

Kemp JE, Evans DM, Augustyn WJ, Ellis AG.

[Invariant antagonistic network structure despite high spatial and temporal turnover of species.](#)

***Ecography* (2016)**

DOI: <http://dx.doi.org/10.1111/ecog.02150>

Copyright:

This is the peer reviewed version of the following article, which has been published in final form at <http://dx.doi.org/10.1111/ecog.02150>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

Date deposited:

14/09/2016

Embargo release date:

29 September 2016

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

Jurene E. Kemp¹, Darren M. Evans^{2,3}, Willem J. Augustyn¹ & Allan G. Ellis¹

¹Botany and Zoology Department, Stellenbosch University, Private Bag X1, Matieland, Stellenbosch 7602, South Africa

²School of Biological, Biomedical and Environmental Sciences, University of Hull, Cottingham Road, Hull HU6 7RX, United Kingdom

³School of Biology, Newcastle University, Newcastle upon Tyne, NE1 7RU, United Kingdom

Corresponding author: Jurene E. Kemp, Botany and Zoology Department, Stellenbosch University, Private Bag X1, Matieland, Stellenbosch 7602, South Africa.

E-mail: jurenekemp@yahoo.com. Fax no: +27 21 8082405

Decision date: 12-Sep-2016

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1111/ecog.02150].

Abstract

Recent work has suggested that emergent ecological network structure exhibits very little spatial or temporal variance despite changes in community composition. However, the changes in network interactions associated with turnover in community composition have seldom been assessed. Here we examine whether changes in ecological networks are best detected by standard emergent network metrics or by assessing internal network changes (i.e. interaction and composition turnover). To eliminate possible spatial or phylogenetic effects, that in large-scale studies may obscure mechanisms structuring networks and interactions, we sampled multiple antagonistic (plant-herbivore) networks for a single diverse plant family (the Restionaceae) in the hyperdiverse Cape Floristic Region. These are the first plant-herbivore networks constructed for this global biodiversity hotspot. We found invariant emergent network structure despite considerable changes in insect and plant composition across communities over time and space. In contrast, there was high interaction turnover between networks. Seasonally, this was driven by turnover in insect species and insect host switching. Spatially, this was driven by simultaneous turnover in plant and insect species, suggesting that many insects are host specific or that both groups exhibit parallel responses to environmental gradients. Spatial interaction turnover was also driven by turnover in plants, showing that many insects can utilise multiple (possibly closely related) hosts and this may create divergent selection gradients that promote insect speciation. Thus we show highly variable interaction fidelity, despite invariant emergent network structure. We suggest that evaluating internal network changes may be more effective at elucidating the processes structuring networks, and many fine-scale changes may be obscured when only calculating emergent network metrics.

Key-words: beta diversity; Cape Floristic Region; network ecology; plant-herbivore interactions; Restionaceae; specialization

Introduction

Ecological networks, which describe the interactions between species and emergent community structure, can provide insight into both contemporary and evolutionary processes that structure and maintain communities (Jordano et al. 2003). Several recent studies have suggested that the structure of both mutualistic and antagonistic networks is relatively invariant spatially and temporally (Olesen et al. 2008, Kaartinen and Roslin 2012, Dáttilo et al. 2013). The emergent structure of some antagonistic networks, for example, does not vary consistently across latitudinal gradients (Morris et al. 2014) and remains constant between years (Kaartinen and Roslin 2012). The relative invariance of antagonistic network structure is somewhat surprising given the ubiquitous spatial and temporal variation in species community composition revealed by beta diversity studies (Novotný and Weiblen 2005, Novotný et al. 2007, Pellissier et al. 2013a, b). This mismatch may either reflect publication bias, where most published antagonistic networks focus on insect-parasitoid interactions and, in contrast, beta diversity studies mostly focus on plants and herbivores, or it may reflect a general trend in ecological communities.

Ecological network structure has been suggested to arise either 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 non-randomly structured through trait mismatches (in terms of both complementary traits and exploitation barriers) that constrain interactions (biological constraint hypothesis - Jordano et al. 2003, Santamaría and Rodríguez-Gironés 2007, Dehling et al. 2016).

Recent work shows that a combination of these hypotheses successfully predicts network metrics, but cannot accurately predict the interactions or interaction frequencies within networks (Santamaría and Rodríguez-Gironés 2007, Vázquez et al. 2009, Olito

and Fox 2014). However, this has mainly been assessed for mutualistic networks and although some attempts have been made to model food web structure (Allesina et al. 2008), whether these findings hold true for antagonistic networks is not known.

Although networks with similar species abundances or similar levels of trait matching may exhibit invariant emergent network structure, differences in interaction identity (i.e. bi trophic interacting pairs) or species composition may still be prevalent and these internal network differences could potentially reveal processes structuring networks.

While some evidence suggests invariant antagonistic network structure, very little is known about how compositional changes affect turnover in interactions of persistent species across communities. For example, changes in available host plants or changes in host plant quality across the landscape could lead to herbivorous insects switching hosts (Jonas and Joern 2008) and possible local adaptation, where insects become divergently adapted to different hosts (Nosil et al. 2002, Winkler et al. 2009, Darwell et al. 2014, Grosman et al. 2015). Novotný (2009) suggested that the beta diversity of interactions can be partitioned into different components responsible for interaction turnover, providing insight into why interactions (i.e. internal network structure) change. Using this approach, Novotný (2009) found high spatial turnover of interactions in tropical plant-herbivore networks driven by high plant species turnover. This method provides insight, beyond that gained from standard network metrics, into the mechanisms underlying network structural stability or lack thereof. For example, in temperate plant-herbivore networks, we might expect strong seasonal changes in interactions (and perhaps even network structure) driven by seasonal niche partitioning and/or host switching. In systems with low seasonal plant species turnover, we expect that if seasonal insect niche partitioning occurs, 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. Poisot et al. (2012) developed a similar method to analyse interaction turnover (based on Sorenson beta diversity rather than the Jaccard index as developed by Novotný (2009)) and emphasized the importance of the information provided by interaction turnover between shared species in elucidating the mechanisms structuring networks. Understanding spatial and temporal changes in interaction turnover can provide insight into the respective roles of evolved host specificity and contemporary interactions in structuring plant-herbivore networks, which cannot be assessed when only evaluating emergent network structure.

