days (using abundance data collected in each site visit), I converted my sampling days to respective Julian days (continuous numerical values across the calendar year) for each elevation zone (Lefevbre et al., 2018). I then used GLMs (specifying Poisson distribution) to assess the relationship between abundance and Julian days of sampling. To establish the abundance peak at each zone among insect taxa, I specified insect abundance as the dependent variable; Julian day and insect taxonomic group at each elevation zone were the fixed factors. I also included the quadratic term of Julian day (JD^2), and I observed two-way interactions between JD and each insect taxon and between JD^2 and each insect taxon. I also computed this using a stepwise model simplification and I removed the non-significant variables or interactions.

Results

A total of 4 912 insect individuals belonging to 253 morphospecies were sampled (Appendix 3a). Beetles constituted 78.5% of the total collection, and far behind, were bees (9.7%), flies (8.9%) and wasps (2.3%). 52 flowering plant species were also recorded across all the elevation zones over the sampling period. The rarefaction curve for flowering plants (Chao = 82.30 ± 16.40, Jackknife2 = 90.63) across the study sites reached an asymptote (Appendix 3b). However, the rarefaction curve for insect species (Chao = 447.95 ± 46.25, Jackknife2 = 470.77) across sampling sites did not reach an asymptote (Appendix 3c), as there were many rare taxa and singletons in the samples.

Overall, there was a significant difference in insect abundance across the elevation zones ($F_{3, 14} = 5.10$, $p = 0.014$). Highest insect abundance was recorded in the middle zone, and lowest in the summit zone (Figure 3.1). There was no significant difference in species richness of insects across elevation zones ($F_{3, 14} = 1.12$, $p = 0.373$).

There was a significant difference in flower abundance across elevation zones ($F_{3, 14} = 3.637$, $p = 0.039$). Highest flower abundance was in the base zone, and lowest in the summit zone (Figure 3.2). There was no significant difference in flowering plant species richness among elevation zones ($F_{3, 14} = 3.186$, $p = 0.057$)

Abundance and richness among insect taxa

There was a significant difference in insect abundance among taxonomic groups at the base, middle, high and summit zone. Beetles made up the dominant group at the three lowest elevations, especially at the middle elevation zone, but beetle abundance declined sharply at the summit, where bees and flies were more abundant (Figure 3.3a). Wasp abundance was the lowest among taxonomic group over all the elevation zones. Across taxonomic group, beetle were the most abundant and this was significantly different from the lowest abundance recorded for wasps at the base zone ($z = -21.49$, $p < 0.0001$, $df = 16$), middle zone (z = -22.90, p < 0.0001, df = 16), high zone (z = -14.03, p < 0.0001, df = 16) and summit zone (z = -5.307, p < 0.0001, df = 8)

Similarly, there was a significant difference in insect species richness among taxonomic groups across all elevation zones. Beetles were the most species rich taxon in the three lowest elevation zones, however, species richness of beetles declined at the summit where flies and bees had higher species richness (Figure 3.3b). Overall, beetle had the highest species richness among insect taxa and this was significantly different from the low species richness recorded for wasps at the base zone (z = -5.434, p < 0.0001, df = 16), middle zone (z = -7.300, p < 0.0001, df = 16), high zone (z = - 5.486, p < 0.0001, df = 16) and summit zone (z = -2.052, p = 0.04, df = 8)

Phenology among taxonomic groups

There was significant difference in species abundance across Julian days and taxonomic groups at the base elevation zone. While bees and beetles showed distinct abundance peaks, flies and wasps showed none. There was no significant difference in abundance of bees and beetles (z = 0.534, p = 0.593, df = 35); however, flowering plants peaked ~6 days earlier than bees (z = -2.611, p = 0.009, df = 35) and ~10 days earlier than beetles (z = -7.800, p < 0.0001, df = 35, Figure 3.4a).

Similarly, at the middle elevation zone, there was a significant difference in species abundance across sampling days and taxonomic group. At this zone, there was significant difference in abundance peaks of bees and beetles (z = -2.096, p = 0.036, df = 35) with bees reaching the peak of abundance 7.5 days before beetles. There was no significant difference in peak abundance of bees and flowering plants (z = -1.876, p = 0.06, df = 35). However, bees peaked in abundance 7.5 days earlier than flowering plants (Figure 3.4b).

There was no significant difference in abundance across sampling days and taxonomic group at the high elevation zone (Figure 3.4c). Although, species abundance was significantly influenced by the interaction between Julian day and insect taxa at the summit, none of the insect taxa showed significant peak abundance over the sampling period (Figure 3.4d).

Species composition

There was a significant difference in species composition of insects across elevation zones (PERMANOVAF = 2.7114, p = 0.001). Species composition at any two of the elevation zones was significantly different. However, the most significant dissimilarity in species composition was among species recorded at the summit and middle elevations, as well as in the summit and base zones (Figure 3.5a). Similarly, there was a significant difference in species composition of flowering plants across the elevation zones (PERMANOVAF = 4.5306, p = 0.001). Flowering plant species composition showed strong differences across any two of the elevation zones (Figure 3.5b).

There was a significant correlation in resemblance matrix of flowering plant composition and flower-visiting insect composition across elevation zones (Rho = 0.138, p = 0.0008)

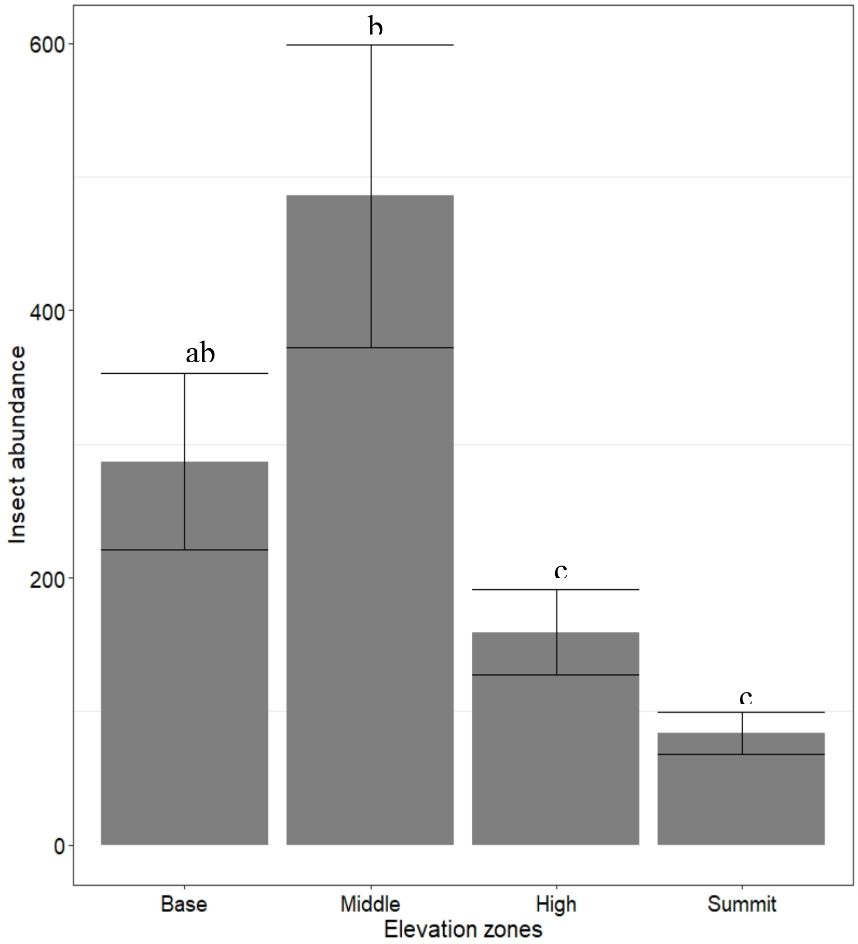

Figure 3.1 Mean insect abundance (±SE) among elevation zones. Bars with common letters are not significantly different at p > 0.05.

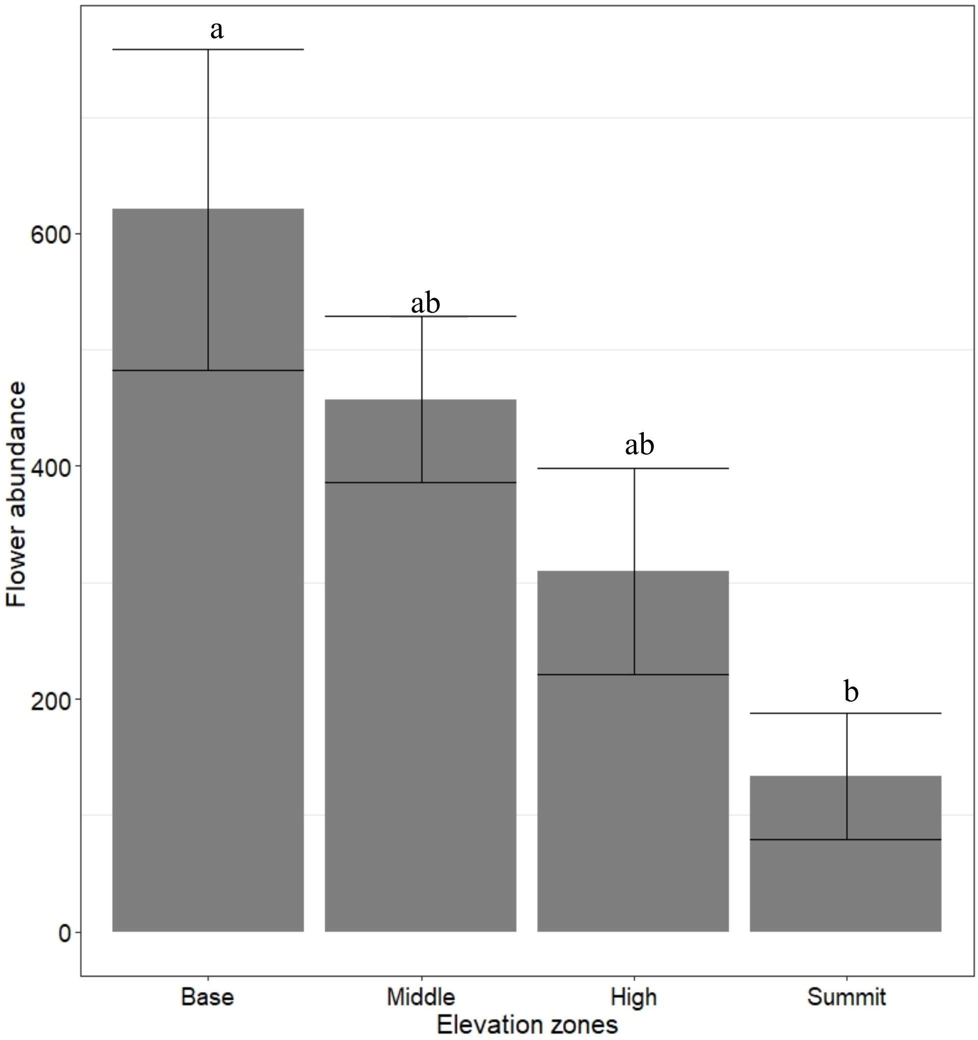

Figure 3.2 Mean flower abundance (±SE) among elevation zones. Bars with common letters are not significantly different at $p > 0.05$.

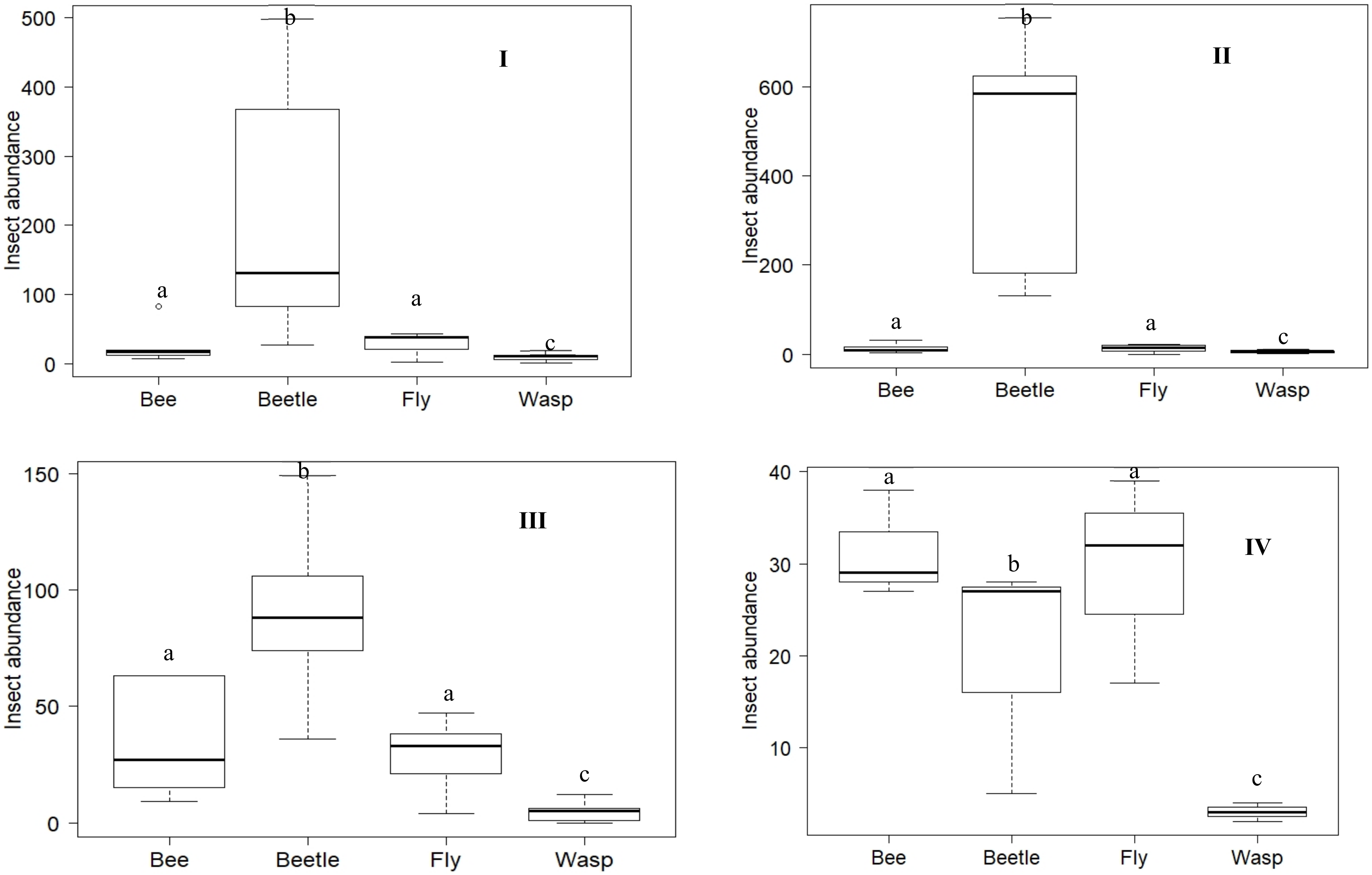

Figure 3.3a. Abundance of flower-visiting insect at the (I) base zone (II) middle zone (III) high zone (IV) summit zone among taxonomic group. Taxon with common letters are not significantly different at $p > 0.05$

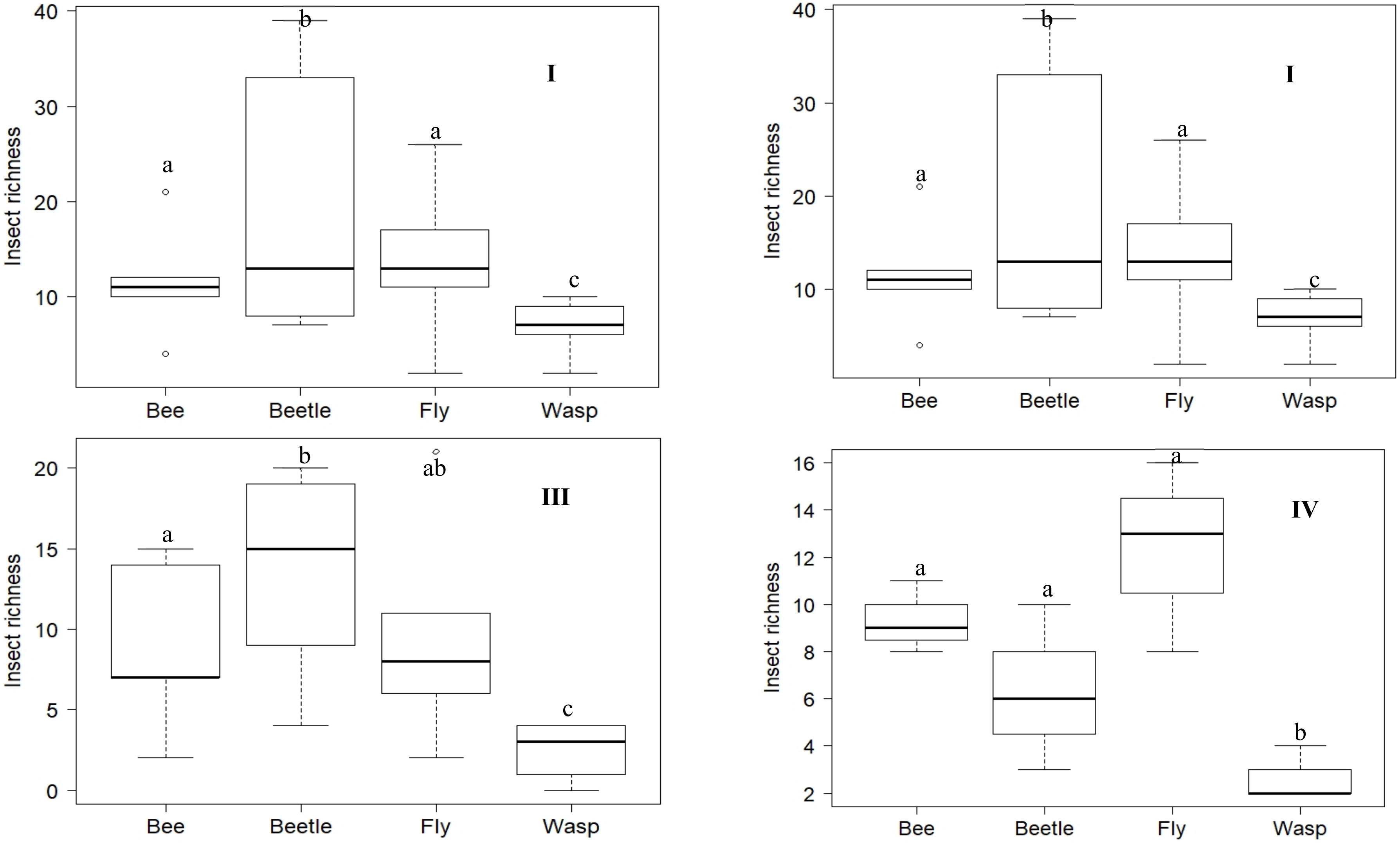

Figure 3.3b. Species richness of flower-visiting insect at the (I) base zone (II) middle zone (III) high zone (IV) summit zone among taxonomic group. Taxon with common letters are not significantly different at $p > 0.05$

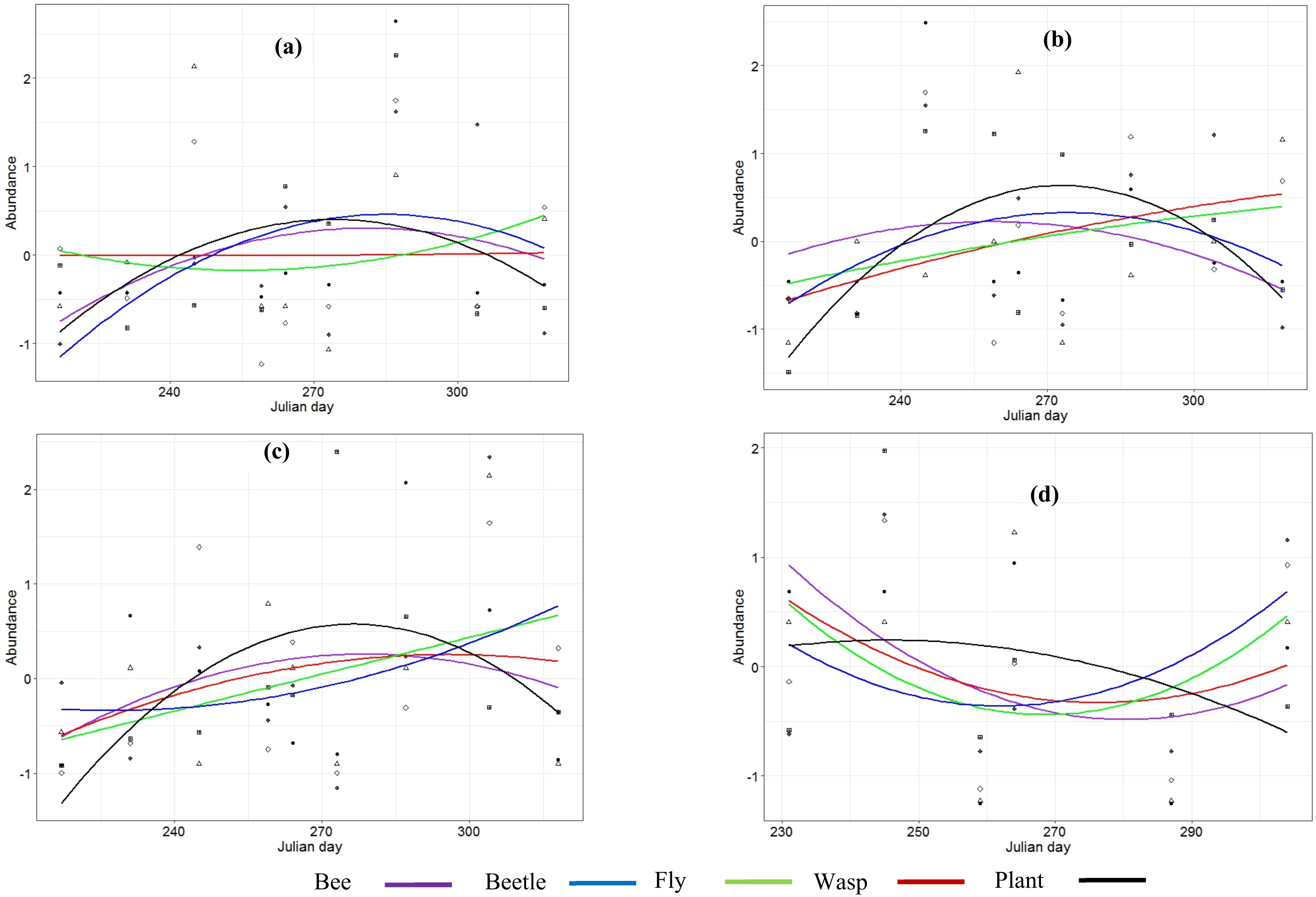

Figure 3.4. Abundance of insect taxa and flowering plant across sampling days in the (a) base zone (b) middle zone (c) high zone (d) summit zone. Lines represent the best models for and the dots represents abundance recorded at each sampling period.

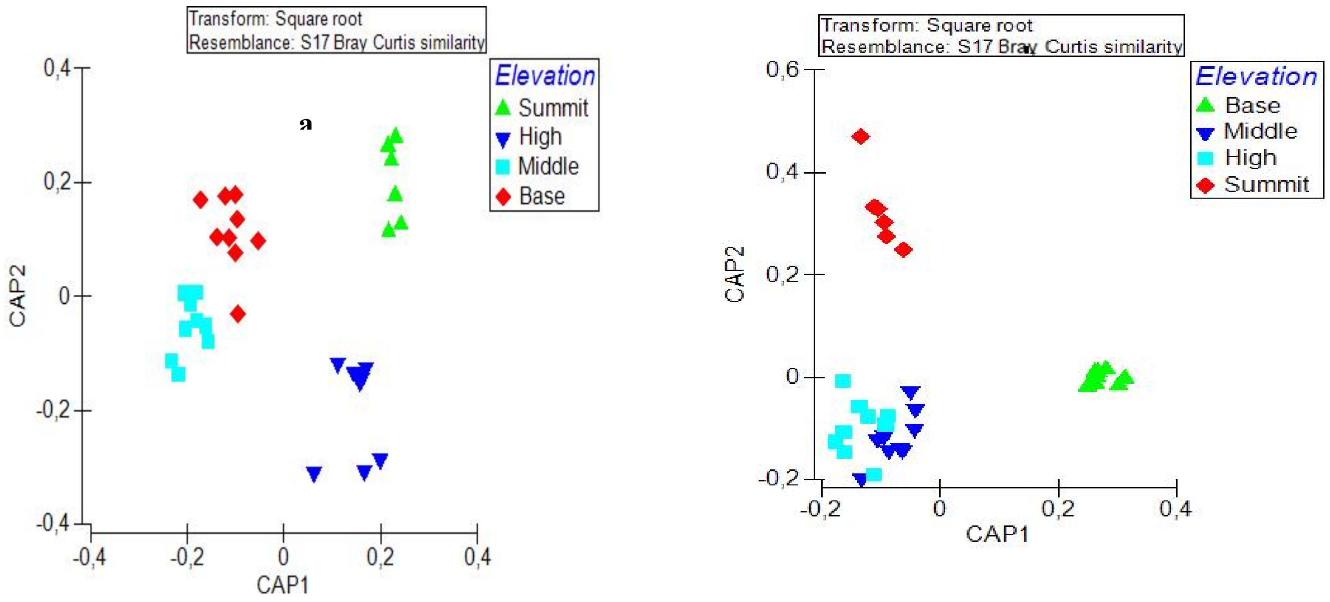

Figure 3.5. Canonical analysis of principal coordinate showing differences in (a) flower visiting insect species composition and (b) flowering plant species composition across elevation zones

Species composition among insect taxa

There was a significant difference in species composition of bees across elevation zones (PERMANOVAF = 2.268, p = 0.003). The most significant difference in species composition was among species in the middle and summit zones, base and summit zones, high and middle zones, as well as high and base zones. There was no significant difference in bee species composition in middle and base zones, and summit and high zones (Figure 3.6a).

Similarly, there was a significant difference in species composition of beetles across the elevation zones (PERMANOVAF = 2.2405, p = 0.001), with the strongest difference being between the summit and middle zone. Species among other zones showed weak differences, with no significant difference in species composition in middle and base zones (Figure 3.6b).

There was no significant difference in species composition of flies across the elevation zones (PERMANOVAF = 1.3217, p = 0.057). There was significant difference in species composition of wasps across the elevation zones (PERMANOVAF = 4.5306, p = 0.001). Pairwise comparison showed a significant difference in wasp species in summit and base zones, high and middle elevation zones, high and base zones, and summit and middle zones. There was no significant difference in species composition among other zone combinations (Figure 3.6c).

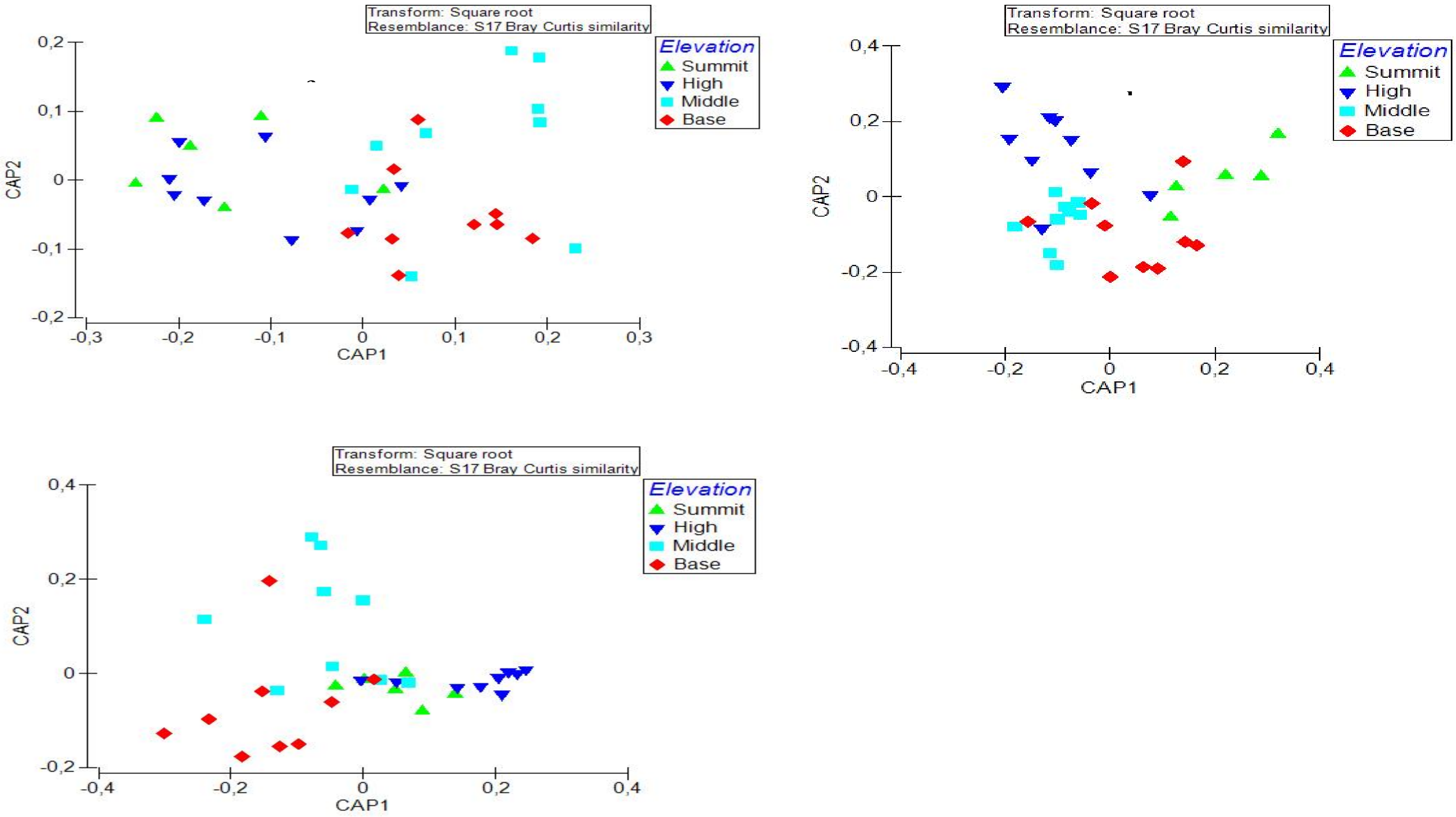

Figure 3.6. Canonical analysis of principal coordinate showing differences in species composition of (a) bees, (b) beetles and (c) wasps across elevation zones

Discussion

Abundance and species richness

This study highlights differences in elevation response among individual groups of flower-visiting insect taxa and flowering plants. Overall, flower abundance peaks at the middle elevation zone, with little or no flowers at the summit. Also, insects generally exhibited a mid-elevational peak in abundance with a major decline above the middle elevation zone. However, changes in environmental conditions across elevation zones shape abundance of insects differently among taxonomic groups.

