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TESE DE DOUTORADO

GRASIELA CASAS

PADRÕES DE DIVERSIDADE DE AVES E REDE DE INTERAÇÃO MUTUALÍSTICA AVE-PLANTA EM MOSAICO FLORESTA-CAMPO

PORTO ALEGRE, AGOSTO DE 2015

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TESE DE DOUTORADO APRESENTADA AO PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA, DO INSTITUTO DE BIOCIÊNCIAS DA UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL, COMO PARTE DOS REQUISITOS PARA OBTENÇÃO DO TÍTULO DE DOUTOR EM ECOLOGIA.

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"Um dia, a menina observando andorinhas a bailar, com os pés no chão e uma pena na mão, queria voar. Então, ela tomou uma decisão: nunca parar de estudar. Hoje, com sonhos nas mãos e entre livros, artigos, amigos e estórias, não se cansa de migrar.

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Grasiela Casas

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RESUMO GERAL

Estudos clássicos com diversidade taxonômica, apesar de serem essenciais, não consideram as diferenças funcionais entre as espécies de uma comunidade. A abordagem considerando atributos funcionais e diversidade funcional vem preenchendo esta lacuna. A compreensão da estrutura e dinâmica de interações mutualísticas também é um elemento essencial em estudos de biodiversidade, permitindo a investigação de mecanismos ecológicos e evolutivos. Porém, a maioria dos estudos com redes de interação disponíveis na bibliografia são pequenas em número de espécies e interações, e é possível que estes dados não tenham sido suficientemente amostrados. Além disto, estudos têm mostrado que muitas métricas utilizadas em análises de rede de interação são sensíveis ao esforço amostral e ao tamanho da rede. Os objetivos desta tese foram: 1) investigar a diversidade taxonômica (DT) e funcional (DF) de aves e os padrões de organização de espécies de aves em comunidades refletindo convergência de atributos (TCAP: *Trait Convergence Assembly Patterns*) ao longo de transições entre floresta e campo; 2) analisar a estrutura de redes de dispersão de sementes de plantas por aves, utilizando as métricas de rede aninhamento, modularidade, conectância e distribuição do grau; 3) desenvolver um método estatístico visando avaliar suficiência amostral para métricas de redes de interação usando o método *bootstrap* de reamostragem com reposição. A composição de espécies de aves diferiu entre os ambientes, indicando uma substituição de espécies ao longo da transição floresta-borda-campo. DT diferiu significativamente somente entre floresta e borda de floresta, enquanto que ambas diferiram significativamente do campo em relação à DF. DT e DF podem indicar diferentes processos de organização de comunidades ao longo de mosaicos floresta-campo. A correlação significativa entre TCAP e o gradiente floresta-campo indica que provavelmente mecanismos de nicho atuam na organização da comunidade de aves, associados a mudanças na estrutura do habitat ao longo da transição floresta-borda-campo agindo como filtros ecológicos. Redes de dispersão de sementes de plantas por aves aparentemente apresentam um processo comum de organização, independentemente das diferenças na intensidade de amostragem e continentes onde as 19 redes utilizadas foram amostradas. Usando reamostragem *bootstrap*, encontramos que suficiência amostral pode ser alcançada com diferentes tamanhos amostrais (número de eventos de interação) para o mesmo conjunto de dados, dependendo da métrica de rede utilizada.

Palavras-chaves: atributo funcional, dispersão de sementes, ecótono, reamostragem *bootstrap.*

