Quantifying patterns and processes of community assembly with co-occurrence analysis

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This thesis is presented for the degree of Doctor of Philosophy, March 2019

March, 2019

For Zsófi, Eszter, Molly, Erik, Changi, Benji, Ezra, and Cricket.

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ABSTRACT

Quantifying community assembly processes and understanding how communities respond to disturbance is a critical goal of ecology. Co-occurrence analysis is an increasingly popular tool that has been used to approach this problem, and quantifies how often a pair of species is found together with respect to a random baseline, usually calculated using possible pairs of species in an assemblage. Despite its potential to provide nuanced pairwise information, practical applications of this approach remain elusive. Associations calculated with co-occurrence do not demonstrate direct biotic interactions. Instead, relationships are often driven by combinations of several mechanisms, which can make associations appear statistically insignificant on the surface and difficult to interpret biologically. In the first chapter of this work, I begin with a short introduction to co-occurrence analysis and outline the challenges and potential advantages associated with it. In the second chapter, I confront these challenges by suggesting that co-occurrence data should be treated as a continuous probabilistic variable. I also demonstrate several ways co-occurrence analysis can untangle biological mechanisms when used in targeted comparative analyses. The third chapter provides an observational study of probabilistic co-occurrence networks, describing their structures and link weights using simulated and empirical examples. In the following chapters, I use two applications of the propose probabilistic framework to answer ecological questions about community responses to disturbance such as extinction and habitat alteration. In chapter 4, I study the end-Pleistocene North American mammal extinction to show how biotic and abiotic regulatory factors can be isolated as components of each co-occurrence. Moreover, I show that community reassembly of the surviving mammals after the extinction

was driven by biotic interactions rather than concurrent climate change. In chapter 5, I demonstrate how co-occurrence analysis can highlight changes in functional trait distribution due to habitat alteration using site inventories of Neotropical birds and bats. The methods presented here can be extended in the future to improve our understanding of multi-taxon association networks and eventually entire ecosystems. Most of the investigated taxa showed a decrease in positive co-occurrences and an increase in negative co-occurrences in response to disturbances. These patterns suggest that anthropogenic disturbance decreases the ability of species to coexist and may reduce the ability of ecosystems to self-regulate via biotic interactions. Thus, the restoration of species interactions may require more attention in conservation and management.

STATEMENT OF ORIGINALITY

I certify that the work in this thesis entitled "*Quantifying patterns and processes of community assembly with co-occurrence analysis.*" has not previously been submitted for a degree nor has it been submitted as part or requirements for a degree to any other university or institution other than Macquarie University.

I also certify that this thesis is an original piece of research and that has been written by me. Any collaboration, help or assistance has been appropriately acknowledged. No Ethics Committee approval was required.

Anikó B. Tóth

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ACKNOWLEDGEMENTS

There are countless people without whom this thesis would never have become a reality, or at the least would have been a miserable and tedious process. I am indebted to the HDR Team and the Department of Biological Sciences, particularly the Admin team, for providing me with the scholarship and facilities to pursue this research. Lara and Julian—you are both champions.

To my advisors: John. The first time we met, we instantly clicked. We spoke the same language, the language of macroecology expressed as R code. I knew then I had made the right decision traveling to Australia to be your student. Thank you for your devotion, loyalty, and patience throughout my PhD. Your office door was always open, whether I needed it once a fortnight or six times a day. In the more difficult moments of my PhD saga, I didn't even need to come to you, because you came to check on me. Throughout the entire process, you trusted me, challenged me, and inspired me in equal measure. You were the perfect advisor and exactly what I needed, and for this I can never thank you enough.

Kate. Your dedication and contributions to this thesis—and to my personal and scientific development—deserve a chapter of their own. You have gone above and beyond the call of duty repeatedly, uncomplainingly, even eagerly. You sacrificed exorbitant amounts of personal time, much of it late at night due to the difference in our time zones, to skype with me, with Pete yelling sage advice from the background. You have this stubborn determination to build my confidence, even when it is shattered, and keep me sane, even when I am not. You taught me that success is not measured in Science papers, but in good,

sound research based on deep curiosity. You taught me to collaborate with openness, respect, candour, humility, and gratitude. You taught me to take responsibility, trust myself, and cherish integrity. You have been the most dedicated and loyal mentor anyone could ever ask for. I would say that I will never forget the things I learned from you, but I am forgetful, and in any case that sounds too final. So instead, I offer my hopes that our teamwork continues for many fruitful years to come.

Drew. Although you only officially became my co-supervisor late in my candidature, your cheerful input and unparalleled understanding of statistics has been an invaluable addition to my advisory team. Thank you for letting me philosophise with you about the nerdiest, most esoteric biostatistical topics over our between-teaching lunch breaks. It really deepened my understanding of the statistical underpinnings of my work.

Kay. You were not one of my official supervisors during my thesis, but our connection is older than that and deserves a mention here. I count myself extraordinarily lucky that Liz and Gene matched me up with you and Kate all those years ago.

To my co-authors in the Evolution of Terrestrial Ecosystems working group: our meetings were always among the highlights of each year. Special thanks to Laura for being the voice of reason on many occasions, and to Nick for offering guidance born from long years of experience.

To the Paleobiology lab at Macquarie, thank you for making me feel at home among you. To Graham, Marissa, and Sarah for showing me the ropes when I was new. To Gabriel for helping me relax. To Glenn, for giving me my first teaching job, to Matt Kosnik, for your endless supply of freely shared chocolate and tea. Tom, Matt Kerr, and Amy, thank you for

listening to my rants and offering your opinions on my increasingly chaotic efforts to plot my results. Zoe, for your encouraging remarks about my writing. To the many people who took a few minutes to listen in the difficult times, I am deeply indebted to you: do not underestimate the power of your words of encouragement.

To the R users group, particularly James, Kate, Anika, Yuri, and Kaja: you gave me a community that I felt happy and comfortable in. My commitment to keeping RUG going is a direct effect of how awesome you guys are to work with. To Brian, for explaining how to version control my thesis with git and patiently putting up with me every time I harassed you with questions about it afterward.

To my friends: Silvia, this whole PhD thing was your idea—you knew me better than I knew myself. Michelle, you were my first true friend in Australia, and you have been with me through thick and thin. I can talk to you about anything, from the finer points of my research to the merits of hazelnut gelato. Timi, thanks for being the organised one who makes sure we actually hang out, and for always inspiring me to try something new, and for supplying me with your decadent homemade desserts (including hazelnut gelato). Kaja, for being my partner-in-crime and taskmaster. To Nick, for reminding me to relax; to Thomas, for keeping me honest, especially when it comes to board game rules; to Bree, for actually visiting me in the Land Down Under and So Ridiculously Far Away; to Andrew, for your persistent interruptions (I probably needed a break anyway); to Harley, for livening up every conversation; to Shorty, for having an inappropriate meme for every situation. To the Posa family: thank you for taking me in when I first arrived in Australia and treating me like family.

To my family: Emese, Steve, Peter, Jess, and family—you gave me a home away from home and a surrogate family that I know I can rely on. I will never be able to repay you for all you have done for me. To Ildikó, Gyuszi, Szilárd, Áron, and Attila, thank you for always having your doors and your hearts open, no matter the situation and no matter the distance. To Endre and Emily, having you and the kids within hailing distance has been a wonderful and rewarding experience. Thank you for everything—the visits, the holiday, the dinners and play dates, the guidance and support, for checking in regularly, and much, much more. I look forward to more of the same.

Ildikó and Emőke, you are the best sisters a girl could ask for, and your endless love and encouragement over the years has a special place in my heart. Ildi, thank you for supplying me with all my favourite workout clothes, and Emő, thanks for sending me on all the best adventures. Mom and Dad: you mean more to me than words can express, and I owe you everything. Thank you for motivating me to follow my passion, supporting me in my decision to come to Australia, and being involved throughout the long process of preparing this thesis. Mom, you are probably the only person on the planet that will ever read my entire thesis voluntarily.

And finally, my deepest gratitude to my partner, Oscar. You've spent hours watching me fidget while I practiced my presentations, and then told me not to fidget. You have made me laugh (and hiccup while laughing) more than anyone I've ever met. You inspire me, push me, and challenge me. You've made my Fear Of Missing Out stronger than my fear of falling. You have experienced the best and the worst of me, and you have fought for me, unwaveringly, through it all. You've broadened my perspective, and taught me more about

life and relationships than I ever could have imagined. You have made these past three years not just a journey in a beautiful tourist destination, but an adventure in paradise. Thank you.

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CHAPTER 1

General Introduction

Natural ecosystems harbour diverse, interacting communities of plants, animals, fungi, and microbes. The processes by which these ecosystems assemble and disassemble are varied and complex, involving environmental influences such as rainfall and interactions between species. Understanding how ecosystems and local communities come to support many coexisting species, and how this changes over space and time is a central focus of macroecology. Such information is vital to both humans and animals, because ecosystems support our very existence: they provide sustenance through intricate mechanisms such as pollination, they decompose, recycle, and circulate nutrients, cleanse toxins, and provide myriad other services that we often take for granted.

In today's rapidly changing world, human influence has altered over 75% of the terrestrial landscape and appropriated most of its resources (Ellis and Ramankutty 2008), caused extinctions that reshaped the faunal body mass distributions of entire continents (Lyons et al. 2004; Lyons and Smith 2013), and downgraded the trophic regulation of global ecosystems (Estes et al. 2011; Ripple et al. 2016). Understanding how assemblages of plants and animals respond to these disturbances over various scales is therefore a pressing question. For example, how might human disturbance alter the resilience of forest bird communities? On long temporal scales, how might survivors of a major extinction event shift their assembly patterns in response to the disappearance of the extinction victims? Is the new community structure of survivors reflected in local diversity or community resilience?

BIOTIC INTERACTIONS AS A DRIVER OF COMMUNITY ASSEMBLY

Increasingly, researchers in ecology, paleoecology, and conservation are expanding their focus to take in complex systems, showing how species relationships and interactions

determine occupancy (Jeffers et al. 2015; Kraft et al. 2015; D'Amen et al. 2018; Serván et al. 2018; Gravel et al. 2018), support ecosystem services (Kaiser-Bunbury et al. 2017), and promote biodiversity (Levine et al. 2017). One study even proposes that environmental filtering should be invoked only when abiotic factors prevent the establishment and growth of a species in the absence of biotic interactions, and suggest a framework to isolate the role of the environment (Kraft et al. 2015). Several studies test frameworks for unravelling the effects of dispersal, environmental filtering, and biotic interactions on species distributions and coexistence (Blois et al. 2014; Weinstein et al. 2017; D'Amen et al. 2018).

Answering these types of questions comes with a unique suite of challenges. First and foremost, our ability to understand the nuances of community structure depends on the data that is available. While the quality of and access to climate and biogeographic data is improving exponentially, local species data must still be collected by field sampling, which takes a tremendous amount of money and effort. Simple occurrence data are much easier to collect than abundance estimates, and are therefore much more readily available. Comparing these sampling types for species monitoring (Joseph et al. 2006; Dibner et al. 2017; Ward et al. 2017) and adapting species assessment methods to work with presence-absence data (e.g. (Valle et al. 2018), is an active area of research. Moreover, direct species interaction data is usually central to understanding the role of interactions in the spatial distribution of species, and this type of data is even more difficult to collect, because it requires direct observation of interactions at local scales.

A second challenge is that there is a range of spatial and temporal scales we need to monitor. Long-term temporal trends are rarely considered in depth, as ongoing monitoring studies are still rare worldwide due to limitations imposed by funding cycles and human

lifetimes (Lindenmayer et al. 2012). Experts have pointed out the critical importance of understanding community change on longer time scales, suggesting that studies integrating modern and palaeoecological analyses should be a priority (Blois et al. 2013; Barnosky et al. 2017). One such study showed that human impacts likely began to alter ecosystems thousands of years ago, suggesting that modern ecology represents a novel state in geologic history (Lyons et al. 2016). Unfortunately, differences in the scale, resolution, collecting method, and taphonomy of paleontological data mean that studies integrating fossil and modern ecology are the exception, not the rule. Furthermore, biotic interaction data for fossil assemblages traditionally comes from indirect evidence such as dental morphology (Pineda-Munoz et al. 2016), herbivory damage on macro-plant fossils (Labandeira and Currano 2013), or predictive models (Pires et al. 2014, 2015). Some types of interactions can only be recorded in the unlikely event that their occurrence was fossilised (e.g. a plant being found with its pollinator or an animal fossilised with its parasites in situ, or fossilised stomach contents).

There is also increasing interest in studying large spatial scales in a search of useful generalisations (McGill 2019). Although there is evidence that biotic interactions influence community assembly at large spatial scales (Araújo and Luoto 2007), it can be difficult to detect their spatial effects across landscapes due to scaling effects (Araújo and Rozenfeld 2013), and direct interaction data collected locally does not scale easily to regions and landscapes. Shifts in community structure may change in opposite ways depending on the spatial scale and resolution of the data (Chase et al. 2018), often making it difficult to draw practical, broadly applicable conclusions.

A third major challenge in scaling up to ecosystem-level analyses is that methods for addressing biological questions in systems with many interacting components are still being

developed. Network analysis is one approach that lends itself intuitively to the study of ecological systems with many interacting components. Studies of biotic interactions, (particularly trophic relationships) utilising some form of network analysis to evaluate the properties of assemblages (particularly stability) have been around for many years (Pimm 1984; Montoya et al. 2006), although recent increases in data availability and computing resources have opened rapid progress in the field (Proulx et al. 2005). Networks have since been used to study how plant-pollinator interactions (Sauve et al. 2015; Kaiser-Bunbury et al. 2017), mutualistic interactions (Rezende et al. 2007; Bascompte and Jordano 2007; Bastolla et al. 2009; Thébault and Fontaine 2010; Suweis et al. 2013; Dáttilo et al. 2013, 2016; Rohr et al. 2014; Welti and Joern 2015; Minoarivelo and Hui 2016), seed-dispersal interactions (Timóteo et al. 2018), and parasitic interactions (Poisot et al. 2012) relate to community structure, dynamics, diversity, and response to disturbances.

Though such research is traditionally limited to examining the role of one type of interaction (e.g. pollination or herbivory), several studies have recently taken steps to analyse the structure and stability of multilayer networks which incorporate multiple interaction types, and have the potential to add spatial and temporal dimensions (Pilosof et al. 2017). For example, several studies examine how the plants on a farm site in the UK connect herbivory and pollination networks (Pocock et al. 2012; Sauve et al. 2015), and another examines modularity in an herbivore-plant-pollinator network from Germany (Astegiano et al. 2017). A series of reviews and theory papers also promote the potential uses of multilayer networks in ecology (Pilosof et al. 2017; García-Callejas et al. 2018; Hutchinson et al. 2019). While biotic interaction networks have proved a popular and fruitful area of research, they still have limitations. Data must be collected on local scales with tremendous effort, and these local

networks do not scale up easily; rather, they must be observed again at different places and times. Moreover, antagonistic interactions that are often fundamental to determining species distributions, such as competition, are difficult to study in network form, although the effect of interspecific competition has been examined in multi-species systems (Jeffers et al. 2015; Ulrich et al. 2017; Elsen et al. 2017; D'Amen et al. 2018). Furthermore, they typically—with the exception of some food webs—focus on bipartite interactions, for instance, a multilayer herbivore-plant-pollinator network can be used to analyse the relationship between pollinators and herbivores and plants, but it ignores the relationships among the plants, among the herbivores (e.g. insects), and among the pollinators (e.g. insects and birds), which constitute entire quadrants of the adjacency matrix that may be subject to confounding competitive, trophic, and parasitic interactions. If we want to understand community assembly on large scales, the spatial outcomes of all possible interactions between species and taxa, as well as their abiotic environment and temporal dynamics, must be taken under consideration, but collecting that much interaction data (including historical and/or fossil data), or even building such models from simulated interactions, seems like an insurmountably complicated task.

CO-OCCURRENCE ANALYSIS

One increasingly popular approach for quantifying community structure that presents a potential solution to the problem is co-occurrence analysis. It uses simple presence-absence data from multiple sites across a study area to quantify the association of each species with each other species in an assemblage. More specifically, it asks whether each pair of species coexists (i.e. occurs together in the same sample) more or less often than expected if they

were randomly distributed. The resulting relationships (called 'associations') can be integrated into a co-occurrence network representing spatial community structure. See Table 1.1 for the definitions of these and other terms that are central to this thesis. Though it is a relatively simple approach, co-occurrence analysis has the potential to reveal a detailed snapshot of community structure for the scales on which it is calculated. Co-occurrence networks can reveal changes in community structure that are more subtle than a decrease in richness or beta-diversity (Kay et al. 2017). They can also elucidate species-specific changes (e.g. does a species become more or less connected to others after a disturbance?) and hint at the role of each species in the greater system (Berry and Widder 2014; Borthagaray et al. 2014). Because of its basic data requirements, co-occurrence analysis can be run on modern as well as paleontological community data (Blois et al. 2014; Lyons et al. 2016). Co-occurrence intrinsically incorporates information about spatial variation in species coexistence, making it an ideal tool for extracting general, consistent patterns from occurrence data. But even more fundamentally, co-occurrence analysis starts with the spatial relationships of all species on a broad scale and works backwards to infer processes and mechanisms. This is advantageous because it does not constrain the assembly mechanisms (i.e. environmental filtering, biotic interactions, dispersal) that may be included and is thus useful for analyses that wish to consider several interacting factors (Freilich et al. 2018). Because it focusses on the observed spatial outcomes of all overlapping processes, it is readily applicable to the real world where unknown or unmeasured variables are often important.

Despite all of its advantages, several studies advise caution on the use of co-occurrence analysis, citing the challenges that still remain. Associations are not interactions, and should not be interpreted as such without additional data (Freilich et al 2018), though

efforts are underway to translate between the spatial patterns of co-occurrence and biotic interactions (Morueta-holme et al. 2016; Harris 2016). In fact, associations are nothing more than statistically postulated relationships which do not have an objective biological interpretation. Associations give important information about the spatial relationships between species, but they do not identify the biological mechanisms driving the associations, and are therefore difficult to utilise in applied situations.

One area where co-occurrence network analysis shows promise is in comparative studies. While it can be difficult to extract biological conclusions about a system from one co-occurrence network, it can be very informative to compare networks across space (i.e. in disturbed and undisturbed sites) or time (i.e. before and after a disturbance event at the same sites). Several studies have used co-occurrence network analysis to study changes in community structure in response to disturbances (Veech 2006; Araújo et al. 2011; Lane et al. 2014; Lyons et al. 2016; Kay et al. 2017). Unfortunately, there are a plethora of ways to calculate co-occurrence, and researchers often use several such metrics to test the robustness of their results. Standardisation of these methodologies is sorely needed, a task that is beyond the scope of this thesis despite a comparison of three methods included in the supplementary materials for Chapter 2. Although co-occurrence studies often report observations about network metrics, many struggle to apply these to test biologically relevant hypotheses. This is partly because the structure of ecological networks varies across interaction types (Thébault and Fontaine 2010) and across space (Poisot et al. 2016), so interpreting changes in network structure has proved challenging (e.g. what does it mean when node centrality decreases?). In addition, little has been done to systematically document and describe the structure of co-occurrence networks for various taxa and spatial scales, leaving us without

baselines for comparison. Finally, studies rarely acknowledge that each individual co-occurrence is a product of several overlapping processes. Existing methods typically categorise pairs according to the most likely driving process, even in cases where they cannot distinguish between them (Blois et al. 2014; D'Amen et al. 2018).

AIMS

The remainder of the current work aims to confront these challenges and demonstrate how co-occurrence can be used to answer biologically important questions about the mechanisms of community assembly, integrate modern and paleoecological data, and identify useful generalisations over regional and continental scales. The second chapter identifies trends in current uses of co-occurrence analysis, and extends and refines current methodologies, highlighting a modelling approach that can shed light on co-occurrence mechanisms. It also introduces the concept of a probabilistic co-occurrence network, which treats the strength of co-occurrence as a continuous variable. Traditional methods use a threshold approach to distinguish significant and random associations, but the approach outlined in this chapter allows researchers to make similar calculations without having to worry that a different threshold might overturn their results. I use an empirical dataset of mammals from East Africa to demonstrate its use for targeting questions about the drivers of community assembly and how it has changed over time.

Table 1.1. A brief glossary of terms that are central to this thesis.

Term	Definition
Coexistence	A general term that is used to describe a relationship between two species in which they are able to co-occur and perhaps even share resources.
Co-occurrence	When two species are found to be present at the same site.
Co-occurrence analysis	A set of statistical calculations which describes the frequency of co-occurrence between every possible pair of species in an assemblage with respect to a random, or expected, baseline. The output of a co-occurrence analysis is generally in the form of a distance matrix.
Network analysis	Any analysis that involves the structure or properties of a network, i.e. a set of nodes connected by edges.
Interaction network	A network in which the nodes are species and edges represent some type of interaction (e.g. biotic interaction such as predation) between species.
Bipartite network	A network in which only the interactions/relationships between two distinct groups of nodes are considered (e.g. the interactions between herbivores and the plants they eat, to the exclusion of any interactions among the plants or among the herbivores). One common example is a plant-pollinator network.
Co-occurrence network	A network in which the edges represent relationships that correspond to the output of a co-occurrence analysis.
Association	A general term for the relationship between a pair of species that is calculated with co-occurrence analysis. An association is a hypothetical statistical construct rather than a biological reality, but may correlate with or indicate the existence of a biological relationship. In this thesis, I argue that such associations should be analysed as probabilities.
Aggregation	An association in which the species pair is found together more often than expected by chance.
Segregation	An association in which the species pair is found together less often than expected by chance.

PRACTICAL APPLICATIONS

The third chapter documents the associations of North American mammal assemblages across the end-Pleistocene, Holocene, and last 2 ka (effectively, the Recent). This covers the critical Pleistocene-Holocene transition interval, which is marked by a mass extinction event that claimed almost all mammals greater than 40 kg (Martin and Wright 1967). The Pleistocene-Holocene transition was also a time of warming climate, driving a reduction of the glaciers from the previous ice age. In this section, I explore how co-occurrence analysis can be used to identify the biotic and abiotic components of species associations and how the extinction event impacted the community structure of survivors. I ask whether changes in abiotic conditions or biotic interactions are responsible for community assembly shifts across the Pleistocene-Holocene transition by comparing mutual niche co-occurrence patterns to overall co-occurrence patterns in pairs of mammals, predicting that both processes play integral roles in community analysis that leaves a detectable signature on the co-occurrence patterns of species pairs.

My fourth chapter tackles another applied problem: the effect of habitat alteration on assemblages of birds and bats in the Central and South American tropics. It presents a comparative analysis of the co-occurrence structures of these taxa in altered and unaltered habitats. Using basic diet information, I demonstrate how co-occurrence analysis can reveal the effect of habitat alteration on the outcomes of resource sharing and competition in Neotropical birds and bats.

Further development and application of these methodologies, with a focus on elucidating biological mechanisms, can have a wide variety of practical conservation

applications. From building a fundamental understanding of community assembly and structure, we will be better able to assess the health of species assemblages. Co-occurrence analysis can also provide a simple way to monitor the effects of conservation and management decisions over time. Eventually, networks encompassing pairwise associations across several taxa (e.g., birds, trees, and insects) may provide similar insights into entire functioning ecosystems.

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CHAPTER 2

Probabilistic co-occurrence analysis for evaluating community assembly processes

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ABSTRACT

Co-occurrence-based associations can be used to detect spatial and temporal changes in community assembly patterns. Most current methods for identifying co-occurrences are based on conventional hypothesis testing, which categorises pairs of species as significantly or randomly associated based on a canonical significance threshold. However, standard network metrics such as modularity and connectivity vary with the choice of threshold. Because co-occurrences are probabilistic relationships, we argue that the probabilistic framework proposed by Poisot *et al.* (2016) is readily applicable to co-occurrence networks, and confers several advantages. We go on to use co-occurrence probabilities as the response variable in a linear modelling approach and present a case study of East African mammals to show how it yields insights into the processes driving community assembly. Carnivores, herbivores, and omnivores present different assembly patterns, with carnivores more likely to exclude one another. Because of variable rates of exclusion across dietary groups, however, competitive exclusion is only weakly detectable in overall community assembly patterns in the presence of other ecological processes. In addition, the rate of exclusion may have decreased over time. Weak links, which are discarded in traditional networks, can be integral to interpreting assemblage structure, especially when there is high compositional turnover or when multiple interacting factors are influencing the co-occurrence of a pair. The probabilistic approach is the logical way forward for co-occurrence analysis.

MAIN TEXT

Introduction

Much of macroecology focuses on explaining the composition and richness of communities across geographic space. Frequently, this question has been approached by modelling species occurrences against environmental variables. A few years ago, the idea that biotic interactions could play an important role in the spatial distribution of species, even on larger spatial scales, began to gain traction in the scientific community (Araújo and Luoto 2007; Araújo and Rozenfeld 2013). The idea was not new: the notion of studying the relationships between the spatial distributions of pairs of species was pioneered by Diamond (1975) based on his observations of bird populations on islands. This work sparked a decades-long debate about whether co-occurrence patterns are meaningful or driven by chance (Connor and Simberloff 1979; Diamond and Gilpin 1982; Gotelli and McCabe 2002).

These developments led to a promising wave of research seeking to integrate the concepts of environmental and ecological niche modelling (Gravel et al. 2018; Dehling and Stouffer 2018). One popular approach to this challenge is to build joint species distribution models (JSDMs), which incorporate residual covariance of species occurrences into environmental distribution models to account for the effect of biotic interactions on species distributions (Pollock et al. 2014). Another method, and the focus of this work, involves analysing pairwise species co-occurrence (Araújo et al. 2011; Steele et al. 2011; Lane et al. 2014; Morueta-holme et al. 2016; Kay et al. 2017). Requiring only presence-absence observations, co-occurrence analysis quantifies the tendency of species to be found at the same sites with respect to a null model. This approach has been used to study the effects of

climate change (Araújo et al. 2011), habitat loss (Lane et al. 2014), interspecific competition (Kamilar and Ledogar 2011), anthropogenic landscape modification (Kay et al. 2017), and events in Earth history (Lyons et al. 2016; Villalobos et al. 2016) on the structures of various assemblages. To face the challenges of a changing world, methods that facilitate bridging the gap between modern and paleontological community data are urgently needed (Blois et al. 2013; Barnosky et al. 2017), and co-occurrence analysis is a possible way forward (Blois et al. 2013).

Despite its potential, co-occurrence methodologies have encountered several obstacles. Many methods exist for the quantification of co-occurrence patterns, and they do not always agree, making information from diverse studies difficult to integrate. A direct biotic interaction between a pair of species cannot be demonstrated with co-occurrence data alone (Freilich et al. 2018). This is in part because the co-occurrence of species pairs can be influenced by factors other than direct interaction, such as shared environmental preferences, dispersal limitation (Blois et al. 2014; Weinstein et al. 2017; D'Amen et al. 2018), phylogenetic distance (Krasnov et al. 2014), and even higher-order interactions with a third species (Levine et al. 2017). Furthermore, the co-occurrence patterns of species pairs can change over space, particularly along ecological gradients (Bar-Massada and Belmaker 2017). How, then, can co-occurrence data best be used to answer real-world biological questions?

In this paper, we tackle three main objectives. We begin with a discussion of current trends in the use of co-occurrence analysis, and point out how the use of significance thresholds has restricted the accuracy of popular approaches. We will then examine an emerging alternative approach to co-occurrence that uses association probabilities, rather than statistically significant associations alone, as the underlying data in analyses. Finally, we

present a case study of a practical application of probabilistic co-occurrence to an example dataset.

Current trends in co-occurrence analysis

Our goal in this section is to discuss trends in recent co-occurrence analyses and highlight their strengths and weaknesses. This is not an exhaustive review; instead, we use a small, diverse sample of recent co-occurrence studies to identify certain commonalities.

Strengths. With the increasing availability of high-performance computing, network analysis has become very popular in ecology (Proulx et al. 2005), and this development is evident in the field of co-occurrence analysis. Co-occurrence networks have been constructed for birds (Araújo et al. 2011; Lane et al. 2014; Tulloch et al. 2016), mammals and amphibians (Araújo et al. 2011), trees (Morueta-holme et al. 2016), animals in general (Borthagaray et al. 2014), microbes (Steele et al. 2011; Barberán et al. 2012; Berry and Widder 2014), and reptiles (Araújo et al. 2011; Kay et al. 2017). This approach involves applying a significance threshold to co-occurrence calculations, which means removing weak associations that do not meet the significance criteria. There are two main advantages of this approach. First, it allows the construction of networks with binary links (yes/no), which can then be described or compared using common metrics of network structure and node characteristics. Second, the number of pairwise associations in an assemblage increases exponentially with the number of species, and this pruning procedure reduces associations to a more manageable number (Morueta-holme et al. 2016), even for highly diverse assemblages.

Another promising trend is the use of co-occurrence-based data as the response variable in linear regression models. Depending on the experimental unit (which can be

species, pairs, or networks), these data can be anything from the strengths of associations to node level metrics such as degree, to network/matrix level metrics such as modularity or nestedness. For example, Kamilar and Ledogar (2011) tested matrix-level checkerboard scores of various primate dietary guilds from ten tropical regions. Berry and Widder (2014) tested the effect of 'keystone-ness' (a measure of species removal effect derived from network simulations based on the Lotka-Volterra model) on various node level metrics, including degree and betweenness centrality, in microbial co-occurrence networks. Morueta-Holme et al. (2016), among several similar analyses, tested the effect of phylogenetic distance and trait distance on the link strength of significant pairs of North American trees. This approach has also been used in biotic interaction networks. For example, Thébault and Fontaine (2010) tested network level metrics against interaction types and noticed that herbivory networks are more modular while pollination networks tend to be nested. The advantage of this approach is that each analysis answers a specific, applicable biological question, as with all of these studies. Specifically, Kamilar and Ledogar (2011) asked whether diffuse interspecific competition for food affects community structure in primates. Berry and Widder (2014) were looking for an easy way to identify keystone species from node characteristics. Morueta-Holme et al. (2016) asked whether similarities or differences in species traits affect the spatial distribution and coexistence of trees.

Weaknesses. Although significance thresholds that are chosen by convention are a familiar concept to most ecologists, co-occurrence analysis requires so many individual calculations that even very low error rates can yield large numbers of false positives and false negatives (Morueta-holme et al. 2016). Furthermore, the choice of significance threshold can strongly influence 'emergent' network properties (Poisot et al. 2016), such as modularity (see Chapter

3). Negative associations tend to be weaker than positive associations (henceforth: segregations and aggregations) in ecological datasets due to the sparseness of occurrence matrices. Therefore, studies that count links or calculate network connectivity usually report more aggregation than segregation (e.g., Veech 2006; Barberán et al. 2012), although segregation can be dominant in some strongly competing communities after controlling for environmental variables and dispersal (e.g., Camarota et al. 2016). As a result, researchers have occasionally gone to great lengths to devise non-arbitrary thresholds for species associations (e.g., Gotelli and Ulrich 2010). However, even this has attracted criticism (e.g., von Gagern et al. 2015).

Finally, binary (yes/no) associations between species are poor descriptors of relationships that vary over space, a feature that has been repeatedly pointed out for biotic interactions (Poisot et al. 2015, 2016) and is certainly applicable to co-occurrence-based associations (Bar-Massada and Belmaker 2017). This is because generating binary associations ignores spatial variation, downgrades the information contained in the data, and has a strong tendency to misrepresent rare events (Poisot et al. 2016).

We propose that co-occurrence analyses should follow Poisot et al. (2016) by shedding significance thresholds and working with the (two-tailed) probability that the species pair's association deviates from random expectations, which we will refer to as the association 'strength'. We discuss three metrics suited to this task in the next section.

Probabilistic co-occurrence analysis

Why is it better? Working with the full distribution of co-occurrence scores confers several distinct advantages. In the traditional approach, pairs of species with weak patterns of co-

occurrence across the landscape (e.g., with a number of mutual occurrences similar to the null expectation) are discarded as random. But co-occurrence patterns over large spatial scales are almost certainly influenced by multiple overlapping processes and variables (Presley et al. 2009, 2010), including environmental filtering, dispersal, and biotic interactions. If several processes are acting in opposition, we would expect species pairs to have weak overall co-occurrence scores, and throwing out weak pairs would compromise our ability to measure those processes, if they happened to be focal to our analysis.

Consider Fig. 2.1, which depicts Holocene occurrences of coyotes (*Canis latrans*) and grey wolves (*Canis lupus*) between 2 and 11 thousand years ago in the conterminous United States. Because mutual and separate occurrences of these two species are both frequent, significance thresholds would discard this association. However, it appears that there may be spatial variation in the co-occurrence of this pair. Coyotes are found throughout the U.S., while wolves occur only in the midwest and west. Where their ranges overlap, coyotes are rarely found without the co-occurrence of wolves. These patterns suggest that there may be environmental and/or dispersal filters that drive differences in the ranges of these two species, while another process (e.g., sharing of prey or low human population density) brings them into close proximity where their ranges overlap. Geographic patterns aside, coyotes and wolves are ecologically similar carnivores that certainly interact (Merkle et al. 2009). Wolves have been known to kill coyotes (Levi and Wilmers 2012), and coyotes routinely scavenge wolf kills for food, resulting in direct competition (Berger and Gese 2007). Although this pair fails the statistical test for non-randomness overall, an analysis of the pair in several smaller regions (e.g. eastern, central, and western USA) could show that the pair has significant patterns of co-occurrence that vary in response to a variable of interest.

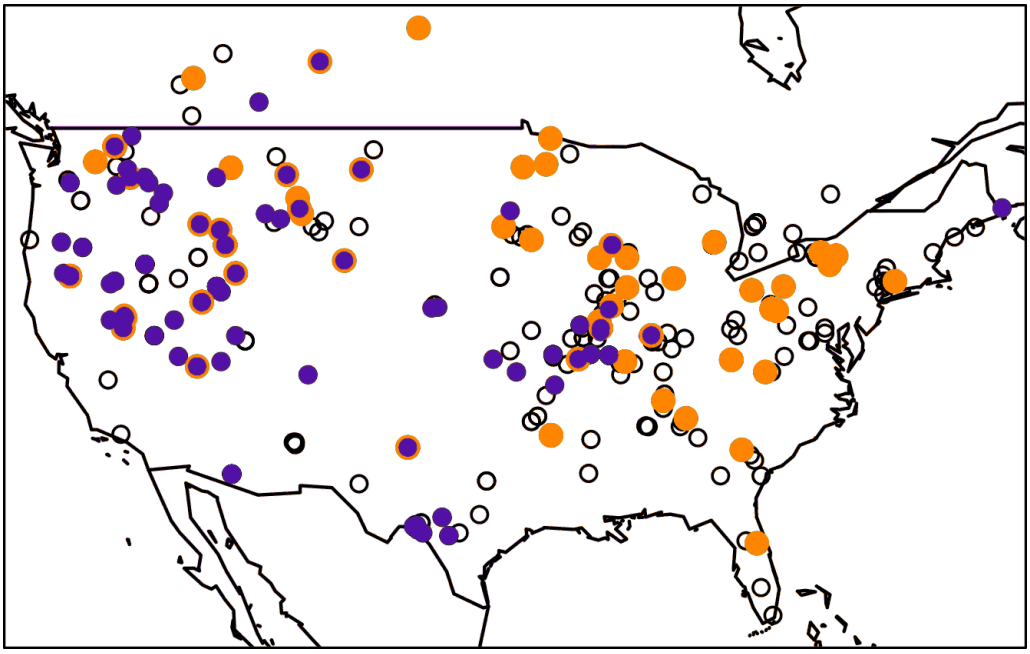

Figure 2.1. Fossil occurrences of coyotes (*Canis latrans*), indicated by filled orange circles, and grey wolves (*Canis lupus*), indicated by filled purple circles, between 2 and 11 thousand years ago in North America. Open black circles indicate sample localities where neither species was sampled. Data extracted from the FAUNMAP II database (Graham and Lundelius 2010).

But the retention of weak associations is also important for other reasons. When searching for mechanisms, explicitly including information about which species do not associate strongly will produce stronger evidence for our conclusions. Consider the primate diet study (Kamilar and Ledogar 2011), which showed that primates within dietary guilds had more checkerboard occurrences than expected by chance, and attributed this to interspecific competition. Based on basic experimental theory, we argue that this analysis would have benefitted greatly from a control, namely, pairs of primates in different diet categories. Demonstrating that pairs of primates competing for food segregate more than pairs that do not compete for food would confer much stronger evidence that food competition is responsible for segregation than merely demonstrating that competing pairs segregate more than the random expectation. After all, we have not shown that non-competing pairs do not

also segregate more than expected by chance (e.g., due to fine gradations in habitat preferences, territoriality, or other ecological processes). To achieve this, the primate study would have had to be run with pairs as the experimental unit, and weak associations would need to be retained so that the 'true' strength of associations in each group could be measured.

In the keystone species study (Berry and Widder 2014), node-level metrics were calculated after a significance threshold was used to remove weak links. Although this is conventionally a perfectly acceptable procedure, our prior discussion of metrics based on binary networks begs the question of whether a different threshold would have yielded different node characteristics. Fortunately, a suite of network metrics tailored to probabilistic data already exists (Poisot et al. 2016), and has been shown to more accurately represent network structure than the analogous measures calculated on binary networks (Poisot et al. 2016). Our third example, the comparison of tree co-occurrence strength to trait and phylogenetic distance (Morueta-holme et al. 2016), used 0s for the strengths of non-significant pairs. Given the high numbers of false positives and negatives in significance-based co-occurrence analysis, however, fitting a linear regression to the continuous data will remove the possibility that pairs are placed in the wrong significance category, and allow pairs that would be false negatives to be taken at face value instead. This is not to say that the modifications suggested here would overturn existing result. However, we do believe they represent improvements in experimental design.

We are aware of two studies that did use a probabilistic approach. Krasnov et al. (2014) examined the association strength of flea species on hosts with respect to phylogenetic distance, and found that closely related species aggregate more than expected. Bar-Massada

and Belmaker (2017) examined the association strength of North American trees with respect to habitat suitability of each species in the pair. They found more segregation when habitat suitability is high, meaning that species tend to segregate from others in the middle of their ranges but become more tolerant of aggregations near their range margins. This study is a powerful illustration of how co-occurrence patterns can change through space, and how these patterns can be explored through a modelling approach.

***Probabilistic co-occurrence metrics*.** To generate probabilistic co-occurrence values, we need a metric that can be calculated from occurrence data and which places species pairs on a continuum from negatively to positively associated based on a null model, and whose output values fall between 0 and 1, such that they can be interpreted as probabilities. Several such metrics already exist. Examples include the mid-P variant of Fisher's Exact Test (FETmP; Berry & Armitage, 1995; Kallio et al., 2011) and the sample-standardised effect size of Fisher's Exact Test (Veech 2013; Arita 2016; Griffith et al. 2016). The standardised effect size of the C-score (Stone and Roberts 1990; Gotelli and Ulrich 2010) is also appropriate (see supplement). Following an adapted version of Gotelli's (2000) methodology for evaluating co-occurrence techniques (see supplementary materials), we tested the performance of each of the three metrics. We use FETmP throughout the remainder of this paper because it assigns reliable weights and is analytical rather than randomization-based. Thus, it is less computationally expensive than calculating the similarly accurate C-Score effect size.

***Implementation*.** One issue remains which we have not yet covered: traditional hypothesis tests have strict assumptions about data independence, normality, and variance of the model variables. The latter two can usually be fulfilled by performing data transformations, but in the case of co-occurrence analysis, pairs of species are necessarily non-independent. This is

an issue which is not yet fully resolved. The North American tree study (Bar-Massada and Belmaker 2017) used an immense dataset that allowed the authors to construct 200 totally independent analyses, thus giving them access to 200 independent data points per species pair. Such a large dataset is not readily available for most systems, particularly given our objective of designing analyses that can be used across fossil data. Usually, we have one distance matrix and we want to test for differences between sections of that matrix. The cell values in such a matrix are non-independent, and therefore a standard test such as a t-test would not be appropriate. The parasite network study (Krasnov et al. 2014) used a Mantel test instead, which is the convention for the comparison of pairwise distance data, but at least one other paper opted not to correct for non-independence, citing the high Type I error rates of Mantel tests (Morueta-Holme et al. 2016). In high-diversity systems with many pairs, small differences may indeed be identified as significant by a Mantel test. However, our observation of empirical networks reveals that the metrics of empirical co-occurrence networks are strongly constrained with respect to the simulated range of possibilities (see supplement), so even relatively small changes may represent biologically meaningful changes in network structure. We suggest that a Mantel test, or permutational ANOVA for categorical data, is a suitable significance test, with the caveat that its performance when using co-occurrence and network data requires additional research, and could require a more stringent significance threshold.

Four examples with association strength

In this section we will illustrate the use of co-occurrence strengths in linear regression models with categorical independent variables, without the need to construct actual

networks. We focus on this application as it forms the basis for the following chapters of this thesis. However, continuous explanatory variables (such as phylogenetic distance) could be used just as easily. This approach can also be extended to node-level or network-level metrics to species or assemblage characteristics as the response variable.

There are four main ways that co-occurrence strengths can be grouped for models. First, species can be grouped based on functional characteristics such as body size, life form, body proportions, or trophic level. Comparing species subsets can answer questions about the differences that functional roles play in the process of community assembly. For instance, do pairs of carnivores tend to co-occur more than pairs of herbivores? Do solitary species form weaker spatial patterns than gregarious species? Comparisons between species subgroups can indicate whether or not the splitting variable has any bearing on community assembly.

Second, pairs of species (rather than the species individually) can be grouped based on the interaction or distance between their respective functional characteristics (Krasnov et al. 2014; Morueta-holme et al. 2016). Comparing subsets of pairs can answer questions about how similarities and differences in species functional roles contribute to community assembly. For instance, competition theory predicts that species that eat different types of food should be able to coexist more readily than species that compete for food in high-diversity areas. Comparing pairs that share a dietary category to those that do not can determine whether this prediction is generally fulfilled across an entire assemblage, and indicate whether competition is an important factor in the assemblage as a whole. Alternately, pairs could be split on whether they have similar or different life history characteristics, sociality, activity times, etc.

Third, biologically relevant subsets of the sites can be used to calculate and compare co-occurrences in an assemblage. Comparing subsets of sites is one way to directly address spatial variation in co-occurrence across a large study area. It can also be used to quantify the effect of climate variables (by contrasting cold sites to warm sites, or wet sites to dry sites, or between major biomes), and to compare the effect of biotic interactions to the effects of abiotic variables (by contrasting co-occurrences calculated from the overlapping climate envelope of a pair to the overall co-occurrence pattern—see Chapter 4).

Each of these comparative splits can be performed using both modern and fossil data, as they require only general categorisations of diet, body size, and climate niche—data that are becoming readily available for many Quaternary fossil assemblages, as well as some deeper-time datasets. The relative influence of subgroups on the assembly patterns of the community can be estimated from the number and/or strength of pairs in each group, as well as their resemblance to the overall pattern.

Finally, and perhaps most importantly, the shifts of these patterns in response to disturbances can be documented by comparing assemblages before and after, or with and without, the presence of the disturbance in question. With enough data, such a hierarchical approach can also be used to explore the interaction of two variables (e.g. does species diversity affect the outcome of interspecific food competition on community assembly?). We will use a case study to demonstrate an application of each of these splitting techniques.

Case study

***Data*.** We used presence-absence data for mammals in East Africa (Tóth et al. 2014a, b). The data set contained 311 mammal species recorded at 38 national parks throughout Kenya and

Tanzania, and each site contained aggregated site inventories for two time intervals: 1888-1950 and 1950-2013. The previously published dataset was carefully compiled from surveys, historical records and museum specimens. It includes body mass and dietary information, which were compiled from the literature for every species. We limited our analysis to the 115 species > 1 kg in average body mass (to reduce computation time and avoid sampling biases associated with small mammals). We calculated co-occurrence probabilities using FETmP for all possible species pairs in this assemblage. We used this dataset to demonstrate simple cases of the four models described above. Because distributions of co-occurrence scores are always centred on 0.5 (representing a perfectly random association) , it is meaningless to compare overall group means. Differences between distributions will occur in the shapes of the two sides of the distribution, the significance of group differences were calculated separately on aggregations and segregations using Mantel tests.

