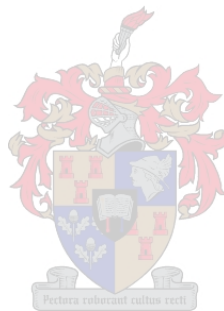


Untangling mechanisms structuring insect diversity patterns in the Cape Floristic Region: the Restionaceae and their herbivores

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Thesis presented in partial fulfilment of the requirements for the degree of Master of Science
at Stellenbosch University

Tesis ingelewer ter gedeeltelike voldoening aan die vereistes vir die graad Magister in
Natuurwetenskappe aan die Universiteit van Stellenbosch

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December 2014

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Signature:

Date: September 2014

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Abstract

Research into the patterns and drivers of insect diversity in the Cape Floristic Region (CFR) lags far behind that of plants. Here I sample insect herbivore communities on a dominant plant family (Restionaceae), and use a spatially nested sampling design and network analysis to evaluate the association between plant and insect diversity in the CFR. I find that plant species richness predicts insect richness better than environmental factors. Turnover in insect communities is strongly associated with turnover in plant (both species and phylogenetic) communities at both local and regional sampling scales, suggesting insect host specificity. Plant communities unsurprisingly show significant turnover at small spatial scales (i.e. communities situated 0.1-3 km apart show significant turnover and may be tied to ecological niches). Insects show a similar pattern, but the decrease in community overlap is more gradual, suggesting many insects can utilise multiple (possibly closely related) hosts while plants are tied to particular niches. The emergent structure of multiple interaction networks is spatially and temporally invariant, despite high compositional change. However, the internal structure of the networks shows variation (i.e. interactions show spatial and temporal turnover). Seasonal interaction turnover is driven by a turnover in herbivores and by herbivore host switching. Spatially the turnover in interactions is driven by simultaneous turnover in both plants and insects, either suggesting that insects are host specific, or that both groups exhibit parallel responses to environmental gradients. Spatial interaction turnover is also driven by a turnover in plants, showing that many insects can utilise multiple (possibly closely related) hosts and have wider distribution ranges than their host plants. Results point toward insect host specificity, but probably not at the species level, as the primary mechanism structuring insect communities associated with the Restionaceae in the CFR.

Opsomming

Navorsing wat verband hou met die patrone en meganismes wat insekdiversiteit in the Kaapse Blommeryk (KBR) hou nie pas met dié van plante nie. In hierdie studie neem ek insekmonsters binne een van die dominante plantfamilies (Restionaceae), en gebruik 'n ruimtelik geneste ontwerp en netwerkanalise om die verbintenis tussen plant- en insekdiversiteit te evalueer. Ek vind plantrykheid voorspel insekrykheid beter as enige omgewingsfaktore. 'n Omset in in insekgemeenskappe is sterk verbind aan 'n omset in plantgemeenskappe (beide spesie en filogenetiese) by beide plaaslik en vir die hele streek. Hierdie dui op insekgasheerspesifisiteit. Plantgemeenskappe wys omset teen kort ruimtelike skale (0.1-3 km). Insekte wys 'n soortgelyke patron, maar die afname in oorvleueling tussen gemeenskappe is meer geredelik. Dit dui dat insek meer as een gasheer kan gebruik, terwyl plante streng tot sekere nisse verbind is. Die ontluikende struktuur van menigde interaksienetwerke wys geen ruimtelike of tydelike variasie nie, ten spyte van hoë gemeenskapsomset. Nietewel, die interne struktuur van die netwerke wys veranderinge (interaksies in netwerke wys omset). Seisonale interaksie-omset kan toegeskryf word aan 'n omset van herbivore en insekgasheerverandering. Ruimtelike interaksie-omset word toegeskryf aan gelyktydige insek- en plantomset, wat óf deur insekgasheerspesifisiteit veroorsaak word óf deur parallele reaksies tot omgewingsveranderinge. Ruimtelike interaksie-omset word ook deur plantomset beïnvloed, wat aandui sommige insekte kan meer as een gasheer benut en insekte het weier verspreidings as hul gasheer. Resultate dui daarop dat insekgasheerspesifisiteit, maar waarskynlik nie op die spesievlak nie, moontlik die primêre meganisme is wat insekgemeenskappe verbind aan die Restionaceae in die KBR struktureer.

Acknowledgments

I thank my supervisor, Prof. Allan Ellis, for patience, guidance, contagious curiosity and always having an open door.

I also thank Dr. Darren Evans for hosting me at the University of Hull with true English hospitality, and to my new friends for making my travels remarkable.

I thank my treasured friends, especially Ydi, Suzette, Hannelize, Kristin and Corneli, for a shared enthusiasm for life, philosophical conversations, hiking, mountains, stargazing, art, wine, tea, music and laughter. And I thank the inhabitants of Room 1019 for distractions, insightful and nonsensical conversations, endless amusement, cake, coffee and academic help.

I thank my parents for their unfailing support and patience.

I acknowledge the funding provided by the NRF through SABI that made the project possible and DAAD-NRF for funding my subsistence. I also acknowledge the European Commission for funding my six month stay in the United Kingdom through the Erasmus Mundus (EMA2SA) program.

South African National Parks (SANParks) and the Western Cape Nature Conservation Board provided permits.

This work has been presented in part at a conference:

Fynbos Forum 2013 – Celebrating Fynbos in a Centenary year

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Chapter 1

Introduction

The global plant-insect diversity relationship

Arthropods associated with plants constitute a major part of the earth's biodiversity (Price 2002) and various studies have investigated the relationship between plant and insect richness (Castagneyrol & Jactel 2012). Since herbivores feed on plants, it is generally accepted that herbivorous insect diversity should increase with an increase in plant diversity (Siemann *et al.* 1998; Lewinsohn & Roslin 2008; Dinnage *et al.* 2012), and this should subsequently also lead to an increase in predacious insect diversity (Castagneyrol & Jactel 2012). However, both the strength of the association between these groups and the key determinants of the relationship, are still debated (Santi *et al.* 2010). Cross-taxon correlates can assist in identifying mechanisms that drive and maintain diversity of interacting groups (Castagneyrol & Jactel 2012).

The exceptional species richness of insects has partly been explained by speciation resulting from evolutionary transitions of specialist herbivores from one host to another (Winkler *et al.* 2009). If insects are host specific, a positive correlation between plant and insect richness is expected (Siemann *et al.* 1998). Insect richness can show a stronger relationship with plant phylogenetic diversity than plant richness if insects are specialised on plants at the generic or family level (Novotný *et al.* 2002) and a community of distantly related plant species will more likely fall within the host range of a larger variety of herbivores (Dinnage *et al.* 2012). Alternatively, a positive association in the species richness of these groups might arise when parallel responses are exhibited to environmental factors. Hawkins & Porter (2003) found that host plant diversity and Californian butterfly diversity were not correlated once environmental variables were accounted for, and Craft *et al.* (2010) showed that while genetic structure of tropical insects is in some cases associated with host specialisation, it often mirrors other landscape gradients. Additional plant properties besides plant community richness, such as plant structure (Axmacher *et al.* 2009) or plant phenophase (Augustyn *et al.* 2013), may influence insect herbivore richness. The effects of all these factors need to be

disentangled if we wish to understand the mechanisms that are driving and maintaining insect diversity.

The change in species composition between communities (beta diversity – Whittaker 1974) can provide insight into mechanisms structuring insect communities when assessed along latitudinal, altitudinal or climatic gradients (Novotný & Weiblen 2005; Ødegaard 2006; Beck *et al.* 2011). Further, associations between plant and insect turnover in community composition can assist in ascertaining whether or not plants and insects are associated through insect host specificity. If insect species turnover is more strongly related to plant species (or plant phylogenetic) turnover than environmental gradients, we can infer insects are host specific. The first evidence was recently provided that shows plant phylogenetic beta diversity structures butterfly phylogenetic beta diversity (Pellissier *et al.* 2013b). A parallel study (Pellissier *et al.* 2013a) showed insect phylogenetic beta diversity is strongly influenced by altitudinal temperature changes and thus environmental filtering also partly structures butterfly communities in the Swiss Alps.

Another approach to determine the drivers of insect diversification and community structure would be to construct interaction networks. Network ecology has mainly focused on the emergent structure of networks, such as the number of hosts per insect (Novotný & Basset 2005), number of herbivores per host (Lewinsohn *et al.* 2005), nestedness (Thébault & Fontaine 2008) and connectance (Tylianakis *et al.* 2007). However, changes within networks, such as composition or interaction turnover, have received much less attention (Novotný 2009). Turnover in community composition or interactions between networks can assist in identifying patterns shaped by processes that structure food webs at a population level (Lewinsohn & Roslin 2008). Interaction networks can show us whether insect diversity is directly dependent on plant diversity through host specialisation or whether insects are responding to environmental gradients. Lewinsohn & Roslin (2008) highlight the importance of simultaneously evaluating plant and herbivore alpha and beta diversity, and also species specificity, in order to determine the drivers of insect diversity. They further emphasise the importance of determining patterns of diversity in regions other than the tropics.

Diversity relationships in the Cape Floristic Region

Tropical studies have shaped our understanding of the association between plant and insect richness (Tylianakis *et al.* 2005; Novotný *et al.* 2007, 2012; Lewinsohn & Roslin 2008), while other hyperdiverse regions, such as the Cape Floristic Region (CFR), have received

much less attention. The Cape Floristic Region of South Africa is a recognised biodiversity hotspot (Myers *et al.* 2000) that contains more than 9000 plant species in 90 000km² (Goldblatt & Manning 2000). The CFR represents a global exception, containing more than twice the plant species richness per unit area than predicted from environmental conditions (Kreft & Jetz 2007). This region provides a unique scenario where plant diversity per area is similar to that of the tropics, but the climatic conditions differ significantly. It allows us to decouple the effects of plant diversity from environmental factors in a way that is not possible in tropical studies.

Plant species richness in the CFR peaks towards the west and declines in the east, and this pattern correlates with geographical changes in rainfall seasonality (e.g. Cowling 1992; Cowling & Lombard 2002). Further, plant species richness is higher in the topographically more complex mountainous areas than in the lowlands (Linder 1991). Speciation in the CFR is correlated with habitat differentiation and at least 80% of sister species pairs in the region exhibit ecological differences (van der Niet & Johnson 2009).

Although patterns of plant diversity are well-established in the CFR (Linder 1991; Cowling & Lombard 2002; Rouget *et al.* 2003; Verboom *et al.* 2009), surprisingly little is known about patterns of herbivorous insect diversity. Insect richness increases with plant richness (Wright & Samways 1998; Pryke & Samways 2008; Procheş *et al.* 2009), but whether insect richness is lower (Johnson 1992; Giliomee 2003) or not (Price *et al.* 1998; Wright & Samways 1998) than expected from the exceptional plant richness, has been much debated. Further, even less is known about patterns of herbivorous insect species turnover across the landscape. Colville (2009) recently showed beetle species turnover in the CFR is related to environmental and plant variables, but studies focusing on mechanisms structuring the distribution of other insect groups are still lacking.

Restionaceae and their herbivores

The African Restionaceae (hereafter restios) belong to the monophyletic Restionoideae subfamily (Briggs & Linder 2000) which contains 350 species (Linder 2003). Restios constitute one of the oldest clades in the CFR, originating approximately 91.5 million years ago (Verboom *et al.* 2009). Restios show strong eco-hydrological niche segregation (Araya *et al.* 2011) and this is associated with the ability of species to produce aerenchyma tissue (Huber & Linder 2012). All plants in this wind-pollinated clade of reed-like plants are dioecious and some show dimorphism between male and female reproductive structures.

While Restionaceae have a typical graminoid growth form, species exhibit substantial differences in plant height, culm diameter and branching of the culms. Restios occur throughout the CFR in habitats that vary in soil type, altitude, groundwater availability, slope, aspect and climate.

A tribe of CFR endemic leafhoppers (Cephalini: Cicadellidae) occur exclusively on Restionaceae and appear to exhibit species-level host specificity (Davies 1988; Prendini & Linder 1998; Augustyn *et al.* 2013). Recent work shows closely related Cephalini species feed on the same Restionaceae tribes (Wiese 2014) and some species track the phenology of their primary hosts (Augustyn *et al.* 2013). Other insects, such as Tropicuchidae, Lentulidae, Chrysomelidae and Fulgoridae, also feed on restios, but levels of specialisation and patterns of turnover in insect communities have not been investigated for these other groups.

Structure of thesis

In this thesis I ask whether insect diversity in the CFR shows an association with plant diversity, and what mechanisms are structuring this correlation. This is done by integrating richness estimates, community ecology and network ecology. I use the Restionaceae, one of the most diverse, abundant and well-studied plant families in the CFR, and its associated herbivores to do this. In Chapter 2, I evaluate the relationship between plant and insect species richness and identify factors that co-vary with this relationship. I also assess the influence of plant phylogenetic diversity on various insect diversity metrics. A spatially nested sampling approach is used to determine whether the plant-insect relationship is present at various sampling scales. The third chapter addresses the relationship between plant and insect community turnover (beta diversity). The same data is used as in Chapter 2 and some overlap in the methods and results sections of these two chapters is thus present. Where the second chapter merely takes the number of species into account, the third chapter takes species identities into account. The spatially nested sampling approach allows me to evaluate the spatial structure of insect community turnover (i.e. I evaluate how much turnover is exhibited between communities separated by various distances) and I determine whether matching patterns are found for plants. Next, I directly assess whether insect beta diversity correlates with plant beta diversity and then use predictive co-correspondence analysis to determine whether plant community composition can predict composition of insect communities. Finally, the effects of environmental variables and other plant components on insect turnover are determined. The fourth and final chapter uses a different sampling

approach and looks at the interactions between plants and insects by constructing bipartite networks. Several standard network metrics are calculated, and the influence of these metrics on changes in insect communities is assessed. The beta diversity of interactions is calculated and this allows me to formulate various hypotheses regarding insect niche-breadth, the size of insect and plant ranges, and insect host switching. I also assess whether insects are randomly selecting the most abundant plant species, choosing plants based on phenophase or picking hosts based on some other plant trait (such as penetrable defences, structural traits, predator avoidance, etc.). This allows me to evaluate what mechanisms that are structuring insect communities in the CFR.

Chapter 2

Plant-insect species richness relationship in a temperate biodiversity hotspot: the Restionaceae and their insect herbivores

Abstract: Globally plant species richness is a significant predictor of insect richness. Whether this is the result of insect diversity responding directly to plant diversity, or both groups responding in similar ways to extrinsic factors, has been much debated. Here I assess this relationship in the Cape Floristic Region (CFR), a biodiversity hotspot. This region has much higher plant diversity than expected from latitude and environmental variables, and this allows us to decouple the effects of plant diversity and extrinsic factors on insect diversity. I quantify diversity relationships at multiple spatial scales for one of the dominant plant families in the CFR, the Restionaceae, and its associated insect herbivore community. Plant and insect diversity are positively correlated at the local scale (10-50 m), but not at the regional scale (50-70 km). This implies that the diversity of local insect assemblages may be directly dependent on plant species, but that the size of the regional insect species pool may not be. Insects often exhibit host specificity at the plant family level, and thus the relationship between plant and insect richness within a single plant family is surprising. It may indicate insects are specialised on plants at fine taxonomic scales. I only find a weak influence of extrinsic variables on this relationship. Comparison of CFR and tropical studies suggests that the ratio of insect species per plant species is significantly lower in the CFR than in the tropics, with the CFR exhibiting ratios similar to other temperate (but plant species poor) regions. The latitudinal decrease in insect diversity might thus be decoupled from the latitudinal trends in plant diversity. Alternately, the low insect richness in the CFR may result from the low plant phylogenetic diversity (i.e. only 33 clades make up more than 50% of plant species).

Introduction

The majority of the world's eukaryotes are terrestrial arthropods (Zhang & Zhan 2011). Speciation resulting from evolutionary transitions of specialist herbivores from one host to another has been inferred as an important mechanism driving arthropod diversity (Winkler *et al.* 2009). If insects are highly specialised on plants, a high diversity of plants should lead to a high diversity of insect herbivores at both the community and regional scales, and thus a positive association between plant and insect richness is expected (e.g. Siemann *et al.* 1998; Castagneyrol & Jactel 2012; Dinnage *et al.* 2012). However, insect host specialisation may be more prevalent in the tropics than temperate zones (Dyer *et al.* 2007) and the slope of the plant-insect richness relationship might thus vary between regions if we expect more insect species per plant species in the tropics due to a finer partitioning of resources (Dyer *et al.* 2007). In contrast, Novotny *et al.* (2006) showed similar numbers of insect herbivore species per area of foliage in phylogenetically comparable tree species in tropical and temperate zones, suggesting that the general decrease in insect diversity with an increase in latitude can be attributed to a latitudinal decrease in plant diversity, rather than a latitudinal change in herbivore specificity. This assumes that insect diversity is directly dependent on plant diversity, and predicts that the ratio of insect to plant species should remain constant across latitudes.

Because insects are often specialised on plants at the generic or family level (Novotný *et al.* 2002), a community of distantly related plant species will more likely fall within the host range of a larger variety of herbivores, resulting in high herbivore diversity in phylogenetically diverse plant communities (Dinnage *et al.* 2012). Castagneyrol *et al.* (2014) suggests the strength of plant-insect richness relationship versus the strength of insect richness and plant phylogenetic relationships is contingent on the patterns of herbivore specialisation, where the relationship between insect richness and plant phylogenetic diversity will be stronger than the alternative if insects are specialised at higher taxonomic levels.

However, the positive correlation between plant and insect diversity is not necessarily the result of a direct association. For example, Hawkins & Porter (2003) found that once environmental variables were controlled for, plant host diversity and Californian butterfly diversity were not correlated. Craft *et al.* (2010) showed that while genetic structure of tropical insects is in some cases associated with host specialisation, it often mirrors other

landscape gradients. These patterns suggest that the plant-insect diversity relationship might arise due to similar responses of both groups to environmental gradients. In addition, properties of plant communities besides species/phylogenetic diversity may influence insect herbivore diversity. For example, Axmacher *et al.* (2009) showed vegetation structure to be an important predictor of geometrid moth richness and Augustyn *et al.* (2013) showed that some endemic insects in the Cape Floristic Region track the phenophases of their host plant species. A range of phenophases in a community may thus lead to a larger variety of insect herbivores. Even a relationship between plant and insect phylogenetic diversity might not indicate a direct association between plant and insect diversity. Turnover in phylogenetic diversity should be high for both plants and insects across ecological gradients, creating a positive relationship between the diversity of these groups.

The majority of studies investigating plant-insect diversity relationships have focused on diverse tropical systems (Erwin 1982; Tylianakis *et al.* 2005; Novotný *et al.* 2006; Whitfeld *et al.* 2012), while other hyperdiverse systems have received much less attention. The Cape Floristic Region (CFR) of South Africa is a recognised biodiversity hotspot (Myers *et al.* 2000) that contains more than 9000 plant species in 90 000 km² (Goldblatt & Manning 2000). While differences in plant species richness between the major floristic kingdoms are minor after controlling for a variety of environmental effects, the CFR represents a clear exception, containing more than twice the plant species richness per unit area than predicted from environmental conditions (Kreft & Jetz 2007). The CFR provides an interesting scenario where plant diversity per area is similar to that of the tropics, but the climatic conditions differ significantly. It allows us to assess whether insect herbivore diversity is directly dependent on plant diversity, or whether other factors have a more profound influence on insect diversity. If insect herbivore diversity is directly dependent on plant diversity, I expect the plant-insect diversity relationship to be similar to that of other plant-rich regions. If, however, insect diversity decreases with an increase in latitude independently of plant diversity, then I expect insect diversity to be lower in the CFR than in the tropics. If this is the case, it is necessary to investigate alternative factors driving insect diversity. The limited work on CFR insect diversity suggests that Cape plant and insect diversity may be positively correlated (Wright & Samways 1998; Procheş & Cowling 2006; Kuhlmann 2009; Procheş *et al.* 2009). Authors, however, disagree on whether Cape insect diversity is high (Price *et al.* 1998; Wright & Samways 1998) or depauperate (Johnson 1992; Giliomee 2003) relative to expectations from the high levels of plant diversity in the region. It has been suggested that

insect diversity in this region is comparable to neighbouring regions (Procheş & Cowling 2006), but insects in these different regions may exhibit varying responses to seasonal changes. By only sampling one season for each region, estimates of total insect richness might be inaccurate.

Here I further explore CFR plant-insect diversity relationships using the species rich Restionaceae and their associated insect herbivores as a model system. This is an ideal system for investigating the links between plant and insect diversity since the Restionaceae support a diverse assemblage of insect herbivores, have fairly uniform growth forms, are a dominant component of CFR vegetation and occur in communities with varying levels of diversity (Dorrat-Haaksma & Linder 2012). The Restionaceae are wind-pollinated, and thus associated insects are likely interacting antagonistically. First, I ask whether herbivore diversity is correlated with plant species and phylogenetic diversity in the Restionaceae system and use a spatially nested sampling design to explore the strength of this relationship at various spatial scales (i.e. local vs. regional). I predict that if insects are specialised on plants at the species level, I should see a strong relationship between plant and insect richness. If insects are specialised at higher taxonomic levels, plant phylogenetic diversity should be a better predictor of insect richness. I expect only a weak relationship between plant and insect richness due to the low phylogenetic diversity created by sampling within a single plant family. Next, I ask which aspects of plant diversity (species, phylogenetic, structural or phenophase diversity) best predict insect diversity and whether these relationships are influenced by environmental factors. Further, I determine whether the relationship found between these groups in the CFR is structured similar to the relationship present in the tropics. If the number of insect species per plant species is not similar to the tropics, I suggest plant and insect diversity may be exhibiting different responses to environmental effects coupled with latitude. Alternately, insect species diversity may rather be dependent on plant phylogenetic diversity (lower in the CFR than in the tropics) or plant size (number of niches per plant).

Methods & Materials

Study system

The African Restionaceae (hereafter restios) is one of the oldest clades in the CFR and originated approximately 91.5 million years ago (Verboom *et al.* 2009). This wind-pollinated monophyletic clade of reed-like plants contains 350 species (Linder 2003); all of which are dioecious and some show dimorphism between male and female reproductive structures. Restio leaves have been reduced to sheaths rolled around the culms at intermittent nodes. While Restionaceae have a typical graminoid growth form, species exhibit substantial differences in plant height, culm diameter and branching of the culms. Restios occur throughout the CFR in habitats that vary in soil type, altitude, groundwater availability, slope, aspect and climate. The high abundance of this group in a variety of habitats makes it ideal for assessing the plant-insect diversity relationship and its correlates in the CFR.