Abundance of beetles showed a distinct response to changes in elevation, which is usually common to this group of anthophilous insects (Gonzalez-Megias et al., 2007; Yu et al., 2013) compared to the relatively stable abundance of bees and flies across elevation zones. While beetle abundance constituted more than 70% of total insects collected at the first three elevation zones, beetle abundance dropped abruptly at the summit where there were high abundance of bees and flies. This supports studies that have shown great sensitivity of beetles to changes in environmental variables across elevation gradient (Bentz et al., 2016; Wardhaugh et al., 2018). This suggests that beetles are a more suitable indicator of elevation shifts and associated changes in environmental variables than the other three taxa. Monkey beetles are important pollinators in this region, contributing to agricultural productivity and ecosystem posterity (Mayer et al., 2006; van Kleunen et al., 2007; Kehinde and Samways, 2014). While the GCFR is a diverse and complex topographic landscape, patterns of beetle abundance observed here suggests limited contribution to pollination of flowering plants at higher elevations. This is a major concern for specialized beetle-pollinated plants at higher elevations. However, the presence of flies and bees across all elevation zones at relatively equal abundance suggests that these taxa are important pollinators of flowering plants especially at elevations where beetles are absent.

Phenology among taxonomic groups at elevation zones

Patterns of temporal phenological peaks in abundance among taxonomic groups differed across elevation zones. At the start of sampling, there was snow in the summit zone, which only began to warm up towards the middle of the sampling season. Steady increase in abundance and richness of insects began towards the end of the sampling season, especially for flies and bees in the summit zone. This likely explains why insects showed no distinct abundance peak period across sampling time compared to other elevation zones.

Bee abundance showed a clear early peak compared to the other taxonomic groups at all elevations. This is important for the pollination of plants that flower early in the season. However, wasps showed a contrasting response to elevation shifts. Compared to bees, wasps showed no distinct peaks in abundance across elevation zones, except the high zone. One possible explanation is differences in ecosystem functional role of each taxon which may shape species distribution across these zones (Hoiss et al., 2012). While most adult wasps feed on nectar and can act as pollinators, they can also act as predators, parasitoids, or even parasites especially at larva stage utilizing ecosystem components differently. Floral and nesting resources may influence wasp abundance and richness across elevation gradient (Morato and Martins, 2006; Abrahamczyk et al., 2011). It is unclear what drove the lack of distinct wasp abundance peaks across elevation zones here. However, while other groups are expected to respond to peak flowering spring season, long-term studies across all seasons might better reflect patterns of wasp abundance distribution across elevation zones.

Beetles peaked in abundance later in the season than bees at all elevation zones. Beetles are sensitive to temperature changes (Bentz et al., 2016) and while bees and flies forage around flowers, monkey beetles here were mostly in the cone of *P. repense* flowers, especially in the high zone. In addition, bees and flies are strongly associated with flower abundance, as most are nectar feeders. However, monkey beetles eat mostly pollen, and sometimes bore into cavities of flowers where they also seek shelter (Goldblatt et al., 1998). Overall, species belonging to different taxonomic group may respond at different rate to environmental conditions which are distributed differentially across elevation gradients (Ovaskainen et al., 2013)

In the GCFR with high plant species specialization (Pauw and Stanway, 2015), some insect pollinated flowering plants here are highly specialized to different species of pollinators. With respect to pollinator taxonomic group, some plants are pollinated only by bees, beetles, wasps or flies. For example, many species of the orchid genus *Disa* in South Africa are specialized to pollination by long-proboscid flies (Goldblatt and Manning, 2000; Johnson, 2006). Also, *Mormodes* orchids are primarily pollinated by orchid bees (*Euglossine*) when the male bees collect oil from the plant (Yam et al., 2009; Hetherington-Rauth and Ramirez, 2015). Reproductive success of the highly specialized flowering plants in this region may be dependent on the peak abundance period of insect pollinators. Since abundance peak period differs among insect pollinator groups as seen here, future studies addressing phenologies of insect pollinators and flowering plants should also assess how variation in abundance peak period of insect pollinators influences reproductive success of highly specialized flowering plants in this region.

This may explain differences in peak periods of flowering plants across elevation zones here. Future studies addressing the phenologies of insect pollinators and flowering plants should consider how flowering times of highly specialized plant species influence the peak periods of associated insect pollinators.

Interestingly however, is the mismatch in peak periods of bee abundance at middle and base elevation zones, which contrasts with that of flowering plants. Bees are the most essential pollinators in several terrestrial ecosystems, and a delay in their appearance may result in some mismatch with the phenology of flowering plants, leading to poor pollination (Visser and Both, 2005; Gordo and Sanz, 2005; Sparks and Collinson, 2007). Bartomeus et al., (2011) found that phenological shifts in bee abundance over a period of 130 years keep pace with flowering plants. As one of the most important pollinator groups in this region, several plants in both natural and agro-ecosystem depend on bees for pollination. However, here I found a dyssynchrony in abundance peaks of bees and flowering plants at the base and middle elevation. Of further concern is the conflicting pattern of difference where bees peaked earlier than flowering plant at middle elevation, but later than flowering plants at the base. Such phenological mismatch between bees and angiosperms can result in poor productivity of flowering plants, especially when the plants are dependent on bees for pollination (Kudo and Ida, 2013). Although, mismatch observed at the middle zone was not statistically significant, a distinct early peak in abundance of bees was observed here. Long-term monitoring of plant and bee phenology may establish the pattern observed at this zone. Contrasting abundance peaks of flowering plants and bees has been associated with climate change (Olliff-Yang and Mesler, 2018). Increasing spring temperature is a critical factor influencing early appearance of bees and flowering plants in most ecosystems (Hegland et al., 2009; Forrest and Thomson, 2011). Mostly, long-term phenological data have shown insects in general to be more sensitive to springtime temperature rise compared to flowering plants per se (Gordo and Sanz., 2005; Parmesan, 2007; Olliff-Yang and Mesler, 2018). While this may be the plausible explanation, the patterns observed here may also be influenced by species seasonal flight duration, which may contrasts with the flowering peak period (Sparks and Collinson, 2007).

The middle elevation zone is rich in flowering plants. However, the base zone is more flower abundant, suggesting mass flowering (Vrdoljak et al. 2016) at the base elevation zone. Since bees are known to track flower abundance (Winfree et al., 2009), bee species may migrate to the base later in the season and this may influence later abundance peaks recorded at the base zone. Possibly also significant, is that I found similar bee species composition in middle and base zones, suggesting indeed some movement between these zones. However overall, there may be a significant threat to pollination success in zones where mismatch in abundance peaks were recorded. Further long-term studies are now essential to understand the crucial factors driving this mismatch, and how it influences species reproductive output across elevation zones.

Differences in species composition

Species composition in all four groups of flower-visiting insects differed significantly across elevation zones. Species in the summit zone segregated distinctly from species in the middle and base zones. Similarly, there was a distinct segregation of species composition of flowering plants across all elevations. Each of the zones is characterized by a distinct vegetation type (Agenbag et al, 2008), suggesting some isolation among elevation zones in terms of flowering plant species. The summit zone here is characterized by dwarf mountain fynbos plants (mostly restios and ericas), most of which are wind pollinated, with the exception of a few insect pollinated flowering plants restricted to this zone. This shows some plants can be successfully pollinated even in the absence of pollinators which are low in abundance at this zone. The summit zone in particular is highly unstable in terms of weather conditions, with extreme weather at different seasons (cold and windy in winter, and hot and dry in summer). While certain plants in this region are adapted to these conditions, small halictid bees and bombyliid flies dominated the insects here that are able to tolerate these conditions.

The resemblance matrix of insect composition correlated significantly with the resemblance matrix of the flowering plants. Although habitat size, in terms of flower abundance, influenced the abundance of flower-visiting insects, habitat quality in terms of flower composition is equally essential, as it is associated with the distribution of flower-visiting insect species across the elevation zones here. This may also support the floral specialization for insect pollinators as some highly specialized flowering plant species have adaptive measures of attracting important pollinators such as the Orchid and Orchid bees (Hetherington-Rauth and Ramirez, 2015). This means at each elevation zone here where other abiotic factors are suitable for both floral and associated fauna, the presence of highly specialized plants may enhance the persistence of associated insect pollinators. This may increase the quality of ecological interactions and delivery of ecosystem functions across elevation zones.

According to Agenbag et al., (2008), the lowest three elevation zones have some common plants species. However, in contrast, species in the summit zone, and which also occurred elsewhere on this mountain, displayed dwarfism such as the invasive *Acacia* sp., and *Protea repense*, which was common in both the summit and other elevation zones. However, I found a higher proportion of flowering plant species common among other elevation zones, including *P. repense, Metalasia muricata, Lobostemon* sp.*, Senecio* sp.1.*, Senecio* sp.2.*, Muraltia* sp.*, Cullumia* sp. etc. While the lowest three elevation zones seem more connected in terms of flowering plant species composition, the peak zone here, although with low plant richness is more distinct in terms of unique plant species. The displacement or loss of a plant species from the summit zone on this mountain could result in a significant loss of interacting insects, most importantly specialist species. This shows the importance of conserving the limited but unique plant species here which may otherwise be prone to temporal loss and local extinction on this mountain.

Conclusion

While it is important to monitor species temporal shifts in response to climate change, elevation shifts may shape this response among various taxa, as seen here. Other studies have shown how insect and flowering plant phenology changes across temporal scales in the context of climate change. Here, for the first time, my results show how zonation across elevation gradients influences the abundance peak period of different taxonomic insect groups, and relative to that of flowering plants. Bee abundance peaked earlier than other taxa, with my results also showing early temporal peaks of bees at higher elevation zones. I also show how insect composition is strongly associated with flowering plant composition across the elevation zones. While low flower diversity at high elevations is driven by climatic factors and other elevation variables, it is also apparent here, that bee abundance declines earlier than flowering plants with increasingly high elevations. This may yield poor bee-pollinated flower diversity as also predicted by previous studies.

The results here are important for global studies addressing how species phenology is affected by climate change over time. There may be large fluctuations across years of climate monitoring. However, the real-time changes across elevation zones may be of great significance. While some studies have shown temporal changes in species phenology at common elevation (Roy and Sparks, 2001; Forister and Shapiro, 2003), monitoring across elevation gradients over time may show some interesting patterns. Importantly, in-depth studies of the impact of climate change on biodiversity should also take into account the pattern of species phenology across elevation zones.

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APPENDIX

Appendix 3a. List of anthophilous insect species

Taxa	Genus	Species/Morphospecies	Family
Beetles	*Amblymelauoplia*	sp.1	Scarabaeidae
Beetles	*Anaspis*	sp.	Scraptiidae
Beetles	*Anisonyx*	*Proletorius*	Scarabaeidae
Beetles	*Anisonyx*	sp.	Scarabaeidae
Beetles	*Anisonyx*	*ursus*	Scarabaeidae
Beetles	*Anthrenus*	*verbasci*	Dermestidae
Beetles	*Apalochrus*	sp.	Melyridae
Beetles	Buprestidae	sp.1	Buprestidae
Beetles	Buprestidae	sp.2	Buprestidae
Beetles	Buprestidae	sp.3	Buprestidae
Beetles	Buprestidae	sp.4	Buprestidae
Beetles	Buprestidae	sp.5	Buprestidae
Beetles	Buprestidae	sp.6	Buprestidae
Beetles	Buprestidae	sp.7	Buprestidae
Beetles	Buprestidae	sp.8	Buprestidae
Beetles	Buprestidae	sp.9	Buprestidae
Beetles	Buprestidae	sp.10	Buprestidae
Beetles	Cantharidae	sp.	Cantharidae
Beetles	Carabidae	sp.	Carabidae
Beetles	*Ceroctis*	*capensis*	Meloidae
Beetles	Chrysomelidae	sp.1	Chrysomelidae
Beetles	Chrysomelidae	sp.2	Chrysomelidae
Beetles	Chrysomelidae	sp.3	Chrysomelidae
Beetles	Cleridae	sp.1	Cleridae
Beetles	Cleridae	sp.2	Cleridae
Beetles	Cleridae	sp.3	Cleridae
Beetles	Cleridae	sp.4	Cleridae
Beetles	Cleridae	sp.5	Cleridae
Beetles	Cleridae	sp.6	Cleridae
Beetles	*Cloniocerus*	*kraussi*	Cerambycidae
Beetles	Coccinellidae	sp.	Coccinellidae
Beetles	Dermestidae	sp.1	Dermestidae
Beetles	Dermestidae	sp.2	Dermestidae
Beetles	*Heterochelus*	sp.	Scarabaeidae
Beetles	*Hippodamia*	*variegata*	Coccinellidae
Beetles	Hoplinii	sp.1	Scarabaeidae
Beetles	Hoplinii	sp.2	Scarabaeidae
Beetles	Hoplinii	sp.3	Scarabaeidae
Beetles	Hoplinii	sp.4	Scarabaeidae
Beetles	Hoplinii	sp.5	Scarabaeidae
Beetles	Hoplinii	sp.6	Scarabaeidae
Beetles	Hoplinii	sp.7	Scarabaeidae
Beetles	Hoplinii	sp.8	Scarabaeidae
Beetles	Hoplinii	sp.9	Scarabaeidae

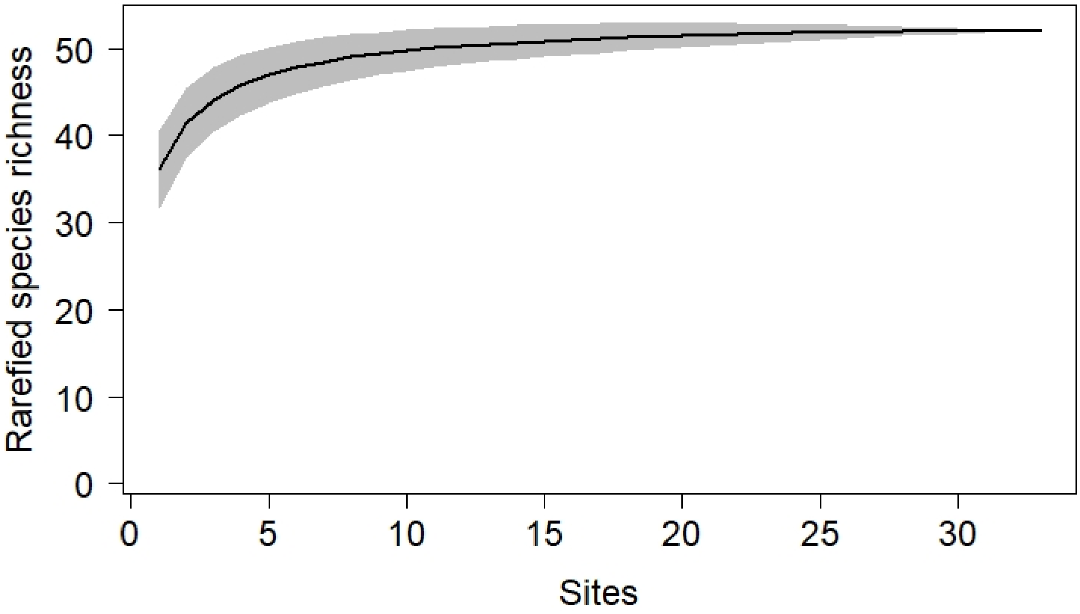
Beetles	Hoplinii	sp.10	Scarabaeidae
Beetles	Hoplinii	sp.11	Scarabaeidae
Beetles	Hoplinii	sp.12	Scarabaeidae
Beetles	Hoplinii	sp.13	Scarabaeidae
Beetles	Hoplinii	sp.14	Scarabaeidae
Beetles	Hoplinii	sp.15	Scarabaeidae
Beetles	Hoplinii	sp.15	Scarabaeidae
Beetles	*Lepitrix*	*dichropus*	Scarabaeidae
Beetles	*Lepitrix*	sp.1	Scarabaeidae
Beetles	*Leucocelis*	*amethysina*	Cetoniinae
Beetles	Meloidae	sp.1	Meloidae
Beetles	Meloidae	sp.2	Meloidae
Beetles	Meloidae	sp.3	Meloidae
Beetles	Meloidae	sp.4	Meloidae
Beetles	Meloidae	sp.5	Meloidae
Beetles	*Melyris*	sp.1	Melyridae
Beetles	*Melyris*	sp.2	Melyridae
Beetles	*Neoeutrapela*	sp.	Tenebrionidae
Beetles	Nitidulidae	sp.1	Nitidulidae
Beetles	Nitidulidae	sp.2	Nitidulidae
Beetles	Nitidulidae	sp.3	Nitidulidae
Beetles	Nitidulidae	sp.4	Nitidulidae
Beetles	*Peritrichia*	*nigrita*	Scarabaeidae
Beetles	*Peritrichia*	sp.1	Scarabaeidae
Beetles	*Promeces*	*longipes*	Cerambycidae
Beetles	Rutelinae	sp.	Scarbaeidae
Beetles	*Trichostetha*	*fascicularis*	Cetoniinae
Beetles	*Trichostetha*	*capensis*	Cetoniinae
Bees	*Allodape*	sp.	Apidae
Bees	*Allodapula*	sp.1	Apidae
Bees	*Andrena*	sp.1	Andrenidae
Bees	*Anthidiini(Plesiamchidium)*	sp.1	Megachilidae
Bees	*Anthidiini*	sp.2	Megachilidae
Bees	*Anthidioma*	sp.1	Megachilidae
Bees	*Anthidioma*	sp.2	Megachilidae
Bees	*Anthophora*	sp.1	Apidae
Bees	*Anthophora*	sp.2	Apidae
Bees	*Anthophora*	sp.3	Apidae
Bees	*Anthophora*	sp.4	Apidae
Bees	*Anthophora*	sp.5	Apidae
Bees	*Anthophora*	sp.6	Apidae
Bees	*Anthophora*	sp.7	Apidae
Bees	*Apis*	*mellifera*	Apidae
Bees	*Brausapis*	sp.	Apidae
Bees	*Lasioglossum*	sp.1	Halictidae
Bees	*Lasioglossum*	sp.11	Halictidae
Bees	*Lasioglossum*	sp.12	Halictidae
Bees	*Lasioglossum*	sp.14	Halictidae

Bees	*Lasioglossum*	sp.10	Halictidae
Bees	*Lasioglossum*	sp.8	Halictidae
Bees	*Lasioglossum*	sp.2	Halictidae
Bees	*Lasioglossum*	sp.3	Halictidae
Bees	*Lasioglossum*	sp.4	Halictidae
Bees	*Lasioglossum*	sp.5	Halictidae
Bees	*Lasioglossum*	sp.13	Halictidae
Bees	*Lasioglossum*	sp.15	Halictidae
Bees	*Lasioglossum*	sp.6	Halictidae
Bees	*Lasioglossum*	sp.7	Halictidae
Bees	*Lasioglossum*	sp.16	Halictidae
Bees	*Lasioglossum*	sp.9	Halictidae
Bees	Megachilidae	sp.	Megachilidae
Bees	*Melitta*	sp.	Melittidae
Bees	Osmiini	sp.4	Megachilidae
Bees	Osmiini	sp.1	Megachilidae
Bees	Osmiini	sp.2	Megachilidae
Bees	Osmiini	sp.3	Megachilidae
Bees	*Pachymelus*	sp.	Apidae
Bees	*Patellapis*	sp.	Halictidae
Bees	*Plesianthidium*	sp.1	Megachilidae
Bees	*Plesianthidium*	sp.2	Megachilidae
Bees	*Pseudoanthidium*	sp.	Megachilidae
Bees	*Redivivoides*	sp.	Melittidae
Bees	*Seladonia*	sp.1	Halictidae
Bees	*Seladonia*	sp.2	Halictidae
Bees	*Seladonia*	sp.3	Halictidae
Bees	*Seladonia*	sp.4	Halictidae
Bees	*Seladonia*	sp.5	Halictidae
Bees	*Tetraloniella*	sp.1	Apidae
Bees	*Tetraloniella*	sp.2	Apidae
Bees	*Xylocopa*	sp.	Apidae
Wasp	Bethylidae	sp.1	Bethylidae
Wasp	Bethylidae	sp.2	Bethylidae
Wasp	Bethylidae	sp.3	Bethylidae
Wasp	Braconidae	sp.1	Braconidae
Wasp	Braconidae	sp.2	Braconidae
Wasp	Braconidae	sp.3	Braconidae
Wasp	Braconidae	sp.4	Braconidae
Wasp	Braconidae	sp.5	Braconidae
Wasp	Braconidae	sp.6	Braconidae
Wasp	Braconidae	sp.7	Braconidae
Wasp	Braconidae	sp.8	Braconidae
Wasp	Chrysididae	sp.1	Chrysididae
Wasp	Chrysididae	sp.2	Chrysididae
Wasp	Eumeninae	sp.1	Vespidae
Wasp	Eumeninae	sp.2	Vespidae
Wasp	Ichneumonidae	sp.1	Ichneumonidae

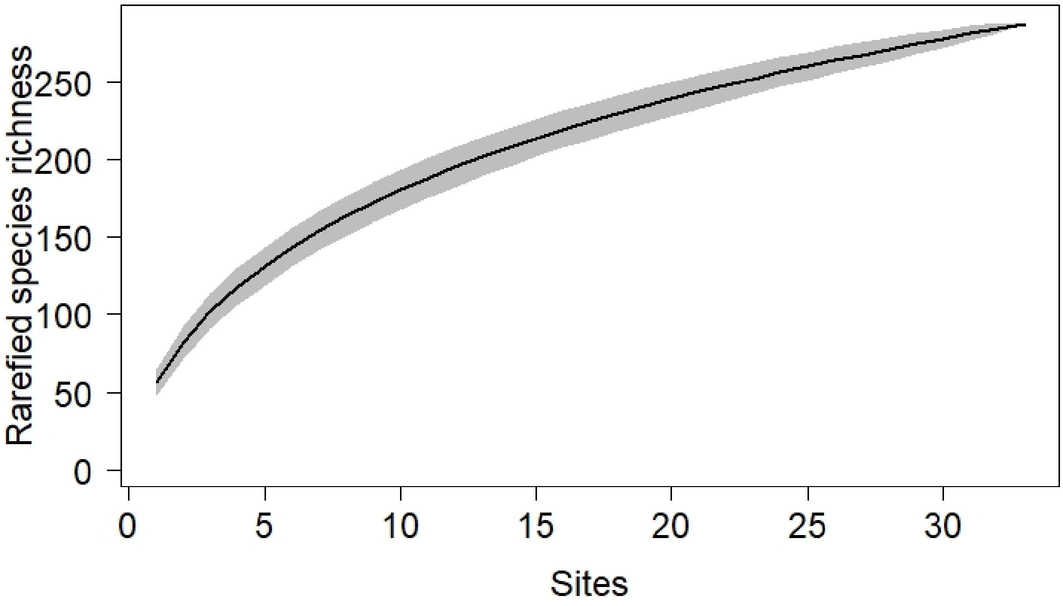
Wasp	Ichneumonidae	sp.2	Ichneumonidae
Wasp	Ichneumonidae	sp.3	Ichneumonidae
Wasp	Ichneumonidae	sp.4	Ichneumonidae
Wasp	Ichneumonidae	sp.5	Ichneumonidae
Wasp	Ichneumonidae	sp.6	Ichneumonidae
Wasp	Ichneumonidae	sp.7	Ichneumonidae
Wasp	Ichneumonidae	sp.8	Ichneumonidae
Wasp	Masarinae	sp.	Vespidae
Wasp	Pompilidae	sp.1	Pompilidae
Wasp	Pompilidae	sp.2	Pompilidae
Wasp	Pompilidae	sp.3	Pompilidae
Wasp	Pompilidae	sp.4	Pompilidae
Wasp	Sphecidae	sp.1	Sphecidae
Wasp	Sphecidae	sp.2	Sphecidae
Wasp	Sphecidae	sp.3	Sphecidae
Wasp	Sphecidae	sp.4	Sphecidae
Wasp	Sphecidae	sp.5	Sphecidae
Wasp	Sphecidae	sp.6	Sphecidae
Wasp	Sphecidae	sp.7	Sphecidae
Wasp	Sphecidae	sp.8	Sphecidae
Wasp	Sphecidae	sp.9	Sphecidae
Wasp	Sphecidae	sp.10	Sphecidae
Wasp	Sphecidae	sp.11	Sphecidae
Wasp	Sphecidae	sp.12	Sphecidae
Wasp	Sphecidae	sp.13	Sphecidae
Wasp	Sphecidae	sp.14	Sphecidae
Wasp	Sphecidae	sp.15	Sphecidae
Wasp	Sphecidae	sp.16	Sphecidae
Wasp	Vespidae	sp.1	Vespidae
Wasp	Vespidae	sp.2	Vespidae
Wasp	Vespidae	sp.3	Vespidae
Fly	Asilidae	sp.1	Asilidae
Fly	Asilidae	sp.1	Asilidae
Fly	Bombyliidae	sp.1	Bombyliidae
Fly	Bombyliidae	sp.2	Bombyliidae
Fly	Bombyliidae	sp.3	Bombyliidae
Fly	Bombyliidae	sp.4	Bombyliidae
Fly	Bombyliidae	sp.5	Bombyliidae
Fly	Bombyliidae	sp.6	Bombyliidae
Fly	Bombyliidae	sp.7	Bombyliidae
Fly	Bombyliidae	sp.8	Bombyliidae
Fly	Bombyliidae	sp.9	Bombyliidae
Fly	Bombyliidae	sp.10	Bombyliidae
Fly	Bombyliidae	sp.11	Bombyliidae
Fly	Bombyliidae	sp.12	Bombyliidae
Fly	Bombyliidae	sp.13	Bombyliidae
Fly	Bombyliidae	sp.14	Bombyliidae
Fly	Calliphora	sp.	Calliphoridae

Fly	Calliphoridae	sp.1	Calliphoridae
Fly	Calliphoridae	sp.2	Calliphoridae
Fly	Calliphoridae	sp.3	Calliphoridae
Fly	Calliphoridae	sp.4	Calliphoridae
Fly	Culicidae	sp.1	Culicidae
Fly	Culicidae	sp.2	Culicidae
Fly	Culicidae	sp.3	Culicidae
Fly	Culicidae	sp.4	Culicidae
Fly	Empididae	sp.1	Empididae
Fly	Empididae	sp.2	Empididae
Fly	*Lasiopyrellia*	sp.	Muscidae
Fly	Muscidae	sp.1	Muscidae
Fly	Muscidae	sp.2	Muscidae
Fly	Muscidae	sp.3	Muscidae
Fly	Muscidae	sp.4	Muscidae
Fly	Muscidae	sp.5	Muscidae
Fly	Muscidae	sp.6	Muscidae
Fly	Muscidae	sp.7	Muscidae
Fly	Muscidae	sp.8	Muscidae
Fly	Muscidae	sp.9	Muscidae
Fly	Muscidae	sp.10	Muscidae
Fly	Muscidae	sp.11	Muscidae
Fly	Muscidae	sp.12	Muscidae
Fly	Muscidae	sp.13	Muscidae
Fly	Muscidae	sp.14	Muscidae
Fly	Muscidae	sp.15	Muscidae
Fly	Muscidae	sp.16	Muscidae
Fly	Muscidae	sp.17	Muscidae
Fly	Muscidae	sp.18	Muscidae
Fly	Mydidae	sp.	Mydidae
Fly	*Pliomelaena*	sp.	Tephritidae
Fly	*Pyrellia*	sp.1	Muscidae
Fly	*Pyrellia*	sp.2	Muscidae
Fly	Sarcophagidae	sp.1	Sarcophagidae
Fly	Sarcophagidae	sp.2	Sarcophagidae
Fly	Sarcophagidae	sp.3	Sarcophagidae
Fly	*Stomoxys*	sp.1	Muscidae
Fly	*Stomoxys*	sp.2	Muscidae
Fly	Syrphidae	sp.1	Syrphidae
Fly	Syrphidae	sp.2	Syrphidae
Fly	Syrphidae	sp.3	Syrphidae
Fly	Syrphidae	sp.4	Syrphidae
Fly	Syrphidae	sp.5	Syrphidae
Fly	Syrphidae	sp.6	Syrphidae
Fly	Syrphidae	sp.7	Syrphidae
Fly	Syrphidae	sp.8	Syrphidae
Fly	Tabanidae	sp.1	Tabanidae
Fly	Tabanidae	sp.2	Tabanidae

Fly	Tabanidae	sp.3	Tabanidae
Fly	Tabanidae	sp.4	Tabanidae
Fly	Tabanidae	sp.5	Tabanidae
Fly	Tabanidae	sp.6	Tabanidae
Fly	Tabanidae	sp.7	Tabanidae
Fly	Tabanidae	sp.8	Tabanidae
Fly	Tabanidae	sp.9	Tabanidae
Fly	Tabanidae	sp.10	Tabanidae
Fly	Tabanidae	sp.11	Tabanidae
Fly	Tachinidae	sp.1	Tachinidae
Fly	Tachinidae	sp.2	Tachinidae
Fly	Tachinidae	sp.3	Tachinidae
Fly	Tachinidae	sp.4	Tachinidae
Fly	Tephritidae	sp.1	Tephritidae
Fly	Tephritidae	sp.2	Tephritidae
Fly	Tephritidae	sp.3	Tephritidae



Appendix 3b. Species richness rarefied curve for flowering plants sampled across study sites. The grey area represents the confidence interval from the standard error of estimates.