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 structure or the turnover of species' interactions. It is thus not known to what extent emergent network structure reflects differences in interaction identities between networks, or what drives these differences. Here, we examine both spatiotemporal turnover in interactions and variation in emergent network structure for a set of plant-herbivore communities within a single biodiverse biome, the Cape Floristic Region (CFR) in South Africa. Limiting the spatial extent of our sampling to this region allows us 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, a temperate

(Mediterranean-like climate) biodiversity hotspot containing more than 9000 plant species in 90 000 km² area (i.e. diversity levels approaching those of the tropics) (Goldblatt and Manning 2000) and extremely high plant beta diversity (Cowling 1990), is no exception. We focused on a single dominant CFR plant family, the Restionaceae (hereafter restios) and its associated herbivores. It has recently been shown that species composition of restio communities (which is highly variable spatially) is a significant determinant of the composition of associated insect herbivore communities (Kemp 2014), which suggests some level of insect specialisation. In addition, insect species composition varies seasonally on restios (Augustyn et al. 2013, Kemp 2014). 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 would lead to turnover in interactions, rather than turnover in species.

This previous work suggests high seasonal and spatial variation in plant and insect community composition in CFR Restionaceae communities (Kemp 2014). Here we examine the turnover of species-interactions across seasons and between sites within seasons, and ask whether these internal network changes (i.e. interaction and composition turnover) are captured by standard network-wide metrics. We expect turnover of interactions driven (1) temporally by turnover in insect species and (2) spatially by turnover in plants. By sampling within a region and within a plant family, we expect to detect mechanisms structuring networks that may be obscured in large-scale studies, whilst also providing new species-interaction data for this unique region.

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 et al. 2003).

The Restionaceae vary considerably in height, culm diameter and branching of the culms, with growth forms ranging from low cushions to 3 m tall reeds. Restios occur throughout the CFR in habitats that vary in soil type, altitude, groundwater availability, slope, aspect and climate. All plants are perennial with species growing (and flowering) throughout the year at different times, and thus the same plants are present for both seasons at each site.

Contrasting results have suggested that insect diversity in the CFR is lower (Johnson 1992, Giliomee 2003) or higher (Price et al. 1998, Wright and Samways 1998) than expected from plant species richness. These differences possibly arose from inadequate sampling and the sampling of different focal groups. Hemiptera form the largest order of herbivores on restios (43% of morphospecies), with the Cephalelini (Cicadellidae) being the dominant group of herbivores on the Restionaceae (10% of insect individuals) (Kemp 2014). 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 (Fig. 1). These were chosen based on similar post-fire vegetation age and high restio species richness, but differed in plant composition. All of the sites are approximately 300 x 300 m in size, and located 8 – 55 km apart.

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 multiple 100 m transects. Sites were sampled twice, once during austral autumn (i.e. March-April 2013), and once six months later during the suggested insect peak season (Pryke and Samways 2008) (i.e. austral spring: September-October 2013). These sampling periods coincide with the two distinct peaks in Cephalelini (dominant restio herbivores) abundance (Augustyn et al. 2013).

Insect sampling

Insects were collected from 80 randomly selected 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. By equally sampling plant species this study is inherently plant-focused, similar to most mutualistic network studies. All of these restios were exhaustively vacuum-sampled for approximately 10 seconds per plant and the nozzle was moved systematically up and down the culms. Restios were subsequently 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 (less than 10 per 1000 plants sampled) were found by search-sampling and no galling or mining insects were present, thus vacuum sampling was deemed an efficient sampling approach in this system.

The Cephalelini (Hemiptera: Cicadellidae) were identified to species in the laboratory 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 sap-

sucking Cephalelini consist of 21 species that are specialized to various restio species (Davies 1988, Prendini 1997, Augustyn et al. 2013), and tend to prefer and perform better on the restios they occur on (Augustyn et al 2016). Other insects were identified to superfamily or family and then sorted into morphospecies since identification keys are not available for the vast majority of insect groups in the CFR. Oliver and Beattie (1996) showed morphospecies to be sufficient surrogates for species, and morphospecies have previously been used in network analysis when keys or expertise were not available (Memmott 1999, Baldock et al. 2011). Since very little is known about the natural history of CFR insects other than the Cephalelini, 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 incidental visitors and also excluded from the dataset. Although most non-herbivorous insect morphospecies were removed from the dataset, feeding trials were not performed and thus some insects might be transient visitors. However, by using weighted networks (outlined below) the effects of these rare non-feeding interactions are mitigated and should not severely obscure real trends in the data. The insect morphospecies collection is housed in the Botany and Zoology department at Stellenbosch University.

Data analysis

Network construction

Insect abundances were weighted by the relative abundance of each plant species at each site prior to network construction. Quantitative plant-herbivore interaction matrices were constructed for each season for each site. Since an equal number of plant individuals were sampled per plant species at each site, as opposed to sampling according to each plant species' abundance, the sampled data do not reflect absolute insect numbers in each community. To adjust for this, the mean number of individuals

of each insect species collected per individual plant was calculated for each plant species at each site. This was then multiplied by the relative abundance (%) of each plant species in the community [(total number of individuals_{insect species A} /number of individual plants sampled_{plant species A}) * relative abundance (%) of plant species A].