Insect diversity in the CFR has been suggested to be low (Johnson 1992), but more recent studies have found high diversity in galling insects (Wright & Samways 1998) and bees (Kuhlmann 2009). The sclerophyllous leaves of CFR plants may act as deterrent to folivores (Giliomee 2003) and the low soil nutrients (leading to low plant nutrients) could favour generalism in herbivorous insects, where insects may switch seasonally between plant species to optimise nutrient intake (Augustyn *et al.* 2013). Alternately, insects may be specialised on a plant species and only be present in the community when nutrient uptake from that plant species is optimal. Leafhopper species in the tribe Cephalelini (Cicadellidae) have been shown to be specialised on Restionaceae taxa (Davies 1988; Prendini & Linder 1998; Augustyn *et al.* 2013).

Sampling design

A spatially nested sampling approach was used (fig. 2.1). Thirty restio-dominated sites were selected for sampling (Appendix - table S2.1). These were situated on three of the major mountain blocks in the southwestern Cape, namely Hottentots-Holland, Kogelberg and the Cape Peninsula, 50-70 km apart. Each mountain block contained two clusters of five sampling sites (15-20 km apart). The five sites in each cluster were situated 100 m to 3 km from one another. Each site consisted of two 10x10 m sampling squares located 10 to 50 m apart. Five 2.5x2.5 m sampling plots were located within each square (the plots were situated in the corners and centre of each square). Each of the three mountains thus consisted of two clusters, ten sites, twenty squares and a hundred plots. This allowed me to explore the plant-herbivore relationship at various spatial scales, i.e. the plot, square, site, cluster, mountain and regional level. Sites with known restio species composition were chosen to allow me to

sample sites which varied in plant species richness, altitude and vegetation age. Plots were sampled twice, once during the suggested insect peak season (Pryke & Samways 2008) (i.e. spring: August-October 2013) and once six months before this (i.e. autumn: March-April 2013). These sampling periods coincide with the two peaks in Cephalelini abundance (Augustyn *et al.* 2013). These will hereafter be referred to as autumn and spring respectively.

Insect sampling and diversity estimation

Insects were collected from all Restionaceae plants occurring in each plot using a modified leaf-blower with a 15 cm diameter nozzle and placed in 70% ethanol. All restios were exhaustively vacuum-sampled for approximately 20 seconds per plant and the nozzle was moved systematically up and down the culms. All restios in each plot were exhaustively vacuum-sampled. Nwokwu & Sanderson (2009) found that using a modified leaf-blower captured more insects than sweep-netting or pitfall trapping, both in terms of richness and abundance. Restios were search-sampled for insects after vacuum sampling to assess the efficiency of vacuum sampling and also to see whether galling/mining insects were present. Extremely few insects were found by search-sampling and no galling or mining insects were present. Insects were identified to superfamily or family and then sorted into morphospecies. Oliver & Beattie (1996) showed morphospecies to be sufficient surrogates for species, especially in estimates of species richness. Samples were matched across seasons. Insect families known to be non-herbivorous were excluded from the dataset. Insect families known to only feed on nectar of plants (absent in restios) were viewed as transient visitors and also excluded from the dataset.

The Cephalelini were identified to species by dissecting male genitalia and using the species descriptions formulated by Davies (1988) and Prendini (1997) and matching specimens to museum collections (Stellenbosch University, Conservation Ecology and Entomology department). Females were matched to males using external morphology and museum specimens. The insect morphospecies collection is housed in the Botany and Zoology department at Stellenbosch University.

Sampling effectiveness was evaluated by constructing individual based species accumulation curves (number of species found per number of individuals sampled) for various sampling scales (i.e. square, site, cluster, mountain, region). Accumulation curves (Appendix – fig. S2.1-S2.2) tended towards saturation and plots (2.5x2.5 m) were deemed an effective sampling unit.

Insect alpha diversity was calculated in terms of Hill numbers (or numbers equivalents) of the Shannon diversity index. This diversity metric has the advantage of exhibiting additivity and is not biased towards rare or common species (Jost 2007). Alpha diversity was calculated for each plot, square, site, cluster, mountain and the entire region.

Plant sampling and components

Restionaceae species occurring in each plot were identified using the online interactive key of Linder (2002). Abundances of each restio were recorded for each plot. Plant height was recorded and each plant was placed in a structural height category: 0-0.5m, 0.5-1m, 1-1.5m, 1.5-2m, >2m. The branching order of plants was recorded as unbranched, branched (each culm branched 1-3 times) or highly branched (each culm branched more than three times). Each plant was placed in a discrete structural group category based on a combination of its height and branching order (e.g. a 0.7m tall unbranched plant would fall in the “Unbranched_0.5-1m” bin). The number of plants in flower in each plot was also recorded.

Plant alpha diversity was calculated in the same manner as insect alpha diversity (based on Hill numbers). Plant phylogenetic diversity (PD) was calculated from the Restionaceae phylogeny (Linder & Bouchenak-Khelladi, unpublished) using the R package *picante* to calculate Faith's PD. Plant structural diversity was calculated using the alpha diversity metric (Hill numbers) for plant structural groups described above. Structural diversity indicates the number (and abundance) of different structural groups or forms in a plot. A monospecific plot with tall highly branched restios would thus score the same as a monospecific plot of short unbranched restios. However, tall or highly branched restios may provide more feeding niches and support a higher diversity of herbivores. Thus I also used a plant structural complexity metric which aimed to capture the relative amount of culm space available to herbivores in each plot. This was calculated for each plot by multiplying the branching score of each plant (where unbranched plants scored 1, branched plants scored 3 and highly branched plants scored 5) by its height, and summing across all plants in a plot. All of the above calculations were repeated for plots, squares, sites, clusters and mountains.

Environmental predictors

The altitude and age of the vegetation after the most recent fire was documented for each square. Altitudes of squares varied between 44 and 968m above sea level. The CFR burns regularly (every 10-15 years) and vegetation in squares ranged in post-fire age from 2 to 20

years. Environmental data was downloaded from Worldclim (Bioclim data, square 46) and climatic variables that showed no variation between squares were excluded. Annual mean temperature, annual precipitation, annual temperature range, maximum temperature of the warmest month and minimum temperature of the coldest month were included.

Data analysis

I first used linear regressions implemented in R (R Core Team 2013) to assess the plant-insect diversity relationship in the Restionaceae system independent of other potential predictors (e.g. vegetation structure, vegetation age, environmental variables, etc.). This allowed direct comparison to previous diversity studies (Wright & Samways 1998; Procheş *et al.* 2009; Novotný *et al.* 2012). I tested for relationships between insect species richness and plant species richness and phylogenetic diversity, both locally (i.e. plot, square, site scale) and regionally (i.e. cluster scale). Insect alpha diversity was also regressed onto plant alpha diversity to test whether plant communities with more even abundances will host insect communities with even abundances. To test whether an increase in plant richness will linearly influence both insect richness and evenness, I also regressed plant richness onto insect alpha diversity.

Next, general linear models (GLMs) were used to assess the influence of the additional plant diversity components (structural diversity, structural complexity and phenophase diversity) and environmental variables on insect species richness. I used the Akaike information criterion (AIC) from stepwise backward elimination to determine which predictor variables should be included in each of the respective GLMs. Model fit was calculated with all predictors included in the model and predictors were then removed one by one to assess whether model fit improved. The model with the best fit was then used for each of the five GLMs. The function “stepwise” in R (R Core Team 2013) was used for this. Response variables (insect species richness) were $\log_{10}(x + 1)$ transformed where necessary to improve normality. I conducted five separate GLMs to explore the influence of environmental variables and additional plant diversity components on the insect and plant species richness relationship, using 1) the full dataset, 2) the spring dataset, 3) the autumn dataset, 4) Coleoptera only, 5) Hemiptera only. This allowed me to determine whether diversity relationships differ between seasons and across the dominant insect orders. Plots were used as input for all GLMs and the significance level was adjusted for multiple testing using Bonferroni correction.

To test for differences in the ratio of insect species to plant species between the CFR and tropical areas, I compiled data from existing studies which assessed the influence of plant diversity on insect diversity. Various combinations of the search terms “insect”, “plant”, “richness”, “relationship”, “diversity”, “Cape Floristic Region” and “tropics” were used to locate tropical studies that relate plant and insect richness. Studies which only assessed a single taxon of insects, were based on experimental treatments or only sampled a vegetation type (rather than report plant species) were excluded, resulting in a set of three appropriate studies from the tropics (Novotný & Basset 2000; Leps *et al.* 2001; Novotný *et al.* 2012) and two studies, in addition to this one, in the CFR (Pryke & Samways 2008; Procheş *et al.* 2009). The ratio of the total number of insect species sampled to total number of plant species sampled for the CFR and tropics was compared with a t-test. Next, similar studies in temperate systems were identified (Stinson & Brown 1983; Novotný 1994, 1995). Due to the limited number of studies that sample entire communities, I included studies sampling temperate Auchenorrhyncha and compared these to my Auchenorrhyncha:plant ratio. If insect to plant ratios in the CFR are closer to other (plant species poor) temperate regions than the tropics with similar diversity to the CFR, it may indicate that regional insect richness is not only dependent on regional plant richness.

Results

Plant and insect composition

A total of 322 herbivorous insect morphospecies were collected (7276 individuals), 221 insect species (3619 individuals) during autumn (March-April 2013), and 195 species (3657 individuals) during spring (August-September 2013). The restio herbivore community was strongly dominated by Hemiptera, both in terms of species richness (42.6% of species) and abundance (58.9% of individuals) (fig. 2.2). The Cephalelini comprised approximately 10% (773 individuals) of the total number of hemipteran individuals sampled and Fulgoroidea comprised 17% (1237 individuals). Although Coleoptera are the largest order of described species globally, they do not dominate this system.

The mean (\pm sd) insect morphospecies richness for plots was 9.47 ± 4.76 (range: 0 – 27), for squares 29.25 ± 10.09 (range: 12 – 60), for sites 44.80 ± 12.80 (range: 26 – 70), for clusters 126.17 ± 9.06 (range: 118 – 144) and for mountains 192.67 ± 22.55 (range: 171 - 216).

The insects were sampled from 5248 Restionaceae plants (55 species; 11 genera). Mean (\pm sd) plant species richness for plots was 3.04 ± 1.80 (range: 1 to 11), for squares 4.48 ± 2.43 (range: 1 to 11), for sites 5.57 ± 2.79 (range: 2 to 14), for clusters 18 ± 7.95 (range: 8 to 29) and for mountains 28.33 ± 12.50 (range: 16 to 41).

Plant-insect diversity relationship

Insect alpha diversity and species richness were always significantly (or nearly significantly) positively associated with plant diversity (alpha diversity, PD and species richness) at the plot (i.e. 2.5x2.5 m sampling unit) and square (10x10 m) scales (table 2.1). However, plant-insect diversity relationships were not significant at larger sampling scales (fig. 2.3; table 2.1). While significant, plant diversity components only explained a maximum of 11% of variance in insect diversity at the square scale. Plant species richness was a stronger predictor of insect species richness than plant phylogenetic diversity.

Additional variance in insect species richness across plots was explained by altitude, some climatic variables (annual precipitation and maximum temperature of the warmest month), post-fire vegetation age and plant structural diversity (table 2.2). Seasonal patterns differed in that during spring plant species richness was the strongest predictor while plant structural diversity was a stronger predictor in autumn. Similarly, Hemipteran richness was most strongly related to plant species richness while Coleopteran richness was predicted by plant structural diversity. Plant species richness or structural diversity were consistently stronger predictors of insect species richness than the environmental variables.

Comparison of insect:plant species ratios between the CFR and the tropics

The mean ratio of the total number of observed insect species to total number of plant species sampled in the tropics was 25.99 (sd = 7.91), which was significantly higher than ratios reported for other CFR studies (mean 3.11, sd = 2.36) ($t = -4.8001$, $df = 2.356$, $p = 0.029$) (table 2.3).

Temperate Auchenorrhyncha species to plant species ratios ranged from 2.24 to 3.33, and my insect surveys reveal a ratio of 1.62. The number of insect species per plant species in the CFR is thus closer to other temperate zones than to tropical zones.

Discussion

A positive relationship between the species richness of Restionaceae plants and their associated herbivorous insect assemblages was observed, confirming previous reports of plant-insect diversity linkage in the CFR (Wright & Samways 1998; Procheş & Cowling 2006; Pryke & Samways 2008; Procheş *et al.* 2009). Other components of plant diversity (i.e. structural diversity) and environmental factors (post-fire vegetation age, altitude) also explained significant amounts of variance in insect species richness and the contribution of these predictors varied between seasons and across the dominant insect orders.

Insects often specialise on plants at the family level (Novotný *et al.* 2002). Thus the positive diversity relationship I demonstrate within a single plant family likely indicates specificity at finer taxonomic levels. This conclusion is further supported by the fact that plant richness is a better predictor of insect richness than plant phylogenetic diversity. However, the large amount of variance ($R^2 = 0.076$) in this plant-insect richness relationship suggests that many insects do not follow this trend. These findings contrast with Procheş *et al.* (2009) who found plant genera and plant phylogenetic diversity to be the strongest predictors of insect diversity in the CFR. Further, insect diversity may rather be dependent on plant composition, where areas with low plant diversity can be associated with high insect diversity and vice versa depending on what plant species are present. Schaffers *et al.* (2008) showed plant species composition to be an important predictor of insect species composition. The effect of plant composition could cause variance in the plant-insect diversity relationship.

The plant-insect diversity relationship was only significant at the smallest sampling scales (<10x10 m). This contrasts with Procheş *et al.* (2009) who found a positive relationship between plant and insect richness up to a 1 km sampling scale in the CFR, although significance was also absent at the regional sampling scale. They suggest a direct relationship between plant and insect diversity at fine spatial scales and an indirect association at broader spatial scales where the diversity of these groups rather become dependent on abiotic variables, immigration, diversification and extinction rates (Procheş *et al.* 2009). Benton (2009) suggested that biotic interactions should shape diversity patterns locally and over short periods of time, and extrinsic factors like climatic and tectonic events should shape regional patterns over longer periods. The Restionaceae and their herbivores could thus be exhibiting different responses to extrinsic factors resulting in breakdown in the relationship at larger

spatial scales. However, I cannot exclude the possibility that lack of significance at larger spatial scales results from reduced statistical power associated with lower sample sizes.

Plant richness continued to positively influence insect richness after accounting for the effects of other components of plant diversity and environmental variables. Insect richness increased with an increase in vegetation age, indicating new insect species continuously colonise an area after a fire. Niche diversity may increase up to a point with an increase in vegetation age, allowing for an increase in insect diversity (Siemann *et al.* 1998). High rainfall and temperature may influence plant nutrients or insects may have limited environmental tolerance, and these environmental factors may hence affect insect richness. Colville (2009) showed that monkey beetle richness in this region is associated with rainfall and temperature, in addition to plant richness, and the strength of influence of these factors varied geographically. The negative influence of altitude on insect richness is similar to what has previously been found in this region (Pryke & Samways 2008). However, this effect is not as strong as expected from global trends (Lewinsohn & Roslin 2008). Surprisingly, the number of plants in flower did not increase insect richness. The wind-pollinated Restionaceae do not produce large attractive flowers and it seems flowering does not attract significantly more herbivores to plants.

Different factors influence Hemiptera and Coleoptera species richness, suggesting different factors could be driving the diversity of different insect orders. The significant influence of plant richness on Hemiptera richness could indicate host specificity in Hemiptera. The Coleoptera, however, seem to be responding to plant structural properties rather than plant diversity, where structurally more diverse plots could provide better hiding places from predators, different food sources and more niches. The respective effects of plant richness and plant structure on insect richness were much more apparent when seasons and insect orders were treated separately. These findings align with Koricheva *et al.* (2000) who showed leafhopper, aphid and beetle abundances exhibit different responses to plant richness. If various insect groups are exhibiting different patterns, these patterns may be obscured when combining all insects during analysis. Interestingly, different factors are influencing insect richness between seasons and the dominant groups in each season may exhibit different responses to the different plant components.

The ratio of insect species to plant species has been suggested to remain constant across latitudes (Novotný *et al.* 2006). Here, however, I show that this is not the case in the CFR.

The ratio of insect species found per plant species in the CFR is lower than in the tropics, and similar to other plant-poor temperate regions. This suggests a decrease in insect species richness with an increase in latitude, independent of plant species richness. The CFR is a global exception with its plant diversity being higher than expected from its latitude (Kreft & Jetz 2007). The insect diversity in the CFR, however, seems to be disproportionately lower than expected from the plant diversity (Giliomee 2003). Different factors may thus be driving the latitudinal decrease in plant and insect richness. The decrease in plant diversity with an increase in latitude has been attributed to climatic conditions, with the number of insect species per plant species remaining constant (Novotný *et al.* 2007). Locally, insect richness correlates with plant richness, but the regional insect species pool is smaller than expected from the plant species pool. The latitudinal differences in the number of insect species per plant species may be confounded by trees being sampled in the tropics and shrubs in the temperate zones. Alternately, the lower than expected insect species richness may result from low plant phylogenetic diversity in the CFR, where only 33 clades constitute 50% of flowering CFR plant species (Linder 2003). If many insects are specialised at the family or genera level, insects should be able to utilise multiple plant species within these clades but not between clades, resulting in lower than expected total insect species richness.

Due to the low abundances of insects in the CFR, it is likely that insect species will be missed if a large number of phylogenetically diverse plant species are sampled in low numbers. This likely explains the lack of saturation of accumulation curves in previous studies of insect diversity in the CFR (Pryke & Samways 2008; Procheş *et al.* 2009). Here, by sampling within a family and sampling high numbers of each plant species, accumulation curves tend toward saturation. This suggests that sampling exhaustively within a plant family, rather than within an area, might be a better approach to assess plant-insect diversity relationships in the CFR. The ratio of insect:plant species I found here is substantially higher than ratios from previous studies in the CFR where many plant families were sampled, and CFR insect richness may thus have been underestimated. Although many large (body length > 5 cm) insects were sampled here, vacuum sampling might favour the collection of small (body length < 5 cm) insects (Doxon *et al.* 2011). Ideally, both sweep-netting and vacuum sampling should be employed to avoid bias.

Conclusion

Insect richness in the CFR shows a positive association with plant richness. Some of the variance in the relationship is explained by filtering by extrinsic factors, such as altitude, post-fire vegetation age and temperature, yet plant richness remained the strongest predictor. The remaining variance might be explained by the role of plant species composition or different insect groups exhibiting different patterns. I find that the CFR has significantly lower insect diversity than expected from plant diversity, and this could either result from the CFR's low plant phylogenetic diversity (i.e. 50% of plant species restricted to 33 clades - Linder 2003), or plants and insects exhibiting different responses to latitudinal gradients.

Table 2.1 Insect-plant diversity relationship at various spatial sampling scales. While the relationship between insect and plant diversity components was always positive, it was only significant at the smaller sampling scales with the most statistical power, and plant diversity components explained a maximum of 11% of variance in insect diversity.

Scale	Insect species richness ~ Plant species richness		Insect species richness ~ Plant species PD		Insect species α diversity ~ Plant richness		Insect species α diversity ~ Plant species α diversity	
	R^2	p	R^2	p	R^2	p	R^2	p
	Plot (n=300)	0.076	<0.001	0.040	<0.001	0.052	<0.001	0.031
Square (n=60)	0.107	<0.001	0.052	0.043	0.046	0.055	0.049	<0.001
Site (n=30)	0.006	0.288	-0.020	0.522	0.084	0.065	0.066	0.092
Cluster (n=6)	0.035	0.338	0.103	0.278	0.047	0.429	0.085	0.292

Table 2.2 Results of GLM models with insect species richness (at the plot level) as the response variable. P-values of all predictors included in each model are shown and significant values after Bonferroni correction are highlighted. The last column depicts model fit (R^2). All effects on insect richness are positive, except altitude, annual temperature range and the maximum temperature of the warmest month, which were negatively related to insect species richness.

Insect richness dataset	Plant components				Environmental components							Model fit
	Plant richness	Structural diversity	Structural complexity	Number of plants in flower	Post-fire vegetation age	Altitude	Annual mean temp	Annual precipitation	Annual temp range	Max temp of warmest month	Min temp of coldest month	R^2
All	<0.001				<0.001	0.040		0.007		0.007		0.17
Autumn	0.056	<0.001		0.150	0.013	0.042		0.002			0.113	0.13
Spring	<0.001				0.007	<0.001		<0.001	0.006			0.30
Hemiptera	<0.001		0.031		0.014	<0.001		<0.001		0.010	0.132	0.21
Coleoptera	0.013	0.005	0.151			<0.001	0.061	0.002	0.140			0.10

Table 2.3 Number of plant species, insect species and the ratio between these for various studies in the tropics and CFR. The number of insect species per plant species is significantly higher in the tropics than the CFR.