Appendix 3c. Species richness rarefied curve for insects sampled across sampling sites. The grey area represents the confidence interval from the standard error of estimates.

Chapter 4

Time since fire strongly influences a range of flower-visiting insects in a fire-prone landscape

Abstract

Drivers of species productivity and ecosystem function are of great ecological significance. In fire-prone ecosystems, dispersion patterns of important pollinator species are driven by the various fire events over space and time. However, different species have varying responses to fire intensity and frequency. Here, I assess how time since last fire influences abundance, composition, and dispersion patterns of flowering plants and anthophiles in a highly fire-prone landscape. I classified study area into age since last fire, which were <1 year (short-term burned), 2-3 years (medium-term burned) and 9-10 years (long-term burned). I collected insect anthophiles using yellow, blue, and white pan traps at sites established in each post-fire class. I also estimated flower abundance at each study site. Overall, anthophile abundance was highest in medium-term burned, while lowest in short-term burned sites. Also, flower abundance was highest at medium-term burned, and lowest in long-term burned sites. Species composition of flowering plants and anthophiles, especially bees and flies, differed significantly across post-fire class. Overall, flower abundance had a significant positive effect on bee abundance. Of all the taxa, bees were the only group that showed dispersion patterns reflecting those of flower abundance. Other factors, such as the requirements for other resources, such as logs, drive the distribution of other anthophile taxa, especially flies. I conclude that fire drives the dispersion patterns of bees through its direct effect on flower abundance and composition in a fire-prone landscape. While high frequency of short-term fire may reduce flower abundance, flower rich areas with moderate time since fire may increase diversity of anthophiles in fire prone landscape.

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Introduction

The preservation of natural habitat for the persistence of important species, especially those responsible for the delivery of ecosystem services is of great importance (Chandler and King, 2011). Flower-visiting insects are critical biotic constituent of terrestrial ecosystems, as some species play crucial ecological roles in the pollination of flowering plants in both natural and agricultural ecosystems (Klein et al., 2007; Potts et al., 2010). Pollination by insects alone increases crop yields globally by 35% (Klein et al., 2007). However, despite the high level of dependence of human food security and ecosystem functions on pollination services (Vanbergen et al., 2013), there is an on-going decrease in the abundance and distribution of insect pollinators (Potts et al., 2010).

Several drivers influence this decline through their impact on flower distribution in natural habitat. Understanding these drivers of species diversity and distribution is pertinent to designing important conservation strategies in disturbed landscapes. Drivers influencing species productivity and their ecological interactions are of great ecological significance (Rosenzweig, 1995). Furthermore, fire is one of the major factors driving floral diversity in many terrestrial ecosystems (New, 2014), and has a positive influence on plant productivity and diversity at various local and landscape scales (Pausas and Ribeiro, 2017).

The Greater Cape Floristic Region (GCFR) biodiversity hotspot is the richest floral kingdom in the world (Myers et al., 2000), and its floral maintenance depends in part on fire events. Fire affects plant reproduction and diversity through complex processes, and although this is still not fully understood, a few processes relating to fire cues have been documented. In legumes with hard seeds, fire helps activate germination by breaking the seed coat through heat from the fire (Sabiiti and Wein, 1987; Saharjo and Watanabe, 1997), allowing water to penetrate into the seed, and enabling germination to begin. Smoke is also a major constituent of fire that also influences germination (Kulkarni et al., 2006; Sparg et al., 2005; Tigabu et al., 2007), as well as the flowering of plants (Keeley, 1993) in fire-prone ecosystems (Staden et al., 2000). In a sensitive fynbos ecosystem like the GCFR, where about 6 000 plant species are endemic, the scleropyllous fynbos vegetation is highly dependent on smoke for germination cues (Brown, 1993). Germination cues from smoke for fynbos plants partly explains the vegetation dynamics (Light et al., 2004).

In addition to direct influence of fire on plant diversity, fire also plays a critical role in the flowering of plants through its influence on other factors mediating plant growth and diversity.

Post-fire conditions influencing plant survival are usually different from pre-fire conditions. There is usually a change in soil composition, especially in microorganism composition and soil structure (Certini, 2005). Also, fire opens up closed habitats through reduction of canopy cover. This increases understorey access to sunlight, which is important for flowering plants (Quintano et al., 2013). Competition is reduced by the impact of fire in ecosystems (Perkins, 2015). Fire mediated by human activities often influences the survival of target species by eliminating competitors in the ecosystem.

Fire history is important in shaping biodiversity composition and distribution in flower rich ecosystems. Changes in fire history across time determine habitat suitability and dispersion patterns of flowering plants and insects in fire-impacted areas (Brown et al., 2017). Most often, insect activities and distribution vary with time since fire (Potts et al., 2006; Pauw, 2007; Geerts et al., 2012), and this is influenced by important environmental factors driving insect diversity in fire-impacted ecosystems. Flowering plants as drivers of anthophilous insect diversity across landscapes has been well documented in relation to fire incidence. For example, while fire drives fynbos communities, short-interval fires may destroy the seed bank, and may eventually result in the loss of fynbos species and associated flower visitors (Vlok and Yeaton, 2000). On the other hand, habitat modification results from impact of fire, so creating patches of heterogeneous landscape (Perry et al., 2011). Fire history also modifies habitat structure, with ground cover often increasing with time since fire. This shapes species distribution based on functional traits influencing nesting requirements. However, while habitat selection is important for the distribution of anthophilous insects, this has received less attention.

The recovery of vegetation post-fire is dependent on the timing of fire events. However, for flower-visiting insects, recolonization may be dependent on the availability of particular flowering plants providing high-quality floral rewards. Oftentimes, activity of adult anthophilous insects is dependent on floral resources used by the insects in the previous season (Potts et al., 2003), and this usually brings about differences in time required for restoration of floral and insect assemblages in flower rich landscapes. This pattern is of great importance for flower-dependent anthophiles, such as bees, as they track flower resources (Winfree et al., 2009). Here, I explore post-fire diversity and composition of different anthophile groups and flowering plants in a fire-prone landscape. I hypothesise that 1) sites with different times since last fire incidence will have varying species composition of flowering plants and anthophiles, this is due to period of germination until flowering which differs among flowering plant species, which will also influence anthophile species with different floral requirements in different post-fire age classes, 2) abundance, diversity and species evenness of flowering plants and anthophilous insects will be highest at sites with intermediate times since fire, since there could be co-occurrence of fire-tolerant and fire-intolerant species during vegetation recovery period, 3) I expect insect taxa with high dependence on floral resources (obligate anthophiles) to be most influenced across post-fire age classes, these are associated with flowering plants which I also expect to vary with post-fire age classes, and 4) distribution patterns of anthophiles will track those of flowering plants across habitats with different post-fire ages.

Materials and methods

The study was conducted in the large natural set-aside areas on adjacent wine farms in the Western Cape Province, South Africa, in the Greater Cape Floristic Region (GCFR) biodiversity hotspot. Bee diversity in the GCFR is exceptionally high, coinciding with that of plants (Kuhlmann, 2005). Two contiguous wine estates were selected (Vergelegen: 34.0764^{o}S, 18.8899^{o}E and Lourensford: 34.0719^{o}S, 18.8886^{o}E). These estates practice biodiversity-friendly agriculture, with extensive areas of the farms devoted to conservation of indigenous biodiversity, where my sites were positioned. The most recent fire in this landscape occurred between December 2016 and February 2017. This mostly occurred on Vergelegen wine estate, leaving a large proportion of Louresnford unburned. This location previously burned in 2014-2015, and the oldest fire in the area was in 2008-2009.

I classified sites based on Time Since last Fire (TSF). Recently-burned sites (TSF=<1 year) were classified as ‘short-term burned’. Patches of vegetation left unburned during the fire incidence in the burned landscape (TSF = 2-3 years) were classified as ‘medium-term burned’. Sites located at Lourensford, where the last fire occurred in 2008-2009 (TSF = 9-10 years), were classified as ‘long-term burned’. In addition, this area is also diverse in topographic position from small hills to valley.

I defined a site as $\geq$ 50 m^2, insects were collected in 6 sites in valleys and hillslopes in each fire class, except in long-term burned, where only three sites were available. Vegetation sampling was done also at every site where I collected insects and also in additional sites mostly at hilltops where I could not erect pan traps for insect collection but observed interactions for the next chapter (Chapter 5). This makes a total of 9 sites within each post-fire class for vegetation sampling. Number of open flowers on each plant species were estimated in five replicates of $2m^2$ plot at each site. Furthermore, I classified vegetation into three major classes (annual plants, short-lived and quick-growth perennial plants, and long-lived, slow growth perennial plants). This was done by accessing ecological information on plant species from the literature.

The recent fire created a mosaic vegetation structure in the burned area, leaving heterogeneous distribution of unburned patches within the burned landscape. Medium-term burned sites were the patches of unburned areas that fit the size of a study site within the short-term burned area (Figure 4.1). The minimum distance between a medium-term and short-term burned sites was 150 m, and minimum distance between sites in the same fire post-age class for medium-term and short-term burned was 250 m. The long-term burned sites are natural areas that border the medium-term and short-term burned sites. The minimum distance between sites located in the long-term burned area was 450 m. Long-term burned area was located 3 000 m away from short-term burned area. Spatial-autocorrelation of insect abundance among study sites was assessed using the Moran I index. Here, Moran I index (0.135 ± 0.095, P= 0.031) showed a weak clustering of insect abundance in sites with similar time since fire.

I used 30 pan traps (10 each of blue, yellow and white bowls) to collect flower-visiting insects at each site. Coloured bowls were half-filled with water, and few drops of liquid detergent were added to reduce water surface tension. Coloured bowls were raised to the immediate vegetation height, and were openly active for 24 hrs. Insects were collected twice at each study site within the period of late August to mid-September, and also within the first 2 weeks of October of 2018 (i.e. early-late spring). Insects acquired in each pan trap were rinsed and kept in 75% ethanol until sorting and identification. The insect groups sampled were bees, beetles, flies and wasps. Insect families included in my analysis are those with roles established as flower-visitors in literature as in previous chapters. Insects were also sorted to morphospecies level using taxonomic guides as in previous chapters.

Statistical analyses

Data collected in the two sampling rounds were pooled and overall abundance for all insect taxa and flowering plants were used in the analysis. To account for sampling adequacy, I estimated species rarefaction curves for flowering plants and anthophile species richness across study sites using the ‘rarefy’ function in ‘vegan’ R package (Oksanen et al., 2006). I assessed the difference in flower and pollinator abundance across post-fire age class using generalised linear model (GLM) in R version 3.4.1 (R core team, 2017) and fitting Poisson distribution. I also used GLM to assess how flower abundance differs among vegetation types (annual plants, short-lived and quick-growth perennial plants, and long-lived, slow growth perennial plants) in different fire class fitting poisson family. Similarly, differences in anthophile abundance among taxonomic group in each fire class was assessed using GLM. Anthophile abundance or flower abundance was specified as the dependent variable with insect taxa or vegetation types as explanatory variable in different models. Furthermore, Z-values for pairwise comparison between treatments in simplified models were recorded where overall significance was observed.

The effect of flower abundance on each anthophile taxonomic group (bees, beetles, flies or wasps) was computed using a Generalised Linear Mixed-Effects Models (GLMM) fitting a Poisson distribution. I specified the abundance of each anthophile taxon as the dependent variable; flower abundance, post-fire age class and their interaction were specified as the explanatory variables. I used backward elimination to highlight the influence of explanatory variables and their interactions. I computed diagnostic checks for possible influential data in my model, one data point from one of my sampling site was high, however the removal of this did not influence my result. For bees, which are the most obligate anthophile/pollinator taxon here, I assessed how bee families responded to differences across post-fire age classes using the ‘glmer’ function specifying Poisson distribution.

I computed Simpson and Shannon diversity indices for species richness and diversity respectively for flowering plants and anthophiles. I also assessed species evenness using the Pielou's evenness index (Mulder et al., 2004). Differences in individual diversity index for anthophiles and flowering plants across post-fire age were assessed. To do this, I used simple ANOVA. Shapiro-Wilk’s test was computed to assess data normality. I also performed Bartlett’s test for homogeneity of variance before data was subjected to ANOVA. Tukey-HSD post hoc test was computed to analyse differences between groups. To understand how frequency of distribution for each anthophile taxon differ across post-fire age, I included evenness index for each taxon as dependent variable in different models and post-fire age class was specified as fixed factor.

Finally, I assessed pollinator and flowering plant species composition across post-fire age class using canonical analysis of principal coordinates (CAP). I then analysed differences in species composition across post-fire age class using PERMANOVA. The PERMANOVA was performed using the Bray-Curtis similarity index obtained from the square-root transformation of abundance data and selecting Type III SS sum of square to account for unbalanced design (Anderson et al., 2008). Data were permuted 999 times for the analysis.

To understand the similarity in the pattern of distribution of flowering plants and anthophile species across post-fire age class, I used the RELATE function in primer 6 software to compare species composition for individual anthophile taxa with flowering plant composition.

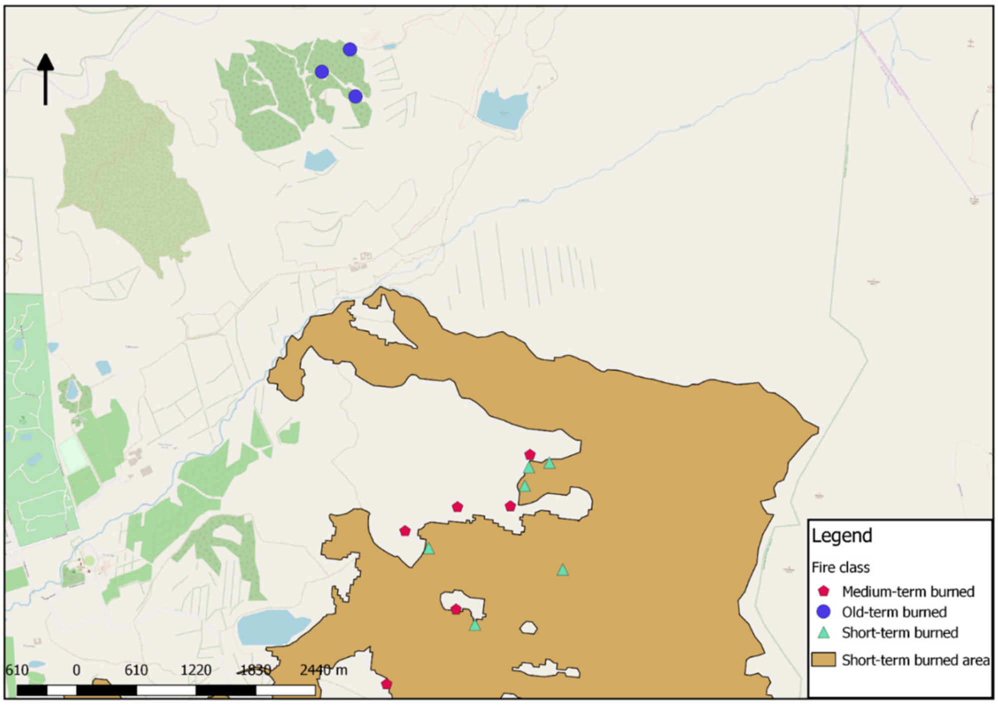

Figure 4.1. Study sites at Lourensford and Vergelegen wine estate showing distribution of sites across fire class area

Results

A total of 9 492 insect individuals, belonging to four taxa (bees, beetles, flies and wasps) and 113 morphospecies were collected over the sampling period (Appendix 4a). Anthophile species estimator indices for all post-fire classes were: Chao = 349.25 ± 94.27, Jackknife2 = 233.75. 61 species were recorded in short-term burned sites with species estimator scores of: Chao = 132.30 ± 35.45, Jackknife2 = 112.23. I found 63 insect species in medium-term burned sites with species estimator score of: Chao = 126.38 ±30.72, Jackknife2 = 113.70. Long-term sites had 50 species with estimator score of: Chao = 101.04 ± 25.97, Jackknife2 = 83.67. Overall, the rarefaction curve for flowering plants across all sites reached an asymptote (Appendix 4b). However, the asymptotic level for the insect curve was less pronounced (Appendix 4c), because of the occurrence of many rare species in my samples, however, this richness did not differ across post-fire age class.

There was a significant difference in flower abundance across post-fire age classes. Highest flower abundance was recorded at the medium-term burned sites, which was significantly different from flower abundance recorded at long-term ($z = -33.16$, $p < 0.0001$, df = 24) and short-term burned sites ($z = -38.32$, $p < 0.00001$, df = 24). In addition, there was a significant difference in flower abundance among vegetation types in different fire class. In short-term burned sites, short-lived perennials had the highest flower abundance with lowest flower abundance recorded for annual plants ($z = -16.467$, $p < 0.0001$, df = 24, Figure 4.2I). Similarly, short-lived perennials had the highest flower abundance in medium-term burned sites and this was significantly different from lowest flower abundance recorded for annual plants ($z = -22.924$, $p < 0.0001$, df = 24, Figure 4.2II). However, in long-term burned sites, while short-lived perennials had the highest flower abundance, long-term perennials had the lowest flower abundance ($z = -9.649$, $p < 0.0001$, df = 24, Figure 4.2III).

There was a significant difference in anthophile abundance across post-fire age class. Highest anthophile abundance was recorded at the medium-term burned sites, and this was significantly different from anthophile abundance recorded at the long-term burned sites ($z = -32.08$, $p < 0.0001$, df =12). In addition, there was a significant difference in anthophile abundance among taxonomic groups in each fire class. Beetles and flies were the most abundant group in short-term fire class with the lowest abundance recorded for wasps ($z = -11.061$, $p < 0.001$, df = 20, Figure 4.3I). Flies were the most abundant in medium-term burned sites and the lowest abundance here was also recorded for wasps ($z = -21.37$, $p < 0.0001$, df = 20, Figure 4.3II). However, in long-term burned sites, beetles had the highest abundance followed by bees and flies and least for wasps ($z = -11.78$, $p < 0.0001$, df = 8, Figure 4.3III).

Effect of flower abundance on insect taxon across fire post-age class

Flower abundance was significantly associated with the abundance of bees ($\chi^2 = 10.496$, $p = 0.01479$, df= 3). There was no significant effect of post-fire age class on bee abundance ($\chi^2 = 8.567$, $p = 0.089$, df = 4). Although the slope of bee-flower abundance relationship was higher at medium-term burned and lower at short-term burned where a negative relationship was also observed ($Z = -2.104$, $p = 0.0354$), there was no overall significant interaction effect of flower abundance and post-fire age class on bee abundance ($\chi^2 = 4.524$, $p = 0.104$, df = 2).

There was no significant effect of flower abundance on the abundance of beetles (χ^2 = 1.580, p = 0.664, df = 3), flies (χ^2 = 2.119, p = 0.548) and wasps (χ^2 = 6.454, p = 0.091, df = 3). In addition, there was no significant effect of post-fire age class on the abundance of beetles (χ^2 = 3.354, p = 0.500, df = 4), flies (χ^2 = 1.149, p = 0.886, df = 4) and wasps (χ^2 = 4.180, p = 0.382, df = 4). In addition, there was no interaction effect of post-fire age class and flower abundance on the abundance of beetles (χ^2 = 0.728, p = 0.695, df = 2) and flies (χ^2 = 1.081, p = 0.582, df = 2) and wasps (χ^2 = 1.578, p = 0.454, df = 2).

Bee family distribution across post-fire class

Overall, Megachilidae (45.47%) accounted for highest proportion of bees followed by Halictidae (33.84%) and Apidae (20.47%). Only one occurrence of Andrenidae was recorded in one of the burned sites. There was a significant difference in the abundance of bees among families in different fire classes. While megachilid bees were highest in abundance in medium- (z = 6.519, p < 0.0001, df = 14) and short-term (z = 6.336, p < 0.0001, df = 14) burned sites, Halictidae (z = 5.118, p < 0.0001, df = 5) and Apidae (z = 4.698, p < 0.0001, df = 5) were higher in long-term burned sites with low abundance of Megachilidae.

Species composition

There was a significant difference in species composition of anthophiles across post-fire age classes (PERMANOVA F = 1.9214, p = 0.036, Figure 4.4a). Species composition at the medium-term burned (t = 1.7403, p = 0.014) and short-term burned sites (t = 1.4668, p = 0.049) were significantly different from species composition at the long-term burned sites. Similarly, there was a significant difference in species composition of flowering plant species sampled across the post-fire age classes (PERMANOVA F = 1.8907, p = 0.002, Figure 4.4b). Species composition of flowering plants at the medium-term burned (t = 1.4175, p = 0.012) and short-term burned sites (t = 1.5615, p = 0.002) was significantly different from species composition at the long-term burned sites. There was no significant difference in species composition of flowering plant between medium-term and short-term burned sites (t = 1.135, p = 0.118). The RELATE function showed no significant relationship between the overall composition of anthophile and flowering plants across post-fire age class (rho =0.081, p = 0.235)

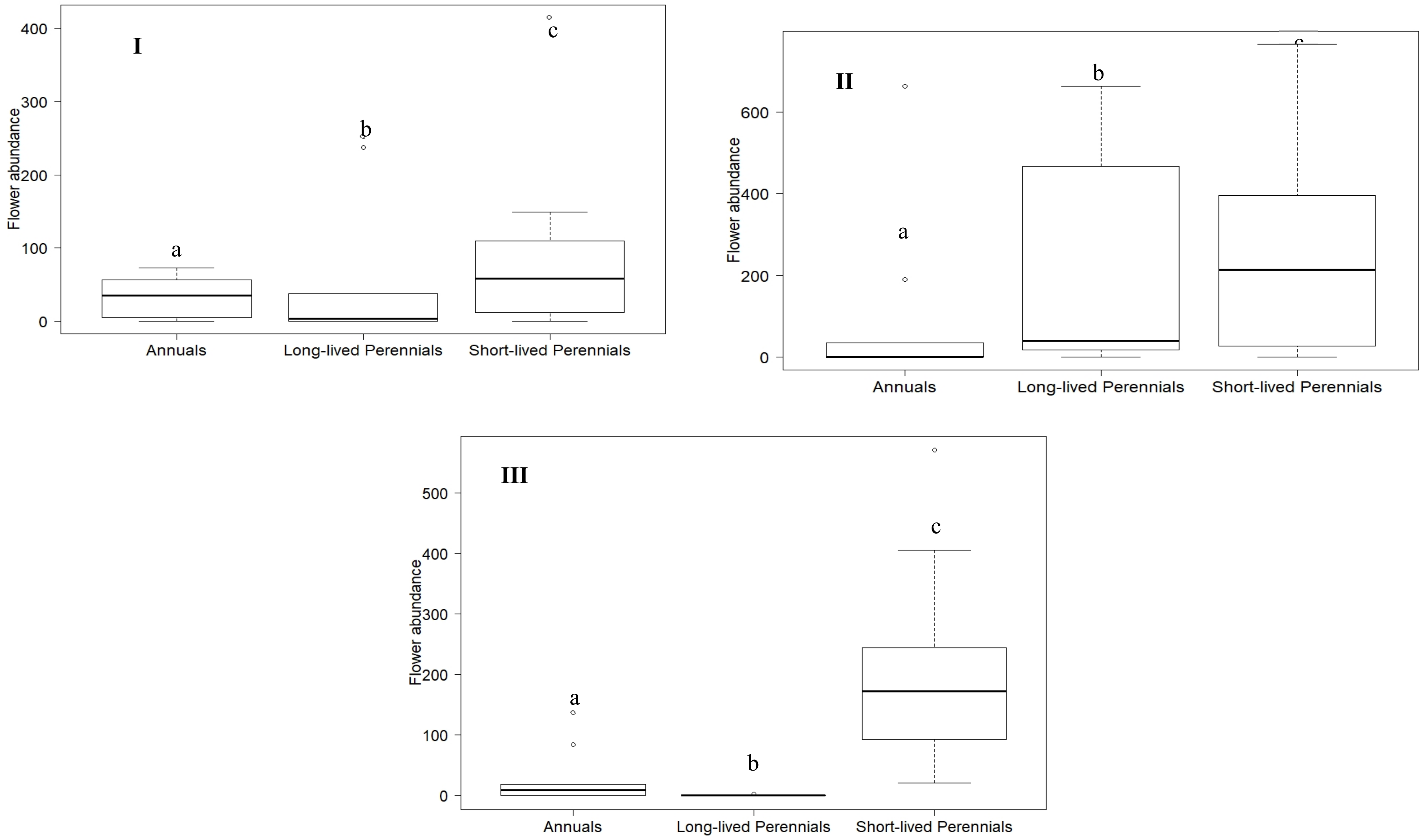

Figure 4.2. Flower abundance among vegetation classes in (I) short-term burned site (II) medium-term burned sites and (III) long-term burned sites. Fire class with common alphabets are not significantly different at $p > 0.05$

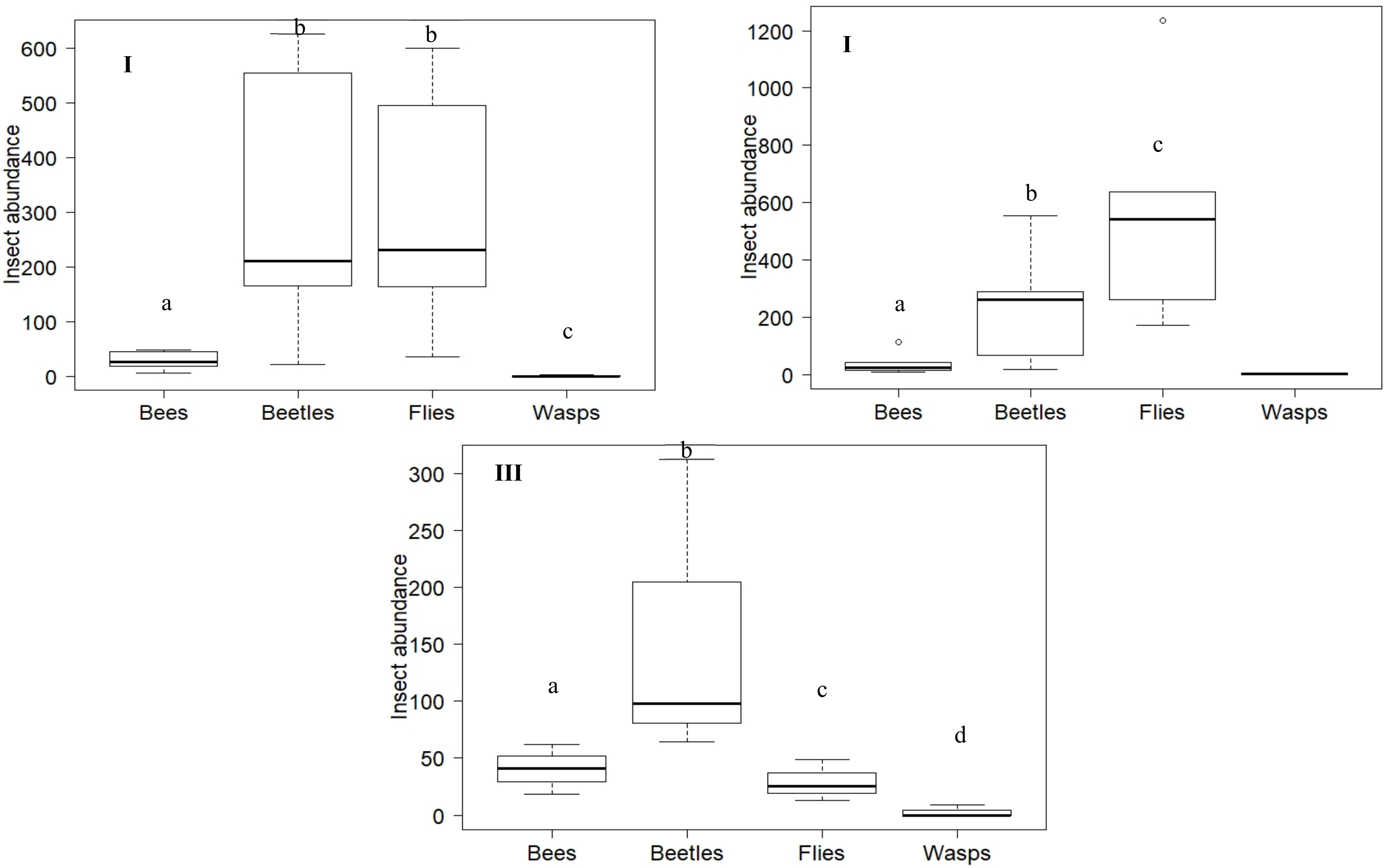

Figure 4.3. Anthophilous insect abundance among taxonomic groups in (I) short-term burned site (II) medium-term burned sites and (III) long-term burned sites. Fire class with common alphabets are not significantly different at $p > 0.05$

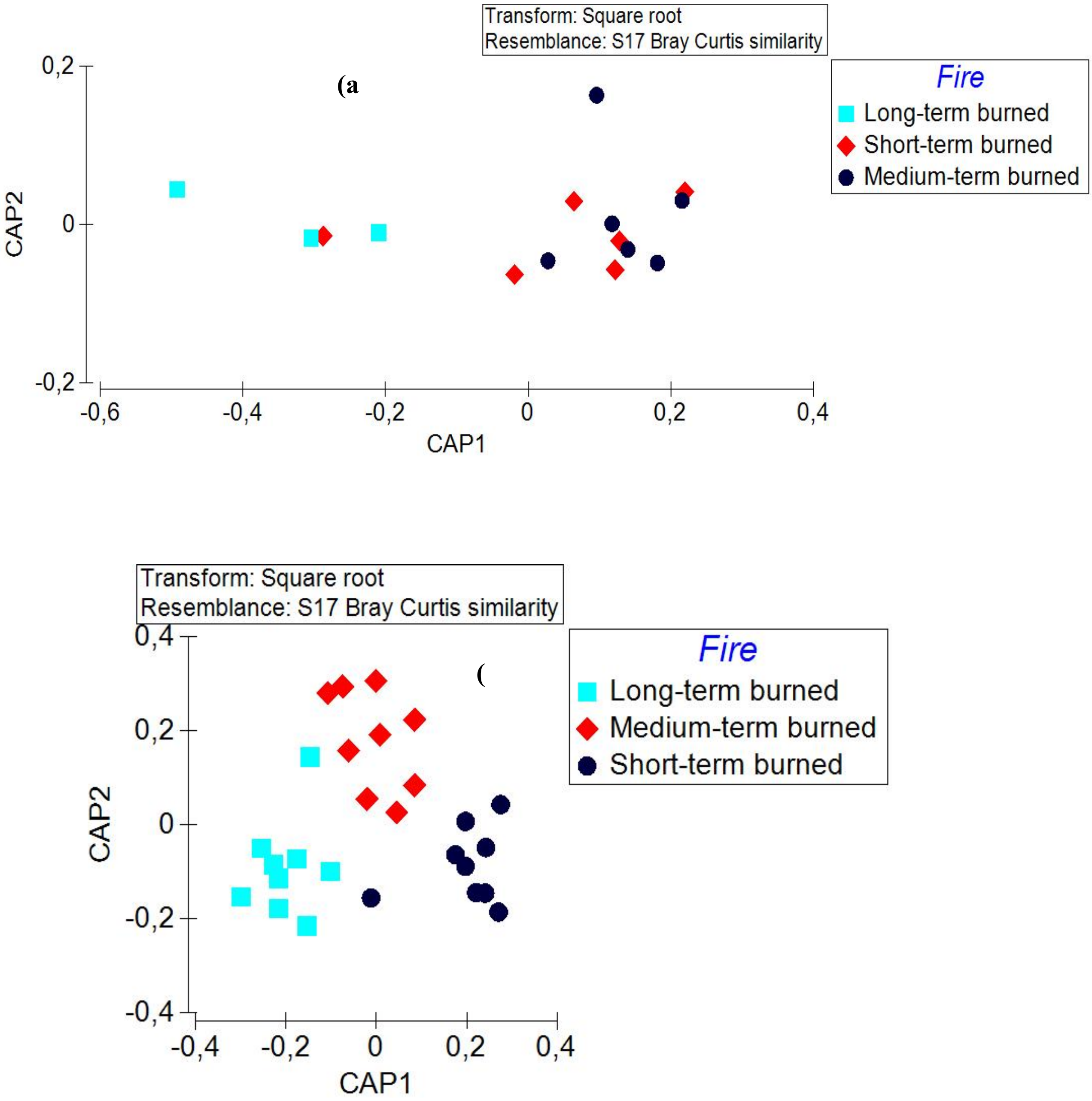

Figure 4.4. Canonical analysis of principal coordinates showing differences in the composition of (a) insect anthophiles and (b) flowering plants across sites in different post-fire age classes

Taxon response to post-fire classes

There was no significant difference in bee species composition across post-fire age classes ($_{\text{PERMANOVA}}F = 1.275$, $p = 0.113$). There was a significant difference in the species composition of flies across the post-fire age classes ($_{\text{PERMANOVA}}F = 3.2298$, $p = 0.008$, Figure 4.5). Between-pairs comparisons showed a significant difference in fly composition between short-term burned and long-term burned sites (t = 1.7037, p = 0.037), and also between medium-term burned and long-term burned sites (t = 2.2289, p = 0.008). There was no significant difference between short-term burned and medium-term burned sites (t = 1.1929, p = 0.202). There was no significant difference in species composition of beetles across the post-fire age classes ($_{\text{PERMANOVA}}F$ = 0.78794, p = 0.651). Similarly, there was no significant difference in species composition of wasps across the post-fire age classes ($_{\text{PERMANOVA}}F$ = 0.99508, p = 0.539).