Interaction strengths were thus weighted by the relative abundance of each plant species in the community. To facilitate network analysis in the bipartite package in R (Dormann et al. 2008), which requires that interaction strengths are integers, all interaction strengths were rescaled by a constant (i.e. multiplied by 100). For instance, if five individual insects of a species were sampled from 80 individuals of a plant species that constituted 15% of the plant community, the total interaction strength would be 94 (i.e. $(5 / 80) * 15 * 100$). This was done separately for each network. Network nodes respectively reflect plant and insect species, and links represent total interaction strengths.

Plant and insect community composition and turnover

To assess overlap of insect species composition between these communities, the Horn similarity index was used following 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.

Network metrics

Standard network metrics (weighted NODF, connectance, vulnerability, generality, linkage density, H_2' , modularity) used frequently in cross-network comparisons were calculated for each season for each site.

Weighted NODF (nestedness based on overlap and decreasing fill) is a nestedness metric that corrects for matrix fill and dimension (Almeido-Neto et al. 2008). A nested network consists of specialist organisms of one group interacting with subsets of

generalist organisms of the other group, and a core of generalists interacting with each other. Furthermore, in a weighted nested network generalist organisms do not only have more interaction partners, but the strength of interactions (i.e. interaction frequencies) is more equally distributed than that of specialist organisms (e.g. if an organism has many weak interactions and one strong interaction, it may be classed as specialist). The nestedness values were compared to that of 500 randomisations using the bipartite package in R (R Core Team 2013) with constrained marginal totals and connectance to assess whether networks are significantly more nested than expected from their size and connectedness. Linkage density is the weighted diversity of interactions per species. Connectance is the realised proportion of possible interactions. H_2' (standardised two-dimensional Shannon entropy) measures network-wide niche partitioning, where 0 represents no niche partitioning and 1 translates into complete niche partitioning (Blüthgen et al. 2006). Strong niche partitioning should result from strong host specificity of network members, and thus this metric is often used as a measure of network-wide specialisation. Generality is the weighted mean number of lower level species (plants) connected to higher level species (insects). Vulnerability is the weighted mean number of higher level species connected to lower level species. Generality and vulnerability represent the diversity of partners each species interacts with and allows us to examine changes in resource-consumer asymmetries. The mean of the vulnerability and the generality of a network equates to the linkage density of that network. 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 QuanBiMod algorithm created by Dormann and Strauss (2014) using the computeModules function in the package bipartite in R (Dormann et al. 2008).

Significance of modularity scores was determined by computing z-scores from null models (Dormann and Strauss 2014).

Comparison of network structure between sites and seasons

Seasonal differences in network-wide metrics (weighted NODF, connectance, vulnerability, generality, linkage density, H_2' , modularity) were assessed separately for each metric using a paired t-test with sites as replicates ($n = 6$ for each test). As results proved non-significant, we treated seasons as replicates in an ANOVA to test for differences between sites for each metric separately (thus 6 sites for each metric and two replicates within each site).

In addition, due to only having two replicates for each site and hence very low statistical power, permutational multivariate analysis of variance (PERMANOVA - Anderson 2001) was implemented with the *adonis* function in the *vegan* package (Oksanen et al. 2016) in R to assess the association between each network metric (separately for each season) and geographic distance between sites ($n = 6$) to ascertain whether network metrics remain constant spatially. To further explore spatial patterns in emergent network structure, sites were divided into two natural clusters consisting of three spatially aggregated sites each (i.e. all sites in a mountain area were considered a cluster - Figure 1). A t-test with seasons as replicates was then used to test for differences between the two clusters (thus $n = 6$) for each metric separately. Tests were performed using R (R Core Team 2013).

Association between emergent network structure and community composition

Next, multiple Mantel tests were implemented to determine whether changes in network metrics are related to changes in insect and plant community composition (Horn similarity calculated according to Jost (2007)). The analysis was performed for each

season and each trophic group separately. Bonferroni corrections were used to adjust critical values (separately for each trophic group).

Interaction turnover between networks and its determinants

To determine whether interactions and internal network structure remain spatially and temporally constant, we quantified interaction turnover. Beta diversity of interactions was calculated spatially within seasons (pairwise between sites) and pairwise for each site between seasons. Following Novotný (2009), interaction diversity was partitioned into the components that drive changes in interactions: B_p = turnover of interaction due to plant species being absent from one network; B_h = turnover of interaction due to herbivore species being absent from one network; B_{ph} = turnover of interaction due to both herbivore and plant species being absent from one network; and B_0 = both plant and herbivore species are present in both networks, but an interaction is only present in one (summarized in Table 1).

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

We 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.

We expect that most of the spatial turnover will be partitioned to B_p if plants are tightly tied to environmental niches or dispersal limited. This expectation stems from the high plant beta diversity in the CFR (Cowling 1990). Turnover in interactions driven by plant turnover 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), and this can

create divergent selection gradients which might promote insect diversification. 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 using 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.

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, and insect richness ranged from 55 to 166. We identified 263 insect morphospecies from 12 orders, including 12 Cephalelini species (745 individuals).

Mean overlap in plant species composition (Horn similarity) between sites was 0.24 (SD = 0.201), showing high spatial plant species turnover. Mean overlap in insect species composition 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. Thus seasonal absence of insect species from local communities does not necessarily imply that they are absent across the region. Both temporal and spatial turnover in insect species community composition is thus high.

Network metrics

Connectance was quite high for all networks with 20-35% of possible interactions being realised and insects tended to interact with few plant species (mean generality = 2.1; SD = 0.20) (Table 2), suggesting insects may be specialised in terms of host use, although it is possible that some of the insect morphospecies may utilize plants outside the Restionaceae. This is further supported by high levels of niche partitioning (mean $H_2' = 0.63$), where many insect species tend to associate with a different subset of the available interaction partners due to strong host specificity. Even though insects showed patterns of niche partitioning, plants were often used by a variety of insects (mean vulnerability = 7.9, SD = 3.8), which is a necessary pattern arising from community asymmetry where the number of plant species are fewer than the number of insect species in each network. All networks for all sites were more nested than expected from network size and connectivity ($p < 0.05$) (Table 2). 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.