Region	Number of plant species	Number of insect species	Insect spp:Plant spp ratio	Study
CFR	440	636	1.45	Procheş <i>et al.</i> 2009
CFR	55	320	5.82	Kemp et al 2014
CFR	105	216	2.06	Pryke & Samways 2008
Tropics	38	865	22.76	Novotny <i>et al.</i> 2012
Tropics	30	606	20.20	Leps <i>et al.</i> 2001
Tropics	30	1050	35.00	Novotny & Basset 2000

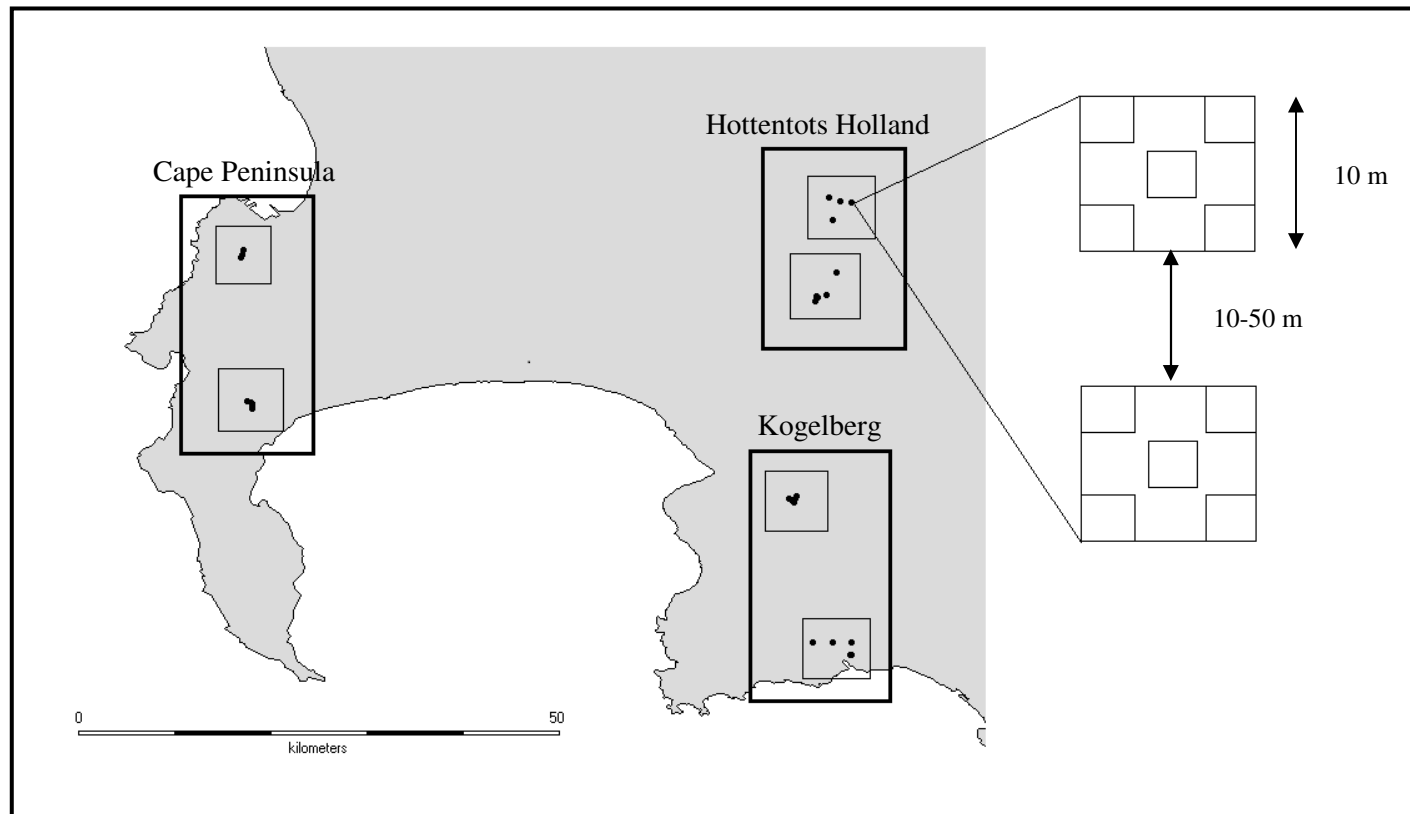


Figure 2.1 A spatially nested sampling design was employed. Thirty sites (represented by black circles) were sampled twice, once in each season of peak insect activity (autumn and spring). Groups of five sites were spatially aggregated to form six clusters (small grey squares). Two clusters were present in each of the three mountain blocks sampled (large rectangles). Sites consisted of two 10x10 m squares situated 10-50 m apart (insert on the right). Each square contained five 2.5x2.5 m plots (four corners and centre of the square). These plots were sampled both for Restionaceae plants and all insect herbivores present on Restionaceae plants.

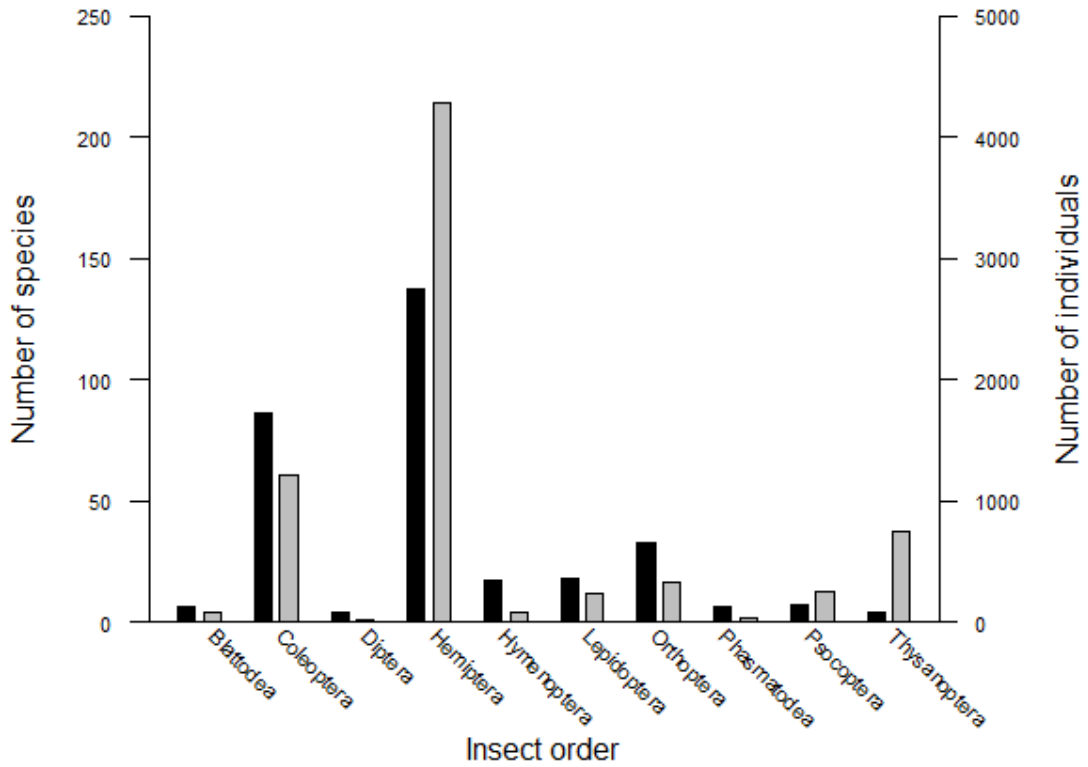


Figure 2.2 Total morphospecies richness (black bars) and abundance (grey bars) for each insect order captured during vacuum sampling surveys of Restionaceae communities across the Cape Floristic Region. Hemiptera dominated herbivore communities on restios. Only herbivorous insects are included.

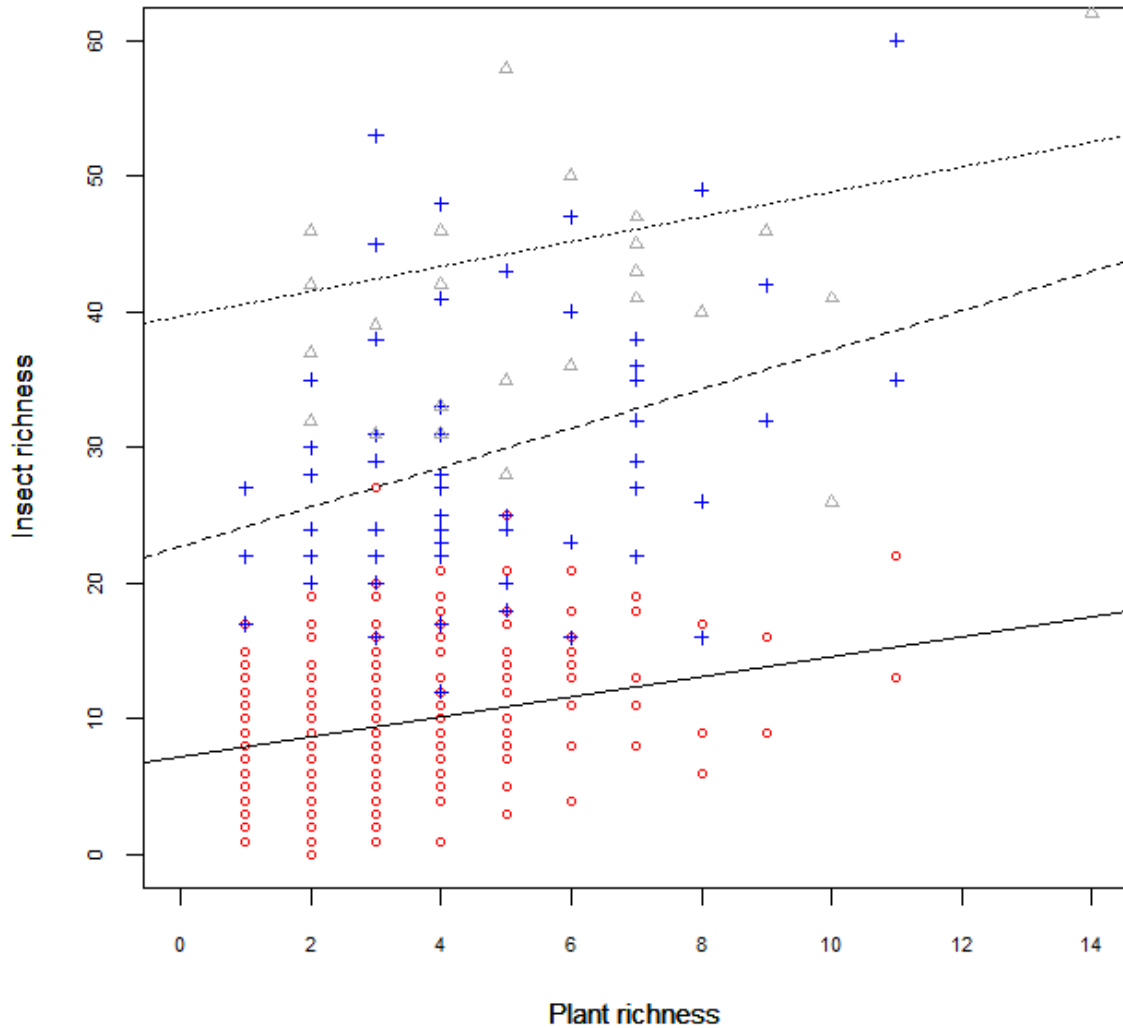


Figure 2.3 Relationship between plant and insect richness at various sampling scales. The plot scale is represented by a solid line and red circles, the square scale is represented by a dashed line and blue crosses, and the site scale is shown by a dotted line and grey triangles. The association is positive at the plot and square scales ($p < 0.001$), but not significant at the site scale.

Chapter 3

Beta diversity of herbivorous insects is coupled to high species and phylogenetic turnover of plant communities across short spatial scales in a biodiversity hotspot

Abstract: Understanding patterns of insect species turnover and specialisation are crucial for estimating global insect species richness. The majority of beta diversity studies focus on tropical systems and hyperdiverse temperate floras have received much less attention. Here I use the Restionaceae, a dominant family in the florally diverse Cape Floristic Region (CFR), and its associated herbivores to characterise the relationship between spatial turnover in plant and insect community composition by using a spatially nested sampling design. I found a positive relationship between insect species and plant (both species and phylogenetic) turnover at all spatial scales and plant communities predict the composition of insect communities, suggesting many insects are specialised and evolutionary transitions between plant hosts might be driving diversity. Plant communities unsurprisingly show significant turnover at small spatial scales (i.e. communities situated 0.1-3 km apart show significant turnover which may be tied to ecological niches). Insects show a similar pattern, but the decrease in community overlap is more gradual, suggesting many insects can utilise multiple (possibly closely related) hosts or are more dispersive than plants, while plants are tied to particular niches. Despite insect abundance and richness remaining relatively constant, seasonal turnover within communities is high and seasonal niche partitioning may be present. The positive association between plant and insect beta diversity at both local and regional scales suggests insect diversity patterns in the CFR are structured by insect host specificity.

Introduction

Despite the global dominance of arthropods (Zhang & Zhan 2011), accurate estimates of arthropod species richness remain elusive. In a seminal paper, Erwin (1982) estimated that global arthropod diversity exceeds 30 million species, under the assumptions that insect herbivores are host-specific and exhibit high levels of beta-diversity. More recent studies suggest a median estimate of 6.1 million arthropod species globally (Novotný *et al.* 2002; Hamilton *et al.* 2013). The uncertainty and variability in global diversity estimates to some extent reflects limited understanding of herbivorous arthropod specialisation and beta diversity, and how these patterns vary across the globe. Understanding the scale of insect compositional turnover and its drivers is thus critical for accurate estimates of global diversity.

Insect species richness can be directly associated with plant richness through speciation resulting from evolutionary transitions of specialist herbivores from one host to another (Winkler *et al.* 2009) and subsequently, species turnover in insect communities can show an association with plant species turnover. Pellissier *et al.* (2013b) recently provided the first evidence showing plant phylogenetic beta diversity structures butterfly phylogenetic beta diversity, and they suggest determining whether traits of importance are phylogenetically conserved can assist in identifying the mechanisms that structure communities. Another approach to assess the influence of plant composition on insect assemblages was developed by Ter Braak & Schaffers (2004), where ordination is used to relate two community data sets to test the influence of the predictor community on the response community. This method, co-correspondence analysis, has been employed to demonstrate that plant species community composition drives arthropod community composition in a semi-natural grassland and heathland (Schaffers *et al.* 2008). These findings indicate patterns of insect distribution mirror patterns of plant distribution, and this association can be explained by insect host specificity.

Alternately, the relationship between plant and insect richness could be indirect, with both groups responding in a similar manner to environmental gradients (Hawkins & Porter 2003). Although Pellissier *et al.* (2013b) showed patterns of plant and insect phylogenetic diversity mirror one another, an additional study (Pellissier *et al.* 2013a) showed insect phylogenetic beta diversity is strongly influenced by temperature. Environmental filtering thus also structures butterfly communities in the Swiss Alps. The relationship between plant and insect

phylogenetic beta diversity might not be a direct association and both groups could be showing high phylogenetic turnover across ecological gradients. Also, Novotny & Basset (2005) showed caterpillars utilising the same host plant species exhibit turnover with altitudinal gradients, highlighting the importance of factors other than host plant availability in determining the distribution of Lepidoptera.

A positive association between plant and insect beta diversity will be present at both local and regional scales if specialised insects are reliant on particular hosts. However, the association may also be structured by turnover in community composition of both groups across environmental gradients. If the relationship is only present at broader spatial scales, both groups may be exhibiting parallel responses along broad environmental or climatic gradients. Also, if broad environmental gradients are driving the changes in insect community composition, I expect a linear association between insect beta diversity and geographic distance. Further, if habitats are isolated and either group's dispersal is limited between habitats, I expect a high number of species confined to a habitat. Geographic barriers limiting dispersal between habitats could then promote allopatric speciation (MacArthur & Wilson 1967). Wright & Samways (1998) suggested insect diversity in the Cape Floristic Region to arise from both evolutionary host shifts and mountains acting as dispersal barriers.

Recent work in the tropics (Novotný & Weiblen 2005; Novotný *et al.* 2006, 2007, 2012) has shaped our understanding of the processes that drive patterns of turnover in herbivorous insects. Much less is known about patterns of insect diversity in floristically diverse temperate areas such as the Cape Floristic Region (CFR). The CFR is a biodiversity hotspot (Myers *et al.* 2000) with levels of plant diversity comparable to the tropics (Goldblatt 1997). Patterns of plant diversity and endemism have been well studied (Linder 1991; Cowling & Lombard 2002; Rouget *et al.* 2003; Verboom *et al.* 2009; Dupont *et al.* 2011), and plant richness in various groups is associated with environmental variables (Linder 1991). The exceptionally high plant diversity has been attributed to steep ecological gradients (Goldblatt & Manning 2000), biotic interactions (Johnson 2010) and relative climatic stability during the Pleistocene and Neogene (Linder *et al.* 2010). Although the association between plant and insect richness has received some attention in the CFR (Wright & Samways 1998; Procheş & Cowling 2006; Pryke & Samways 2008; Kuhlmann 2009; Procheş *et al.* 2009), less is known about the spatial patterns of insect turnover and the mechanisms structuring these patterns. Colville (2009) addressed this deficiency by modelling spatial monkey beetle community turnover and assessing the influence of environmental and plant community changes. Spatial

beetle turnover was strongly related to environmental factors and weakly influenced by plant variables. Studies assessing patterns and drivers of community turnover in other insects in the CFR are still lacking.

Here I aim to identify drivers of spatial changes in insect community composition in the CFR. I use the Restionaceae, one of the most diverse, abundant and well-studied plant families in the CFR, and its associated herbivores to determine the spatial structure and correlates of herbivorous insect species turnover. First, I ask what the temporal structure of insect species turnover is (i.e. do insect communities show seasonal turnover) and does this vary with sampling scale. Next, I evaluate the spatial structure of insect turnover (i.e. how different are communities separated by various distances) and I assess whether this pattern mirrors plant turnover patterns. The relationship between changes in insect community composition (beta diversity) and changes in plant community composition is evaluated, and I determine whether this is a direct association or one structured by parallel responses to environmental gradients.

Methods & Materials

Study system

The Cape Floristic Region (CFR) is a biodiversity hotspot situated in South Africa with approximately 9000 plant species occurring in less than 90 000 km² (Goldblatt & Manning 2000). The fynbos region is characterised by the combined presence of Proteaceae, Ericaceae and Restionaceae. The African Restionaceae (hereafter restios) originated approximately 91.5 million years ago and is one of the oldest clades in the CFR (Verboom *et al.* 2009). This wind-pollinated monophyletic clade of reed-like plants contains 350 species (Linder 2003); all of which are dioecious and some show dimorphism between male and female reproductive structures. Restios occur throughout the CFR in habitats that vary in soil type, altitude, groundwater availability, slope, aspect and climate. These plants are often associated with particular eco-hydrological niches (Araya *et al.* 2011) and thus often exhibit species turnover over small scales. Their high abundance in a variety of habitats and high compositional turnover make the Restionaceae ideal for assessing the plant-insect beta diversity relationship and its correlates in the CFR.

Insect diversity in the CFR has been suggested to be low (Johnson 1992; Giliomee 2003), but more recent studies have found high diversity in galling insects (Wright & Samways 1998)

and bees (Kuhlmann 2009). Augustyn *et al.* (2013) showed that some Cephalelini (Cicadellidae) species (the dominant herbivores on restios) track the phenophases of their host plants. The Cephalelini have been shown to be specialised on Restionaceae species (Davies 1988; Prendini 1997; Augustyn *et al.* 2013) and thus spatial turnover in restio species should lead to a turnover in Cephalelini species. If insects track the phenophases of their host plants, seasonal turnover is expected. Pryke & Samways (2008) showed insect abundance and richness remain the same between seasons, but insect community compositional turnover was not assessed. Colville (2009) showed CFR beetle diversity is correlated with both environmental and plant variables.

Sampling design

A spatially nested sampling approach was used. Thirty restio-dominated sites were selected for sampling (Appendix – table S2.1) on three of the major mountain blocks in the southwestern Cape, namely Hottentots-Holland, Kogelberg and the Cape Peninsula (50 to 70 km apart). Each mountain block contained two sets of five spatially aggregated sites (100 m to 3 km apart). I refer to these aggregations as ‘clusters’ and clusters within a mountain were located 15 to 20 km apart. Each site consisted of two 10x10 m squares located 10 to 50 m apart. Five 2.5x2.5 m plots were located within each square (the plots were situated in the corners and centre of each square). Each of the three mountain blocks thus consisted of two clusters, ten sites, twenty squares and a hundred plots. This allowed me to explore correspondence between changes in plant and herbivore community composition at various spatial scales, i.e. the plot, square, site, cluster, mountain and regional level. Sites with known restio species richness were chosen to allow me to sample sites which varied in plant species richness, altitude and vegetation age. The altitude and post-fire vegetation age was documented for each square. Altitudes of squares varied between 44 and 968m above sea level. The CFR burns regularly (every 10-15 years) and vegetation in squares ranged in age from 2 to 20 years. Groundwater availability was recorded for each site, where sites were categorised as well-drained, seepage or streambank. Plots were sampled twice, once during the suggested insect peak season (Pryke & Samways 2008) (i.e. spring: August-October 2013) and once six months before this (i.e. autumn: March-April 2013). These sampling periods coincide with the two distinct peaks in Cephalelini abundance (Augustyn *et al.* 2013). These will hereafter be referred to as autumn and spring respectively.

Insect sampling and diversity estimation

A modified leaf-blower (nozzle with 15 cm diameter) was used to sample insects from all Restionaceae plants occurring in each plot. All restios were exhaustively vacuum-sampled for approximately 20 seconds per plant and the nozzle was moved systematically up and down the culms. Insects were placed in 70% ethanol. Nwokwu & Sanderson (2009) found that using a modified leaf-blower captured more insects than sweep-netting or pitfall trapping, both in terms of richness and abundance. Also, search-sampling revealed extremely few insects were missed by vacuum sampling and no galling or mining insects were present, thus vacuum sampling was deemed as an efficient sampling approach in this system. Insects were identified to superfamily or family and then sorted to morphospecies. Oliver & Beattie (1996) showed morphospecies to be sufficient surrogates for species, especially in estimates of species richness and turnover. Insect families known to be non-herbivorous were excluded from the dataset. Insect families known to only feed on nectar of plants (absent in restios) were viewed as transient visitors and also excluded from the dataset.

The Cephalelini were identified to species by dissecting male genitalia and using the species descriptions formulated by Davies (1988) and Prendini (1997) and matching specimens to those in a museum collection (Stellenbosch University, Conservation Ecology and Entomology department). Females were matched to males using external morphology and museum specimens. The insect morphospecies collection is housed in the Botany and Zoology department at Stellenbosch University.

Individual-based accumulation curves were constructed for various sampling scales (Appendix – fig. S2.1-S2.2). Although accumulation curves built from plot samples (i.e. 2.5x2.5 m) tended towards saturation at the square (i.e. 10x10 m) scale, I opted to use squares as the lowest level of input to these analyses due to high variability in degree of saturation of accumulation curves across squares. Accumulation curves built from plot samples reached saturation at the regional scale and sampling effort was deemed sufficient.

Beta diversity was calculated using the package *vegetarian* in R (R Core Team 2013). The Horn similarity index was used as advised by Jost (2007) to be the only overlap measure that is not disproportionately biased towards rare or common species. This index is considered a true overlap measure that shows the proportion of species shared between samples. Beta diversity here thus refers to the overlap of effective species between effective compositional units (Tuomisto 2010). The Horn similarity index is defined as:

$${}^1D_{\beta} = (\ln 2 - H_{\beta\text{Shan}}) / \ln 2$$

where $H_{\beta\text{Shan}}$ is Shannon entropy based on Hill numbers and beta diversity is thus independent of alpha diversity (see Jost 2007). When the index approaches 1, compositional overlap between communities is high.

Insect species lists with abundances were created for squares, sites, clusters and mountains separately. Horn similarities were then calculated pairwise between all squares to create a similarity matrix. This was repeated for sites, clusters and mountains respectively to create a similarity matrix for each spatial scale. The function `sim.table` in the R package `vegetarian` was used for this. Additionally, geographic distance matrices were constructed at the square and site levels using the function `earth.dist` in the R package `fossil`.

The similarity of insect communities across seasons was calculated for each square, site, cluster, mountain and the entire region respectively. If insect species are present year-round but move around within the region, I expect seasonal overlap to be high regionally (mountain and region scales) and low locally (square and site scales).