Diversity among post-fire classes

There was no significant difference in overall Simpson ($F_{2,\ 12}$ = 0.167, p = 0.848) and Shannon ($F_{2,\ 12}$ = 1.997, p = 0.178) diversity of anthophiles across post-fire age. Similarly, there was no significant difference in Simpson ($F_{2,\ 24}$ = 0.600, p = 0.557) and Shannon ($F_{2,\ 24}$ = 2.712, p = 0.086) diversity indices for flowering plant across post-fire age. Also, there was no significant difference is overall evenness diversity index for flowering plant ($F_{2,\ 24}$ = 1.37, p = 0.273) and anthophiles ($F_{2,\ 12}$ = 2.721, p = 0.106) across post-fire age.

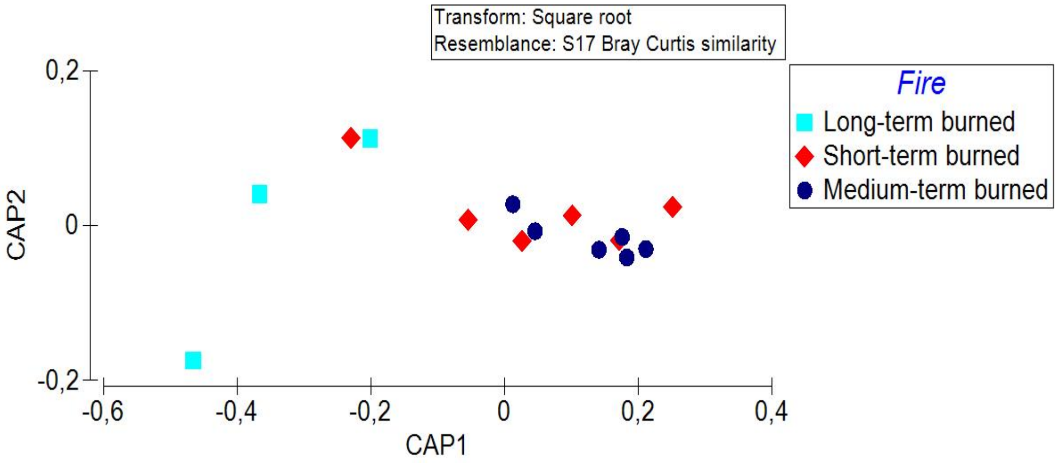

Figure 4.5. Canonical analysis of principal coordinates showing differences in the composition of flies across sites in different post-fire age classes

However, overall average species evenness for anthophiles (0.467) and for flowering plants (0.706) were moderately high. This showed that assemblages were fairly even in frequency of different species.

Evenness among post-fire classes

Overall mean species evenness for anthophiles was highest for bees (0.721±0.039), followed by beetles (0.566±0.045), wasps (0.289±0.112), and flies (0.244±0.063). Flies were the only taxon that showed a significant difference in species evenness across the post-fire age classes ($F_{2, 12} = 5.011$, $p = 0.0262$). Highest evenness was recorded at long-term burned sites, and least at medium-term burned sites (Fig 8). There was no significant difference in the evenness diversity index of bees ($F_{2, 12} = 0.423$, $p = 0.666$), beetles ($F_{2, 12} = 1.292$, $p = 0.31$) and wasps ($F_{2, 12} = 0.45$, $p = 0.648$) across post-fire age classes.

Discussion

Fire influences the distribution patterns in terms of diversity and composition of flowering plants and the various anthophile taxonomic groups. Varying responses of different ecological communities in fire-impacted areas is often associated with uneven spread of fire across a heterogeneous landscape (Suding and Gross 2006, Pausas and Verdú 2008, Myers and Harms 2011, Myers et al. 2015). Overall, short-lived perennial plants were highly successful in terms of flower abundance compared to other groups. Most of the perennial plants, such as *Phylica buxifolia, Protea laurifolia, Oesteospermum* sp. and *Lobostemon dorothea*, are important flowering plants in the GCFR, and provide quality floral reward for endemic insect anthophiles and birds (Anderson et al., 2014). Some of these plants take flowering cues from smoke, and then resprout during the next flowering season after fire (Staden et al., 2000). However, for slow growing, long-lived perennials, such as *P. laurifolia*, the germination from seed to first flowering after fire takes an average of seven years (Notten, 2009). This explains why flower abundance was low for slow growing, long-lived perennial plants. In area prone to fire, while short-lived perennials and annuals could recover within a short time, longer period of recovery of long-lived perennials may explain the low flower abundance of these plants especially in areas with high fire frequency.

Similar to flower abundance across post-fire age classes, anthophile abundance was also highest at medium-term burned. However, while flower abundance was lowest at short-term burned sites, lowest abundance of anthophiles was recorded at long-term burned sites. This illustrates that flower distribution does not entirely track anthophile distribution across the post-fire age classes. Contrary to my hypothesis, other factors may be responsible for the distribution of anthophiles across post-fire age classes. One possible explanation is habitat selection on the part of different anthophile taxa. Flies dominated anthophile abundance in my study. Furthermore, fly composition was different between long-term burned and short-term burned sites. Flies were most abundant in medium-term burned and short-term burned sites, and lowest at long-term burned sites. This was contrary to the abundance of flowers among the post-fire age classes. Although flies can be important pollinators of flowering plants (Larson et al., 2001), in comparison with other anthophiles, most flies are also carrion feeders and may be driven by factors apart from flower availability. *Drosophila* spp. made up about 40% of my entire fly samples, and these flies breed in decaying logs and plant branches (Offenberger and Klarenberg, 1992). In my study, they are predominantly associated with short-term burned and medium-term burned sites with poor representation at long-term burned sites. The entire short-term burned landscape has heterogeneous distribution of decaying burned logs and twigs, which may be the major factor driving their dispersion patterns in this region compared to the great reduction of flies at the long-term burned sites.

Bees were very different from flies. They tracked flower abundance across post-fire age classes, with their highest abundance at long-term burned and medium-term burned sites, while lowest at short-term burned sites. In short, bees followed flowering plant dispersion patterns. This could be a mass movement of bees into the medium-term burned and long-term burned sites during the fire events, with the presence of more flowers here providing quality nectar rewards, which may drive this pattern (Wojcik, 2011). The presence of important nectar and pollen rewarding flowering plants here may drive the distribution of bees in the absence of other possible environmental factors which may influence species distribution. In response to natural fire, flower abundance across fire post-age class seems to be an important influence for bee abundance across sites with different time since fire.

Unlike other taxonomic groups, where other ecological roles, such as parasitism and predation, have been discussed, several studies have shown pollination as the major functional role of bees in most ecosystems through their dependence on floral rewards. This also explains the differences in bee composition with distinct segregation of bee species among post-fire age classes. According to Van Nuland et al. (2013), flower abundance was the mechanism by which fire indirectly influences anthophile visitation. Overall, this implies that over a period of succession in a heterogeneous fire mosaic landscape, the quality and abundance of flowering plants providing floral resources for anthophiles, especially bees, and the flower distribution patterns play a major role also in the distibution patterns of insect anthophiles.

Bees also differed significantly in response to time since fire among families. Overall, megachilid bees are highest in abundance, however, this pattern was not observed across all post-fire age class. While megachilidae was highest in abundance in medium- and short-term burned, my results also showed highest abundance for apidae and halictidae in long-term burned. This shows that species-specific factors may be driving the distribution of bee in different families across fire post-age class. This could be dietary requirements, habitat selection, body size, specialization habit, tongue length among other factors. Several studies have shown how bee functional groups may influence their distribution across landscape (Winfree et al., 2007; Hoehn et al., 2008; Munyuli, 2012). However, studies showing how bee functional groups respond to time since fire are few. Fire regime, most especially fire frequency, filters biotic assemblage composition and abundance through its direct impact (Keeley et al., 2011). While I show here how habitat modification in terms of fire influences the distribution of bees, future studies should address how species-specific requirements among families influence bee distribution across post-fire age classes.

Evenness and diversity

Simpson and Shannon diversity indices here showed no significant pattern for species richness and diversity across fire classes, or among insect groups and flowering plants. However, I found some differences in species evenness, especially among insect groups. Species richness and evenness are different diversity components, and the two do not necessarily show the same pattern of response when used to assess differences in ecosystems (Wilsey and Stirling, 2007). Overall, flowering plant and anthophile evenness indices are moderately high, suggesting that the frequency distribution is moderately even among species (Smith and Wilson, 1996).

Species diversity response to disturbance is complex. However, a decline in species richness may lead to an offset in the distribution of different species and components of biodiversity in disturbed landscapes. Yeboah et al. (2016) showed a complex response by species diversity and evenness in a tree stand relative to time since last fire. My results show that annual plants such as *Oxalis* spp. and a few perennial Asteraceae regrew quickly after fire, and dominated the entire landscape. I however recorded very low flower abundance of bushy *Protea* spp., *Leucadendron* spp., which are important for anthophile foraging and only a few other long lived perennials. The implication of this is that the full complement of anthophiles depends largely on plants with very low frequency of flowering. These long-lived fynbos plants are an important source of nectar for insect anthophiles, as well as for certain birds. Anthophilous insects, especially the specialised group, may compete for the low resources of a few rich nectar rewarding plant available across my post-fire age classes. This may overtime lead to temporal loss of these insect groups that are unable to find a replacement for the limited rich nectar flower across my study area.

Bees showed the highest species evenness, followed by beetles. However, for flies and wasps, evenness was moderately low, illustrating differences in frequency of occurrence of species of these two groups. Here, as *Drosophila* spp. constituted almost 40% of all the flies collected, they created a major imbalance in the frequency of occurrence of flies. Wasps on the other hand, had very low abundance, with many rarely captured species. Across post-fire age classes, flies showed significant differences in species evenness. Highest evenness was recorded at long-term burned sites, and lowest at short-term burned and medium-term burned sites. One possible explanation for this is the mosaic nature of the burned landscape, with medium-term burned areas acting as refuge patches across areas impacted by fire. As mentioned above, flies may also be driven by decayed burned logs at my sites. However, these logs were in patches, and not evenly distributed across the landscape. This may explain a varying frequency of occurrence of fly species among sites across the heterogeneous burned landscape.

Conclusions

The effect of fire on the abundance of bees was influenced by flower abundance. In addition, other species-specific factors appeared to be influencing the distribution among families, especially for bees and flies. Overall flower abundance declined in association with a short-interval fire frequency, and this was especially pronounced among long-lived perennial plants. While other anthophile taxonomic groups, aside from bees, may not be influenced much by the fire regimes, bees are mostly obligate flower-visitors, and are highly associated with flower distribution pattern. Thus, flower rich area with moderate time since fire incidence may increase the diversity of bees in fire prone areas.

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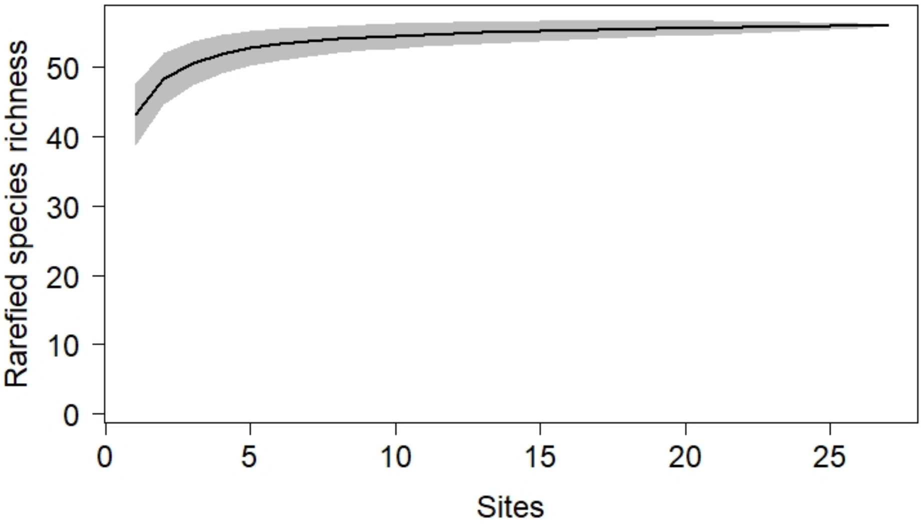
APPENDIX

Appendix 4a. List of pollinator species

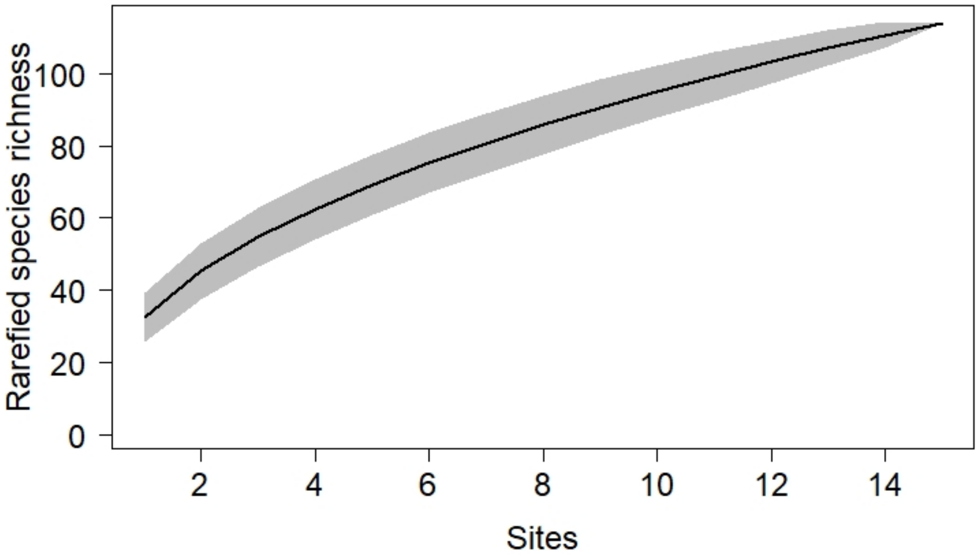
Taxa	Family	Genus	Species/Morphospecies
Bee	Halictidae	*Halictus*	sp.1
Bee	Halictidae	*Halictus*	sp.2
Bee	Halictidae	*Halictus*	sp.3
Bee	Halictidae	*Halictus*	sp.4
Bee	Halictidae	*Halictus*	sp.5
Bee	Halictidae	*Halictus*	sp.6
Bee	Halictidae	*Halictus*	sp.7
Bee	Halictidae	*Halictus*	sp.8
Bee	Halictidae	*Halictus*	sp.9
Bee	Halictidae	Halictidae	sp.1
Bee	Halictidae	Halictidae	sp.2
Bee	Halictidae	Halictidae	sp.3
Bee	Halictidae	Halictidae	sp.4
Bee	Halictidae	Halictidae	sp.5
Bee	Halictidae	Halictidae	sp.6
Bee	Halictidae	Halictidae	sp.7
Bee	Halictidae	Halictidae	sp.8
Bee	Halictidae	Halictidae	sp.9
Bee	Halictidae	Halictidae	sp.10
Bee	Halictidae	Halictidae	sp.11
Bee	Halictidae	Halictidae	sp.12
Bee	Halictidae	Halictidae	sp.13
Bee	Halictidae	Halictidae	sp.14
Bee	Halictidae	Halictidae	sp.15
Bee	Halictidae	Halictidae	sp.16
Bee	Halictidae	Halictidae	sp.17
Bee	Halictidae	Halictidae	sp.18
Bee	Halictidae	Halictidae	sp.19
Bee	Halictidae	Halictidae	sp.20
Bee	Halictidae	Halictidae	sp.21
Bee	Halictidae	Halictidae	sp.22
Bee	Halictidae	Halictidae	sp.23
Bee	Halictidae	Halictidae	sp.24
Bee	Halictidae	*Nomia*	sp.1
Bee	Halictidae	*Nomia*	sp.2
Bee	Halictidae	*Nomia*	sp.3
Bee	Halictidae	*Nomia*	sp.4
Bee	Halictidae	*Nomia*	sp.5
Bee	Halictidae	*Nomia*	sp.6
Bee	Halictidae	*Lasioglossum*	sp.1
Bee	Halictidae	*Lasioglossum*	sp.2
Bee	Halictidae	*Lasioglossum*	sp.3
Bee	Halictidae	*Lasioglossum*	sp.4
Bee	Halictidae	*Lasioglossum*	sp.5
Bee	Halictidae	*Lasioglossum*	sp.6
Bee	Halictidae	*Patellapis*	sp.1
Bee	Halictidae	*Patellapis*	sp.2
Bee	Halictidae	*Patellapis*	sp.3
Bee	Halictidae	*Pseudapis*	sp.1
Bee	Halictidae	*Pseudapis*	sp.2
Bee	Halictidae	*Pseudapis*	sp.3

Bee	Megachilidae	*Lithurgus*	sp.1
Bee	Megachilidae	*Lithurgus*	sp.2
Bee	Megachilidae	*Lithurgus*	sp.3
Bee	Megachilidae	*Megachile*	sp.1
Bee	Apidae	*Anthophora*	sp.1
Bee	Apidae	*Anthophora*	sp.2
Bee	Apidae	*Amegilla*	sp.1
Bee	Apidae	*Amegilla*	sp.2
Bee	Andrenidae	*Andrena*	sp.
Bee	Apidae	*Apis*	*mellifera*
Bee	Apidae	*Allodapula*	sp.
Bee	Apidae	Apidae	sp.1
Bee	Apidae	Apidae	sp.2
Beetle	Scarabaeidae	*Hedybius*	sp.
Beetle	Scarabaeidae	*Hedybius*	sp.2
Beetle	Scarabaeidae	*Peritrichia*	sp.
Beetle	Scarabaeidae	*Anisonyx*	*ursus*
Beetle	Scarabaeidae	*Pachycnema*	sp.1
Beetle	Scarabaeidae	*Pachycnema*	sp.2
Beetle	Scarabaeidae	*Pachycnema*	sp.3
Beetle	Scarabaeidae	Hoplinii	sp.
Beetle	Scarabaeidae	Scarabaeidae	sp.1
Beetle	Scarabaeidae	Scarabaeidae	sp.2
Beetle	Scarabaeidae	Scarabaeidae	sp.3
Beetle	Cerambycidae	Cerambycidae	sp.
Beetle	Coccinellidae	Coccinellidae	sp.1
Beetle	Coccinellidae	Coccinellidae	sp.2
Beetle	Coccinellidae	Coccinellidae	sp.3
Beetle	Meloidae	Meloinae	sp.
Beetle	Curculionidae	Scolytinae	sp.1
Fly	Drosophilidae	*Drosophila*	*melanogaster*
Fly	Muscidae	Muscidae	sp.1
Fly	Muscidae	Muscidae	sp.2
Fly	Syrphidae	Syrphidae	sp.1
Fly	Syrphidae	Syrphidae	sp.2
Fly	Syrphidae	Syrphidae	sp.3
Fly	Tabanidae	Tabanidae	sp.1
Fly	Tabanidae	Tabanidae	sp.2
Fly	Tabanidae	Tabanidae	sp.3
Fly	Tephritidae	Tephritidae	sp.
Fly	Bombyliidae	Bombyliidae	sp.1
Fly	Bombyliidae	Bombyliidae	sp.2
Fly	Calliphoridae	*Lucilia*	*sericata*
Fly	Calliphoridae	Calliphoridae	sp.1
Fly	Calliphoridae	Calliphoridae	sp.2
Fly	Culicidae	Culicidae	sp.
Fly	Asilidae	Asilidae	sp.1
Fly	Asilidae	Asilidae	sp.2
Wasp	Sphecidae	Sphecidae	sp.1
Wasp	Sphecidae	Sphecidae	sp.2
Wasp	Sphecidae	Sphecidae	sp.3
Wasp	Sphecidae	Sphecidae	sp.4
Wasp	Pompilidae	Pompilidae	sp.1
Wasp	Pompilidae	Pompilidae	sp.2
Wasp	Braconidae	Braconidae	sp.1
Wasp	Braconidae	Braconidae	sp.2
Wasp	Masaridae	Masaridae	sp.
Wasp	Crabronidae	*Tachysphex*	sp.1

Wasp	Crabronidae	*Tachysphex*	sp.2
Wasp	Scoliidae	Scoliidae	sp.1
Wasp	Scoliidae	Scoliidae	sp.2
Wasp	Ichneumonoidea	Ichneumonoidea	sp.



Appendix 4b. Species richness rarefied curve for flowering plants sampled across study sites. The grey area represents the confidence interval from the standard error of estimates.



Appendix 4c. Species richness rarefied curve for insects sampled across sampling sites. The grey area represents the confidence interval from the standard error of estimates.

Chapter 5

Refuges from fire maintain plant-pollinator interaction networks

Abstract

Fire is an important disturbance factor in many terrestrial ecosystems, leading to landscape transformation in fire-prone areas. Species in mutualistic interactions are often highly sensitive to disturbances like fire events, but the degree and complexity of their responses are unclear. I use bipartite insect-flower interaction networks across a recently burned landscape to explore how plant-pollinator interaction networks respond to a recent major fire event at the landscape level, and where fire refuges were present. I also investigate the effectiveness of these refuges at different elevations (valley to hilltop) for the conservation of displaced flower-visiting insects during fire events. Then I explore how the degree of specialization of flower-visiting insects changes across habitats with different levels of fire impact. I did this in natural areas in the Greater Cape Floristic Region (GCFR) biodiversity hotspot, which is species rich in plants and pollinators. Bees and beetles were the most frequent pollinators in interactions, followed by wasps and flies. Highest interaction activity was in the fire refuges, and least in burned areas. Interactions also tracked flower abundance, which was highest in fire refuges in the valley, and lowest in burned areas. Interactions consisted mostly of specialized flower-visitors, especially in refuge areas. The interaction network and species specialization were lowest in burned areas. However, species common to at least two fire classes showed no significant difference in species specialization. I conclude that flower-rich fire refuges sustain plant-pollinator interactions, especially those involving specialized species, in fire-disturbed landscape. This may be an important shelter for specialized pollinator species at the time that the burned landscape goes through regrowth and succession as part of ecosystem recovery process after a major fire event.

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Introduction

Fire is a critical disturbance factor in many terrestrial ecosystems (New, 2014). It is especially prevalent through the recent increase in human-induced landscape transformation and rapid climate change, especially in Mediterranean-type ecosystems, where warmer and drier conditions are increasingly prevalent (Syphard et al., 2009; Bowman et al., 2011; Archibald et al., 2012; Steel et al., 2015). Immediate impact of fire usually results in high mortality of resident species, which increases with intensity and frequency of fire (Adeney et al., 2006; Silveira et al., 2010; Bennett et al., 2016). Flower-visiting insects, especially the less mobile species, are greatly affected by fire in natural landscapes. However, species functional traits may influence survival during fire; for example, while zoophagous and phytophagous arthropods are highly resilient to the effects of fire, mortality was higher for ground-litter saprophagous species (Moretti et al., 2006). Also, specialist bee species decline more than generalists in freshly-burned habitat (Peralta et al. 2017). In addition, long-term recolonization of burned habitat may be affected by transformation processes of the habitat, as the newly transformed habitat may yield different species composition. Over the post-fire period, fire usually transforms landscapes into more open habitat, which may change species composition over time (Case and Staver, 2017). This is seen in South Africa, where the composition of the butterfly community changed over the period of recovery following a major fire event (Pryke and Samways, 2009; Yekwayo et al. 2018).

Most studies on fire show a positive influence of fire on flowering plant diversity and abundance of insect pollinators (Bond and Scott, 2010; Lamont and Downes, 2011; Ponisio et al., 2016). While this is important for the long-term biodiversity succession in fire-disturbed ecosystems, there is concern for the immediate species response during and after fire. Potts et al. (2003) showed a time lag of 2 years for burned area to reach full recovery and a flowering peak. Immediately after fire, decrease in pollinator abundance and floral resources is expected in burned areas, yielding a temporal decline in plant-pollinator interactions. During this time, while flowering plants are burned down, mobile insect pollinators seek refuge in areas not impacted by fire. Refuges are areas in an ecosystem where a disturbance affecting a larger region did not take place. As a consequence, they can buffer the effect of transformation events in natural landscapes (Mackey et al., 2002). Despite the great importance of refuges on the recovery process and resilience of populations, they are rarely studied (Robinson et al., 2013). In fire-prone areas, patches of vegetation that escape the absolute impact of fire can serve as refuges for individuals of certain insect species (Bradstock et al., 2005; Perera et al., 2007; Burton et al., 2008; Castro et al., 2010). However, for a patch to function effectively as a refuge, it must provide enough floral and nesting resources for survival of the locally lost or displaced flower-visiting insect species (Watson et al., 2012; Brown et al., 2016). As fire ultimately leads to temporal displacement of flower-visiting insects during the fire event, refuge patches are essential for local survival and even persistence of flower-visiting insects.

Insect pollinators forage in areas close to their nest (Gathmann & Tscharntke, 2002; Schweitzer et al., 2012). The location of important fire refuges in the disturbed landscape may be critical for the presence of specific pollinator species in fire-disturbed landscape. While some large bees can visit vegetation patches for floral resources over long distances, ground dwelling and less mobile groups may require nesting resources within patches around the burned area (Steffan-Dewenter, 2002). In addition to site-specific abiotic components, such as nutrient availability, canopy cover may influence the effectiveness of fire refuges and play a significant role in the conservation of insect pollinators during fire disturbance. For example, changes across elevation may influence flowering plant distribution, with plants at higher elevations having reduced growth (Boscutti et al., 2018) and low species richness (Jacquemyn et al., 2005).

Most times, pollinators are displaced from areas of few flowers at high elevations to flower rich lower elevations (Rahbek, 2005; Guo et al., 2013; Lara-Romero et al., 2019). Unlike hilltops, valleys sometimes contain riparian corridors with rich vegetation, and these rich vegetation can be effective nest provision and forage site for some insect pollinators, especially bees (Mader et al., 2011). In the context of fire refuges, it is expected that areas of sufficient requirements for nesting will make a better refuges during fire disturbance. However, there is little information on the effectiveness of fire refuges across heterogeneous topographic landscapes.

Network metrics are used to describe properties of interaction networks. Most of the metrics are standardized ways of explaining the contribution of individual species and communities in a network leading to the success of interactions and delivery of ecosystem functions. For example, there is a simple approach to estimating species specialization in a network where it is possible to directly link a species to all interacting partners by observation (Johnson and Steiner, 2003; Ollerton et al., 2007). However, this approach is limited by not taking into account estimates of resource diversity. A more inclusive index for species specialization is index d', which takes into account diversity of interacting partners and their importance in a network based on observed and expected interacting frequencies (Blüthgen et al., 2006).