Comparison of metrics between sites and seasons

‘This article is protected by copyright. All rights reserved.’

Paired t-tests comparing networks between seasons with sites as replicates ($n = 6$) revealed no significant differences between seasons (Fig. 2; Supplementary material Appendix 1, Table A1). There are thus no consistent changes in the emergent network structure between spring and autumn.

ANOVA and Tukey's HSD test comparing networks spatially with seasons as replicates revealed that two sites (SB & MR) differed significantly from each other in linkage density ($F_{5,6} = 6.108$, $p = 0.02$) and vulnerability ($F_{5,6} = 6.226$, $p = 0.02$), but no other significant differences between sites were present (Supplementary material Appendix 1, Table A2). Similarly, no association between changes in geographic distance and changes in network metrics was found (PERMANOVA, d.f. = 6, $p = 1.00$). Further, t-tests revealed no significant differences in emergent network structure between two geographically separated clusters of sites (Supplementary material Appendix 1, Table A3). Very limited spatial difference in network structure is thus present, but no seasonal difference (Table 2; Supplementary material Appendix 1, Figs. A1-6).

Association between emergent network structure and community composition

Changes in only one of the metrics (generality) were linearly associated with changes in insect community composition in autumn after adjusting for multiple tests (Mantel $r = 0.87$, $p = 0.003$), but since this trend was not present in another season or trophic level, and may thus be an artefact. No further associations were present (Supplementary material Appendix 1, Table A4). This is not surprising since virtually no significant differences were found between sites for any network metrics.

Interaction turnover and its determinants

Interaction turnover between seasons was high (mean $B_{cc} = 0.90$, $SD = 0.04$) and was mostly driven by 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 high (mean $B_{cc} = 0.97$, $SD = 0.04$), and was driven by turnover of plants (mean $B_p = 0.37$, $SD = 0.13$) and turnover in both plants and insects (mean $B_{ph} = 0.39$, $SD = 0.13$). Turnover in interactions due to turnover in herbivores (mean $B_h = 0.12$, $SD = 0.11$) and interaction rewiring (mean $B_0 = 0.08$, $SD = 0.08$) had a smaller influence on total spatial interaction turnover.

Both total interaction turnover (B_{cc} : $R^2 = 0.19$, $p = 0.009$) and simultaneous plant and herbivore turnover (B_{ph} : $R^2 = 0.28$, $p = 0.002$) (Fig. 3) increased with distance separating sites. In contrast, geographic distance is negatively related to herbivore turnover in the absence of plant turnover (B_h : $R^2 = 0.16$, $p = 0.015$) and also negatively related to interaction turnover with no species turnover (B_0 : $R^2 = 0.29$, $p = 0.001$).

Discussion

We constructed the first plant-herbivore networks for the CFR and revealed spatially and temporally invariant emergent network structure, suggesting that changes in insect and plant community composition are not associated with changes in network structure. However, we detected high spatial and seasonal interaction turnover despite consistent emergent network structure. Seasonally, interaction turnover is driven by turnover in insect herbivores and herbivore host switching. Spatially, interaction turnover is driven by turnover in plants and simultaneous plant and herbivore turnover, suggesting host specificity might structure network assembly in our system. This is supported by high niche partitioning ($H2'$) values (0.39-0.79) and the fact that insects were on average associated with very few plant species (generality = 2.11).

Overall, the plant-herbivore network structure remained 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 (Karttinen and Roslin 2012) networks. Here, we excluded possible latitudinal

and phylogenetic effects that may obscure the mechanisms structuring networks by sampling within a region and within a plant family, and still found invariant network structure (although our low statistical power limits inferences). 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 species interact despite changes in diversity (Morris et al. 2014). Our findings align with these large-scale studies and suggest that neither large nor fine scale differences in environmental factors or species compositional change are influencing emergent network structure. When evaluating mechanisms that structure communities, it may be more useful to assess changes in internal network structure and drivers of interaction turnover rather than using emergent network structure metrics alone. Canard et al. (2014) showed species abundances can predict network metrics, suggesting their host-parasitoid networks are to some extent neutrally structured. However, species abundances could not predict the distribution of interactions across species within networks, showing networks may exhibit some non-neutral structuring that is not captured by standard network metrics (Canard et al. 2014). Similarly, Morris et al. (2014) suggest that much of the variation in network metrics across networks could be an artifact arising from the dependence of metrics on network size, and Vázquez et al. (2009) showed that although abundance and phenology predict emergent network structure, they fail to predict the occurrence or frequency of pairwise interactions. We should thus be cautious inferring mechanisms structuring ecological systems from emergent network structure, and changes in interaction fidelity or distribution may provide more valuable insights.

Despite invariant emergent structure of plant-herbivore networks, interactions showed both spatial and temporal turnover. Seasonal interaction turnover resulted from seasonal insect turnover, or interestingly, because seasonally persistent species tended to switch interaction partners between seasons. Some insects with similar ecological traits or needs exhibit temporal niche partitioning resulting from competition and thus coexist by being active at different times of the day or year (Wolda and Fisk 1981, Tylianakis et al. 2005, Augustyn et al. 2013). Saavedra et al. (2016) recently showed temporal interaction turnover dampens the effect of changes in community composition (i.e. the removal or addition of species) on the likelihood of the persistence of predator-prey communities. 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 and 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. Our results suggest that most of the insect species on restios seem to follow this pattern of different insect species occurring in the different peak insect seasons, rather than exhibiting seasonal host switching.