Species split: *Do herbivores, omnivores, and carnivores have different effects on community assembly?* Using the "DIET2" data field in the East African dataset, we organised species into carnivores (including "meat", "piscivore", "bone", "invrt", and combinations thereof), omnivores (including "p_dom", "a_dom", "mixed", and "fruit_meat"), and herbivores (including "gr", "br", "fruit", "root", "seed", "fungi", and combinations thereof). We then compared associations where both species fell into the same group (i.e., carnivore-carnivore, omnivore-omnivore, and herbivore-herbivore pairs). Omnivores do not represent an ecologically cohesive unit (Pineda-Munoz and Alroy 2014), but we use it here because we are characterising only very broad trophic patterns.

Pair split: *Does food competition cause exclusion in East African mammals?* We split pairs into two groups depending on whether or not they were in the same specific diet category (e.g., invertivore, piscivore, frugivore, etc.). Though pairs in the same diet group do not necessarily compete, they are more likely to compete for food than pairs in different diet categories, and this competition is likely to be more intense. Thus, if pairs in the same diet category have more or stronger segregations, we can hypothesize that food-based competitive exclusion is an important factor in community assembly. The pair split is distinct from the species split because it uses the difference or similarity of the traits in a species pair, rather than the actual trait categories, as the levels of the response variable.

Site split: *Have community assembly patterns changed over time in East Africa?* We split the sites into historical and modern time intervals that were provided in the dataset, and removed empty rows (i.e., if species did not occur in a given time interval). Co-occurrence probabilities were re-calculated for the intervals. The pair and species splits used samples from both time intervals, and therefore their results are subject to time averaging, but including sites from both ~60-year time intervals in one analysis makes their results more reliable. This is because in order to receive a strong aggregation, a pair needs to have aggregated in both time intervals. If the spatial association of the pair changed over time (e.g., it segregates in the historical time interval but aggregates in the recent), then the time-averaged score for the pair would be weaker. Therefore, strong associations derived from datasets encompassing multiple finer time intervals represent patterns that are both temporally and spatially consistent.

Hierarchical split: *Did the outcome of interspecific competition change over time?* This is an example of hierarchical splitting, where the data are first split by time interval, and then split

by whether or not the species in the pair use the same food source. If the outcome of interspecific competition (e.g. exclusion) changes over time, we expect a shift in the associations of same-diet and different-diet pairs between the regions. We performed permutational ANOVAs on each model to determine p-values.

Results

Do herbivores, omnivores, and carnivores have different effects on community assembly?

East African omnivores formed stronger segregations than herbivores and carnivores (Fig. 2.2). Herbivores had the weakest segregations, and carnivores were in between. Aggregation strengths were similar between the three groups. Carnivores aggregated the most often (79.6% of pairs aggregated) followed by herbivores (70.5% of pairs aggregated), followed by omnivores (62.8% of pairs aggregated). Therefore, omnivores had the strongest and most frequent segregations. Differences between aggregation ($p = 0.0037$) and segregation ($p < 0.0001$) were significant across all three groups.

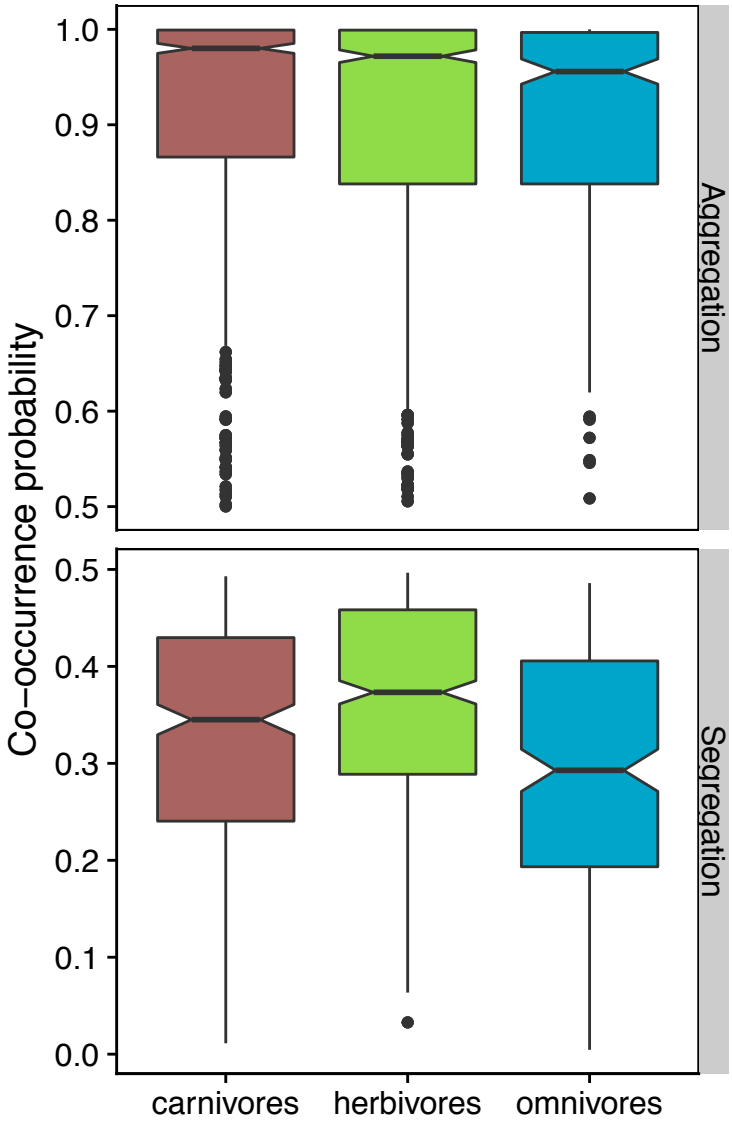

Figure 2.2. Strength of aggregations and segregations in carnivore-carnivore, herbivore-herbivore, and omnivore-omnivore pairs in East Africa. Cross-guild pairs are not depicted. Values near 1 represent strong aggregations, values near 0.5 indicate random associations (i.e., species distributions are indistinguishable from random expectations), and values closer to 0 indicate strong segregations.

Does food competition cause exclusion in East African mammals? Species pairs that had differing diets were equally likely to segregate as species having the same diet (Fig. 2.3). Segregations and aggregations are both slightly stronger for species pairs that have the same diet, but the differences were not significant (p_{agg} = 0.31, p_{seg} = 0.1109).

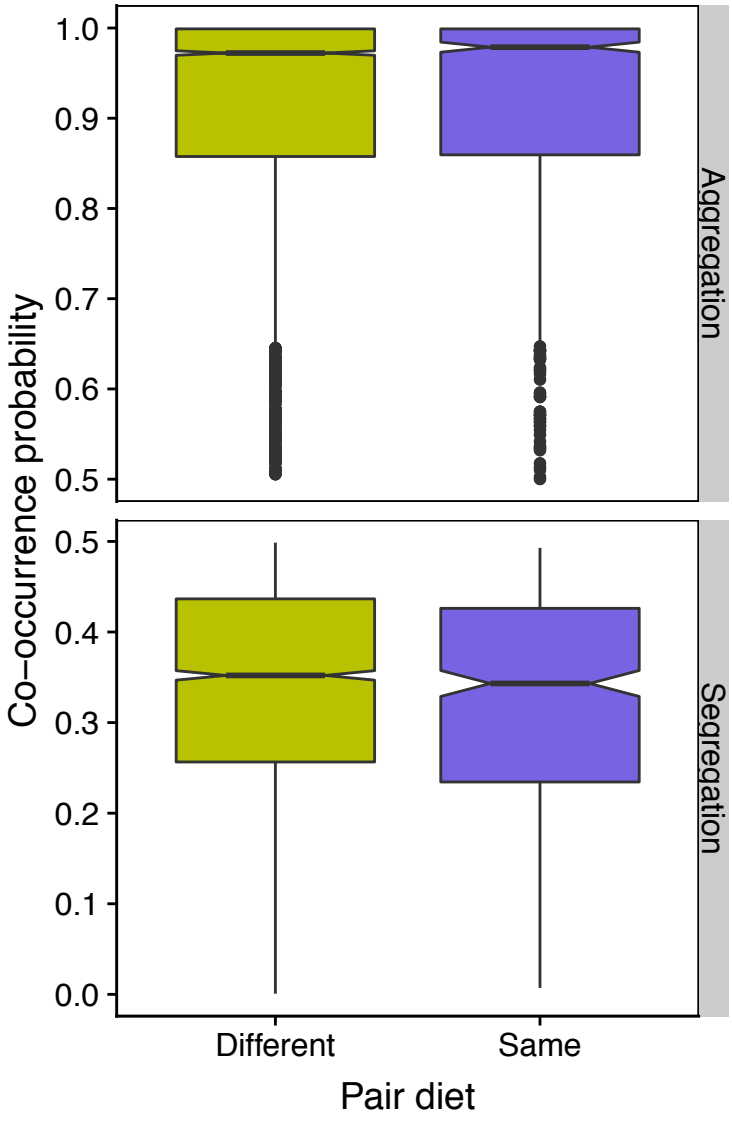

Figure 2.3. Strength of aggregations and segregations in species pairs with different diets versus same diets in East Africa. Diet categories correspond to "DIET2" column in the dataset, comprising detailed diet categories. Values near 1 represent strong aggregations, values near 0.5 indicate random associations (i.e., species distributions are indistinguishable from random expectations), and values closer to 0 indicate strong segregations.

Have community assembly patterns changed over time in East Africa? Aggregations became much more common going from the historical to the recent time interval (62.9% to 76.7% of pairs). Additionally aggregations became stronger while segregations stayed the same ($p_{agg} < 0.0001$, $p_{seg} = 0.594$; Fig. 2.4). As occupancy distributions are closely linked to

co-occurrence (see supplement, Fig. 2.8), difference in sampling across time intervals can influence these results. In this dataset, there was only 2.2% difference in matrix fill between the two time intervals, suggesting that sampling was reasonably consistent over time.

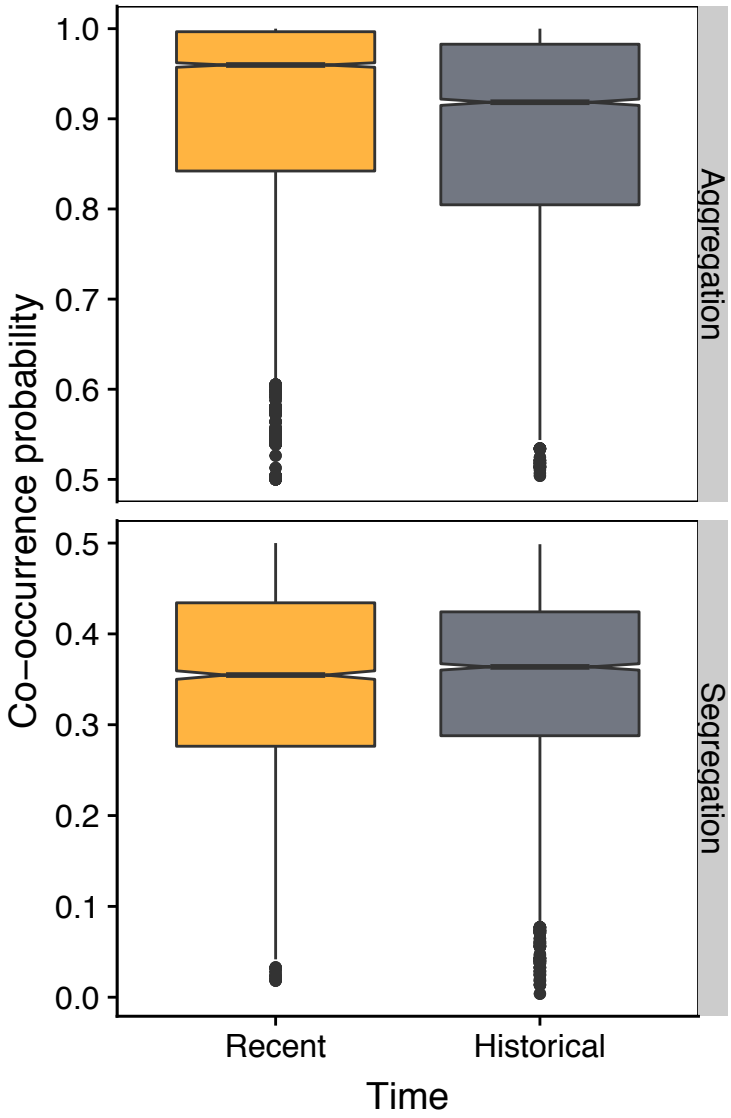

Figure 2.4. Strength of aggregations and segregations in recent (1950-2013) and historical (1888-1950) mammal pairs in East Africa. Values near 1 represent strong aggregations, values near 0.5 indicate random associations (i.e., species distributions are indistinguishable from random expectations), and values closer to 0 indicate strong segregations.

Did the outcome of interspecific competition change over time? Pairs in the same diet category became more likely to co-occur in the recent time interval compared to the historical time interval. Aggregations went from representing 65.8% of pairs to 81.0% of pairs, and aggregations on average became stronger ($p_{agg} < 0.0001$) while segregations remained the same ($p_{seg} = 0.876$; Fig. 2.5). Pairs with a different diet also became more likely to co-occur, with aggregations going from 62.6% to 76.2% of pairs. Aggregations ($p_{agg} < 0.0001$) became stronger and segregations did not change significantly ($p_{seg} = 0.506$). In short, the same effects happened to pairs in both different- and same-diet groups (Fig. 2.5).

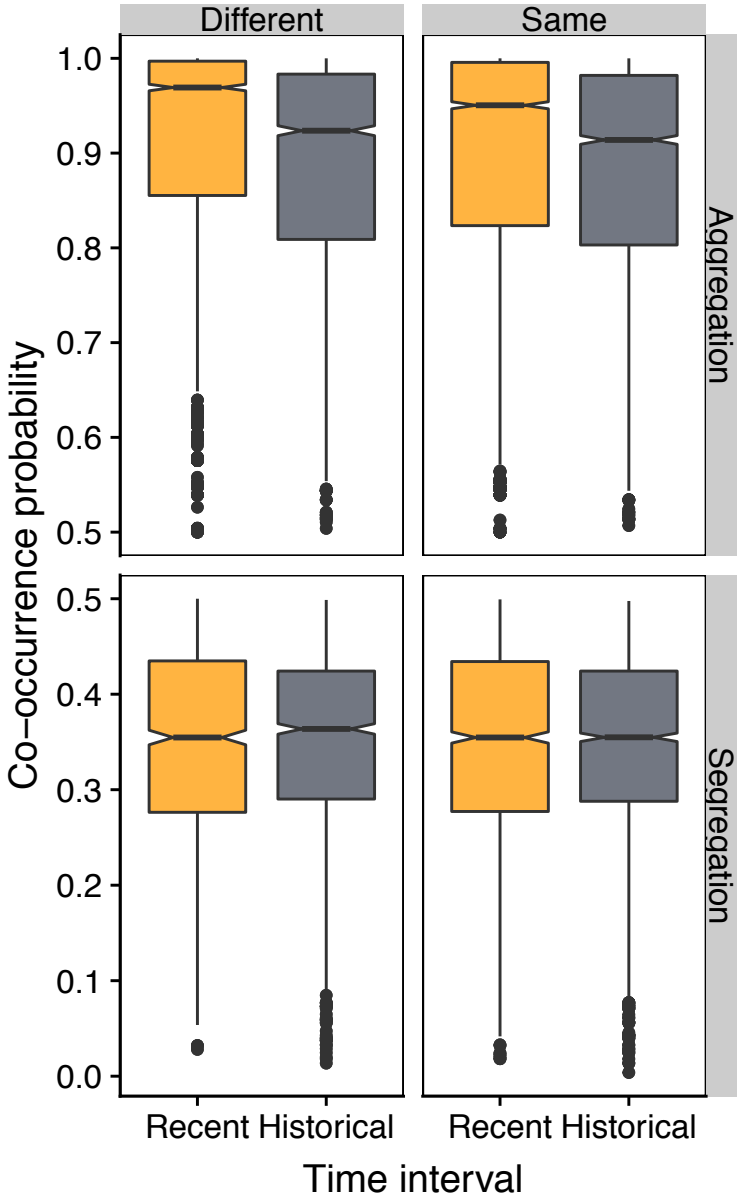

Figure 2.5. Strength of aggregations and segregations in recent (1950-2013) and historical pairs (1888-1950) in East Africa, split by whether they have the same diet or different diets. Values near 1 represent strong aggregations, values near 0.5 indicate random associations (i.e., species distributions are indistinguishable from random expectations), and values closer to 0 indicate strong segregations.

Discussion

Case study. The results of our case study lend insights into the community assembly of East African mammals. Carnivores are the most likely to aggregate, and their aggregations are stronger than those of herbivores and omnivores. Aggregations occur when species occur

together at some subset of the sites and are mutually absent at others. For African ecosystems, this suggests that large-herbivore-dominated savannas such as the Serengeti act as a congregation point for large predators and carnivorous scavengers. Herbivores aggregate almost as strongly as carnivores, as savannas support a large biodiversity of grazing and browsing artiodactyls and perissodactyls.

Interestingly, carnivores and herbivores have very different segregation strengths, which may indicate that interspecific competition is more likely to result in exclusion at individual sites for carnivores. Indeed, the potential for carnivore competition is relatively well-documented (Caro and Stoner 2003), and a more recent study showed that previously accepted temporal partitioning in competing carnivores is much weaker than expected, suggesting carnivores are "starvation driven" and are willing to risk direct competition to survive (Cozzi et al. 2012). It is important to note, however, that competition that does not result in exclusion may cause abundances to covary negatively, but still allow mutual presence (aggregation) as opposed to segregation, and this may partly account for why both aggregations and segregations are relatively strong in carnivores. Herbivores (particularly large ungulates), by contrast, have recently been shown to be surprisingly adept at resource partitioning (Kleynhans et al. 2011; Kartzinel et al. 2015), and even older studies typically found equal if not more evidence for facilitation (such as protection from predators in mixed herds) than competitive interactions (Sinclair 1985; Arsenault and Owen-Smith 2002). This ecological difference between herbivores and carnivores is reflected in the outcome of local community assembly.

Omnivores exhibit stronger and more frequent segregations than carnivores and herbivores. This group is comprised of diverse opportunistic taxa with varied diets including

jackals, most primates, civets, porcupines, genets, and wild pigs. Because omnivores commonly live in a wide variety of environments, such as forests, savannas, and woodlands, it is reasonable to suggest that habitat preferences are at least partly responsible for the prevalence and strength of segregations in this group, and the weakness of aggregations. This could be tested with further data splits (e.g., splitting sites into open and closed habitats should reduce segregation within groups). Although competitive interactions are probably reduced by the wide variety of food sources used by omnivores (e.g., insects, leaves, fruit, bark, roots, small prey such as lizards, etc), it is still possible that related species, such as primates, experience competitive exclusion to some degree (Kamilar and Ledogar 2011). Carnivores have a higher proportion and strength of aggregations than omnivores (Fig. 2.2), and this suggests that more carnivore pairs prefer the same habitats (perhaps because environmental filtering causes them to aggregate), whereas fewer omnivore pairs prefer the same habitats. As pairs are non-independent, carnivore segregations are thus more likely to be a result of competitive exclusion, while omnivore segregations likely reflect a combination of exclusion and environmental filtering.

In our split of same-diet and different-diet pairs, we saw no significant difference across the two groups (Fig. 2.3). Though same-diet pairs are theoretically more likely to compete than pairs with different diets, some species in the same categories actually partition resources (Kleynhans et al. 2011) and facilitate one another (Arsenault and Owen-Smith 2002). While competition causes a detectable increase in segregation strength in some subgroups of same-diet pairs (e.g. carnivores, as discussed previously), other factors, such as environmental filtering and mutualistic interactions, likely play similar roles across both groups, and this makes the spatial effect of food competition undetectable in the overall data.

Our temporal split of the data indicated that aggregations became much stronger and more common in the recent compared to the historical interval. Although we cannot tell why this is the case based on our simple analysis, further split analyses of these data have the potential to shed light on this question by asking which functional groups (diet, activity time, sociality, etc.) were most strongly affected over time. We could also hierarchically split sites based on measures of anthropogenic disturbance (e.g., human population density) or climate change (e.g., the amount of change in average temperature or precipitation) to further explore why this shift in community assembly occurred.

Our hierarchical split of same- and different-diet pairs across time intervals shows that both same- and different-diet pairs increased their aggregation strength and frequency across the time interval. These results suggest that increasing aggregation in the recent time interval is not related to food competition.

The probabilistic approach. We have shown how the co-occurrence scores generated by the probabilistic approach can be used in a modelling framework that allows the exploration of how ecological variables affect community assembly outcomes. In this paper, we used only proportion and strength of aggregations and segregations, but this framework can easily be extended to compare properties of network nodes and 'emergent' network properties (such as the modularity and nestedness metrics proposed by Poisot *et al.* (2016) across groups. By contrast, binary networks are likely to understate the likelihood of rare events and overstate the importance of common events in the spatial structuring of communities, and therefore have the potential to introduce systematic biases (Poisot et al. 2016).

Based on analyses of binary networks, there is growing evidence that disturbance causes rearrangement of network structure (e.g., Lane et al., 2014; Kay et al., 2017). Probabilistic networks provide a holistic and accurate view of co-occurrence network structure. This is not to say that probabilistic and binary networks will most often disagree. Instead, the advantage of using probabilistic networks instead of binary networks is that they eliminate the possibility that results can be overturned by changing the significance threshold. Moreover, probabilistic networks do not ignore the underappreciated fact that weak associations can capture biologically meaningful properties of ecological networks. This is particularly true when a matrix is sparse, which is likely to result in a high proportion of weak associations, especially segregations. Recent research using co-occurrence analysis has been making progress in the right direction. In our view, turning to probabilistic metrics that take weak links at face value would represent a paradigm shift that would impart substantial improvements for co-occurrence models. Models using probabilistic associations can be more useful, as they allow for making more explicit comparisons between pairs, taxa, or assemblages; more robust, because they do not rely on significance thresholds; more realistic, as they treat associations as probabilities rather than concrete biological entities; more detailed, because they are represented by numerical rather than categorical data; and more interpretable, reflecting the way co-occurrence patterns can vary across space and time. Acknowledging and leveraging the probabilistic nature of species associations is therefore the logical next step for co-occurrence analysis.

Conclusions. Our case study highlights how various categorizations of the data can yield information about what drives co-occurrence in species pairs. In particular, ecological differences between carnivores, herbivores, and omnivores influence the extent to which

biotic interactions such as competition and facilitation are detectable in community assembly outcomes.

As researchers increasingly utilise co-occurrence networks to understand community assembly over large spatiotemporal scales, it is important to ensure that their methodologies are appropriate to the nature of the underlying data. Probabilistic co-occurrence analysis produces networks that are more robust and more biologically interpretable. The modelling approach examined in this paper provides advantages to any research employing comparative co-occurrence analysis, and can help elucidate the spatial and temporal impacts of disturbance, climate change, management actions, and extinctions on communities of any reasonably well-sampled taxa.

ACKNOWLEDGEMENTS

We wish to thank the Biology Department at Macquarie University for supporting this research. We thank Laura Soul for her constructive comments on the manuscript.

SUPPLEMENTARY MATERIALS

Probabilistic co-occurrence metrics

***Fisher's Exact Test mid-P variant*.** This metric is used as a co-occurrence measure for frequentist inference (Berry and Armitage 1995; Kallio et al. 2011), but can be used as a weighting metric. It is based on Fisher's Exact Test (FET) computes the probability that *k* sites

out of a total of *N* sites will contain both species A and species B, given the incidence of each species

$$P(agg) = \left(\sum_{\substack{\max(A+B-N,0) \\ \leq k \leq \mathrm{X}}} \frac{\binom{A}{k}\binom{N-A}{B-k}}{\binom{N}{B}} \right)$$

where *A* is the number of occurrences of species A, *B* is the number of occurrences of species B, *k* is a hypothetical number of sites where species A and B could occur together where max(*A*+*B*-*N*, 0) ≤ *k* ≤ min(A, B), and *X* is the observed number of sites where the species co-occur. This can be used for inference by treating whichever tail probability is smaller as a p-value for significance testing (not including the probability of the observed value). If the upper tail is the smaller tail, the association is positive, and if the lower tail probability is smaller, the association is negative (Fig. 2.6a and b). By contrast, the mid-P variant of FET (FETmP) creates a continuous, normalised metric by splitting the probablity of the observed co-occurrence value equally between the tail probabilities, ensuring they add up to 1 (Fig. 2.6c).

$$P(agg) = \left(\sum_{\substack{\max(A+B-N,0) \\ \leq k \leq \min(\mathrm{A},\mathrm{B})}} \frac{\binom{A}{k}\binom{N-A}{B-k}}{\binom{N}{B}} \right) - \frac{\binom{A}{X}\binom{N-A}{B-X}}{2\binom{N}{B}}$$

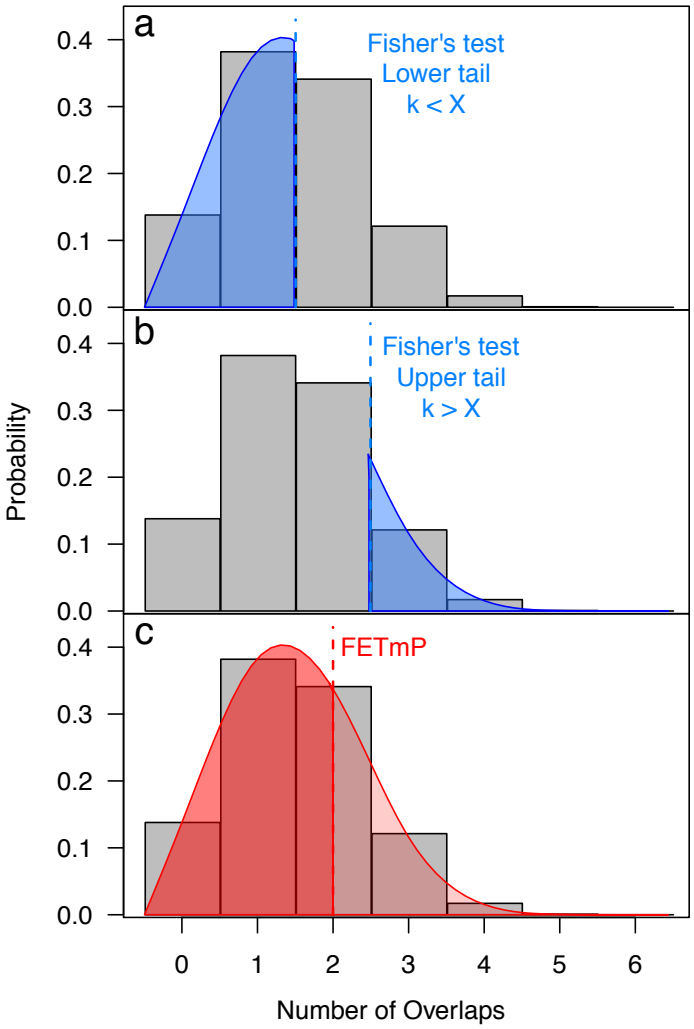

Figure 2.6. Probability distribution of overlaps when considering two species with 6 occurrences each in 24 sites. (a) Lower tail of Fisher's exact test (FET), (b) Upper tail of FET, and (c) weight assigned by FETmP where the observed number of overlaps X = 2. In this situation, FET effect size would classify the pair as random based on the upper tail value of 0.139 (non-significant), whereas FETmP used as a continuous metric would assign a weight of 0.69, a very weak positive association. If the observed number of overlaps was 4 instead, FET might assign a significant aggregation based on the upper tail probability of 0.018 (depending of the cut-off is 0.05 or 0.01), and FETmP would assign a weight of 0.99.

The co-occurrence weight is then simply the lower tail (including half the observed probability), which will be small when pairs are negatively associated and large when they are positively associated, but will never take the exact values of 0 or 1 because of the mid-P split.

The equation produces middling values for observations that approach expected overlap values. See Berry and Armitage (1995) for a discussion of mid-P confidence intervals.

Fisher's Exact Test effect size (observed – expected). Another way to use FET to calculate a continuous metric is to use the effect size as defined by Veech (2013). By this definition, the expected number of shared sites for any pair of species is sum(P_j x j), where 0 ≤ j ≤ min(occurrences of species A, occurrences of species B) and P_j is the probability of observing that overlap (i.e. one point in the discrete probability distribution). By taking the difference between our observed and expected overlaps, we will get a value that ranges between negative and positive infinity, though the magnitude is limited by the number of sites. As it happens, the output weights of FET effect size and z-scores of FETmP are closely and linearly related (Fig. 2.7, left).

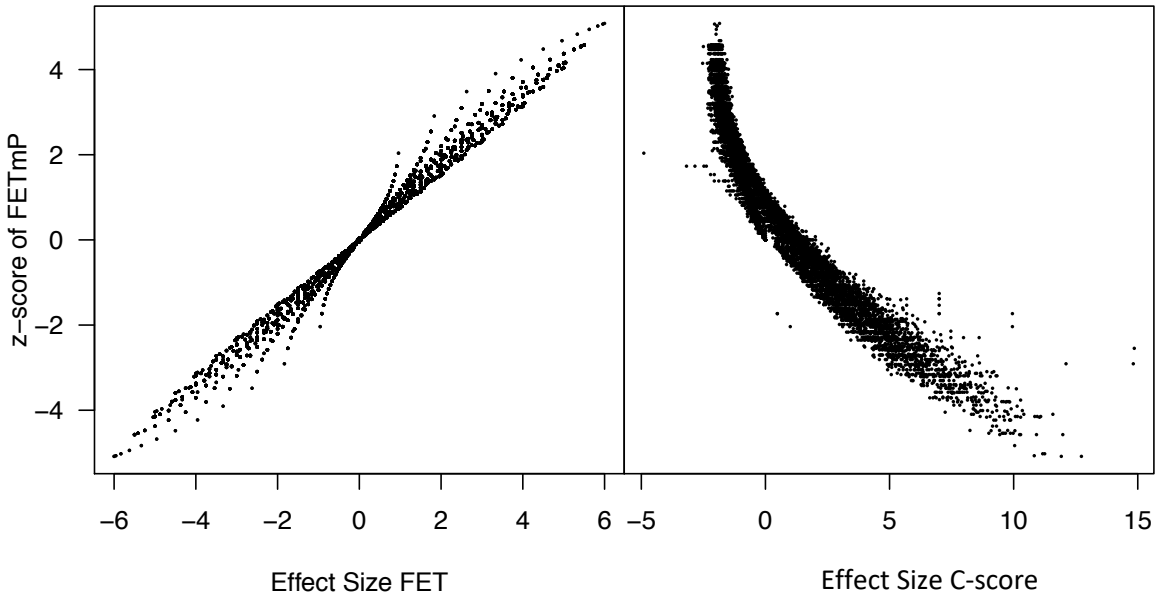

Figure 2.7. Relationship between z-score of FETmP, FET effect size, and C-Score effect size. Points are pairs of species from an artificial 24-site matrix that displays all the possible combinations of co-occurrence patterns (192 species/rows).

C-score effect size (observed - expected). A third possible continuous metric is the effect size of the C-score, as developed by Stone and Roberts (1990). The normalized C-score is defined as

$$C_{AB} = \frac{(A - X)(B - X)}{AB}$$

and takes values 0 to 1 inclusive, where 0 indicates complete overlap and 1 indicates no overlaps. The PAIRS program (Ulrich, 2008; Gotelli & Ulrich, 2010) calculates the pairwise C-scores for a specified number of randomized matrices shuffled using an algorithm that keeps marginal totals constant (Gotelli 2000). It then compares the null distribution of C-scores for each pair to the observed C-score in the original matrix to determine the effect size. This procedure also returns somewhat similar values to FETmP and FET effect size, although the relationship is not linear (Fig. 2.7, right). Interestingly, the effect size of the C-score seems to separate out negative associations more strongly than positive associations. One disadvantage of this method is that randomisations with fixed marginal totals are a computationally expensive process and take a long time to run for diverse systems (e.g. more than 150 species). If any kind of subsampling or bootstrapping procedure is required, these randomisations can become prohibitively time-consuming.

Error testing

We compared the accuracy and detection power of FETmP z-score, FET effect size, and C-score effect size. Although it is somewhat counterintuitive to test for error rates when our goal is to test each metric as a descriptive measure, applying a threshold and testing for false positives and false negatives (i.e. treating them as hypothesis testing methods) can help determine whether pairs are being assigned appropriate scores. We determined accuracy

(Type I error) by testing for false positives on random matrices. We used a highly structured and systematically degraded Diamond and Gilpin-type matrix to test the detection power (Type II error) of each metric (Diamond & Gilpin, 1982; Gotelli, 2000).

Accuracy. We ran FET effect size and FETmP on 72 random matrices with varied numbers of sites (10-300), numbers of species (10-400) and fill (5-30%). Each matrix was constructed by randomly sampling the global abundance distribution of mist-netted bats from the Ecological Register (http://ecoregister.org) (Alroy 2015), to mimic natural occupancy distributions. Since the matrices are random, an ideal test will detect no strong aggregations or segregations and be unaffected by sample size, richness, and matrix fill. More specifically, the percentage of the pairs above any α-level should not exceed theα-level itself. We also ran PAIRS using F-F randomization on 30 of the unstructured datasets, and compared these to the FET outputs. Running PAIRS on all of the random test matrices was not feasible due to time constraints.

***Detection power*.** We tested the detection power of each algorithm using methodology adapted from Gotelli (2000). We began with a blocked matrix with two perfectly segregated groups of perfectly co-occurring species (Gotelli 2000), and systematically degraded the presences, performing the pairwise calculations FET effect size and FETmP at each stage using an α of 0.01. Gotelli (2000) used a random swap for each row, but we merely removed presences, because this mimics decreased sampling. Because the test matrix is highly structured, every pair is perfectly aggregated or segregated, allowing us to measure loss of detection as the sampling becomes poorer. Ideally, degradation does not affect the detection rate for approximately half of the steps, after which noise takes over and most pairs rapidly become randomly associated (Gotelli 2000). We recorded the degradation tolerated by the matrix before pairs began appearing as random above the α-level, a measure we will call

"tolerance". We repeated the analysis for a series of matrices with 30 to 300 sites by 60 species and 20 to 300 species by 100 sites. We ran PAIRS on a systematically degraded matrix with 60 species and 100 sites, and compare detection power across the three methods.

Results of Accuracy and detection power. The comparisons between FET effect size, PAIRS, and FETmP showed that FET effect size consistently produces somewhat higher rates of false positives than the other two metrics, especially with low matrix fill or low richness (Fig. 2.8). Although FETmP did respond to sampling and matrix fill, it consistently had error levels below the α-level (Fig. 2.8). C-score effect size also produced low error rates (Fig. 2.8). There was no difference in the detection power of the three metrics when the Bayesian filter in PAIRS was not used; when it was used, it weakened the detection power of PAIRS (Fig. 2.9). Overall, FETmP performed slightly better than FET effect size and equally as well as PAIRS.

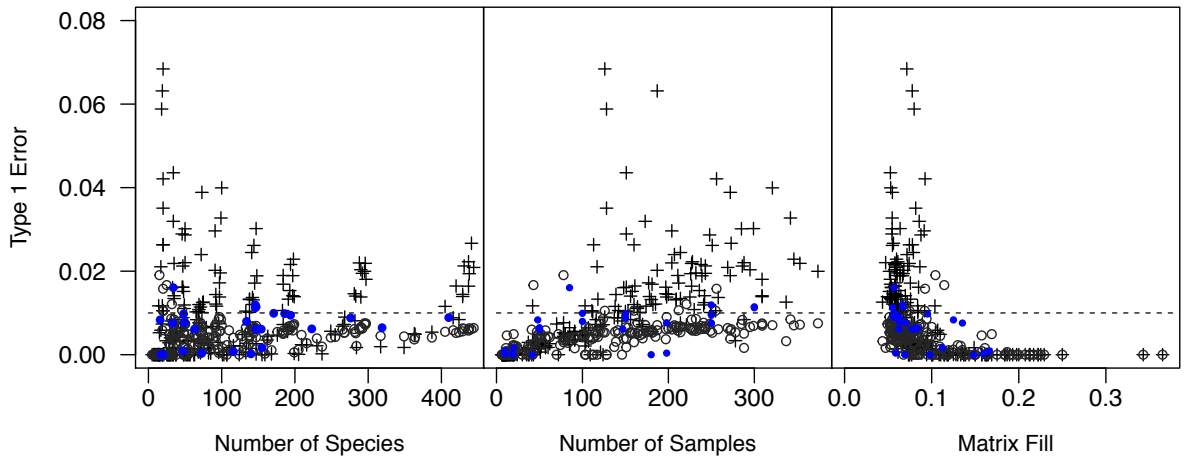

Figure 2.8. Scatterplots showing the rate of Type I error (false positive rate) produced by applying Fisher's exact test effect size (crosses), FETmP (open circles), and C-score effect size (filled blue circles) to random datasets. The dashed line indicates the α-level (0.01). FET has error rates above the α-level that are influenced by sample size and matrix fill while FETmP and C-score values are consistently below the α-level.

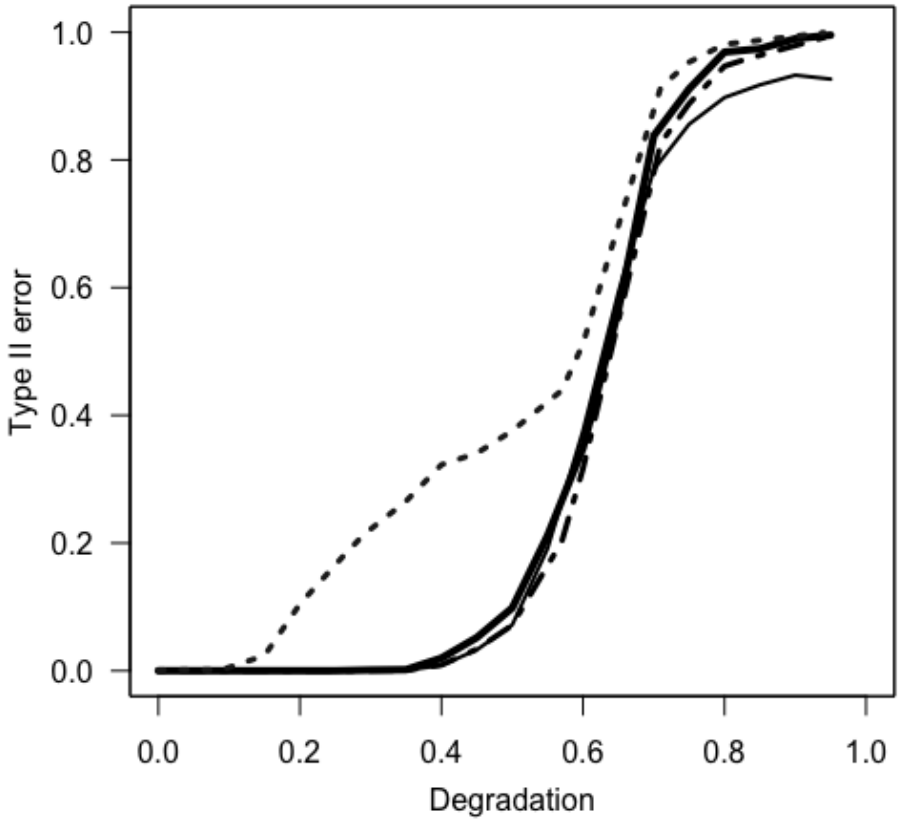

Figure 2.9. A typical run of the Type II error degradation test (60 species by 100 samples), showing the Type II error contrasted with the degree of degradation for FET effect size (thin solid line), FETmP (thick solid line), C-score effect size (dashed line), and C-score effect size with Bayesian significance threshold (dotted line), calculated on a perfectly structured checkerboard matrix. The plot resembles the ideal curve depicted in Gotelli (2000, Fig. 2.6). FET, FETmP, and PAIRS z-scores provide nearly identical detection power, while the Bayesian significance threshold loses power with minimal degradation.

Accuracy. FET effect size shows sensitivity to richness, sample size, and the matrix fill of the random datasets. The rate of Type I error increases with matrix fill and decreases with sample size. Overall, the Type I errors generated by FET vary between 0% and 7% (mean = 1%), and are above the α-level in 41% of random matrices (Fig. 2.8). Although the error rates are not too high, they are higher than previously reported (Veech 2013). Because of the discrepancy, we checked the results using the matrix parameters described in Veech (2013), including a high matrix fill and a peaked abundance distribution. The results confirm that FET effect size

is sensitive to the abundance distribution used to produce the test matrices, so the Type I error generated by FET effect size for real datasets may be higher in empirical datasets with many rare and a few common species than originally estimated on test matrices with peaked abundance distributions. FETmP is only mildly affected by the shape of abundance distributions, and produces values that meet our expectations for an accurate measure. Though low matrix fill and increased sample counts mildly affect the outputs, the proportion of values meeting the α-level never rises above the α-level. The average Type I error rate over all FETmP matrices was 0.0040, significantly lower than the error generated by FET effect size. C-Score effect size produces comparable Type I error rates (mean = 0.0014; Fig. 2.8).

Detection power. The Type II error of FETmP not deviate strongly from that of FET effect size (Fig. 2.9). Type II error in both tests follows the ideal hypothetical curve (Gotelli 2000). At low sample sizes (30 samples), both tests have a degradation tolerance of 10-15%. At high sample sizes (200-300 samples), they exhibit a tolerance > 50%. C-score effect size produces error rates comparable to FET effect size and FETmP. Fig. 2.9 depicts the degradation curves in all three scenarios, plus a curve for the Bayesian significance threshold applied to the C-Score via the PAIRS program, added for comparison, in a typical run (60 species and 100 samples), which demonstrates just under 40% tolerance for the former three.

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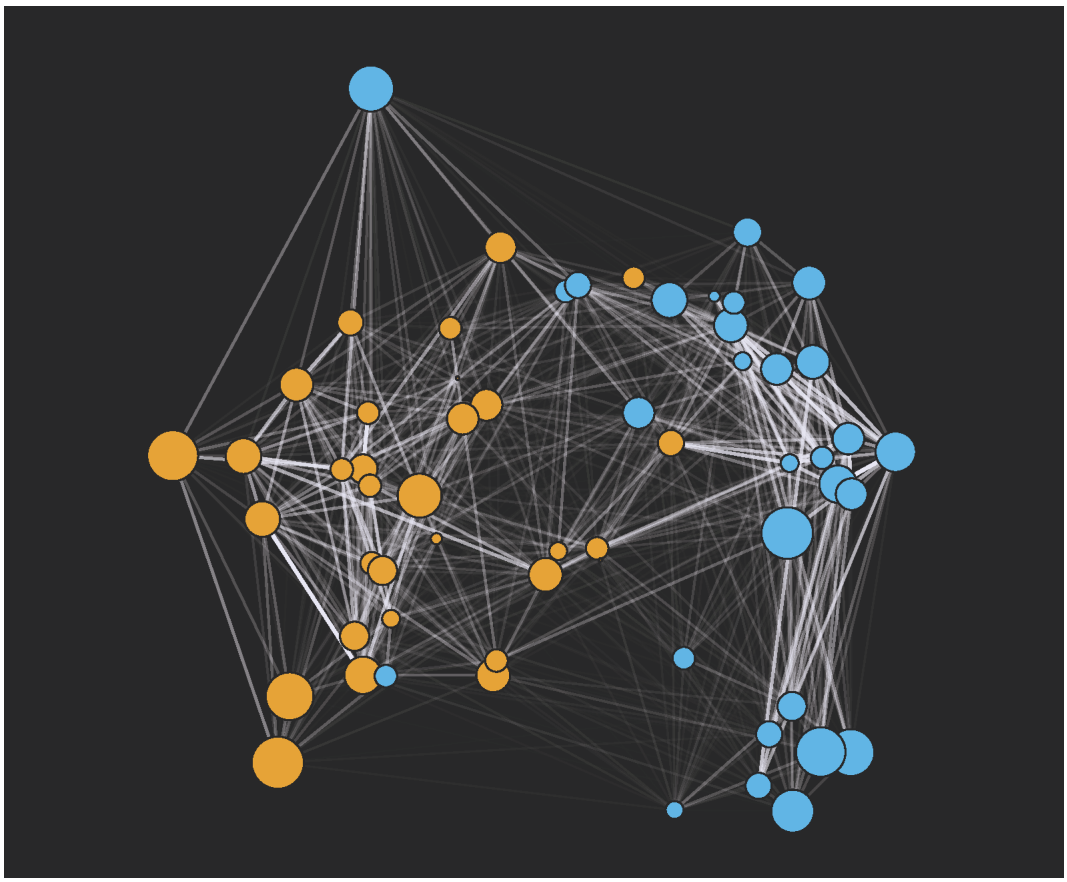
CHAPTER 3

Probabilistic and binary co-occurrence networks for simulated and empirical assemblages: a comparative analysis



Simulated network visualisation, by Anikó Tóth

Authors: Anikó B. Tóth, John Alroy, and Laura C. Soul

ABSTRACT

The study of probabilistic networks of co-occurrence is in its infancy. While it is a promising method for exploring the patterns and processes driving community assembly, there is much that is not known about probabilistic co-occurrence analysis, including how it compares to binary approaches, how it behaves under simulation, and the properties of its output for empirical assemblages. The purpose of this chapter is to undertake an observational and comparative study of probabilistic and binary co-occurrence metrics that begins to answer these questions. We find that weak links that are typically discarded by binary network constructions have an important role in differentiating between various types of simulated network structures. We also observe that networks constructed for empirical assemblages only weakly resemble simulated structures, even though the structures are simple and obvious. This finding suggests that existing models of community structure are too simplistic. Finally, we observe that empirical assemblages exhibit probabilistic co-occurrence structures much more similar to one another than to simulated structures, suggesting that empirical co-occurrence structures are governed by rigid assembly patterns that link disparate taxa and apply across a substantial range of spatial scales.