Overall, network metrics can be applied to explore community structure, especially for mutualistic species. While abundance and distribution of interacting partners may be relatively stable in less disturbed areas, fire disturbance in fire-prone landscapes may facilitate fluctuations in species abundance and distribution. Over time, more generalized species become increasingly abundant in areas with frequent fires with short-term intervals (Peralta et al., 2017). Consequently, interactions consisting of more specialized species in burned areas may face a breakdown (García et al, 2018). For example, a specialist pollinator may be forced to explore other available floral resources in a smaller refuge patch when it is displaced from its extensive habitat.

Despite the high impact of fire in changing natural landscapes and community interactions, there is little information on the response of plant-pollinator interactions to fire events, and how fire refuges alter species response to fire. Here, I explore how plant-pollinator interaction networks respond to recent fire at the landscape level where fire refuges are present. I also investigate the effectiveness of these refuges at different elevations for the conservation of displaced flower-visiting insects during fire events. Refuges may have an important relationship with elevation and rugosity of landscape. While there is no information on how topographic features influence fire refuge, features such as river in the valley, rocks and vegetation at hillslope or hill tops may contribute to the leaving of areas that escape being burned.

I undertake this study in the flower- and pollinator-rich Greater Cape Floristic Region (GCFR) biodiversity hotspot. I also explore how the degree of specialization of flower-visiting insects changes across habitats with different levels of fire impact. To this end, I compile information from observations on bipartite insect-flower interaction networks from visitation to flowers by important flower-visiting insects across a recently burned landscape. I hypothesize that: i) like most disturbance events, the direct impact of fire is expected to aid the emigration of species from burned to unburned area, thereby, I expect highest abundance of flowering plants and highest interaction frequency in unburned compared to burned and refuge habitats; ii) geographical valleys are often nutrient rich with streams running along them, and may have more flowering plant species that may act as refuges in the valley, and are therefore more effective in sustaining interactions compared to those on hilltops, and iii) since flower-visiting insects are associated with flowering plants, more specialised networks will be observed in unburned habitats with high flower abundance.

Materials and methods

The study was conducted in the large natural set-aside areas on wine farms in the Western Cape Province, South Africa, in the Greater Cape Floristic Region (GCFR) biodiversity hotspot. Bee diversity in the GCFR is exceptionally high, coinciding with that of plants (Kuhlmann, 2005). Two adjacent wine estates were selected (Vergelegen: 34.0764°S, 18.8899°E and Lourensford: 34.0719°S, 18.8886°E). These estates practice biodiversity-friendly agriculture, with extensive areas of the farms devoted to conservation of indigenous biodiversity, where my sites were positioned. The landscape varies in topography, with sites available in valleys, on hill slopes, and on hilltops. These natural areas on the estates burned, but left refuges December 2016-February 2017.

I classified my sampling sites into those in refuges (2 years since last fire), burned (6 months since last fire), and unburned areas (9-10 years since last fire) (Appendix 5a). I collected insects using pan traps from some of these sites and estimated flower abundance from all of the sites in chapter 4. These sites are however named differently here to fulfil the aim of this chapter. Refuge sites were defined as patches ≥ 50m^2 and of unburned vegetation within the burned matrix. Unburned sites were those in extensive natural areas that were beyond the fire front. Sites were selected in the valleys (≤ 200 m a.s.l.), on hill slopes (200-350 m a.s.l.), and on hilltops (400-450 m a.s.l.). For every valley site, I also sampled matching hill slope and hilltop sites. Plant-pollinator interactions were recorded at 27 sites across the fire categories late August-November 2017.

A total of nine sites (3 sites per elevation), each of 50 m^2, were in each of the refuges, burned areas, and also unburned areas (i.e. 9 sites × 3 fire classes = 27). Burned and refuge sites were selected in pairs ≥100 m apart from the edge, which in turn, were 0.9-3 km from the unburned sites. For every burned site, I selected the closest refuge patch that matched the size of a study site (i.e. 50 m^2) in each elevation category. The refuge and burned sites in each elevation category were visited on the same sampling day, and observation time was altered between fire classes in the second visit.

I observed plant-pollinator interactions in five replicates of 2 m^2 subplot within each 50 m^2 plot as in previous chapters. Timed observation of insect activity was standardized to 10 min/2 m^2 plot to avoid over-emphasizing the specialization of flowering plants (Gibson et al., 2011), reducing sampling bias from variables such as flower abundance. During this time, an interaction was noted when an insect visited the stamen of a plant.

Flower-visiting insects were identified in the field, or caught for later identification as morphospecies using identification guilds as in previous chapters. Five replicates per 2 m^2 sampling unit within each site yielded a total of 50 min observation time per site per sampling period. Every site was visited twice, with a total of 100 min observations per site, which were pooled as a single interaction network. Flower abundance of each plant species was estimated in each 2 m^2 plot where insect activities were observed. A flower unit is defined here as the unit from which a honeybee-sized insect will fly to the next unit rather than crawl (Dicks et al., 2002). I also estimated flower area of display for each flowering plant species. Area of floral display was determined for each open flowering plant species by measuring the diameter of 1-10 flowers per plant species. Areas of flowers with circular outline was estimated using πr^2 and L x B for those flowers with a more rectangular surface outline. A flower with visible depth, such as that of *Protea repens*, was estimated using $2\pi r^2 d + \pi r^2$. The mean flower area for a plant species, together with the total abundance of flowers, was used to estimate the plant flower area per site.

Statistical analyses

Interaction matrices for plant-pollinator interaction networks were compiled for each site. Data were analyzed using the bipartite package (Dormann et al., 2008) in R as in previous chapters. Network- and species-level indices were computed for each of the 27 networks: connectance, weighted nestedness (NODF), network specialization (H_2'), normalized degree (ND) and species specialization (d’). Network and species specialization indices were selected for this purpose, as these are insensitive to diversity of interacting partners (Schleuning et al., 2012), and so are suitable for this kind of study where effect of fire is expected to influence flowering plant diversity across my study sites.

To account for biases in estimates of interaction metrics, especially specialization which could result from differences in activities or attractiveness of interacting partners, I employed null models for the quantitative network metrics, based on the observed number of interactions for a species in a given network (using the Patefield algorithm: Dormann et al., 2009). I computed 100 null models for each network, and calculated z-scores for each network metric (i.e. differences between observed and mean null model index values, divided by the standard deviation of the null model values). The application of null models here reduces the biases in estimating network indices, especially with differences in number of interactions across my study sites.

To assess the differences in interaction frequency, abundance of flowering plants, and flower area across fire classes, and elevation, I used generalized linear model (GLM) with fire class and elevation category as fixed variables in different models. Similarly, I assessed how flower abundance and area of display influenced the pattern of interaction frequency across elevation and fire class using a GLM. In addition, to understand how z-scores of network metrics change across elevation and fire classes, I used GLMs, with fire class and elevation as predictors. I computed a PERMANOVA to analyse the difference in species composition of pollinators observed in interaction among fire class and elevation. Analyses were carried out using the packages lme4 and vegan.

To understand how interactions of pollinators are structured by availability of interacting partner across fire classes, I used the Normalised Degree function (ND) in the bipartite package. ND shows the degree of generalization of pollinator species through the sum of links scaled by the number of possible partners for individual species in a network. Here, I computed the relationship between interaction frequency and the ND of each species, and I observed how this changed across fire class. I used a generalized linear mixed effect model (GLMM) for this purpose using the ‘lme4’ package in R (Bates et al., 2014). I specified species as a random factor, to assess the confounding effect of different ND and interaction values of the same species in different fire class.

Finally, to understand the degree of specialization of individual species in the network across fire class, I computed each species' d'-value. Species specialization index (d') was used to measure the degree of discrimination of a species from random selection of partners in a network. Index d' is constructed in such a way that it measures specialization in terms relative to the other pollinators and resource abundances (Blüthgen et al. 2006). I assessed how observed d'-values changed across fire class and elevation using a linear mixed effect model, specifying species as a random factor. I also used the z-scores of d'-value from the null model following the same approach. Then, to assess whether species change the degree of specialization in different fire classes, I selected species common to at least two fire classes, and I assessed how their d'-values changed across fire class.

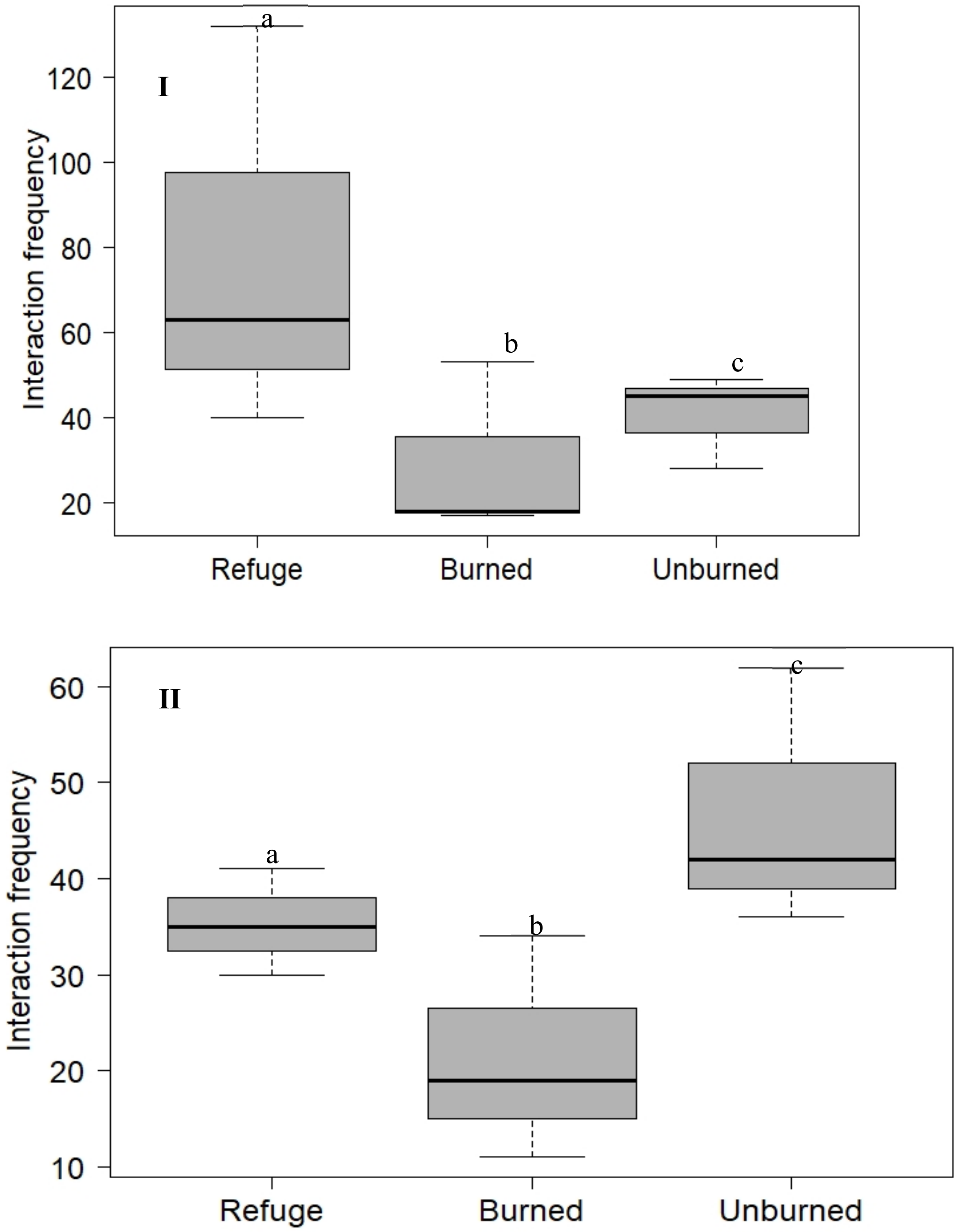
Results

A total of 1 176 interactions were recorded among 67 insect (Appendix 5b) and 56 plant species (Appendix 5c). Interactions consisted of bees (55.6%), beetles (25.94%), flies (17.09%), and wasps (1.53%). There was a significant difference in overall interaction frequency among fire classes. Highest interaction was observed in fire refuges which was significantly different from the lowest interactions observed in burned areas ($z = -9.524$, $p < 0.001$, df = 24, Figure 5.1). There was no significant difference in interaction frequency across elevation. In addition, there was a significant difference in interaction frequency observed among fire classes at each elevation category. Interaction frequency was significantly highest at the refuge in the valley ($z = 7.860$, $p < 0.001$, df = 6) and hillslope ($z = 2.185$, $p = 0.029$, df = 6), however, unburned sites had highest interaction at the hilltop ($z = 2.864$, $p = 0.029$, df = 6, Figure 5.1).

There was a significant difference in flower abundance across fire classes. Highest flower abundance was observed in fire refuges, while lowest in burned areas ($z = -29.45$, $p < 0.001$, df = 24, Figure 5.2I). Also, there was a significant difference in flower abundance across elevation. Flower abundance was highest in the valley, and lowest on the hilltop ($z = -16.77$, $p < 0.001$, df = 24, Figure 5.2II). However, there was no significant difference in flower area of display across fire class or elevation. Also, there was no significant difference in flowering plant and pollinator species richness across fire class or elevation.

There was no significant difference in species composition of pollinator assemblages across fire class ($F_{2, 24}$ = 1.0668, p = 0.347) or elevation ($F_{2, 24}$ = 1.1123, p = 0.273). In addition, there was no significant difference in flowering plant composition across elevation ($F_{2, 24}$ = 1.1163, p = 0.211). However, there was a significant difference in species composition of flowering plants across fire class ($F_{2, 24}$ = 1.4611, $p < 0.01$).

Overall, flower abundance ($F_{21, 22}$ = 85.92, $p < 0.001$) and flower area ($F_{21, 22}$ = 13.14, $p < 0.001$) significantly influenced pollinator activities. However, while interaction between flower abundance and topography was significantly associated with pollinator activity ($F_{21, 23}$ = 5.79, $p < 0.01$), pollinator activity was not significantly associated with the interaction between flower abundance and fire class ($F_{21, 23}$ = 1.08, p = 0.34). In case of the relationship between pollinator activity and flower area, there was a significant association of pollinator activity with the interaction between flower area and fire class ($F_{21, 23}$ = 12.07, $p< 0.001$). However, there was no significant association between pollinator activity and interaction between flower area and topography ($F_{21, 23}$ = 1.27, p = 0.28)


I
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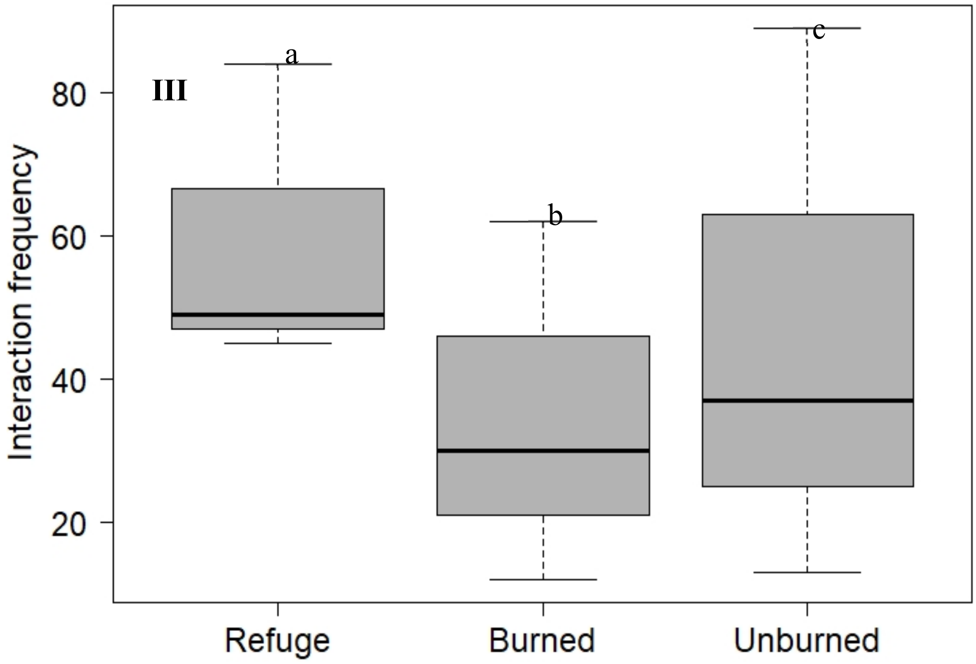
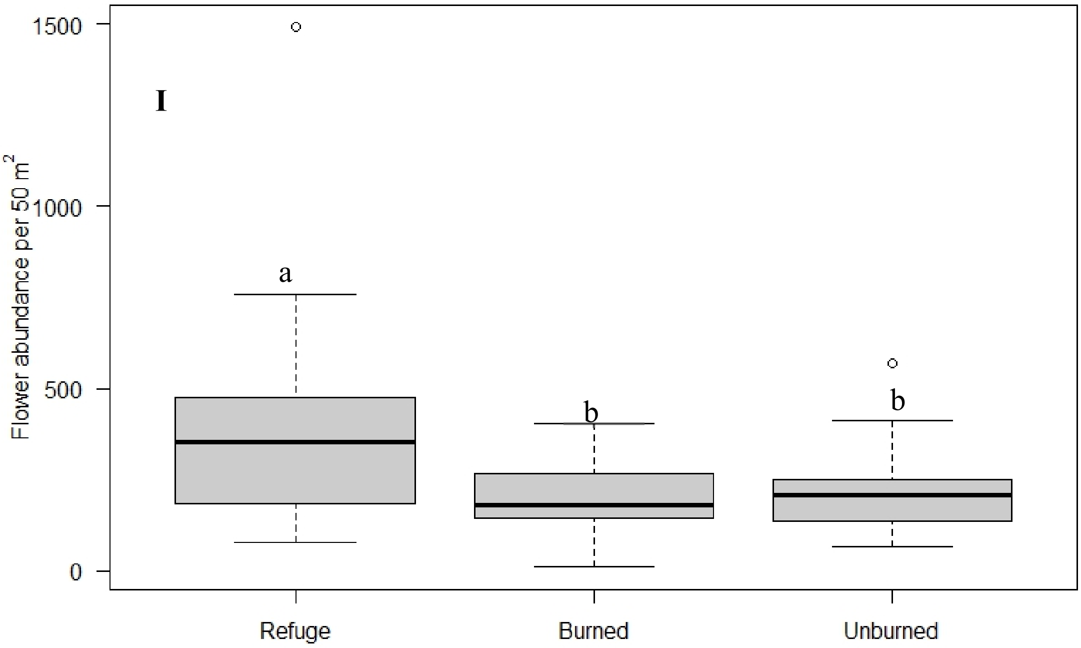


Figure 5.1. Interaction frequency among fire classes in (I) valley, (II) hilltop and (III) hillslope. Fire class with common alphabets are not significantly different at p > 0.05



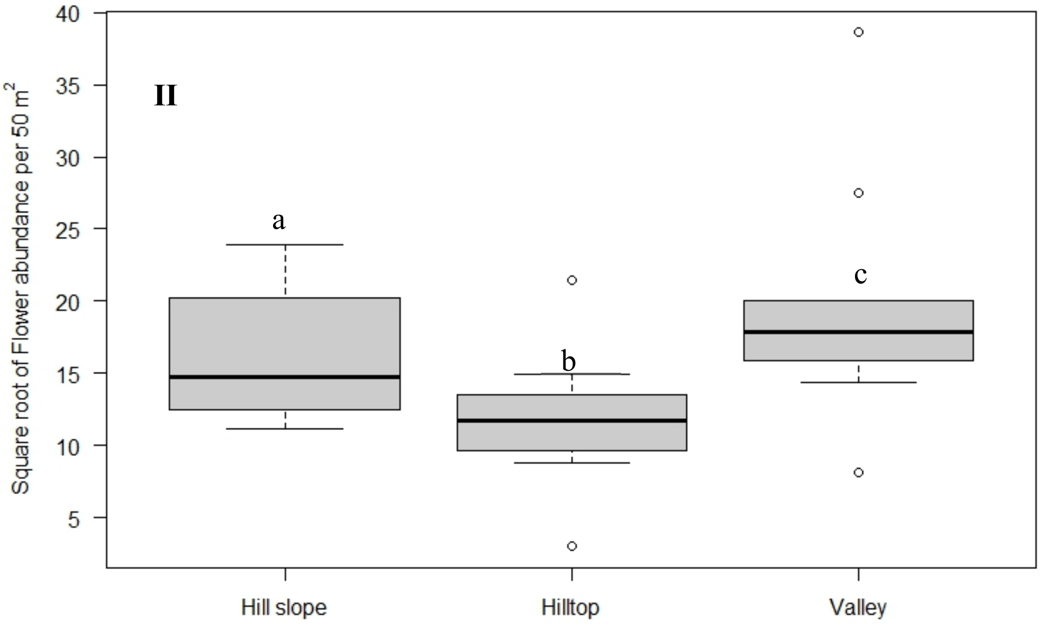


Figure 5.2. Flower abundance across (I) fire classes and (II) elevation category. Fire class with common alphabets are not significantly different at $p > 0.05$

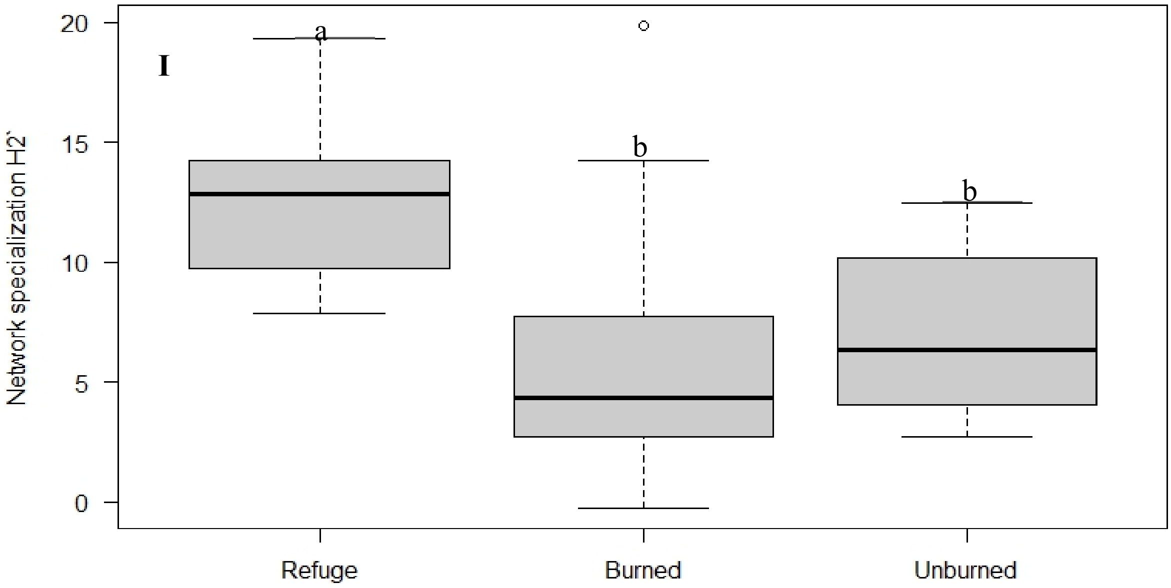
The average network specialization (H_2') value across the 27 study sites was high (mean = 0.736, standard deviation = 0.214). There was a significant difference in z-scores of network specialization (H_2') among fire classes ($F_{2,\,24} = 4.30$, p=0.025). Highest network specialization was at refuge sites, and this was significantly different from the lowest H_2' at the burned sites (Figure 5.3I). However, H_2' was not significantly different across elevation. Weighted nestedness (NODF) also differed significantly among the fire classes ($F_{2,\,24} = 5.581$, p = 0.01). Networks at refuge sites were less nested than those at burned sites (Figure 5.3II). However, there were no significant differences in NODF across elevation. There were no significant differences in network connectance across fire and elevation classes ($p > 0.05$).

Species-level specialization

Overall mean of per-flower-visiting species d' (mean=0.407, standard deviation= 0.323) indicates that the flower-visiting insects were moderately specialized. Flowering plants on the other hand, were highly specialized with overall mean per-species d' (mean = 0.972, standard deviation = 0.167). There was no significant difference in d'-value across study sites in different fire classes ($F_{2,\,210} = 2.913$, p = 0.0565). However, after correction by the null model, I found a significant difference in d'-value across fire classes ($F_{2, 210}$ = 7.123, p = 0.001). Highest z-scores for d'-values was observed at the refuge sites, and lowest at the burned sites.

When I compared, for common flower-visiting insects, their specialization in the three fire classes, I found no significant differences among fire classes ($F_{2, 77}$=1.983, p = 0.145) or elevation category ($F_{2, 77}$ = 1.083, p = 0.344).

There was a significant difference in pollinator normalized degree (ND) across fire class ($F_{2, 97}$ = 29.89, p < 0.001). ND was highest in unburned sites, followed by burned sites, and lowest at refuge sites (Figure 5.4). There was also a significant relationship between species interaction and generalization across fire classes (χ^2 = 12.723, p = 0.002, df = 2). ND values was highest for pollinators involved in interactions at unburned sites, and lowest for interactions at refuge sites.



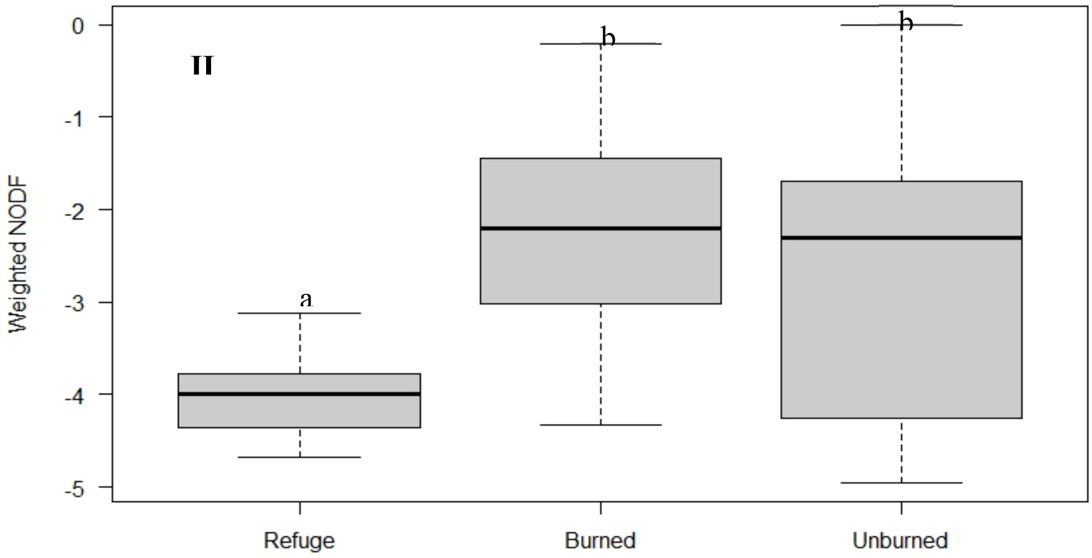


Figure 5.3. Z-scores of (I) network specialization (H_2') and (II) weighted nestedness (NODF) across fire classes. Fire class with common alphabets are not significantly different at $p > 0.05$

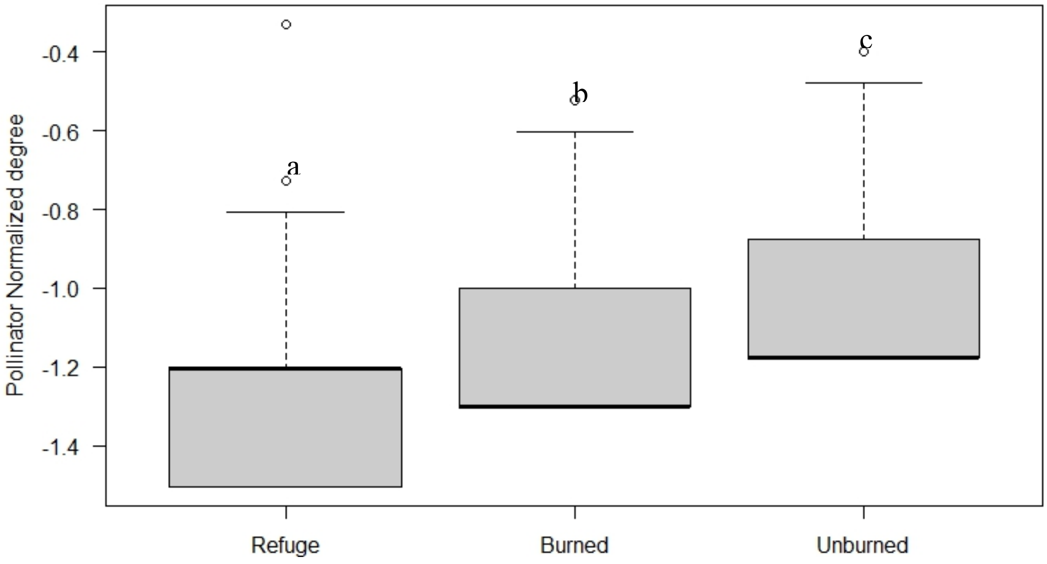


Figure 5.4: **Normalized degree (ND) across fire classes. Fire class with common alphabets are not significantly** different at $p > 0.05$

Discussion

The influence of fire across the landscape is usually uneven. Remnant patches are left behind after fire, creating a mosaic of biodiversity. I found that fire refuges had the highest flower resources, and plant-pollinator interactions, compared to the recently burned areas, and also compared to the unburned areas beyond the fire front.

Overall, abundant floral resources, especially for mass flowering plants, were important for the high species interaction observed in the refuges. While generalization (quantified as normalized degree, ND) was high in unburned and burned sites, more specialized species were involved in interactions in fire refuges. This is surprising, as one would have expected a less discriminating behaviour as more individuals visit flowers ('scramble competition'), compared to the lower-density unburned sites. However, species response to environmental stress such as fire is apparently complex. This may be explained by several factors, which I now discuss.

Interaction frequency and species abundance

Fire can impact plant-pollinator interactions in several ways, most of which hinge on resource availability in fire-prone landscapes (Brown et al., 2013). For a site to be an effective refuge, there must be sufficient nesting and floral resources for the survival and persistence of flower-visiting insects (Robinson et al., 2013). While high flower abundance drives insect activities across elevation, surface area of flowers influences insect visitation activities across fire classes, as seen here.