ABSTRACT

Classic studies on taxonomic diversity, though essential, do not consider the functional differences between species in a community. Studies using functional traits and functional diversity are filling this gap. Understanding the structure and dynamics of mutualistic interactions is also essential for biodiversity studies and allows the investigation of ecological and evolutionary mechanisms. However, most networks published are small in the number of species and interactions, and they are likely to be under-sampled. In addition, studies have demonstrated that many network metrics are sensitive to both sampling effort and network size. The aims of this thesis were: 1) to investigate bird taxonomic diversity (TD), functional diversity (FD), and patterns of trait convergence (TCAP: Trait Convergence Assembly Patterns) across forest-grassland transitions; 2) to analyse the structure of seed-dispersal networks between plants and birds using the metrics of nestedness, modularity, connectance and degree distribution; 3) to develop a statistical framework to assess sampling sufficiency for some of the most widely used metrics in network ecology, based on methods of bootstrap resampling. Bird species composition indicated species turnover between forest, forest edge and grassland. Regarding TD, only forest and edges differed. FD was significantly different between grassland and forest, and between grassland and edges. TD and FD responded differently to environmental change from forest to grassland, since they may capture different processes of community assembly along such transitions. Trait-convergence assembly patterns indicated niche mechanisms underlying assembly of bird communities, linked to changes in habitat structure across forest-edge-grassland transitions acting as ecological filters. Seed dispersal mutualistic networks apparently show a common assembly process regardless differences in sampling methodology or continents where the 19 networks were sampled. Using bootstrap resampling we found that sampling sufficiency can be reached at different sample sizes (number of interaction events) for the same dataset, depending on the metric of interest.

Keywords: bootstrap resampling, ecotone, functional traits, seed dispersal.

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INTRODUÇÃO GERAL

Por que espécies são abundantes como são? Por que elas ocorrem neste e não naquele lugar? Como as comunidades se organizam no tempo e no espaço? Quais os processos ecológicos que governam a estrutura de comunidades? Tais questionamentos e a busca por processos ecológicos através dos padrões é o que mais instiga pesquisadores em ecologia de comunidades, e consequentemente, é o principal foco da presente tese.

Uma das abordagens teóricas acerca da compreensão destes padrões inclui padrões de organização de comunidades ou regras de montagem (*assembly rules*). Neste contexto, o principal objetivo é predizer qual subconjunto do *pool* total de espécies de uma dada região ocorrerá em um habitat específico (Diaz et al. 1999). Diamond (1975) descreveu como as interações bióticas influenciam a limitação da composição de espécies em escala local, tanto por exclusão competitiva (Gause 1934) quanto por limitação de similaridade (MacArthur e Levins 1967). De acordo com a limitação de similaridade, espécies com uso de recurso e atributos funcionais semelhantes competirão, e para que coexistam, é preciso haver dissimilaridade entre estas ou complementaridade.

Condições ambientais agem como filtros, permitindo que somente espécies com determinadas características ecológicas e fisiológicas se estabeleçam nestes locais (Zobel 1997). Cada indivíduo, de acordo com a teoria do nicho (Grinnell 1917, Gause 1934), se estabelecerá somente em habitats onde as condições ambientais locais forem propícias à sua sobrevivência e reprodução. Por outro lado, segundo a teoria neutra (Hubbell 2001), as comunidades ecológicas são estruturadas por deriva (estocasticidade demográfica), com todos os indivíduos de uma comunidade possuindo igual probabilidade de reproduzir, morrer ou migrar (Hubbell 2005). No entanto, uma teoria não exclui a outra, e ambas podem agir na estruturação de comunidades (Gravel et al. 2006).

A partir da perspectiva de nicho, a organização das comunidades envolve convergência e divergência de atributos das espécies (Pillar et al. 2009). O padrão de organização a partir da convergência de atributos (TCAP: *Trait Convergence Assembly Patterns*) está relacionado à capacidade das espécies em transpor os filtros ambientais, e consequentemente, as espécies que coocorrem em uma dada comunidade tendem a apresentar similaridade na expressão de determinados atributos (Keddy 1992, Weiher et al. 1998, Pillar e Duarte 2010). Por outro lado, a limitação de similaridade leva a padrões de organização de divergência de atributos entre espécies (TDAP: *Trait Divergence Assembly Patterns*) (Macarthur e Levins 1967, Pillar e Duarte 2010). Pillar et al. (2009) apresentam um método para discriminar TCAP e TDAP nas comunidades em relação a gradientes ecológicos, baseado em correlações e correlações parciais de matrizes descritas por espécies, atributos e variáveis ambientais. TCAP pode ser identificado quando áreas vizinhas ao longo de um gradiente ecológico apresentam espécies com similaridade nos atributos, e mudanças nos mesmos podem estar relacionadas a este gradiente (Pillar et al. 2009).