Plant sampling and diversity estimation

The online interactive key of Linder (Linder 2002) was used to identify Restionaceae species occurring in each plot. Abundances were recorded for each plot as well as plant height. Each plant was then placed in a height category: 0-0.5m, 0.5-1m, 1-1.5m, 1.5-2m, >2m. The branching order of plants was recorded as unbranched, branched (each culm branched 1-3 times) or highly branched (each culm branched more than three times). Each plant was placed in a discrete structural group category based on a combination of its height and branching order (e.g. a 0.7m tall unbranched plant would fall in the “Unbranched_0.5-1m” bin).

Plant structural diversity was then calculated for each square in terms of Hill numbers of the Shannon diversity index. Plant structural complexity was calculated as an additional metric to quantify the structure of plants in a square. The structural diversity variable shows the number of different structural groups in a square (accounting for relative abundances), and a monospecific square with tall highly branched restios will thus score the same as a monospecific square of tall unbranched restios. However, tall or highly branched restios could possibly provide more feeding niches and support a higher diversity of herbivores, or provide more spaces to hide from predators. Structural complexity was calculated for each

square by assigning a value to each plant based on its branching order, where unbranched plants scored 1, branched plants scored 3 and highly branched plants scored 5. The branching score of each plant was multiplied by its actual height, and a total value for all plants in a square was then calculated as a metric of available culm space (i.e. structural complexity).

Horn similarity matrices were constructed for plant species and plant genera respectively for each spatial scale, in the same manner as described for insects. Further, similarity matrices were constructed for each respective spatial scale for plant phylogenetic beta diversity (PhyloSor Index - Bryant *et al.* 2008) using the Restionaceae phylogeny (Linder & Bouchenak-Khelladi, unpublished) with function *phylosor* in the package *picante* in R (R Core Team 2013). PhyloSor is a modification of the Sorensen similarity index which quantifies phylogenetic similarity of communities as the proportion of shared phylogenetic branch-lengths between two samples. If the length of shared branches is high, communities comprise phylogenetically closely related taxa.

Data analysis

Seasonal beta diversity

To investigate spatial patterns of seasonality in insect communities, seasonal turnover in insect species was compared between sampling scales using an ANOVA and a Tukey HSD post-hoc test. Horn similarity between seasonal samples was used as dependent variable and spatial scale (i.e. square, site, cluster, mountain) was used as the independent variable.

Spatial scale of turnover

To determine the spatial scale of turnover in morphospecies composition of insect communities, the square-level beta diversity matrix was partitioned into various independent spatial components. I thus calculated insect morphospecies turnover of (1) squares within sites (10 – 50 m scale), (2) squares between sites within clusters (0.1 – 3 km scale), (3) squares between clusters within mountains (15 – 20 km scale) and (4) squares between mountains (50 – 70 km scale). Non-parametric Kruskal-Wallis ANOVAs were conducted to test for differences in Horn similarity values at various spatial scales, followed by appropriate post-hoc tests. Data from both seasonal surveys were combined for these analyses. This allowed me to assess how different insect communities in squares are at various separation distances. The spatial component of turnover in plant species composition was investigated in an identical fashion. Next, Wilcoxon paired tests were used to assess whether turnover was

similar for plants and insects for each respective spatial scale. P-values were adjusted accordingly (Bonferroni corrections).

Insect beta diversity and its correlates

To test whether the change in insect species composition shows a linear relationship with geographical distance or with a change in plant species composition, Mantel tests were conducted in R (R Core Team 2013) using the square-level similarity matrices described previously. A linear association between geographic distance and changes in insect community composition could indicate insect communities are structured by broad abiotic gradients. The similarity matrix for insect morphospecies was correlated with beta diversity matrices of plant species, plant genera, plant phylogenetic overlap and geographic distance respectively. The influence of geographic distance on plant turnover was also assessed. These analyses were repeated for the site and cluster scales to determine whether relationships are present at higher spatial scales. The effect of geographic distance was not assessed at the cluster scale since distances between squares within clusters varied and the centre point of each cluster will not provide an accurate measure of distance between clusters.

The effects of environmental factors and plant structure on insect species turnover were assessed using permutational multivariate analysis of variance (PERMANOVA - Anderson 2001) with the *adonis* function in the *vegan* package in R (R Core Team 2013). This approach allowed me to assess whether association between insect and plant turnover is related to both groups responding in a similar manner to extrinsic factors. The insect beta diversity matrix (square-level) was used as dependent variable and the influence of post-fire vegetation age, altitude, plant structural diversity and plant structural complexity were tested as independent variables. The effects of post-fire vegetation age, altitude and groundwater availability were assessed for plant turnover.

Testing for a direct association between plant and insect community composition

The Mantel approach employed above merely provides an indication of an association between changes in plant and insect community composition, but it provides no indication of causality. Thus I also tested whether insect community composition can be predicted by plant community composition. Predictive co-correspondence analysis (predictive CoCA – Ter Braak & Schaffers 2004) was used to assess the influence of plant species composition, plant genera composition and plant structural group composition on various levels of insect

composition (see below). Co-correspondence analysis is an ordination method that relates two types of communities (e.g. plants and herbivores). It first identifies the number of significant ordination axes using a permutation approach and then uses a cross-validation test to assess the validity of the model. Values larger than zero validate the model (i.e. confirm that the composition of one community can be predicted from the composition of the other), and higher values indicate a better fit.

Insect composition was evaluated at six different levels: (1) insect order composition; (2) Hemiptera family composition; (3) Coleoptera family composition; (4) Cephalelini species composition; (5) Hemiptera morphospecies composition and (6) Coleoptera morphospecies composition. Hemiptera and Coleoptera were singled out due their dominance in terms of both species richness and abundance (Chapter 2). The Cephalelini were assessed separately due to their dominance as herbivores on restios and our knowledge of their life history. Data were $\log_{10}(y + 1)$ transformed to increase normality and analysed at the square level (n=60). The R package cocorresp was used for this. I could not use the entire morphospecies dataset in this analysis as co-correspondence analysis loses its predictive power when the number of axes included in the cross-validators fit model is more than the number of sites (Ter Braak & Schaffers 2004). Following Schaffers *et al.* (2008), it was decided to rather partition the dataset into meaningful groups.

Results

Plant and insect composition

A total of 322 herbivorous insect morphospecies were collected (7276 individuals), 221 insect species (3619 individuals) during the first sampling season (March-April 2013), and 195 herbivorous insects (3657 individuals) during the second sampling season (August-September 2013). Mean (\pm sd) Horn similarity was 0.23 (\pm 0.13) between squares, 0.28 (\pm 0.11) between sites, 0.48 (\pm 0.09) between clusters and 0.60 (\pm 0.03) between mountains. The Cape Peninsula had 38 unique insect morphospecies, the Hottentots-Holland mountain 64 unique species and Kogelberg 26 unique species.

A total of 55 Restionaceae species from 11 genera were sampled. Mean (\pm sd) Horn similarity was 0.09 (\pm 0.18) between squares, 0.09 (\pm 0.15) between sites, 0.24 (\pm 0.11) between clusters and 0.39 (\pm 0.06) between mountains.

Seasonal turnover in insect community composition

Insect morphospecies overlap between seasons was generally low at all sampling scales (${}^1D_\beta < 0.5$, fig. 3.1) suggesting substantial seasonal change in insect community composition. Although estimates of seasonal similarity were higher at the larger spatial scales (mountain: mean ${}^1D_\beta = 0.42 \pm 0.12$; cluster: mean ${}^1D_\beta = 0.42 \pm 0.17$; site: mean ${}^1D_\beta = 0.29 \pm 0.17$; square: mean ${}^1D_\beta = 0.25 \pm 0.17$) these differences were not significant ($F = 2.673$, $df = 3$, $p > 0.05$) (fig. 3.1).

Spatial scale of compositional turnover

Insect morphospecies overlap between 10x10 m sampling squares decreased significantly at each successive sampling scale (i.e. as the distance between sampling units increased) (KW chi-square = 209.46, $df = 3$, $p < 0.001$, fig. 3.2). This is not a linear decrease, and could thus not be identified using Mantel tests. The pattern was similar for plants (KW chi-squared = 84.32, $df = 3$, $p < 0.001$), except that community similarity did not decrease between the mountain and region levels (fig. 3.3).

For both insects and plants the largest decrease in compositional overlap between 10x10 m squares was evident between the site (i.e. 10-50 m apart) and the cluster (0.1-3 km apart) level. Plant communities showed significantly higher overlap than insects at the site level ($V = 120$, $p < 0.001$), but significantly lower overlap at all other levels ($p < 0.001$). Thus, even though plant and insect compositional overlap at different spatial scales show similar patterns, the decrease between sites and clusters is very sudden in plants and more gradual in insects.

Correlates of beta diversity

Mantel tests showed that neither insect ($r_{\text{square}} = -0.35$, $p_{\text{square}} = 1.00$; $r_{\text{site}} = -0.22$, $p_{\text{site}} = 1.00$) nor plant ($r_{\text{square}} = -0.21$, $p_{\text{square}} = 1.00$; $r_{\text{site}} = -0.19$, $p_{\text{site}} = 1.00$) beta diversity was linearly correlated with geographic distance between sampling units (table 3.1). Instead, insect morphospecies turnover at the lower sampling scales ($r_{\text{square}} = 0.34$, $p_{\text{square}} < 0.01$; $r_{\text{site}} = 0.22$, $p_{\text{site}} < 0.01$) was significantly correlated with turnover in plant species. The correlation between turnover in insect morphospecies and plant genera was significant at the square level ($r = 0.18$, $p < 0.01$), but not at the broader sampling units (site & cluster). Insect species turnover correlates significantly with plant phylogenetic turnover at all spatial scales ($r_{\text{square}} = 0.38$, $p_{\text{square}} < 0.01$; $r_{\text{site}} = 0.38$; $p_{\text{site}} < 0.01$; $r_{\text{cluster}} = 0.64$, $p_{\text{cluster}} < 0.03$).

In contrast to the significant association between plant and insect compositional turnover suggested by Mantel tests, insect compositional turnover was not significantly influenced by altitude, vegetation age or plant structure ($p > 0.05$ for all variables in PERMANOVA). Turnover in plants species composition was also not influenced by any of the parameters I assessed.

Predicting insect community composition from plant composition

The co-correspondence analysis showed plant species composition was a significant predictor of Coleoptera, Hemiptera and Cephalelini species composition, where plant species composition predicted 15% of Coleoptera community composition, 10% of Hemiptera community composition and 19% of Cephalelini community composition (table 3.2). Cephalelini species composition was also predicted well by plant structural group composition (24%). The composition of Hemiptera families is weakly predicted by plant genera composition.

Discussion

Results reveal that changes in the species composition of insect herbivore communities track changes in plant species community composition and plant phylogenetic turnover. This pattern did not result from parallel responses of plant and insect species to any of the environmental factors I measured. In addition I show that turnover in both plant and insect community composition does not occur gradually across the landscape, nor is it primarily associated with major geographical barriers (e.g. mountain ranges). Instead species turnover in insect communities tracks near complete turnover of plant communities over very short spatial scales (0.1-3 km) in the CFR. The positive association between insect and plant beta diversity at both local and regional scales suggests that insect diversity patterns in the CFR are strongly structured by insect host specialisation. While similarity of species composition of herbivore communities was significantly associated with plant species compositional similarity, phylogenetic similarity of plant communities was consistently the strongest correlate at all spatial scales.

These results align with other recent studies demonstrating that plant lineage turnover is a primary determinant of insect beta diversity along ecological gradients (Pellissier *et al.* 2013a, 2013b). It also supports the notion that insect herbivores tend to either be specialised

on plant hosts at a taxonomic level higher than the species level (Novotný *et al.* 2002), or that insects are selecting host plants on the basis of certain traits that tend to be phylogenetically conserved (Ødegaard 2006; Whitfeld *et al.* 2012). While little is known about host specialisation in the vast majority of CFR insect herbivores, studies on the Cephalelini leafhoppers, which these surveys reveal are the dominant herbivores on the Restionaceae, suggest strong host specialisation (Davies 1988; Prendini 1997; Augustyn *et al.* 2013). Augustyn *et al.* (2013) showed species level ecological specialisation of Cephalelini within local communities. However, subsequent phylogenetic work and regional host use surveys suggest that Cephalelini species are evolutionarily specialised at higher taxonomic levels (at the Restionaceae subtribe level or on lineages within subtribes) (Wiese 2014). Host specialisation is the likely mechanism underlying our ability to predict Cephalelini species composition from restio species composition using predictive co-correspondence analysis (Table 3.2). Restio species composition was also a significant predictor of both Coleoptera and Hemiptera species composition, suggesting that these groups might exhibit similar host specialisation patterns to the Cephalelini in the CFR.

Additionally, I did not find an effect of any other variables on insect community turnover and the PERMANOVAs further suggest that the turnover in both insect and plant communities are not responses to environmental gradients. However, the stepwise decrease in insect community overlap with an increase in spatial scale could indicate responses to broader environmental gradients or could point towards allopatric speciation (also supported by presence of unique insect species on each mountain range). Although these results support Pellissier *et al.* (2013b)'s findings, where patterns of Lepidoptera distribution mirror those of their host plants, a parallel study showed turnover in Lepidoptera communities to also be strongly influenced by altitudinal changes in temperature (Pellissier *et al.* 2013a), a pattern which is well established for insects (Novotný & Weiblen 2005). The absence of altitudinal effects on insect compositional turnover in this study is thus surprising, but this may simply be due to the absence of a linear association with insect turnover or the limited extent of altitudinal range sampled (44 - 968m above sea level). Wright & Samways (1998) also found no effect of elevation on gall-forming insect turnover in the CFR and they attribute this to sampling across a short altitudinal gradient (1100m). Post-fire vegetation age does not influence insect composition – insects will recolonise an area based on plant species composition and young vegetation may have fewer insect species or lower abundances, but not different species than expected. These findings coincide with Schaffers *et al.* (2008) who

found insect assemblages to be best predicted by plant species composition, and only weakly predicted by plant structure or abiotic factors. Using plant species as a predictor encompasses various other factors, such as plant structural diversity (Axmacher *et al.* 2009), secondary metabolites and nutrient availability (Jonas & Joern 2008), and I show here that plant structural composition also partly predicts insect species composition in the CFR. Only weak support for mechanisms other than specialisation is thus found for structuring these communities.

Similar to plant communities, insect communities show turnover at small spatial scales (i.e. 0.1-3 km), and this could promote allopatric speciation. The small spatial scale at which plants show turnover is not surprising; Restionaceae species are often tied to micro-hydrological and ecological niches (Araya *et al.* 2011). However, the spatial patterns of insect and plant turnover do not match exactly: the change in insect turnover is more gradual than the sudden change in plant species turnover. The low abundances of insects may explain the lower overlap of insects compared to plants at the site level, where new insect species are more likely to be encountered between squares within sites than new plants species. Also, insects may be less dispersal limited than plants or less tightly tied to their niches than plants (i.e. insects can survive on multiple hosts or in various environmental conditions). As discussed previously, insects should be able to utilise phylogenetically closely related hosts. Novotny *et al.* (2007) showed herbivorous insect communities in tropical lowland rainforests are not structured by dispersal limitation, and even host specific caterpillars are able to utilise multiple closely related hosts. However, in contrast to my findings, Novotny *et al.* (2007) found low beta diversity of herbivorous insects, but this might be associated with plants also showing low turnover or the herbivores studied. Colville (2009) also demonstrated that turnover between CFR monkey beetle communities is not explained by geographic distance, but rather by plant variables and environmental factors.

Less than half of insect species are present in both seasons and seasonal niche partitioning might be present. A trend of increase in seasonal overlap between communities with an increase in spatial scale could indicate geographic movement of insects between seasons or seasonal host switching. Jonas & Joern (2008) showed mixed-feeding grasshoppers can selectively feed on structurally and chemically diverse plants to balance their nutrient needs, and CFR insects may thus be switching hosts or moving around to obtain adequate nutrients. However, Augustyn *et al.* (2013) did not find this for the dominant Cephalelini. Species within this group do not switch hosts seasonally, but rather occur only in one season and

remain specialised onto a particular host or group of hosts. This trend can alternately be caused by the increasing likelihood of rare species being encountered with an increase in sampling scale, or certain species that show seasonality having some individuals that survive to endure another season in low abundances.

Turnover in plants species composition was also not influenced by any of the parameters I assessed. However, this is probably due to these parameters being too coarse to assess fine-scale changes driving plant species turnover. Sites of both high and low diversity were deliberately selected at various altitudes and post-fire vegetation ages which could also mask the effects of these variables on plant diversity and composition. This approach, however, allowed me to decouple the effects of altitude and post-fire vegetation age on insect composition from the effects of plant composition. My main interest lay in assessing drivers of insect community composition rather than that of plants, which have been studied extensively (Linder 1991).

Conclusion

Insect communities associated with Restionaceae can be predicted by plant phylogenetic and plant species distributions, indicating many insects are host specific and evolutionary transitions between plant hosts might be driving diversity. Both plants and insects show turnover across small spatial scales, and allopatric speciation might thus influence insect diversification. High numbers of insects confined to each mountain also suggest allopatric speciation may partly be driving insect diversity in the CFR. Seasonal turnover in insect assemblages is high and some temporal niche partitioning could be present.

Table 3.1 R statistics and p-values from Mantel tests correlating beta diversity (Horn similarity) of insects (or plants) and various predictors at the square, site and cluster spatial scales. Plant phylogenetic beta diversity is positively correlated with insect species beta diversity at all spatial scales. No linear association between insect or plant beta diversity and geographic distance is present.

		Square (n=60)		Site (n=30)		Cluster (n=6)	
		r	p	r	p	r	p
Herbivore species	Plant species	0.341	0.001	0.219	0.001	-0.471	0.963
Herbivore species	Plant genera	0.183	0.001	0.076	0.141	0.038	0.423
Herbivore species	Plant phylogenetic	0.379	0.001	0.383	0.001	0.637	0.025
Herbivore species	Geographic distance	-0.348	1.000	-0.219	1.000	N.A.	N.A.
Plant species	Geographic distance	-0.206	1.000	-0.118	0.984	N.A.	N.A.

Table 3.2 Results from co-correspondence analysis of plant and insect composition. The number of significant ordination axes and cross-validated fit values (i.e. % of insect composition predicted by plant composition) are indicated. Cross-validated fit values larger than zero validate the model, and higher values indicate a better model fit. Values smaller than zero are indicated here as “not validated”. If no significant axes were identified, the cross-validated fit was not calculated.

Insect level	Plant level	Nr. sign. axes	Cross-val. fit
Orders	Plant species	1	not validated
Orders	Plant genera	1	not validated
Orders	Plant structural groups	2	9.931
Hemiptera families	Plant species	0	N.A.
Hemiptera families	Plant genera	1	1.208
Hemiptera families	Plant structural groups	1	not validated
Coleoptera families	Plant species	0	N.A.
Coleoptera families	Plant genera	0	N.A.
Coleoptera families	Plant structural groups	0	N.A.
Cephalelini	Plant species	2	18.883
Cephalelini	Plant genera	1	not validated
Cephalelini	Plant structural groups	4	23.684
Hemiptera species	Plant species	3	10.115
Hemiptera species	Plant genera	2	not validated
Hemiptera species	Plant structural groups	3	1.385
Coleoptera species	Plant species	2	15.077
Coleoptera species	Plant genera	2	0.286
Coleoptera species	Plant structural groups	3	3.534

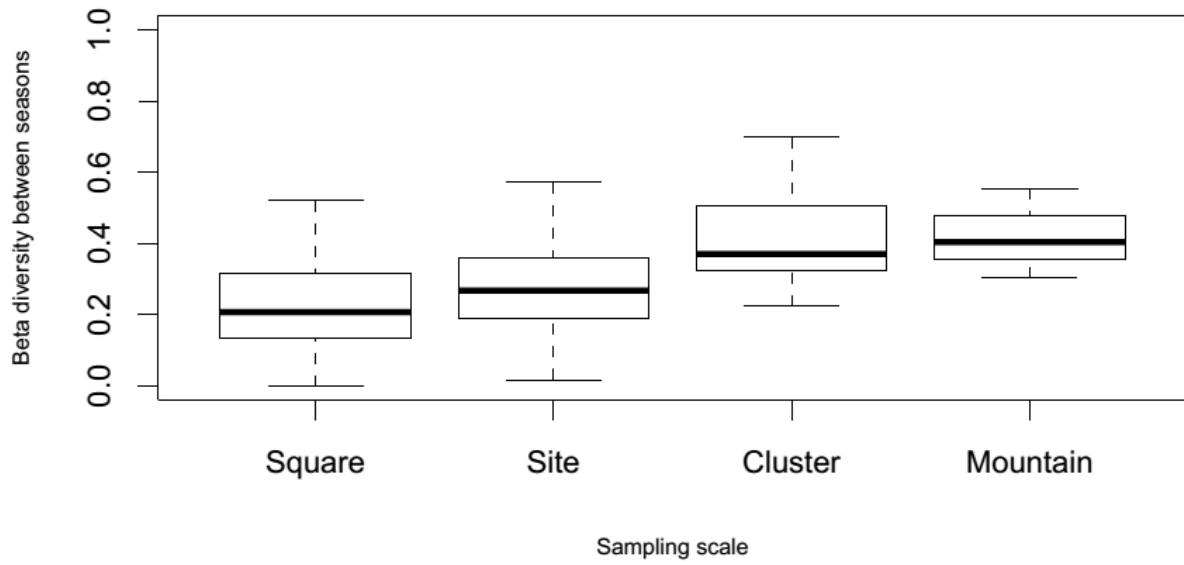


Figure 3.1 Low overlap (Horn similarity) of insect species composition between seasons at various sampling scales. When the index approaches 1, overlap between communities is high. Whiskers depict minimum and maximum values, and boxes show medians and quartiles. No significant differences are present.