Although flower abundance plays a significant role in pollinator visitation, mass flowering, which is an essential feature of most flowering plant species of the GCFR, increases pollinator activity in this highly diverse hotspot (Vrodjilak et al., 2016; Simaika et al., 2018). In my study, flower abundance was highest in the refuges, and lowest at burned and unburned sites. The difference between refuge (two years fire history) and unburned areas (9-10 years fire interval) in my study is consistent with most studies on the impact of fire on flowering plant distribution. For example, Mola and Williams (2018) found a more prolonged time of interaction in recently burned areas where floral abundance persisted for a longer period of time compared to the unburned places. Also, Campbell et al. (2007) illustrated how pollinator abundance and richness increases with reduced canopy of natural areas and increased understorey vegetation. Here, I found that the unburned habitat with fire interval of ten years was overgrown with more shrubs, weeds, and less visible flower units. However, not only did the refuges not have enough time to regenerate, these areas are relatively open, with fewer shrubs and without tree canopy, unlike the unburned areas. Flowering plants on Mt Carmel national reserve in Israel reached peak flowering two years after fire, so increasing pollinator diversity. However, this peak steadily declined over the next 50 years (Potts et al., 2003). This emphasizes the importance of flower-rich open habitat in the conservation of flower-visiting insects and their important role in ecological interactions (Holzschuh et al., 2007; Carvalheiro et al., 2011; Vrdoljak et al., 2016).

The difference between the burned and the refuge areas in terms of flower resource abundance may in turn, also be linked to the time taken for resource re-distribution in this area. The burned habitat was sampled six months after the fire incidence, when most flowering plants here at this time are at an early emerging period. Full flower regeneration is essential for the visitation of associated pollinator in fire-impacted habitat (Potts et al., 2003). This is the key driver of low flower abundance and interaction in the burned area compared to the flower-rich refuge habitats. Overall, this shows the importance of nearby rich refuges, where insect pollinators can seek floral requirements, until full regeneration of the burned habitat following fire disturbance.

Network and species specialization

My results showed high network specialization in refuges in comparison with unburned and burned sites. Similarly, more specialized species were present in interactions in the refuge networks compared to unburned and burned networks. Also, species in two or more fire classes had similar d’-values, implying fire class did not alter species specialization behaviour in their response to the changes caused by fire. This means that specialized species then can remain associated with the most preferred flowers at sites with high flower abundance. However, at sites with limited floral resources, flower-visiting insect species cannot afford to be selective in seeking their most preferred flowers. This pattern was also observed by Peralta et al. (2017), where fewer specialist species were found in burned sites with low flower abundance. Similarly, Plowman et al. (2017) recorded a breakdown in interaction networks and reduced network specialization with a decrease in interacting partners. In my study, normalized degree, which explains species ability to establish links with multiple interacting partners, was highest at unburned and burned sites. This is also supported by high ND values for species in interactions at unburned and burned sites compared to fire refuges.

These findings emphasize the importance of fire refuges as a shelter for displaced pollinators, especially the specialized species with limited range of floral resources. Furthermore, resource availability plays an essential role in the persistence of specialized species at refuge sites. This implies that it is essential for refuges to be rich in required flowering plant species necessary for interacting insect species, especially specialists, while the burned area recovers from the effects of fire.

Species composition of insects involved in interactions was not significantly different among fire classes. Although I could not assess community composition pre-fire across study sites, the largely similar insect species composition involved in interactions across fire classes supports the possibility of movement of insects among burned, refuge, and unburned sites. While ground-dwelling pollinators may find burned habitat most suitable as a result of less ground cover (Potts et al., 2005), species here are likely to use power flight to reach other sites (refuges and unburned) in search of suitable floral resources. This is expected to affect the pollinator network and species specialization across fire classes.

Overall, mean network specialization (H_2') was high in my study area, supporting high community specialization of plant-pollinator interaction networks in the GCFR (Pauw and Stanway, 2015). However, the low species specialization (d') in my study may be linked to depleted resources resulting from fire disturbance. Since H_2' and d' values are linked in interaction networks (Blüthgen et al., 2006), the high network specialization observed here can be influenced by the high d' value for flowering plants compared to lesser mean d' value of flower-visiting insects. This also supports Pauw and Stanway (2015), where higher d' values was recorded for flowering plants in this region compared to visiting pollinators. Although overall I recorded few interactions, especially in burned areas, the d' and H_2' metrics are less sensitive to sampling efforts and diversity of interacting partners (Schleuning et al., 2012)

The difference in species specialization (d') among fire class may be attributed to competition among flower-visiting insect species, especially in habitat with low or few flower resources. The burned sites here were sampled six months after fire incidence, and this area had the first set of re-sprouting flowering plants, but in low abundance compared to refuges. This would increase competition of resident insect pollinators in this area for the scanty resources. Exclusivity of interactions among individual species is more prominent in habitats with more interacting partners, yielding higher species specialization (d') values (Pauw and Stanway, 2015) than were seen here.

Globally, a trend of higher specialization in the species rich tropics has been reported with a decline towards temperate areas (Dyer et al., 2007; Dalsgaard et al., 2009; Pauw, 2013). This matches the limited resources in the burned areas here influencing the less specialized species. This means that over time, with less resources at the burned sites, the refuges may serve as an alternative for more specialized species until burned sites regrow with more floral resources. Species specialization (d') values for common species across the fire classes was not significantly different. This shows that species remain in a specialized relationship with associated flowers across all fire classes. Flower-visiting insects, especially solitary bees, find floral resources in areas close to their nesting sites (Gathmann & Tscharntke, 2002). This could also be influenced by mobility of the various insect species, with large bees foraging >3 km, while small solitary bees seek floral resources <500 m of their nesting sites (Steffan-Dewenter & Tscharntke, 1999; Steffan-Dewenter, 2002). Since I found no significant differences in flowering plant species richness across fire categories, this means that specialized insect species find their preferred flowers within their maximum flight distance.

Interaction network nestedness and species distribution

I found networks to be more nested at burned sites, and least at refuge sites. Unlike H_2', where interacting species can be selective and retain unique partners, nestedness showed that generalists and specialists in my interaction networks share similar resources (Spiesman and Inouye, 2013). In habitats with high network nestedness, poorly-linked and rare species are able to secure interaction partners, as these are linked to more stable components of the network (Bascompte et al., 2003; Memmott et al., 2004; Gibson et al., 2006). Although, it is difficult to interpret nestedness in small networks (Olesen et al., 2007), my null models nevertheless corrected for this effect. The more nested networks at the burned sites, especially those located on hilltops, may be linked to low flower abundance and less resources for flower-visiting insects. This increases the opportunity for generalist and specialist species to interact with the same flower partner in the network. This also suggests that the presence of flowering plant species is able to maintain such interactions with insect mutualists in burned and less favorable habitat. Indeed, well-linked drought-resistant plant species are important to community resilience and network persistence during harsh conditions in the environment (Lance et al., 2017).

Conclusions

Reducing biodiversity loss involves understanding how different components of natural landscapes can be optimized for the conservation of biodiversity and ecological processes during transformation events. Refuges can be part of this loss reduction, with my fire refuges being, in effect, temporary holding areas into which the flower-visiting insects can retreat while the burned matrix goes through regrowth and succession as part of natural ecosystem recovery. This is likely to be a process that has been honed for many millennia in fire-prone systems such as the GCFR. It is also promoted by the cragginess of the topography in this system, which provides natural fire refuge areas. Conservation of flower-visiting insects, along with much other biodiversity (Pryke & Samways, 2012a, 2012b; Yekwayo et al. 2018), appears to be naturally adapted to these retreats from fire, enabling populations to survive in patches even when much of the area burns. In turn, it is conceivable in evolutionary terms that this has not only contributed to the generation of high flower diversity in the area, but also that of their insect mutualists.

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Appendix

Appendix 5a: Details of study sites (coordinates) classification across fire class and elevation.

Location	Coordinates	Fire class	Elevation
Vergelegen Wine Estate	S: 34.079 E: 18.933611	Burned	Valley
	S: 34.065917 E: 18.937778	Burned	Valley
	S: 34.068722 E: 18.931889	Burned	Valley
	S: 34.085167 E: 18.924444	Refuge	Valley
	S: 34.067917 E: 18.936389	Refuge	Valley
	S: 34.068417 E: 18.930556	Refuge	Valley
	S: 34.063611 E: 18.940278	Burned	Hilltop
	S: 34.064194 E: 18.938056	Burned	Hilltop
	S: 34.073278 E: 18.942778	Burned	Hilltop
	S: 34.063056 E: 18.938056	Refuge	Hilltop
	S: 34.077778 E: 18.931389	Refuge	Hilltop
	S: 34.062417 E: 18.943917	Refuge	Hilltop
	S: 34.072389 E: 18.927778	Burned	Hillslope
	S: 34.082722 E: 18.950306	Burned	Hillslope
	S: 34.076556 E: 18.937417	Burned	Hillslope
	S: 34.071028 E: 18.925000	Refuge	Hillslope
	S: 34.075056 E: 18.935083	Refuge	Hillslope
	S: 34.068889 E: 18.933250	Refuge	Hillslope
Lourensford Wine Estate	S: 34.034986 E: 18.931656	Unburned	Valley
	S: 34.035128 E: 18.928052	Unburned	Valley
	S: 34.033634 E: 18.934317	Unburned	Valley
	S: 34.025576 E: 18.899001	Unburned	Hilltop
	S: 34.029722 E: 18.911389	Unburned	Hilltop
	S: 34.027453 E: 18.914271	Unburned	Hilltop
	S: 34.030366 E: 18.916533	Unburned	Hillslope

	S: 34.031701 E: 18.915333	Unburned	Hillslope
	S: 34.031744 E: 18.915995	Unburned	Hillslope

Appendix 5b. List of flower-visiting insect species

Taxa	Family	Genus	Species/Morphospecies
Bee	Halictidae	*Halictus*	sp.1
Bee	Halictidae	*Halictus*	sp.2
Bee	Halictidae	*Halictus*	sp.3
Bee	Halictidae	*Halictus*	sp.4
Bee	Halictidae	*Halictus*	sp.5
Bee	Halictidae	*Halictus*	sp.6
Bee	Halictidae	*Halictus*	sp.7
Bee	Halictidae	*Lasioglossum*	sp.1
Bee	Halictidae	*Lasioglossum*	sp.2
Bee	Colletidae	*Colletes*	sp.1
Bee	Colletidae	*Colletes*	sp.2
Bee	Apidae	Apidae	sp.1
Bee	Megachilidae	*Megachile*	sp.1
Bee	Apidae	*Anthophora*	sp.1
Bee	Apidae	*Anthophora*	sp.2
Bee	Apidae	*Amegilla*	sp.
Bee	Apidae	*Apis*	*mellifera*
Bee	Apidae	Apidae	sp.2
Bee	Apidae	*Xylocopa*	sp.1
Bee	Apidae	*Xylocopa*	sp.2
Bee	Apidae	*Xylocopa*	sp.3
Bee	Apidae	*Xylocopa*	sp.4
Bee	Apidae	*Xylocopa*	sp.5
Bee	Apidae	*Xylocopa*	sp.6
Beetle	Cerambycidae	Cerambycidae	sp.1
Beetle	Cerambycidae	Cerambycidae	sp.2
Beetle	Cerambycidae	Cerambycidae	sp.3
Beetle	Cerambycidae	Cerambycidae	sp.4
Beetle	Cerambycidae	Cerambycidae	sp.5
Beetle	Cerambycidae	Cerambycidae	sp.6
Beetle	Cerambycidae	*Typocerus*	sp.
Beetle	Scarabaeidae	*Hedybius*	sp.
Beetle	Scarabaeidae	*Peritrichia*	sp.1
Beetle	Scarabaeidae	*Peritrichia*	sp.2
Beetle	Scarabaeidae	*Peritrichia*	sp.3
Beetle	Scarabaeidae	*Clania*	*glenlyonensis*
Beetle	Scarabaeidae	*Anisonyx*	*ursus*
Beetle	Scarabaeidae	Scarabaeidae	sp.1
Beetle	Scarabaeidae	Scarabaeidae	sp.2
Beetle	Scarabaeidae	Scarabaeidae	sp.3
Beetle	Scarabaeidae	Scarabaeidae	sp.4
Beetle	Scarabaeidae	Scarabaeidae	sp.5
Beetle	Coccinellidae	Coccinellidae	sp.
Beetle	Meloidae	Meloinae	sp.
Fly	Drosophilidae	*Drosophila*	*melanogaster*
Fly	Tephritidae	Tephritidae	sp.
Fly	Muscidae	*Ophyra*	sp.

Fly	Muscidae	Muscidae	sp.1
Fly	Muscidae	Muscidae	sp.2
Fly	Syrphidae	Syrphidae	sp.1
Fly	Syrphidae	Syrphidae	sp.2
Fly	Tabanidae	Tabanidae	sp.
Fly	Bombyliidae	Bombyliidae	sp.1
Fly	Bombyliidae	Bombyliidae	sp.2
Fly	Bombyliidae	Bombyliidae	sp.3
Fly	Calliphoridae	*Lucilia*	*sericata*
Fly	Calliphoridae	Calliphoridae	sp.1
Fly	Calliphoridae	Calliphoridae	sp.2
Fly	Culicidae	Culicidae	sp.
Fly	Asilidae	Asilidae	sp.
Wasp	Sphecidae	Sphecidae	sp.1
Wasp	Sphecidae	Sphecidae	sp.2
Wasp	Sphecidae	Sphecidae	sp.3
Wasp	Vespidae	Vespidae	sp.1
Wasp	Pompilidae	Pompilidae	sp.
Wasp	Icheumonidae	Ichneumonidae	sp.1
Wasp	Icheumonidae	Ichneumonidae	sp.2

Appendix 5c. List of plant species

Family	Genus	Species/Morphospecies
Asteraceae	*Heterolepsis*	*aliena*
Asteraceae	*Euryops*	sp.1
Asteraceae	*Gerbera*	*linnaei*
Asteraceae	*Berkheya*	*herbacea*
Asteraceae	*Helichrysum*	*moeserianum*
Asteraceae	*Helichrysum*	sp.2
Asteraceae	*Helichrysum*	sp.3
Asteraceae	*Anthanasia*	*crithinifolia*
Asteraceae	*Senecio*	sp.
Asteraceae	*Artotheca*	*calendula*
Asteraceae	*Cotula*	*turbinata*
Asteraceae	*Metalasia*	*muricata*
Asteraceae	*Osteospermum*	sp.
Asteraceae	*Felicia*	sp.
Asteraceae	*Hymenolepsis*	*crithmoides*
Asteraceae	*Helichrysum*	*cymosum*
Asteraceae	*Osteospermum*	sp.2
Asteraceae	*Ursinia*	*punctata*
Asteraceae	*Senecio*	sp.2
Asteraceae	*Tanacetum*	sp.
Asteraceae	*Euryops*	sp.
Boraginaceae	*Lobostemon*	*dorotheae*
Bruniaceae	*Brunia*	*laevis*
Bruniaceae	*Berzelia*	*abrotanoides*
Campanulaceae	*Lobelia*	sp.
Campanulaceae	*Lobelia*	sp.2
Ericaceae	*Erica*	sp.1
Ericaceae	*Erica*	*labialis*
Fabaceae	*Acacia*	sp.
Fabaceae	*Podalyria*	*myrtillifolia*
Fabaceae	*Psorelea*	sp.
Fabaceae	*Aspalathus*	sp.
Fabaceae	*Aspalathus*	sp.2
Fabaceae	*Aspalathus*	sp.3

Fabaceae	*Aspalathus*	sp.4
Iridaceae	*Morea*	sp.1
Iridaceae	*Watsonia*	*laccata*
Iridaceae	*Morea*	sp.2
Iridaceae	*Bobartia*	*indica*
Iridaceae	*Ixia*	*odorata*
Iridaceae	*Ixia*	*scillaris*
Iridaceae	*Dietes*	sp.
Iridaceae	*Morea*	sp.
Montiniaceae	*Montinia*	sp.
Oxalidaceae	*Oxalis*	*obtusa*
Oxalidaceae	*Oxalis*	*purpurea*
Oxalidaceae	*Oxalis*	*luteola*
Oxalidaceae	*Oxalis*	sp.3
Polygalaceae	*Muraltia*	*heisteria*
Proteaceae	*Leucadendron*	*salignum*
Proteaceae	*Protea*	*laurifolia*
Scrophulariaceae	*Pseudoselago*	*spuria*
Scrophulariaceae	*Oftia*	*africana*
Scrophulariaceae	*Selago*	*corymbosa*
Rutaceae	*Coleonema*	sp.
Rhamnaceae	*Phylica*	*buxifolia*

Chapter 6

Trade-offs between retaining stands of alien trees and the conservation of indigenous flowering plants, pollinators and rare vertebrates in a biodiversity hotspot

Abstract

Limited access to sunlight reduces plant productivity and foraging activities of pollinators. This can occur when alien trees shade out native flowering plants. In a conservation context, the immediate reaction is to remove the impoverishing effect of the alien tree canopy. But there can be trade-offs to this conservation action, as when alien tree stands provide scarce resources for vertebrates and pollinators of conservation concern. I investigate these trade-offs here in a natural open landscape at different level of invasion. I also assess how different insect pollinator groups respond to shading from young, small, open canopy alien pine trees vs. older, larger, closed canopy pine trees retained as refuges for certain rare vertebrates. I used coloured pan traps to sample pollinator species diversity within and at various distances from the pine stands of both sizes of trees in a matrix of low, sclerophyllous, natural vegetation. I then assess how plant-pollinator interactions differ according to position on the landscape using bipartite network plots. Overall, flower and pollinator abundance were higher in small pines. Furthermore, flower and pollinator abundance were higher outside pine stands, but decreased in the pine interior, down to zero flowers in the tall pine understorey. Also, pollinator species composition varied with distance from pine understorey in the case of tall pines. Interaction frequency also decreased towards both pine stand interiors. The flower-pollinator interactions associated with small pines were more specialized than those associated with tall pines. Significantly, large-sized xylocopid bees, bombyliid flies, paper and spider wasps were associated with unique interactions among tall pines only. I conclude that while pine tree age and increasing development of a continuous canopy reduces the success of native flowering plant species, and a collapse of the natural interaction networks, alien pine stands nevertheless are important for some insect species that require trees for nesting, as it is for vertebrates that require wooded retreats in this open landscape which is so short of large tree resources.

Introduction

Biological invasion is one of the leading drivers of biodiversity loss and extinction in many terrestrial ecosystems (Mooney and Hobbs, 2000). This is highly pronounced in sensitive biodiversity hotspots like the Greater Cape Floristic Region (GCFR) with typically low and sclerophyllous fynbos vegetation and limited trees (Van Wilgen et al. 2008). Most invasive conifer trees like pines have the intrinsic capacity to colonize new areas due to their rapid growth and biomass increase, most especially in ecosystems with low tree diversity (Higgins and Richardson, 1998; Richardson et al., 1994). This is one of the leading characteristic features of this region yielding high susceptibility to pine invasion (Van Wilgen et al., 2008). Pines are the most commonly used tree species for plantations in the GCFR. Some of these pines are now invasive, and have successfully become an integral part of invaded terrestrial ecosystems (Richardson, 1998).

Alien pine (*Pinus* spp.) trees (‘pines’) can rapidly colonize and invade ecosystems and displace local resident species, with natural grasslands being transformed into conifer forests in a short time (Harding, 2001), and also influencing the delivery of ecosystem services through important ecological interactions. Rapid replacement of native vegetation with alien conifers has a direct effect on local biodiversity and ecosystem processes (de Abreu and Durigan, 2011; Rolon et al., 2011). While little is known on how pine invasion influences ecological interactions, pine invasion may play a unique role in driving pollinator distribution through its direct impact on loss of flowering plants since pollinators are known to track flowering plants (Winfree et al., 2009). This is of great concern in the GCFR of South Africa where most native shade intolerant species have been lost to pine invasion as much of the region is transformed into conifer forests (Richardson et al., 1994).

Shading is one of the most prominent means by which pine trees influence species diversity and composition of native flowering plants and insects (Franzese et al., 2016). The ecological ability of pine trees to out-shade the native grassland and shrub-like vegetation community encourages the rapid conversion of native vegetation into a conifer forests (Richardson 1998). Pine trees are fast growing with extensive canopy cover in highly dense area, thereby reducing light accessibility to understorey native vegetation. Low irradiance yields differences in air temperature and vapour pressure, all of which influence plant photosynthesis, respiration and stomata conductance (Niinemets and Valladares 2004; Sellin et al. 2010). Overall, growth of understorey native vegetation is impaired with increase in the density of pine trees and shade level. Subsequently, important interactors that depend largely on the diversity of the native flowering plants may be affected indirectly by pine invasion through the loss of flowering plants. This creates a ripple effect across multiple ecosystem processes starting with the loss of native flowering plants to the shading effect. For example, pollinators are known to track flowering plant diversity (Ebeling et al., 2008), and in an ecosystem when the basic floral requirement is lacking, subsequent loss of interacting pollinators is imminent. At community level, such loss of interacting partners may be irreplaceable, especially for highly specialized species where floral preference is restricted. Understanding how shade drives the decline of important plant-pollinator interactions, especially those involving highly sensitive specialized species in invaded areas, is key to implementing adequate conservation measures in invaded area.

In addition to the indirect effect of pine trees on pollinator diversity and their interaction with native plants, shade from pine trees may also directly influence the distribution pattern of pollinators and their activities in invaded areas (Liow et al., 2001; Kilkenny and Galloway, 2008). Most insect pollinators, especially bees, are more active in open areas with less trees and canopy cover. Flowering plants in open, sunlit areas receive 8-11 times more visitation from pollinators than those in shaded forest patches (Cao et al., 2017). This is because insects require high temperatures for flight and foraging activities (Kilkenny and Galloway, 2008). In addition, these pollinators respond to visual cues, some of which are restricted in low light environment (Kelber et al., 2005). Overall, reduced light accessibility influences the foraging ability of pollinators directly and may hinder the delivery of important service of pollination of native flowering plants in shaded understorey.

While the significant loss of flowering plant and pollinators to tree invasion is evident, the presence of trees in ecosystems is also a major requirement for nesting by certain pollinating insects and other wildlife (Gardenfors, 2010, Homyack et al., 2005). Some pollinating wasps, bees and beetles are wood nesters, creating nests in trees or log cavities (Michener, 2007; Orr et al., 2015). Some of these species nest in the trees or burrow into the cool understorey or trees and search for flowers in areas within their foraging range (Steffan-Dewenter & Tscharntke, 1999; Steffan-Dewenter, 2002). Thus, in a sensitive ecosystem like the GCFR, with its many endemic pollinators, some of which create nests in trees or dead wood e.g 'carpenter bees', an understanding of critically short resources is required to maintain effective populations of local populations requiring wood.

I aim here to determine how different taxa of flower-visiting insects ('pollinators') respond to canopy size of alien pine trees. I hypothesize that 1) flower abundance will vary according to level of direct sunlight vs. shade reaching the understorey plant community due to the importance of light for plant and flower development (McConnaughay and Coleman, 1999), 2) pollinator species richness and abundance will change according to the changes in the understorey plant communities, since insect pollinators are known to track flower distribution (Ebeling et al., 2008; Winfree et al., 2009) 3) Since shade reduces understorey temperature (Valladares et al., 2016), insect foraging activity is expected to be influenced by temperature change with higher number of plant-pollinator interactions occurring outside pine areas compared with the closed canopy understorey and 4) degree of network specialisation will vary from the interior to outside the canopy area, since highly specialised insect species may decline rapidly in areas with loss of flowering plants (Biesmeijer et al., 2006)

Materials and methods

The study was conducted in the large natural set-aside areas on an extensive wine farm, Lourensford (34.0719ºS, 18.8886ºE) in the Western Cape Province, South Africa, in the Greater Cape Floristic Region (GCFR) biodiversity hotspot. The natural matrix also included two major protected areas (The Helderberg Nature Reserve and the Hottentot-Hollands Nature Reserve), as well as being in a biosphere reserve (Cape Winelands Biosphere Reserve). The wine estate practices biodiversity-friendly agriculture, with extensive areas of the farm devoted to conservation of indigenous biodiversity, where my sites were positioned. However, the farm is still invaded by pine trees, most of which started as a plantation but over time, were abandoned, and are now a distinct pine forest with a largely continuous canopy cover. This invaded but contained area is now set aside specifically for the conservation of important animals of conservation concern. The area is off limits to the public, and receives no/minimum human intervention. I refer to insects collected here as 'pollinators' as 1) they come into contact with the stamens and accumulate pollen, and 2) to avoid including the many species that visit flowers for thermoregulation and as tourists or as predators of the pollinators. However, I recognize that I have not proved pollination through to seed set.

The alien trees in the study area were at different stages of growth. Most of the trees were young, with a height of 4-7 m ('small'), and enabled some development of a flowering plant understorey. Other pine trees were >10 m high ('tall'), with well-developed canopies and little or no understorey vegetation. I estimated the height of pine trees using approaches in Samways et al. (2010). The pine stands I chose were in a natural vegetation matrix, and specifically retained for the conservation of certain mammals such as Cape baboon (*Papio ursinus*), Cape leopard (*Panthera pardus melanotica*), and Honey badger (*Mellivora capensis*), as well as for some reptiles. I computed Moran I index to assess spatial autocorrelation for observed insect abundance pattern among study sites established in each pine tree size category. Moran I index (-0.414 ± 0.19, P = 0.262) showed no significant correlation among study sites in each pine category which support independence of study sites.

I selected pine stands which were either small pines or were tall pines. Pine stands belonging to different size category (Small vs Tall) were distributed randomly across the invaded landscape. These stands were within a near-natural matrix of sclerophyllous vegetation. Four sampling locations was established in each pine tree size category. Sampling location was classified as 1) in the pine stand interior, 2) at the edge of the pine stand/natural fynbos, 3) at 30 m from the edge of the pine stand into the natural matrix, and 4) at 60 m from the edge of the pine stand into the natural matrix. Three replicates of each sampling location was established in each pine tree size category. A 50 m^2 sampling sites was established in each sampling location, this gives a total of 12 sites (1 site per replicate of location) in tall pine and 12 sites in small pine area distributed across sampling locations (Study design details in Appendix 6a).

I observed plant-pollinator interactions (September – November 2017) in five replicates of 2 m^2 observation sampling units (OSUs) at each 50 m^2 sampling site in each sampling location (i.e. a total of 24 x 5 = 120 OSUs). OSUs were about 10 m apart within each sampling site, and exact position of each was chosen to maximize the number of flowering plants within each of the 24 sampling locations. Observation of plant-pollinator interaction was conducted twice on each study site. Observation was carried out between 9:30 and 12:00 in the first site visit and between 13:00 and 15:00 in the second visit. Interactions from both site visits were pooled for statistical analysis.

During each site visit, timed observation of insect activity was standardized to 10 min/2m^2 in each OSU to avoid over-emphasizing the specialization of flowering plants (Gibson et al., 2011), and to reduce sampling bias from variables such as flower abundance. During this time, an interaction was noted when an insect visited and interacted with the stamens of the flower unit. Five replicates per 2 m^2 OSU within each site yielded a total of 50 min observation time per sampling site per visit, which were pooled as a single interaction network. Pollinators were identified in the field, or caught for later identification as morphospecies.

At each sampling period, I also estimated flower abundance for each plant species in the individual 2 m^2 OSUs where insect activities were observed. Here, I counted and recorded all open flowers for each plant species. Flower unit was defined as the visible unit of an inflorescence of a plant from which a honeybee-sized insect will fly to the next unit rather than crawl (Dicks et al., 2002). I also pooled these data across all plots to account for flower abundance and richness at each sampling location.

After each observation, I also used 12 pan traps each of colour blue, white and yellow to estimate abundances of pollinators at each sampling location. This makes up 144 bowls for each of the two pine size classes. Coloured bowls (2 000 ml) were half filled with water and a little liquid detergent, and were raised to the general indigenous vegetation height in the immediate area. I left the pan traps on site for 24 hrs, after which insects collected were rinsed and stored in 70% ethanol until sorting and identification. Collection with pan traps was only conducted once at each study site during sampling period. Sampling was conducted on days with no rainfall, minimum cloud cover and wind (usually ≤ 6km/h).

I estimated the percentage shade cover over the understorey of pine interior at each observation period for plant-pollinator interaction. I did this by measuring the average diameter of patches of illuminated area across the understorey at each site visit. I divided this value by 50 m (edge length of a sampling location) and multiplied by 100%. This gave the average area that had access to sunlight at each of my sampling locations, and I estimated the percentage shade cover from this value.

Statistical analyses

Web structure for plant-pollinator interaction networks were compiled for each site. Data were analyzed using the bipartite package in R (Dormann *et al.,* 2008) version 3.4.1 (R core team, 2017). Network-level specialization (H_2') was computed for each of the 21 networks (no interactions were observed in sites located at the interior of tall pines). To account for differences in activity, abundance, or attractiveness of different species in my study, I developed null models for the network specialization (H_2') index, based on the observed number of interactions for a species in a given network (using the Patefield algorithm: Dormann et al., 2009). I computed 100 replicates of null models for each network, and calculated z-scores for H_2' metric value (i.e. differences between observed and mean null model index values, divided by the standard deviation of the null model values).

The z-scores of H_2' was compared among distances (sampling locations) from pine edge in each category of pine size using simple ANOVA with data following normal distribution (Shapiro-Wilk's test). Tukey HSD post-hoc test was computed to observe pairwise comparison among groups. I used Generalised Linear Model (GLM) to compare abundance and species richness of flowering plants and pollinators in pan traps among location category and pine size. Abundance and species richness were the dependent variables; location and pine size category were explanatory variables. I also assessed how dependent variables here differ across sampling location in each pine category in different models. I also included flower abundance in the model as a predictor of insect abundance. Then I assessed the response of pollinator abundance to the interaction between flower abundance and pine size class and also between flower abundance and sampling location.