MAIN TEXT

Introduction

Co-occurrence analysis has been around for many years, but rarely has it been applied in a probabilistic manner. Traditionally, studies using co-occurrence analysis have focused on binary (yes/no) implementations based on frequentist significance testing.

Recently, Poisot et al. (2016) introduced a probabilistic network approach for networks of biotic interactions. The previous chapter makes a case for why the probabilistic approach is also highly applicable for co-occurrence networks, and gives several examples highlighting how its use can improve models that aim to understand how co-occurrence is related to biological measures of community structure and diversity.

Unfortunately, only a few studies (Krasnov et al. 2014; Bar-Massada and Belmaker 2017) have explored the possibility of using co-occurrence analysis in a probabilistic manner (i.e. by using co-occurrence weights as a numerical response variable in models). Very little is known about how co-occurrence weights behave for simulated and empirical datasets. For instance, the shape of their distributions are not well documented, nor to what extent this property can be expected to vary from dataset to dataset.

Ecological network analysis is new enough that we are still in the process of observing and documenting network structures (Proulx et al. 2005), and this is especially true for co-occurrence networks. This is a critical part of the research process that constructs a knowledge foundation on which to build future studies. Without such baselines it can be difficult to translate network metrics into applicable biological conclusions. Modularity in particular has many interpretations that depend on the type of network (Morueta-holme et al. 2016). Thus, observing and comparing between different types of networks is a vital topic, and it is starting to receive attention (Thébault and Fontaine 2010; Steele et al. 2011). Of several studies that have constructed and analysed co-occurrence networks, many settled on describing the networks, using their observations to identify future research questions (Steele et al. 2011; Barberán et al. 2012; Borthagaray et al. 2014; Lane et al. 2014; Kay et al. 2017).

Much of the co-occurrence literature compares the structures of communities through time or across space. Researchers have used binary co-occurrence networks to compare communities before and after disturbances or management actions (Tulloch et al. 2016) and inside and outside managed areas or modified habitats (Lane et al. 2014; Kay et al. 2017), and to compare the network properties of different taxa under changing climate conditions (Araújo et al. 2011). Often, network measurements such as node degree and centrality (Araújo et al. 2011; Berry and Widder 2014), counts of positive to negative associations (Veech 2006; Villalobos et al. 2016), connectivity (Kay et al. 2017), and modularity (Barberán et al. 2012; Kay et al. 2017) are used as a basis for these comparisons. However, it is not known how network metrics calculated from binary versus probabilistic networks compare to one another.

The value of observational studies for establishing baselines is frequently underestimated. This chapter uses a series of simulated and empirical datasets to build both binary and probabilistic co-occurrence networks. It then goes on to compare common metrics between the two network types, explore the properties of probabilistic co-occurrence weights, and compare probabilistic networks of simulated and empirical datasets. Our aim is to document and compare co-occurrence structures across various spatial scales, taxa, and community types so that future research can proceed with a basic understanding of the behaviour of probabilistic co-occurrence distributions, networks, and metrics under various scenarios.

Materials and Methods

Simulated Data. To facilitate the interpretation of empirical co-occurrence networks, we created five synthetic datasets of 60 species by 60 sites containing previously published hypothetical assemblage structures. The assemblage structures we used are randomly dispersed and compartmented gradients (Hoagland & Collins, 1997; Leibold & Mikkelson, 2002) and single- or multi-compartmented nested patterns, shown in Fig. 3.2 (Patterson and Atmar 1986; Lewinsohn et al. 2006). Each structure can be formed by one or more biological mechanisms (Connor and Simberloff 1979; Gilpin and Diamond 1982; Leibold and Mikkelson 2002; Presley et al. 2010). To these we added noise in the form of five random swaps per row and column, simply to make the matrices more realistic.

***Empirical Data*.** We downloaded seven empirical datasets having various spatial scales and including different taxa from the Ecological Register (Alroy 2015). Details of the retrieval of each dataset are recorded in Appendix 1. After downloading, we removed observations represented by a single individual and species represented by a single sample to avoid low detection rates.

***Construction of Binary Networks*.** We constructed a series of binary co-occurrence networks for our empirical datasets using two existing inferential methods, Fisher's Exact Test (FET: Veech, 2013; Arita, 2016) and F-F randomization on C-Scores (Stone & Roberts, 1990) in the software program PAIRS (Gotelli and Ulrich 2010).

Binary Network Metrics. Varying the significance threshold (α) for co-occurrence scores can alter whether any particular link between a species pair is present in a binary network. Network measurements that depend on link presence and absence may therefore vary depending on the choice of α. We assessed the behaviour of commonly used binary network

metrics in response to changing α-levels using binary networks created from our empirical datasets. We pruned the binary networks of the empirical datasets with an increasingly stringent α-level. At each step, we calculated three basic properties of each network. (1) Connectivity: the percentage of links realised, calculated with $C = 2e/n\ (n - 1)$ where e is the number of links and n is the number of nodes (species). (2) Modularity: the degree of compartmentation (clustering) in a network. We used the leading eigenvector method (Newman 2006) from the igraph package in R (Csardi and Nepusz 2006; R Core Team 2015) to compute modularity, using only significant positive associations. (3) The ratio of negative associations. We report the effect of α-level on these three properties of each network, and on the relationships amongst them. Finally, we generated binary networks for each matrix with the threshold set to 0.05, for comparison with probabilistic network metrics.

***Construction of Probabilistic Networks*.** We generated probabilistic networks for our simulated datasets and for the empirical datasets from the Ecological Register. For simulated datasets, we calculated pairwise co-occurrence scores using the mid-P variant of Fisher's Exact Test (Kallio et al. 2011) and used these to generate probabilistic networks. The number of samples in a dataset can affect the outcome of association weights; in particular, datasets with more samples have higher connectivity (i.e. stronger associations) (Kay et al. 2017). Therefore, to create probabilistic networks for the empirical datasets, we subsampled each matrix to the same number of sites without replacement and repeated the process 100 times, calculating co-occurrence scores on each iteration. We plotted cumulative link weight distributions across all iterations to reflect the frequency with which each pair appears in the subsamples.

Probabilistic Network Metrics. We calculated connectivity for each network, using the sum of aggregation strengths divided by the total number of links, and repeating for segregations. Association strengths were scaled between -1 and 1 for this procedure (so that strong segregations result in high negative connectivity). We also calculated modularity for each probabilistic network. We used the "spin glass" clustering algorithm (Newman and Girvan 2003; Reichardt and Bornholdt 2006) with *z*-scores of association weights because it uses positive and negative links together. The spin glass algorithm is implemented in the igraph package in R (Csardi and Nepusz 2006; R Core Team 2015).

Comparative Analyses. We conducted comparisons between: (1) probabilistic and traditional binary network metrics for empirical and simulated datasets; (2) probabilistic co-occurrence weight distributions and network metrics across simulated matrices; and (3) distributions and network metrics across empirical matrices. To compare empirical datasets, we used the mean of the measurements taken on subsamples, and compared the datasets in pairs where either the spatial location or the focal taxon was held constant. We compared 1) bats from North America and southern Brazil; 2) mammals from the New World and Indomalaya; 3) rodents and bats from southern Brazil; and 4) trees and scarabs from North America. We used this pairwise approach to control for differences in the input datasets, which helped us interpret variation in output co-occurrence distributions.

***Visualisation*.** We also generated network plots based on each simulated matrix for comparison to empirical matrices. We follow Lewinsohn *et al*. (2006) in using an ordination approach to visualize assemblage structure. We suggest using the *z*-scores of the co-occurrence weights as a distance matrix in a principal coordinates analysis (PCoA). This is different from a traditional principal components analysis because the pairwise distances are

not, say, Euclidean distances, but instead scores that reflect the deviation between observed and random patterns. We plot each graph separately with the aggregations and segregations represented as lines, with thickness and transparency varied to indicate association strength.

Results

Effect of thresholds on binary metrics. The values for the modularity of each binary empirical network varied unpredictably across the different α-levels for both inferential methods (Figs. 3.1A-B). Connectivity necessarily decreases as α increases for any network. However, the relative connectivity values among the various networks also change arbitrarily when implementing FET (Fig 3.1C). The values generated by the PAIRS program are more consistent, but it is clear that the relative connectivity of the different datasets can change with the threshold (Fig. 3.1D), and this uncertainty translates to uncertainty in biological interpretations. For example, the threshold used in PAIRS influences whether North American or Southern Brazilian bats have a higher connectivity (Fig. 3.1D). At higher thresholds, North American bats have stronger connectivity, but lower thresholds cause Southern Brazilian bats to have higher connectivity. It is therefore unclear which assemblage has interactions that influence spatial distribution patterns more strongly. The percentage of significant pairs that were negatively associated tended to decrease with α-level, suggesting that positive associations are often stronger than negative ones. However, this does not always hold true, and again the relationships among the networks change as α increases (Figs. 3.1E and 3.1F). Our tree dataset had stable measurements across α-levels. However, because the measurements for networks of animal data change with α, so do the comparisons between animal and tree networks.

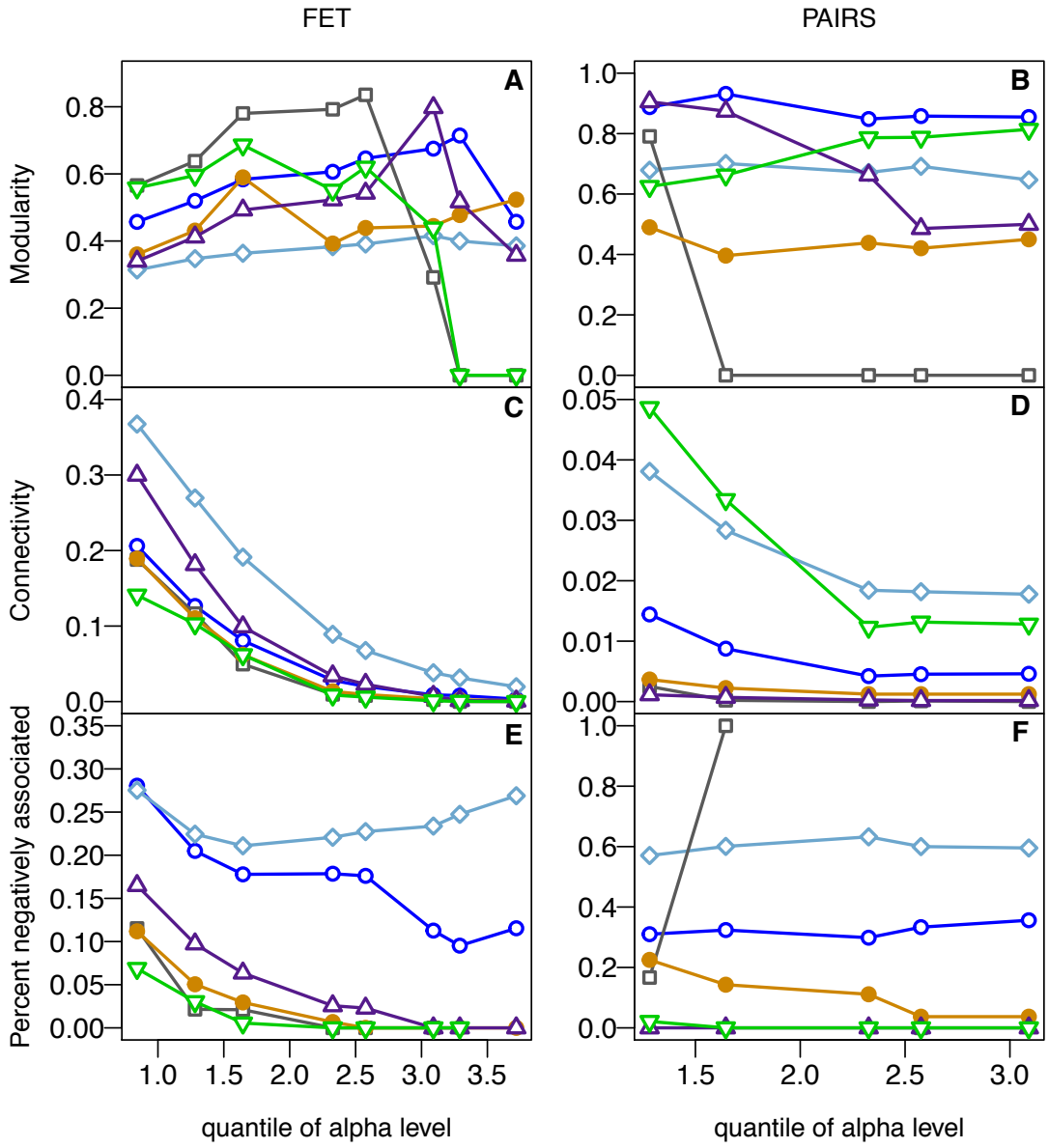


Figure 3.1. Response of modularity, connectivity, and percent negatively associated pairs to changing the significance threshold (New World mammals = circles, North American scarabs = squares, North American trees = dots, North American bats = diamonds, southern Brazilian bats = triangles, and southern Brazilian rodents = inverted triangles). Datasets are from the Ecological Register (Alroy 2015). Panels A, C, and E show results for Fisher's Exact Test (Veech 2013), while B, D, and F show results of PAIRS (Gotelli and Ulrich 2010). Missing data points indicate that no significant links remain at that threshold. Squares are partly obscured under triangles in panel d, and triangles are obscured under inverted triangles in panel f. Note that quantile of alpha level is used on the x-axis for readability, and a quantile of 1.96 corresponds to the usual significance threshold of 0.05.

Binary vs probabilistic metrics. Network metrics calculated on binary and probabilistic networks are related and behave somewhat predictably. Probabilistic connectivity measures consistently yield greater connectivity values than binary measures for both positive and negative links (Fig. 3.2 B-C). The ratio between them is particularly high for empirical networks, where most links weights are weak and tend to be discarded in binary network construction. Modularity values are typically higher for binary networks, and the correlation between them is not tight (Fig. 3.2 A).

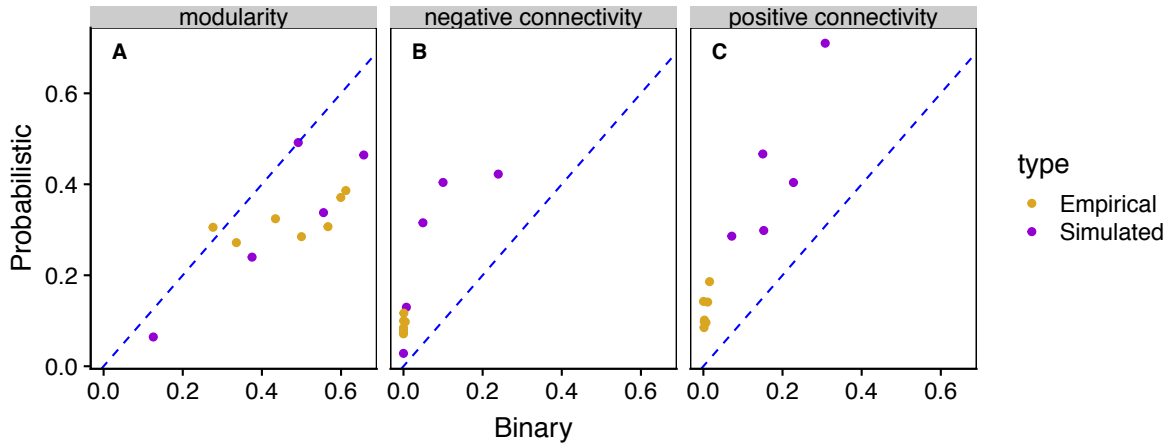


Figure 3.2. Relationship between network metrics calculated on binary versus probabilistic networks. Empirical network results are plotted in yellow while simulated networks are plotted in violet. The line of unity is shown.

Probabilistic network metrics. We present simulated network link weight distributions as a set of three comparisons between related simulations (nested against compartmented-nested, three compartments against single gradient, and square compartments against compartmented-nested structures). Simulated matrices generally produced bimodal co-occurrence probability distributions (Fig. 3.3) because matrices were deliberately constructed with strong aggregations and segregations. Compartmentation produced stronger bimodality

than graded turnover (i.e. an increase in the strength of both aggregations and segregations), and segregations were proportionally more frequent with an increasing number of compartments. Nested structures produced positive associations (Fig. 3.3). When compartments were nested, strong aggregations and weak segregations resulted. Network graphs visually represented network structure (Fig. 3.4), and provide a simple baseline for comparison.

Table 3.1. Pairwise counts, positive, negative and total connectivity, and modularity results for each of the simulated datasets using probabilistic networks (grps = number of clusters, mod = spin glass modularity value).

Dataset	Number of pairs			Weighted Connectance			Modularity	
	Positive	Negative	Total	Positive	Negative	Total	grps	mod
Nested	1662	108	1770	0.738	0.028	0.766	4	0.048
Two nested compartments	1170	600	1770	0.392	0.160	0.552	2	0.254
Two-compartment gradient	869	901	1770	0.433	0.454	0.887	2	0.493
Three-compartment gradient	751	1019	1770	0.309	0.430	0.739	3	0.450
Smooth gradient	813	957	1770	0.274	0.315	0.589	3	0.352

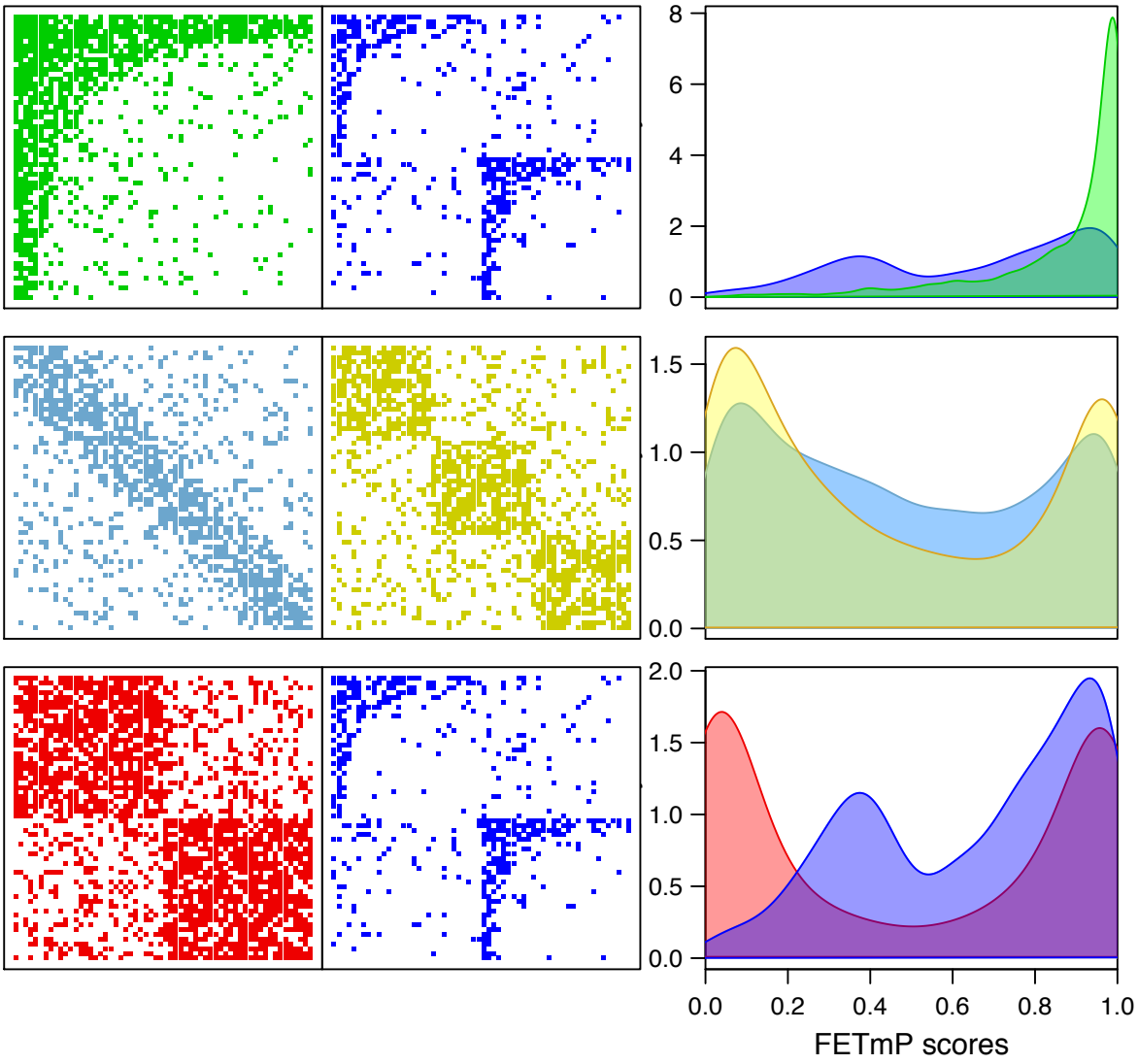


Figure 3.3. Comparisons between pairs of test matrices (left), and their association strength density distributions (right). Colours of the simulated datasets match their association strength density distributions, which indicate the strength and relative frequency of co-occurrence scores. FETmP scores > 0.5 signal positive associations while scores < 0.5 signal negative associations.

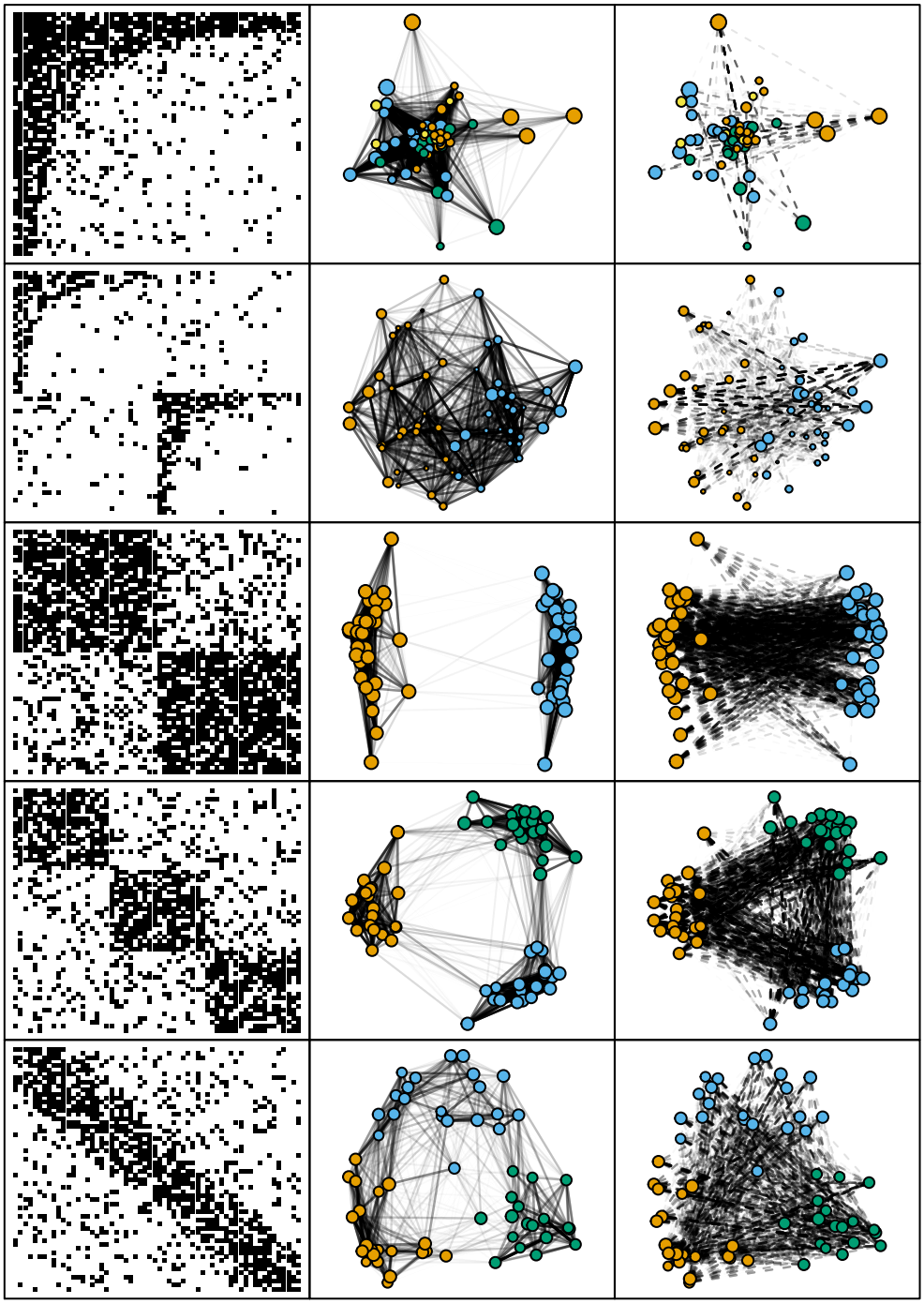


Figure 3.4. Network PCA plots of positive (centre) and negative (right) associations for each simulated dataset (left). Colours indicate clusters identified by the spin glass algorithm. Node size indicates species occurrence frequency.

Observations of empirical networks

Description of networks. Our analysis of the empirical datasets revealed that ecological regional assemblages share some striking similarities, despite differences in spatial extent, location, and taxonomic composition. For instance, they tend to be strongly dominated by weak segregations (numbers just under 0.5: Fig. 3.5). Aggregations are fewer in number and tend to be stronger (numbers greater than 0.5: Fig 3.5). This difference in aggregation and segregation frequencies is not surprising, as ecological matrices with many samples are typically sparse, making weak negative associations likely. Furthermore, all assemblages had stronger positive than negative connectivity, although the ratios of positive to negative connectivity varied (Table 3.2). Modularity of empirical networks was constrained in a tight range, between 0.3 and 0.41 (Table 3.2).

Table 3.2. Pairwise counts, positive, negative and total connectivity, and modularity (grps = number of clusters, mod = spin glass modularity value) results for each of the seven empirical test datasets from the Ecological Register based on probabilistic networks, calculated on the subsample level and reported as means of the subsamples. Note that the total number of pairs is less than the total possible because subsamples do not always include all species.

Dataset	**Number of pairs**			**Connectivity**			**Modularity**	
	Positive	Negative	Total	Positive	Negative	Total	grps	mod
North American Bats	919	2185	3104	0.207	0.135	0.342	4.19	0.328
Southern Brazilian Bats	819	2210	3029	0.189	0.111	0.300	4.13	0.323
Southern Brazilian Rodents	319	1768	2087	0.116	0.099	0.215	7.30	0.408
North American Scarabs	1234	6494	7728	0.118	0.124	0.242	4.54	0.394
Eastern North American Trees	1220	4521	5741	0.140	0.104	0.245	5.42	0.305
New World Mammals	641	2326	2967	0.134	0.121	0.254	4.63	0.351
Indomalayan Mammals	1344	3650	4994	0.201	0.129	0.330	4.77	0.325

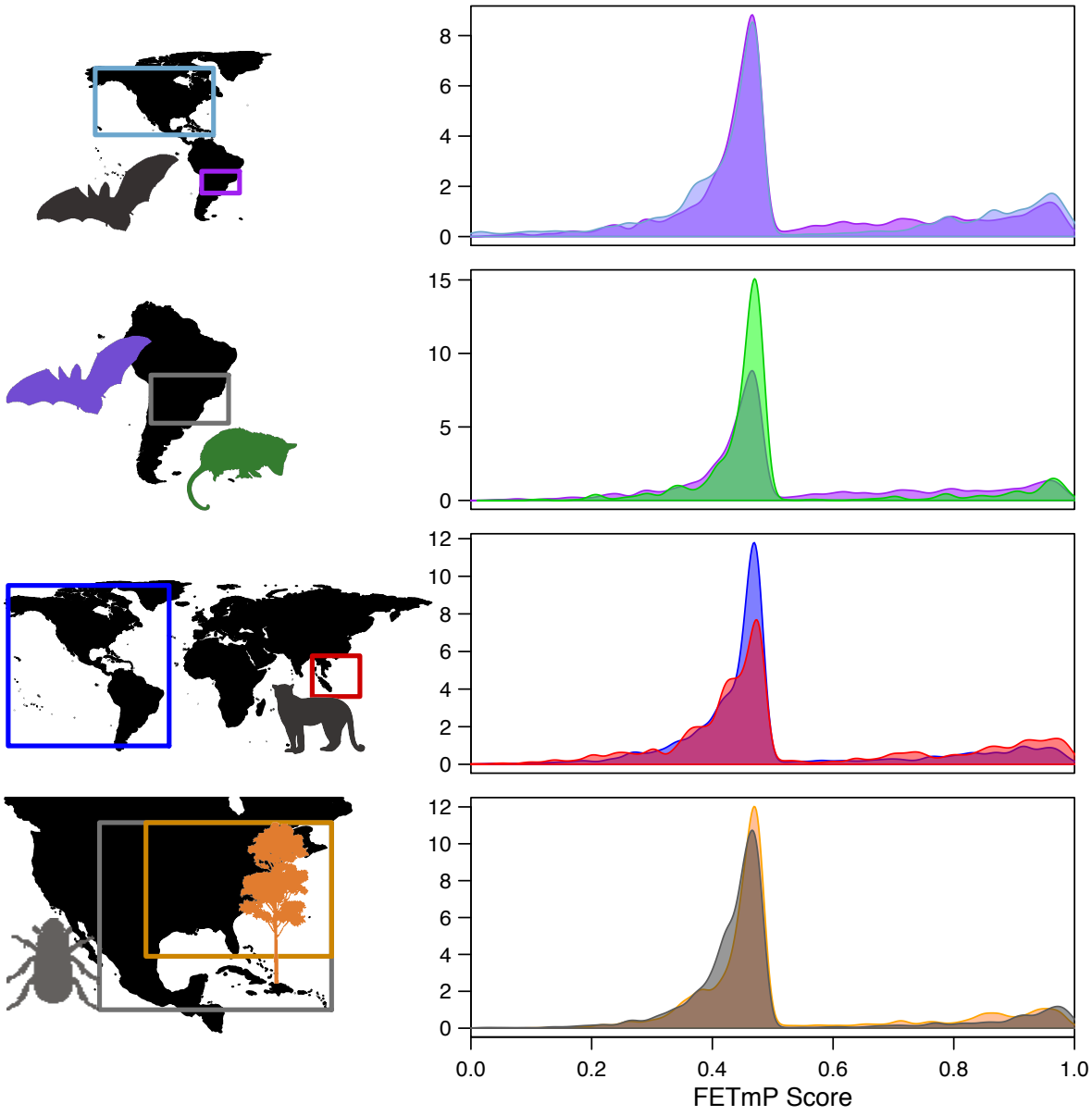


Figure 3.5. Association strength density distribution comparisons between various empirical datasets, which are specified on the left. Colours on the plots match the colour of either the taxon or spatial extent of the corresponding dataset. Values closer to 1 constitute strong positive associations and values closer to 0 constitute strong negative associations. Values near 0.5 are weak associations.

In the comparison of association strengths between our two bat datasets, North American bats had more associations with strong weights, suggesting that they form well-separated clusters. This is supported by the network visualisations (Fig. 3.6) and modularity calculations (Table 3.2). Both bat assemblages have high ratios of positive

connectivity to negative, suggesting that they assemble in nested communities and supporting a nested-compartmented assembly structure for North American bats. In our comparison between bat and rodent communities in southern Brazil, rodents had very low total connectivity, and pairs were dominated by weak negative associations, strong positive associations and very high modularity, suggesting high spatial turnover rates and insular local assemblages.

The two mammal camera trap datasets produced similar network structures (Fig. 3.6). The Indomalayan dataset had stronger positive associations, higher total connectivity, and lower modularity. This combination of features suggests nested structures that are weakly separated in space or overlapping. The North American dataset had positive to negative connectivity ratio close to 1, and a modularity of 0.35, features that are similar to the smooth gradient simulated matrix. This is compatible with our a priori knowledge of the data, which encompasses 100 degrees of latitude and is therefore expected to exhibit gradual turnover of species along this broad gradient.

Our final comparison, between trees and scarabs from North America, shows that scarabs exhibit slightly stronger associations (Fig. 3.5) and much higher modularity (Table 3.2, Fig. 3.6), indicating a more clustered structure.

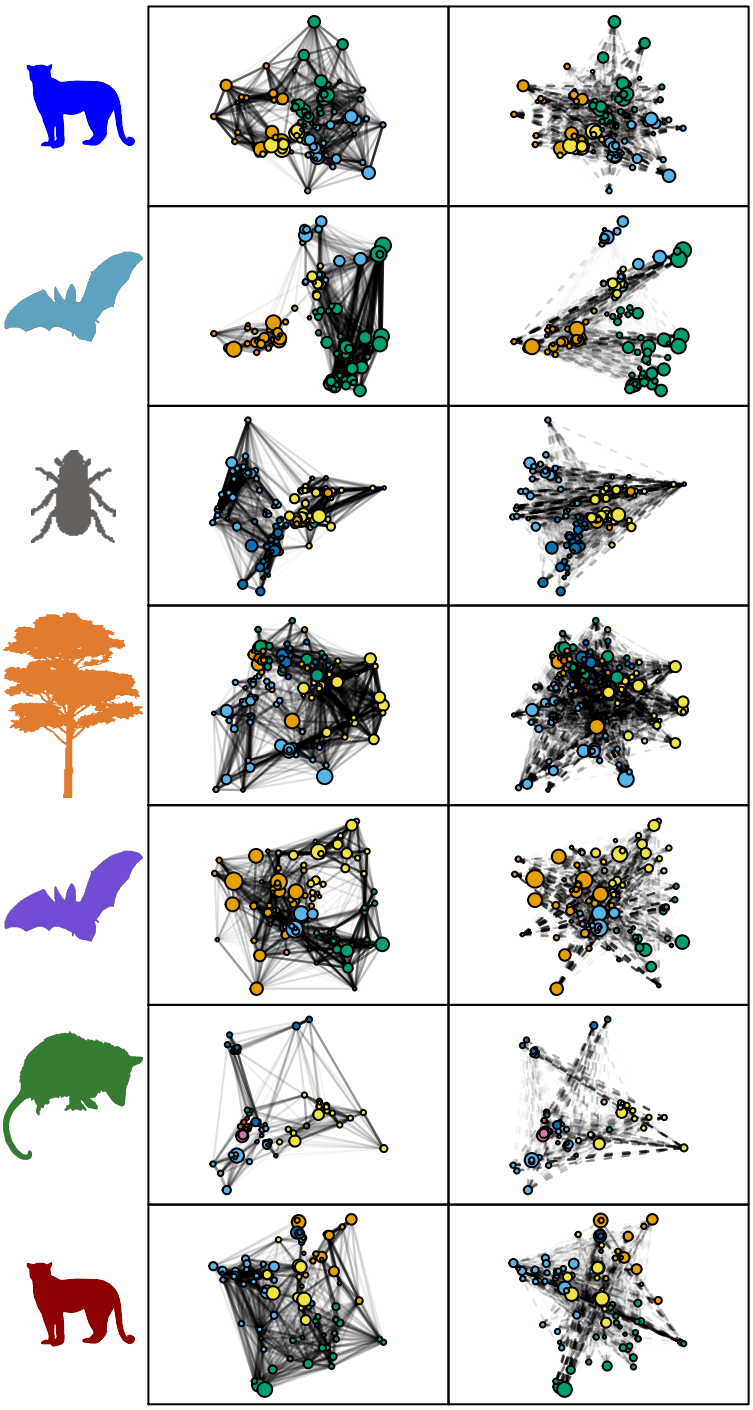


Figure 3.6. Network PCA plots of positive (centre) and negative (right) associations for each empirical dataset, indicated by icons (left; colours match Fig. 3.4). Node colours indicate clusters identified by the spin glass algorithm using mean z-scores for each pair, and node size indicates species occurrence frequency. Note that the placement of nodes is the same in positive and negative plots because the PCoA is calculated on all links at once. Positive and negative links are depicted separately for visual clarity.

Discussion

Binary metrics. The choice of an α-level can affect network-level metrics in ways that in turn affect biological interpretations. This issue extends to node-level determinations such as degree (the number of links connected to a node) and betweenness (proposed as identifiers of keystone species: Berry & Widder, 2014), which are also strongly controlled by α. In short, measurements of network properties using presence and absence of associations do not necessarily yield any information that is inherent to the network—except perhaps in isolated cases—nor do they elucidate any robust relationships between different networks. This property greatly limits the usefulness of full-network metrics as comparative tools. At best, pruning links from co-occurrence networks degrades the information contained in the network, and at worst, it could lead to misleading conclusions.

Simulated networks. Our simulated networks served to provide a baseline that illustrates how hypothetical network structures differ in link weight distributions and common network metrics. Related simulated structures (e.g. nested vs nested-compartmented matrices) often have similar link weight distributions, differing primarily in the density of weaker links. This suggests that weak links may sometimes contain important information about community co-occurrence structure.

Empirical networks. Several patterns emerged from our observation of empirical probabilistic co-occurrence networks. Despite the diversity of datasets, all of our matrices had more negative than positive associations. This appears to be a universal property at larger (landscape and continental) spatial scales. Total connectivity and modularity also fell within limited ranges compared to simulated networks, with a few notable exceptions. The bat

datasets tended to have much higher positive connectivity than negative connectivity, which suggests bats may be more adept at sharing space despite their high diversity. The only other animal dataset to have a similarly high ratio is the Indomalayan mammal dataset, which represents a system that has highly concentrated diversity. In sharp contrast, we observed low total connectivity of rodent communities in southern Brazil, coupled with the highest modularity. It appears that rodents share space with a limited number of other species, producing a high-turnover, low-diversity assembly pattern with distinguishable community types (clusters in Fig. 3.6), perhaps as a result of habitat specialisation. This would suggest that niche partitioning strategies differ between bats and rodents, namely, rodents fill niches limited in physical space, whereas bats may partition a different limiting resource, such as food type.

The high connectivity of our Indomalayan mammal dataset indicates that species are more affected by one another than in the North American mammal dataset. The structural differences between these mammal communities probably reflect the disparity in spatial scale: the New World mammal positive associations are weaker because species can disperse vast distances and are not affected by the distributions of other species. The Indomalayan dataset has much less land area and high richness, so its associations are more likely to reflect fine niche partitioning and biotic interactions. Our only plant dataset, for Eastern North American trees, has low total connectivity and also the lowest modularity. It may be worth exploring whether this difference is common to plant-animal comparisons, for instance, whether the sessile nature of plants causes them to form more loosely defined community types. It would also be interesting to examine the effect of seed dispersal strategy on network modularity of plants.

Some unexpected features emerged in our empirical analyses. Our empirical datasets generally did not replicate the association strength distributions of the hypothetical assemblage structures, even though their metrics indicated similarities to hypothetical structures. A specific example is that even though the North American mammal dataset had very similar ratios to the hypothetical smooth gradient, its association strength distribution is more similar to the other empirical datasets than it is to our hypothetical smooth gradient. It seems likely that the patterns in large-scale empirical datasets are more complex than accepted assemblage structure models. Previous studies of metacommunity structure have recognised the existence of assemblages with multiple driving variables (Leibold and Mikkelson 2002; Presley et al. 2010), but such patterns have not been extensively studied in empirical datasets. An emerging literature is describing a multi-dimensional network framework that includes multiple types of interactions (Pilosof et al. 2017; García-Callejas et al. 2018). Because probabilistic co-occurrence networks include all possible pairwise relationships, it is possible that probabilistic networks represent a collapsed multidimensional network (i.e. a network in which multiple processes and interaction types, usually separated out into several layers of networks, are conflated into a single network), and perhaps future research should involve a multidimensional approach.

ACKNOWLEDGEMENTS

The authors thank the Evolution of Terrestrial Ecosystems (ETE) working group for discussions that improved the paper. We thank Nick Gotelli for his insightful guidance on co-occurrence analysis. Support for this project was provided by Macquarie University. LCS was supported by a Peter Buck Deep Time postdoctoral fellowship from the Smithsonian's National Museum of Natural History. ABT was supported in part by the ETE Program of the

Smithsonian's National Museum of Natural History and NSF-DEB-1257625. This is ETE publication 379.

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CHAPTER 4

Reorganisation of surviving mammal communities after the end-Pleistocene megafaunal extinction

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A formatted version of this manuscript has been accepted for publication in Science.

ABSTRACT

Large mammals are at high risk of extinction globally. To understand the consequences of their demise for community assembly, we tracked community structure through the end-Pleistocene megafaunal extinction in North America. We decomposed the effects of biotic and abiotic factors by analysing co-occurrence within the mutual ranges of species pairs. Although shifting climate drove an increase in niche overlap, co-occurrence decreased, signalling shifts in biotic interactions. Furthermore, the effect of abiotic factors on co-occurrence remained constant over time, while the effect of biotic factors decreased. Biotic factors apparently played a key role in continental-scale community assembly before the extinctions. Specifically, large mammals likely promoted co-occurrence in the Pleistocene, and their loss contributed to the modern assembly pattern in which co-occurrence frequently falls below random expectations.

Tóth, A. B., Lyons, S. K., Barr, W. A., Behrensmeyer, A. K., Blois, J. L., Bobe, R., Davis, M., Du, A., Eronen, J. T., Faith, J. T., Fraser, D., Gotelli, N. J., Graves, G. R., Jukar, A. M., Miller, J. H., Pineda-Munoz, S., Soul, L. C., Villaseñor, A., & Alroy, J. (2019). Reorganization of surviving mammal communities after the end-Pleistocene megafaunal extinction. Science, 365(6459), 1305-1308. https://doi.org/10.1126/science.aaw1605

MAIN TEXT

Human activities have put extant large-bodied mammals at high risk of extinction (Davidson et al. 2009), and their eventual loss may have severe ecological repercussions. For example, the loss of ecosystem engineers such as megaherbivores has the capacity to alter entire landscapes (Zimov et al. 1995; Estes et al. 2011; Ripple et al. 2016). Such human-mediated extinctions will have impacts lasting far beyond our lifetimes, making it important to examine long-term records of past extinctions in order to forecast the consequences of current biodiversity loss. A key example is the catastrophic and approximately synchronous (Faith and Surovell 2009) extinction of large mammals, including mammoths and saber-toothed cats, at the end of the Late Pleistocene in North America (Barnosky et al. 2017). The rich and highly resolved Pleistocene and Holocene fossil record provides a unique opportunity to explore how extinction alters communities.

The causes of Pleistocene extinctions have been debated for decades (Lyons et al. 2004; Koch and Barnosky 2006). In light of the current biodiversity crisis, recent work has focused on understanding their ecological and evolutionary legacies instead (Malhi et al. 2016). A compelling picture of ecological transformation across the continents has emerged, including the disappearance of the mammoth steppe (Zimov et al. 1995), changes in vegetation and fire regimes (Gill et al. 2009; Johnson 2009; Rule et al. 2012), loss of functional groups (Davis 2017), loss or rearrangement of interactions (Lyons et al. 2016; Galetti et al. 2017), and shifts in global biogeochemistry (Smith et al. 2016a) and biophysical feedback systems (Doughty et al. 2016). However, empirical studies of changes in mammal community structure, including the extinction of most species over 40 kg (Lyons et al. 2004), have often

been centered on individual fossil deposits (Smith et al. 2016b) or particular taxa (e.g. (Pardi and Smith 2016; Hayward et al. 2016) but see (Lyons 2005; Plotnick et al. 2016)).