Spatial changes in interactions were mostly driven by either turnover in plant species only or by simultaneous changes in insect and plant species occurrence. High turnover in plant species only could indicate insects are widespread and can utilize multiple, possibly closely related, hosts. Interestingly, if insects use different hosts in different locations, this may lead to local adaptation and possibly speciation (Nosil et al. 2002,

Winkler et al. 2009, Darwell et al. 2014, Grosman et al. 2015). Simultaneous changes in insect and plant species occurrence showed distance decay, where nearby sites 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. We know that the African Restionaceae are tightly tied to eco-hydrological niches (Araya et al. 2011) and show high spatial turnover across short distances (Kemp 2014), and thus the sharp turnover in interactions associated with particular plants is expected. In contrast, both interaction rewiring (B_0) and interaction turnover due to the absence of herbivores (B_h) decrease with an increase in distance. However, both these are contingent on the same plant species being present across communities and this trend can thus be attributed to non-overlapping plant species composition in geographically distant communities. Further, average spatial interaction rewiring was low, indicating many insects tend to utilize a potential host whenever it is available. The high interaction turnover due to plant species turnover shows many insects can utilize multiple hosts, and insect species possibly consist of locally adapted populations (Fox and Morrow 1981).

These patterns of interaction turnover for an entire community in a temperate region are surprisingly similar to the findings of Novotný (2009) who assessed tropical Lepidoptera food webs. He also found evidence for interaction turnover associated with geographic distance driven by a simultaneous change in plant and insect turnover. Lepidoptera in that tropical system exhibit host specificity and do not show dispersal limitation (Novotný 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 (Novotný 2009). Novotný et al.

(2006) suggested levels of insect herbivore specialisation do not differ between latitudes and here we show that the patterns caused by geographic changes in the internal structure of interaction networks (i.e. interaction turnover) in these temperate networks are similar to the patterns found by Novotný (2009) in tropical networks. Interestingly, Carstensen et al. (2014) found a slightly different trend for pollination networks, where all turnover components were positively related to geographic distance and Trøjelsgaard et al. (2015) similarly found a breakdown in partner fidelity with an increase in geographic distance, and thus patterns of interaction turnover may differ between network types (i.e. mutualistic vs. antagonistic).

Further, the mean network niche partitioning (H_2') 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). Although we know from previous work (Kemp 2014) that plant community composition partly predicts insect community composition in the Restionaceae, a pattern which likely results from some level of host specificity, it is quite surprising that we found such high levels of specialisation and niche partitioning when sampling herbivores from a single plant family. We further detected non-random modularity and nestedness patterns in these networks. Augustyn et al. (2016) showed restio leafhoppers (Cephalelini) exhibited strong modularity and niche partitioning structured solely by preference-performance linkage, where different leafhoppers prefer different groups of restios on which they perform best. The non-random nestedness patterns suggest specialist insects interact with subsets of the plant species which generalist interact with. This may indicate that certain plant species tend to have fewer chemical defences or are nutritionally more favourable to insects, and these plants are then utilized by a large variety of insect species. It also suggests that the most generalist insects can utilize plant species that few other insects can feed on.

The interpretation of the observed patterns, however, is limited by the low statistical power, and further inquiry is required to determine whether these trends (i.e. invariance of network structure) hold when comparing a larger number of networks spatially or temporally. Our networks were thoroughly sampled and this should decrease variance in the estimated metrics, i.e. the calculated metrics should reflect “real” results, and thus differences (or the lack thereof) between networks likely reflect true patterns. A further caveat lies in assuming all insects included in the dataset were feeding on the plants. This is likely to underestimate niche partitioning (for which we found strong support, i.e. high H_2' values and significant modularity), but the effects of this should be mitigated by excluding non-herbivorous groups from the dataset and using weighted networks in the analyses.

Although sampling at a small scale allowed us to start elucidating mechanisms that structure antagonistic ecological networks, sampling of other taxa within this region is needed to determine the generality of our findings beyond the Restionaceae. We show here that by examining the turnover of interactions, and not merely the emergent network structure, the processes shaping interactions may become apparent. Although our findings are similar to what has been found in the tropics, we suggest that the turnover of interactions should be evaluated in other regions to establish whether our results are applicable globally. We suggest that caution should be taken in pooling spatial data, even if emergent network properties seem similar. Future work should further determine what species traits are associated with interaction turnover and ideally species traits should be incorporated into networks, asking whether interaction strength is influenced by other factors such as insect size, plant phenophase or plant secondary metabolites (Violle et al. 2007, Bolnick et al. 2011). Also, interaction turnover in linked networks (i.e. networks of networks incorporating herbivores, pollinators,

microorganisms - Pocock et al. (2012), Evans et al. (2013)) could provide insight into both the direct and indirect mechanisms structuring multitrophic networks, and reveal the reciprocal effects of mutualistic and antagonistic interactions on interaction fidelity.

Conclusion

In summary, we found invariant spatial and temporal emergent network structure despite substantial changes in insect community composition and interaction fidelity. Interactions show high turnover, driven seasonally by turnover in insect species and insect host switching. Spatially the turnover in interactions is driven by turnover in both plants and insects, suggesting either insects are host specific or both groups are responding to similar environmental gradients. Variance in the distribution and identity of interactions between networks may be overlooked when only calculating emergent network metrics. We suggest that metrics quantifying emergent network properties may not be as effective as metrics quantifying changes in internal network composition (i.e. interaction turnover, distribution of specialisation, distribution of strong links, etc.) in identifying mechanisms that structure ecological systems.

Acknowledgements

We thank three anonymous reviewers for insightful comments on improving the quality of the manuscript. Funding was provided by the South African National Research Foundation (South African Biodiversity Initiative) and Stellenbosch University (to AGE) and the Erasmus Mundus (EMA2SA) program (to JEK). Cape Nature (permit 0028-AAA008-00111) and SANParks provided permits for this work.