I also observed the influence of light accessibility on insect and flowering plant species richness and abundance (GLM) and specifying Poisson distribution in a separate model. I computed model simplification through a backward selection process for all models to assess the effect of explanatory variables or interactions. I included only one variable per model where a pair of variable was highly correlated at (R > 0.6 and p < 0.05), this was observed between the pair of light accessibility and sampling location.

I assessed pollinator species composition in both small and tall pine areas using canonical analysis of principal coordinates (CAP). I then analysed differences in species composition across sampling locations from the pine interior using Permanova in both small and tall pine stands. To assess sampling adequacy, I estimated species rarefaction curves for flowering plants and pollinators across all study sites using the 'vegan' package in R (Oksanen et al., 2006).

Results

A total of 80 insect pollinator morphospecies (Appendix 6b), in four orders, and made up of 1 144 individuals were collected in pan traps over the sampling period. A total of 65 species were collected at sites with small pines, and 36 species at sites with tall pines. Only 17 insect species were unique to tall pine areas, while 45 species were only in small pine areas, and 19 species common to both categories. Overall pollinator species estimator indices for all sites were : Chao = 136.89 ± 22.88, Jackknife2 = 147.91. A total of 29 species of flowering plants was recorded with species estimator indices: Chao = 39.37 ± 7.28, Jackknife2 = 47.26. Overall, the rarefaction curve for flowering plants across all sites reached a near asymptote (Appendix 6c). However, the asymptote for the pollinator curve was not reached (Appendix 6d) because of the occurrence of many rare species in my samples.

Overall, there was a significant difference in insect abundance among to pine tree size category (Small vs Tall pine stands). Higher abundance was recorded in the small pine tree stands, and abundance was lower for tall pine tree stands (z = -9.850, p<0.0001, df = 22, Figure 6.1I). In addition, there was a significant difference in insect abundance across sampling location in each pine size category. While abundance was highest at 30 m away from pine stand edge in the case of small pine trees, highest abundance was recorded 60 m away from pine edge in the tall pine tree stands (Figure 6.1II). There was a significant positive effect of percentage light accessibility on pollinator abundance in both small and big pine tree areas (z = 4984, p < 0.0001, df = 22).

There was a significant difference in insect species richness between pine size classes. Higher insect richness was observed among the small pine trees compared to the tall pine trees (z = 3.296, p = 0.0009, df = 22, Figure 6.2). There was no significant difference in insect species richness across sampling locations in each pine tree size category. Furthermore, there was no significant effect of light accessibility on insect species richness (z = 1.789, p > 0.074, df = 20).

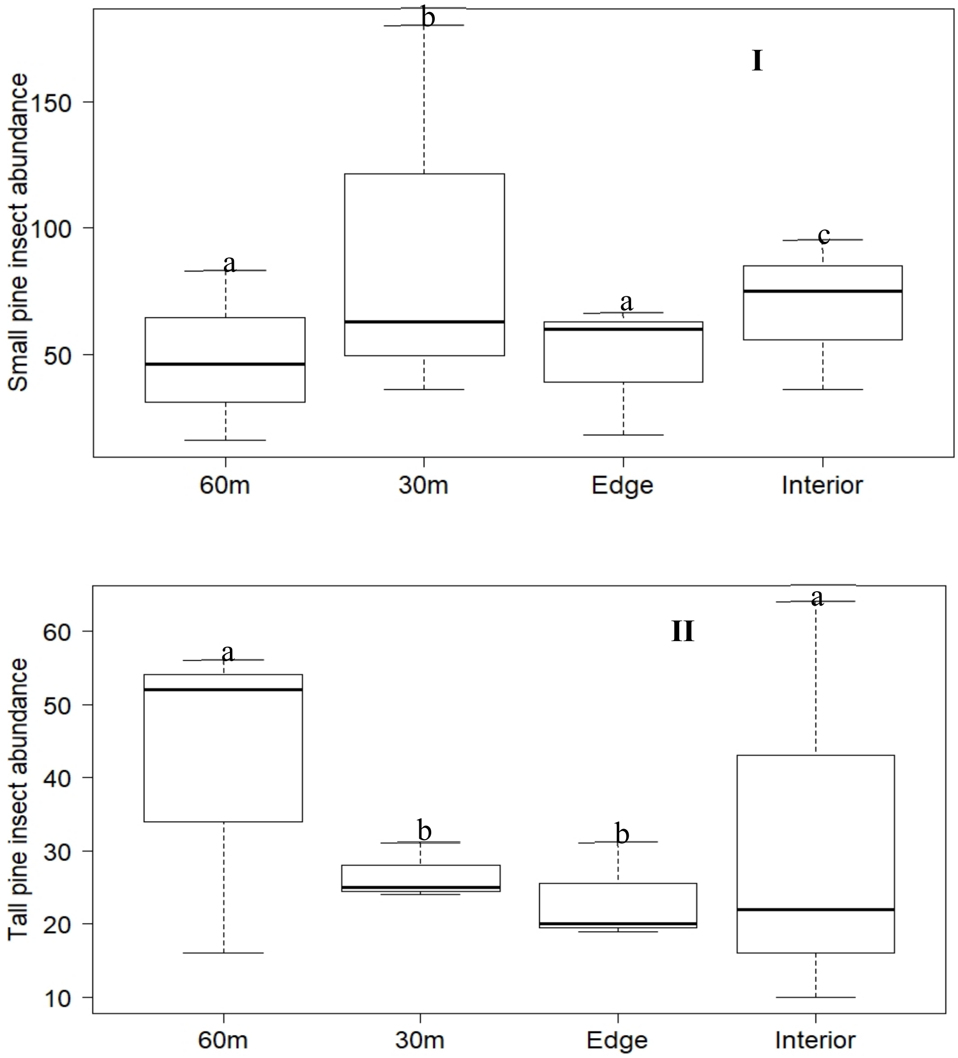


Figure 6.1. Abundance of pollinator across sampling locations in (I) small pine tree stands and (II) tall pine tree stands. Sampling locations with common alphabets are not significantly different at p > 0.05

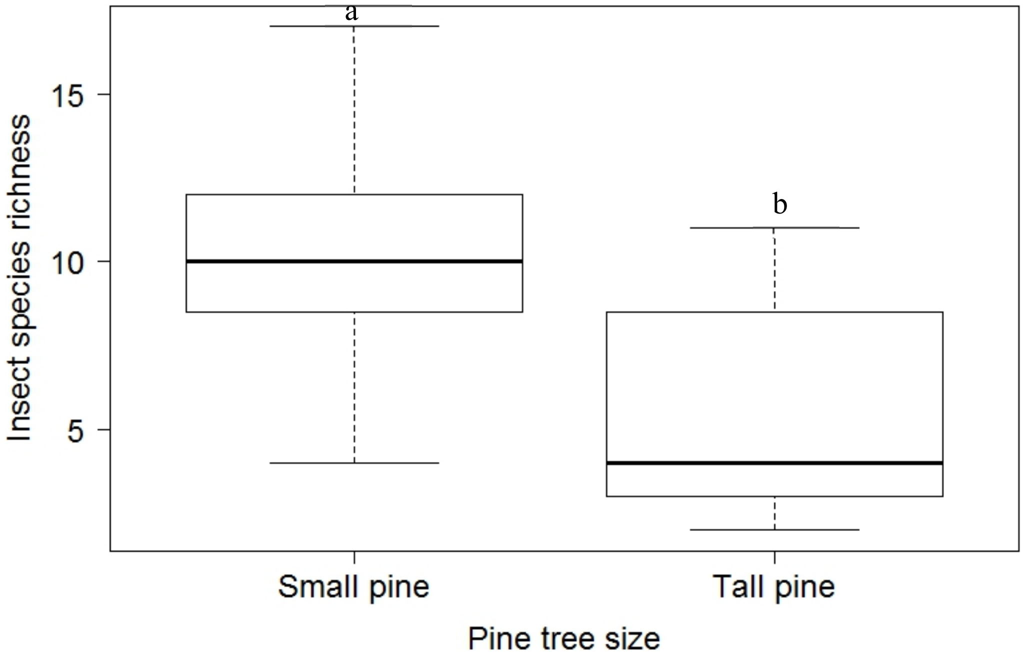


Figure 6.2. Species richness of pollinator among pine tree size. Pine size with common alphabets are not significantly different at $p > 0.05$

There was a significant difference in flower abundance between pine tree size class. Higher flower abundance was observed in small pine tree stands, and this was significantly different from lower flower abundance at small pine tree stands ($z = -22.789$, $p<0.0001$, df = 22, Figure 6.3II). There was also a significant difference in overall flower abundance across sampling locations. Highest flower abundance was observed at the 30 m from the edge of pine tree stands, and this differed significantly from the lowest abundance observed in pine stand interiors ($z = -12.992$, $p<0.00001$, df = 20, Figure 6.3II). While flower abundance was highest at 30 m from pine edge in both pine size categories, zero flower was recorded in the interiors of tall pine areas. There was a significant positive effect of light accessibility on flower abundance across distance along transects in both small and tall pine areas ($z = 16.24$, $p<0.00001$, df = 22).

There was a significant difference in species richness of flowering plant between pine tree size class. Higher species richness was observed overall in small pine tree stands, with lower species richness in tall pine tree stands ($z = -3.459$, $p= 0.0026$, df = 22, Figure 6.4). There was also a significant difference in species richness of flowering plants across sampling locations.

Highest species richness of flowering plant was observed at 30 m from the edge of pine trees, and this was significantly different from the lowest species richness in the pine interiors (z = -4.316, p = 0.0004, df = 20). There was a significant effect of light accessibility on species richness of flowering plant (z = 3.064, p = 0.0022, df = 22).

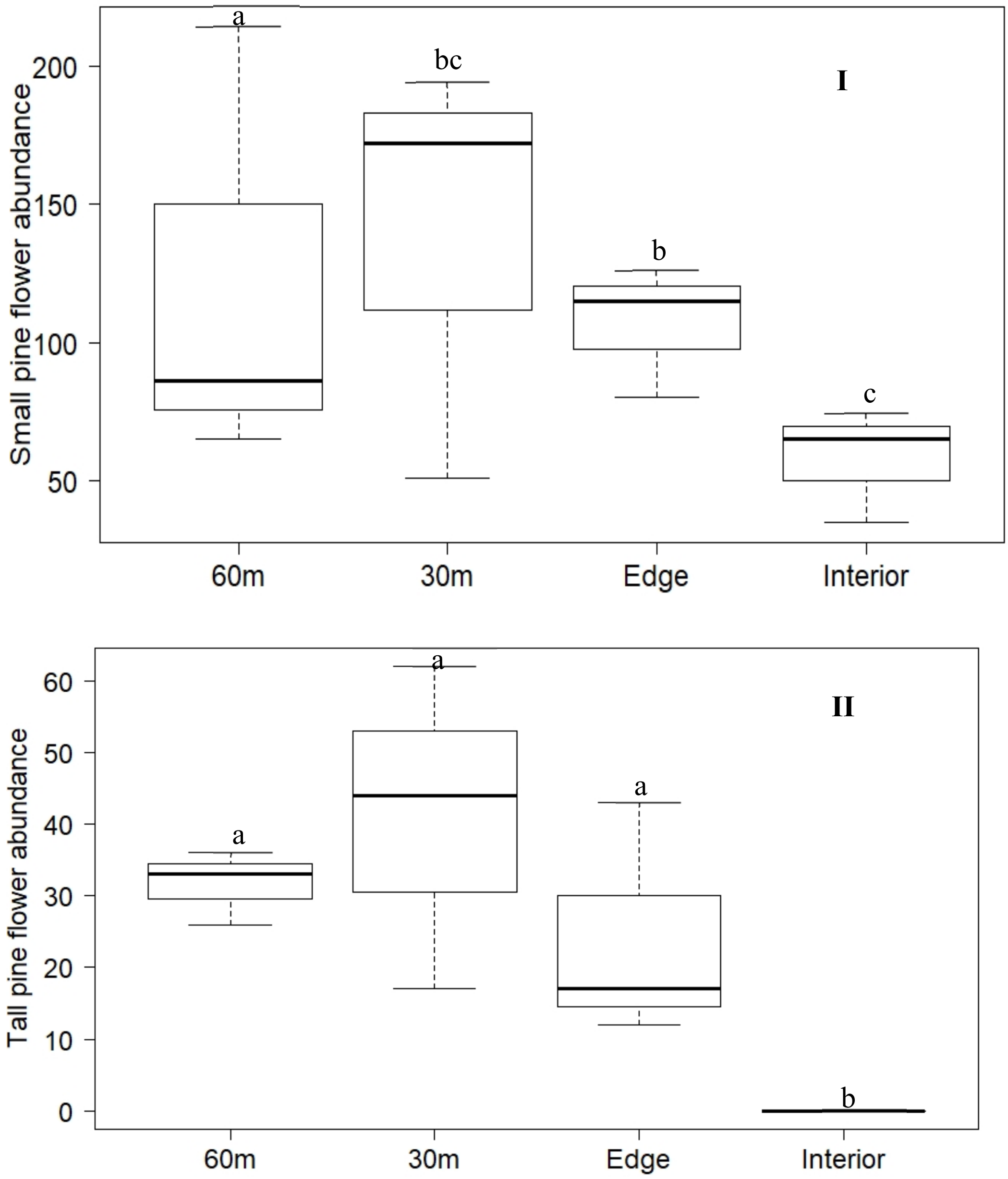


Figure 6.3. Abundance of flowering plants across sampling locations in (I) small pine tree stands and (II) tall pine tree stands. Sampling locations with common alphabets are not significantly different at $p > 0.05$

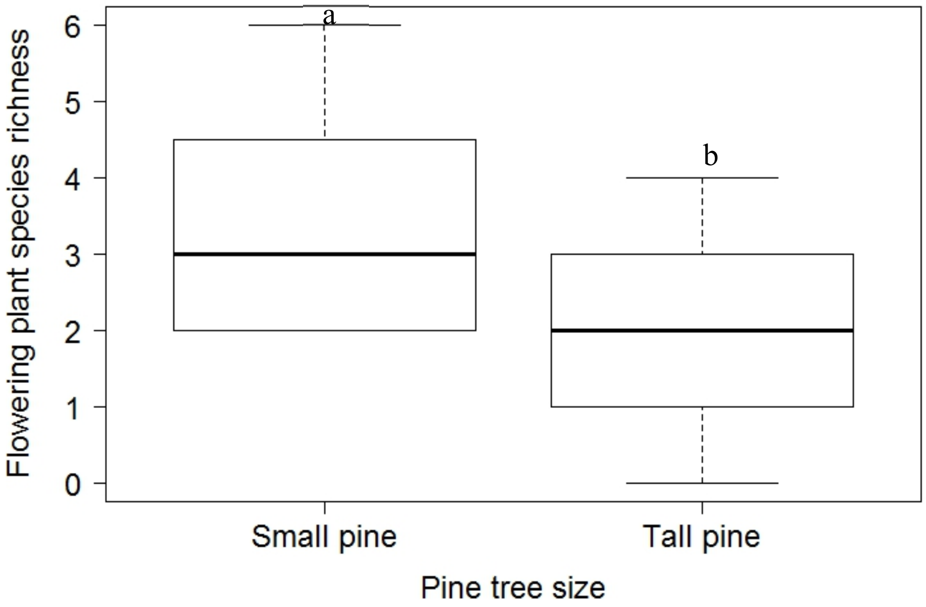


Figure 6.4. Mean flowering plant species richness (±SE) among pine tree size. Pine size with common alphabets are not significantly different at $p > 0.05$

Effect of flowering plant on pollinator abundance

Pollinator abundance was significantly associated with flower abundance ($z = 12.85$, $p < 0.0001$). Pollinator abundance was also significantly associated with the interaction between pine size and flower abundance ($\chi^2 = 34.45$, $p < 0.0001$, df = 2). Pollinators were more associated with flowering plants in small pine tree stands compared to tall pine tree stands. Similarly, Pollinator abundance was significantly associated with the interaction between sampling location and flower abundance ($\chi^2 = 40.80$, $p < 0.0001$, df = 2). Pollinators were strongly associated with flowering plants at 30 m from the pine tree edge in both pine class.

Pollinator species composition

There was a significant difference in insect species composition across sampling locations (interior through to 60 m into natural matrix) in tall pine areas (PERMANOVA F = 2.045, p= 0.013, Figure 6.5). The pairwise comparison showed weak separation in species composition across sampling distances, and there were no significant difference in species composition at any two sampling locations in tall pine tree areas. There was no significant difference in species composition across sampling locations (interior through to 60 m into natural matrix) in the small pine tree areas (PERMANOVA F = 1.0791, p = 0.314).

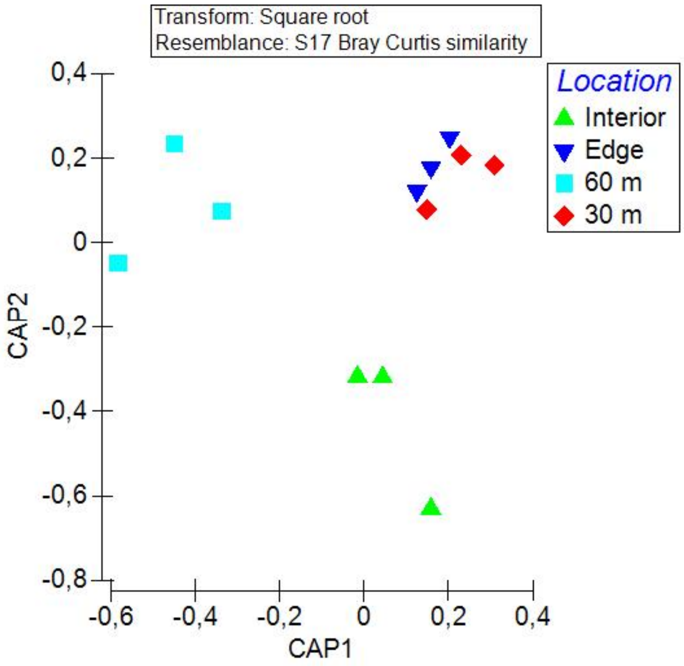
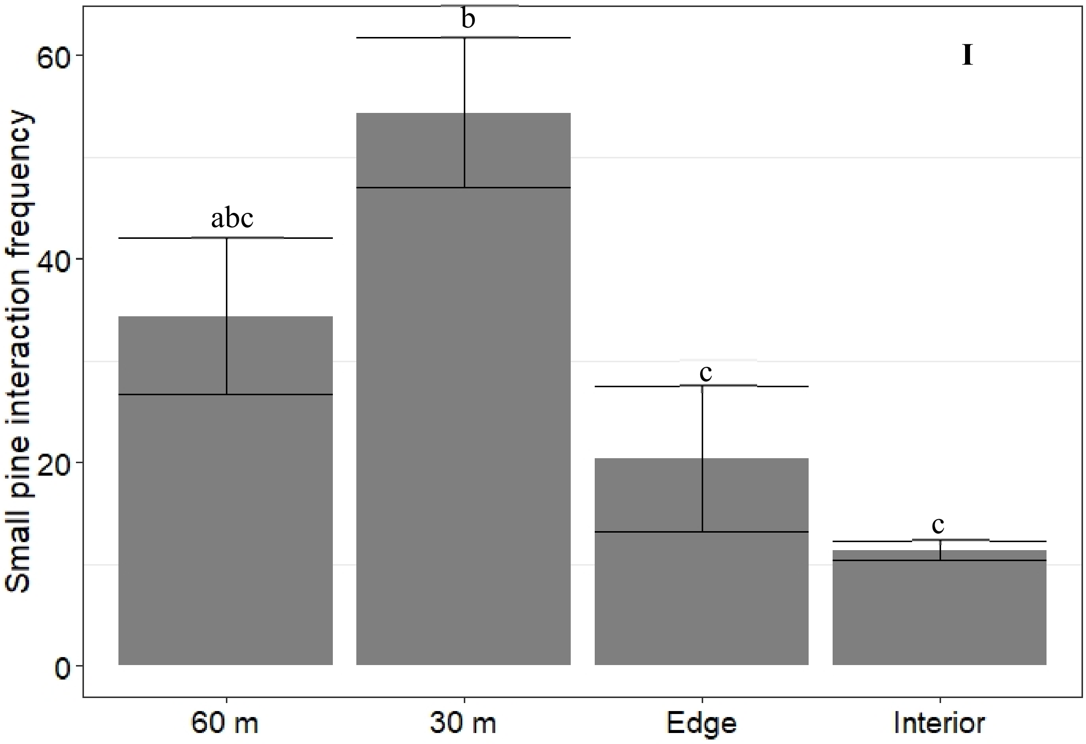


Figure 6.5. Canonical analysis of principal coordinates showing differences in pollinator species composition across sampling locations in tall pine stands

Insect-flower interaction specialization

A total of 501 interactions were observed between 45 insect and 29 plant species. 25 insect species were unique in interactions associated with small pine trees, while 12 species were only associated with large pine trees. Large-sized xylocopid bees, bombyliid flies, spider hunting wasps, and paper wasps were unique in interactions in association with tall pine tree stands only (Appendix 6e). Of the total interactions, bees constituted 66.6%, followed by beetles (20.6%), and far behind, were flies (9.8%) and wasps (3%). Because of absence of flowering plants in the tall pine tree stand interiors, no interactions were observed there. There was no significant difference in insect species richness in interactions across whole transects associated with both the small and tall pine stands (excluding the tall pine interior). There was a significant difference in number of interactions across locations associated with small pine trees ($F_{3,8}$ = 8.519, p= 0.007). Highest number of interactions were recorded at 30 m into the natural matrix, and the least interactions in the small pine stand interiors (Figure 6.6I). Similarly, there was a significant difference in number of interactions across locations associated with tall pine trees ($F_{2,6} = 8.794$, p= 0.017). Highest number of interactions was recorded at sites 30 m from the edge of small pines, and least interactions at the edge of tall pine areas (excluding the zero value for tall pine interiors) (Figure 6.6II)

Network specialization (H_2') differed with pine tree size (i.e. small vs. tall trees) ($F_{1, 16} = 9.797$, p= 0.006). Higher H_2' values were recorded from locations associated with small pines, compared to those associated with tall ones (Figure 6.7). Furthermore, there was a significant difference in H_2' along distances associated small pines ($F_{3, 8} = 6.566$, p=0.015). Highest H_2' values was recorded 30 m into the natural matrix, and least in the small pine tree stand interior (Figure 6.8). However, there was no significant difference in H_2' across all locations associated with tall pines (excluding tall pine interior) ($F_{2, 6} = 0.081$, p=0.923)



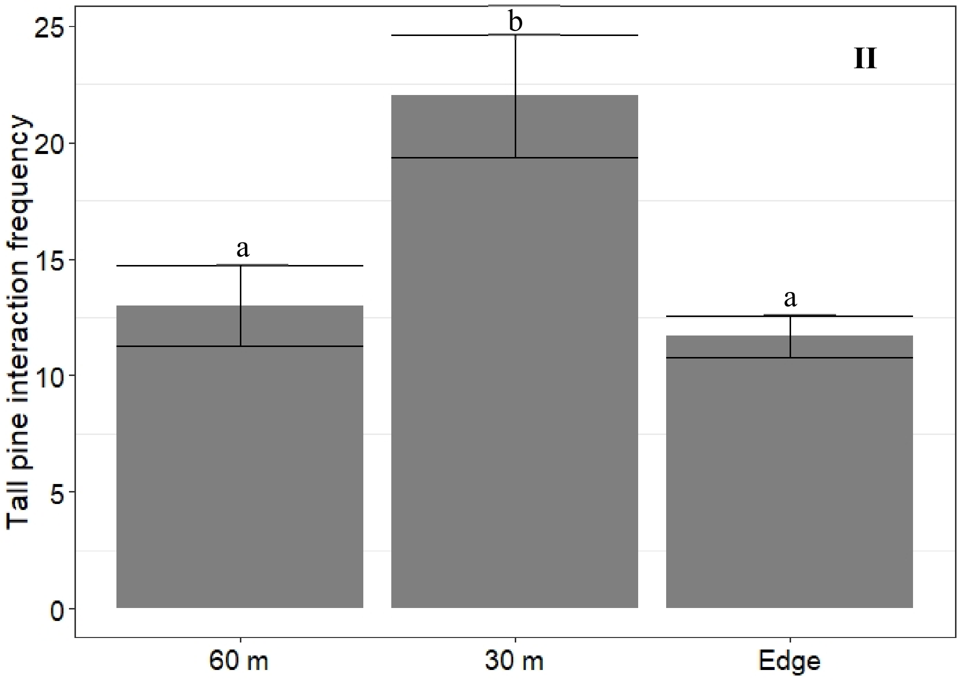


Figure 6.6. Mean (±SE) interaction frequency along sampling locations associated with (I) small pine tree stands and (II) tall pine tree stands. Bars with common alphabets are not significantly different at $p < 0.05$

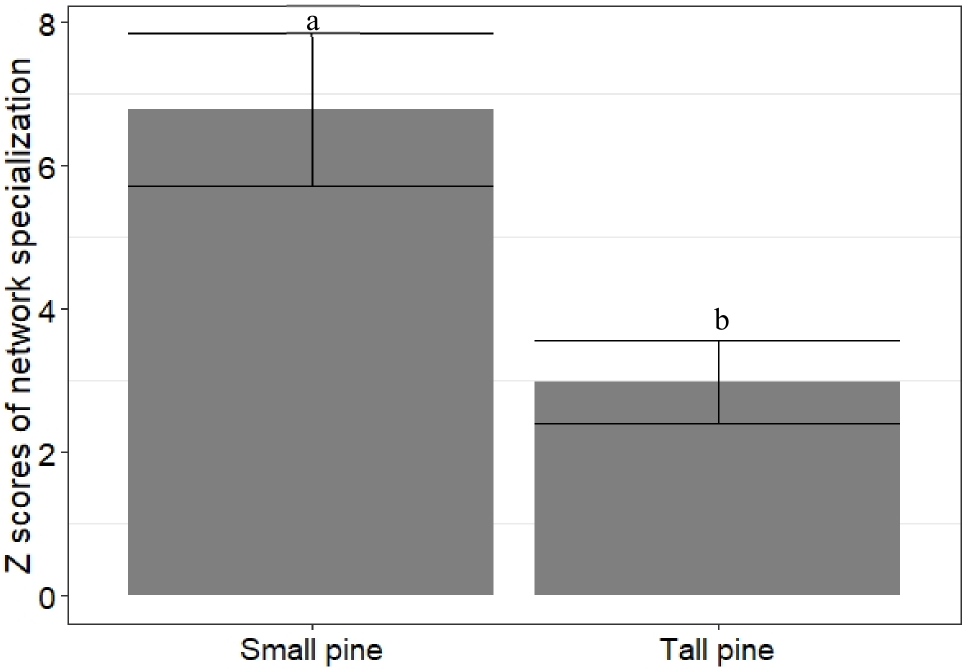


Figure 6.7. Mean (±SE) z-scores of H_2' between pine tree size class. Bars with common alphabets are not significantly different at $p < 0.05$

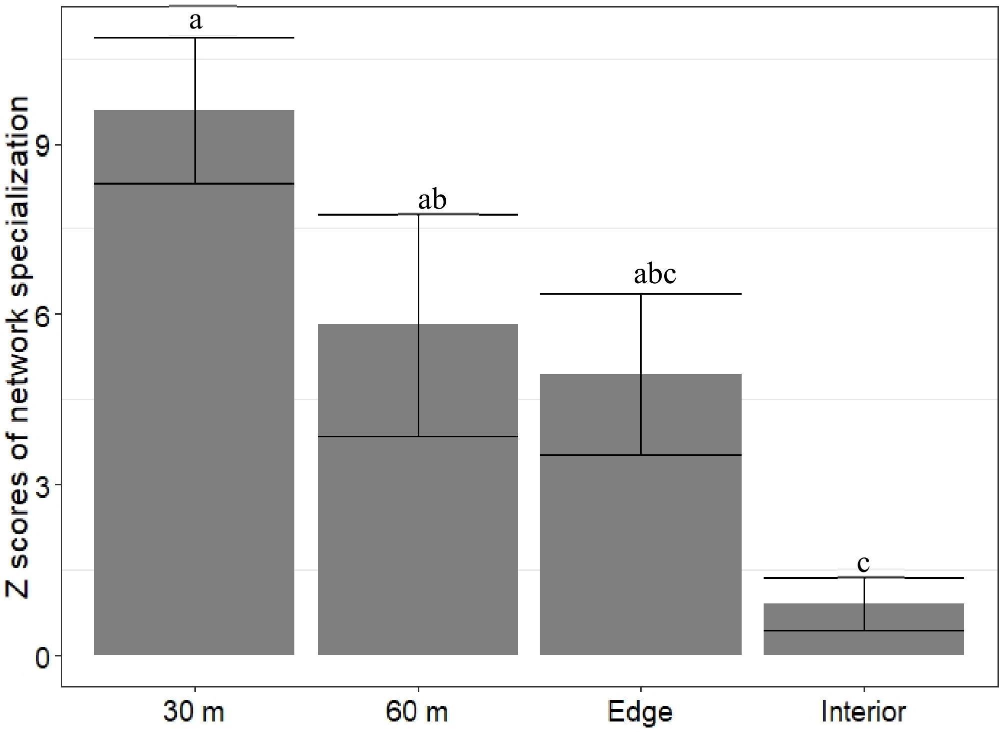


Figure 6.8. Mean (±SE) z-scores of H_2' along sampling locations in small pine tree stands. Bars with common alphabets are not significantly different at $p < 0.05$

Discussion

I found that flowering plants declined greatly in association with tall pine tree stands, with no flowers at all in the stand interiors. Although I did not test whether sunlight influences flowering plant reproductive output here, some studies have shown the importance of direct sunlight for sustaining the quality and abundance of flowering plant in natural ecosystems (Pierson et al., 1990; Leege and Murphy, 2001). The small pine tree stands had reduced shade in the interior, with plants having more access to sunlight than in tall pine areas. However, in both pine tree size categories, flowering plant abundance and richness increased away from the pine stand interiors in the direction of sunlit areas. Flowering plants require high irradiance to photosynthesize (Sellin et al. 2010). This means with increased pine height and canopy level, flowering shrubs, most of which are endemic to the GCFR are being shaded out as observed in the tall pine interiors here. As light is a critical factor for resource uptake in low vegetation communities (Jucker et al. 2015; Zhang et al. 2015), increased level of pine invasion in this region drives loss of local flowering plant communities, as seen here, and this may lead to extinction of important species in this region of high endemism.