Here we examine community assembly patterns of surviving large mammals across the Pleistocene-Holocene transition using occupancy, niche size, and patterns of species co-occurrence. We examined end-Pleistocene (21-11 ka), Holocene (11-2 ka), and Recent (2-0 ka) (see supplement for details) mammal occurrence data (Fig. 4.1) drawn from the FAUNMAP II database (Graham and Lundelius 2010), comprising 93 species (> 1kg). Only survivor-survivor pairs were analysed to ensure that community changes were not simply a result of reduced diversity or lost associations involving extinct species. Every possible species pair received an association weight that quantifies how strongly the two co-occur. We refer to a species pair as aggregated when the species occur together more often than expected by chance, and segregated when they co-occur less often than expected. Segregations receive negative weights. Broad shifts in community assembly may be influenced by both extinction and climate change. We estimate the contributions of these two factors by isolating the relative effects of abiotic and biotic changes on the association of each survivor-survivor pair across this interval.

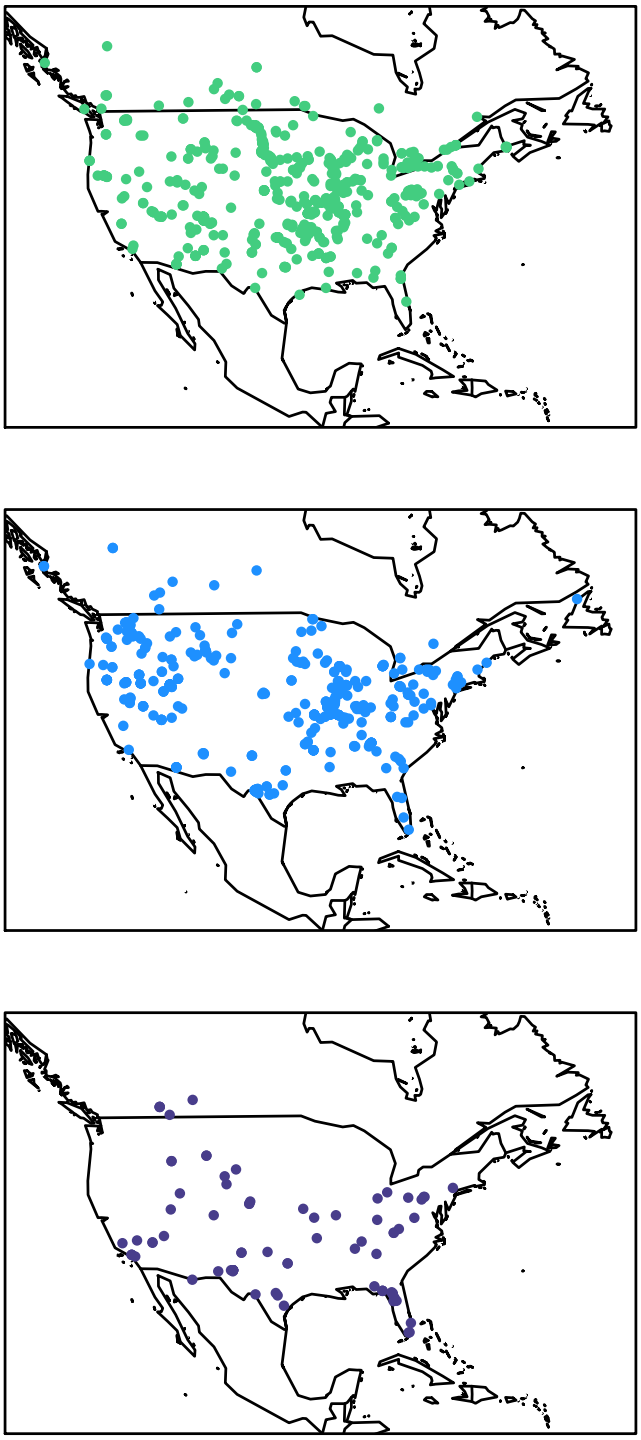


Figure 4.1. Maps of sites including large (>1 kg) mammals that fall within the Recent (top: 535 sites), Holocene (middle: 381 sites), and end-late Pleistocene (bottom: 78 sites).

Species associations are caused by a combination of abiotic and biotic drivers, which can be differentiated by first establishing species' geographic and environmental constraints.

Geographic envelopes were constructed using Lambert azimuthal equal area projected coordinates. The climatic envelope of each species was calculated from mean annual temperature, precipitation, temperature seasonality and precipitation seasonality of sites falling within the species' geographic envelopes. Climate estimates were extracted from downscaled paleoclimate simulations (Z. Liu et al. 2009; Veloz et al. 2012) and z-transformed. All envelopes were calculated with Blonder's hypervolumes (Blonder et al. 2014). The set of sites falling within both geographic and climatic envelopes (Fig. 4.2) was defined as the potential range of each species. The potential range represents sites where the occurrence of a species is not constrained by climate or dispersal ability. We also calculated background climatic and geographic hypervolumes for each species in each time interval to quantify how much of the available geographic and environmental space is being occupied by each species (see supplement).

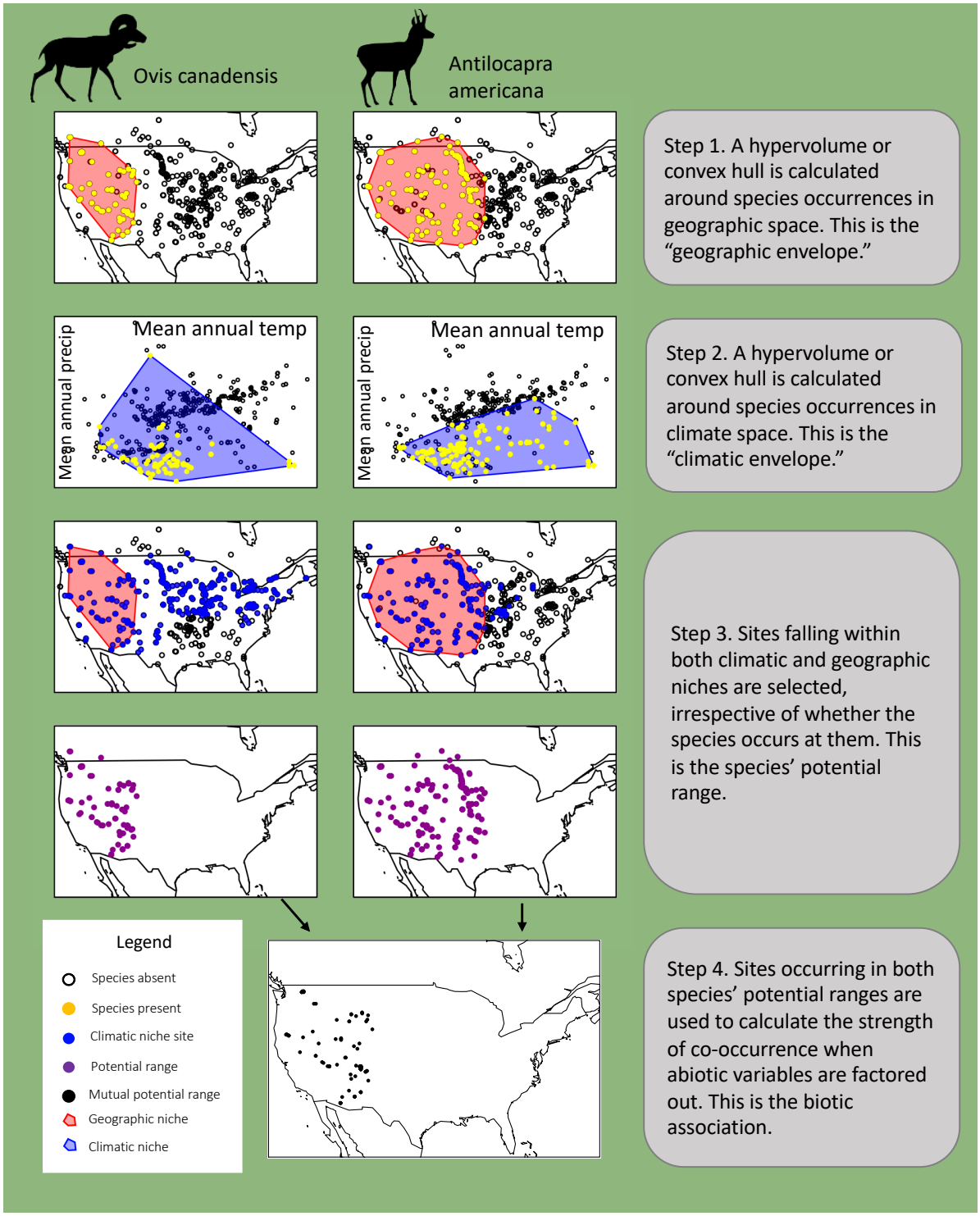
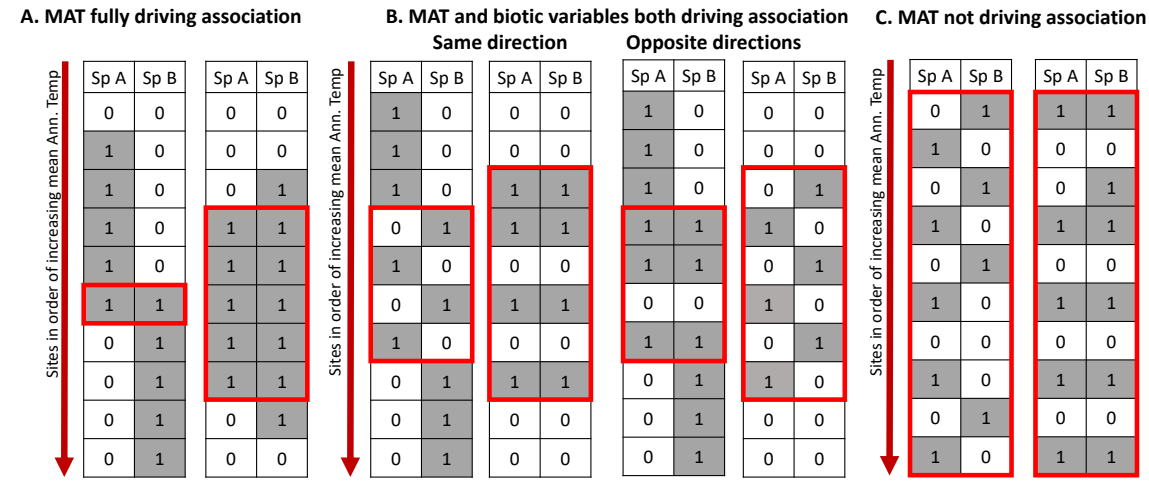


Figure 4.2. Calculation of mutual potential range sites for an example pair. This schematic illustrates the process of selecting the mutual potential range sites of an example pair. Mutual potential range sites are used to calculate the biotic component of associations.

We calculated the strength and direction of pairwise co-occurrence of species pairs with the mid-P variant of Fisher's Exact Test, which provides an association weight for each pair (Kallio et al. 2011). We then individually calculated biotic and abiotic components of co-occurrence, such that the sum of the association weights of these two components equals the original association weight (Fig. 4.3). We did this by calculating the association weight within the mutual potential range (i.e., the sites remaining after accounting for abiotic limits for both species), which represents the component of each association regulated by biotic factors. The abiotic component was defined as the difference between the full association and its biotic component (see supplement). The abiotic component of a pair received a positive association weight if species have similar niches, and negative if their niches were disparate (Fig. 4.3). The biotic and abiotic components of a pair may have the same or opposite signs, and when the latter occurs the full association weight may be close to 0 (Fig. 4.3). Using this framework, we evaluated changes in co-occurrence patterns and their components across the Pleistocene-Holocene transition and into the Recent.



Full	-1.6	1.75	-2.3	2.8	-0.6	-1.05	-2.3	2.3
Biotic	0	0	-1.4	1.8	1.2	-1.96	-2.3	2.3
Abiotic	-1.6	1.75	-0.9	1.0	-1.8	0.9	0	0

Figure 4.3. Explanation of the mutual potential range calculation. Example calculations of biotic and abiotic components across a hypothetical set of sites, simplified to one abiotic variable for clarity (sites are ordered vertically in order of increasing mean annual temperature). Three scenarios are depicted for segregation (first table of each pair) and aggregation (second table in each pair). The second scenario has two additional tables depicting hybrid situations. (A) Mean annual temperature is responsible for the association. (B) Mean annual temperature is partially responsible for the association. (C) Mean annual temperature does not explain the association. The association weight is calculated first for the full set of sites and then for the mutual potential range (red rectangle). In scenario (A), the biotic score is strongly reduced in absolute value (compare full to biotic association weight). In (B) the score is somewhat reduced or flipped when biotic and abiotic regulators are acting in the same direction. When regulators are acting in opposite directions, there is no predictable pattern but component scores have opposite signs and may be stronger (absolute value) than the full association weight. In (C) the score is not reduced, and it may increase. Associations observed only within the mutual potential ranges cannot be attributed to the abiotic variables being tested.

Across the Pleistocene-Holocene transition, common surviving species became even more common and rare species remained the same or became rarer (Fig. 4.4A). There were no significant changes in occupancy patterns between the Holocene and the Recent (Fig. 4.4B). Extinction victims had smaller climatic niches and geographic ranges than survivors in the end-Pleistocene (Fig. 4.5). On average, climatic and geographic envelopes of surviving species expanded from the end-Pleistocene to the Holocene, even when compared to background variation (i.e., as a proportion of the total space each species could potentially occupy; Fig. 4.5B-C)

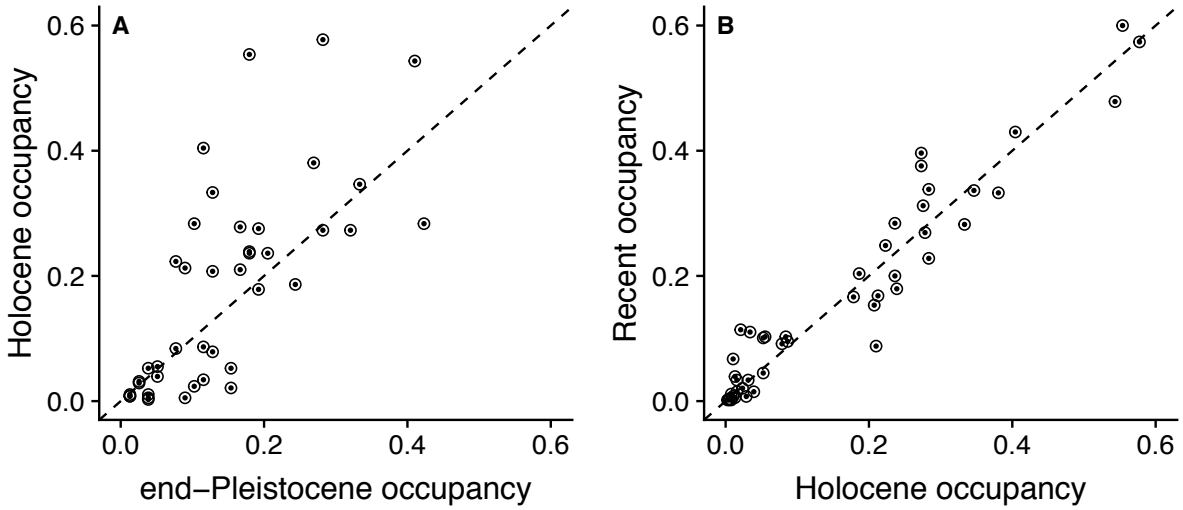


Figure 4.4. Occupancy (fraction of sites occupied) changes for survivors over the two time interval transitions: (A) end-Pleistocene to Holocene (n = 44) and (B) Holocene to Recent (n = 45). Points represent species, plotted over the line of unity. Only species sampled in consecutive time intervals are shown.

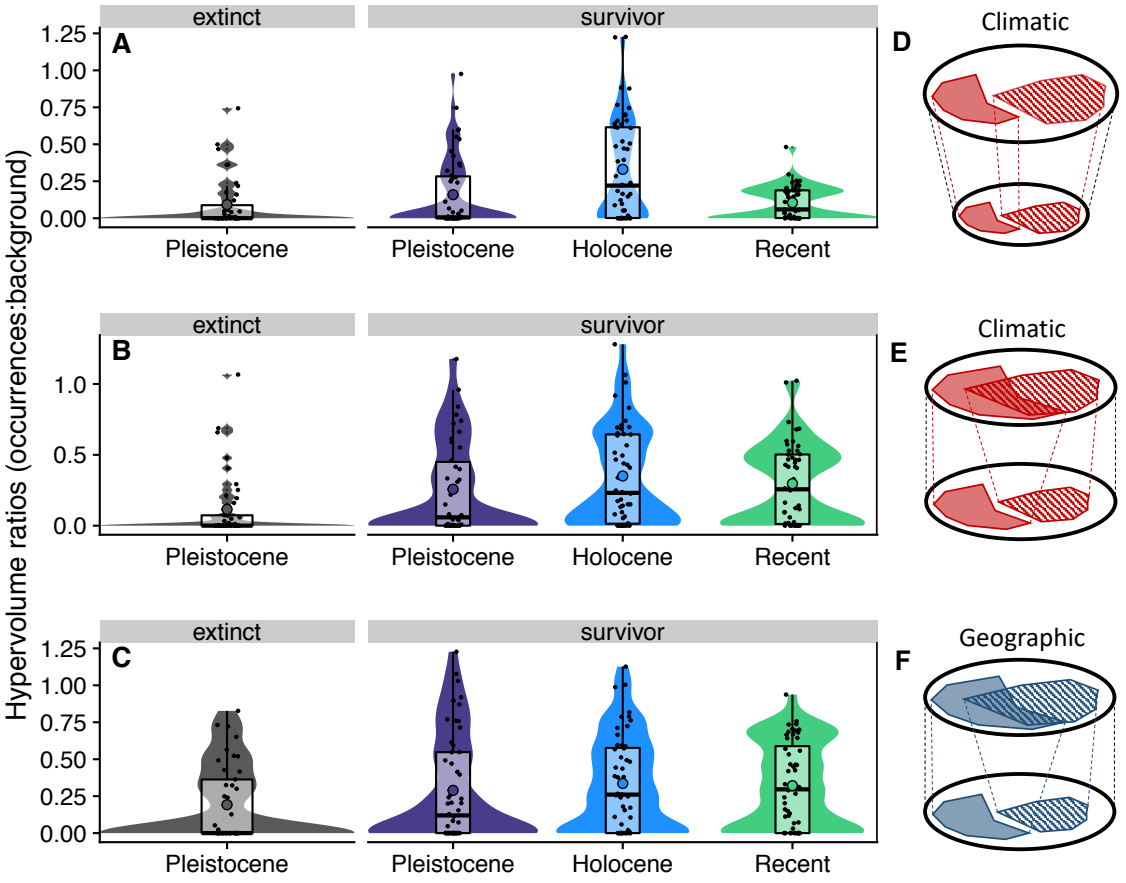


Figure 4.5. Increases in niche overlap. Climatic (A-B) and geographic (C) envelopes of species are compared to pooled climate envelopes (A) and background envelopes (B-C) in each time interval. In (A) larger ratios correspond with larger niches because niche space expands, as illustrated by oval sizes in (D); in (B) and (C) larger ratios result from proportionately higher fill that causes increased niche overlap (E-F). In (A-C), each shaded distribution sums to an area of 1; circles are means. In (D-F), shared polygons represent hypothetical species niches.

Aggregations were dominant for survivor pairs in the end-Pleistocene, and segregations increased in the Holocene and Recent (Fig. 4.6E-F). There was also a marked decrease in association weights for aggregations and an increase for segregations over the Pleistocene-Holocene transition (Fig. 4.6K-L). We considered and ruled out several confounding factors such as sampling and dating biases (see supplement). Observed occupancy changes between the time intervals predict stronger associations and increased

proportion of aggregations (see supplement). While this may partially explain why segregations became stronger, it cannot explain the increase in proportion of segregations or the decrease in aggregation strengths. When associations were split into their biotic and abiotic components, end-Pleistocene associations calculated within mutual potential ranges of pairs (i.e., biotic associations) were also dominated by aggregations, which diminished in both mean weight (Fig. 4.6I) and as a proportion of the pairs (Fig. 4.6C) across the Pleistocene-Holocene transition, while segregations increased in mean weight and proportionally (Figs. 4.6J and 4.6D). Abiotic associations (i.e., the difference between the full association and the biotic association) exhibited the opposite pattern (Figs. 4.6A-B, G-H). Note that associations due to abiotic components were typically segregations while those due to biotic components were typically aggregations, and this pattern was greatly weakened but not overturned by the trends described above.

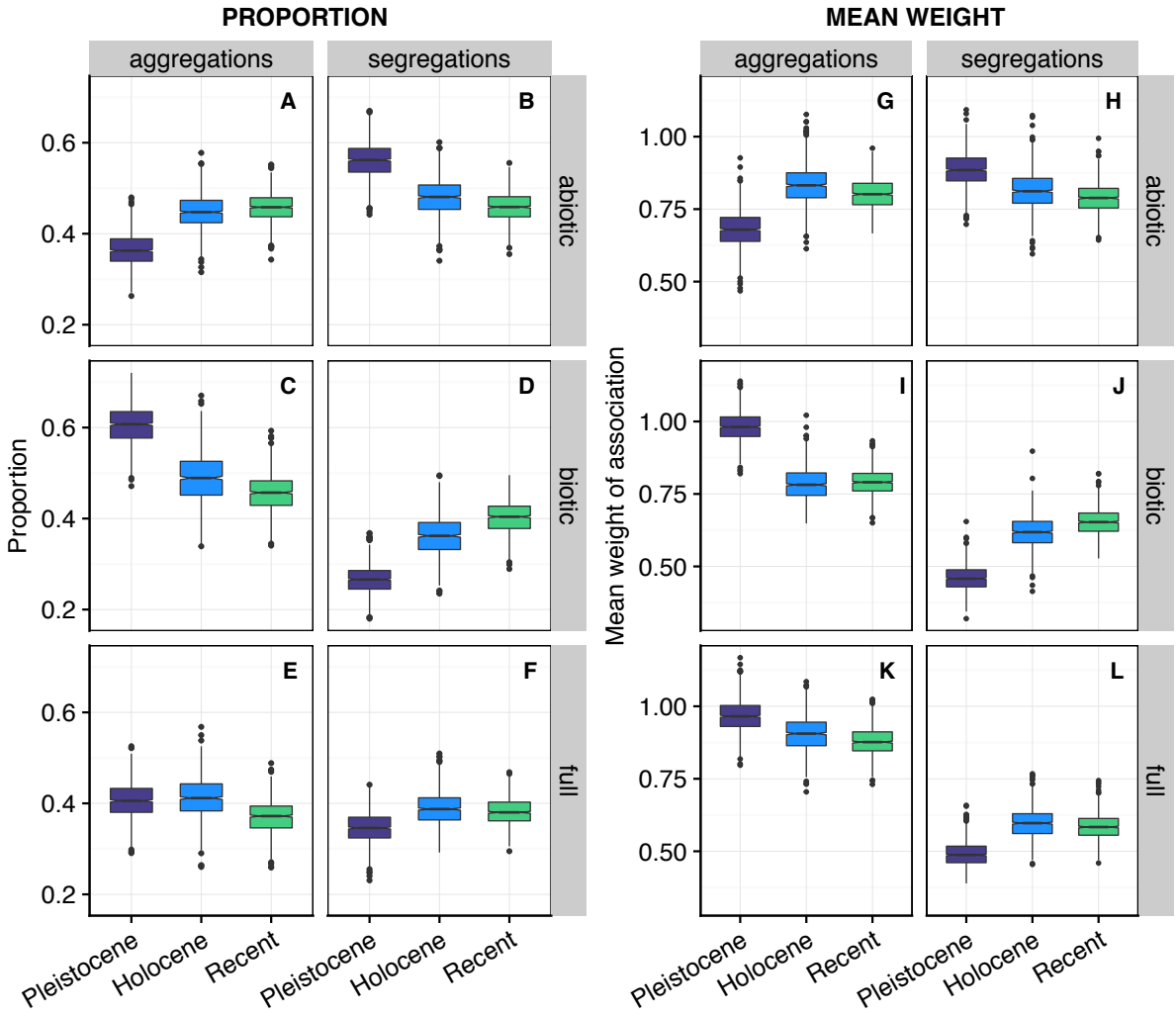


Figure 4.6. Proportion of aggregations (left) and segregations (right) for abiotic components (A-B), biotic components (C-D), and full associations (E-F) in each subsample (n = 1000). Only pairs with at least 10 sites falling within their mutual niche are included.

The Pleistocene-Holocene transition was characterized by substantial changes in occupancy (Fig. 4.4), niche size (Fig. 4.5), and association patterns (Fig. 4.6). The fact that survivors of the extinction exhibited larger potential ranges than the victims (Fig. 4.5) is consistent with the concept that specialists with narrow ranges are at higher risk of extinction (Davidson et al. 2009). The expansion of climatic niche fill in the Holocene may reflect the filling of empty niche space after competitive release.

The overall shift toward segregations starting in the Holocene resulted from changes in the relative effects of the biotic and abiotic components of species co-occurrence. Increasing climatic and geographic niche fill (Fig. 4.4) drives increasing potential range overlap between pairs in the Holocene (Fig. 4.7), and this caused the shift toward aggregations in abiotic associations. In contrast, co-occurrence decreased within mutual potential ranges (i.e. biotic associations; Fig. 4.6C-D). All else being equal, these opposing forces might have annulled any trend in the full associations. We observe a trend, however, because of the change in the relative importance of biotic and abiotic factors, which can be quantified using the average magnitude (absolute value) of association weights within each component. Species responses to environmental factors contributed consistently to community assembly over time despite the dramatic climatic changes driving species dispersal over this interval (Graham et al. 1996), while co-occurrence patterns due to biotic interactions diminished after the end-Pleistocene (Fig. 4.8). This loss of biotic regulation contributes to the segregation pattern, as biotic interactions tend to promote aggregations (compare Fig. 4.6C and 4.6D). Thus, the decrease in co-occurrence was driven by the combined effects of weakening biotic associations and a decrease in the tendency of biotic associations to be aggregated. Therefore, shifting biotic factors (i.e. the loss of the megafauna or the advent of humans), not climate change, were responsible for the ecological upheaval.

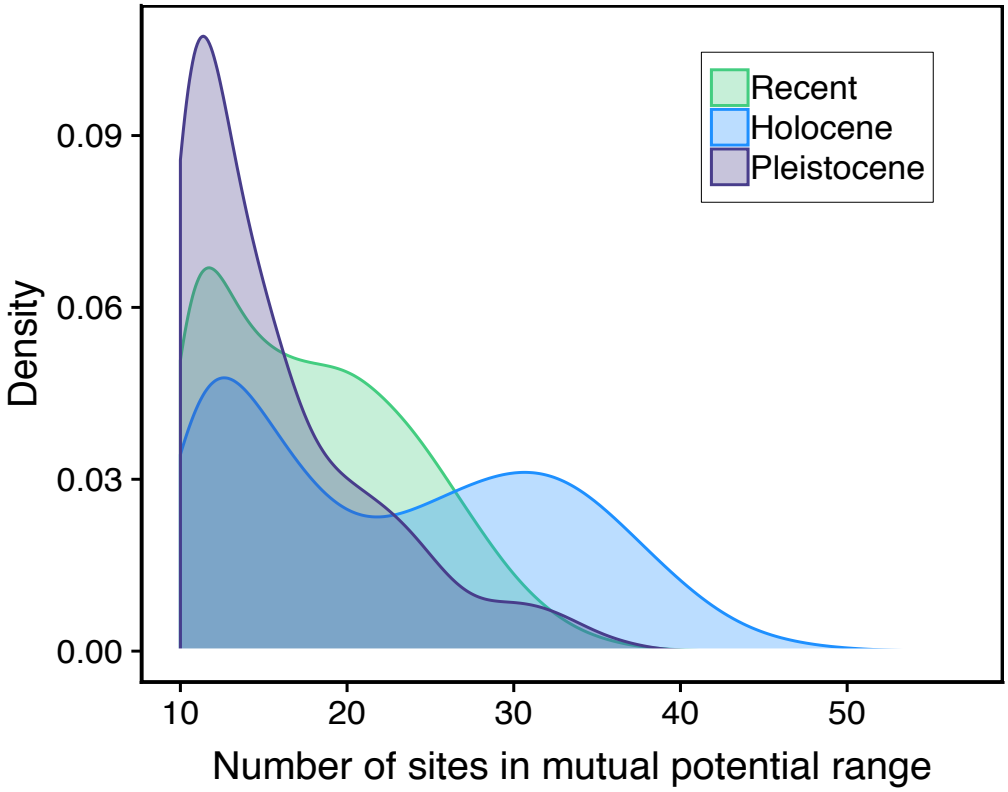


Figure 4.7. Number of sites in the mutual niche M_{ij} of each pair over the three time intervals for pairs with at least 10 mutual niche sites. Mutual niches are calculated on subsamples of 60 sites to standardize sampling, so 60 is the maximum overlap. All else being equal, an increase in the number of mutual niche sites causes an increase in the strength of abiotic aggregations and a decrease in the strength of abiotic segregations, because it results in more mutual absences in the full set and fewer sites where one species in the pair occurs without the other.

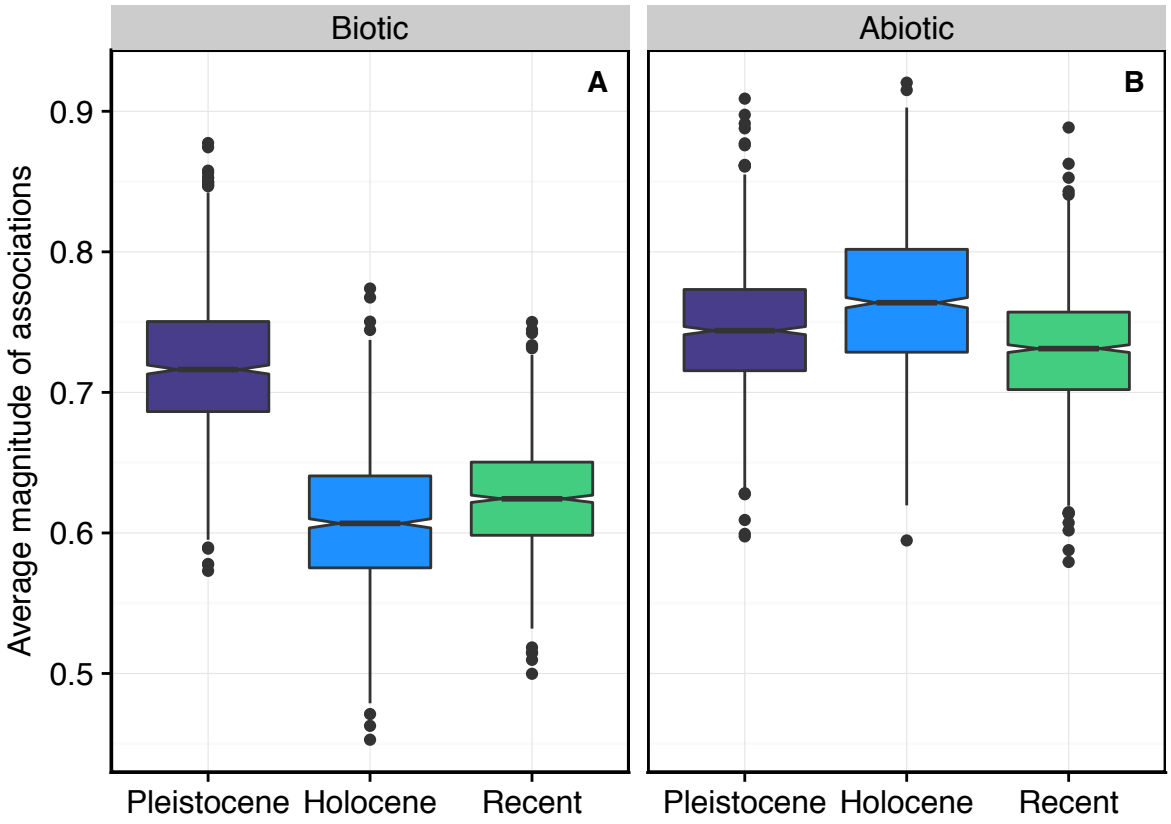


Figure 4.8. Absolute values of associations weights, broadly representing the relative importance of biotic and abiotic components for overall community assembly patterns. Boxplots represent the variation among subsamples (n = 1000).

It is difficult to determine from our results whether the change in survivor co-occurrence is a direct result of the loss of survivor-victim interactions, more indirectly due to the loss of megafauna in their role as ecosystem engineers, or other contemporaneous changes such as increasing human impacts. Pleistocene predators were often more specialized (Van Valkenburgh et al. 2004), and their loss may have allowed survivors to consume a wider range of prey species, reducing the need to co-occur strongly with a primary prey species and thus weakening aggregations. In addition, the loss of large-bodied prey could have caused prey-shifting to more abundant smaller-bodied mammals and thus reduced fidelity to any particular prey species (Hayward et al. 2016). Segregations also increased in

abundance and magnitude within mutual potential ranges. One potential explanation is that the loss of predators and competitors increased the abundances of survivors in a rapid competitive release scenario (Alroy 2001) that eventually led to enhanced competition and increased exclusion from mutual niche sites.

Contemporary loss of keystone species causes direct and indirect effects on other species and communities (Beschta and Ripple 2009; Estes et al. 2011; Ripple et al. 2015) via the loss of biotic interactions. These include top-down biotic processes (Estes et al. 2011), higher-order interactions (i.e., a third species affecting the interaction of two others) (Levine et al. 2017), ecosystem engineering, pollination, pest control, and nutrient cycling (Doughty et al. 2016). Such loss often results in reduced biodiversity and degradation of ecosystem health. The extinction of the megafauna may have caused substantial shifts in the biotic drivers of community assembly via similar pathways, particularly via the loss of top-down control and the liberation of resources. The trend away from aggregations is crucial because it has been suggested that coexistence enhances biodiversity through the emergence of higher-order interactions (Levine et al. 2017), and biodiversity is a central focus of modern conservation efforts.

The end-Pleistocene extinction caused measurable, lasting effects on the dynamics of mammal communities that go beyond simple biodiversity loss. Our analysis suggests that it disrupted a network of species interactions that supported high levels of aggregation, leading to a modern fauna in which continent-wide species associations are now regulated more strongly by climate and dispersal limitation and are characterised increasingly by segregation. We find that biotic mechanisms such as species interactions and range dynamics once played a measurable role in mammal community assembly by consistently affecting how species

co-occur on continental scales. Remaining species interactions among survivors likely take place opportunistically, on smaller scales, or within shorter timeframes. Overall, we find that biotic mechanisms now play a reduced role in species co-occurrences on a continental spatial scale, and this shift was most likely driven by the extinction of the Pleistocene megafauna.

ACKNOWLEDGMENTS

We thank the palaeobiology lab group at Macquarie University for discussions that improved this paper. Support for this research was provided by a National Museum of Natural History Program grant to the Evolution of Terrestrial Ecosystems Program (ETE) and NSF-DEB 1257625. This is ETE publication #382. ABT was supported by an iMQRES scholarship from Macquarie University.

SUPPLEMENTARY MATERIALS

Materials and Methods

Data. We obtained species-by-site tables of mammal occurrences from the FAUNMAP II database (Graham and Lundelius 2010), which documents fossil localities across North America (excluding Mexico; Fig. 4.1). Using the pre-existing FAUNMAP epoch classifications, we extracted sites from the Holocene (HOLO) and Late Pleistocene (LPLE, PLEI). A site is defined as an entity having a unique combination of the fields 'analysis unit' and 'machine number', so different strata from the same locality were treated as separate sites (see supplemental section on time averaging for a discussion of the effects of site duration). We did not use sites whose FAUNMAP epoch classifications were HOPL (i.e., not clearly Holocene or Pleistocene). Although dating of individual sites within FAUNMAP is often imprecise, this treatment ensured that the Holocene and Pleistocene species clearly represent pre- and post-

extinction faunas (i.e. communities with extinct megafauna and communities without them), despite some minor inconsistencies in dating boundaries. Finally, we removed indeterminate species and marine species such as cetaceans, sirenians, pinnipeds, and sea otters.

We obtained mean body mass estimates for fully identified species from the MOM 3.0 database (Smith et al. 2003), and removed species with mass estimates less than 1 kg, which excluded bats from the analysis. We did this to focus our main analysis on larger species, but also to avoid biases associated with sampling methods, which differ for small and large mammals. From the resulting species-by-site tables (one for each time interval), we removed sites that were above a latitude of 60˚N in order to avoid artificial biogeographic biases introduced by the large unsampled area separating Alaska and the Yukon from the rest of the sites.

We then discarded sites with fewer than five species to exclude samples which are unlikely to reflect the original communities and to increase the computational speed and accuracy of community analyses. Including a large number of sites with only one or a few species would result in very low matrix fill, and therefore artificially cause co-occurrence results to be biased heavily toward negative associations.

We downloaded the locality data table from FAUNMAP and extracted metadata on our remaining sites. We calibrated all uncalibrated ^{14}C dates with the IntCal13 calibration curve (Niu et al. 2013; Reimer et al. 2013) in OxCal (Bronk Ramsey 1995). The dates of sites estimated with other methods were left unchanged. This is unlikely to bias our analyses because the sites were grouped into broad intervals for our analyses, where the most important distinction was the separation of pre- and post-extinction faunas. We left the

Pleistocene data in their original FAUNMAP epoch categories, but we divided the Holocene epoch into Holocene and Recent time intervals, setting the boundary at a maximum calendar date of 2 ka. We limited the end-Pleistocene interval to sites whose mean age was younger than 21 ka. This is the oldest interval that has associated climate simulations, and taking this step also accounts for bias associated with time averaging by establishing roughly equal temporal durations for the end-Pleistocene (9.3 ka) and the Holocene time intervals (9.7 ka).

Climates were inferred from the CCSM3 paleoclimate simulations (Z. Liu et al. 2009), which were downscaled to 0.5˚ x 0.5˚ at 1000-year intervals from 0 to 21 ka (Veloz et al. 2012). We extracted mean annual precipitation (MAP) and mean annual temperature (MAT) for each site, matching the mean calibrated calendar ages of sites to the corresponding climate inferences. We excluded sites for which we could not estimate climate (i.e., sites assigned to a single epoch in FAUNMAP but lacking dates).

The final dataset had the following time intervals: end-Pleistocene (21-11 ka), Holocene (11-2 ka), and Recent (2-0 ka). The general properties of our final dataset are listed in Table 4.1, and their locations are mapped in Fig. 4.1. The end-Pleistocene dataset includes 83 species, the Holocene 48 species, and the Recent 48 species (Table 3.1), and 45 of the latter are sampled in both the Recent and Holocene, for a total of 51 surviving species. The discrepancy between Holocene and Recent data sets is due to less common species not being sampled in both time intervals. Of the end-Pleistocene species, 45 survived and 53 went extinct (i.e., they were not sampled in the Holocene and Recent and are listed as extinct in the recent literature). The only contentious extinction was that of *Martes nobilis,* which was considered an extinct species distinct from *M. americana* in this study (Hughes 2009). The taxonomy of extinct mammals does not affect our analysis, as we only compared associations

among surviving species. For instance, there is a lack of consensus about the splitting of extinct *Equus* species, but it is widely accepted that only one species, *E. ferus*, is still extant. Finally, it is widely known that some extinct species may have survived briefly into the Holocene (Dale Guthrie 2004). Because we wished to compare surviving faunas pre- and post-extinction, the sites in our time intervals represent pre- and post-extinction faunas, even at the expense of precise date boundaries. In other words, Holocene-dated sites that include any remnants of megafauna were not included in our Holocene dataset. The dataset represents 105 species in total. With the exception of the niche space analyses, which included a category for extinction victims, the central analyses in this manuscript are focused on community changes of the survivors across the three time intervals. This is because we were interested in how changes over the extinction interval affected the community structure of extant species.

Table 3.1. Basic properties of the dataset used in this paper, split by time interval. Note that overlaps in duration are a result of including loosely dated sites whose age ranges place their maximum age estimates outside of the accepted boundaries of their time intervals. Specifically, eight Holocene sites have maximum age estimates older than 11 ka.

Epoch	Sites	Species	Matrix fill	Duration (by Max Age)	Occupancy Range	Occupancy Median/Mean
Recent	535	48	0.160	2.0-0 ka	0.600	0.103 / 0.160
Holocene	381	48	0.155	13.1-2 ka	0.577	0.081 / 0.155
End-Pleistocene	78	83	0.117	21-11 ka	0.423	0.090 / 0.117

Niche space analysis. Understanding the breadth of the geographic extent and climatic niche of each species helps to interpret how abiotic factors affected the co-occurrence

structure of the assemblage pre- and post-extinction. We estimated the climatic and geographic envelopes of each species, and then compared the sizes of these envelopes to several types of backgrounds.

We estimated the realized climatic envelope (*C*) and geographic envelope (*G*) of each species *i* in each time interval in two ways. First, we used Blonder's hypervolume package (Blonder et al. 2014) in R to construct a hypervolumes with the geographic coordinates (projected into the Lambert azimuthal equal area projection and z-transformed to make them more comparable to climatic envelopes) of each occurrence of species *i*. These hypervolumes represent the geographic envelope G_i of each species. We repeated the process to make climatic envelopes C_i using z-transformed mean annual precipitation, mean annual temperature, precipitation seasonality, and temperature seasonality, extracted for each site from the temporally corresponding layer in a previously published, downscaled paleoclimate simulation (Z. Liu et al. 2009; Veloz et al. 2012). Mean annual precipitation and precipitation seasonality were also square root transformed before calculating z-scores. Climatic envelopes were calculated only from sites falling within the geographic envelope of each species in each time interval. We then compared the sizes of these climate envelopes to three background variations.

First, to investigate changes in the degree of niche infilling over time, we calculated background climate from the absences within each species geographic niche within each time interval (background 1). This comparison explores the extent to which species filled the climatic niche that they accessed in each time interval.

Second, to investigate changes in the degree of potential niche infilling over time, we calculated a background climate from the pooled absences within the geographic envelope of each species across all three time intervals (background 2). This comparison asks: in each time interval, to what extent did species fill the climatic niche that they accessed over all time intervals? This also addresses the question of whether, objectively, the niches of species expanded or contracted, whether or not the background variability changed. We expect time intervals with larger overall climatic variability to exhibit larger niche sizes.

Third, to investigate changes in the degree of available niche fill over time, we calculated background climate from the absences of each species within each time interval (background 3). This comparison investigates the extent to which species filled the climatic niche that they had the ability to access within each time interval. Mammal species, particularly larger-bodied mammal species, are very mobile and are easily able to disperse thousands of kilometres over time intervals as long as the ones in this study. Environmental and biotic factors, not physical boundaries or physiological limitations, would have constrained their realized geographic envelopes. The purpose of calculating background this way was to understand how much climate space was utilized by each species with respect to the total unused available background, because this influences the degree of overlap in the potential ranges of species, which has bearing on the abiotic component of the co-occurrence analysis.

We did not include geographic and climatic variables in the same hypervolumes because geographic coordinates are often collinear with climatic variables, and this restricts the hypervolumes to a hyperplane, strongly affecting volume calculations. Including collinear variables in the same hypervolume is not recommended by the authors (Blonder et

al. 2014). Furthermore, species are not biologically confined to particular combinations of climate and geographic location. Species should disperse to any sites with suitable climatic conditions as long as they are able to reach them.

We repeated this process using simple convex hulls instead of hypervolumes to calculate geographic, climatic, and seasonality envelopes. Geographic range was estimated for each species *i* by calculating the area of the convex hull G_i around the locations (plotted using the Lambert azimuthal equal area projection and z-transformed) where the species occurred. Climatic envelope was estimated by calculating the area of the convex hull C_i around occurrences plotted by their mean annual temperature in ˚C and square root transformed mean annual precipitation in mm/year, taken for each site from the temporally corresponding layer the downscaled paleoclimate simulations (Z. Liu et al. 2009; Veloz et al. 2012) and z-transformed. Seasonality envelope was estimated by taking the area of the convex hull S_i around the occurrences of each species plotted by z-transformed temperature and precipitation seasonality (from the same source as mean annual climate data).

We compared estimated geographic envelope and climate envelope areas for victims of the extinction in the end-Pleistocene and survivors across the three time intervals. In the main text, we present the areas of the hypervolume-based geographic envelopes with respect to unoccupied geographic space, and the volumes of climatic envelopes with respect to backgrounds 2 and 3 described above (Fig. 4.5). We present the raw hypervolume sizes and background 1 in Fig 4.9. All of the convex hull results (including raw areas and areas with respect to background variation) are presented in Figs. 4.10 and 4.11.