References

- Allesina, S. et al. 2008. A general model for food web structure. - *Science* 320: 658–661.
- Almeido-Neto, M. et al. 2008. A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement. - *Oikos* 117: 1227–1239.
- Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. - *Austral Ecol.* 26: 32–46.
- Araya, Y. N. et al. 2011. A fundamental, eco-hydrological basis for niche segregation in plant communities. - *New Phytol.* 189: 253–258.
- Augustyn, W. J. et al. 2013. Specialised host-use and phenophase tracking in restio leafhoppers (Cicadellidae: Cephalelini) in the Cape Floristic Region. - *J. Insect Conserv.* 17: 1267–1274.
- Augustyn W.J. et al. 2016. Experimental evidence for fundamental, and not realised, niche partitioning in a plant-herbivore community interaction network. – *J. Anim. Ecol.* 85: 994-1003.
- Baldock, K. C. R. et al. 2011. Daily temporal structure in African savanna flower visitation networks and consequences for network sampling. - *Ecology* 92: 687–698.
- Blüthgen, N. et al. 2006. Measuring specialization in species interaction networks. - *BMC Ecol.* 6(9).
- Bolnick, D. I. et al. 2011. Why intraspecific trait variation matters in community ecology. - *Trends Ecol. Evol.* 26: 183–192.
- Canard, E. F. et al. 2014. Empirical evaluation of neutral interactions in host-parasite networks. - *Am. Nat.* 183: 468–479.

- Carstensen, D. W. et al. 2014. Beta Diversity of Plant-Pollinator Networks and the Spatial Turnover of Pairwise Interactions. - *PLoS One* 9(11):e112903.
- Cowling, R. M. 1990. Diversity components in a species-rich area of the Cape Floristic Region. - *J. Veg. Sci.* 1: 699–710.
- Darwell, C. T. et al. 2014. The roles of geography and founder effects in promoting host-associated differentiation in the generalist bogus yucca moth *Prodoxus decipiens*. - *J. Evol. Biol.* 27: 2706–2718.
- Dáttilo, W. et al. 2013. Spatial structure of ant-plant mutualistic networks. - *Oikos* 122: 1643–1648.
- Davies, D. M. 1988. Leafhoppers (Homoptera: Cicadellidae) associated with the Restionaceae, 1. The tribe Cephalelini (Ulopinae). - *J. Entomol. Soc. South. Afr.* 51: 31–64.
- Dehling, D. M. et al. 2016. Morphology predicts species' functional roles and their degree of specialization in plant – frugivore interactions. - *Proc. R. Soc. B Biol. Sci.* 283: 20152444.
- Dormann, C. F. and Strauss, R. 2014. A method for detecting modules in quantitative bipartite networks (P Peres-Neto, Ed.). - *Methods Ecol. Evol.* 5: 90–98.
- Dormann, C. F. et al. 2008. Introducing the bipartite Package: Analysing Ecological Networks. - *R news* 8: 8–11.
- Dupont, Y. L. et al. 2009. Spatio-temporal variation in the structure of pollination networks. - *Oikos* 118: 1261–1269.
- Evans, D. M. et al. 2013. The robustness of a network of ecological networks to habitat loss. - *Ecol. Lett.* 16: 844–852.

- Fox, L. R. and Morrow, P. 1981. Specialization: Species Property or Local Phenomenon? - *Science* 211: 887–893.
- Giliomee, J. H. 2003. Insect diversity in the Cape Floristic Region. - *Afr. J. Ecol.* 41: 237–244.
- Grosman, A. H. et al. 2015. No adaptation of a herbivore to a novel host but loss of adaptation to its native host. - *Sci. Rep.* 5: 16211.
- Johnson, S. D. 1992. Plant animal relationships. - In: Cowling, R. M. (ed), *The Ecology of Fynbos: Fire, Nutrients and Diversity*. Oxford University Press, pp.175-205.
- Jonas, J. L. and Joern, A. 2008. Host-plant quality alters grass/forb consumption by a mixed-feeding insect herbivore, *Melanoplus bivittatus* (Orthoptera: Acrididae). - *Ecol. Entomol.* 33: 546–554.
- Jordano, P. et al. 2003. Invariant properties in coevolutionary networks of plant – animal interactions. - *Ecol. Lett.* 6: 69–81.
- Jost, L. 2007. Partitioning diversity into independent alpha and beta components. - *Ecology* 88: 2427–2439.
- Kaartinen, R. and Roslin, T. 2012. High temporal consistency in quantitative food web structure in the face of extreme species turnover. - *Oikos* 121: 1771–1782.
- Kemp, J. E. 2014. Untangling mechanisms structuring insect diversity patterns in the Cape Floristic Region : the Restionaceae and their herbivores. MSc Thesis, available: <http://scholar.sun.ac.za/handle/10019.1/96067>.
- Linder, H. P. et al. 2003. Contrasting patterns of radiation in African and Australian Restionaceae. - *Evolution.* 57: 2688–2702.