Avaliações de padrões de organização de comunidades podem ser feitas com base na composição de espécies (Diamond 1975) ou pelos atributos funcionais das espécies (Pillar et al. 2009). Atributos funcionais de aves vêm sendo utilizados para acessar a resposta funcional a diferentes tipos de mudanças ecossistêmicas (Vandewalle et al. 2010), e para realizar predições sobre mudanças na diversidade biológica e funcional em resposta às modificações do habitat (Hausner et al. 2003). Assim, a abordagem de atributos funcionais e diversidade funcional vêm crescendo nos últimos anos com objetivo de responder a questões ecológicas (Mason e de Bello 2013). A diversidade funcional é um parâmetro que leva em consideração as diferenças funcionais entre as espécies de uma comunidade, ou seja, considera a variação dos atributos funcionais (Tilman et al. 2007).

Apesar de estudos clássicos com diversidade taxonômica e riqueza de espécies serem essenciais, eles podem ser insuficientes em capturar as interações ocorrendo no ecossistema, porque geralmente assumem que todas as espécies são igualmente distintas em relação a suas influências sobre relações ecológicas (Mouchet et al. 2010). Recentes estudos sugerem que atributos funcionais são mais eficazes em predizer os efeitos de mudanças globais sobre serviços ecossistêmicos quando comparado com a diversidade de espécies (Cadotte et al. 2011), e que ambos podem capturar diferentes processos de organização de comunidades ao longo de gradientes ecológicos (Bernard-Verdier et al. 2013, Janeček et al. 2013).

Este estudo foi realizado em um gradiente ecológico de mosaicos floresta-campo no sul do Brasil, facilitando a investigação de padrões de comunidades de aves e a importância das restrições ambientais impostas por filtros na transição floresta-campo (na presente tese foi utilizada a categorização dos tipos de habitats como um gradiente de estrutura do habitat). O Sul do Brasil está situado em uma zona de transição entre vegetação tropical ao norte e vegetação de clima temperado ao sul (Overbeck et al. 2007). No Rio Grande do Sul, os ecossistemas campestres formam zonas de transição (ecótonos) com Florestas Ombrófilas Mistas (floresta com Araucária) e Estacionais. Os campos sulinos caracterizavam o sul do Brasil bem antes da expansão das formações florestais, e estudos palinológicos demonstraram que a formação de mosaicos campo-floresta foi fortemente determinada por alterações climáticas do Quaternário (Behling and Pillar 2007).

O primeiro capítulo da tese, intitulado **Diversidade de aves e padrões de organização de comunidades em mosaicos floresta-campo** (*Bird diversity and community assembly patterns in forest-grassland mosaics*), tem como objetivo investigar a diversidade taxonômica (DT) e funcional (DF) ao longo da floresta, borda e campo, e se DT e DF responderão de forma similar à transição destes ambientes. Também foram selecionados um conjunto de atributos de aves que maximizem a expressão de padrões de organização de espécies de aves em comunidades refletindo convergência de atributos (TCAP: *Trait Convergence Assembly Patterns*) relacionado a mudanças no habitat da floresta para o campo.

O estudo de redes de interações ecológicas é uma importante ferramenta em ecologia de comunidades, pois auxilia na compreensão da resistência e dinâmica de comunidades, e manutenção da biodiversidade (Bascompte et al. 2006, Montoya et al. 2006). O estudo de redes de interação pode ser considerado então parte do estudo de biodiversidade, cujo foco principal é analisar a estrutura e robustez da rede, permitindo a procura por mecanismos ecológicos e evolutivos.