The potential range of each species was then calculated as the intersection of the climate and geographic envelopes for that species across the entire time interval (Fig. 4.2). The set of sites in the potential range of each species (P_i) thus consists of all the sites where the species occurred, plus any additional sites that fall within both G_i and C_i, thereby ensuring that they are environmentally suitable and geographically accessible for species *i*. The collection of sites within the potential ranges of species were used later to conduct the analysis of mutual potential range co-occurrence. Potential ranges based on hypervolumes are used in the main text, but the same analyses using convex hulls are included in the supplementary figures.

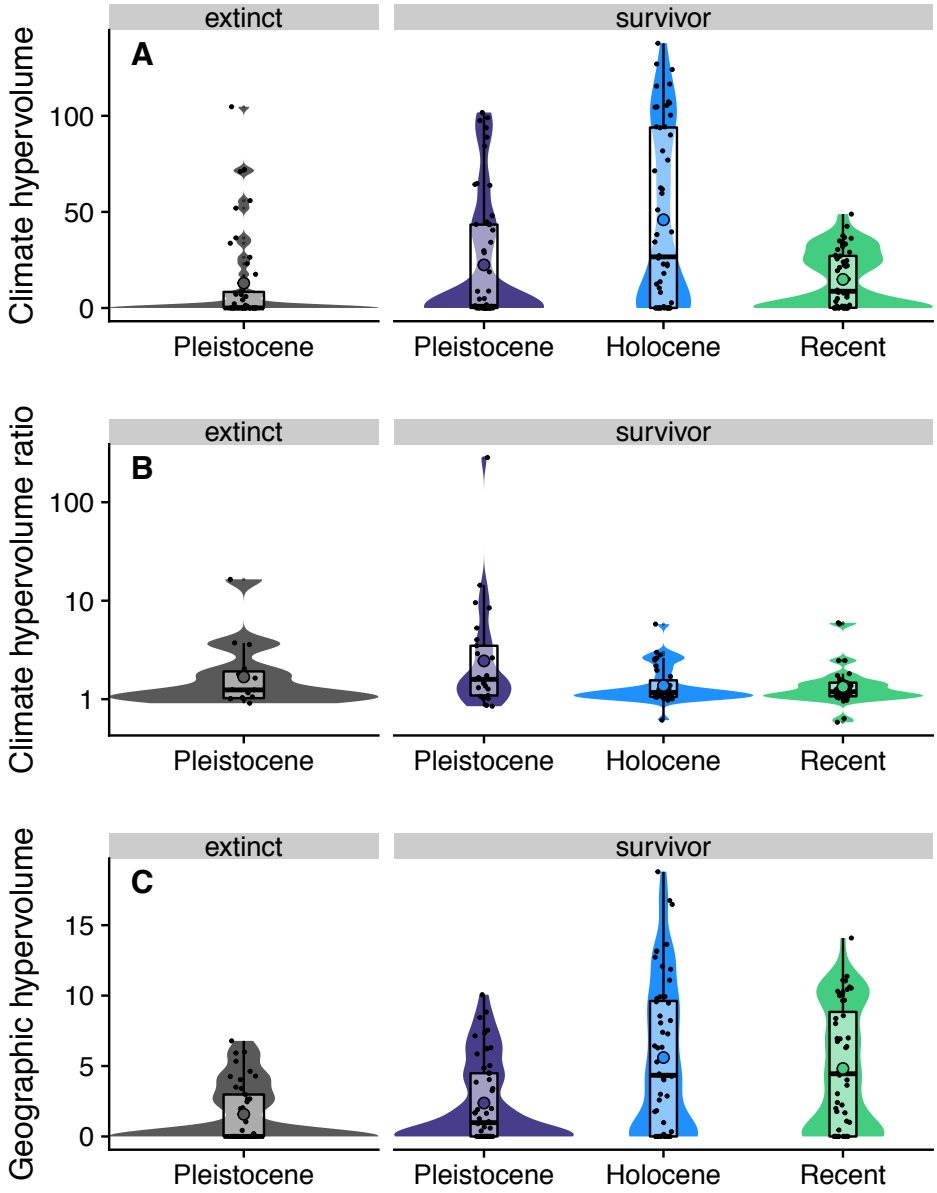


Figure 4.9 Niche hypervolumes. (A) Climatic envelope of each species calculated from occupied sites inside its geographic hypervolume in each time interval. (B) Climatic envelopes in A as a ratio of the climate envelope calculated from unoccupied sites inside each species' geographic hypervolume in each time interval. (C) Geographic envelope of each species by time interval. Species climatic hypervolumes include mean annual temperature, mean annual precipitation, temperature seasonality, and precipitation seasonality. Geographic hypervolumes are constructed from projected equal-area geographic coordinates. Shaded areas represent density distributions with a total area of 1.

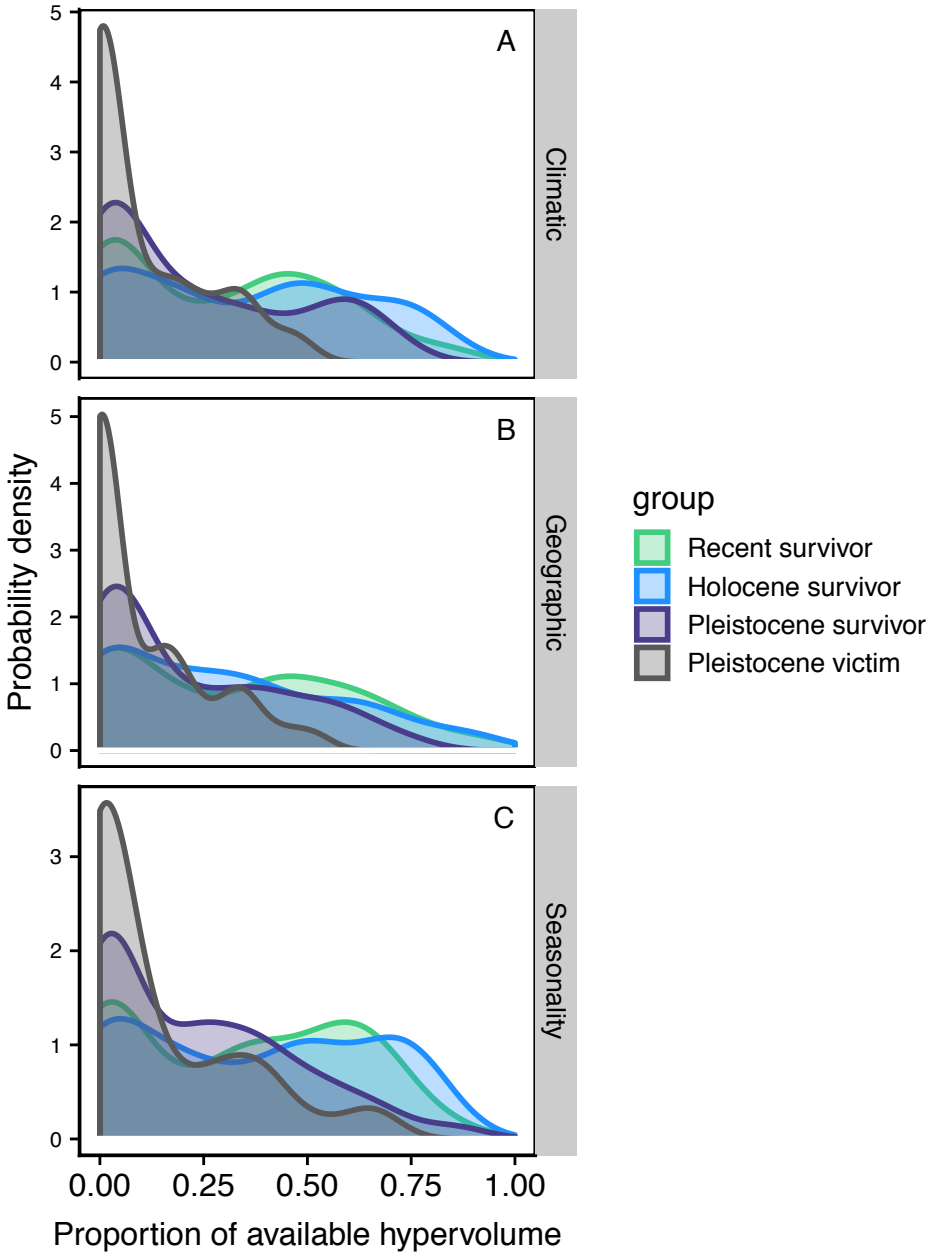


Figure 4.10. Relative niche areas**.** Density distribution of geographic, climatic, and seasonality convex hull areas, as a proportion of total background variability in each time interval.

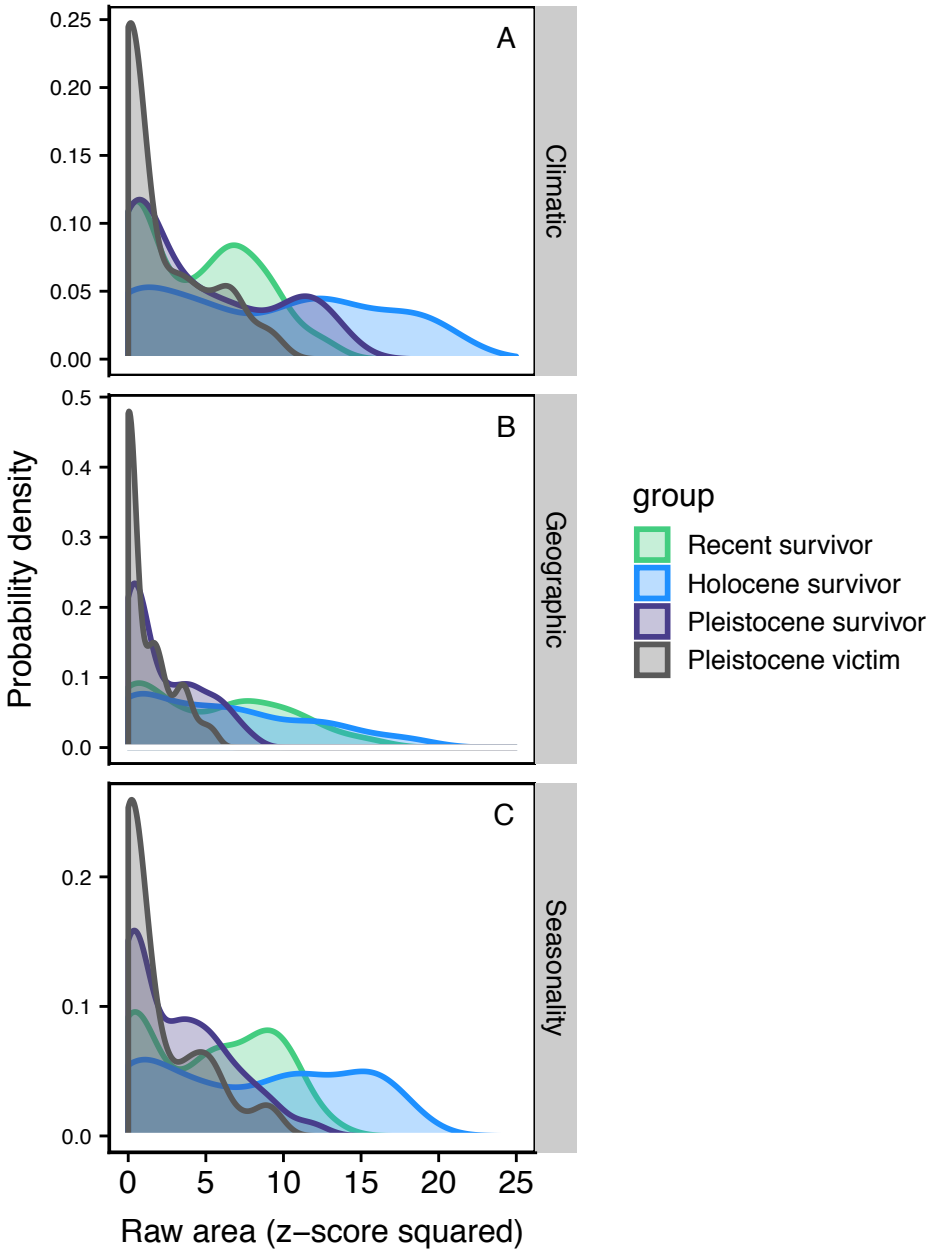


Figure 4.11. Raw niche areas. Density distribution of climatic, geographic, and seasonality envelope areas in the time intervals, calculated with convex hulls. The raw areas are displayed, without accounting for background variation.

Co-occurrence analysis. Interactions between species are recognized as crucial to community assembly (Blois et al. 2013). Co-occurrence analysis quantifies the association between species pairs based on the frequency with which they are found at the same sites, and has increasingly been used to characterize the structure and dynamics of communities (Araújo and Luoto 2007; Barberán et al. 2012; Lane et al. 2014; Lyons et al. 2016; Villalobos et al. 2016; Ulrich et al. 2017). To remove the confounding effect of sample size, we subsampled (without replacement) the species-by-site occurrence matrices (one each for end-Pleistocene, Holocene, and Recent) to 60 sites, computed pairwise Fisher's Exact Test mid-P variant (FETmP) co-occurrence scores (Berry and Armitage 1995; Kallio et al. 2011) for all possible pairs in each subsample, and repeated this process 1000 times. Note that the null model for this analysis is included in the calculation of the mid-P variant of Fisher's Exact test, described in the next paragraph. The random subsampling procedure was used to standardize sample size across the time intervals, not to build a null model.

The mid-P variant of Fisher's exact test is a continuous descriptive metric that is based on Fisher's exact test and uses analytical probabilities of co-occurrence to describe associations, based on species occupancy and the number of samples. It is the analytical equivalent of a fixed-equiprobable null model, which was used here for efficiency and to avoid under-randomization. Unfortunately, as of yet there is no equivalent analytical solution to a fixed-fixed randomization null model. Fisher's exact test was recently repurposed as an analytical approach for categorizing species associations as positive, negative, or random (Veech 2013). We used the mid-P variant of Fisher's exact test because we were interested in a continuous metric that can be compared across associations rather than categorizing and

counting association types using a significance threshold. The mid-P variant of Fisher's exact test describes the probability of two species co-occurring at any number of sites, given their frequencies of occurrence and the number of sites (based on simple combinatorics). The metric comparatively scores the strength of each association on a continuous scale by splitting the probability distribution at the observed number of mutual occurrences for each pair. This produces values near 0.5 for pairs that co-occur the expected number of times, values > 0.5 for species that are positively associated (or aggregated), and values < 0.5 for pairs that are negatively associated (or segregated). The output scores vary between 0 and 1, non-inclusive. Raw scores are then transformed to z-scores using the base R function qnorm, which are useful because they vary from positive to negative infinity, placing random scores near 0. One advantage of z-scores is that they emphasize variations in strong interactions (e.g. the difference between 0.990 and 0.999 is given more weight than the difference between 0.790 and 0.799 because this difference is harder to achieve mathematically). They also provide a way to subtract association scores (which we do in our separation of biotic and abiotic variables, see next section), because they are unbounded, while the raw scores are restricted to values between 0 and 1. Recently, Harris (Harris 2016) introduced new methods for inferring indirect effects and networks of species interactions from co-occurrence data. However, Harris' method assumes that all species in the analysis are potentially interacting indirectly through a connected network (e.g. three-way interactions involving the regulation of associations by third parties). The null model analysis used here is a simpler measure of the degree of direct pairwise positive and negative associations. We will refer to the z-transformed output of the mid-P variant of Fisher's exact test as association weights, and as

aggregation and segregation weights when referring to the weights of positive and negative associations.

Mutual niche co-occurrence analysis. To determine the strength of abiotic effects on associations over time, we ran a co-occurrence analysis on potential range sites shared between each species in a given pair (termed mutual potential range, M_{ij}). We used the potential ranges previously estimated for each species (P_i). For each pair, we extracted the subset of sites falling within the potential ranges of both species in the pair ($M_{ij} = P_i \cap P_j$) (Kallio et al. 2011).

Because sample size can affect the outcome of association weight calculations, we included only pairs that had at least 10 sites in their mutual potential ranges (enough to perform a co-occurrence calculation, but not so many as to exclude a majority of pairs). The original association weights were recalculated using 10 randomly chosen sites from the current subsample of 60 sites, as described above in the co-occurrence analysis section, and the mutual potential range associations were calculated using 10 sites randomly chosen from within the mutual sites present in the subsample (8 and 12 sites were also tried with no substantive change in the results). The original associations were then separated into two components, such that the association weights of the components sum to the original association weight: (1) the biotic component, calculated using sites in each pair's mutual potential range (because associations within mutual potential ranges cannot be driven by dispersal ability or the climate variables used to calculate their limits, see Fig. 4.3); and (2) the abiotic component, computed by calculating the difference between the original association and its biotic component. We treat the biotic and abiotic components as species pair

associations in their own right, and the sum of their weights is equivalent to the association weight of the full associations.

The biotic component includes potential mechanisms such as direct and indirect biotic interactions and range dynamics. The biotic component can also be impacted by selection between time intervals. The abiotic component is strictly a result of the factors used to calculate the mutual niche space (geographic coordinates, mean annual precipitation, mean annual temperature, and seasonality variables). In theory, this means that the biotic component could also be affected by a climate variable that we have not factored out in this analysis. However, for such a variable to have an appreciable effect on our results within the mutual potential ranges of species pairs, it would have to impact species occurrence consistently across the continent, in a fashion comparable to mean annual precipitation or mean annual temperature without covarying with them. The association weight and abundance of aggregations and segregations for each component was compared with those of the full associations.

If the included abiotic variables (mean annual temperature, mean annual precipitation, seasonality of temperature and precipitation, and geographic coordinates) strongly regulate the association of a given pair, the association within the mutual potential range will be weaker than the original association strength because the sites included in the recomputed mid-P variant of Fisher's exact test will no longer harbor that abiotic signal. For example, if two species are segregated due to disparate climate preferences, the original association will be negative, but species should associate randomly inside of the mutual potential range, indicating that climate fully accounts for the association. If a pair aggregates in sites with high mean annual temperature, using the mutual potential range (i.e.,

removing the sites with low mean annual temperature where neither species occurs) will reduce the strength of the original aggregation by removing mutual absences. The reasoning for the mutual niche analysis is summarized visually in Fig. 4.3.

The comparison between the original and mutual potential range association scores identifies three broad categories of pairs, corresponding to the three panels in Fig. 4.3. In panel (A), Abiotic variables fully explain the spatial relationship of the pair. (B) Abiotic and biotic variables both influence the spatial relationship of the pair. (C) Abiotic variables do not explain the spatial relationship of the pair. In the first case, the association within the mutual potential range is random and its z-score is close to zero. In this scenario, the original score minus the biotic score will be close to the original score, indicating that abiotic factors are driving the relationship (Fig. 4.3A). If biotic and abiotic variables are both contributing to the relationship, then the biotic component will be estimated by the weight calculated from the mutual potential range, and the difference between the original and biotic weights will indicate the extent of abiotic component (Fig. 4.3B). If biotic and abiotic components work in the same direction (e.g. both cause segregation), then their absolute values will add up to the absolute value of the original score. However, it is possible for the biotic and abiotic components to drive spatial patterns in opposing directions. If this is the case, a positive and negative value may yield an overall association that appears weak (association z-score close to zero; Fig. 4.3B). In the final case, the biotic component will be almost the same as the original association, indicating either that the pattern is only evident inside the mutual niche, or that the mutual niche is almost as large as the full potential range of both species (Fig. 4.3C). Either way, the abiotic component will be left with a number close to zero when the biotic score is subtracted from the original.

The analysis revealed that the use of mutual potential range sites causes many original association strengths to decrease or flip, indicating abiotic regulation of community assembly. This is the most common case in all three time intervals. However, the end-Pleistocene aggregations were stronger and more frequent when abiotic variables were factored out, suggesting enhanced biotic regulation of co-occurrence when the megafauna were still alive (Fig. 4.3A-B, Fig. 4.3A-B).

Confounding factors

Statistical confounding factors. Measurements of co-occurrence can be influenced by certain characteristics of the input matrix. The most common confounding factors are the number of sites and the variance in sampling intensity. Sampling intensity can in turn influence apparent occupancy of species and the richness of sites in a dataset. Of course, biological changes in occupancy and richness also control co-occurrences patterns and should be interpreted as a real signal. Co-occurrence analyses attempting to compare associations calculated from data with varying number of sites and sampling intensity should take measures to account for each of these factors.

***Number of samples*.** Time periods with more sites yield stronger statistical power and may artificially create stronger associations relative to assemblages with fewer sites. We employed a subsampling procedure to ensure that the same number of sites was used for the calculation of pairwise associations we wished to compare (e.g., association strength distributions across time intervals). To compare full associations across time intervals, we subsampled each time interval to the same number of sites and repeated the process 1000 times. The number of sites in each subsample is somewhat arbitrary, but should be no

greater than the number of sites in the least-sampled interval (Pleistocene), as subsampling was run without replacement. Sub-sampling below the sample size of our least-sampled interval allows us to estimate variance of our results within all sampled intervals. We ran our analyses fixing the subsample size at 48, 60, and 72 (the latter is the total number of sites available in the end-Pleistocene). The subsample number did not substantively change our results. Although there were differences in the weights of individual pairs, this translated to only very small changes in the proportions and average weights of aggregations and segregations in each subsample. The overall temporal patterns (i.e. increase in segregation weight and proportion in biotic associations and the opposite in abiotic associations) remained constant. The analyses presented in the main text are based on subsamples of 60 sites. The figures for the co-occurrence analysis with 48 and 72 sites in each subsample were so similar to our main text results (i.e. Figs 3 and 4) that we felt it would be redundant to include them here.

Sampling variance. Variance in the sampling intensity between time intervals can also create artificial differences. In particular, sampling intensity affects the marginal totals of a data matrix (occupancy = row sums and site richness = column sums). The closer the occupancies of species in a pair are to 50%, the stronger the power of co-occurrence tests. Increased sampling intensity resulting in higher site richness may, but does not necessarily, lead to overestimation of positive associations in comparison to a dataset with lower sampling intensity. It is difficult to determine the extent to which site richness and species occupancy patterns reflect biological changes vs. sampling effects. However, we can estimate the effects that occupancy and richness have on the outcome of associations by running randomization analyses (i.e., null models) with these features fixed (i.e. fixed row sums or column sums).

If the outcome of the randomizations differs from the outcome of the empirical dataset, we can assume that the distribution of species pair associations is not an artefact of the marginal totals of the matrix, regardless of whether or not the marginal totals are influenced by sampling intensity.

We implemented a fixed-equiprobable randomization (Gotelli 2000) of the subsampled matrices (R package EcoSimR (Nicholas J. Gotelli 2015)), which preserves species occupancy while randomizing the actual sites of occurrence. We also implemented an equiprobable-fixed randomization which preserves site richness instead. We avoided fixed-row and fixed-column sum randomizations to avoid the problem of under-randomization (Colwell and Winkler 1984; Jonsson 2001). Association weights for each pair were extracted from the randomized matrices. We examined the density distributions of randomized associations over time to establish expectations. See Fig. 4.12 for an explanation of how to interpret density distributions. The difference in occupancy over the time intervals (or any sampling bias causing apparent occupancy shifts) predicted the strengthening of association scores (a decrease in the height of the centre peak and increases in the left and right peaks). However, these factors do not explain the shift toward negative associations that is the central result of this paper (Fig. 4.13c). They also predict an increase in the proportion of aggregations, which is opposite to the results in this paper. Site richness predicted slightly stronger positive associations in the end-Pleistocene, but was unable to predict the excess of strong positive associations in this interval and the excess of strong negative associations in the subsequent intervals (Fig. 4.13).

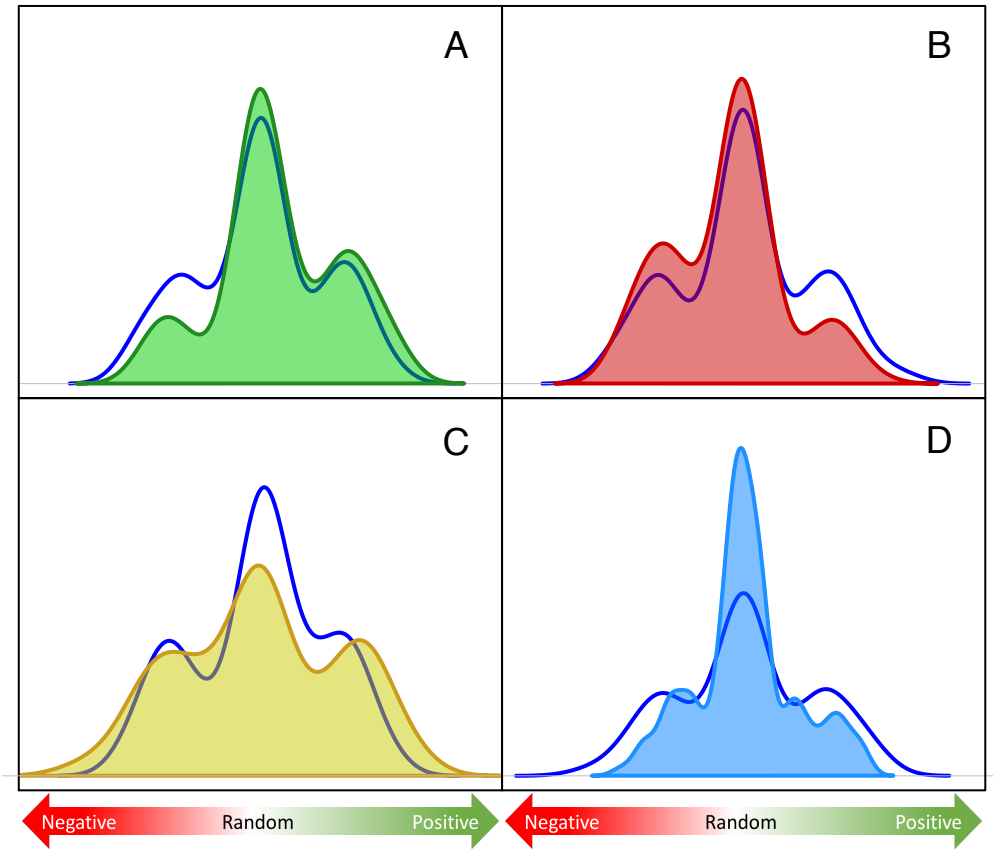


Figure 4.12. Expectations for the density distribution of association scores in the presence of systematic community shifts in a hypothetical structured assemblage. Dark blue line indicates a baseline expectation for the distribution of co-occurrence scores, with weak associations falling in the center peak, and strong positive and negative associations represented by the right and left hand peaks, respectively. Panels show the expected change in the case of a hypothetical shift toward (A) positive associations; (B) negative associations; (C) stronger associations; and (D) weaker associations. Note that the center peak does not move on the x-axis; changes are indicated by a combination of shifts in the left and right peaks or changes in the height of any peak.

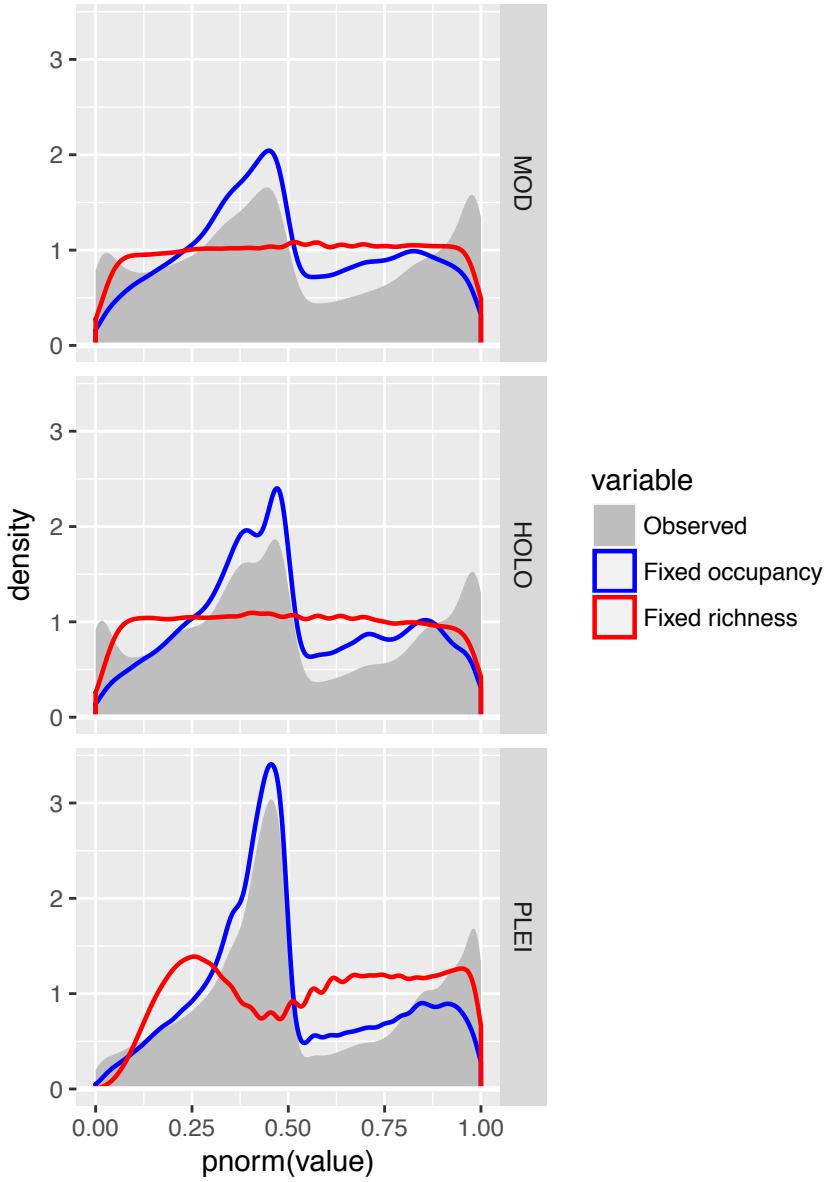


Figure 4.13. Kernel density plots of large mammal association FETmP weight distributions within the end-Pleistocene (bottom), Holocene (middle), and Recent (top) when matrices are randomized using fixed species occupancy with equiprobable site richness (blue) and equiprobable species occupancy with fixed site richness (red) randomization. This indicates the expected result if association scores purely result from changes in occupancy or site richness, since row and column sums are fixed, respectively. Shaded gray areas represent the observed distribution of association weights in each time interval.

Ideally, we would like to know whether and to what extent occupancy and site richness have changed biologically (versus from sampling inconsistencies) over our time intervals. There are two ways we can approach this question: Qualitatively, we can check for

alignment with *a priori* knowledge about each time interval. For example, previous research shows that there was competitive release in the Holocene as a result of the extinctions (Alroy 2001). This suggests that survivors should increase in abundance and occupancy in the Holocene. We observe an increase in the occupancy of common species, which aligns with this prediction. We also know that the end-Pleistocene had much higher gamma diversity than the later time intervals due to the presence of megafaunal species across the continent. As such, we should be unsurprised to find end-Pleistocene sites have a higher maximum richness.

A quantitative way to evaluate the effects of sampling is to estimate the number of species occurrences missing in our datasets and evaluate in missing data through time. To do this, we first used correspondence analysis and coherence (Burroughs and Brower 1982; Leibold and Mikkelson 2002) of the assemblages to estimate the number of false negatives: the number of species occurrences missing from our datasets that should likely be presences. Previous research has supported the notion that species are often arrayed according to several overlapping gradients (Presley et al. 2009), and as such, species absences at sites surrounded by presences (when the sites are ordered according to an environmental gradient) are unlikely to represent true absences. We counted embedded absences (absences surrounded by presences) with the sites arranged according to each of the first three axes of the correspondence analysis (because the eigenvalues of the correspondence analysis indicated three dimensions of organization). This was done separately for each of the three orderings. We then evaluated how many of the embedded absences were found all three times (triply absent), which mostly likely represent false absences (false negatives). The count of triply embedded absences divided by the sum of presences plus embedded absences is

then the percent of the estimated total occurrences that is missing (false negatives). False negatives were most common in the recent (46.97%), lower in the Holocene (43.41%), and lowest in the end-Pleistocene (30.17%). Occupancy changes are in the opposite direction than we would expect from these results, including increases in the occupancies of several common species in the Holocene and Recent. Thus, we conclude that the occupancy changes are driven by biological mechanisms (not statistical artefacts), and therefore that our results are not being driven by sampling. In sum, changes in marginal totals cannot fully explain our results, and changes over time of marginal totals in our data are unlikely to be caused by sampling biases. Therefore, any influences of marginal totals on our results are probably biological rather than artefacts of sampling. Matrix fill, which can influence the strength of associations, was lowest in the end-Pleistocene and highest in the Recent. Nonetheless, the rate of false negatives was lowest in the end-Pleistocene interval. This suggests that our conclusion that marginal totals have changed biologically rather than by sampling biases applies to matrix fill as well. Furthermore, lower matrix fill should cause weaker associations, so change in matrix fill cannot explain the directional shift that is the focus of this paper.

Occupancy. The distribution of mammalian occupancy changed from the end-Pleistocene to the Holocene (Fig. 4.14). The end-Pleistocene was characterized by many species having low- to mid-occupancy. The high number of species with the lowest occupancies partially persisted into the Holocene, but the number of species at medium occupancies decreased dramatically, with most surviving species moving toward occupancies above 20% or below 5%. The Holocene occupancy distribution also expanded to include higher-occupancy species (end-Pleistocene, Holocene, and Recent maximum occupancies are 43, 55, and 59%, respectively), while occupancies of rarer species decreased (Fig. 4.14). As a result, the median occupancy

dropped from 7.0% in the end-Pleistocene to 4.1% in the Holocene, but the mean occupancy increased from 9.3% to 12.8%. The correlation in occupancy of species that survived across consecutive intervals was highly significant ($P < 0.001$) for both transitions (end-Pleistocene to Holocene, Holocene to Recent), but end-Pleistocene occupancy only partly explained variation in Holocene occupancy ($r^2 = 0.60$; Fig. 4.14A), while Recent occupancies closely tracked Holocene occupancies ($r^2 = 0.93$; Fig. 4.14B).

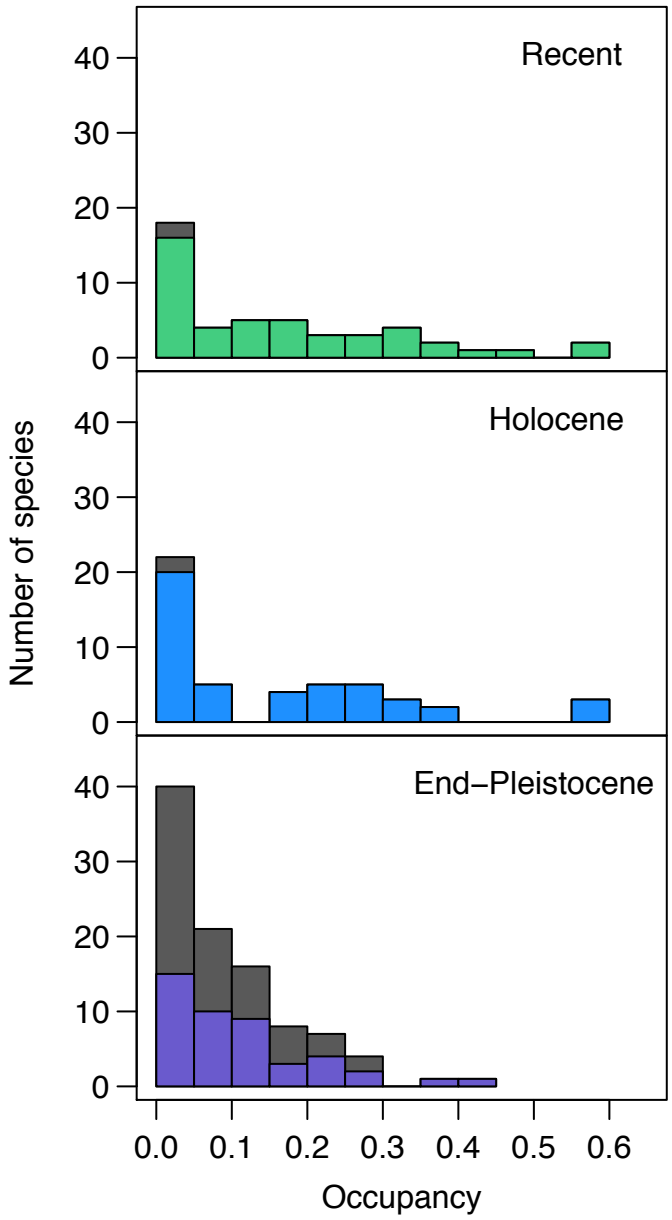


Figure 4.14. Occupancy distributions of mammals (>1 kg) in the Recent, Holocene, and end-Pleistocene. Gray bars represent species that are recorded only within one time interval. For the Holocene and Recent intervals these are rare or low-density species that are not commonly sampled, while for the end-Pleistocene all are extinct megafauna.

Taphonomy. The depositional environment of the sites in each time interval could influence the results of this study, especially if these environments are differentially represented in the three time periods. The sites come from a variety of depositional systems, including cave sites

and several types of surface assemblages. Cave or karst bone assemblages are formed in many different ways and may be dominated by smaller-bodied mammals as a result of accumulation by owls (Andrews 1990), or because of the relatively small size of species that usually inhabit caves (e.g., bats). Many cave sites also include larger animals, however. The subset of FAUNMAP sites used in this paper includes several dozen cave assemblages with 10 or more species of mammals larger than 1 kg, and these are distributed across all three time intervals.

If cave assemblages in our study are biased toward smaller mammals, a time interval with a higher proportion of cave sites might be expected to exhibit weaker aggregations. This is because many smaller mammal species typically have lower occupancies (i.e. smaller ranges) than larger mammals (Brown 1995; Plotnick et al. 2016). Therefore, they might not associate strongly with other species. If the Holocene and Recent have more cave sites than the Pleistocene, then taphonomy could drive the observed pattern toward weaker apparent positive association. We find that 40% of the end-Pleistocene sites and 51% of the Holocene sites are caves, while only 19% of Recent sites are caves. Although the Holocene does have slightly more cave sites than the end-Pleistocene, the Recent has many fewer. Nonetheless, the Holocene and Recent produce similar co-occurrence results that differ from end-Pleistocene co-occurrences. This indicates that taphonomic processes resulting in body size biases in cave fossil assemblages have not affected our results.

Biogeography. Maps of our Pleistocene, Holocene, and Recent sites are presented in Fig. 4.1. The sites in all time intervals are restricted to the US and southern Canada. The density of sites is higher in the younger time intervals, but this is accounted for by our subsampling protocol, which summarized results from 1000 runs in which each time interval is

subsampled to the same number of sites. Visual inspection of the maps reveals that there is a lack of Pleistocene sites in the northern mid-west and northwest of the US, resulting in a reduction of the total geographic area covered by our sites in the end-Pleistocene interval (6.6 million km^2, estimated with a minimum convex polygon, disregarding coastlines) compared to the Holocene (13.9 million km^2) and Recent (11.7 million km^2) intervals. These areas were covered by glaciers in the Pleistocene, so if the change in extent caused changes in co-occurrence, it should be interpreted as a real biological signal and is factored into our analysis of abiotic variables. We use hypervolumes and convex hulls on species occurrences to estimate species niches in this study, and the presence of glaciers covering entire regions could cause large over-estimates of species geographic ranges in the end-Pleistocene. However, because end-Pleistocene ranges are consistently smaller than Holocene and Recent ranges, correction for this factor would only strengthen our results. Survivors and victims in the end-Pleistocene are subject to the same biases, and so their comparison should not be systematically biased by the presence of glaciers. Finally, the mutual potential range analysis does not suffer from bias imposed by glacier coverage, as there are no sites in these areas, and thus they cannot be counted in the mutual niche of any pair.

The distribution of geographic ranges across the time intervals correlates with the total amount of area available in each time interval (compare areas cited above with Figs. 4.9C and 4.11B), and a similar relationship is evident with the four-dimensional climate envelope hypervolumes (31.1, 84.5, and 27.6 z^4 for the end-Pleistocene, Holocene, and Recent, respectively; compare with Fig. 4.9A). It is possible that changes in niche breadth were simply a result of retreating glaciers and climate change, but such a simple explanation would only be supported in the unlikely event that all niche expansions take advantage of

new niche space only. When geographic and climatic envelopes were plotted as a ratio of available background variation (Figs. 4.4 and 4.9B), the pattern of expanding Holocene niches was still evident. This indicates that Holocene mammals filled a larger percent of the available climate space than the same species in the end-Pleistocene. Such expansions suggest that the competitive release scenario we discussed in our main text allowed some species to fill newly available niche space as well as existing niche space in the absence of competition or predation from extinct end-Pleistocene megafauna and is consistent with the central message of this paper.

Time averaging. There are three related types of time averaging that could affect the outcome of this analysis: (a) Because increases in time-averaging may correspond to increases in richness, the number of species recovered may be increased in sites with a longer duration between minimum and maximum ages. (b) Binning sites from different times into broader time intervals place sites that may not be strictly contemporaneous in the same matrix, and (c) the three time intervals explored here (end-late Pleistocene, Holocene, Recent) are not the same duration. These three types of time averaging are each addressed below.

If sites have minimum and maximum dates that are farther apart, time-averaging may cause some species to appear at the same sites that did not truly co-occur. This would cause species pairs to appear more aggregated in intervals where sites have broader age ranges. End-Pleistocene sites had an average duration of 5418 years, Holocene sites had an average duration of 2877 years, and Recent sites had an average duration of 385 years. We do see more frequent and stronger aggregations in the end-Pleistocene, which has the least precisely dated sites. To address this potential bias, we ran a version of the co-occurrence analyses from which we removed end-Pleistocene sites with higher richness than the richest site in the

Recent interval with a duration less than 400 years. This ensures that all site faunas could have accumulated in 400 year period (maximum 21 species). The analysis yielded similar results (Fig. 4.15) to those presented in the main text.

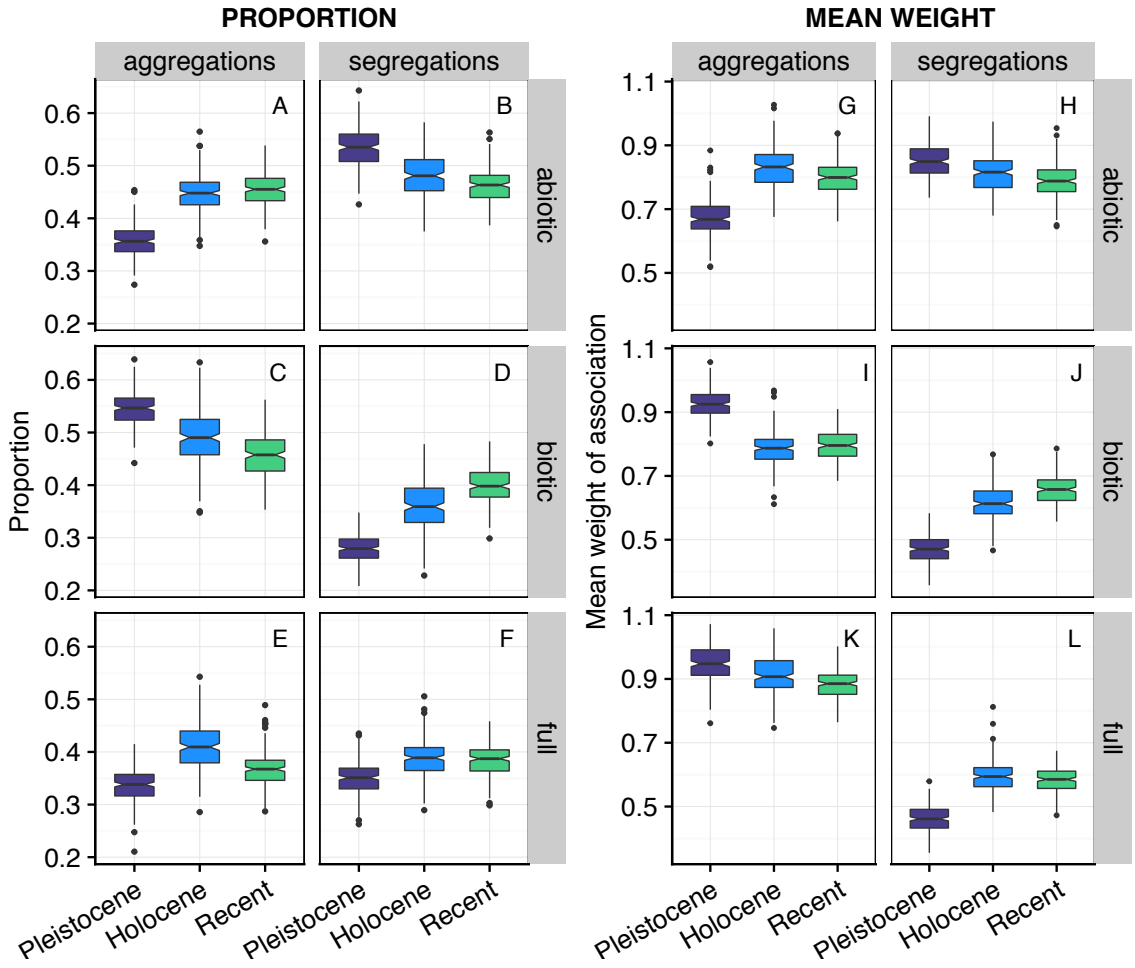


Figure 4.15. Results of reduced richness analysis to test for systematic time averaging bias. Proportion (left) and mean weight (right) of aggregations and segregations for abiotic components (top), biotic components (middle), and full associations (bottom) when end-Pleistocene sites with more than 21 species are removed. Boxplots represent variation over subsamples (n= 200). Only pairs with at least 10 sites falling within their mutual potential range are included and all associations are calculated with exactly 10 sites.