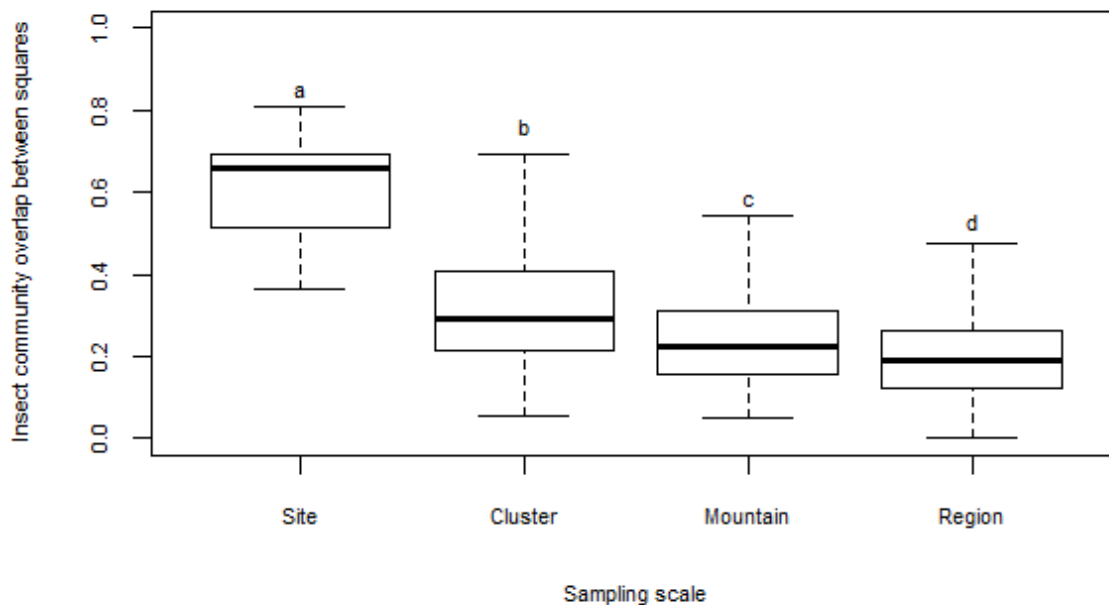


Figure 3.2 Insect morphospecies overlap (Horn similarity) between sampling units (10X10 m squares) at different spatial scales. When the index approaches 1, overlap between communities is high. The site scale indicates turnover between squares at a site (i.e. separated by 10-50 m), the cluster scale indicates turnover between squares across sites within a cluster (0.1-3 km apart), the mountain scale indicates turnover between squares across clusters within a mountain (15-20 km apart) and the region scale indicates turnover between squares across mountains within the entire region (50-70 km apart). Whiskers depict minimum and maximum values, and boxes show medians and quartiles.

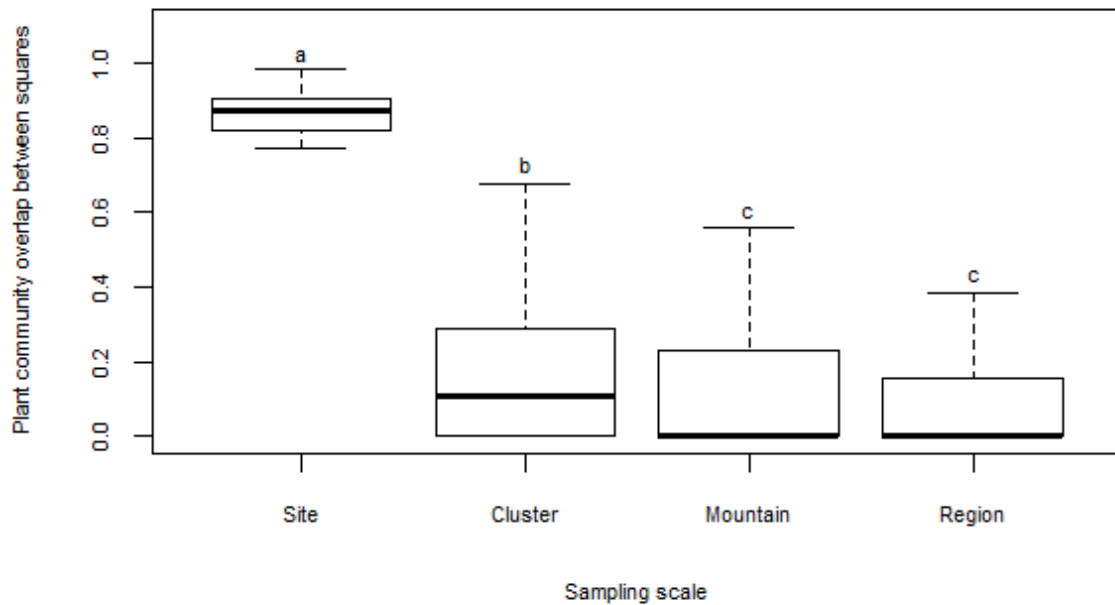


Figure 3.3 Plant species overlap (Horn similarity) between sampling units (10X10 m squares) at different spatial scales. When the index approaches 1, overlap between communities is high. The site scale indicates turnover between squares at a site (i.e. separated by 10-50 m), the cluster scale indicates turnover between squares across sites within a cluster (0.1-3 km apart), the mountain scale indicates turnover between squares across clusters within a mountain (15-20 km apart) and the region scale indicates turnover between squares across mountains within the entire region (50-70 km apart). Whiskers depict minimum and maximum values, and boxes show medians and quartiles.

Chapter 4

Invariant antagonistic network structure despite high spatial and temporal turnover of species and their interactions in a biodiversity hotspot

Abstract: Recent work has suggested emergent network structure exhibits very little spatial or temporal variance despite changes in community composition. The changes in interactions associated with changes in community composition have not been assessed. Here I sample multiple antagonistic (plant-herbivore) networks within a plant family and within a region (Cape Floristic Region) to eliminate possible spatial or phylogenetic effects that, in large-scale studies, may obscure mechanisms that structure networks and interactions. I find invariant spatial and temporal emergent network structure despite changes in insect and plant community composition. In contrast, interactions show high turnover, driven seasonally by turnover in insect species and insect host switching. Spatially the turnover in interactions is driven by simultaneous turnover in both plant and insect species, either suggesting that insects are host specific or that both groups exhibit parallel responses to environmental gradients. Spatial interaction turnover is also driven by a turnover in plants, showing that many insects can utilise multiple (possibly closely related) hosts and have wider distribution ranges than some of their host plants. Insects are preferentially selecting favourable plants, and these choices do not seem to be related to plant phenophase or plant abundance. I thus show highly variable internal network structure with changes in community composition, despite invariant emergent network structure.

Introduction

Several recent studies have suggested that the structure of both mutualistic and antagonistic networks is relatively invariant spatially and temporally (Lewis *et al.* 2002; Kaartinen & Roslin 2012; Rasmussen *et al.* 2013). In a meta-analysis, Morris *et al.* (2014) showed emergent antagonistic network structure does not vary consistently across latitudinal gradients and Kaartinen & Roslin (2012) showed antagonistic network structure remains constant between years. The lack of differences in network structure is somewhat surprising given the ubiquitous spatial and temporal variation in community species composition revealed by beta diversity studies (Novotný & Weiblen 2005; Novotný *et al.* 2007; Pellissier *et al.* 2013a, 2013b).

Network structure has been suggested to arise because interactions occur randomly in proportion to relative species abundances (neutrality hypothesis - Vázquez *et al.* 2009; Canard *et al.* 2014), or alternatively because interactions are structured through trait mismatches and subsequent interaction constraints (biological constraint hypothesis - Jordano *et al.* 2003; Santamaría & Rodríguez-Gironés 2007). Recent work shows that a combination of these hypotheses successfully predicts network metrics, but cannot accurately predict the interactions or interaction frequencies (Vázquez *et al.* 2009; Olito & Fox 2014). Network structure, but not interactions, can thus be predicted, and this may contribute to emergent network structure being invariant despite changes in species composition in space and time.

While evidence suggests invariant network structure, very little is known about how compositional changes affect turnover in interactions of persistent species across communities. Novotny (2009) suggested that the beta diversity of interactions can be partitioned into different components, providing insight into why interactions change. Using this approach, Novotny (2009) found high spatial turnover of interactions in tropical food webs driven by high plant species turnover. This method provides insight, beyond that gained from standard network metrics, into the mechanisms underlying network stability or lack thereof. For example, in temperate plant-herbivore networks, we might expect strong seasonal changes in interactions (and perhaps network structure) driven by seasonal niche partitioning and/or host switching. If seasonal niche partitioning is present, beta diversity should be largely driven by insect species turnover, while under host switching we would expect seasonal changes in the links formed by individual species. However, even if

interactions and species show turnover, the overall structure of the network may still remain similar between seasons.

If species composition and interactions change spatially and temporally, we would also expect a change in topologically important species in networks (i.e. species with the most interactions). Network structure is strongly affected by topologically important species and identifying them provides insight into the association between individual species and network properties (Jordán *et al.* 2008; Sazima *et al.* 2010). By conserving the most important species, the network structure is most likely to be retained, preventing collapse under disturbance or species loss (Dunne *et al.* 2002a). Studies aiming to identify topologically important species usually do not take temporal fluctuation in species communities into account. These fluctuations could, for example, lead to different plants being the dominant food resource at different times if insects are selecting plants based on phenophase and nutrient availability. Thus all plant species that act as important resource species through time should be identified and conserved. An alternative approach to identifying important species is to simulate consecutive extinctions of hosts and subsequently determine how robust a network is to compositional change (Evans *et al.* 2013).

To our knowledge no studies have simultaneously assessed spatial and temporal variation in antagonistic networks in relation to compositional change, both in terms of emergent network properties or interaction structure. Here I do this for a set of plant-herbivore communities within a single biodiverse biome, the Cape Floristic Region in South Africa. Limiting the spatial extent of our sampling allows me to ask whether the invariant network structure suggested by larger scale studies (Dupont *et al.* 2009; Morris *et al.* 2014) holds at small spatial scales, as mechanisms structuring networks might be obscured when assessing differences between phylogenetically divergent communities across the globe.

Because of the onerous sampling required, interaction networks are not often constructed for hyperdiverse regions where considerable turnover in species and interactions over short spatial scales is likely. South Africa's Cape Floristic Region (CFR), a temperate biodiversity hotspot containing more than 9000 plant species in 90 000 km² area (i.e. diversity levels approaching those of the tropics) (Goldblatt & Manning 2000), is no exception. For practical reasons I focus on a single dominant CFR plant family (the Restionaceae, hereafter restios) and its associated insect herbivores. It has recently been shown that species composition of restio communities (which varies spatially) is a significant determinant of the composition of

associated insect herbivore communities (Chapter 3), which suggests some level of insect specialisation. In addition, insect species composition varies seasonally on restios (Chapter 3). This could result from specialised insect species tracking the seasonal phenology of their hosts (as shown by Augustyn *et al.* (2013) for endemic restio-leafhoppers, the Cephalelini). It is also possible that insects that are present throughout the year may switch hosts which may lead to a turnover in interactions, rather than a turnover in species.

From previous work (Chapter 3), I expect high seasonal and spatial changes in plant and insect community composition, and I investigate whether these compositional changes are associated with changes in network metrics. I also examine the turnover of interactions across seasons and between sites within seasons. I expect a turnover of interactions driven (1) temporally by a turnover in insect species and (2) spatially by a turnover in plants. Further, I ascertain whether topologically important plant species remain the same between seasons and predict that these should vary across seasons with a variation in plant phenophase. Since these are the first host-herbivore interaction networks constructed for the CFR, I also assess how robust the system is to plant species loss.

Methods & Materials

Study system

One of the dominant families in the CFR, the African Restionaceae, is a wind-pollinated monophyletic clade of reed-like plants that contains 350 species (Linder 2003). A multitude of growth forms exist within the Restionaceae, where variation can be found in height, culm diameter and branching of the culms. Restios occur throughout the CFR in habitats that vary in soil type, altitude, groundwater availability, slope, aspect and climate. All plants are perennial and thus the same plants are present for both seasons at each site.

Contrasting results have suggested insect diversity in the CFR to be low (Johnson 1992; Giliomee 2003) or high (Price *et al.* 1998; Wright & Samways 1998). The low soil nutrients (leading to low plant nutrients) could favour generalism in herbivorous insects or insects may switch seasonally between plant species to optimise nutrient intake (see Augustyn *et al.* 2013). Alternately, insects may be specialised on a plant species and only be present in the community when nutrient uptake from that plant species is optimal.

Sampling design

Six sites with diverse restio communities were selected in the south western CFR (table 4.1). These were chosen based on their high levels of restio diversity and similar post-fire vegetation age. All of the sites are approximately 300x300m in size.

Insect sampling

Insects were collected from 80 equally sized individual plants of each Restionaceae species occurring at each site using a modified leaf-blower with a 15 cm diameter nozzle and placed in 70% ethanol. All of these restios were exhaustively vacuum-sampled for approximately 20 seconds per plant and the nozzle was moved systematically up and down the culms. Restios were search-sampled for insects after vacuum sampling to assess the efficiency of vacuum sampling and also to see whether galling/mining insects were present. Extremely few insects were found by search-sampling and no galling or mining insects were present, thus vacuum sampling was deemed as an efficient sampling approach in this system. Nwoku & Sanderson (2009) found that using a modified leaf-blower captured more insects than sweep-netting or pitfall trapping, both in terms of richness and abundance, in dense and open habitats. Sites were sampled twice, once during the suggested insect peak season (Pryke & Samways 2008) (i.e. spring: August-October 2013) and once six months before this (i.e. autumn: March-April 2013). These sampling periods coincide with the two distinct peaks in Cephalelini abundance (Augustyn *et al.* 2013). Insects were identified to superfamily or family and then sorted in to morphospecies. Oliver & Beattie (1996) showed morphospecies to be sufficient surrogates for species. Insect families known to be non-herbivorous were excluded from the dataset. Insect families known to only feed on nectar of plants (absent in restios) were viewed as visitors and also excluded from the dataset.

The Cephalelini were identified to species by dissecting male genitalia and using the species descriptions formulated by Davies (1988) and Prendini (1997) and matching specimens to those in a museum collection (Stellenbosch University, Conservation Ecology and Entomology department). Females were matched to males using external morphology and museum specimens. The insect morphospecies collection is housed in the Botany and Zoology department at Stellenbosch University.

Plant sampling

Restionaceae occurring at each site were identified using the online interactive key of Linder (2002). The relative abundance of each plant species was determined for each site by recording the occurrence of plant species along six to ten 100m transects.

Data analysis

Network construction

Insect abundances were weighted by the relative abundance of each restio species at each site prior to network construction. Quantitative plant-herbivore interaction matrices were constructed for each season for each site. Matrix columns represented herbivore species, rows represent plant species and cells represent interaction frequency (number of individuals sampled of each insect morphospecies on each plant species).

Comparison of network structure and species composition between sites and seasons

Standard network metrics (weighted NODF, connectance, vulnerability, generality, linkage density, H_2' , modularity) used frequently in cross-network comparisons (Tylianakis *et al.* 2007; Kaartinen & Roslin 2012; Morris *et al.* 2014) were calculated for each season for each site to quantify emergent network structure

Weighted NODF (nestedness based on decreasing fill) is a nestedness metric that corrects for matrix fill and dimension (Almeido-Neto *et al.* 2008). A nested network consists of the most specialist organisms of one group interacting with the most generalist of the other group. Linkage density is the weighted diversity of interactions per species. Connectance is the realised proportion of possible interactions (Dunne *et al.* 2002a). H_2' measures network-wide specialisation and niche partitioning, where 0 represents no specialisation and 1 translates into full specialisation. Generality is the weighted mean number of lower level species (plants) connected to higher level species (insects). Vulnerability is the reverse of this (weighted mean number of higher level species connected to lower level species). This represents the diversity of partners each species interacts with. The nestedness values were compared to that of 99 randomisations using the `permatfull` function in the package `vegan` in R (R Core Team 2013) with no constraints on rows or columns to assess whether networks are significantly more nested than expected from their size and connectedness. Weighted modularity (the degree to which a network can be divided into modules, where more

interactions are present within a module than between modules) was determined for each network using the QuaBiMod algorithm created by Dormann & Strauss (2014) using the computeModules function in the package bipartite in R. Significance of the identified modules was determined by computing z-scores from null models (Dormann & Strauss 2014).

Spatial and seasonal differences of metrics (weighted NODF, connectance, vulnerability, generality, linkage density, H2', modularity) were simultaneously assessed with two-way analysis of variance (ANOVA) and subsequent Tukey HSD post-hoc test for each metric separately with sites and seasons as predictor variables. Due to only having two replicates for each site and the subsequent low statistical power, permutational multivariate analysis of variance (PERMANOVA - Anderson 2001) was implemented with the adonis function in the vegan package in R to assess the association between network metrics and geographic distance to ascertain whether network metrics change spatially. Next, seasonal changes in the network metrics were evaluated separately using paired t-tests for each metric and treating sites as replicates. Tests were performed using R (R Core Team 2013).

To assess overlap of insect species composition between these communities, the Horn similarity index was used as recommended by Jost (2007) to construct similarity matrices. Community overlap was first calculated for sites within each respective season and then pairwise for each site between seasons.

Next, PERMANOVA was implemented to assess the influence of network metrics on spatial insect species turnover. This approach uses a permutation test to partition distance matrices among sources of variation and fits linear models, and allowed me to assess whether changes in network metrics are related to changes in insect community composition. The Horn similarity matrix of species overlap between sites (described above) was used as response variable and the network metrics were used as predictor variables. The analysis was performed for each season separately.

Interaction turnover between networks

To determine whether interactions and internal network structure remain constant spatially and temporally, I quantified interaction turnover. Beta diversity of interactions was calculated spatially within seasons (pairwise between sites) and pairwise for each site between seasons.

Following Novotny (2009), interaction diversity was partitioned into the components that drive changes in interactions:

B_p = a turnover of interaction due to plant species being absent from one network;

B_h = a turnover of interaction due to herbivore species being absent from one network;

B_{ph} = a turnover of interaction due to both herbivore and plant species being absent from one network;

B_0 = both plant and herbivore species are present in both networks, but an interaction is only present in one.

The total Jaccard turnover of interactions (B_{cc}) was also calculated. Values range between 0 and 1, where high values indicate high turnover.

I expect seasonal interaction turnover to be attributed to B_h if insect communities exhibit high seasonal turnover and possible seasonal niche partitioning. Seasonal interaction turnover will be attributed to B_0 if insects are switching hosts between seasons. B_p and B_{ph} will be zero since plants persist seasonally.

I hypothesise that most of the spatial turnover will be partitioned to B_p if plants are tightly tied to environmental niches or dispersal limited. It also indicates insect distribution is not tightly tied to plant distribution and insects can utilise multiple hosts (herbivores are present in the community despite the absence of the plant species they interacted with in a previous community). B_h will constitute most of the partitioned turnover when herbivore assemblages exhibit dispersal limitation or are filtered by certain environmental conditions, such as temperature, precipitation or wind speeds. Herbivores are thus absent from the community despite the presence of potential host plants. B_{ph} will constitute most of the partitioned turnover when herbivores track the distribution of plants and insect niche space is determined by host plants, or when both groups exhibit parallel responses to environmental gradients. B_0 should be high spatially when insects have wide host-use breadth and the quality of hosts varies in space.

Each component's relationship with geographic distance was tested with linear models. A relationship between B_{ph} and geographic distance should occur when pairs of plant and insect distributions correspond (through parallel responses to environmental conditions or through specialisation) and are limited (either by dispersal or adaptation). An increase in B_h with

geographic distance would be expected when insects have narrower ranges than their plant hosts, and factors influencing insect ranges change gradually. An increase in B_p with geographic distance would be expected when plants have narrow ranges and insects can utilise multiple (possibly closely related) hosts. Factors limiting plant ranges should change gradually across the landscape.

Comparison of topologically important species between networks

The topological importance of plant species was determined using unweighted degree, where degree is a measure of the number of links a species has. Weighted degree is based on the number of individuals a species interacts with, whereas unweighted degree quantifies the diversity of interactions without bias towards rare or common species and thus indicates the number of species using a plant as host. Since this is an antagonistic system, I was only interested in identifying the plant species that support the most insect species. To determine whether the plant species with the highest diversity of interactions is the most abundant plant species at each site, plant unweighted degree was determined for each site for each season and regressed onto relative plant abundance. This allowed me to evaluate if insects are utilising the most abundant plant species or insects are preferentially exploiting certain plants. Next, the two plant species with the highest unweighted degree were identified for each network to determine whether topologically important plants vary between seasons at sites.

Robustness

I modelled the robustness (the tolerance of the network to species extinctions - Dunne *et al.* 2002b) of each of the insect species in the entire region (networks of all sites combined) and for the two separate seasons (combination of sites for autumn and spring separately). Three robustness analyses were conducted by simulating plant extinctions in three ways: (1) plant species were randomly removed from the network and insects became extinct when all plants they occur on were removed (simulating random plant extinctions), (2) plant species with the highest degree were removed first followed by sequentially lower degrees (simulating the most extreme scenario for insect extinction) and (3) plants with lowest abundances were removed first followed by sequentially higher plant abundances (simulating the most likely situation involving extinction of the rarest plant species first). Analyses were conducted in R (R Core Team 2013) with the bipartite package. Robustness values were compared with a two-way analysis of variance (ANOVA) to assess the differences in robustness between datasets (i.e. autumn, spring, combination of seasons) and extinction methods.

Results

Plant and insect community composition and turnover

In total 8923 individual insects (3885 individuals in autumn and 5038 in spring) were sampled from 39 plant species. The number of plant species per site ranged from 6 to 10. I identified 263 insect morphospecies from 12 orders and 12 Cephalelini species.

Mean insect species overlap (Horn similarity) between sites in autumn was 0.32 (sd = 0.087) and in spring 0.37 (sd = 0.167). Mean pairwise insect species overlap for sites between seasons was 0.37 (sd = 0.173). However, insect overlap between seasons for the entire region was higher at 0.50. Insects may thus still be present in the region even if they are locally absent or rare species are more likely to be encountered with increased sampling effort. Both temporal and spatial turnover in insect species community composition is thus high (Appendix - tables S4.1 & S4.2).

Network metrics

Connectance was low for all networks with only 20-35% of possible interactions being realised. This might be associated with the high levels of specialisation (mean $H2' = 0.63$). All networks, except one, were more nested than expected from network size and connectivity. Five of the six sites were significantly modular in autumn and three sites in spring. During autumn, three of the significantly modular sites consisted of two modules with one of these comprising a single plant species. This trend was not observed during spring. The change in modularity and species composition within modules is the only difference observed between networks spatially and temporally.

Comparison of metrics between sites and seasons

The two-way ANOVA and Tukey HSD showed that only two sites (SB & MR) differed spatially from one another in linkage density (df = 5, $F = 6.53$, $p = 0.03$) and vulnerability (df = 5, $F = 5.96$, $p = 0.04$). Limited spatial differences in network structure are thus present, but no seasonal differences (Appendix - fig. S4.1-S4.6).

Similarly, no association between changes in geographic distance and changes in network metrics were found (PERMANOVA, df = 6, $p = 1.00$).