Redes de interação ecológica são representadas pela interação (links) entre espécies (nós). As interações entre organismos podem ser antagônicas, na qual os organismos de espécies diferentes se prejudicam (na predação, por exemplo), ou mutualísticas, em que ambas saem ganhando, aumentando a chance de sobrevivência e reprodução (polinização e dispersão de sementes, por exemplo). Como a grande maioria das plantas dos trópicos precisa da ajuda de animais para a polinização e a dispersão de sementes (Howe e Smallwood 1982), esses mutualismos são essenciais. Pode-se dizer que as interações mutualísticas geram serviços ambientais. A ideia de inserir um capítulo na tese com dispersão de sementes surgiu devido à área de estudo englobar mosaicos de floresta-campo, onde principalmente aves dispersoras são importantes no processo de expansão florestal. Estas aves contribuem na fase inicial de nucleação de árvores, pois transportam diásporos da floresta para o campo (Duarte et al. 2006). A primeira pergunta para o surgimento do capítulo dois foi: ‘quem dispersa o quê’ e ‘quais as espécies de aves e plantas são mais importantes na dispersão?’. Para responder a estas questões, duas redes de interação ave-planta foram coletadas em duas regiões diferentes no estado do Rio Grande do Sul.

Como a busca por processos ecológicos através dos padrões é o que mais instiga pesquisadores em ecologia, outras perguntas então surgiram relacionadas a este capítulo: qual é a estrutura da rede de interação coletada nestas áreas de mosaicos floresta-campo? O padrão encontrado nas redes coletadas é o mesmo comparando outras redes de interação ave-planta coletadas em outros continentes? O que se encontra na literatura é que processos coevolutivos entre redes mutualísticas e antagônicas podem divergir, e consequentemente, a estrutura destes tipos de redes ecológicas serão também diferentes (Lewinsohn et al. 2006). No entanto, para redes mutualísticas, principalmente comparando interação planta-polinizador e dispersor, espera-se que tais redes apresentem uma estrutura em comum. Por exemplo, em dois estudos clássicos comparando redes de polinização e dispersão, Bascompte et al. (2003) e Jordano et al. (2003), encontraram que redes mutualísticas são geralmente aninhadas, isto é, espécies especialistas (com poucas interações) interagem com um subconjunto de espécies que também interagem com as espécies generalistas (espécies com muitas interações). Redes mutualísticas são geralmente caracterizadas por poucas espécies supergeneralistas, sendo que a maioria das espécies apresentam poucas interações.

Algumas redes mutualísticas também são modulares, ou seja, um subgrupo de espécies (módulos) interagem mais entre si do que com espécies de outros subgrupos.

Em redes de interação planta-polinizador, a modularidade aumenta a estabilidade da rede, pois distúrbios em cascatas parecem se dissipar lentamente em uma rede modular do que em redes não modulares (Olesen et al. 2007). Estudos prévios também encontraram modularidade em redes de dispersão de sementes ave-planta (Mello et al. 2011, Vidal et al. 2014), apesar deste padrão ter sido pouco investigado em redes de dispersão de sementes. Inclusive, em comparação com números de estudos realizados com redes mutualísticas, polinização é bem mais estudada comparado a redes de interação planta-dispersor (Miranda et al. 2013).

O segundo capítulo da tese, intitulado **Estrutura de redes de dispersão de sementes de plantas por aves** (*Structure of seed-dispersal networks between birds and plants*), teve como objetivo analisar a estrutura de redes de dispersão de sementes ave- planta, utilizando as métricas de rede: aninhamento, modularidade, conectância (proporção de links observados na rede de interação relativo aos links possíveis) e distribuição do grau (probabilidade de encontrar uma espécie com um determinado número de interações). Além das duas redes coletadas em mosaicos floresta-campo no estado do Rio Grande do Sul, outras 17 redes foram analisadas para acessar padrões de redes mutualísticas entre ave e planta, incluindo uma rede de outro pesquisador também coletada no estado. O índice de importância foi utilizado para verificar quais foram as aves e as plantas mais importantes das redes, mas somente com aquelas coletadas nesta tese.