If sites that are not strictly concurrent are analysed as parts of a single interval, the pattern of presences and absences that drive the association results must be consistent

temporally (within the interval) as well as spatially to receive strong weights (i.e. high positive or negative Fisher's Exact Test mid-P z-scores). In other words, the binned time interval represents a spatiotemporal relationship for each pair rather than a strictly spatial one, and the association must be occurring in space as well as time to exhibit a strong pattern. In a practical sense, this is true for any fossil dataset, because most fossil assemblages take time to accumulate. In practice, the association weights calculated for a time interval of any given duration represent the spatial relationship of pairs over that duration, averaging out any variations in the relationship that may have occurred over time. Thus, if associations do change over the duration of the binned interval, time averaging could cause such pairs to appear random. The more dynamic the association pattern, the weaker we expect associations to be. The longer the time interval, the more likely that it encompasses temporal changes in associations and thus the weaker we expect the pairs to be. If this scenario was driving our results, we would expect the end-Pleistocene and the Holocene to exhibit weaker associations than the Recent, but we observe that the Holocene and Recent have similar associations.

Finally, the Holocene and end-Pleistocene intervals have roughly the same duration (9.7 and 9.3 ka, respectively) while the Recent interval is much shorter (2 ka). Despite this, the greatest differences in co-occurrence are observed over the Pleistocene to Holocene transition. This indicates that time averaging is not responsible for the patterns detected in our analyses. Nonetheless, to firmly rule out the idea that time averaging is responsible for the aggregations observed in the end-Pleistocene, we ran our co-occurrence analyses again with sites older than 18 ka excluded, effectively reducing the duration of the end-Pleistocene interval to 7 ka. The temporal co-occurrence patterns in Fig. 4.6 were unchanged. This analysis

demonstrates that longer time-averaged intervals do not necessarily translate to stronger aggregations.

Radiocarbon dating. Radiocarbon dates can be compromised by contamination of modern carbon (Zazula et al. 2014, 2017). The influence of contamination becomes larger with the increasing age of the dated sample (i.e., a 1-2 ka discrepancy for 0-10 ka sites and 2-4 ka discrepancy for 10-25 ka sites with 2% contamination). The potential inaccuracies in our radiocarbon dates may cause our sites to be aligned with the incorrect climate layers in our niche analyses, and this mismatch may be more frequent in the Pleistocene dataset than the Holocene and Recent datasets. Therefore, differences in the abiotic signal in the end-Pleistocene might be caused by incorrect ^{14}C dates. However, the direction of the dating inaccuracy caused by contamination is consistently toward a younger age, typically meaning that the true site age is 0-4 ka older than its calculated age. To address this issue, we ran both of our abiotic analyses with coarsened climate data, where each site was assigned climate means averaged across its apparent CCSM3 1000-year interval and the three intervals preceding it. Because Pleistocene sites are most likely to be dated incorrectly, this approach gives Pleistocene sites a much greater chance of having accurate—if less precise—climate estimates. Simultaneously, it weakens the accuracy and precision of Holocene and Recent climate estimates (i.e., because Holocene sites are not likely to have more than 1 ka discrepancy, and therefore were probably assigned the correct climates to begin with), thus placing the time intervals on more equal footing.

It is also possible that accurate dating of sites might be degraded by the procedures used by FAUNMAP, e.g., assigning the same dates to loosely associated macrofossils or using bulk dating methods. Therefore, it is possible that the dataset contains random instead of

systematic bias in dating errors. To address this issue, we randomly selected either the minimum or maximum age of each site to align with climate layers and re-ran the niche analyses, repeating the process five times. This approach is highly conservative because it shows whether or not the dating uncertainties have the capacity to overturn our results. By randomly choosing the minimum or maximum age of each site, we may also shuffle sites along the environmental gradient, and this can influence the outcome of the estimated niche spaces. Neither analysis changed our results. This sensitivity analysis suggests that spatial gradients in climate are relatively stable even when large shifts are occurring over time (e.g. site A is always warmer/colder than site B), lending robustness to these results.

Supplementary results

Relative magnitude of biotic vs. abiotic components. We compared the average magnitude of each component to establish its relative importance in community assembly. Both biotic and abiotic components were important in all time intervals, but the magnitude of the biotic component decreased from the Pleistocene to the Holocene and stayed consistent into the Recent (Fig. 4.8). The relative magnitude of biotic and abiotic components reinforces the conclusion that abiotic control of community assembly processes is important but did not vary strongly over time. By contrast, the importance of the biotic component decreases after the Pleistocene. This result suggests that biotic factors structured end-Pleistocene communities to a greater degree than subsequent time intervals, and this partly drove the loss of aggregations in the following intervals.

Excluded survivor-survivor pairs. The results presented in the main text are based on pairs with at least 10 mutual niche sites. However, the pairs excluded by this threshold are

theoretically separated by abiotic factors, namely, differential habitat preferences or inability to disperse into one another's niches. Thus, it may be informative to examine how many pairs do not have sufficient overlapping potential ranges in each time interval. The percent of pairs excluded was 67.2, 68.5, and 58.4 for the end-Pleistocene, Holocene, and Recent, respectively. This percent is calculated on subsamples of pairs, so this means that the average pair was excluded roughly 60% of the time it was present in the 1000 iterations. These numbers suggest potential range overlap increased in the recent. This agrees with the increasing climate-driven aggregation and decreasing segregation we observe in the results for pairs that do have 10 or more sites in their mutual potential ranges (Fig. 3.6A-B), thus strengthening our results.

Dating sensitivity analyses. The results of the dating sensitivity analysis were not substantively different from the results presented in the main text. The original analysis used 1000-year climate layers to assign climate variables to each site. The median (rather than mean) magnitude of aggregations and segregations is presented in Fig. 4.16, to ensure that our results are not influenced by the distributions of association scores, which may be strongly skewed when aggregations and segregations are plotted separately. We present our results for climates assigned according to 4000-year averages in Fig. 4.17. Note that the average weight of aggregations decreases in the original associations and in the biotic component, while the abundance and weight of segregations increases. The opposite pattern is evident in the abiotic component, and this is consistent with the results presented in the main text. The results do not change when sites are assigned climates from their minimum or maximum date at random (Fig. 4.18). Interestingly, the interplay between abiotic and biotic factors removes the decrease in the proportion of aggregations that we observed in the main results. This

indicates that climate and biotic interactions take more equal opposing roles that cancel one another out in the overall spatial pattern, supporting the notion that these sensitivity analyses capture the role of climate more strongly than the 1000-year layers (i.e. because the abiotic signal was somewhat overpowered in the main results, but not here). In Figs. 4.19 and 4.20, the average relative association weights for biotic and abiotic factors are presented when calculated with 4000-year averages and minimum/maximum dates, respectively. The decrease in biotic regulation over the Pleistocene-Holocene transition is upheld. However, it appears that abiotic regulation may also have decreased slightly. Although the patterns in the full associations seem to have been weakened slightly, it is still clear how they were formed: (1) a decrease in the importance of biotic regulation with respect to abiotic regulation, and (2) a shift of biotic regulation toward segregations.

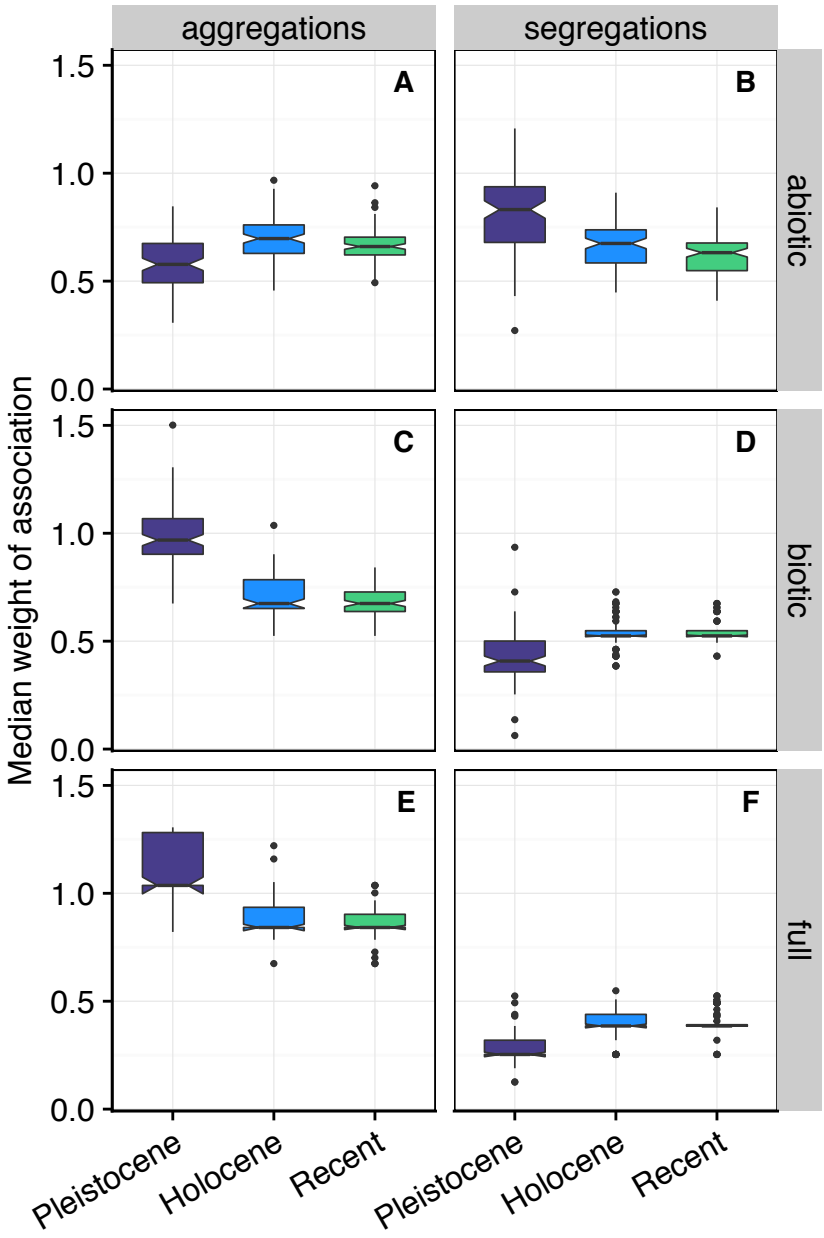


Figure 4.16. Median weight of aggregations (left) and segregations (right) for abiotic components (A-B), biotic components (C-D), and observed full association (E-F) in each subsample (n = 100). Only pairs with at least 10 sites falling within their mutual niche are included.

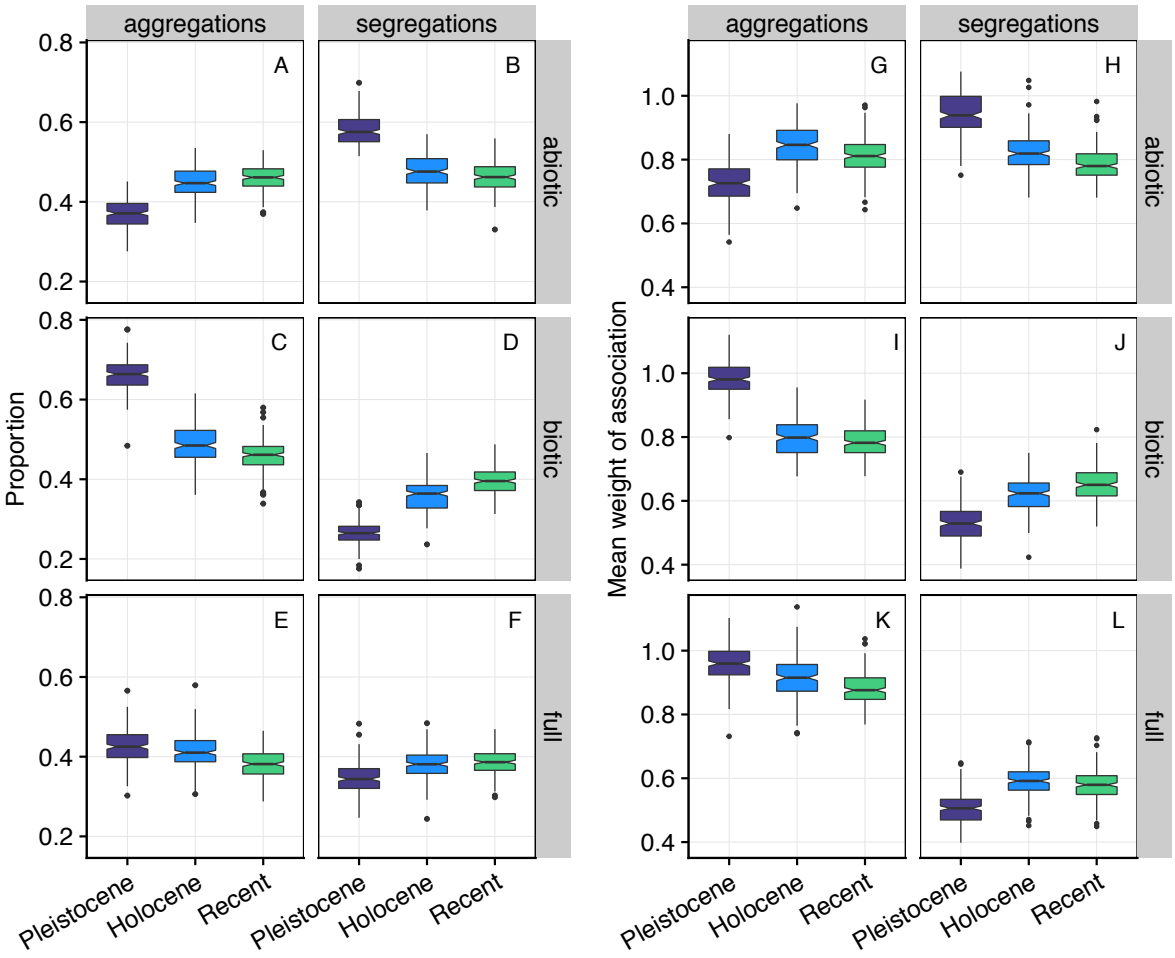


Figure 4.17. Proportion (left) and mean weight (right) of aggregations and segregations for abiotic components (top), biotic components (middle), and full associations (bottom) when climates are averaged over 4 ka. Boxplots represent variation over subsamples (n= 200). Only pairs with at least 10 sites falling within their mutual niche are included and all associations are calculated with exactly 10 sites

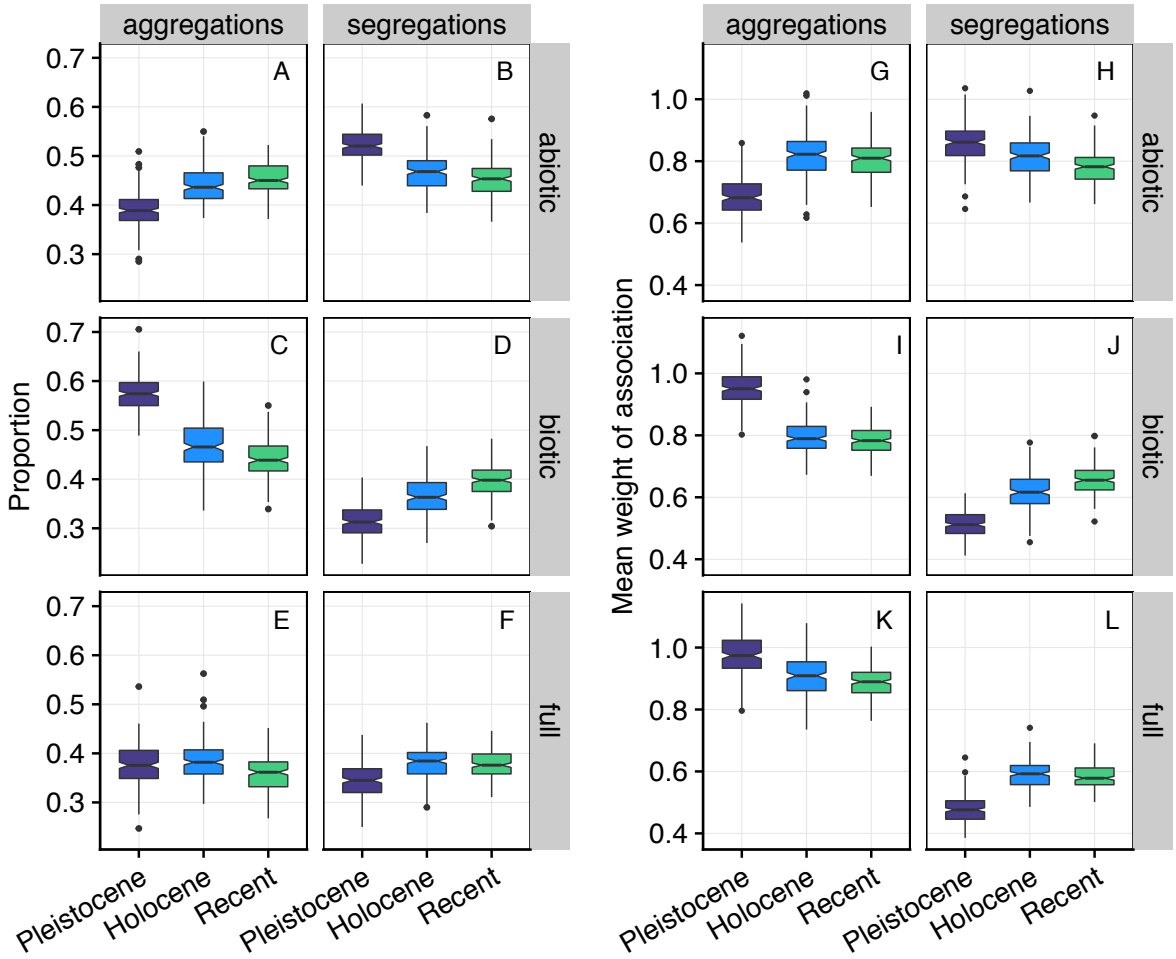


Figure 4.18. Proportion (A-F) and magnitude (G-L) of aggregations and segregations for abiotic components (top), biotic components (middle), and full associations (bottom) when climates are calculated by randomly chosen minimum or maximum calibrated age. Boxplots represent variation over subsamples (n= 200). Only pairs with at least 10 sites falling within their mutual niche are included.

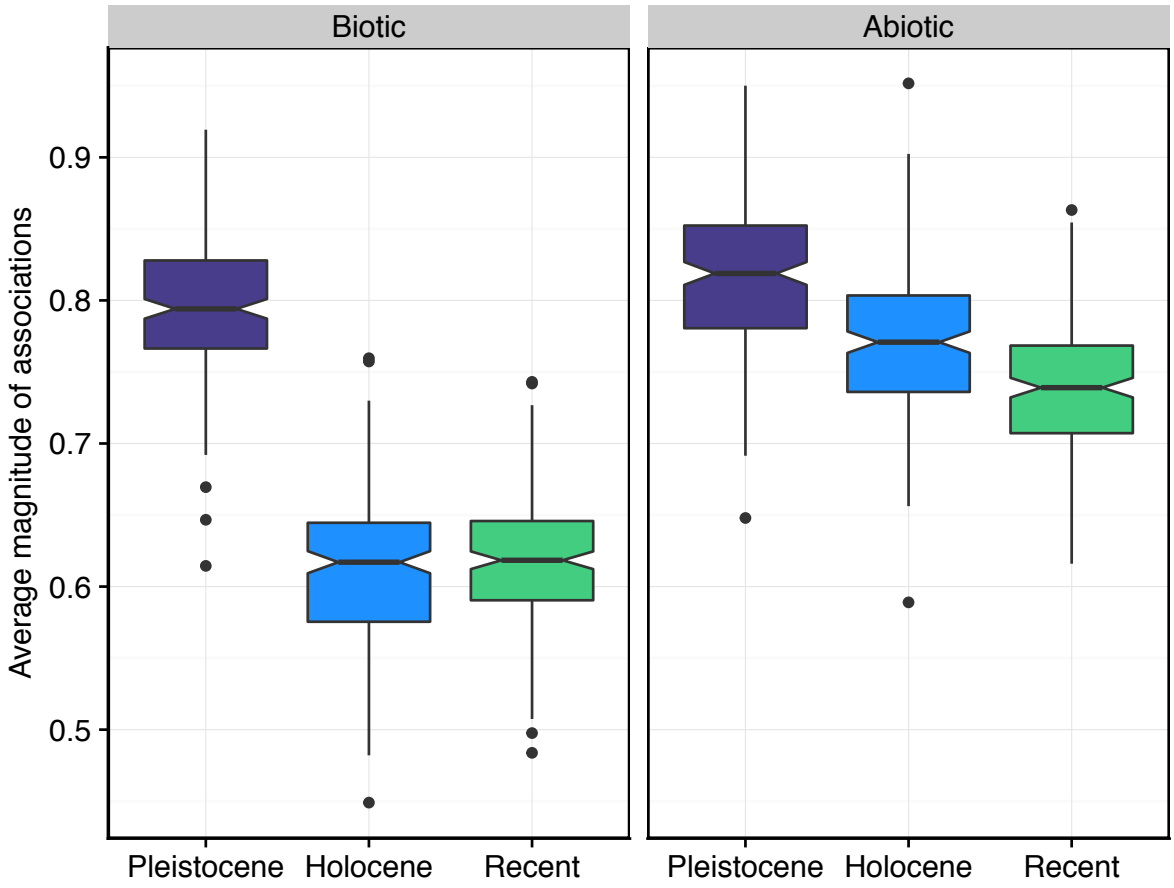


Figure 4.19. Average magnitude of biotic and abiotic associations over the three time intervals, broadly representing the relative importance of these two components for overall community assembly patterns, when climates are assigned using 4000-year averages. Boxplots represent the variation among subsamples (n = 200).

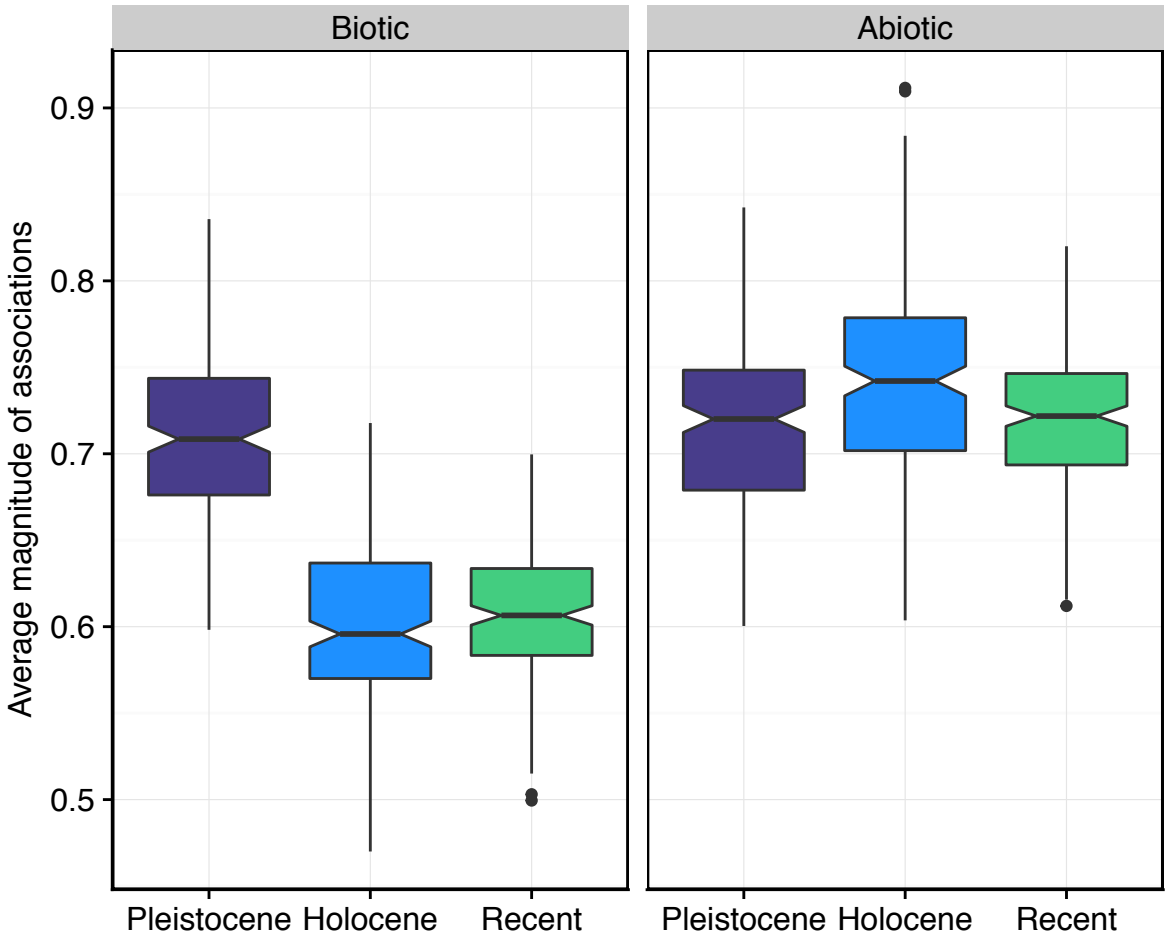


Figure 4.20. Average magnitude of biotic and abiotic associations over the three time intervals, broadly representing the relative importance of these two components for overall community assembly patterns, when climates are assigned using 1000-year averages corresponding to minimum or maximum site age (chosen randomly). Boxplots represent the variation among subsamples (n = 200).

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CHAPTER 5

Habitat alteration reduces food competition and local trait diversity in Neotropical bats and birds

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ABSTRACT

Anthropogenic habitat destruction is one of the greatest threats facing biodiversity. It is increasingly recognized that conservation and monitoring efforts should target not just species but communities and ecosystems. Traditional measures of communities include species richness and beta diversity, but they are somewhat limited because species must become extirpated or extinct before changes can be observed. Co-occurrence analysis is an increasingly popular method that can be used to detect more subtle changes in community structure. We calculated richness and beta-diversity for Neotropical assemblages of birds and bats at altered and unaltered sites. We also calculated co-occurrence scores for all possible pairs of species in each assemblage. Using a comparative approach, we quantified how habitat alteration affected patterns of co-occurrence in pairs with varying degrees of diet overlap. We found that pairs with the same diets co-occurred more in altered habitats, while pairs with related or different diets co-occurred less, even though alpha diversity and composition were not significantly different. Our results suggest that altered habitats provide abundant food sources at predictable places and times, reducing the incidence of exclusion and causing aggregation within particular dietary groups. However, this also means that sites are less likely to support multiple species from several dietary groups, compromising local trait diversity and possibly functional redundancy.

MAIN TEXT

Introduction

Landscape-scale habitat alteration by humans is one of the foremost threats facing species today (Newbold et al. 2015). More than three-quarters of Earth's non-frozen land area has been altered or settled by humans in some capacity (Sanderson et al. 2002; Ellis and Ramankutty 2008), encompassing almost 90% of global net primary productivity as well as 80% of global tree cover (Ellis and Ramankutty 2008). Thus, the quantification of changes in local communities of species as a result of human disturbance has become a major goal of ecology and conservation science.

One traditional approach to analysing changes in communities is estimating alpha diversity (i.e. richness). Shifts in the composition, as opposed to the richness, of communities are also well-documented (McKinney 2006; Dornelas et al. 2014; Tóth et al. 2014) and have received increasing attention. The consequence of shifting community composition in response to habitat alteration is often a reduction in beta diversity, particularly when sensitive and specialist species are extirpated while adaptive generalists or human-cultivated species become increasingly dominant (McKinney and Lockwood 1999; McKinney 2006; Tóth et al. 2014).

However, alpha- and beta-diversity metrics provide only broad summary statistics of community change without suggesting mechanistic links to the observed patterns. Ecosystem change is often reflected in turnover of species over time rather than simple reductions in alpha diversity (Magurran et al. 2018). If multiple species respond to a disturbance differently, community assembly patterns may undergo shifts that are not detectable by examining the

richness or spatial turnover of the assemblage. Magurran *et al.* (2016) stress that changes in composition, particularly when it results in homogenization, may eventually compromise ecosystem function. Documenting and examining more subtle changes in community assembly is therefore critical, even before species are extirpated. Therefore, it is increasingly clear that the focus of conservation, management, and monitoring efforts should also be on the biotic interactions that underlie ecosystem function and community assembly. The degree to which biotic interactions have been disrupted is one of the five criteria proposed by Keith (2015) for assessing the risk level of ecosystems, and has been adopted by the IUCN Red List of Ecosystems (Keith et al. 2015).

Competition is a commonly recognised biotic interaction that is backed up by a foundation in ecological theory. Although it has not escaped substantial criticism, the notion that competition is an important driver of community assembly amassed much empirical support in its early years (Schoener 1983), and evidence has continued to mount. A considerable proportion of this body of literature is devoted to tropical birds (Terborgh and Weske 1975; Remsen and Graves IV 1995; Jankowski et al. 2010; Freed and Cann 2014; Weinstein and Graham 2016), but competition in other taxa, such as primates (Kamilar and Ledogar 2011), has also been examined. Based on competition theory, we expect competing species to either coexist in a dynamic equillibrium such that their abundances covary negatively through time, or to exclude one another, i.e., be found together less often than species which do not compete (Kamilar and Ledogar 2011). Studies evaluating the effects of human disturbance on the outcome of interspecific competition, however, are scarce.

One way to look more closely at differences in community assembly is to calculate the co-occurrence patterns of all pairwise combinations of species. This approach has been used for

decades (Diamond 1975), and has recently been used to examine community assembly of species under human disturbance (Lane et al. 2014; Kay et al. 2017), changing climate conditions (Araújo and Luoto 2007), across environmental gradients (Bar-Massada and Belmaker 2017), and across short (Tulloch et al. 2016) and long timescales (Lyons et al. 2016). Such methods can detect whether species occur together more often than expected (aggregation) or less often than expected (segregation) by chance. They can also assign a value, or weight, to represent how strongly the pattern is supported by the data available. The resulting associations can then be examined singly or as a whole, represented by a network data structure where the nodes are species and the links (edges) represent their co-occurrence patterns.

Unfortunately, many previous studies have sometimes struggled to provide applicable biological interpretations for their observations of changing network structure for a variety of reasons. Natural network topology may be variable (Thébault and Fontaine 2010), and interpreting changes in networks in the context of disturbances is still difficult. Another challenge is that associations calculated on the basis of co-occurrence do not have an objective biological interpretation. Instead, much of what we know about network dynamics is derived from biological interactions such as plant-pollinator networks and food webs. Despite recent attempts to clarify the relationship between co-occurrence and biotic interactions (Cazelles et al. 2016; Harris 2016), it is still not known how measured properties of the network and its nodes (representing species) may be interpreted when the network edges do not necessarily imply direct interactions (Freilich et al. 2018). For example, a highly connected insect in a plant-pollinator food web is a generalist that pollinates a variety of plants. A highly connected rodent in a co-occurrence network may be a specialist that always

occurs on mountaintops with several other mountaintop-adapted species, but it could also be a keystone species that facilitates the occurrence of many others in some other way. In fact, most associations are likely driven by a combination of factors (e.g., shared habitat preferences, direct and indirect biotic interactions, and/or mutual dispersal barriers), and distinguishing between these – or quantifying their respective contributions – is one of the current challenges associated with this approach (Blois et al. 2014; Weinstein et al. 2017).

In this paper, we evaluate the effect of habitat alteration on the outcomes of competition for food in Neotropical birds and bats. We use comparisons between targeted groupings of the dataset to provide biological interpretations of changing co-occurrence patterns. We begin by examining the alpha richness and beta diversity of communities of Neotropical birds and bats under altered versus unaltered conditions. Then, we calculate pairwise species co-occurrence scores to look at changes in community structure after habitat alteration, using unaltered sites as controls. Based on categorical information about diets, we ask whether co-occurrence patterns are different for pairs that share food sources and those that do not. If species in the same guild are more aggregated than pairs in different diet guilds, it means they are partitioning the shared resource or able to coexist despite some competition. If they are more segregated, this means they may be excluding one another. We report marked changes in co-occurrence that are indifferent to changes in richness and composition.

Materials and Methods

Abundance data were downloaded from the Ecological Register (http://ecoregister.org) on 30 July 2018 for mistnetted bats and birds from the Neotropics,

defined as all areas in the western hemisphere south of the Tropic of Cancer (23.44˚N). The sites were sourced from a total of 88 bat studies and 90 bird studies. Samples were combined if they were within the same 11.1 x 11.1 km^2 equal-area grid cell, sourced from the same study, and represented the same habitat and alteration category. We also downloaded Register metadata for the species and sites in the abundance tables, including coordinates for sites and dietary guild assignments for species. Guild assignments were based on recently published primary literature. Four bird sites from altered habitats were added to improve the spatial coverage of this category (Flores et al. 2001; Arteaga and Moya 2002; Vidaurre et al. 2006; Villegas and Garitano-Zavala 2010). Sampling methods and reporting was standard across the samples, as all were mist-netted samples with consistent mesh size, harp net samples were excluded, and almost all nets were placed at ground level. Duration of sampling did vary among datasets, but this variation was present across all subsequent splits of the data. The full dataset consisted of 56,149 bat and 46,264 bird individuals representing 219 bat and 1299 bird species from 124 and 81 sites, respectively. Maps of the sites are plotted in Fig. 5.1 (top row).

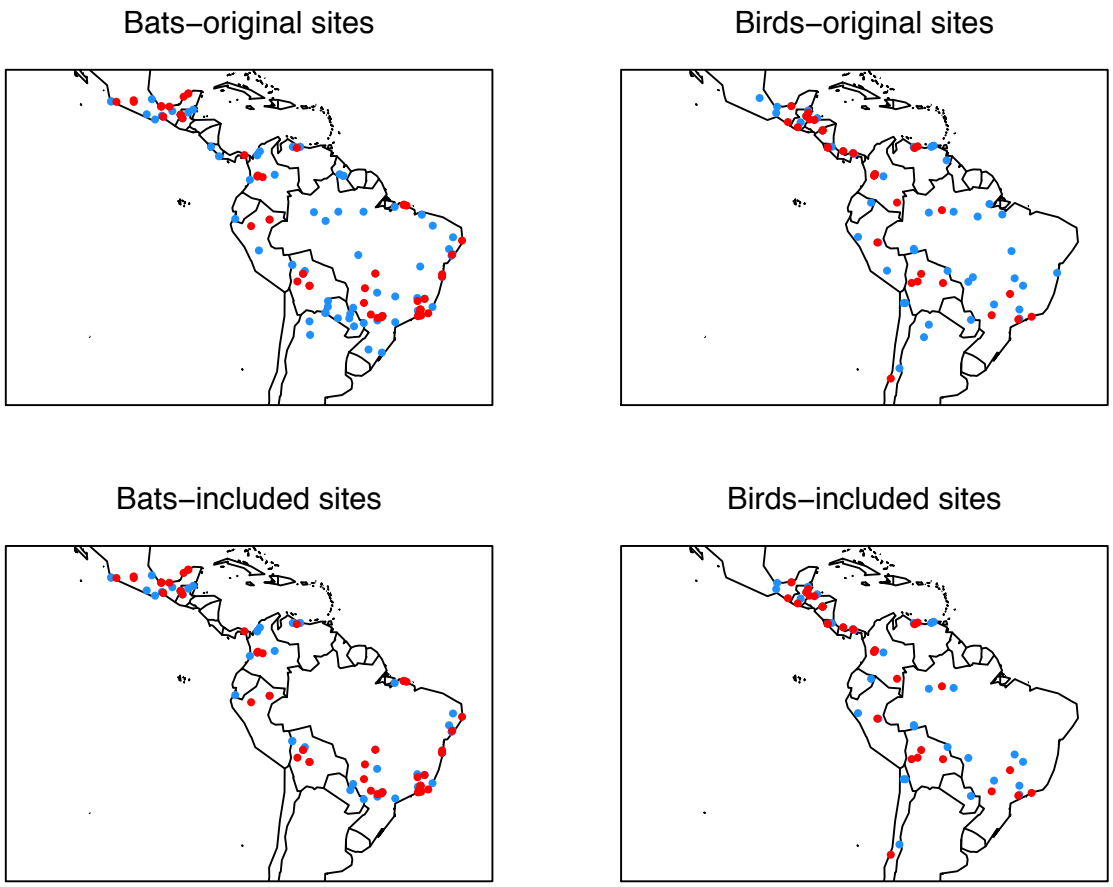

Figure 5.1. Maps of Neotropical bat and bird sites before (top) and after (bottom) biogeographic matching, with altered sites represented in red and unaltered sites represented in blue.

Richness and Beta diversity. We estimated richness for bats and birds in altered and unaltered sites using the squares diversity extrapolator (Alroy 2018) and Chao1 (Chao 1984) on the raw abundance data for all sites. The split was based on the 'altered.habitat' field in the site metadata table, which is empty if the site is unaltered and filled with land use category if the site is altered. Altered sites included cropland, disturbed forest, forest fragment (required to be no larger than 1 km^2 in size), pasture, plantation, rural, secondary forest, suburban, and urban categories, although the latter two comprise only a small minority of sites. A finer split of site types was not possible due to the low number of sites in

most of the altered subcategories. Altered sites are composed primarily of forest fragments, secondary forest, and disturbed forest. Bats had 73 unaltered and 51 altered sites while birds had 48 unaltered sites and 33 altered sites. The abundance data were converted to a presence-absence format. The spatial coverage of altered sites was a subset of the larger spatial area covered by unaltered sites. To minimize the effects of biogeography on our results, we removed unaltered sites that were farther from the nearest altered site than the most isolated altered site was from its nearest unaltered site (see supplement), resulting in roughly the same spatial coverage for both site types. The resulting dataset had 1,798 occurrences of bats and 3,316 occurrences of birds at 98 and 70 sites, respectively (Fig. 5.1, bottom row). We then calculated the beta diversity between all pairs of sites for bats and birds separately and plotted the distributions of values, separating them into groups by each pair's habitat: altered-altered, unaltered-unaltered, and unaltered-altered. We used the modified Forbes similarity index (Forbes 1907; Alroy 2015a) as the similarity measure for beta diversity. Finally, we ran a principal coordinates analysis (PCoA) on the sites using the R function cmdscale in the stats package to check for compositional shifts between altered and unaltered habitats. We used Forbes distances (one minus Forbes similarity values) in ordination analyses because this approach has been shown to minimize sampling biases and outperforms the widely-used Dice-Sorenson coefficient (Alroy 2015b). The Forbes similarity index is F = a (n + sqrt n)/[a (n + sqrt n) + 3/2 b c] where a is the number of species in both sites, b is the number of species unique to the first site, c is the number of species unique to the second site, and n = a + b + c. We tested for significant shifts in the site centroids on the two primary PCoA axes using a permutation test.

Co-occurrence analysis. We separated the presence-absence data into altered and unaltered habitat types. We classified each species in the dataset into one of two categories: shared (species occurring in both habitat types) and unique (species occurring in only one habitat type). Comparing the co-occurrence patterns of unique species between altered and unaltered sites can elucidate whether species exclusive to altered sites are filling similar or different structural roles as species that have potentially been extirpated from altered sites, although failure to sample may explain many such absences. Comparisons of shared species co-occurrences, on the other hand, measure differences that are not caused by local extirpation, sampling failure, or turnover between unaltered and altered sites, but by changes in occupancy across sites. The final dataset was comprised of 202 bat and 1181 bird species in 47 and 37 unaltered and 51 and 33 altered sites, respectively. We calculated the co-occurrence score of every pairwise combination of species using the mid-P variant of Fisher's Exact Test (Kallio et al. 2011). Co-occurrence metrics are influenced by the number of sites used to calculate them, so in comparative analyses co-occurrence scores should be calculated using the same number of sites. Because altered and unaltered categories had different numbers of sites, we subsampled each matrix down to the same number of sites (equal to five-sixths of the available sites in the least sampled site type for each taxon) and ran co-occurrence analysis on the subsampled matrices. This allows for many combinations while keeping enough sites to run a meaningful analysis. We repeated the subsampling process 100 times, and used subsamples as the experimental unit in subsequent analyses.

Functional Analysis. We used dietary information for bats and birds from the Ecological Register to analyse our co-occurrence results (Table 4.1). We selected all pairs with complete dietary information and split them by the degree to which they shared food sources.

Same-guild pairs had to share primary and secondary sources of food, but the order of these could be reversed (e.g., a frugivore-nectarivore and a nectarivore-frugivore). Related-guild pairs shared one food source and had at least one non-shared food source (e.g., frugivores and insectivore-frugivores), and different-guild pairs had no shared food sources. Although species sharing food sources are not necessarily competing, pairs with more dietary overlap have a higher probability of experiencing competition for food, while different-guild pairs have no chance of competing for food. Therefore, this comparison evaluates whether habitat alteration changes the spatial outcomes of food competition from the patterns observed in unaltered habitats.

Table 4.1. number of species per diet guild for bats and birds.

Life form	Guild	Abbreviation	Count
bat	carnivore-insectivore	CI	22
bat	frugivore	F	69
bat	frugivore-insectivore	FI	1
bat	frugivore-nectarivore	FN	3
bat	insectivore	I	80
bat	insectivore-nectarivore	IN	2
bat	nectarivore	N	21
bat	sanguinivore	S	3
bird	carnivore	C	16
bird	carnivore-insectivore	CI	10
bird	frugivore	F	96
bird	frugivore-granivore	FG	43
bird	frugivore-insectivore	FI	251
bird	frugivore-nectarivore	FN	8
bird	granivore	G	24
bird	insectivore	I	459
bird	insectivore-granivore	IG	17
bird	insectivore-nectarivore	IN	54
bird	nectarivore	N	40

When analysing associations, it is helpful to picture the distribution of association strengths as falling along a continuum of co-occurrence between segregated and aggregated pairs, with random or weakly associated pairs in the middle. Segregated pairs co-occur less than expected and receive negative weights while aggregated pairs co-occur more than expected and receive positive weights. In this framework, a net change in co-occurrence across altered versus unaltered sites can create several patterns: (a) an increase in the proportion and strength of aggregations indicates more co-occurrence; (b) an increase in the proportion and strength of segregations indicates less co-occurrence; (c) increase in the strength of both aggregations and segregations and signals that species are more tightly linked to the occurrence of their pairs; and (d) a decrease in the strength of all associations indicates that pairs are closer to being randomly distributed with respect to one another. Complex restructuring of links can also lead to other combinations that lack a clear pattern. The net difference in co-occurrence patterns associated with altered versus unaltered habitats can suggest biological mechanisms for the shifts in co-occurrence. For instance, reduced co-occurrence in same-guild pairs compared to different-guild pairs suggests that competitive exclusion has increased, because fewer species that share resources are co-occurring. Increased co-occurrence of same-guild pairs means that particular altered sites (i.e., those that provide the requisite dietary resource) can support the coexistence of more species in the same dietary group more often. Tighter co-occurrence can be interpreted as indicating that species form clusters, likely due to an increase in the spatial segregation of food resources introduced by habitat alteration (e.g., pastures provide an abundance of one type of food and plantations provide other types). Relaxed associations indicate that the

spatial structure of food resources is less defined in altered habitats, and species are dispersing more randomly or becoming very widespread.