- Memmott, J. 1999. The structure of a plant-pollinator food web. - *Ecol. Lett.* 2: 276–280.
- Morris, R. J. et al. 2014. Antagonistic interaction networks are structured independently of latitude and host guild. - *Ecol. Lett.* 17: 340–349.
- Nosil, P. et al. 2002. Host-plant adaptation drives the parallel evolution of reproductive isolation. - *Nature* 417: 440–443.
- Novotný, V. 2009. Beta diversity of plant-insect food webs in tropical forests: a conceptual framework. - *Insect Conserv. Divers.* 2: 5–9.
- Novotný, V. and Weiblen, G. D. 2005. From communities to continents: beta diversity of herbivorous insects. - *Ann. Zool. Fennici* 42: 463–475.
- Novotný, V. et al. 2006. Why are there so many species of herbivorous insects in tropical rainforests? - *Science* 313: 1115–1118.
- Novotný, V. et al. 2007. Low beta diversity of herbivorous insects in tropical forests. - *Nature* 448: 692–695.
- Oksanen, J. et al. 2016. *vegan: Community Ecology Package*. R package version 2.0-10.
- Olesen, J. M. et al. 2008. Temporal dynamics in a pollination network. - *Ecology* 89: 1573–1582.
- Olito, C. and Fox, J. W. 2014. Species traits and abundances predict metrics of plant-pollinator network structure, but not pairwise interactions. - *Oikos*: EV1–9.
- Oliver, I. and Beattie, A. J. 1996. Invertebrate morphospecies as surrogates for species: a case study. - *Conserv. Biol.* 10: 99–109.
- Pellissier, L. et al. 2013a. Turnover of plant lineages shapes herbivore phylogenetic beta

diversity along ecological gradients. - *Ecol. Lett.* 16: 600–608.

Pellissier, L. et al. 2013b. Phylogenetic alpha and beta diversities of butterfly communities correlate with climate in the western Swiss Alps. - *Ecography*. 36: 541–550.

Pocock, M. J. O. et al. 2012. The robustness and restoration of a network of ecological networks. - *Science* 335: 973–977.

Poisot, T. et al. 2012. The dissimilarity of species interaction networks. - *Ecol. Lett.* 15: 1353–1361.

Prendini, L. 1997. Two new host-restricted restioid leafhoppers of the genus *Cephalelus* Percheron (Cicadellidae: Cephalelini), with descriptions of the females of *C. brevipilus* Davies, *C. daviesi* and *C. rawsonia* Davies. - *African Entomol.* 5: 273–281.

Price, P. W. et al. 1998. Global patterns in local number of insect galling species. - *J. Biogeogr.* 25: 581–591.

Pryke, J. S. and Samways, M. J. 2008. Conservation of invertebrate biodiversity on a mountain in a global biodiversity hotspot, Cape Floral Region. - *Biodivers. Conserv.* 17: 3027–3043.

R Core Team 2013. R: A language and environment for statistical computing.

Saavedra, S. et al. 2016. Seasonal species interactions minimize the impact of species turnover on the likelihood of community persistence. - *Ecology* 97: 865–873.

Santamaría, L. and Rodríguez-Gironés, M. A. 2007. Linkage rules for plant-pollinator networks: trait complementarity or exploitation barriers? - *PLoS Biol.* 5: e31.

Trøjelsgaard, K. et al. 2015. Geographical variation in mutualistic networks : similarity ,

turnover and partner fidelity. *Proc. R. Soc. B.* 282: 20142925.

Tylianakis, J. M. et al. 2005. Spatiotemporal variation in the diversity of Hymenoptera across a tropical habitat gradient. - *Ecology* 86: 3296–3302.

Vázquez, D. P. et al. 2009. Evaluating multiple determinants of the structure of plant-animal mutualistic networks. - *Ecology* 90: 2039–2046.

Violle, C. et al. 2007. Let the concept of trait be functional! - *Oikos* 116: 882–892.

Winkler, I. S. et al. 2009. Repeated climate-linked host shifts have promoted diversification in a temperate clade of leaf-mining flies. - *Proc. Natl. Acad. Sci. U. S. A.* 106: 18103–18108.

Wolda, B. Y. H. and Fisk, F. W. 1981. Seasonality of tropical insects. II. Blattaria in Panama. - *J. Anim. Ecol.* 50: 827–838.

Wright, M. G. and Samways, M. J. 1998. Insect species richness tracking plant species richness in a diverse flora: gall-insects in the Cape Floristic Region, South Africa. - *Oecologia* 115: 427–433.

Table Legends

Table 1 Total interaction turnover (B_{cc}) can be partitioned into four components that drive the turnover of interactions. If most of the turnover is partitioned to a particular component, certain predictions about the mechanisms structuring communities can be made.

Metric	Definition	Prediction
B_{ph}	Turnover of interaction due to both herbivore and plant species being absent from one network	Herbivores track the distribution of plants and are specialized to host plants, or both groups exhibit parallel responses to environmental gradients
B_p	Turnover of interaction due to plant species being absent from one network	Plants are tightly tied to environmental niches. Herbivores can use multiple hosts, and are possibly locally adapted to different plant species
B_h	Turnover of interaction due to herbivore species being absent from one network	Herbivores are absent despite presence of potential host plants. Herbivores exhibit dispersal limitation or are filtered by environmental conditions
B_0	Both plant and herbivore species are present in both networks, but an interaction is only present in one	Herbivores have wide host-use breadth and the quality of hosts varies spatially

Accepted Article

Table 2 Metrics quantifying 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 (*) and those significantly lower than expected are indicated with a (+).

Site (season)	Linkage density		Weighted NODF		Connectance		Generality		Vulnerability		H2'		Modularity (Q)	
	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	0.10*	0.13*
MR	7.14	8.84	29.11*	23.32*	0.35	0.28	2.00	1.63	12.28	16.06	0.46	0.64	0.09*	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	0.02*	0.20*
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	0.09*	<0.01 ⁺
SB	2.66	3.00	29.31*	19.14*	0.31	0.23	1.94	1.46	3.39	4.54	0.65	0.76	0.50*	0.30*
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	0.07*	0.29*

Accepted Article

Figure Legends

Figure 1 (A) Six sites in the southwestern mountains of the Cape Floristic Region were sampled during austral autumn and spring. Circles indicate two distinct mountain areas into which sites clustered. (B) An insect herbivore (Lentulidae) clings to a restio (*Restionaceae*) culm whilst feeding.