As redes coletadas foram muito pequenas, totalizando 43 espécies de aves e plantas nos municípios de Santana da Boa Vista e Herval, e apenas 16 espécies em Jaquirana e Cambará do Sul. Para a menor rede, o aninhamento não foi significativo e também não foi possível estimar a distribuição do grau. Devido aos resultados desta segunda rede e ao seu tamanho, iniciou-se uma discussão sobre suficiência amostral em redes de interação. Será que esta rede não é aninhada ou o não aninhamento foi devido ao tamanho amostral? Além disto, uma questão muito abordada pelo grupo do laboratório “Ecologia Quantitativa” é: suficiente para quê? A menor rede coletada pode não ter sido suficiente para a métrica “aninhamento”, mas foi suficiente para “modularidade”?

Amostrar uma considerável parte da diversidade de interações é um esforço intenso, e é provável que a maioria dos dados que se tem na literatura não tenham sido suficientemente amostrados. Chacoff et al. (2012) realizaram uma intensa amostragem, mas detectaram menos de 60% do potencial de interações. Em relação a redes de dispersão de sementes, a maioria das redes publicadas são pequenas e, provavelmente, insuficientemente amostradas. Além do tamanho pequeno da maioria das redes de interação ave-planta, muitas métricas de rede são sensíveis ao esforço amostral e ao tamanho da rede (Dormann et al. 2009). Olesen et al. (2007) encontrou uma relação entre o tamanho de redes planta-polinizador com aninhamento e modularidade. A conectância apresentou uma correlação negativa com o tamanho da rede (Mello et al. 2011). Bascompte et al. (2003) encontraram que, para redes planta-frugívoro e planta-polinizador, acima de 50 espécies, todas as redes de interação foram significativamente aninhadas. Consequentemente, estudos que apresentam um baixo esforço amostral precisam ser interpretados com cautela (Rivera-Hutinel et al. 2012).

A teoria estatística objetiva responder três perguntas: a) como os dados devem ser coletados; b) como devem ser analisados; e c) quão preciso são os dados. A terceira questão faz parte do processo conhecido como inferência estatística (Efron e Tibshirani 1993), e foi um dos objetivos do terceiro capítulo da tese, utilizando o método de reamostragem com reposição *bootstrap* (Efron 1979, Efron e Tibshirani

1993, Pillar 1998). O método *bootstrap* parte do princípio que a distribuição dos valores observados em uma amostra é o melhor indicativo da distribuição no universo amostral em que a amostra foi coletada. A reamostragem no método ocorre com reposição, imitando a reamostragem do universo amostral.

Tendo em vista a influência do tamanho amostral nas métricas de rede e que a maioria das redes de interação ave-planta são pequenas, no terceiro capítulo, intitulado **Avaliação de suficiência amostral em métricas de redes de interação utilizando *bootstrap*** (*Assessing sampling sufficiency of network metrics using bootstrap*), o objetivo foi desenvolver um método estatístico visando avaliar suficiência amostral para algumas das mais utilizadas métricas de redes de interação, com o método de reamostragem com reposição *bootstrap*. Foram utilizadas três redes quantitativas de interação ave-planta (que inclui a frequência da interação) como exemplo, e as métricas conectância, aninhamento e modularidade.

Área de estudo

Esta tese fez parte do projeto SISBIOTA (Biodiversidade dos campos e dos ecótonos campo-floresta no Sul do Brasil: bases ecológicas para sua conservação e uso sustentável). A tese se enquadrou em um dos objetivos da rede de pesquisa do projeto: a identificação de padrões taxonômicos, funcionais e filogenéticos de organização de espécies da flora e da fauna em comunidades biológicas características dos campos sulinos e ecossistemas florestais associados. As áreas de ecótono (mosaicos floresta-campo) pertencentes ao projeto foram localizadas nos seguintes municípios: Cambará do Sul, Jaquirana, São Francisco de Paula (região fisiográfica Campos de Cima da Serra), Encruzilhada, Santana da Boa Vista, Herval (Serra do Sudeste), Santana do Livramento, Santo Antônio das Missões e São Francisco de

Assis (Campanha).