For each analysis, we report differences in the proportion and strength of aggregations and segregations between altered and unaltered habitats. We distinguish between shifts in association strengths driven by pairs that are shared between site types and pairs that are unique to one site type. We present our results by pairing the subsamples of altered and unaltered sites randomly and plotting group means (e.g., mean strength of aggregated unique pairs) in scatterplots with unaltered means on the x-axis and altered means on the y-axis. We chose this presentation of the data for clarity and readability, as any random pairing of subsamples would result in a similar plot. Side-by-side boxplots of the same results are provided in the supplement for reference (Figs. 5.S1-5.S4).

Results

Richness and Beta Diversity. Based on Chao 1 and squares, altered sites have unchanged alpha richness compared to unaltered sites (squares p-values = 0.905 and 0.55, and Chao 1 p-values = 0.562 and 0.586 for bats and birds, respectively). Average richness for bat samples in unaltered habitats was 23.37 (squares) and 25.17 (Chao1), and this changed to 21.48 (squares) and 26.34 (Chao 1) in altered habitats. Average richness for bird samples was 59.13 (squares) and 68.49 (Chao 1) in unaltered habitats, and 57.59 (squares) and 64.53 (Chao 1) in altered habitats. For bats and birds, respectively, 38% and 49% of species increased their occupancies in altered habitats, and of these, 34% and 55%, respectively, appeared only in altered habitats. Overall, however, 62% of all bat and 51% of all bird species decreased their occupancies in altered habitats, and of these, 40% and 71% disappeared altogether. There

are a relatively large number of species that were sampled only in altered habitats (13% of bats and 27% of birds). These species likely do exist in unaltered habitats. However, as we took measures to standardize sampling between altered and unaltered sites, these species may represent a group whose abundances have been substantially increased in altered sites, such as vampire bats (Delpietro et al. 1992). The converse may be true for some species that only occur in unaltered sites.

Altered sites had the lowest beta-diversity in bats, decreasing significantly from unaltered sites ($p = 0.002$). Birds did not exhibit a significant change in beta-diversity between habitat types ($p = 0.9$). Bats had on average about four times higher similarity across sites, indicating that spatial turnover in birds is much higher and suggesting the two taxa have very different community structures. This is unsurprising because Neotropical bats frequently have geographic ranges spanning almost the entire realm, unlike Neotropical birds.

Fig. 5.2 depicts the altered and unaltered sites plotted by the first two principal coordinates. The permutation tests detected no significant compositional differences between altered and unaltered sites (p-values = 0.189 for bats and 0.127 for birds). This indicates that the considerable faunal turnover observed across altered and unaltered sites is not atypical, even across sites in the same category.

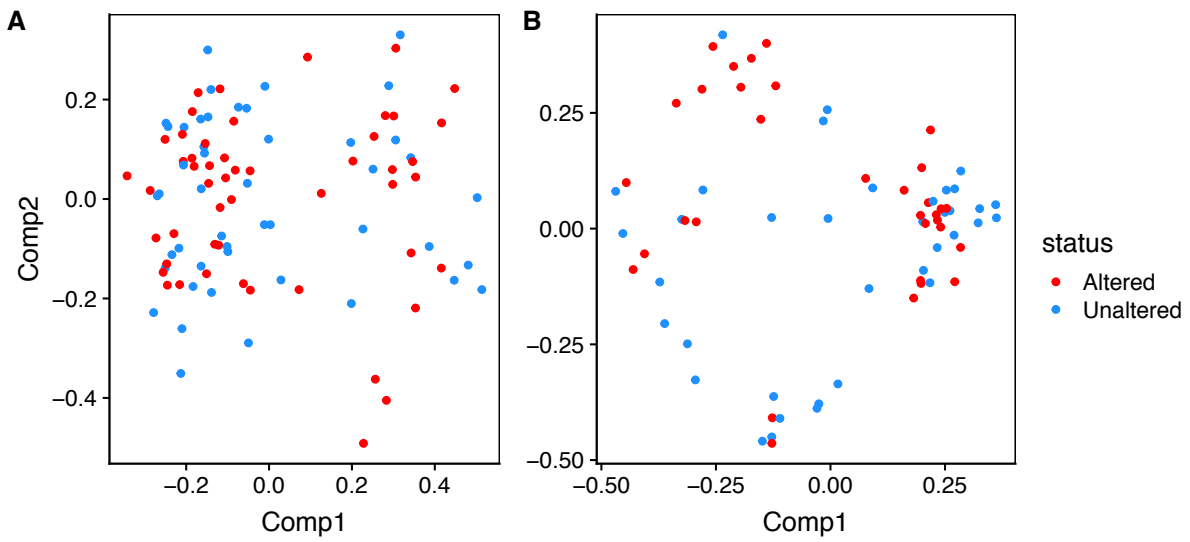

Figure 5.2. Principal coordinates analysis of altered and unaltered sites for bats (A) and birds (B). There is no significant difference between the composition of altered and unaltered sites for either taxon, based on a permutation test of the site type centroids (p-values = 0.181 and 0.137 for bats and birds, respectively).

Functional Co-occurrence Analysis– Bats. The proportion of aggregations exhibited by bats is higher in unaltered sites and lower in altered sites. This is true for all groups except same-guild unique pairs, meaning pairs that are unique to altered habitats aggregate more often than same-guild pairs that are unique to unaltered habitats (Fig. 5.3).

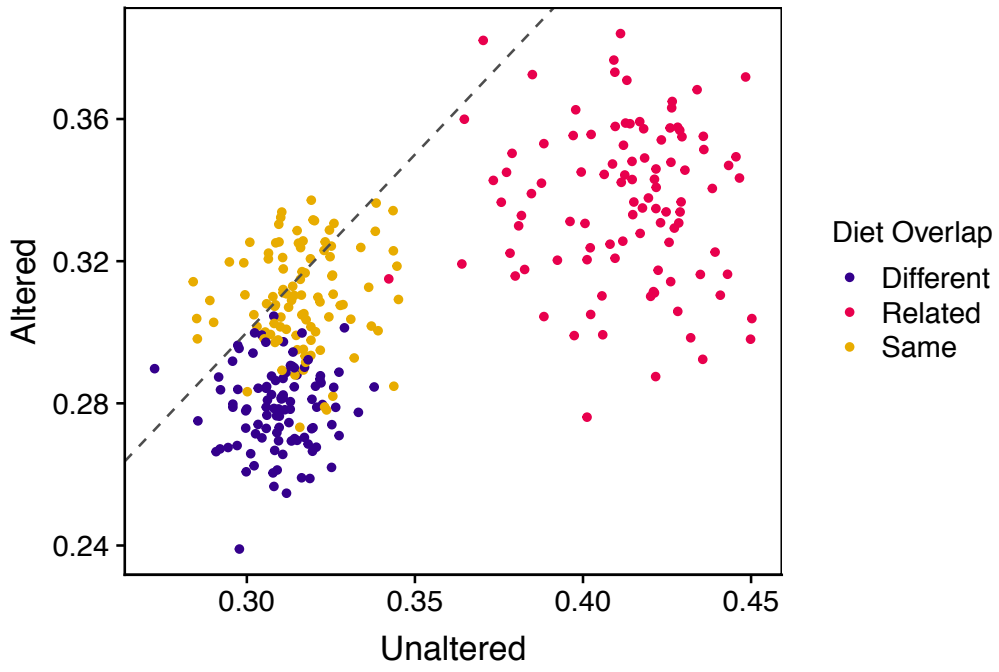

Figure 5.3. Proportion aggregated pairs in altered versus unaltered habitats for pairs of bats, separated based on degree of dietary overlap. The dashed line is the line of unity. The points represent subsamples. Samples falling above the line had a higher proportion aggregations in altered habitats, and samples falling below the line had a lower proportion of aggregations in altered habitats than unaltered habitats.

At unaltered sites, bat pairs have stronger aggregations the more their diet overlaps, with same-guild pairs exhibiting particularly strong aggregations (Fig. 5.4A). The pattern is stronger at altered sites, where different- and related-diet pairs have weaker or unchanged aggregation strength, while same-guild pairs have even stronger aggregations (Fig. 5.4A). Segregation strength is approximately equal across different, related, and same-diet pairs in unaltered sites (Fig. 5.4). Altered sites exhibit stronger segregations in all three diet overlap categories, but especially in same-guild pairs, and this pattern is driven by unique pairs (i.e., pairs unique to altered habitats segregate more strongly than pairs unique to unaltered habitats, such that compositional turnover between altered and unaltered habitats drives the increase in segregation strength; Fig 4.4B).

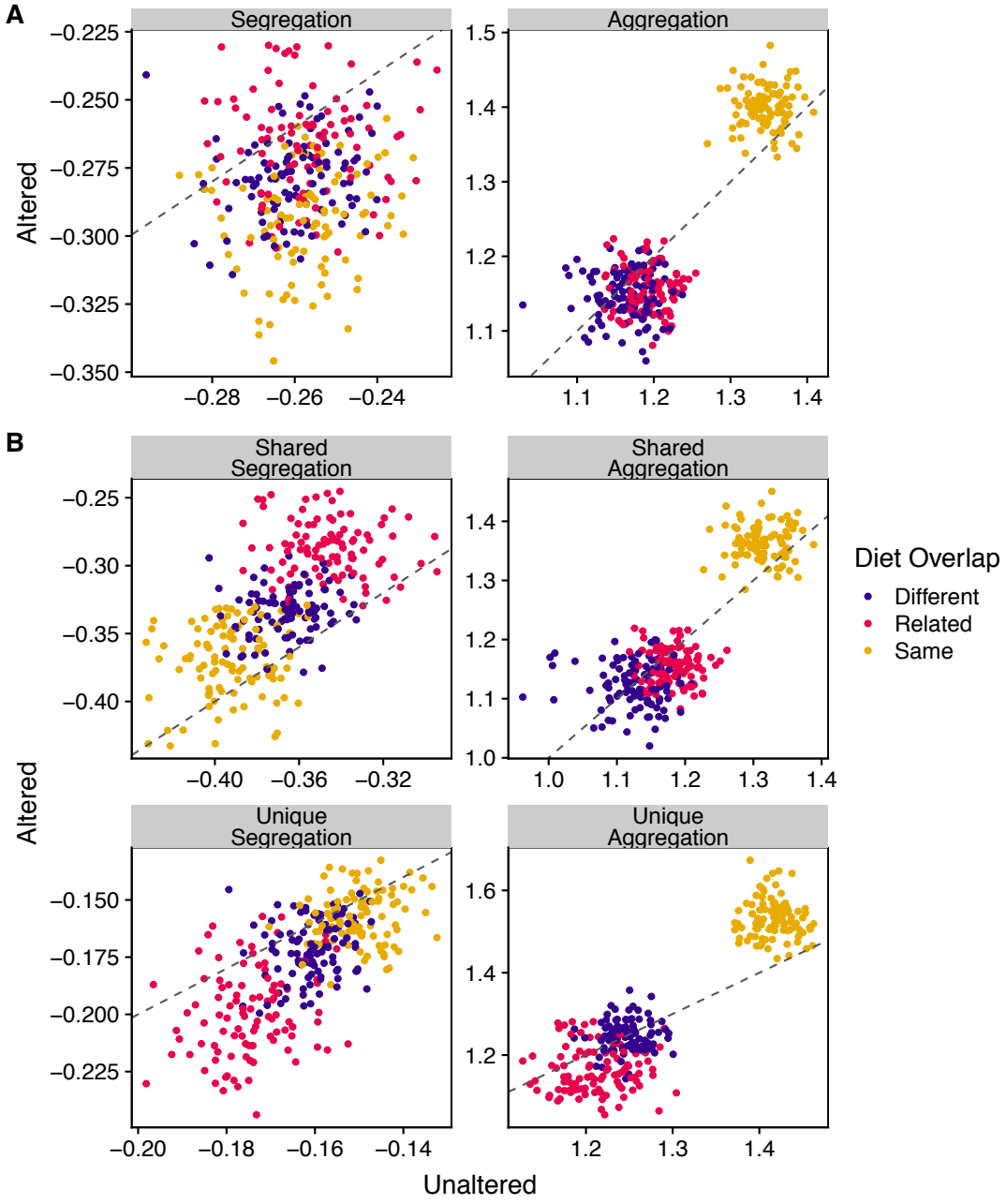

Figure 5.4. Mean magnitude of aggregations and segregations in pairs of bats, grouped based on degree of dietary overlap, with altered sites plotted against unaltered sites. The points represent subsamples. The dashed line is the line of unity. Samples falling below the line exhibit less co-occurrence (stronger segregations or weaker aggregations) in altered sites, while samples falling above the line exhibit more co-occurrence (stronger aggregations or weaker segregations) in altered sites compared to unaltered sites.

Functional Co-occurrence Analysis– Birds. At unaltered sites, bird pairs with different diets segregate most often and related-guild pairs aggregate most often. This pattern is driven by

both shared and unique pairs. Bird pairs are more likely to aggregate across the board in altered sites versus unaltered sites (Fig. 5.5), but the relationship between diet overlap categories does not change. Shared pairs in particular tend to aggregate more at altered sites than unaltered sites, and the likelihood of aggregation increases with dietary overlap.

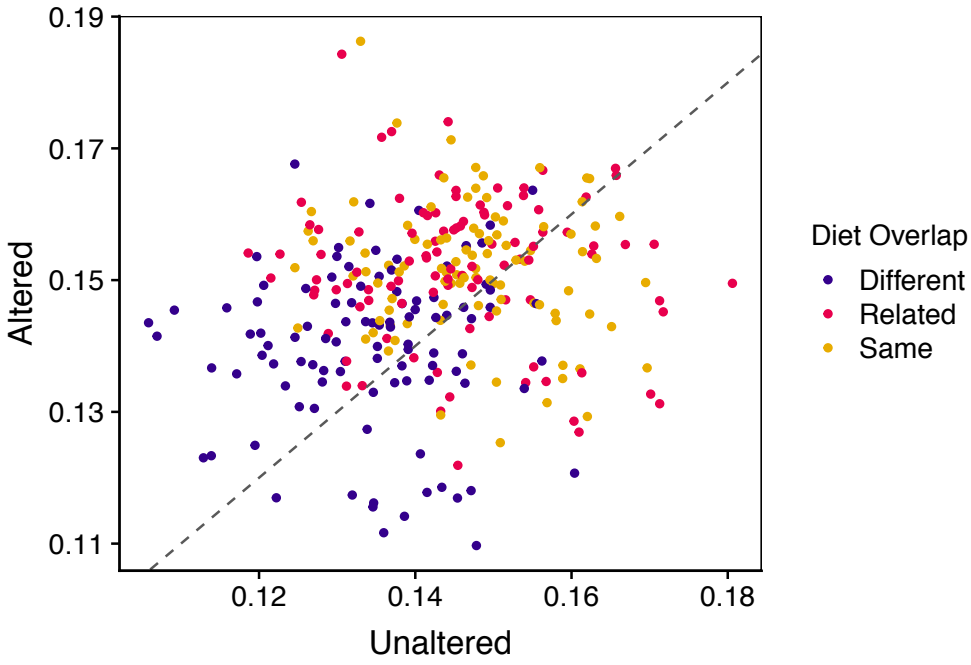

Figure 5.5. Proportion aggregated pairs in altered versus unaltered habitats for pairs of birds, separated based on degree of dietary overlap. The dashed line is the line of unity. The points represent subsamples.

Alteration causes weaker co-occurrence (stronger segregations and/or weaker aggregations) in different-guild and related-guild pairs (Fig. 5.6A), particularly in unique pairs (Fig. 5.6B). Pairs that share at least one food source co-occur more strongly (stronger aggregations and weaker segregations) in altered versus unaltered sites. The difference in the response of diet categories to alteration is driven by pairs unique to each site type (i.e., changes in community assembly associated with faunal turnover), because shared pairs,

which tend to occur more commonly, actually have more co-occurrences in all diet overlap categories, which is different from the pattern in the overall data (Fig 4.6B).

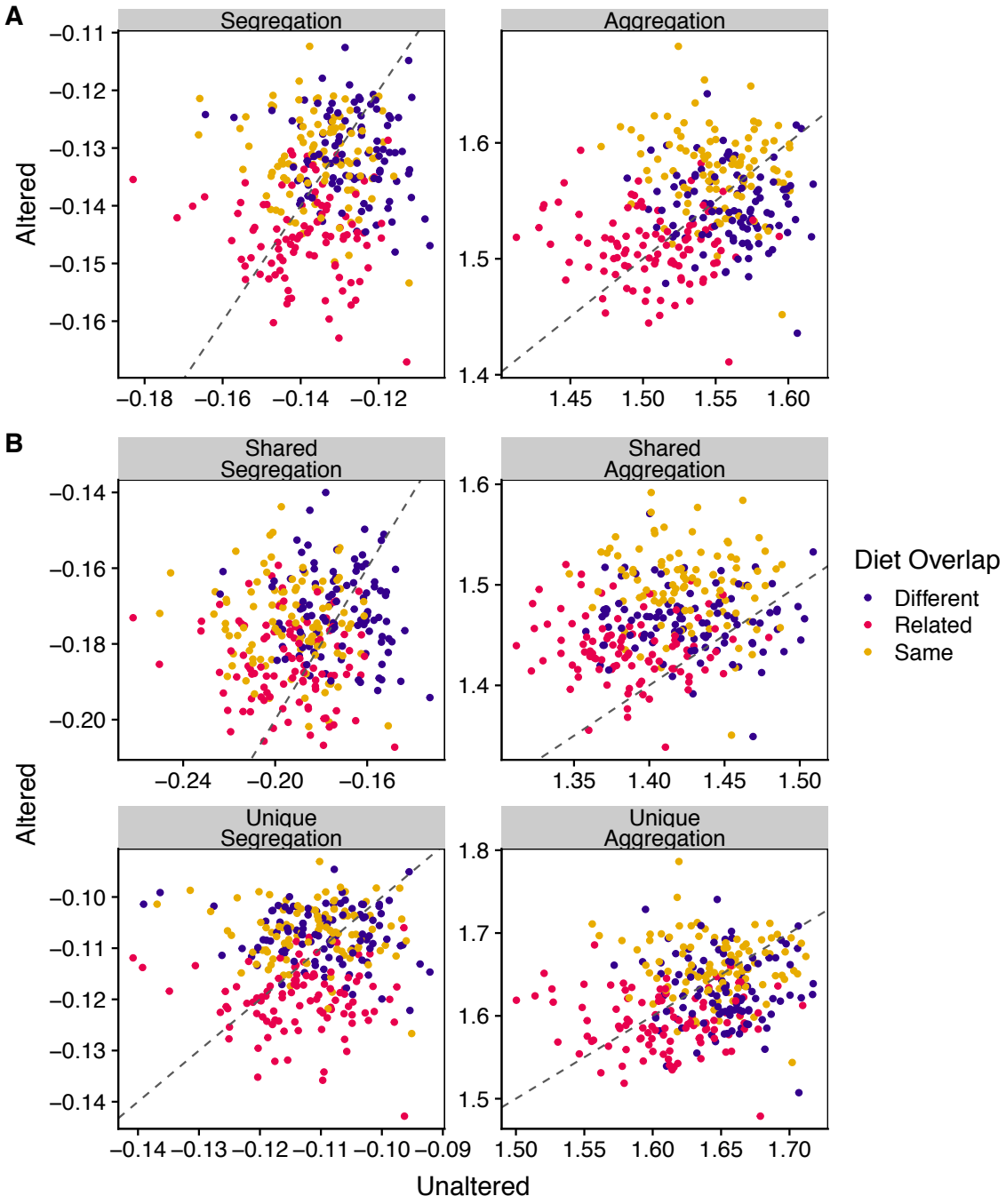

Figure 5.6. Mean magnitude of aggregations and segregations in pairs of birds, grouped based on degree of dietary overlap, with altered sites plotted against unaltered sites. The points represent subsamples. The dashed line is the line of unity.

Functional Co-occurrence Analysis– Comparison. In summary, both bat and bird pairs exhibit more co-occurrence (more aggregations as well as stronger aggregations) in altered habitats than unaltered habitats when sharing food sources, and less co-occurrence when not sharing food sources. Although habitat alteration does not substantially alter the general relationships between diet-overlap groups in either taxon, it causes the existing patterns in bats to become stronger. Finally, unique pairs (i.e., pairs occurring in only one habitat type because of compositional turnover) are more likely to dominate the overall patterns.

Discussion

In this study we examined the effect of habitat alteration on the community assembly of Neotropical bats and birds. Unaltered and altered habitats did not differ significantly in species richness, nor did we find differences in composition between unaltered and altered habitats. This suggests that biogeographic location is more important in determining compositional turnover than habitat alteration. However, beta diversity was lower in altered habitats for bats, similar to the results of Kay *et al.* (2017) for lizards in Australia. This paper therefore joins a long parade of studies showing that habitat alteration can cause homogenisation rather than reducing richness (McKinney and Lockwood 1999; McKinney 2006; Trentanovi et al. 2013; Dornelas et al. 2014; Tóth et al. 2014; Knop 2015). We found this pattern even though our altered sites encompassed a wide variety of habitat types ranging from urban to secondary forest. Such homogenisation is typically driven by the expansion of a few cosmopolitan species while rare and sensitive species are extirpated (McKinney and Lockwood 1999; Tóth et al. 2014). It is possible that compositional similarities between altered and unaltered sites are a result of extinction debt (i.e. the concept that some

species trapped in diminishing fragments will go locally extinct but have not yet done so), however, bats and birds are such mobile species that this explanation does not seem likely.

Differences in association strength between altered and unaltered sites were largely dominated by reduced co-occurrence (stronger segregations and weaker aggregations) in the presence of alteration, except in pairs that shared the same diet sources. Previous studies have focused on the number or proportion of significant aggregations and segregations, showing a decrease in the incidence of aggregations in mammals (Lyons et al. 2016; Smith et al. 2016) and fossil pollen (Blois et al. 2014; Lyons et al. 2016) in the presence of disturbance. Similarly, a loss of aggregations is seen in modern Australian lizards (Kay et al. 2017) in modified sites and birds in forest plantings (Lane et al. 2014). Unlike most previous work, we evaluated both the incidence and the strength of associations using all associations, not just those above a significance threshold. Although our results are compatible with previous findings, our investigation makes it clear that the picture is more complex than a loss of aggregations. Bat pairs had fewer aggregations in altered versus unaltered habitats and these aggregations were also weaker, except in same-guild pairs. Bird pairs had more aggregations, but these were again weaker in all except same-guild pairs.

Our functional analysis hints at an explanation for the observed differences. In birds, the relationship between different-, related-, and same-guild pairs is similar between altered versus unaltered habitats, but all groups have a higher incidence of aggregation in altered habitats. This suggests that some other variable, such as competition for breeding territories, or some type of interaction entirely, is more instrumental than diet in structuring bird communities in the wild. The fact that bat pairs had more and stronger segregations irrespective of dietary overlap suggests that altered habitats increase the incidence of

exclusion, but this is not driven by competition for food. Food resource sharing does influence the effect of habitat alteration on aggregations, however, because aggregations are stronger in same-diet pairs and weaker in the others, increasing the existing difference between them in both taxa.

Even in unaltered habitats, same-guild bat pairs aggregate much more strongly than different-guild pairs (Fig. 5.4), suggesting that far from excluding one another, competing species of bats are able to coexist easily in the wild. We do not believe this result is a sampling artefact because the Ecological Register intentionally excludes literature that did not fully document bat faunas because, for example, it only discussed phyllostomids. If our pattern is real, it makes sense because bats often share roosts with other species (Swift and Racey 1983) and have been shown to partition microhabitats and foraging times (Aldridge and Rautenbach 1987; Nicholls and Racey 2006), which allows them to overlap broadly on the trophic dimension (Kingston et al. 2000). The changes associated with habitat alteration actually enhance this pattern, suggesting that certain properties of altered habitats actively aggregate particular diet guilds in space (e.g. to particular types of sites) or in time (e.g., seasonal fruiting of a particular tree that is frequently planted). Birds segregate much more often than bats in general, but co-occurrence is nonetheless stronger for same-guild pairs in altered habitats (Fig. 5.6), suggesting that a similar mechanism may be in play for both taxa.

Strong aggregations form when species occur together at a relatively small number of sites and are both absent from other sites. Vampire bats have been shown to feed preferentially on the blood of livestock (Delpietro et al. 1992; Bobrowiec et al. 2015), suggesting they co-occur in farms and ranches (Becker et al. 2018), even though they are relatively rare in the wild. Although there are only a few species of sanguinivore bats in our

data, carnivorous bats (22 species) co-occur disproportionately in plantations and forest fragments. Frugivorous bats (69 species) co-occur primarily in plantations and rural sites, suburban sites support the highest proportion of insectivore bats (80 species), and nectarivores (21 species) co-occur disproportionately in plantations (Fig. 5.7). Different-guild pairs might segregate due to this spatial structure in food resources, and likewise for related-guild pairs, if their non-shared food resources are not available in the same sites. Interestingly, insectivores and nectarivores disproportionately segregate, suggesting that competitive exclusion is stronger in altered habitats for these feeding guilds, but also that insect and nectar food resources may be available in a broader range of altered site types.

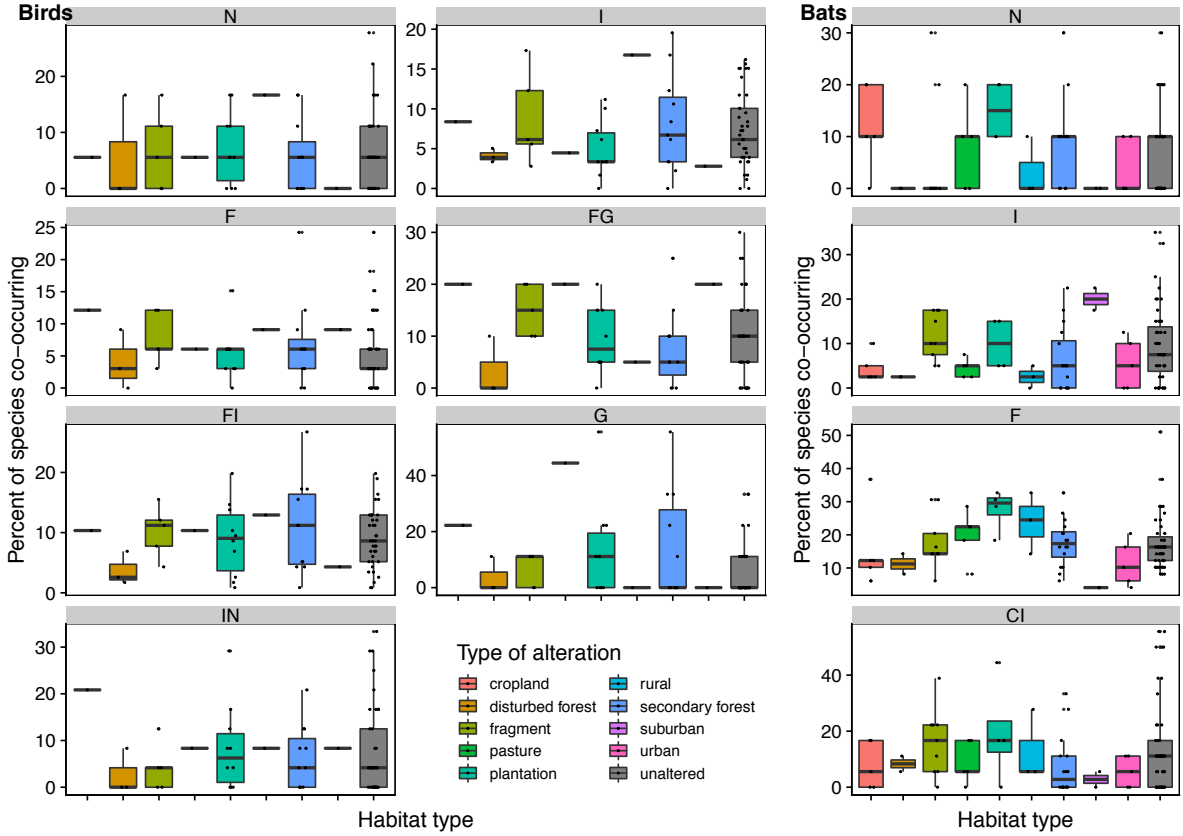

Figure 5.7. Percent of species in bird (left and center) and bat (right) guilds, represented by panels, that co-occur at individual altered sites by type of alteration. Each point is an altered or unaltered site. There are no bird sites in the suburban category. Key to panels: C = carnivore; F = frugivore; I = insectivore; N = nectarivore; and G = granivore. Multiple letters indicate mixed feeders.

The overall differences in the data are often dominated by the pattern established by unique pairs. This means that species pairs sampled only in altered habitats do not substitute the community structural roles of pairs that have been extirpated and remain only in unaltered habitats. In particular, pairs unique to altered habitats appear to co-occur less often than their counterparts in unaltered habitats, partially driving the stronger segregations and weaker aggregations in the different- and related-diet categories for both taxa. This is despite the fact that pairs conserved between the two habitat types often aggregate more in altered habitats.

Our findings suggest that exclusion from interspecific competition for food is weaker or absent in altered sites compared to unaltered sites, probably due the predictability and reliability of abundant food sources. However, they also indicate that species apparently segregate by dietary guild to a greater extent than in unaltered sites, and this may reduce the dietary diversity of local communities in altered habitats, because it reduces the co-occurrence of pairs with different diets. It is possible that the aggregation of one dietary group at a site reduces trait diversity and redundancy of other guilds for that site through competition for other resources, such as roosting sites, breeding territory, or foraging time.

Conclusion

Our approach confirms that there are indeed marked differences in the community assembly of bats and birds as a result of habitat alteration, even though richness and habitat composition were not significantly different. Most pairs that share food sources exhibit stronger aggregations in altered habitats, suggesting that changes in community assembly are more strongly influenced by the spatial distribution of food resources in altered sites than in the wild. This implies that habitat alteration reduces competitive exclusion for food, particularly in bats. Unfortunately, this leads to reduced co-occurrence of functionally diverse pairs, as evidenced by the decreasing co-occurrence of pairs with different food sources. This is consistent with previous work suggesting that human disturbances are reducing aggregations in non-volant mammals (Lyons et al. 2016) and reptiles (Kay et al. 2017). Reduced competition and exclusion between species with similar diets ultimately results decreased diversity of dietary groups at altered sites.

ACKNOWLEDGEMENTS

The authors would like to thank the members of the Macquarie University Palaeobiology Lab, whose discussions helped facilitate the improvement of this manuscript. ABT was supported by an iMQRES scholarship at Macquarie University.

SUPPLEMENTARY MATERIALS

Methods

Biogeographic correction. The geographic layout of sites can influence the results of co-occurrence analysis. For instance, if sites are clustered in two regions with no sites in between, there is a good chance that the co-occurrence structure of the assemblage will reflect this clustered layout by exhibiting strong aggregations within the clusters and segregations between clusters. Ideally, we would like to have even sampling over our entire study area, but when this is not possible, we must at least make sure that any comparisons are not influenced by geographic sampling bias. One way to achieve this is to ensure that the sites used to calculate co-occurrences have roughly the same geographic coverage when comparing co-occurrences across different types of sites or time periods.

In this paper, the study extent covers Central and South America south of the Tropic of Cancer. This includes large swathes of the Amazon in Brazil where there are no samples in altered habitats. As a result, the unaltered sites cover more area than the altered sites, and the geographic coverage of the latter is nested within that of the former. To correct for these biases, we removed the unaltered sites covering areas that were not covered by altered sites using the following algorithm. (1) We measured the distance to the closest altered site for all unaltered sites and vice versa. (2) We found the altered site farthest from its closest unaltered

site. (3) We removed all unaltered sites whose closest altered neighbour was farther than the distance in step 2. Note that this approach only works because the geographic coverages is nested; site types that are offset in space would require a different approach. Our final sites are plotted in Fig. 5.1.

Guild-by-guild analysis. The main text of this study compares pairs that have the same diet, related diets, and different diets. To understand the differences between individual dietary guilds in terms of response to habitat alteration, we used our co-occurrence results for same-diet pairs to compute the proportion and strength of aggregations and segregations within each dietary guild separately. This analysis can shed light on which dietary guilds are strongly affected by alterations and which dietary guilds are responsible for the overall patterns. Because some guilds are poorly represented, we restricted this analysis to dietary guilds that have an average of at least 50 pairs per subsample per habitat type in total (counting both shared and unique pairs), which is roughly equivalent to at least 10 species showing up regularly in each habitat in each subsample. We plotted the proportion and magnitude of associations of species in altered habitats against those in unaltered habitats.

Analysis of alteration types. To gain more insight into the aggregation of same-guild pairs in altered habitats, we calculated the percent of species in each guild that co-occur at individual sites (e.g., 5% of bat carnivore species occur at site A, etc.). We plotted the sites by alteration type to examine the influence of alteration on species aggregation. Although we do not have enough sites of each alteration type to draw any definitive conclusions, this analysis can suggest reasons why certain guilds might co-occur more in altered sites and provide questions for future research.

Results

Guild-by-guild analysis. Same-guild pairs in different dietary categories unsurprisingly showed a variety of responses to habitat alteration. As expected, the largest guilds dominated the overall pattern. In bats, insectivores and frugivores are the largest guilds. In altered sites, both guilds showed a higher proportion of segregations for shared pairs but a higher proportion of aggregations for unique pairs (Fig. 5.8). Bat carnivore pairs aggregated more than pairs in any other guild in unaltered sites, but their aggregations were substantially less prevalent in altered sites. Nectarivores aggregated less than the other guilds in unaltered sites, but aggregations were more common in altered sites. Although frugivores and insectivores have stronger aggregations in altered sites versus unaltered sites, which drives the overall pattern, both unique nectarivores and shared carnivores had weaker aggregations. Stronger average segregations observed in altered sites are mainly driven by nectarivores and unique insectivores (i.e., turnover in insectivore species: Fig. 5.9).

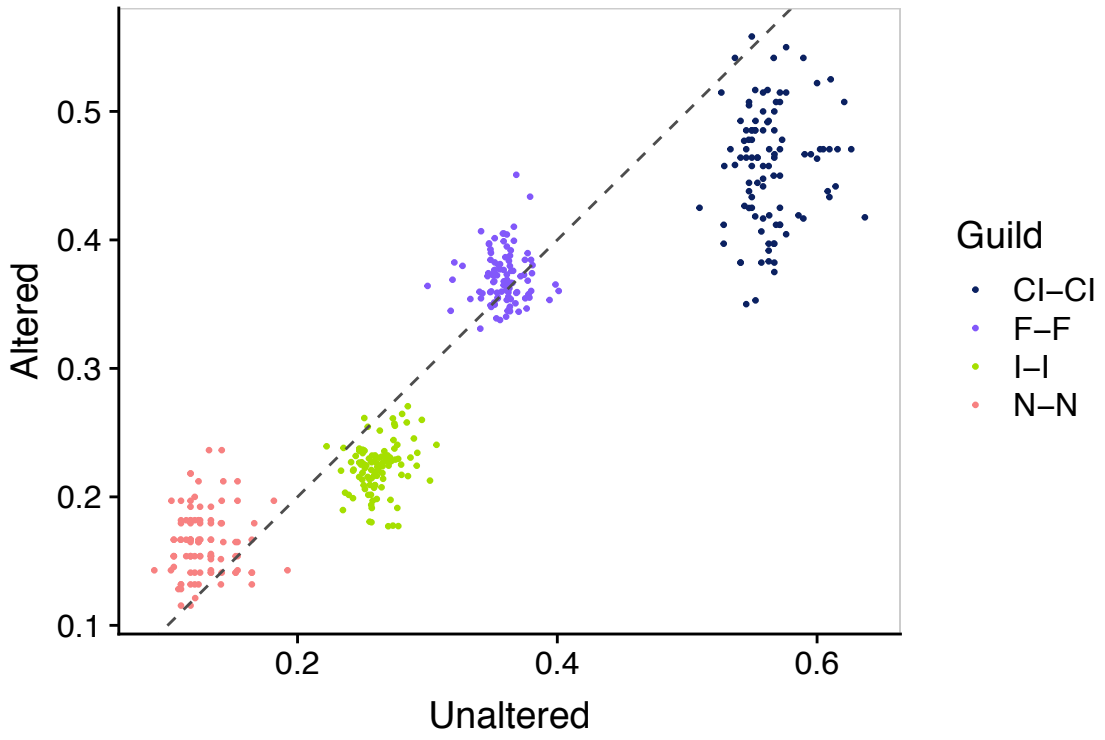

Figure 5.8. Proportion of pairs that are aggregated in altered versus unaltered habitats for pairs of bats that have the same dietary sources, by guild. The dashed line is the line of unity. The points represent the appropriate pairs from the subsamples (i.e. each subsample is plotted four times, once for its pairs in each guild). Samples falling above the line have a higher proportion of aggregations in altered habitats, and samples falling below the line have a lower proportion of aggregations in altered habitats. Key: CI = carnivore-insectivore; F = frugivore; I = insectivore; N = nectarivore.

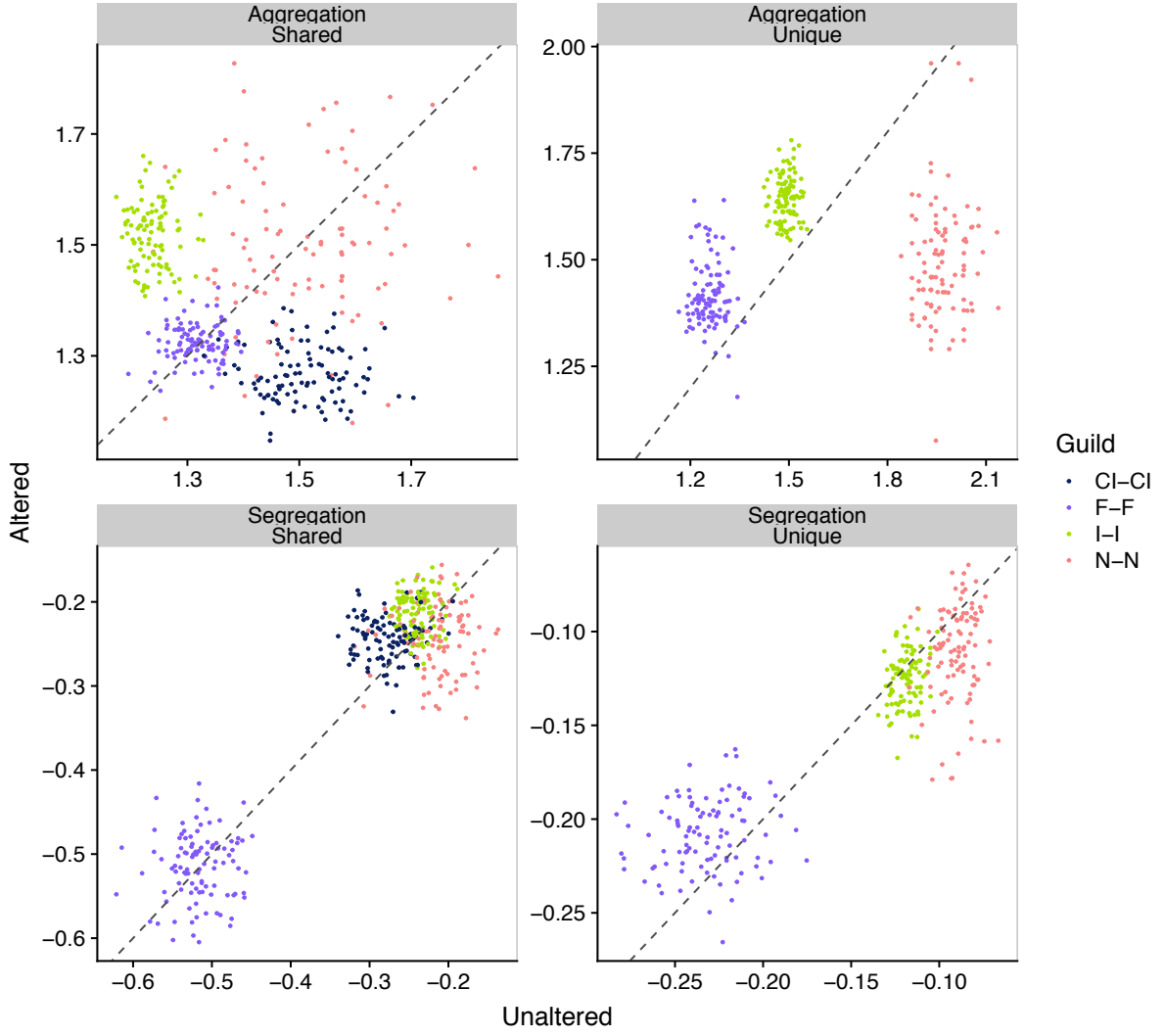

Figure 5.9. Mean magnitude of aggregations and segregations in pairs of bat species that share the same dietary sources, grouped into diet categories, with altered subsamples plotted against unaltered subsamples. The points represent subsamples. The dashed line is the line of unity. Samples falling below the line exhibit less co-occurrence (stronger segregations or weaker aggregations) in altered sites, while samples falling above the line exhibit more co-occurrence (stronger aggregations or weaker segregations) in altered sites compared to unaltered sites. Key: CI = carnivore-insectivore; F = frugivore; I = insectivore; N = nectarivore.

In birds, the largest guilds were insectivores and frugivore-insectivore mixed feeders. Insectivores showed no difference in proportion of aggregations to segregations, but frugivore-insectivore pairs had more aggregations in altered habitats (Fig 4.10). Two other guilds, granivores and nectarivores, also tended toward more aggregations in altered

habitats, and the opposite was true for the three remaining guilds (frugivores, frugivore-granivores, and insectivore-nectarivores). Interestingly, almost every bird guild showed overall less co-occurrence (stronger segregations or weaker aggregations) in altered sites, particularly in unique pairs (Fig 4.11). Only insectivore and nectarivore bird pairs tended toward more co-occurrence (stronger aggregations and weaker segregations) with any consistency, and this trend in insectivores dominates the overall pattern, due to the large number of insectivore bird species.

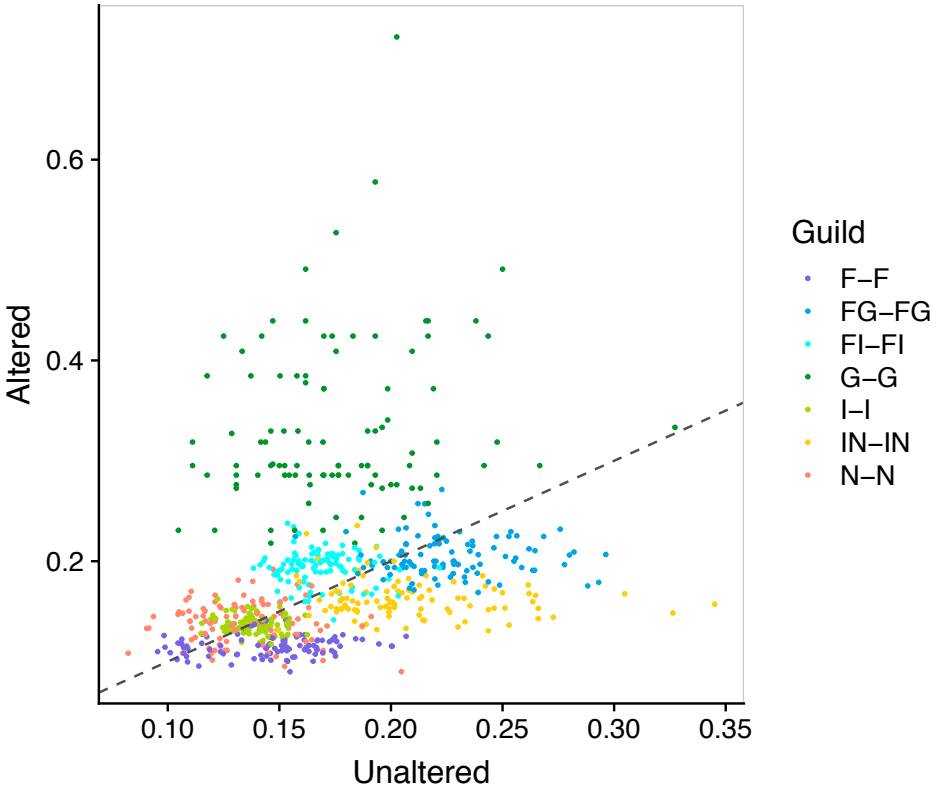

Figure 5.10. Proportion of aggregated pairs in altered versus unaltered habitats for pairs of bird species that have the same dietary sources, by guild. The dashed line is the line of unity. The points represent each dietary guild within subsamples (i.e. each subsample is plotted seven times, once for its pairs in each guild). Subsamples falling above the line have a higher proportion aggregations in altered habitats, and samples falling below the line have a lower proportion of aggregations in altered habitats. Key: C = carnivore; F = frugivore; G = granivore; I = insectivore; N = nectarivore; multiple letters indicate mixed feeders.