Percentage light accessibility through shades from alien trees was significantly associated with pollinator abundance in this study. While light accessibility may have had no influence on insect species richness, there was a positive effect of light on total insect abundance. Insects generally require warm temperature to fulfil ecosystem function and ecological roles (Totland 2001; Kilkenny and Galloway, 2008; Cao et al., 2017). Since shade is directly linked to temperature with understorey cooler during the day (Tielbörger and Kadmon, 2000; Liow et al., 2001; Valladares et al., 2016,), this impact on insect distribution, as seen here, may be at the detriment of insect pollinated plants in the pine stand interiors (Totland 2001). This may also be highly pronounced in the tall pine tree stands where insect abundance was lower. While pollination service delivery at the level of the understorey may be reduced due to reduced light during the day, other ecosystem functions may be successful. For example, insect-herbivory interactions increase greatly in cool understories with reduced light access compared to open sunlit areas (Baraza et al., 2004). To understand how shade from pine invasion influences ecosystem multi-functionality and dynamics, research focused on multi-trophic interactions should be conducted especially in sensitive biodiversity hotspots like the GCFR.

While only insect abundance was associated with percentage light, both abundance and species richness of flowering plant was associated with percentage light accessibility at the understorey. It would seem here that flowering plant abundance and richness among pine size category are more susceptible to the strong influence of shade of the alien pine trees than are insect pollinators. Although shade as a factor may not impact species richness of pollinators directly, nevertheless, the indirect effect of shade through loss of flowering plant species is a critical concern in terms of potential loss of endemic pollinator species in pine-invaded areas, as also seen from the association between flowering plants and pollinator abundance in my result. This is also supported by Andrieu et al., (2018), who found that flower cover is the main factor driving bee abundance with little or no effect of edge factors. Thus, in a region with different levels of invasion and canopy cover, conservation action should be targeted towards ameliorating direct effect of shade on understorey flowering plants, as this in turn, may lead to the recovery of insect pollinators.

Tall pine trees did not have a completely negative influence on the pollinators, with 17 species even unique to tall pine stands, and 19 species common to both small and tall pine tree stands.

Among the species common to both areas, these species may seek nesting sites in trees, which are much more readily available among tall pine trees than in the natural sclerophyllous fynbos vegetation. Also supporting this contention is my CAP analysis, which showed greater segregation of species in the interior of tall pine tree stands. It would seem there is a distinct difference between communities in the interiors of the pine stands compared to outside, and in the case of both small and tall pine trees. Although overall insect abundance was highest at 30 m for small pine tree stands, and 60 m for tall pine, the second highest abundance was recorded in the pine stand interiors in both cases. It appears that while species recorded outside pine areas may be mediated by high flower abundance, other factors, such as nest requirements, may also determine the distribution of species recorded in the interior.

Wood resources can often be a limiting resource for many taxa, leading to many species being threatened, as their nesting resources are in short supply (Tikkanen et al., 2006; Gardenfors, 2010). About 30% of solitary bees and some aculeate wasps are wood nesters dwelling in hollows of tree branches or in dead logs (Westerfelt et al., 2015; Rubene et al., 2015). Eltz et al. (2003) recommended retaining many large trees to conserve the stingless bee populations that nest in large tree hollows. In an ecosystem like the natural vegetation fynbos, where natural vegetation is shrub-like, with very few trees, presence of tall pine trees may encourage cavity nesters and other species of flower visitors that require large trees for nesting. Some large insects such as the xylocopid bees, which make burrows in old trees, may nest in the trees here, yet forage in neighboring areas where there are floral resources (Steffan-Dewenter & Tscharntke, 1999; Steffan-Dewenter, 2002).

Interaction networks and specialization

As expected, bees were the most dominant interactors with flowering plants. Interactions track flowering plant abundance most closely, i.e. with the highest interaction frequency in association with the small pine tree stands compared to the reduced interaction frequency in the tall pine tree stands. These interactions follow the same trend observed for flowering plant abundance. For the insects, like the plants, it appears as if access to direct sunlight is extremely important. Bees, which are the most frequent insect group in insect-flower interactions, and also the dominant pollinator in this region, require optimum temperature and light intensity for foraging activities (Vicens and Bosch, 2000; Clarke and Robert, 2018). Furthermore, bees can find it difficult to locate their nest entrance in dim light, which discourages foraging activities (Kelber et al., 2005). This is a major problem, especially for small-sized bees such as the solitary bees, with Streinzer et al. (2016) finding a negative relationship between body size and light intensity threshold in bees. The small proportion of interactions that I recorded in tall pine trees could be because of these limitations. Rare, small-sized *Lasioglossum* and other solitary bees in the tall pines, also may be at higher risk of displacement from these habitats compared to the larger *Xylocopa* and *Megachile* species.

Network specialization was highest away from the pine canopy, but decreased at the edge of the small pine stand and its interior. The situation was even more extreme in the case of the tall pine trees, where network specialization was severely reduced. While pine tree shade reduces productivity of flowering plants and the activities of pollinators, the situation is acute for pollinators with restricted floral preferences. Network specialization here showed poor partition in networks among tall pine trees, with insect species behaving more as generalists compared to in the small pine tree stands, which had about three times better partition. Although I cannot be completely certain that there were more specialists in the small pine areas, owing to such low visitation frequency and the presence of several rare species (Williams, 2005), the presence of more flower resources in open areas, especially in small pines, means better chances for species to be associated with their most preferred flower. At tall pine edges, with few flowers, I observed more generalized interactions. With scarce resources, insect pollinators, especially those with restricted range of floral preference may need to fly farther away from the tall pine areas in search of suitable resources.

One major finding here is that most species unique to tall pine areas were large-sized flower visitors, such as xylocopid bees, spider wasps, paper wasps, and bombyliid flies. According to Gottlieb et al. (2005), most species of xylocopid bees nest in dead or soft wood, except the subgenus *Proxylocopa* which nests in the soil. Paper wasps are also known to be closely associated with tree branches for nesting (Yamane and Ito, 1994). Little is known about the nesting habit of bombyliid flies. However, gravid females breed in the nest of the host (Westerfelt et al., 2015). With the exception of generalist xylocopid bees, which are largely dependent on floral resources from different plant species (Keasar, 2010), wasps and flies have wide range of dietary requirements, which include plant nectar, through which they function as pollinators. These insects are largely generalist in nature, and are well adapted to the tall pine tree areas here. Although there were no interactions within the tall pine understorey due to absence of flowering plants, these insects nevertheless, may seek refuge in the tall pine tree canopy (tree dwellers) or understorey (ground dwellers).

Conclusion and management implications

My findings largely support the four hypotheses: that 1) flower abundance varies according to level of direct sunlight vs. shade reaching the understorey plant community, and declines with increasing shade, 2) pollinator abundance also changes in the same direction as understorey plant communities with increasing level of sunlight along sampling distance from the interior of the pine areas, there was also a shift in community composition to large-sized more generalized species away from small-sized insect species in associations with tall pine trees, 3) higher number of plant-pollinator interactions occur outside pine canopies compared to the interior, and 4) more specialized plant-pollinator networks occur outside pine canopies than under these canopies.

This shows that there is great merit in removing pine trees at an early stage of growth before they become too large, too damaging, and difficult to remove. Plant invasion continues to adversely impact biodiversity, especially in lowland vegetation areas where invasive trees can dominate. This has both a direct and indirect effect on pollinating insect activity. I show here that pine tree age and increasing development of a continuous canopy reduces the success of native flowering plant species, and is associated with a great change in natural interaction networks. Also, with the sensitivity of the GCFR as a region with a high proportion of endemic species, species temporal loss may be happening in the invaded parts of this ecosystem, especially among species with a narrow habitat and resource preferences in terms of particular floral resources. I strongly recommend restoration management actions in areas with high densities of pine trees.

However, there is strong rider here when I consider the conservation of biodiversity overall. Firstly, the pine tree stands were in effect, set-aside, contained, areas for vertebrate conservation. In other words, invasion had been halted, and these small stands were left as refuges for these vertebrates. Secondly, and something I was not expecting, is that the large-sized, highly mobile insect species also benefitted, not because of flowers but because of potential nesting sites, and like the vertebrates, had the best of both local worlds. In the case of many of the pollinators, flowers outside the pine interior stand for food, and pine trees within for nesting. While the footprint of pines adversely affected the pollinating insects, these pine stands were in confined area within the landscape and are bordered by natural habitats, including two major protected areas (The Helderberg Nature Reserve and the Hottentot-Hollands Nature Reserve) as well as being in a biosphere reserve (Cape Winelands Biosphere Reserve). These points are of great significance when considering whether invasive alien trees are harmful or not. In sum, small and contained alien pine stands confer some distinct benefit. However, these stands must not be so extensive that they cannot be managed.

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Appendix

Appendix 6a. Details of study sites (coordinates) classification and sampling.

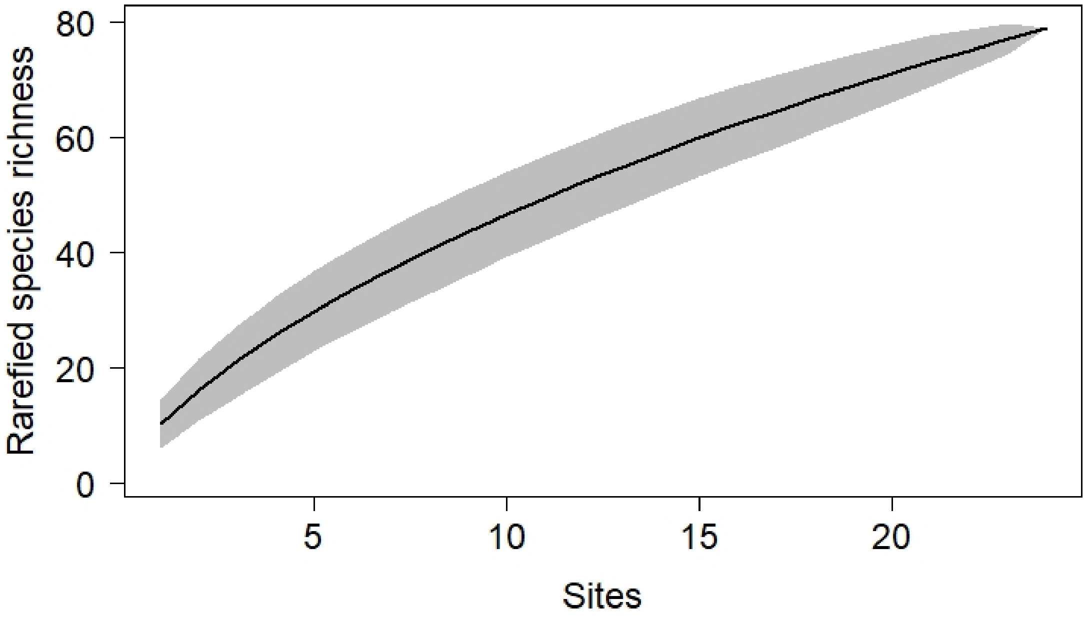
Coordinates	Pine size	Location/Site	Number of pan traps	Total minutes for plant-pollinator interaction observation (2 visits)
S: 34.030366 E: 18.91653	Tall Pine	Interior	12	100
		Edge	12	100
		30 m	12	100
		60 m	12	100
S: 34.032357 E: 18.91518	Small Pine	Interior	12	100
		Edge	12	100
		30 m	12	100
		60 m	12	100
S: 34.027453 E: 18.914271	Tall Pine	Interior	12	100
		Edge	12	100
		30 m	12	100
		60 m	12	100
S: 34.031701 E: 18.915333	Small Pine	Interior	12	100
		Edge	12	100
		30 m	12	100
		60 m	12	100
S: 34.02992 E: 18.91303	Tall Pine	Interior	12	100
		Edge	12	100
		30 m	12	100
		60 m	12	100
S: 34.029722 E: 18.911389	Small Pine	Interior	12	100
		Edge	12	100
		30 m	12	100
		60 m	12	100

Appendix 6b. List of pollinator species

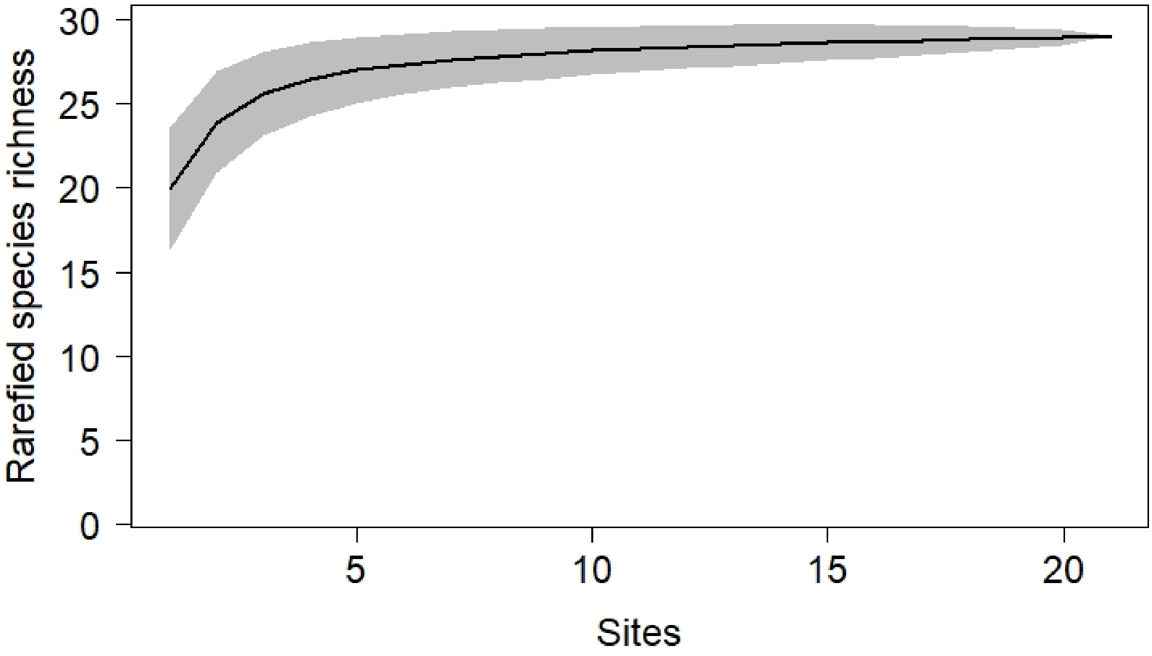
Taxa	Family	Genus	Species/Morphospecies
Bee	Halictidae	*Halictus*	sp.1
Bee	Halictidae	*Halictus*	sp.2
Bee	Halictidae	*Halictus*	sp.3
Bee	Halictidae	*Halictus*	sp.4
Bee	Halictidae	*Halictus*	sp.5
Bee	Halictidae	*Halictus*	sp.6
Bee	Halictidae	*Halictus*	sp.7
Bee	Halictidae	*Halictus*	sp.8
Bee	Halictidae	*Halictus*	sp.9
Bee	Halictidae	*Halictus*	sp.10
Bee	Halictidae	*Halictus*	sp.11
Bee	Halictidae	*Halictus*	sp.12
Bee	Halictidae	Halictidae	sp.1
Bee	Halictidae	Halictidae	sp.2
Bee	Halictidae	Halictidae	sp.3
Bee	Halictidae	Halictidae	sp.4
Bee	Halictidae	Halictidae	sp.5
Bee	Halictidae	Halictidae	sp.6
Bee	Halictidae	Halictidae	sp.7
Bee	Halictidae	*Lasioglossum*	sp.1
Bee	Halictidae	*Lasioglossum*	sp.2
Bee	Halictidae	*Patellapis*	sp.1
Bee	Halictidae	*Patellapis*	sp.2
Bee	Halictidae	*Patellapis*	sp.3
Bee	Halictidae	*Patellapis*	sp.4
Bee	Halictidae	*Patellapis(Zonalictus)*	sp.
Bee	Halictidae	*Patellapis(Chaetalictus)*	sp.
Bee	Halictidae	*Pseudapis*	sp.1
Bee	Halictidae	*Pseudapis*	sp.2
Bee	Colletidae	*Colletes*	sp.
Bee	Megachilidae	*Lithurgus*	sp.
Bee	Apidae	*Anthophora*	sp.
Bee	Apidae	*Apis*	*mellifera*
Bee	Apidae	*Allodapula*	sp.1
Bee	Apidae	*Allodapula*	*melanopus*
Bee	Apidae	Apidae	sp.
Bee	Apidae	*Xylocopa*	*albifrons*
Beetle	Scarabaeidae	*Hedybius*	sp.
Beetle	Scarabaeidae	*Peritrichia*	sp.
Beetle	Scarabaeidae	*Anisonyx*	*ursus*
Beetle	Scarabaeidae	*Pachycnema*	sp.1
Beetle	Scarabaeidae	*Pachycnema*	sp.2
Beetle	Scarabaeidae	Scarabaeidae	sp.1
Beetle	Scarabaeidae	Scarabaeidae	sp.2
Beetle	Scarabaeidae	Scarabaeidae	sp.3
Beetle	Coccinellidae	Coccinellidae	sp.1
Beetle	Coccinellidae	Coccinellidae	sp.2
Beetle	Meloidae	Meloinae	sp.
Beetle	Curculionidae	Scolytidae	sp.1
Beetle	Curculionidae	Scolytidae	sp.2
Beetle	Curculionidae	Scolytidae	sp.3
Fly	Drosophilidae	*Drosophila*	*melanogaster*
Fly	Tephritidae	Tephritidae	sp.1
Fly	Muscidae	Muscidae	sp.1

Fly	Muscidae	Muscidae	sp.2
Fly	Muscidae	Muscidae	sp.3
Fly	Syrphidae	Syrphidae	sp.1
Fly	Syrphidae	Syrphidae	sp.2
Fly	Tabanidae	Tabanidae	sp.1
Fly	Tabanidae	Tabanidae	sp.2
Fly	Tabanidae	Tabanidae	sp.3
Fly	Bombyliidae	Bombyliidae	sp.
Fly	Calliphoridae	*Lucilia*	*sericata*
Fly	Culicidae	Culicidae	sp.
Wasp.	Sphecidae	Sphecidae	sp.1
Wasp.	Sphecidae	Sphecidae	sp.2
Wasp.	Sphecidae	Sphecidae	sp.3
Wasp.	Sphecidae	Sphecidae	sp.4
Wasp	Vespidae	Vespidae	sp.
Wasp	Pompilidae	Pompilidae	sp.1
Wasp	Pompilidae	Pompilidae	sp.2
Wasp	Braconidae	Braconidae	sp.1
Wasp	Braconidae	Braconidae	sp.2
Wasp	Masaridae	Masaridae	sp.
Wasp	Crabronidae	*Tachysphex*	sp.1
Wasp	Crabonidae	*Tachysphex*	sp.2
Wasp	Crabonidae	*Tachysphex*	sp.3
Wasp	Scoliidae	Scoliidae	sp.1
Wasp	Scoliidae	Scoliidae	sp.2
Wasp	Scoliidae	Scoliidae	sp.3
Wasp	Scoliidae	Scoliidae	sp.4

Appendix 6c. Species richness rarefied curve for flowering plants sampled across study sites. The grey area represents the confidence interval from the standard error of estimates.



Appendix 6d. Species richness rarefied curve for pollinators collected across sampling sites. The grey area represents the confidence interval from the standard error of estimates.



Appendix 6e. Pollinator species in interactions associated with different pine tree sizes.

Taxa	Family	Genus	Species/Morphospecies	Small pine	Tall pine
Bee	Halictidae	*Halictus*	sp.1	-	
Bee	Halictidae	*Halictus*	sp.2		-
Bee	Halictidae	*Halictus*	sp.3	-	
Bee	Halictidae	Halictidae	sp.1	-	
Bee	Halictidae	Halictidae	sp.2	-	
Bee	Halictidae	Halictidae	sp.3	-	
Bee	Halictidae	Halictidae	sp.4	-	
Bee	Halictidae	Halictidae	sp.5	-	
Bee	Halictidae	Halictidae	sp.6		-
Bee	Halictidae	Halictidae	sp.7		-
Bee	Halictidae	*Lasioglossum*	sp.1	-	-
Bee	Halictidae	*Lasioglossum*	sp.2		-
Bee	Halictidae	*Lasioglossum*	sp.3	-	
Bee	Colletidae	*Colletes*	sp.	-	
Bee	Megachilidae	*Megachile*	sp.	-	
Bee	Anthophoridae	*Anthophora*	sp.	-	
Bee	Anthophoridae	*Anthophora*	sp.2	-	
Bee	Anthophoridae	*Anthophora*	sp.3	-	
Bee	Anthophoridae	*Anthophora*	sp.4	-	
Bee	Anthophoridae	*Amegilla*	sp.	-	
Bee	Apidae	*Xylocopa*	sp.1		-
Bee	Apidae	*Xylocopa*	sp.2		-
Bee	Apidae	*Apis*	*mellifera*	-	-
Beetle	Scarabaeidae	*Hedybius*	sp.	-	-
Beetle	Scarabaeidae	*Peritrichia*	sp.	-	-
Beetle	Scarabaeidae	*Anisomyx*	sp.	-	-
Beetle	Scarabaeidae	*Pachycnema*	sp.	-	
Beetle	Scarabaeidae	Scarabaeidae	sp.	-	
Beetle	Meloidae	Meloidae	sp.1	-	-
Beetle	Meloidae	Meloidae	sp.2	-	
Beetle	Coccinellidae	Coccinellidae	sp.	-	
Beetle	Cerambycidae	Cerambycidae	sp.	-	
Fly	Syrphidae	Syrphidae	sp.1	-	
Fly	Syrphidae	Syrphidae	sp.2	-	
Fly	Muscidae	Muscidae	sp.1	-	-
Fly	Muscidae	Muscidae	sp.2		-
Fly	Muscidae	Muscidae	sp.3	-	
Fly	Calliphoridae	*Lucilia*	*sericata*	-	-
Fly	Bombyliidae	Bombyliidae	sp.		-
Wasp	Sphecidae	Sphecidae	sp.1		-
Wasp	Sphecidae	Sphecidae	sp.2	-	
Wasp	Masaridae	Masaridae	sp.1	-	
Wasp	Masaridae	Masaridae	sp.2		-
Wasp	Vespidae	Vespidae	sp.		-
Wasp	Pompilidae	Pompilidae	sp.		-

Chapter 7

General conclusion and recommendations

Global change and subsequent transformed ecosystems are having a major impact on biodiversity. This is particularly evident among species' ecological interactions, leading to cascading effects across multiple trophic levels. While most studies have shown how landscape transformation influences species distribution patterns, studies focusing on response of ecological interactions to these changes are still relatively few. This is especially the case for the Greater Cape Floristic Region (GCFR), with its high level of endemism and important interactions between plants and pollinators (Khulmann, 2005). It is therefore essential to understand how flowering plants and their interactions with insect pollinators respond to important drivers of environmental change which are pertinent to this region, and hence this study.

Plant-pollinator interactions are fundamental for providing essential pollination ecosystem services. This is not only critical for the maintenance of ecosystems globally, but is also essential in mediating the challenge of food security associated with growing human population. Furthermore, these interactions are as essential for stemming biodiversity loss as they are for meeting the needs of humans. However, landscape transformations influence pollinator and plant distribution and this may also affect the delivery of pollination services, especially among specialized species. This may be critical here in the GCFR where honeybees are responsible for the pollination of about 27% of herbs, 44% of shrubs and 28% of trees (Hepburn and Radloff, 2013)

I reported in Chapter 2 that an increase in elevation in the GCFR is associated with a decline in temperature and flowering plant diversity. These changes led to a breakdown in interaction networks involving bees and beetles, but not those involving flies and wasps. Bees and beetles are more associated with changes in air temperature and flower indices across elevation zones may be prone to temporal loss in interaction following the decline of interacting partner.

Mountains are often important biodiversity hotspots with several high elevation specialized pollinators and flowering plants (Spehn et al., 2010). However, this often species rich montane ecosystem is fragile in species distribution in interaction across elevation zones with the lowest representative of interacting partners at the summit. Although most flowering plant species at this high elevation are pollinated by wind, thereby reducing the chances of loss of flowering plants, however, some insects that are resilient to the conditions at high elevations, especially the specialized species may be at risk of possible future loss in the absence of interacting partners. While species which are highly adapted to cold and often wet conditions at the summit such as flies (Lázaro et al., 2008) may be at less risk of temporal displacement, some halictids which are abundant at the summit zone in my study and also obligate flower feeders may be at risk of future loss in areas with low diversity of nectar rewarding flowering plants.

Lower elevation zones in this study were rich in flowering plants and insect pollinators, as shown in Chapters 2 and 3. Highest abundance and species richness of flowering plants, and pollinators, as well as their interactions, were recorded in the middle elevation zone in particular, which is also an ecotone. This further emphasizes the importance of an ecotone as area of great conservation interest. However, while abundance peaks of insect pollinators track those of flowering plants at each elevation zone, bees peak seasonally earlier in abundance compared to flowering plants at the middle (ecotone) elevation zone. This suggests a phenological mismatch between bees and interacting flowering plants at this zone. Also of importance is the differential phenological response among insect taxonomic group across elevation zone. This suggests species-specific traits playing a major role in influencing species phenology across elevation zone. In addition, species may have different response to varying environmental conditions across elevation gradient (Ovaskainen et al., 2013). Assessing species distribution patterns across elevation zones shows the middle zone (ecotone) to be an area with highest number of interacting species and interactions. There is now, however, a necessity for assessing species temporal (phenological) response and how life history traits of different groups mediate species phenology towards conserving the sensitive montane ecosystems as with ongoing global climate change. While I encourage active conservation efforts, especially those directed at reducing the impact of climate change, I also encourage long-term future monitoring of species interactions, phenology, and productivity, especially at the ecotone to assess the effect of conservation efforts.

In Chapter 4, I showed how fire frequency influences assemblages and distribution of pollinators and flowering plant species. Flower abundance was lowest in the short-term burned areas, especially for long-lived perennials, while highest in the medium-term burned areas. Fire also structured the community composition and distribution of insect pollinators, especially bees, through its direct effect on flowering plants. Bees are highly dependent on quality floral rewards. However, other environmental factors such as nesting requirements seem to be driving the distribution of other taxa, especially flies. One would have expected areas with longer fire history to have the highest flower abundance. Yet, this was not the case here. Thus, while immediate impact of fire may bring about loss of important flowering plants and insect pollinators, as seen here, fire is also required to improve quality of flowers through reduction of shade from canopy cover. According to the literature, most *Protea* species require fire frequency of about 10-15 years for optimum reproduction (Van Wilgen and Forsyth, 1992). This means that it is necessary to put into practice controlled burning of overgrown natural areas, which is essential for rejuvenating the floral resources that will also indirectly increase pollinator visitation in this area.

It is also important to note how fire influences species nesting requirements, as also seen in Chapter 4. Although floral resource availability plays crucial role in bee dispersal patterns, nest requirements are also of great significance. Megachilid bees mostly build their nests with leaves and soil particles, and so these bees require exposed soil surfaces and plant material for effective nesting, which are available in short-term burned and medium-term burned sites, thereby driving high abundance of megachilid bees here. On the other hand, other bee families, especially the Halictidae and Apidae, some of which require logs or tree branches for nesting, were most abundant in the long-term burned areas. Thus, management practice that takes into account species nest requirements should be considered, especially during controlled burning for conservation purposes.

In addition, fire frequency also influences plant-pollinator interaction networks, as seen in Chapter 5. Here, I also showed how fire refuges may alleviate the immediate impact of fire in a fire-prone landscape. In this chapter, I showed that recently burned habitat was limited in flower resources, which yielded more generalized interactions compared to refuge patches. Surprisingly, there was also a refuge patch effect, accommodating more specialized species than the unburned natural areas.

Refuge patches in burned landscapes are overlooked important ecosystem physical elements. This suggests that conservation actions should include controlled burning for provision of flower-rich refuges in the burned landscape. In landscapes with complex topography, like the GCFR, more efforts should be focused on fire refuges in the valley, as these are more effective for the persistence of insect pollinators, especially the specialized species, while burned areas are recovering.

In Chapter 6, I showed how alien pine trees influence plant-pollinator interactions, as well as their distribution across landscape at different stages of invasion. Invasion is one of the most prominent challenges facing biodiversity globally, and greatly influences assemblages and ecosystem function in different ways. Competition for resources between native and invasive species is highly pronounced in invaded areas, especially in the GCFR. As most endemic plant species in the GCFR are shrub-like, invasive trees, such as pines, are highly successful through their ability to out-shade native undergrowth. However, it is imperative to understand how important plant-pollinator interactions are affected through the indirect shading impact of alien trees on native flowering plants.

More interactions were observed in areas during early invasion with small pine trees compared to areas that were invaded by high-density tall pine trees. Specialized insect pollinators also had higher number of interactions in small-sized pine tree areas. However, overall interactions, especially those involving specialized insect pollinators, were more frequent with increased distance outside the pine trees. This emphasizes the importance of restoration efforts, especially in areas with tall pines. Overall, I conclude that pine trees should be removed at the early invasion stage to reduce further loss of important native species, as well as their interactions.

While it is essential to reduce the impact of pine trees on native species through restoration, it is also important to take into account the positive impact of these invasive pines, especially for insect groups that depend on them for nesting. Some large-sized wasps, bees and flies were only found in tall pine areas, where the trees are important as nesting sites. In addition, this area with tall pine trees is also aimed at preserving some mammals of conservation concern, some of which are endemic to this region. There is therefore a thin line in making conservation decisions regarding these alien trees, which, while invasive, can also provide important resources both for certain insects and certain mammals.

In conclusion, while this study has quantified the effect of these drivers of landscape transformation on important plant-pollinator interactions, there is urgent need for future studies addressing species productivity in the face of growing environmental threats, especially in sensitive biodiversity hotspots like the GCFR. More attention should be given to species-level analysis addressing pollinator importance and their effectiveness in interactions with flowering plants. This is of great importance for identifying species vulnerable to temporal loss in this region of high endemism, where loss of a species also means global extinction.

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