A definição das unidades amostrais foi estabelecida mediante um delineamento amostral comum para os diferentes grupos biológicos em que foram selecionadas Unidades Amostrais de Paisagem (UAPs) de tamanho 2x2 km e, dentro destas, Unidades Amostrais na Escala Local (UALs) com 70x70 m. Para tanto, adotou-se uma abordagem sistemática e padronizada de escolha das unidades amostrais, combinando estratificação e aleatorização, a partir do conhecimento espacializado sobre a distribuição atual e pretérita dos campos e ecótonos no Rio Grande do Sul.

Dentro de cada UAP de ecótono foram estabelecidas cinco UALs (Figura 1). Três UALs foram estabelecidas sobre área de campo (70x70 m), preferencialmente sem evidência de colonização por indivíduos lenhosos florestais. As outras duas UALs foram alocadas em áreas de borda floresta-campo, que apresentavam evidências de expansão da floresta sobre o campo (Figura 2). Cada UAL de borda floresta-campo foi composta por duas parcelas contíguas de 70x70 m cada, sendo uma parcela orientada para o interior da área predominantemente campestre e a outra parcela orientada para o interior da área florestal (Figura 1).

A amostragem da avifauna para o primeiro capítulo foi realizada em todas as nove áreas na escala de paisagem (UAP), mas quando possível, no interior ou próximo das UALs do projeto. Os pontos de escuta para amostragem da avifauna sempre foram realizados com no mínimo 100 m de distância da borda da floresta, e consequentemente, fora das UALs florestais. Também foi criada para a tese mais uma UAL em área de borda floresta-campo. A captura da avifauna para coleta de sementes no segundo capítulo foi realizada em quatro das nove áreas de ecótono: Jaquirana, Cambará do Sul (Campos de Cima da Serra), Santana da Boa Vista e Herval (Serra do Sudeste). As 16 redes de neblina foram alocadas no interior da floresta (fora das

UALs florestais) e na borda da floresta. Foram utilizadas oito redes de neblina em cada ambiente.

Figura 1. Exemplo de demarcação das unidades amostrais em áreas de ecótonos pertencente ao projeto SISBIOTA. Ao fundo, imagem de satélite do aplicativo Google Earth. As linhas brancas delimitam a UAP (Unidades Amostral de Paisagem) e as UALs.