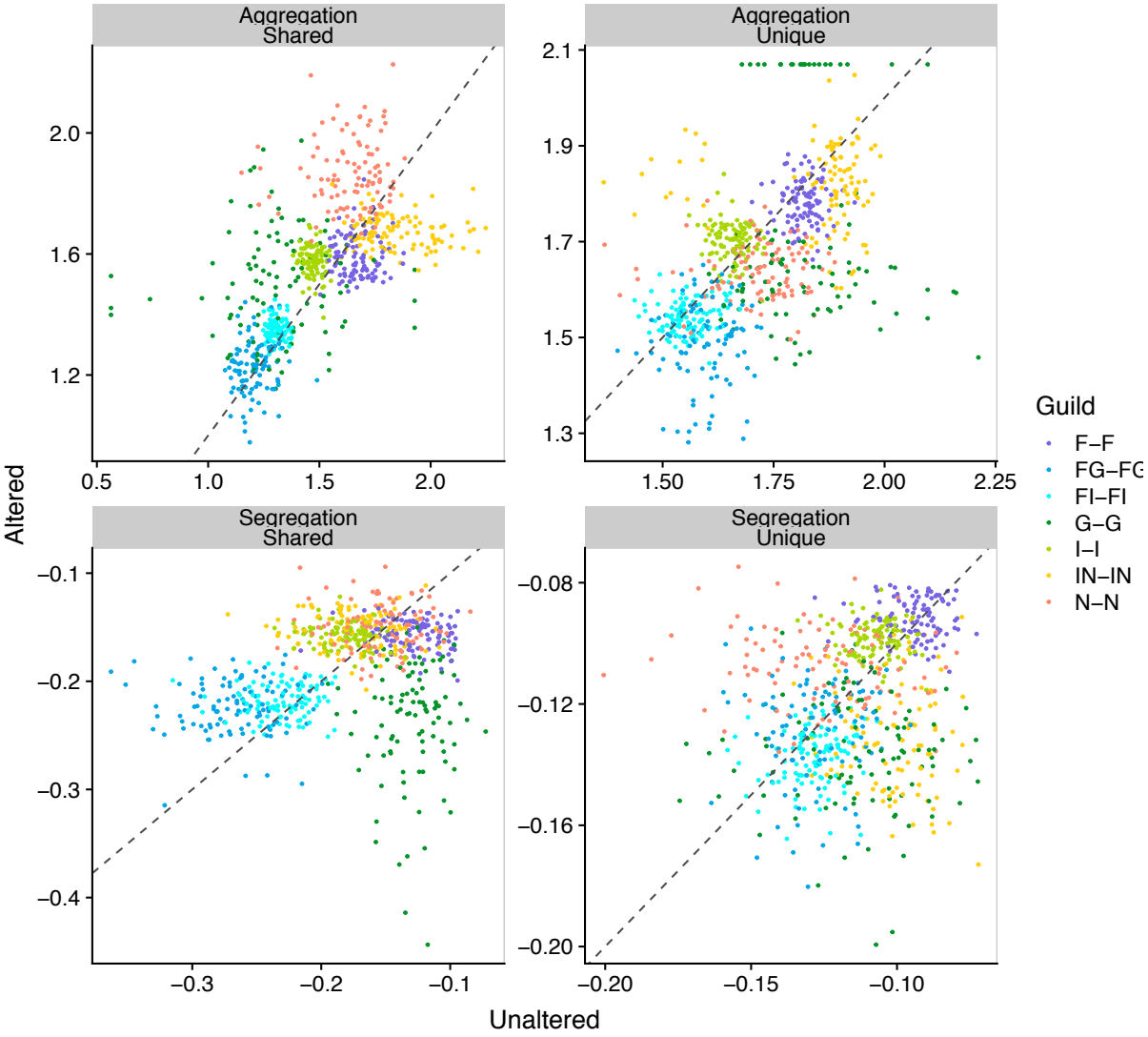

Figure 5.11. Mean magnitude of aggregations and segregations in pairs of bird species that share the same dietary sources, grouped into diet categories, with altered subsamples plotted against unaltered subsamples. The dashed line is the line of unity. The points represent subsamples. Subsamples falling below the line exhibit less co-occurrence (stronger segregations or weaker aggregations) in altered sites, while subsamples falling above the line exhibit more co-occurrence (stronger aggregations or weaker segregations) in altered sites compared to unaltered sites. Key: C = carnivore; F = frugivore; I = insectivore; N = nectarivore; and S = sanguinivore. Multiple letters indicate mixed feeders.

Analysis of alteration types. Many of our alteration categories included only one or two sites, which is insufficient to draw definitive conclusions about how bat and bird guilds aggregate over the altered landscape. However, our results, plotted in Fig. 5.7, suggest that plantations

are an important aggregation point for bats, particularly frugivores and nectarivores. Although only two suburban sites are included, these sites both contain more than 15% of bat insectivores, and very few other species. The data for site types are even sparser for birds, but the primary habitat types are forest fragment, secondary forest, disturbed forest, and plantation. These site types vary in their importance to individual bird guilds. Birds with fruit or nectar in their diets co-occurred in plantations and fragments, while insectivores were most strongly aggregated in secondary forest. Disturbed forest typically contained a low percentage of each dietary guild. Secondary forest and forest fragment sites appear more instrumental for the co-occurrence of same-guild birds than bats (Fig. 5.7).

Discussion

The results of the guild-by-guild analysis show that the increase in co-occurrence between same-guild pairs is not universal across all dietary guilds. In fact, much of the analysis is dominated by the patterns of insectivore pairs in both taxa. As insectivores are the most speciose guild in both bats and birds (by far in the latter), the prevalence of insectivore-based patterns in the data should be considered biologically relevant. However, diversity of life history traits depends upon the co-occurrence of a variety of taxa, and functional redundancy depends on the ability of many same-guild taxa to co-occur. Based on this analysis, many guilds from both taxa have lost the ability to form strong, frequent aggregations, suggesting that functional redundancy at altered sites is being lost for particular functional groups.

Our main analyses suggest that different-guild and even related-guild pairs co-occur more weakly at altered sites. We interpret this to mean that functional diversity, i.e. the ability of species with diverse functional roles to coexist, has been reduced at altered sites in

the Neotropics. According to the guild-by-guild analysis, functional redundancy for many groups may also be affected.

Supplementary figures

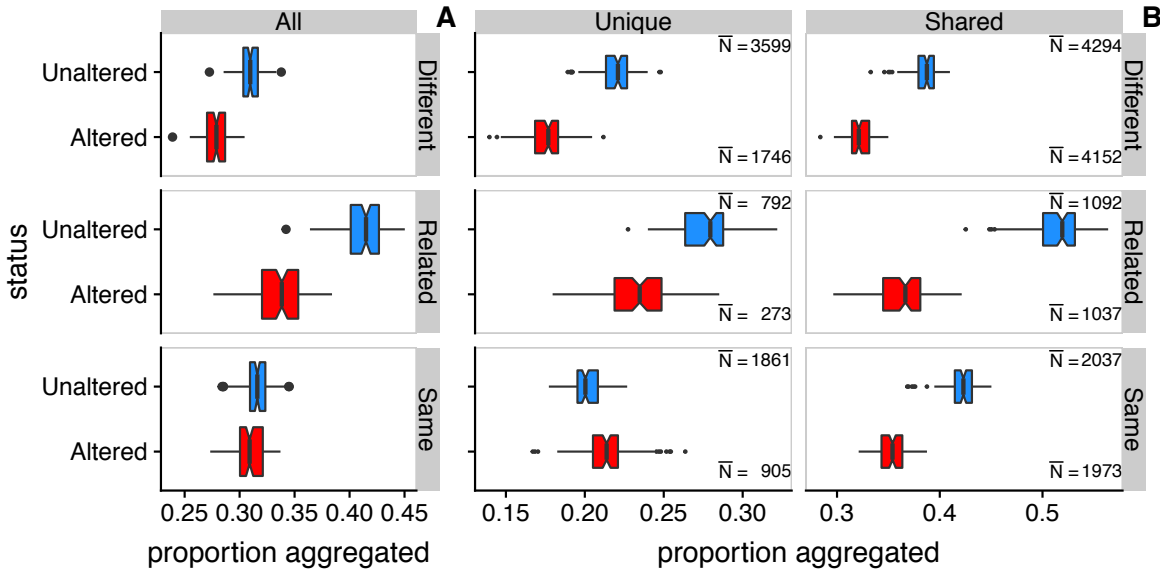

Figure 5.S1. Percent aggregated pairs in altered and unaltered habitats for bats, separated by the degree to which diets are shared by each species pair. Each group is further separated based on whether it is found in both altered and unaltered habitats (shared) or only in one (unique), distinguishing between community change that is or is not driven by compositional turnover. Points represent subsamples, and the average number of pairs per subsample in each category is displayed within each panel.

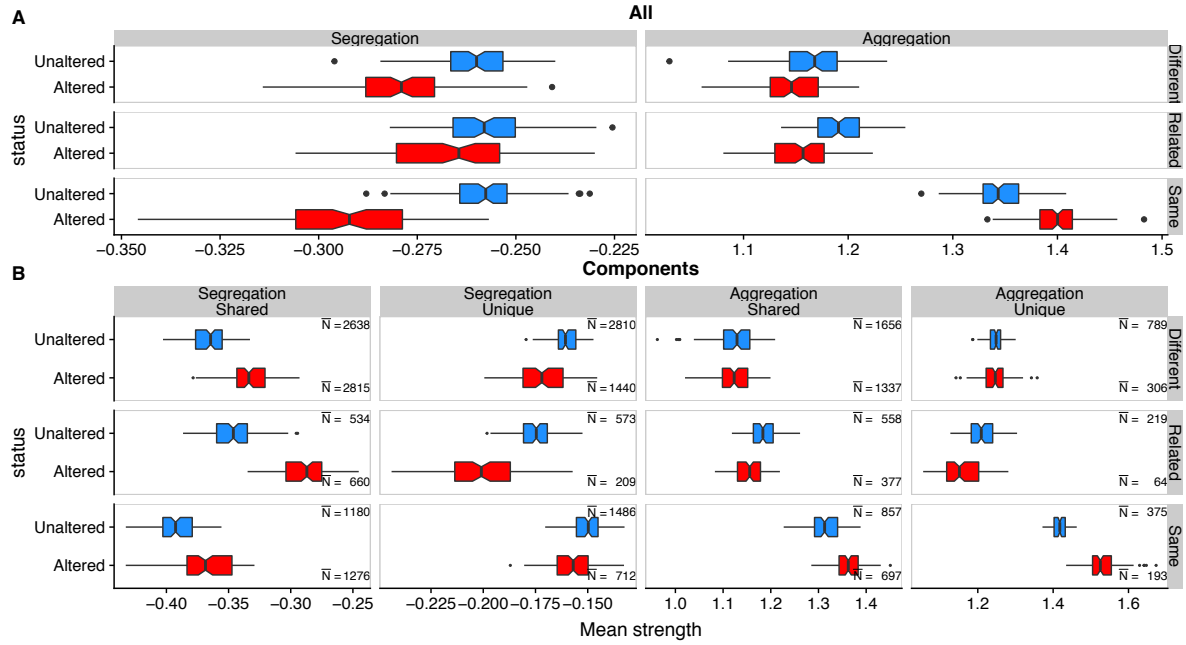

Figure 5.S2. Strength of aggregations and segregations in altered (red) and unaltered (blue) habitats for bats, separated by the degree to which diets are shared by each species pair (A). Each group is further separated based on whether it is found in both altered and unaltered habitats (shared) or only in one (unique), distinguishing between community change that is or is not driven by compositional turnover (B). Points toward the right represent stronger aggregations or weaker segregations, which is an increase in co-occurrence. Points toward the left represent stronger segregations or weaker aggregations, which constitutes a decrease in co-occurrence.

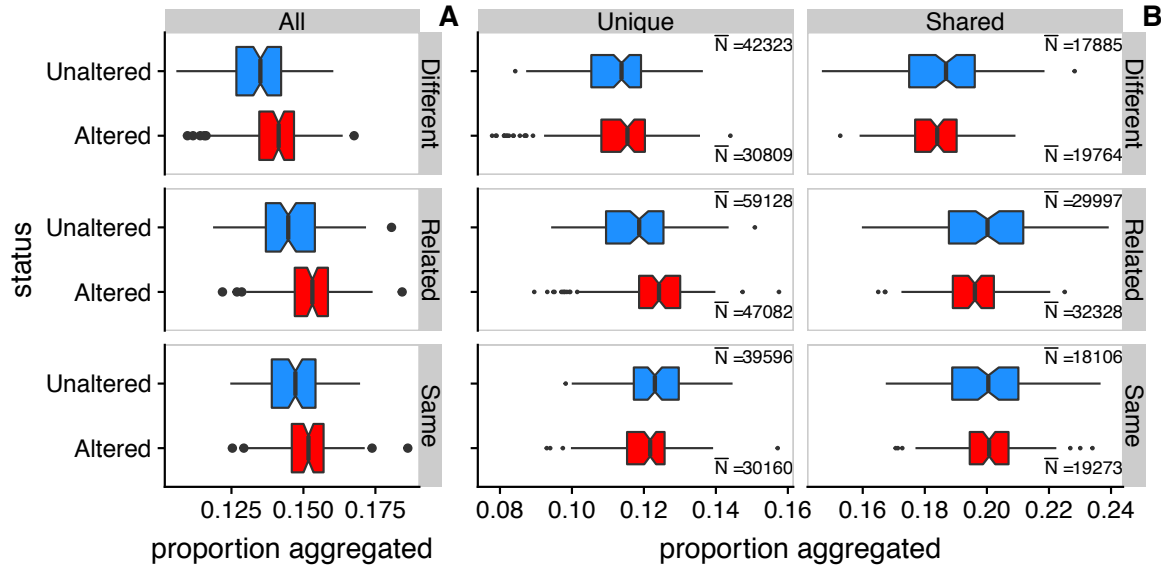

Figure 5.S3. Percent aggregated pairs in altered and unaltered habitats for birds, separated by the degree to which diets are shared by each species pair. See caption of Fig. 5.S1 for details

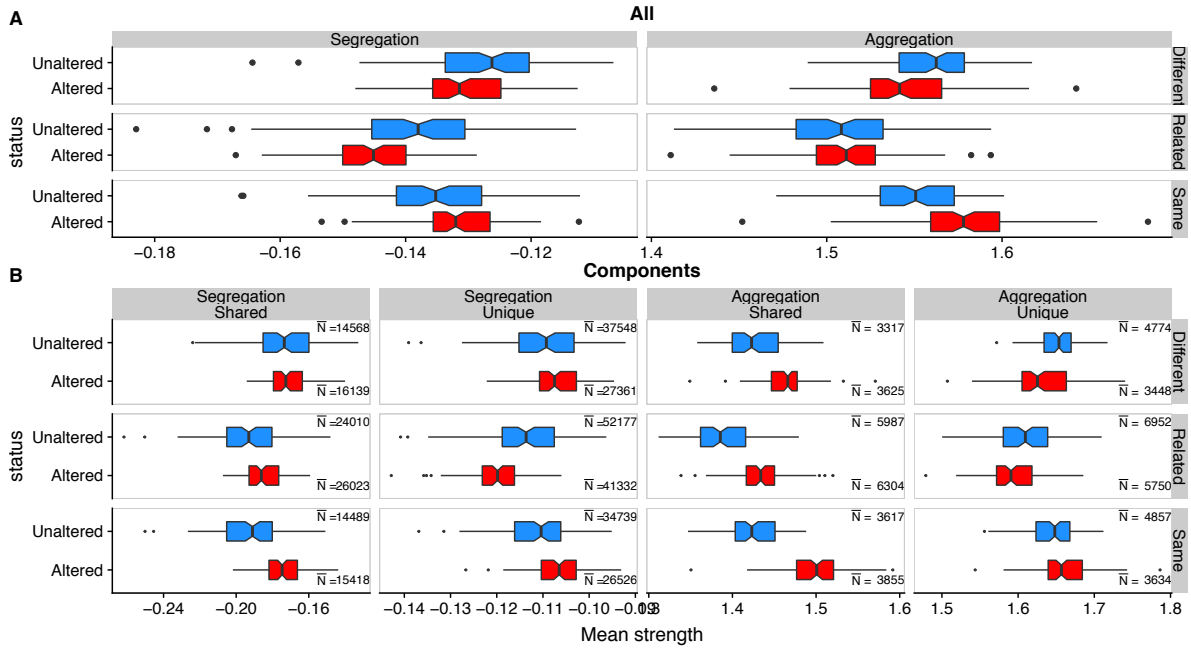

Figure 5.S4. Strength of aggregations and segregations in altered and unaltered habitats for birds, separated by the degree of shared dietary sources between the species pair. See caption of Fig. 5.S2 for details.

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CHAPTER 6

Synthesis

MAIN TEXT

Recap

The previous chapters have introduced a comparative, probabilistic approach to co-occurrence analysis and applied it to answer two distinct questions about the biological mechanisms of community assembly. The first chapter presented an introduction to ecological networks and co-occurrence analysis. The second chapter outlined some challenges involved in co-occurrence analysis and illustrated how a comparative approach to probabilistic co-occurrences can provide answers to meaningful biological questions about community assembly. The third chapter is an observational study of simulated and empirical co-occurrence networks that provides a side-by-side comparison of binary vs. probabilistic network metrics as well as an assessment of the similarities between simulated and empirical network structures. The fourth chapter was a practical application of the comparative approach that observed changes in mammal community assembly over a critical extinction interval. The fifth chapter compared various dietary groupings of Neotropical bats and birds in altered and unaltered habitats to explore the effects of human land use on the outcomes of interspecific competition.

Four steps to conservation

The use of comparative co-occurrence analysis for improving applied conservation and management of dynamic ecosystems requires a four-step process (Fig. 6.1). As modern ecological network analysis is tied to relatively recent advances in computing, we are still in the process of documenting and observing ecological networks (Proulx et al. 2005). Therefore,

first, co-occurrence patterns must be observed and documented for target taxa at various scales to establish a baseline and the normal range of variation. Systems minimally impacted by anthropogenic factors should be studied here.

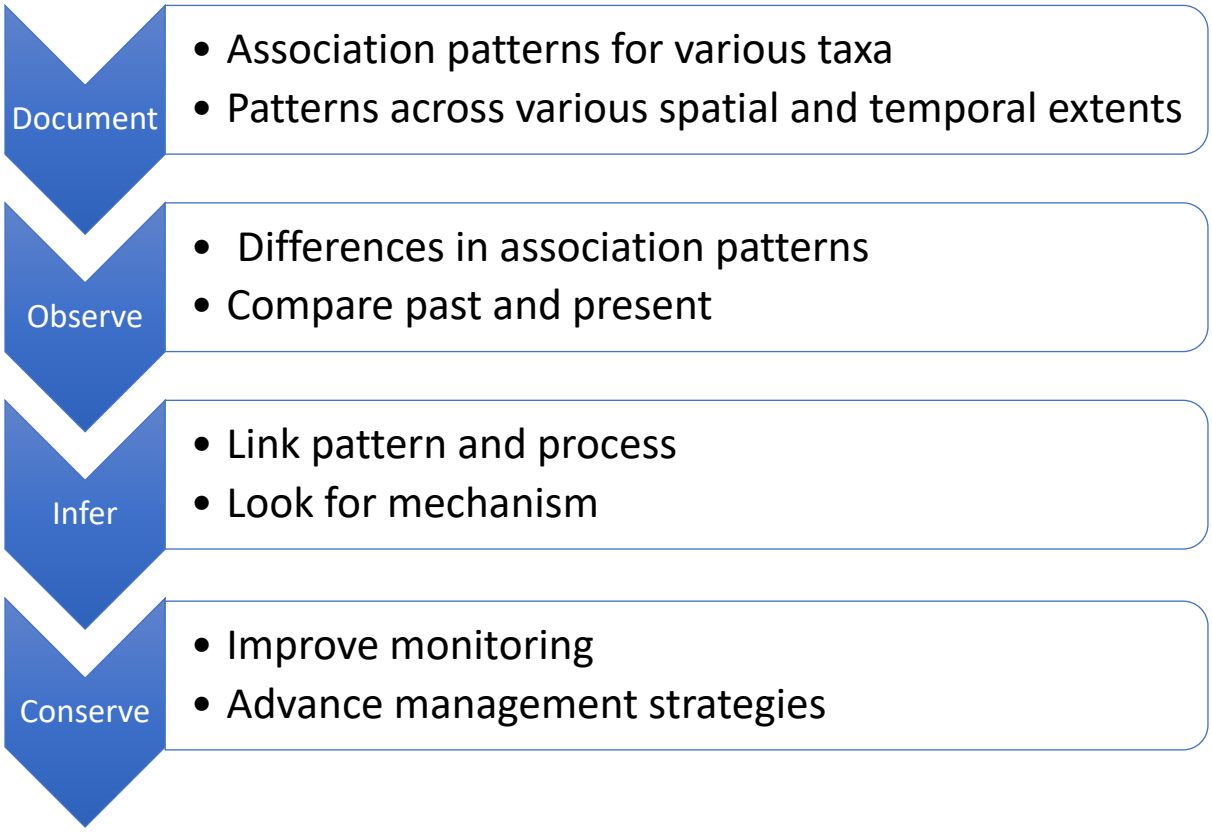

Figure 6.1. The process of using co-occurrence data to improve conservation management consists of four steps. This work addresses the first three steps directly. Continued monitoring of managed systems over time in the fashion demonstrated here would make it possible to achieve step 4, improving conservation management.

In chapters 2-5 of this work, co-occurrences were documented for communities of large-bodied non-volant mammals, bats, trees, insects, and birds, in some cases for various spatial and temporal extents. The results of these analyses suggest that—although ecological networks have already been shown to have a variety of topologies (Thébault and Fontaine 2010)—the co-occurrence structure of empirical communities is strongly constrained with

respect to the full range of possibilities observed in simulated matrices. To illustrate this, we plotted the probabilistic connectivity (Poisot et al. 2016) of co-occurrence networks for aggregations against segregations (Fig. 6.2) using the empirical datasets in chapters 2-5 of this work, in addition to several other datasets from the Ecological Register (http://ecoregister.org). We see that networks derived from empirical matrices fall in a small section near the centre of the connectivity plot, whereas networks derived from simplistic simulated matrices cover much of the possible plot area (Fig. 6.2). As a result, even seemingly small shifts in co-occurrence distributions can actually signal significant changes in community assembly patterns. This property is at least partially tied to numerical properties of empirical matrices such as low matrix fill and the presence of many rare and a few common species, which are relatively consistent across taxa.

The effect of spatial and temporal resolution on co-occurrences is still largely unknown, though it has been suggested that they do not necessarily behave as expected, for instance, decrease in temporal resolution does not necessarily cause an increase in aggregations (Lyons et al. 2016b). Chapter 5 of this thesis illustrates that co-occurrence patterns do not necessarily correlate with temporal extent either, because the younger two time intervals with very different temporal extents had more in common than the longer of these with the oldest time interval, which had a similar temporal extent. These findings suggest that co-occurrence analysis may be robust to reasonable variation in grain and extent and manages to capture biological patterns instead.

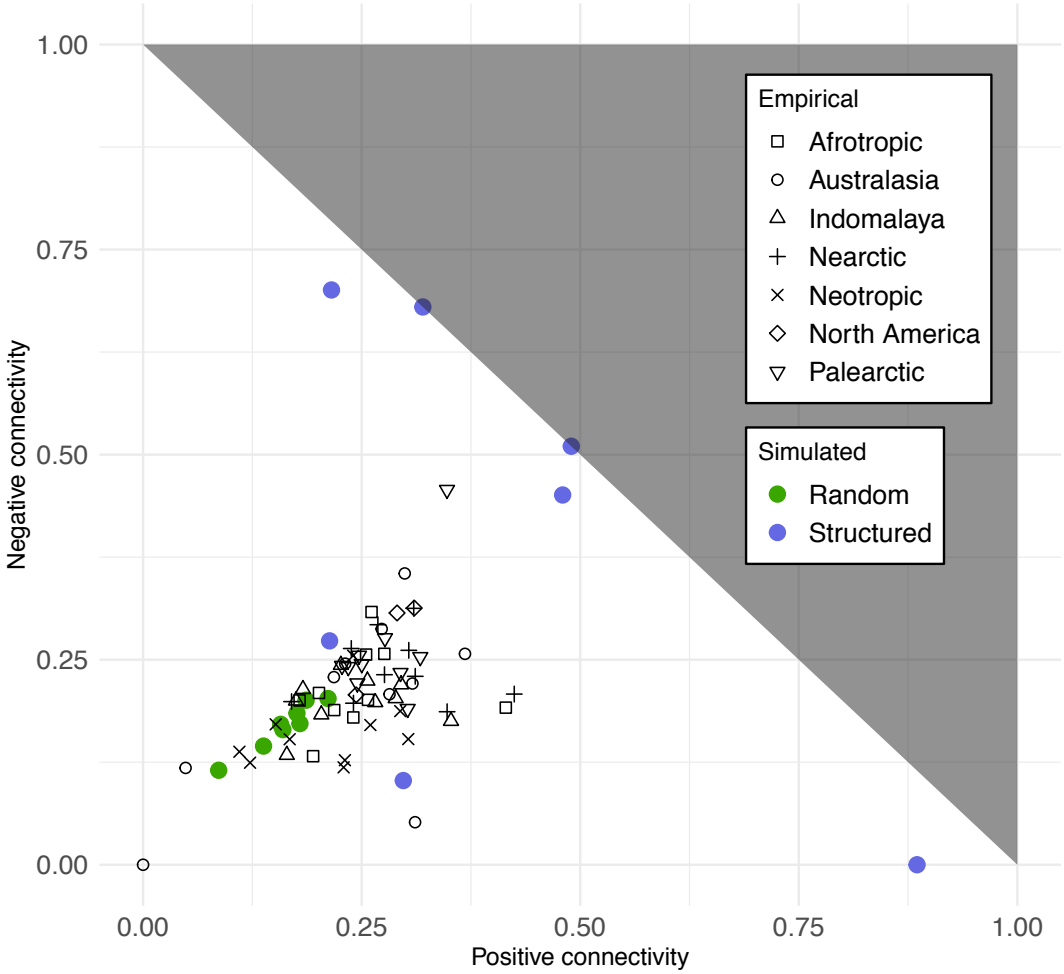

Figure 6.2. Probabilistic connectivity of aggregations plotted against probabilistic connectivity of segregations for the empirical matrices used in this work, and additional empirical datasets from the Ecological Register, including datasets documenting ants, bats, birds, butterflies, frogs, mammals, mosquitoes, scarab beetles, small mammals, and trees (not differentiated in legend) from different biogeographic realms. These are plotted along with a series of simulated datasets, including structured and unstructured (random) matrices with realistic occupancy distributions. The simulated structured matrices are based on community theory (see Chapter 3), and illustrate the theoretically possible range of connectivity combinations for co-occurrence networks. The gray triangle indicates the mathematically impossible section of the parameter space.

The second step is to observe changes in co-occurrence patterns over space and time, keeping in mind that modern communities may already be significantly different from those occurring before anthropogenic activities became widespread. This was one of the goals of chapter 4 of this work, in which North American large mammal co-occurrences were

compared before and after a major human-driven extinction event. This and other previous work (Lyons et al. 2016) suggests that the segregation-dominated assemblages of surviving species seen today could be an anthropogenic phenomenon that emerged in the last 10,000 years. In North American mammals, it appears that the shift toward segregation is a result of changes in biotic interactions, most likely the loss of top-down control by apex predators and of competition with large herbivores. A possible avenue of future research would be to repeat these analyses for the Pleistocene megafaunal extinction event on other continents. Another important area of inquiry would be to observe community shifts across non-anthropogenic critical intervals, such as the Palaeocene-Eocene Thermal Maximum (PETM) or the mass extinctions at the Cretaceous-Paleogene boundary (KPG), which would help to establish whether or not human disturbances bring about novel community assembly patterns, i.e. whether other critical intervals also precipitated increases in the prevalence of segregations, or another pattern.

The third step, and the major goal of this work, was to link observed changes to biological mechanisms, such as dispersal barriers, habitat preferences, and biotic interactions. The idea that many factors are simultaneously at work cannot be discounted, and thus we must find ways to determine how each one contributes to community assembly. The approach outlined in chapter 2 of this work is specifically targeted at identifying the interplay of mechanisms that assembles communities.

In chapters 4 and 5, I used two example systems to demonstrate how the comparative approach can answer targeted biological questions. In chapter 4, I compared the effects of biotic and abiotic factors on the community assembly of survivors of the end-Pleistocene extinction. Critically, I demonstrated that the observed shifts in community structure are

driven by a decrease in biotic regulation, rather than the climatic changes that occurred simultaneously. The pattern of changes suggests that the extinction ultimately resulted in increased competitive exclusion. This could have been driven by removal of competition and top-down control that led to increases in abundance. These results imply that mammal assemblages impacted by the Pleistocene mass extinction may be irreversibly altered by human interference above and beyond the permanent loss of extinct megafauna. This chapter builds on previous work describing changes in co-occurrence over deep time (Lyons et al. 2016a; Smith et al. 2016; Villalobos et al. 2016) by focusing explicitly on this critical interval.

In chapter 5, I assessed whether the spatial outcomes of interspecific food competition is influenced by human habitat alteration. I showed that the ability of altered habitats to support the coexistence of multiple species with different functional traits is diminished, but species with similar functional traits co-occurred more. This result suggests that altered communities, while still rich in species numbers, are losing functional diversity.

Given enough data, this approach could be implemented in many ways. For example, one could compare co-occurrence patterns in different types of altered habitat to determine whether alteration has differential effects on co-occurrence. One could split species using other functional characteristics, such as foraging mode in bats or degree of territoriality in birds, to test whether these traits are more instrumental in competitive exclusion than dietary guild assignments. Pairs could be split based on phylogenetic distance or differences in body size. Sites from different regions could be compared for a more spatially explicit analysis (i.e., to compare assembly patterns across space). Communities of plants with and without a target invasive species could be compared to see how invaders influence community structure.

Observing co-occurrence patterns and figuring out why they shift will improve our understanding of assemblages over regional and continental spatial scales. Monitoring changes in co-occurrence over time or comparing managed and unmanaged assemblages can help detect and evaluate the outcomes of management strategies. Drawing comparisons with relatively undisturbed areas could provide conservation targets for managing disturbed areas such as plantations or suburbs. Node-level properties of co-occurrence networks could provide potential options for actively replacing extirpated species with surrogates possessing similar roles in community structure. Because co-occurrence analysis only requires presence-absence data, it provides highly nuanced information even if there have not yet been more obvious changes like a decrease in richness due to extirpation.

Understanding ecosystems

Scaling up. Co-occurrence analysis can be applied at any spatial scale, and provides generalisations for the scale on which it was calculated. These generalisations can shift or be broken down if the analysis is performed on smaller scales, allowing us to measure spatial variation in species associations and understand the processes that combine to create the overarching pattern. Macroecology seeks to identify useful generalisations at large spatial scales (McGill 2019), and co-occurrence analysis provides a way to recognise and understand such patterns in species distributions.

A hierarchy of complexity. Another major long-term goal of macroecology is to understand how biotic and abiotic factors interact to govern functioning ecosystems. To achieve this, we must focus on understanding the connections between increasingly diverse and numerous interacting elements. Traditionally, much of ecology research has tended to be species-

centric, directed at understanding the behaviour of species, their distributions, and their roles in their ecosystems. This type of research is a logical first step and produces necessary foundational knowledge to describing the biosphere. It is still a central area of effort today, although the focus has shifted to a systematic biodiversity census with the aim of ecological risk assessment through efforts such as the IUCN Red List of Threatened Species. But higher-level questions about ecosystem function have also been in the literature for many decades. Interactions between species, particularly trophic and competitive relationships between single pairs of species, were an active area of research throughout the 1900s, as researchers tried to document how one species can affect others. The keystone species concept (Paine 1969) was born from the stability-complexity debate when Paine (1969) observed that the structure, and even the physical appearance, of two disparate marine communities were both strongly affected by the abundance of a single species of predatory starfish, despite rich biodiversity, and that there was no evidence of a similar effect for other species in these communities. The community assembly of birds on island archipelagos was instrumental in the development of the idea that competitive interactions across an entire assemblage, "diffuse competition" may be more applicable to the real world than the myriad studies examining competition between two species (Diamond 1970, 1975). Today, these ideas are being extended by the joint species distribution modelling (JSDM) literature, which incorporates the co-occurrence of other species—in addition to abiotic environmental variables—in predictive models of species distributions (Ovaskainen and Soininen 2011; Pollock et al. 2014; Warton et al. 2015).

While competitive models typically focused on species within a single taxon (e.g. birds), early research on food webs explored interactions across multiple taxa, typically

in marine and aquatic systems where several taxa (e.g. fish, gastropods, bivalves, echinoderms, etc.) make up reasonably small local food webs (Paine 1966; Pimm 1980). Eventually, more research began to explicitly emphasise how various biotic interactions link assemblages of two otherwise unrelated taxa, heralding the emergence of modern network ecology (Proulx et al. 2005; Montoya et al. 2006). These studies often use bipartite networks, focusing on the interactions between two taxa, not interactions within each taxon. Recently, research on multi-taxon interaction networks involving three or more taxa or functional groups (e.g. plants, herbivores, and their parasites) has begun to gain more traction (Pilosof et al. 2017; Astegiano et al. 2017; Hutchinson et al. 2019). A huge number of interactions occur across taxa (e.g. parasitism, commensalisms, herbivory, pollination, mutualism), so two species are often indirectly connected through interactions with a third, and all three may be in different taxa and functional groups. These multilayer networks allow us to analyse interaction networks across as well as within several taxa; essentially a multi-taxon unipartite network, which considers all possible relationships. Network layers can also correspond to different spatial locations and temporal intervals.

Co-occurrence analysis has been with us for most of the journey. Now, it offers a way to study all the possible relationships between any taxa, across space and time, by starting with the spatial outcomes of all driving processes, and working backwards to distinguish the driving mechanisms, all without a priori data about direct interactions. The four-step process outlined above provides a way forward for studying each level of the hierarchy described above, which is illustrated in Fig. 6.3. This thesis demonstrates the first three steps of this procedure and draws conclusions that can inform our development of the final step. Applications of the framework outlined in these chapters may eventually answer deeply

complex ecological questions, such as how do species co-occurrence patterns change along gradients of spatial resolution and extent? How do members of myriad different taxa share and partition space in ecosystems? How does this reflect their interactions and contributions to ecosystem function? How do interactions encourage local diversity and sustain complex food chains? How do ecosystems provide ecosystem services? How do ecosystems assemble and disassemble? And what can we do to ensure we do not destroy these processes?

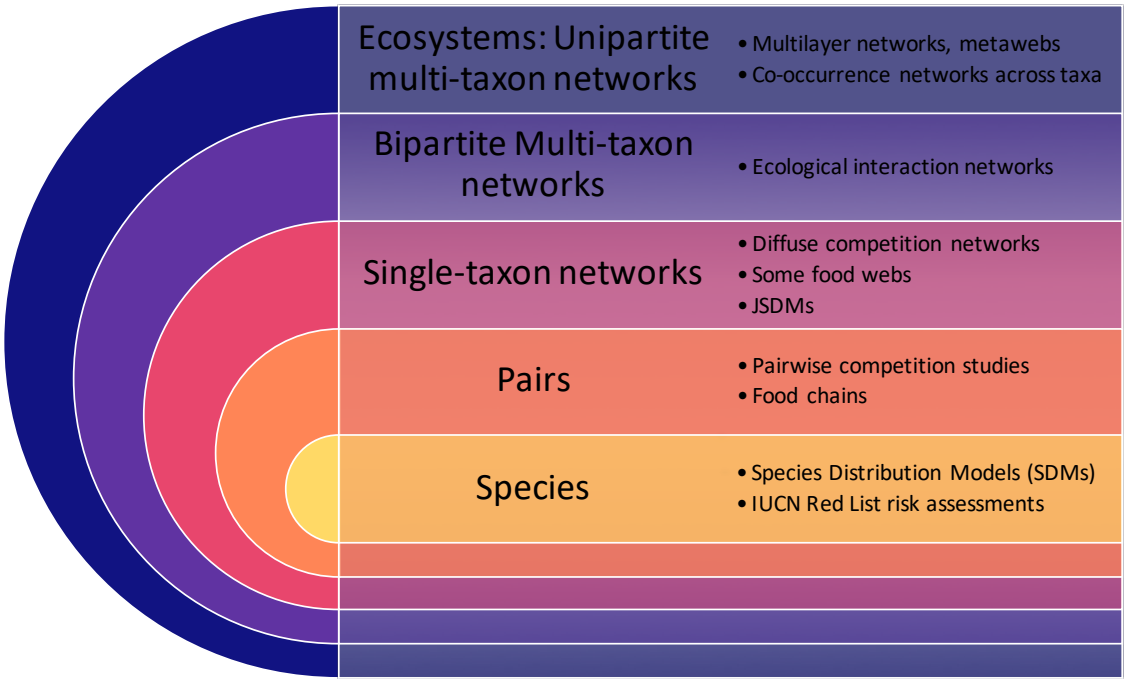

Figure 6.3. The hierarchy of interactions that make up the biosphere. Individual species and their pairwise interactions are basic units of community assembly. To understand ecosystems, however, we must focus future attention on multi-taxon networks, and consider relationships within and across taxa, including both biotic and abiotic factors. Co-occurrence analysis can be used across all levels of this hierarchy, as well as over long temporal scales using fossil occurrences.

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APPENDIX

DATA AND CODE AVAILABILITY

Chapter 2

- Code relating to Chapter 2 can be found at https://github.com/anikobtoth/FCW.
- Empirical East African Mammals data for Kenya (Tóth et al. 2014) is available from http://www.esapubs.org/archive/ecol/E095/150/metadata.php.
- Full dataset including Tanzanian data is available on https://github.com/anikobtoth/FCW. All other empirical data can be downloaded from the download page of the Ecological Register (Alroy 2015) at http://ecoregister.org using the following search parameters.

Chapter 3

- Code and simulated data relating to Chapter 3 can be found at https://github.com/anikobtoth/FCW.
- Empirical data can be downloaded from the download page of the Ecological Register (Alroy 2015) at http://ecoregister.org using the following search parameters.

Table 1. Empirical datasets from the Ecological Register (http://ecoregister.org). The table details the search criteria entered into the download form. Limits = the latitudinal and longitudinal range in degrees.

Dataset	Limits (degrees)				Included sampling methods	Minimum indiv. per sample
	North	South	East	West		
North American Bats	-	15	-60	-	-	50
South Brazilian Bats	-15	-33	-38	-70	-	50
South Brazil Rodents	-15	-33	-38	-70	-	50
North American Scarabs	-	15	-60	-110	-	50
E. North American Trees	50	25	-60	-100	-	50
New World Mammals[†]	-	-	-30	-	camera	-
Indochinese Mammals[†]	23	-10	130	90	camera	30

[†] Does not include bats. Ecological Register taxon search criteria include carnivores, primates, rodents, ungulates, other large mammals, and other small mammals.

Chapter 4

- Code and curated data relating to Chapter 3 can be found at https://github.com/anikobtoth/Megafauna.
- The raw fossil data were originally downloaded from FAUNMAP II (Graham and Lundelius 2010), and can be accessed from https://ucmp.berkeley.edu/faunmap/use/datadownload.html

- CCSM3 climate simulations (Veloz et al. 2012, Blois et al. 2013) are available from https://nelson.wisc.edu/ccr/resources/paleoclimate.php
- Body mass data (Smith et al. 2003) can be downloaded from http://www.esapubs.org/archive/ecol/E084/094/

Chapter 5

- Code and curated data relating to Chapter 4 can be found at
- https://github.com/anikobtoth/HabitatAlteration
- The raw empirical data can be downloaded from the download page of the Ecological Register (Alroy 2015) at http://ecoregister.org using the following search parameters.

Table 2. Empirical datasets from the Ecological Register (http://ecoregister.org). The table details the search criteria entered into the download form. Limits = the latitudinal and longitudinal range in degrees.

Dataset	Limits (degrees)				Included sampling methods	Repeat samples
	North	South	East	West		
Bats	23.5	-	-30	-	mist	Lump in small cells
Birds	23.5	-	-30	-	mist	Lump in small cells

CONFERENCE PRESENTATIONS AND INVITED TALKS DURING CANDIDATURE

2019 **Tóth AB**, Lyons SK, ETE working group, and Alroy J. Reassembly of surviving mammalian communities after the end-Pleistocene mass extinction. 9th Biennial conference of International Biogeography Society, Málaga, Spain. Jan. 8- 12. (Oral presentation)

2019 Pineda-Muñoz S*, **Tóth AB**, Lyons SK, Wang Y, McGuire J. Changes in North American mammal niche preferences from the late Pleistocene to the present. 9th Biennial conference of International Biogeography Society, Málaga, Spain. Jan. 8-12.

2018 **Tóth AB*,** Lyons SK, and Alroy J. Evaluating community assembly over space and time using co-occurrence network analysis. University of Nebraska, Lincoln. Oct. 26. (Invited talk)

2018 **Tóth AB*,** Lyons SK, ETE working group, and Alroy J. End-Pleistocene megafaunal extinction caused a fundamental shift in mammal species interactions. Annual meeting of the Society of Vertebrate Paleontologists, Albuquerque, NM. Oct. 17-20. (Invited talk).

2018 Pineda-Muñoz S*, Lyons SK, **Tóth AB**, and Behrensmeyer AK. Lessons in designing databases for examining paleocommunities through geological time. 78th Annual meeting of the Society of Vertebrate Paleontologists, Albuquerque, NM. Oct. 17-20.

2018 Fraser DL*, Villaseñor A, Balk M, Eronen JT, **Tóth AB**, ETE Working Group, Behrensmeyer AK, and Lyons SK**.** Profound prehistoric biotic homogenization of North America. 78th Annual meeting of the Society of Vertebrate Paleontologists, Albuquerque, NM. Oct. 17-20.

2017 Pineda-Muñoz S*, Jukar AM, **ETE working group** and Lyons SK. Human impact on North American mammal faunas from the Pleistocene. 102nd Annual meeting of the Ecological Society of America, Portland, OR, August 2017

2016 Pineda-Muñoz S*, Jukar AM, **ETE working group** and Lyons SK. Human impact on North American Mammal faunas. 76th Annual Meeting of the Society of Vertebrate Paleontology, Salt Lake City, Utah.

2016 **Tóth AB*.,** Soul L, Eronen JT, Lyons SK, Behrensmeyer AK, and Pineda- Muñoz S. Stability of empirical mammal co-occurrence networks over paleontological timescales. 76th Annual Meeting of the Society of Vertebrate Paleontology, Salt Lake City, Utah.

* indicates speaker

ADDITIONAL PROJECTS I HAVE BEEN INVOLVED IN DURING CANDIDATURE

Lyons SK, Miller JH, Amatangelo KL, Behrensmeyer AK, Bercovici A, Blois JL, Davis M, DiMichele WA, Du A, Eronen JT, Faith JT, Graves GR, Jud N, Labandeira CC, Looy CV, McGill B, Patterson D, Pineda-Muñoz S, Potts R, Riddle B, Terry RC, **Tóth AB,** Ulrich W, Villaseñor A, Wing SL, Anderson H, Anderson J, and Gotelli NJ. 2016. Reply to How foreign is the past. *Nature* **538**, doi:10.1038/nature20097.

Lyons SK, Miller JH, **Tóth AB,** Amatangelo KL, Behrensmeyer AK, Bercovici A, Blois JL, Davis M, DiMichele WA, Du A, Eronen JT, Faith JT, Graves GR, Jud N, Labandeira CC, Looy CV, McGill B, Patterson D, Pineda-Muñoz S, Potts R, Riddle B, Terry RC, Ulrich W, Villaseñor A, Wing SL, Anderson H, Anderson J, and Gotelli NJ. 2016. Reply to Questioning Holocene community shifts. *Nature* **537,** doi:10.1038/nature19110

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