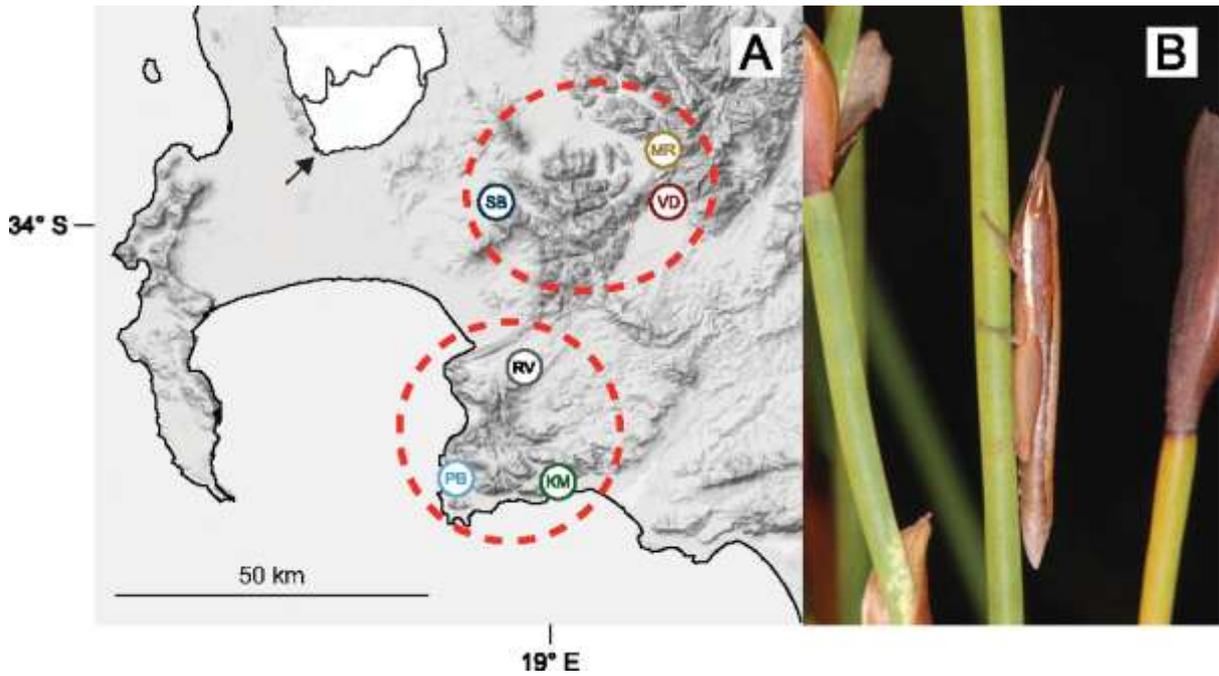


Figure 2 Linkage density, weighted NODF, connectance, generality, vulnerability, H2' (network-wide niche partitioning) and modularity between autumn and spring, and the different sites are indicated with different colours. No significant differences are present in means between seasons (Supplementary material Appendix 1, Table A1).

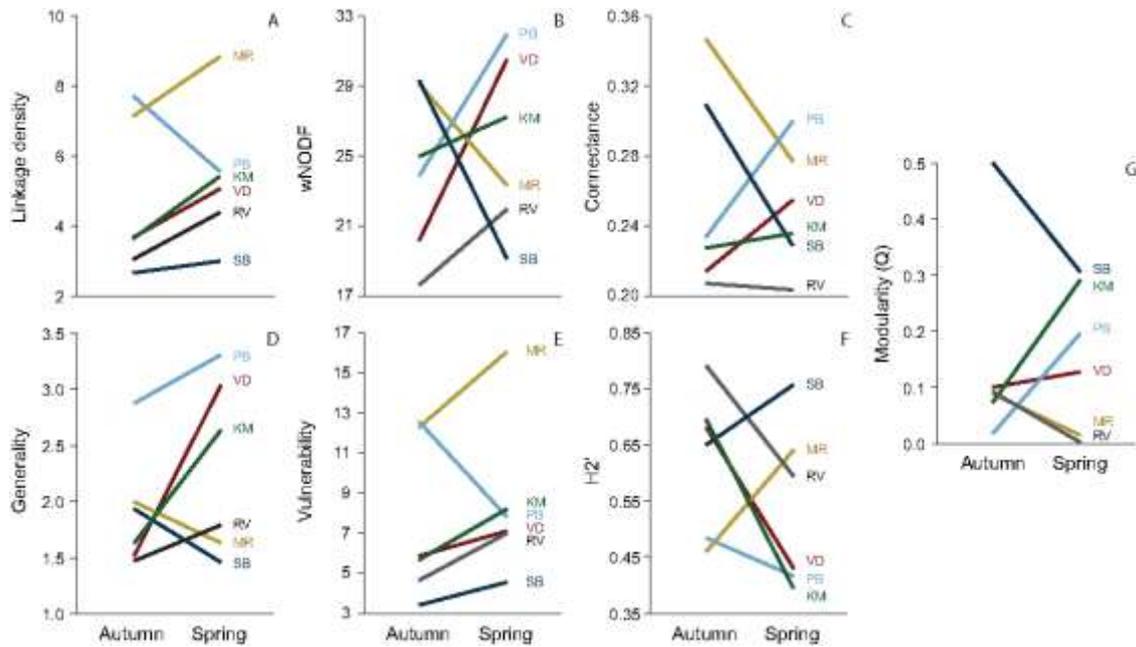


Figure 3 Regression between interaction turnover and geographic distance. All relationships shown are significant, except B_p . As distance between sites increases, more turnover is attributed to both plants and herbivores being absent (B_{ph}). This suggests plant distribution is limited by dispersal 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 .