Also, paired t-tests comparing seasonal networks revealed no significant differences between seasons for connectance ($df = 5$, $t = -0.89$, $p = 0.42$), $H2'$ ($df = 5$, $t = 1.04$, $p = 0.35$), linkage density ($df = 5$, $t = -0.97$, $p = 0.38$), vulnerability ($df = 5$, $t = -0.73$, $p = 0.50$), generality ($df = 5$, $t = -0.87$, $p = 0.42$), modularity ($df = 5$, $t = -1.85$, $p = 0.12$) and weighted NODF ($df = 5$, $t = -0.89$, $p = 0.42$) (fig. 4.1). Network structure thus remains constant for each site between seasons.

None of the network metrics were associated with changes in insect species turnover for either season (PERMANOVA, $df = 6$, $p > 0.05$). This is not surprising since no significant differences were found between sites for any network metrics.

Interaction turnover

Interaction turnover between seasons was high (mean $B_{cc} = 0.90$, $sd = 0.04$) and was mostly driven by a turnover in herbivores (mean $B_h = 0.63$, $sd = 0.10$). A large proportion of herbivores also switched hosts seasonally (mean $B_o = 0.27$, $sd = 0.08$). Spatially, turnover in interactions within each season was driven by turnover of plants (mean $B_p = 0.37$, $sd = 0.13$) and turnover in both plants and insects (mean $B_{ph} = 0.39$, $sd = 0.13$) (fig. 4.2).

Geographic distance shows a positive linear relationship with total interaction turnover ($R^2 = 0.19$, $p = 0.009$) and with simultaneous plant and herbivore turnover ($R^2 = 0.28$, $p = 0.002$). Geographic distance is negatively related to herbivore turnover ($R^2 = 0.16$, $p = 0.015$) and also negatively related to interaction turnover with no species turnover ($R^2 = 0.29$, $p = 0.001$).

Comparison of topologically important species between networks

Unweighted degree did not show a linear association with plant abundance ($df = 94$, $F = 1.218$, $p = 0.27$). Insects thus do not seem to merely make use of the most abundant species, but preferentially select particular plant species at a site to feed on.

For all except one site (KM), at least one of the two topologically most important species remained constant between seasons (table 4.2). For three sites (VD, RV, PB) both of the topologically most important species remained the same between seasons.

Robustness

Robustness values for the combination of the seasons are significantly higher than for autumn ($p = 0.002$) and spring separately ($p = 0.001$). When plants with the highest degree are

eliminated from the community first, robustness is significantly lower than either random ($p = 0.006$) or abundance-based ($p = 0.002$) extinctions (Appendix - fig. S4.7-S4.9). The scenario where plants are removed randomly did not differ significantly from abundance-based extinctions ($p = 0.129$).

Discussion

This study reveals spatially and temporally invariant emergent network structure and suggests that changes in insect and plant community composition are not associated with changes in various network metrics. In contrast, I detect high spatial and seasonal interaction turnover despite constant emergent network structure. Interaction turnover is driven seasonally by a turnover in herbivores and herbivore host switching. Spatially, interaction turnover is driven by a turnover in plants and simultaneous plant and herbivore turnover, suggesting host specificity might structure network assembly. Also, plants with the largest variety of interactions at each site are not the most abundant plants. Insects are thus preferentially selecting favourable plants based on specialisation, predator avoidance or food source quality, causing some plants with low abundances to have many interaction partners.

Restio-herbivore network structure remains constant both spatially and temporally, despite a high turnover in insect and plant species composition. These results align with recent studies of both pollination (Dupont *et al.* 2009) and host-parasitoid (Lewis *et al.* 2002; Kaartinen & Roslin 2012) networks. Here I exclude possible latitudinal and phylogenetic effects that may obscure the mechanisms structuring networks by sampling within a region and within a plant family, and still invariant network structure is observed. The mechanisms behind this property of networks have not been thoroughly assessed, but it is suggested that species are replaced by topologically similar species in compositionally different communities (Dupont *et al.* 2009) or that basic rules may determine how antagonists interact despite changes in diversity (Morris *et al.* 2014). My findings align with large-scale studies (Dupont *et al.* 2009; Morris *et al.* 2014) and suggest that neither large nor fine scale differences in environmental factors are influencing emergent network structure and when evaluating mechanisms that structure communities, it may be more useful to assess drivers of interaction turnover.

Despite invariant emergent network structure, interactions showed both spatial and temporal turnover. Turnover in interactions between sites mostly resulted from either turnover in plant

species only (i.e. insects are widespread and can utilise multiple, possibly closely related, hosts) or turnover in plant-herbivore species pairs. The latter could reflect specialist insects exhibiting similar distributions to their hosts or both groups responding to the same environmental factors. In contrast, seasonal interaction turnover resulted from seasonal insect turnover, or interestingly, because seasonally persistent species tended to switch interaction partners between seasons. Insects with similar ecological traits or needs can avoid competition and coexist by being active at different times of the day or year (Wolda & Fisk 1981; Tylianakis *et al.* 2005) and thus partition niches temporally. The high seasonal turnover in insects indicates some seasonal niche partitioning which may have resulted from evolutionary host switches between seasons. This, however, requires further investigation. Insect host switching may arise when the nutritional quality of the plants change seasonally and insects are selectively feeding on structurally and chemically diverse plants to balance their nutrient needs (Jonas & Joern 2008). However, seasonal host switching is not present in the dominant group of restio herbivores, the Cephalelini (Augustyn *et al.* 2013). The Cephalelini rather exhibit two separate seasonal peaks in abundance, with individual species not present in both of those peaks. My results suggest that most of the insect species on restios seem to follow this pattern rather than exhibiting seasonal host switching.

Spatial changes in interactions were mostly driven by simultaneous changes in insect and plant communities. Nearby sites thus shared many pairs of interacting plant and insect species which were absent from more distant sites. This pattern can either be explained by host specific insects that are associated with dispersal limited or niche constrained plants, or by both insects and plants exhibiting parallel responses to environmental gradients. B_0 and B_h decreases with an increase in distance due to the absence of overlapping plants in geographically distant communities (both B_0 and B_h are contingent on plant species being present in both communities). These patterns of interaction turnover for an entire community in a temperate region are surprisingly similar to the findings of Novotny (2009) who assessed tropical Lepidoptera food webs. They also found evidence for interaction turnover associated with geographic distance driven by a simultaneous change in plant and insect turnover. Lepidoptera in this tropical system exhibit host specificity and should not show dispersal limitation (Novotny *et al.* 2007), and the association between geographic distance and simultaneous turnover in insects and plants thus likely arises from host specific herbivores associated with dispersal limited plants (Novotny 2009). Novotny *et al.* (2006) suggested levels of specialisation do not differ between latitudes and here I show that not only may

specialisation levels be similar between latitudes, but the patterns caused by geographic changes in the internal structure of interaction networks may also be similar. This suggests networks may be consistently structured across latitudes (Morris *et al.* 2014).

Further, the mean network specialisation ($H2'$) in our networks was similar to what Morris *et al.* (2014) found in host-parasitoid networks across latitudes (global mean = 0.65, this study = 0.63). It is quite surprising that I found these high levels of specialisation and niche partitioning when sampling herbivores from a single plant family. However, many rare species and low abundances might have led to high $H2'$ estimates (Blüthgen *et al.* 2008), but this should be the case in the networks used in Morris *et al.* (2014) as well. Low phylogenetic diversity should lead to low observed specialisation if insects are specialised at higher taxonomic levels, where an insect may utilise a range of closely related plant species. The high $H2'$ values indicate many insects on the Restionaceae are specialised at finer taxonomic scales and have narrow host use ranges. From previous work (Chapter 3), we know plant community composition predicts insect community composition in the Restionaceae, a pattern which likely results from host specificity.

Insect species on the Restionaceae seem to preferentially feed on favourable plant species rather than merely feeding on the most abundant plant species present. In this nutrient poor region it may be important for insects to utilise plant species that have sufficient nutrient levels, which might be associated with plant phenophase (Jonas & Joern 2008). Alternately, they may be utilising plants with penetrable herbivore defences or those that provide predator-free space. However, the topologically most important species remained constant between seasons and many insects thus make host choices regardless of plant phenophase. Other factors, such as predator-free space and penetrable defences, may remain constant between seasons and insects might be selecting plants on these traits rather than nutrients. Ideally, the nutrient content of these plants need to be measured and compared to insect abundances and richness associated with each plant species. The robustness analyses also indicated the plants with the highest degree are favoured disproportionately more than others, and the loss of these plants is more detrimental to the community than random plant species loss.

Conclusion

In summary, I find invariant spatial and temporal emergent network structure despite changes in insect community composition. Interactions show high turnover, driven seasonally by

turnover in insect species and insect host switching. Spatially the turnover in interactions is driven by a turnover in both plants and insects, suggesting either insects are host specific or both groups are responding to similar environmental gradients. Insects are preferentially selecting favourable plants, and these choices do not seem to be related to plant phenophase.

Table 4.1 Locations of sites used for networks, including plant and insect species richness.

Site	Latitude	Longitude	Plant richness	Insect richness
Kleinmond (KM)	-34.332	18.9959	8	85
Mont Rochelle (MR)	-33.898	19.1579	7	105
Pringle Bay (PB)	-34.326	18.8400	10	166
Rockview dam (RV)	-34.188	18.9431	8	87
Stellenbosch mountain (SB)	-33.887	18.9007	6	55
Villiersdorp (VD)	-33.970	19.1669	10	101

Table 4.2 Values of the various metrics calculated to quantify network structure for all sites for each season (spring – S, autumn – A). Weighted NODF and modularity values that are significantly higher ($p < 0.05$) than expected from network size and connectance are indicated with a (*).

Site (season)	Linkage density		Weighted NODF		connectance		generality		vulnerability		H2'		modularity	
	A	S	A	S	A	S	A	S	A	S	A	S	A	S
KM	3.68	5.07	20.16*	30.52*	0.21	0.26	1.51	3.04	5.84	7.09	0.68	0.43	<0.01*	0.02
MR	7.14	8.84	29.13*	23.32*	0.35	0.28	2.00	1.63	12.28	16.06	0.46	0.64	<0.01*	0.01
PB	7.72	5.55	23.86*	31.96*	0.23	0.30	2.88	3.31	12.56	7.80	0.48	0.41	0.01*	0.01*
RV	3.05	4.39	17.62*	21.96*	0.21	0.20	1.47	1.79	4.62	6.98	0.79	0.59	0.08*	0.25
SB	2.66	3.00	29.32	19.14*	0.31	0.23	1.94	1.46	3.39	4.54	0.65	0.76	0.03*	0.01*
VD	3.64	5.42	24.98*	27.24*	0.23	0.24	1.63	2.64	5.65	8.20	0.70	0.39	0.01	0.24*

Table 4.3 The two plant species with the highest unweighted degree (number of insect species interacting with a plant species) for each site in autumn and spring respectively. For all except one site (KM), at least one of the two topologically most important species remained constant between seasons.

Site	Autumn	Spring
KM	<i>Elegia filacea</i>	<i>Mastersiella digitata</i>
	<i>Elegia stokoei</i>	<i>Restio parvispiculus</i>
MR	<i>Hypodiscus aristatus</i>	<i>Staberoha cernua</i>
	<i>Staberoha cernua</i>	<i>Restio sieberi</i>
PB	<i>Staberoha vaginata</i>	<i>Hypodiscus aristatus</i>
	<i>Hypodiscus aristatus</i>	<i>Staberoha vaginata</i>
RV	<i>Restio parvispiculus</i>	<i>Elegia filacea</i>
	<i>Elegia filacea</i>	<i>Restio parvispiculus</i>
SB	<i>Elegia neesii</i>	<i>Restio sieberi</i>
	<i>Hypodiscus aristatus</i>	<i>Hypodiscus aristatus</i>
VD	<i>Restio capensis</i>	<i>Restio capensis</i>
	<i>Restio curviramis</i>	<i>Restio curviramis</i>

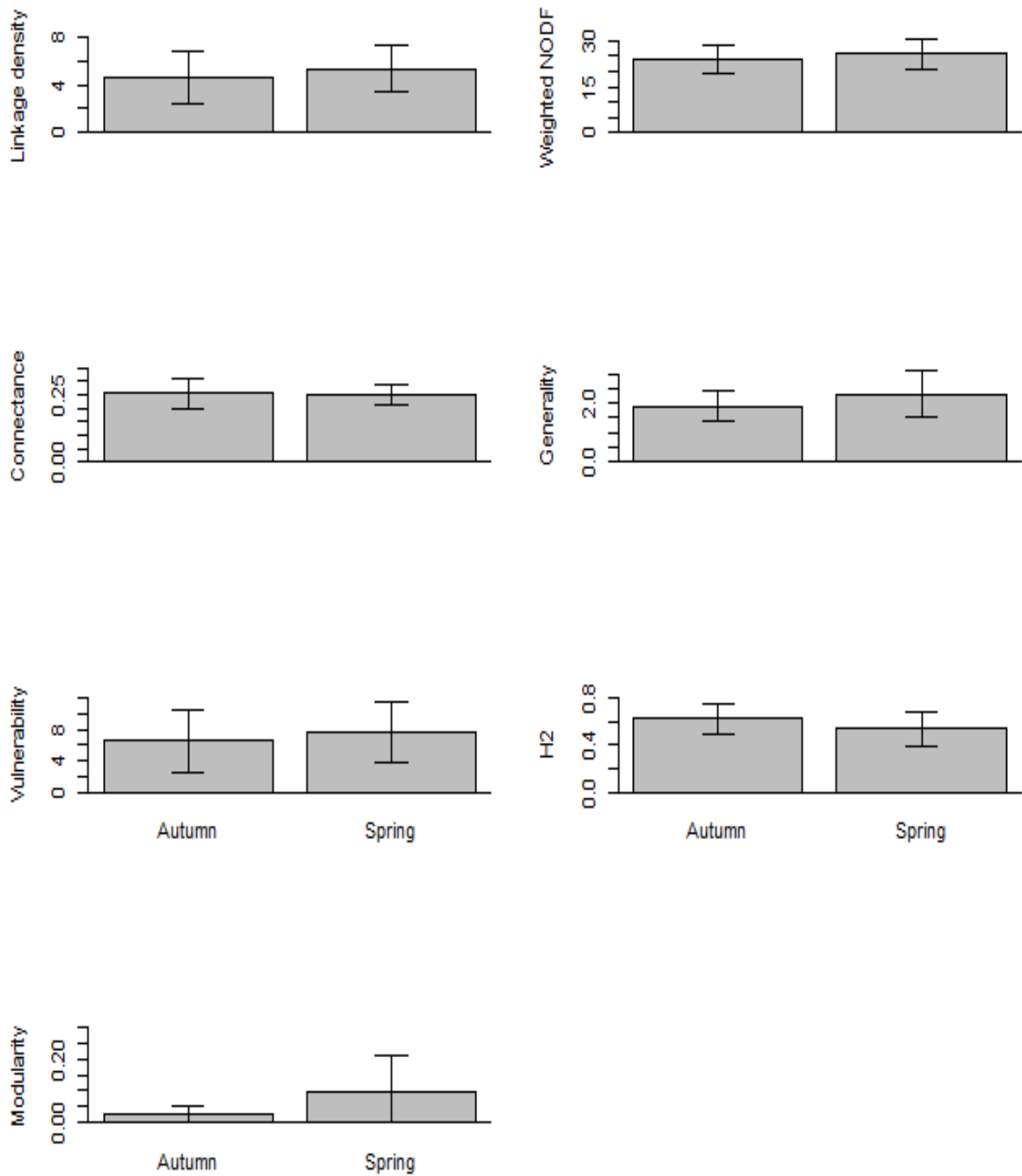


Figure 4.1 Linkage density, weighted NODF, connectance, generality, vulnerability, H2 (network-wide specialisation) and modularity between autumn and spring. No significant differences are present between seasons. Arrows depict standard deviation.

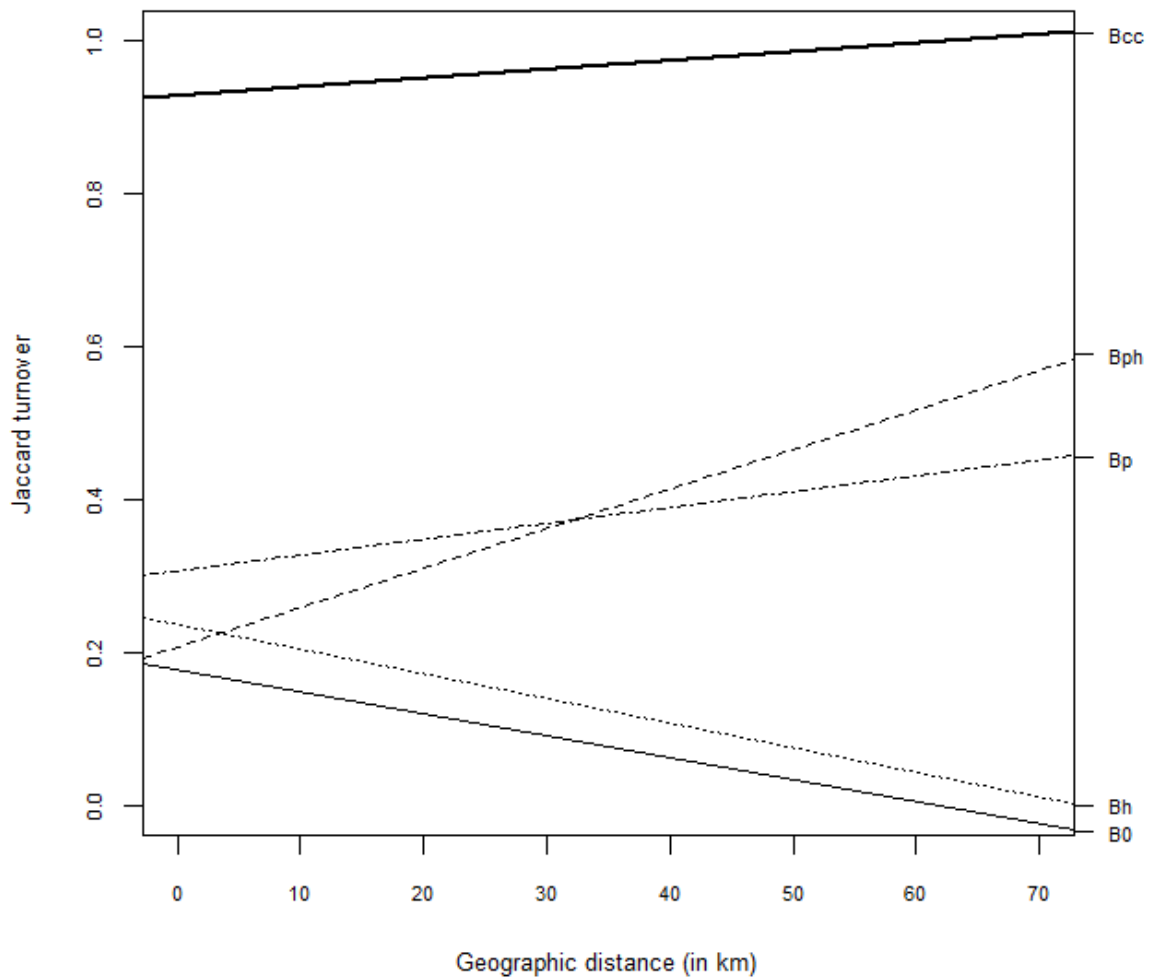


Figure 4.2 Regression between interaction turnover and geographic distance. All relationships shown are significant, except B_p . As distance between sites increases, more turnover is attributed to both plants and herbivores being absent (B_{ph}). This suggests plant distribution is limited by dispersal and insects have narrow host-use ranges. Also, sites situated further apart tend to show less network turnover due to only herbivores being absent (B_h). It must be noted that the decrease in interaction beta diversity attributed to only the absence of herbivores with an increase in geographic distance does not mean lower insect turnover. A larger proportion of turnover is simply partitioned to both insects and plants being absent. Also, when plants show complete turnover, interaction turnover cannot be partitioned to B_h or B_0 .

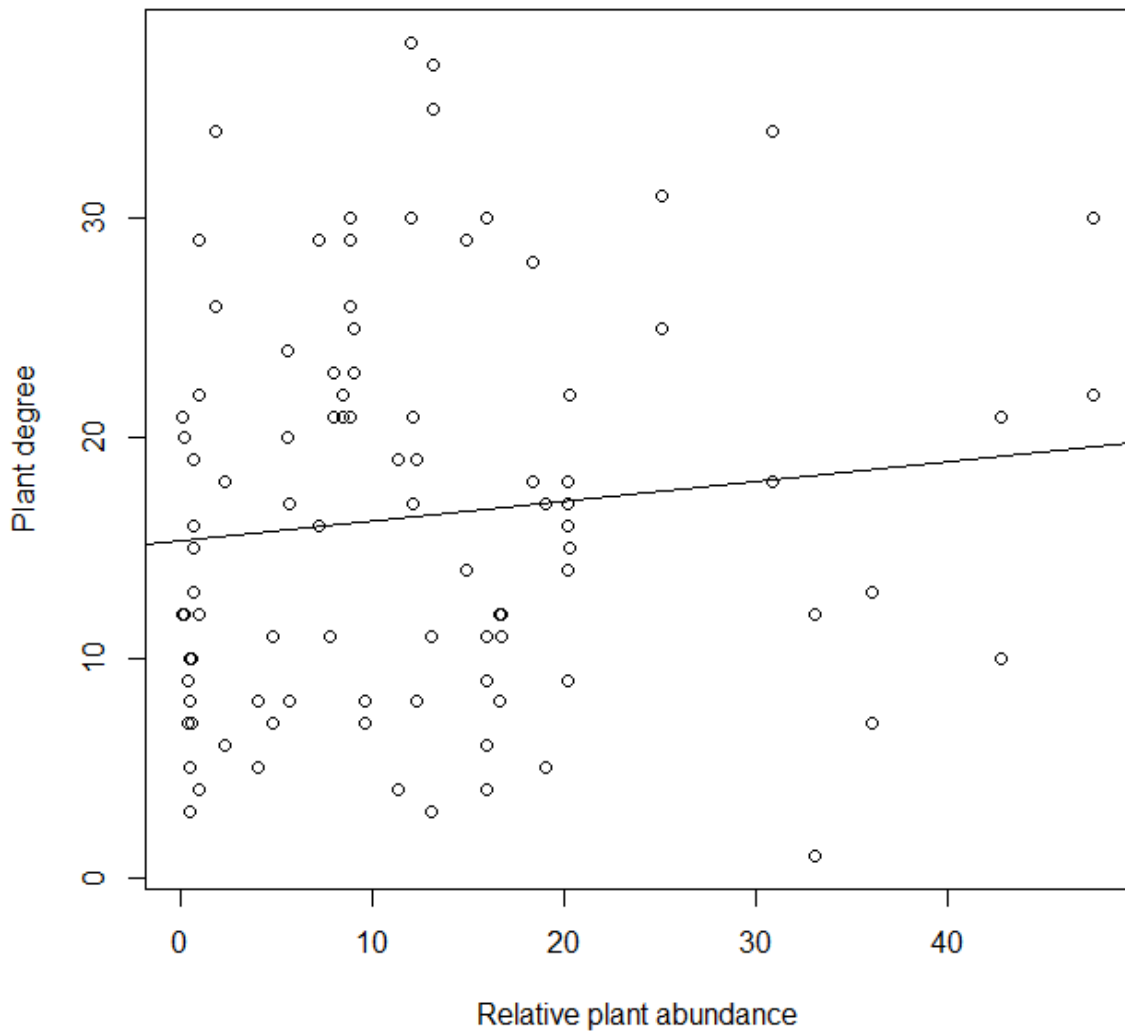


Figure 4.3 No association is present between unweighted degree (number of insect species each plant species interacts with) and the relative abundance of a plant species at a site ($R^2 = 0.002$, $p = 0.27$).

Thesis conclusion

In this thesis I investigated the mechanisms that structure the association between plant and insect communities in the CFR. I did this by integrating richness estimates, community ecology and network ecology. All three approaches implemented here point toward a direct association between plant and insect communities which is mainly driven by insect host specificity. This is supported by the positive association between plant and insect richness found in Chapter 2, with other variables only exhibiting weak effects on insect richness. A positive correlation between plant and insect community turnover was present in Chapter 3 and plant community composition successfully predicted insect community composition. Other variables did not account for changes in insect community composition. Also, the spatial structure of plant and insect community turnover showed analogous patterns. In Chapter 4, insect host specificity was supported by high H2' (network-wide specialisation) values and interaction turnover driven primarily by simultaneous plant and insect turnover. Although environmental effects should not be ignored, my findings suggest that they are not the primary factors structuring insect communities associated with the Restionaceae.

Results further suggest that many insects utilise multiple phylogenetically closely related host plants. Both Chapters 3 and 4 show many insects have wider distribution ranges than plants, and insects might thus be less dispersal limited or have broader niche-breadths than plants (i.e. plants are tightly associated with ecological niches and insects can utilise multiple closely related hosts). In Chapter 3, insect beta diversity is correlated best with plant phylogenetic beta diversity at all spatial scales, also supporting the notion that insects can feed on closely related hosts. My results align with tropical studies that suggest insects can utilise multiple closely related hosts and that the global megadiversity of insects primarily arose from insect host specificity and evolutionary shifts between hosts.

Further, I show that despite the positive association between plant and insect richness, insect richness is lower than expected from the exceptionally high regional plant richness. This may result from plant diversity being restricted to a few lineages with insects specialised at higher taxonomic levels (i.e. insects utilise multiple closely related hosts within these lineages).

By exhaustively sampling within a plant family, accumulation curves tended to saturation – previously unobserved in CFR insect studies. This minimised the effects of sampling error on

statistical analyses and by sampling within a widespread and species rich plant family, I was able to untangle the various factors influencing insect occurrence. Further, the spatially nested sampling approach assisted in determining whether or not both groups exhibit parallel responses to environmental gradients, and to what extent patterns of insect diversity are tracking patterns of plant diversity at various spatial scales. The network analyses allowed a direct assessment of insect host specificity and interaction turnover. Also, high seasonal insect species turnover and seasonal host switching was detected, and thus sampling insects in only one season will lead to insect richness being underestimated. Thorough and structured sampling thus assisted in partly elucidating the mechanisms that shape insect communities in the CFR. Further studies are needed on other CFR plant families to determine the generality of these results and I suggest that similar approaches are implemented.

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Appendix

Table S2.1 GPS coordinates of the centre point of each square (n = 60).

Square	Lat	Lon
1	-33.9032	19.15747
2	-33.9033	19.15729
3	-33.9018	19.15827
4	-33.9019	19.15794
5	-33.8982	19.15782
6	-33.8983	19.15791
7	-33.9203	19.15849
8	-33.9206	19.15853
9	-33.9697	19.16671
10	-33.9702	19.16686
11	-33.991	18.99107
12	-33.991	18.99107
13	-33.9947	19.00474
14	-33.9946	19.00484
15	-33.9926	18.99718
16	-33.9925	18.99739
17	-33.9931	18.97574
18	-33.9932	18.97556
19	-33.9966	18.9831
20	-33.9967	18.98288
21	-34.3203	18.9642
22	-34.3204	18.96425
23	-34.3214	18.96459
24	-34.3211	18.96496
25	-34.3214	18.96112
26	-34.3213	18.96093
27	-34.3331	18.9978
28	-34.3329	18.99774
29	-34.3322	18.99587
30	-34.3321	18.99591
31	-34.1823	18.94534
32	-34.1826	18.94561
33	-34.184	18.94438
34	-34.1842	18.94438
35	-34.1877	18.94315
36	-34.1879	18.94314
37	-34.1862	18.94059
38	-34.1862	18.94033
39	-34.1843	18.93807
40	-34.1844	18.93767
41	-34.0929	18.42223
42	-34.0927	18.42226

43	-34.0943	18.42414
44	-34.0944	18.42416
45	-34.0961	18.42484
46	-34.0961	18.42463
47	-34.0985	18.42883
48	-34.0983	18.42874
49	-34.0916	18.42527
50	-34.0917	18.42511
51	-33.9486	18.42804
52	-33.9485	18.428
53	-33.9537	18.4274
54	-33.9532	18.42735
55	-33.9558	18.42014
56	-33.9557	18.42031
57	-33.9558	18.41777
58	-33.9559	18.41764
59	-33.9584	18.4174
60	-33.9532	18.41733

Table S4.1 Horn similarity values between sites in autumn.

	KM	MR	PB	RV	SB	VD
KM						
MR	0.33					
PB	0.49	0.40				
RV	0.31	0.25	0.39			
SB	0.28	0.33	0.49	0.28		
VD	0.21	0.31	0.32	0.23	0.23	

Table S4.2 Horn similarity values between sites in spring.

	KM	MR	PB	RV	SB	VD
KM						
MR	0.29					
PB	0.83	0.41				
RV	0.26	0.28	0.23			
SB	0.55	0.39	0.60	0.50		
VD	0.32	0.34	0.33	0.11	0.26	

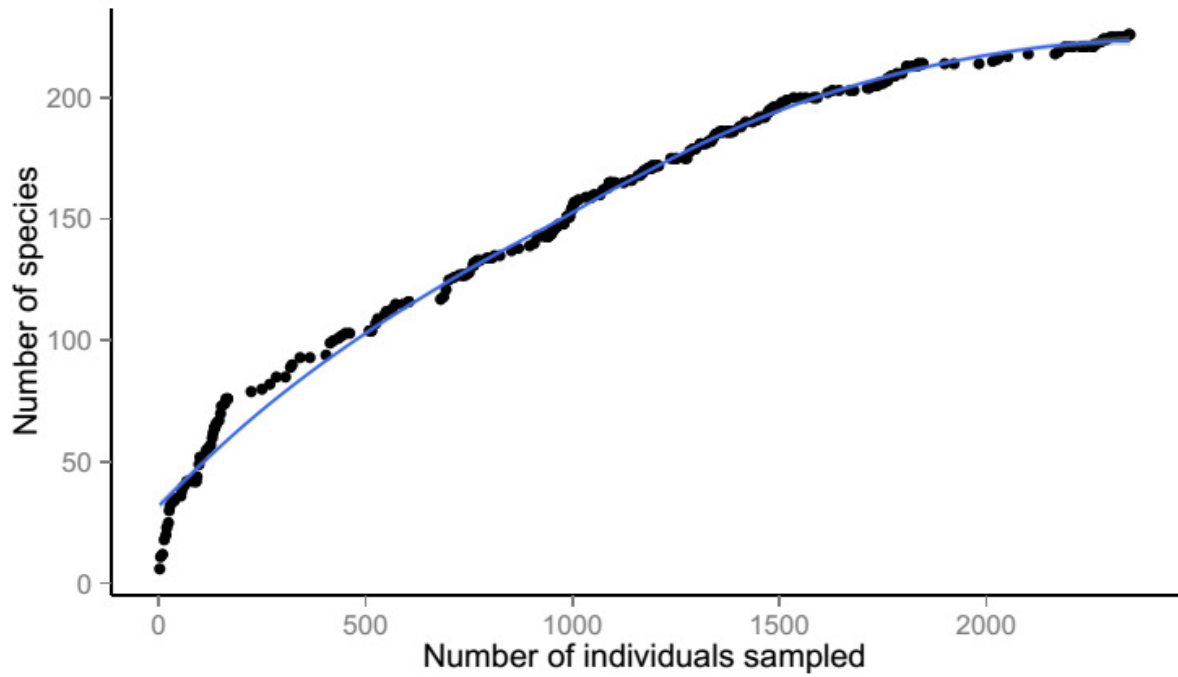


Figure S2.1 Individual-based accumulation curve constructed for autumn. Fitted curve is based on locally weighted scatterplot smoothing (LOESS).

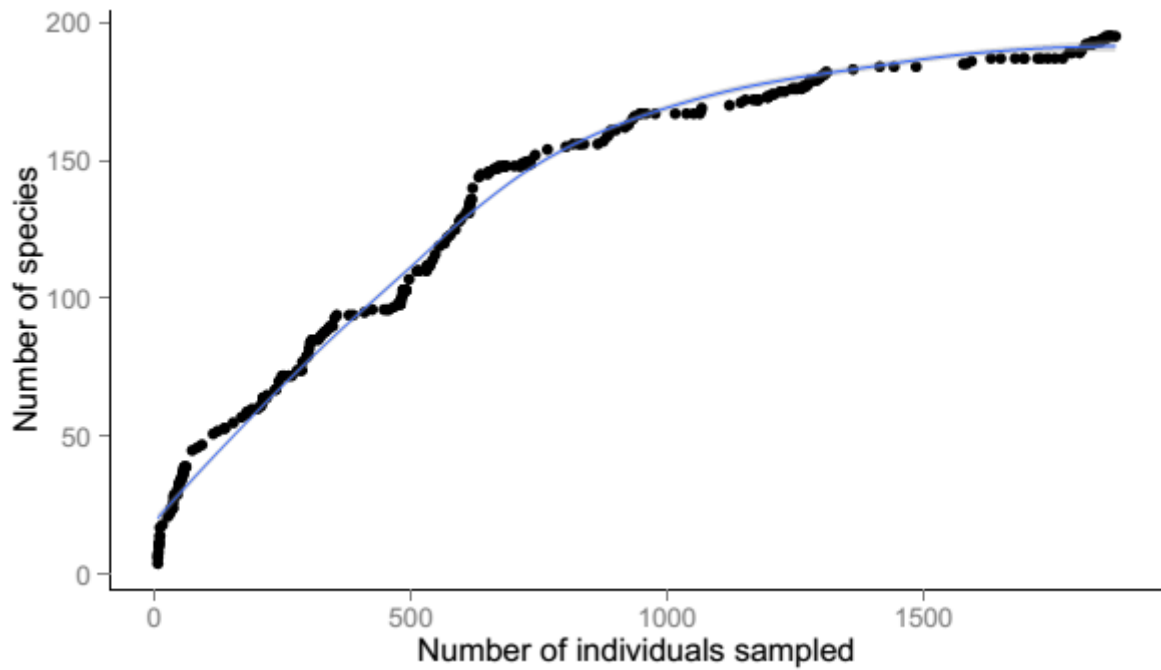


Figure S2.2 Individual-based accumulation curve constructed for spring. Fitted curve is based on locally weighted scatterplot smoothing (LOESS).

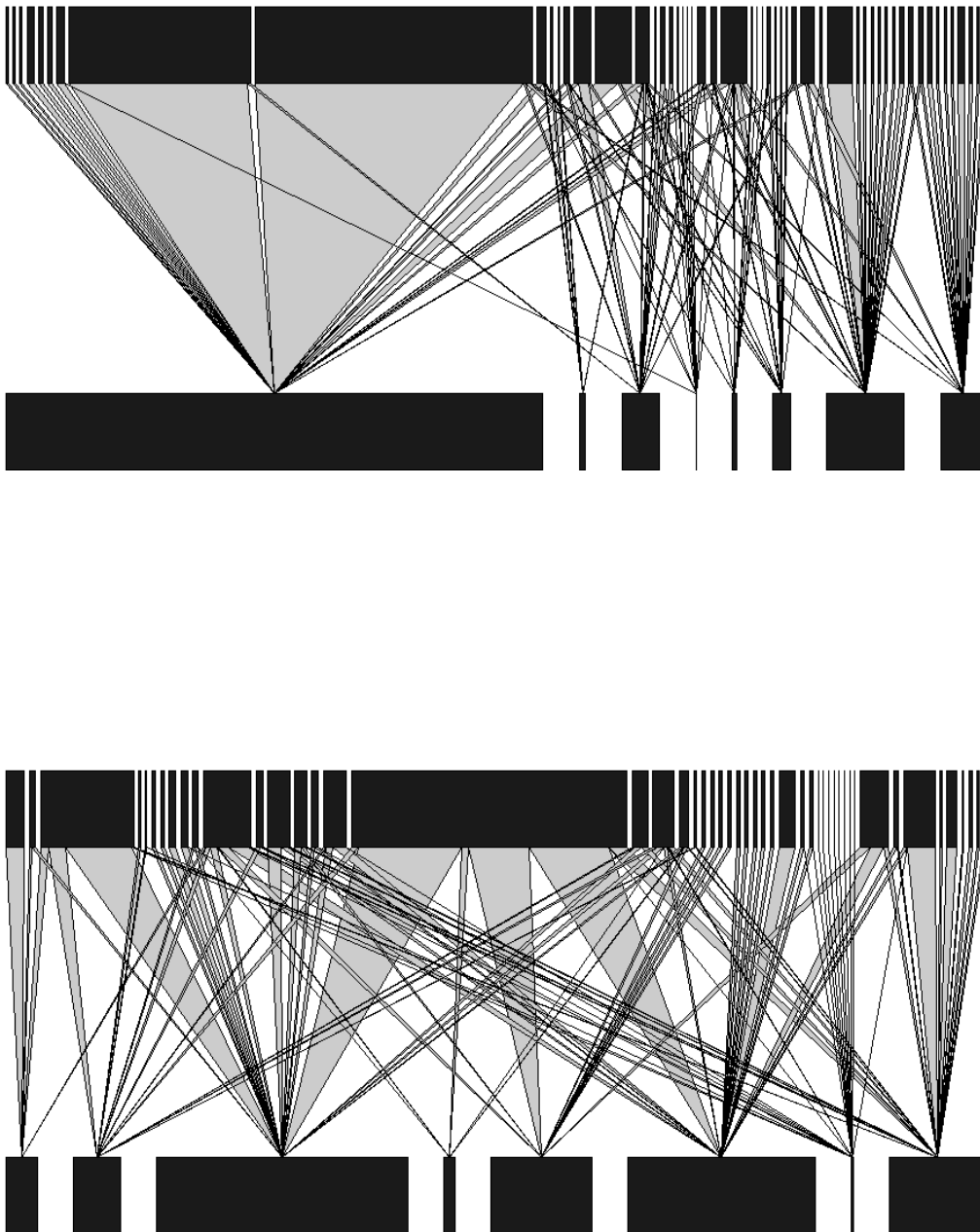


Figure S4.1 Interaction networks for KM for each season are shown. The top figure depicts the autumn network and the bottom figure shows the corresponding spring network. The lower level in each network represents plants and the upper level herbivores.

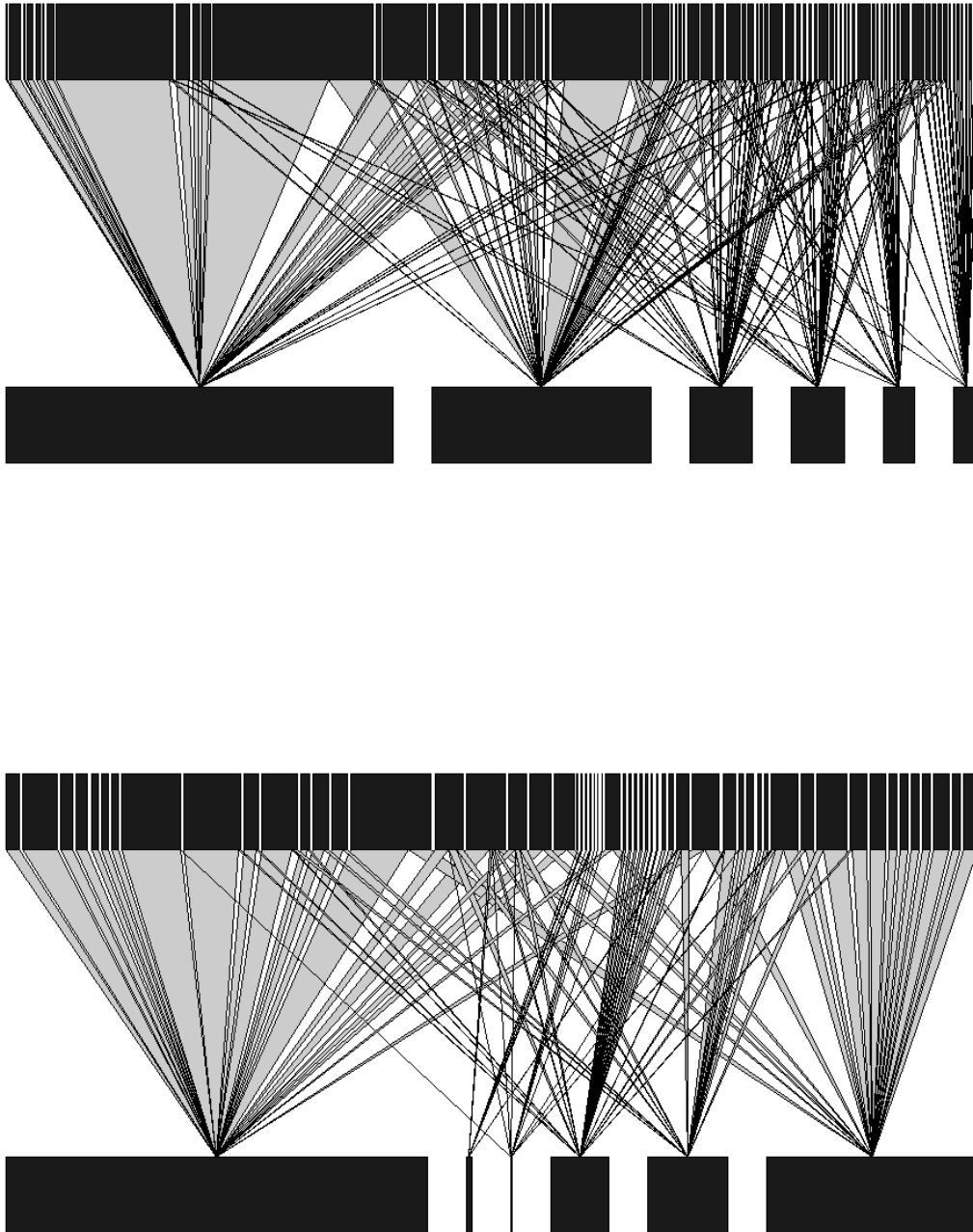


Figure S4.2 Interaction networks for MR for each season are shown. The top figure depicts the autumn network and the bottom figure shows the corresponding spring network. The lower level in each network represents plants and the upper level herbivores.

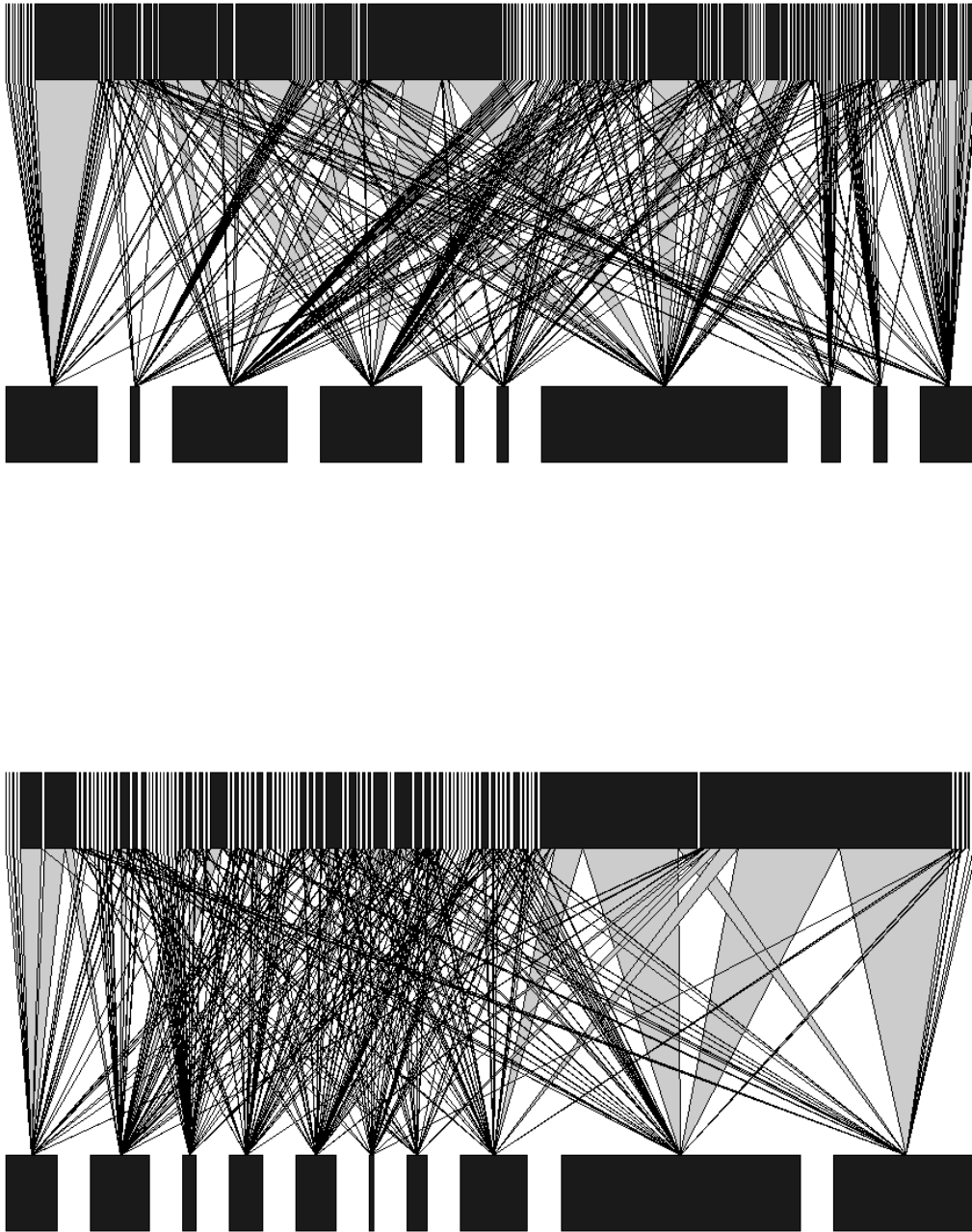


Figure S4.3 Interaction networks for PB for each season are shown. The top figure depicts the autumn network and the bottom figure shows the corresponding spring network. The lower level in each network represents plants and the upper level herbivores.

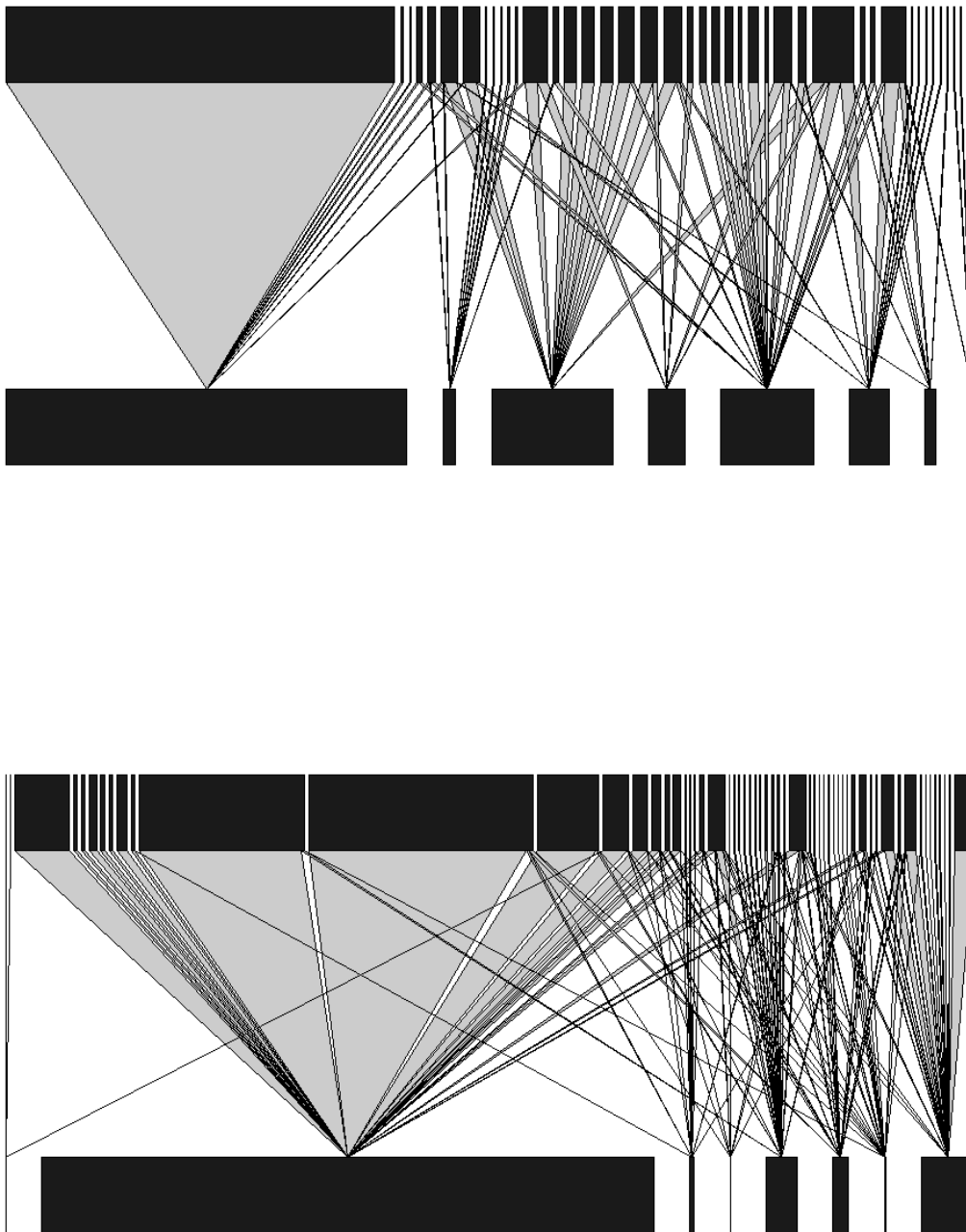


Figure S4.4 Interaction networks for RV for each season are shown. The top figure depicts the autumn network and the bottom figure shows the corresponding spring network. The lower level in each network represents plants and the upper level herbivores.

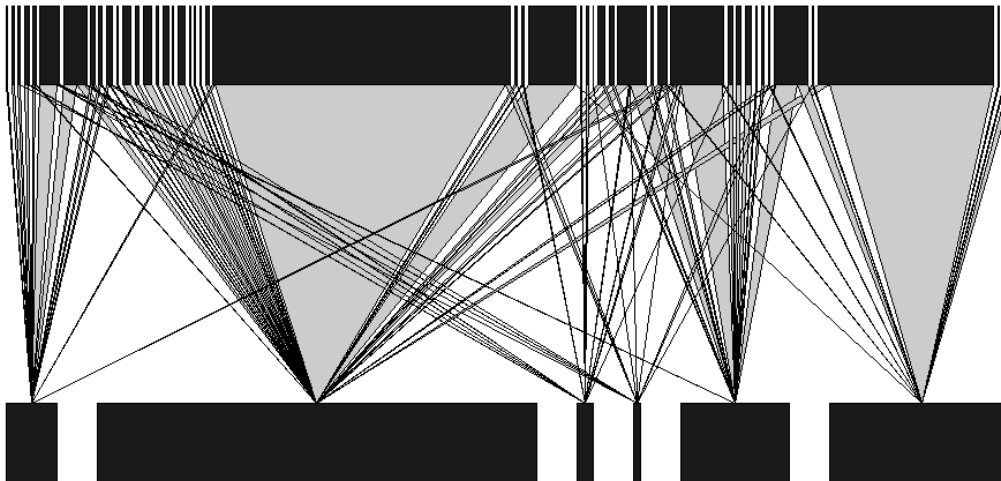
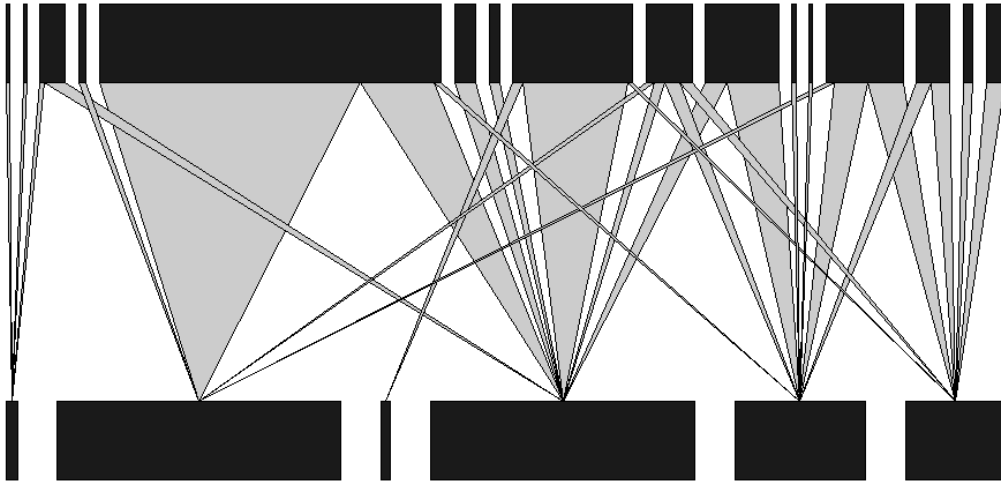


Figure S4.5 Interaction networks for SB for each season are shown. The top figure depicts the autumn network and the bottom figure shows the corresponding spring network. The lower level in each network represents plants and the upper level herbivores.

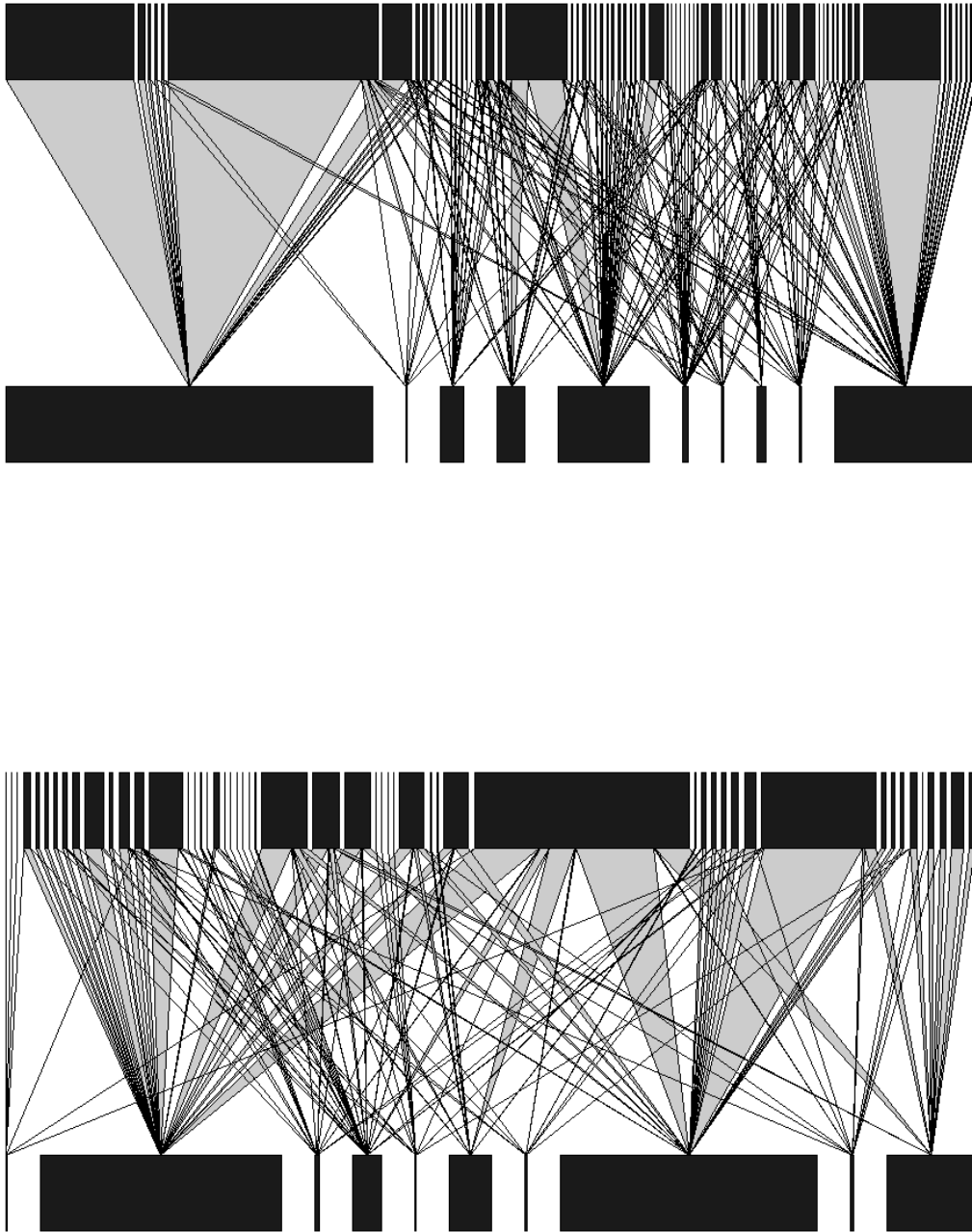


Figure S4.6 Interaction networks for VD for each season are shown. The top figure depicts the autumn network and the bottom figure shows the corresponding spring network. The lower level in each network represents plants and the upper level herbivores.

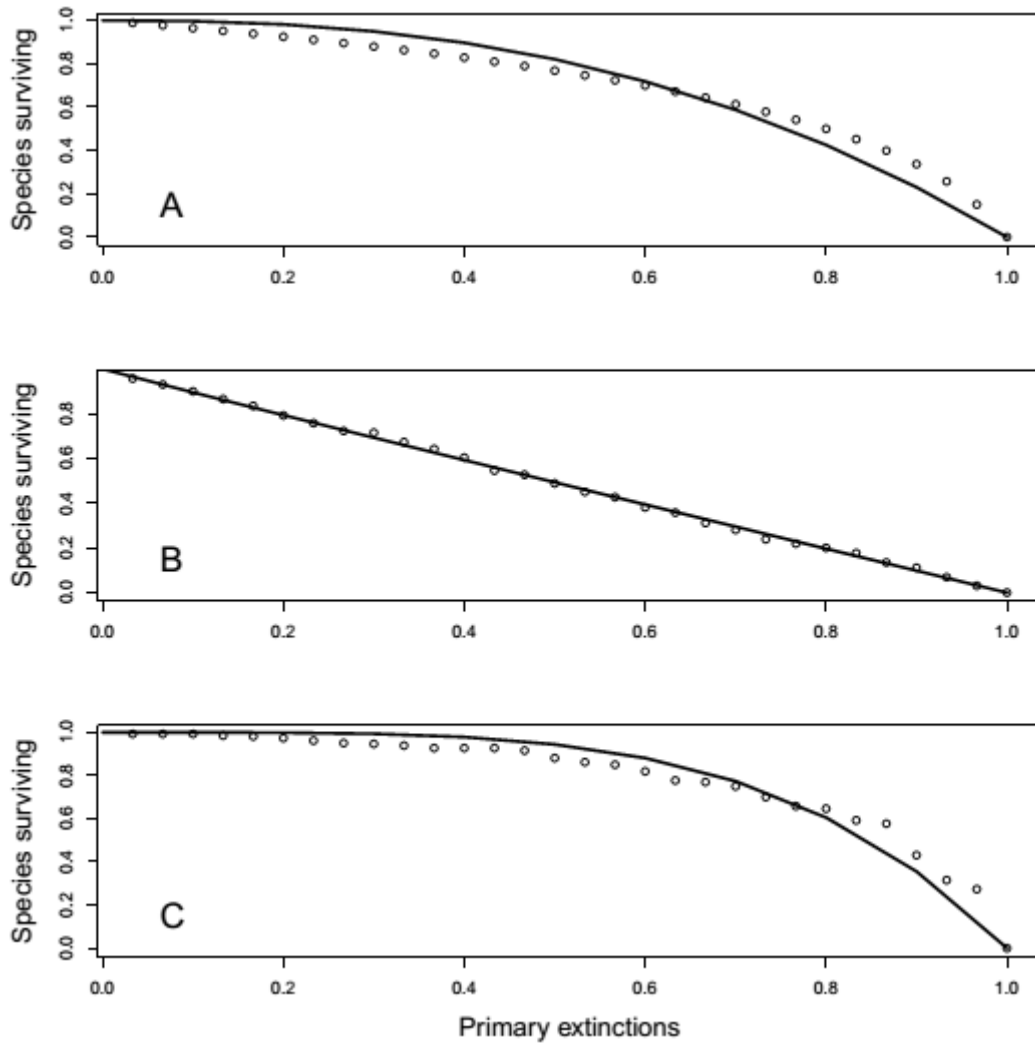


Figure S4.7 Robustness curves for the combined network (seasons and sites combined) where (A) plants are eliminated randomly, (B) the plants with the highest degree (most interactions) are eliminated first and (C) the plants with the lowest abundances are eliminated first. Insect species surviving are shown on the y-axis. Circles represent the calculated number of species surviving per plant species eliminated and lines represent a line of best-fit.

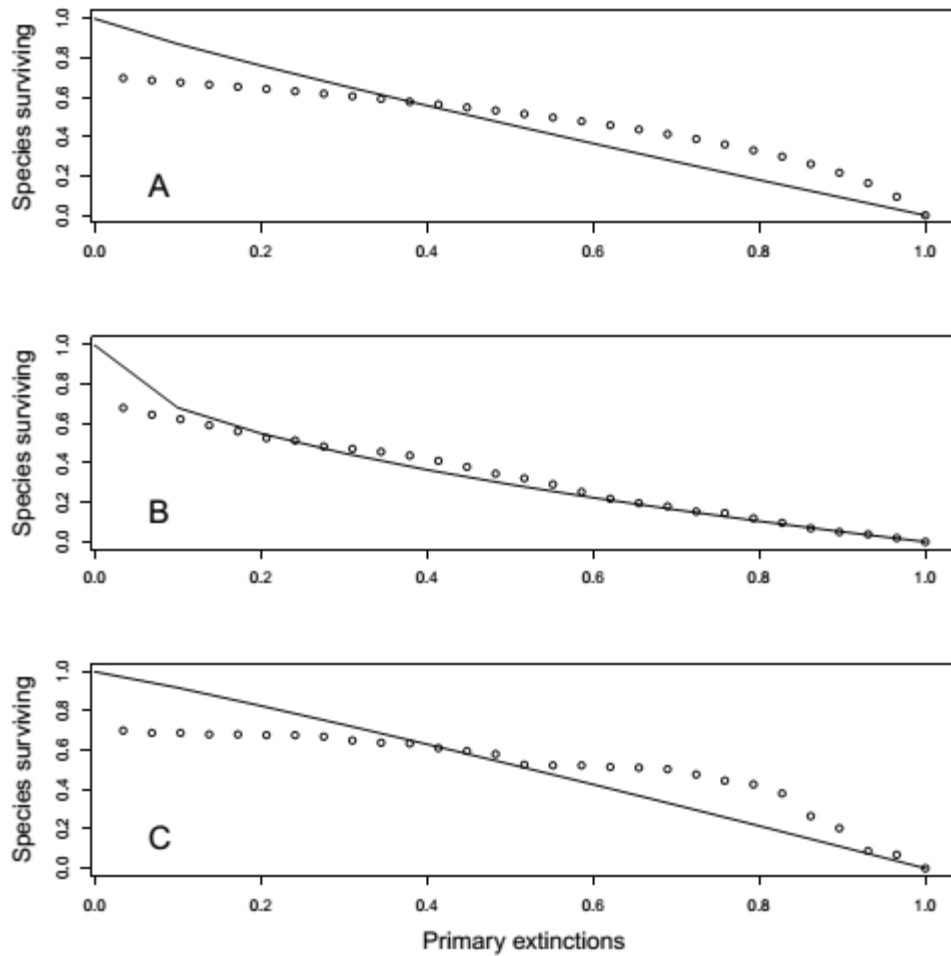


Figure S4.8 Robustness curves for the combined autumn network (sites combined) where (A) plants are eliminated randomly, (B) the plants with the highest degree (most interactions) are eliminated first and (C) the plants with the lowest abundances are eliminated first. Insect species surviving are shown on the y-axis. Circles represent the calculated number of species surviving per plant species eliminated and lines represent a line of best-fit.

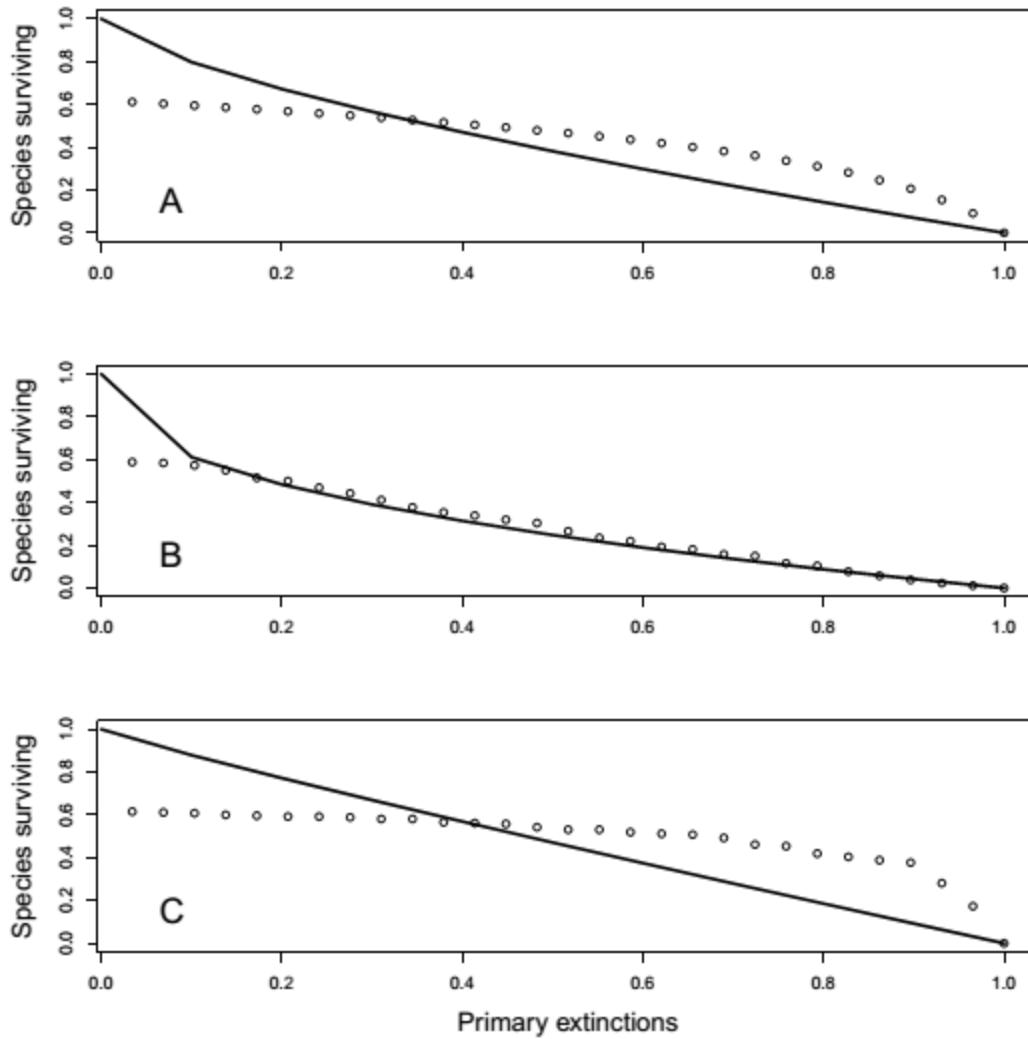


Figure S4.9 Robustness curves for the combined spring network (sites combined) where (A) plants are eliminated randomly, (B) the plants with the highest degree (most interactions) are eliminated first and (C) the plants with the lowest abundances are eliminated first. Insect species surviving are shown on the y-axis. Circles represent the calculated number of species surviving per plant species eliminated and lines represent a line of best-fit.