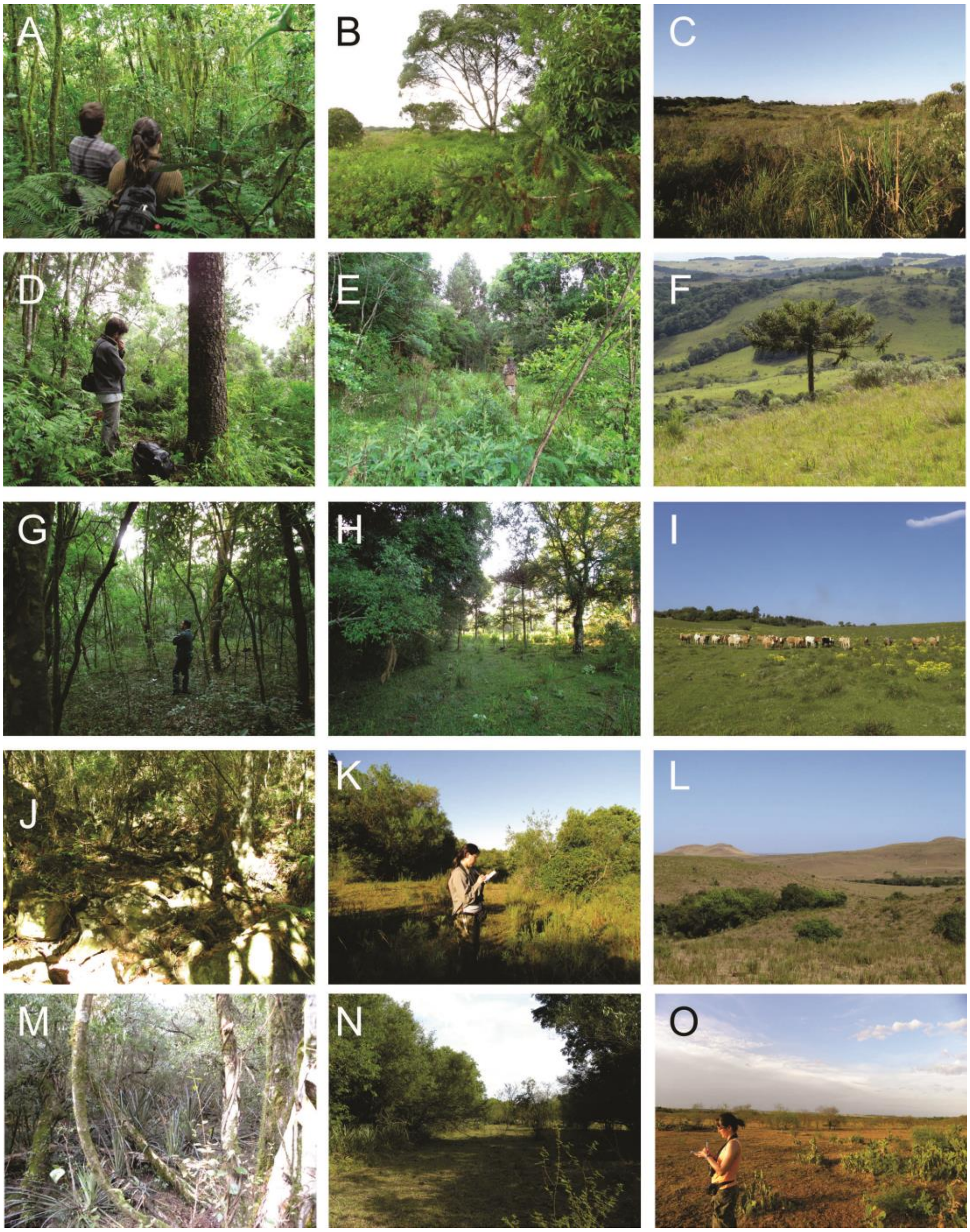

Figura 2. Fisionomia da vegetação nas UALs borda floresta-campo e campo do projeto SISBIOTA no Rio Grande do Sul. A região fisiográfica Campos de Cima da Serra está representada pelas figuras A a F; Serra do Sudeste de G a I; e Campanha de J a O.

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CHAPTER 1

BIRD DIVERSITY AND COMMUNITY ASSEMBLY PATTERNS IN FOREST-GRASSLAND MOSAICS

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Bird diversity and community assembly patterns in forest-grassland mosaics

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Abstract: Classic studies on taxonomic diversity, although they are essential, do not consider the functional differences between species in a community. Studies using functional traits and functional diversity are filling this gap. Our aim was to investigate how bird taxonomic diversity (TD) and functional diversity (FD) vary across forest-grassland ecotones. We selected sets of traits (through an iterative algorithm) that maximize the expression of patterns of trait convergence related to environments variable of habitat types. For the quantitative survey of the avifauna, we used the point count method in nine areas located in Southern Brazil. We used morphological, dietary, foraging substrate and behavioural traits. Bird composition was different between forest, forest edge and grassland. Regarding TD, only forest and edges differed. FD was significantly different between grassland and forest, and between grassland and edges. The similar FD between forest and edges was influenced by the small differences in vegetation structure, which are much more evident in comparison with grasslands. Grassland encompassed the highest FD in comparison with forest and edges, as well as the highest number of exclusive species. Among all traits, seven maximized the correlation between functional community patterns and the habitat types: beak curvature, claw of digit three, mid rectrix, diet grains and invertebrate, gleaning foraging method and vegetation foraging substrate. The TD and FD responded differently to environmental change from forest to grassland, and our use of both taxonomic and a functional diversity approaches was useful to conclude that these two facets of diversity may capture different processes of community assembly along such transitions. Trait-convergence assembly patterns indicated niche mechanisms underlying assembly of bird communities, and differences in environmental variables across forest-edge-grassland habitats are acting as ecological filters.

Key words: avifauna, ecological filters, ecotone, functional traits, species composition.

Introduction

Many studies are emerging using functional traits and functional diversity to assess ecological questions (Mason and de Bello 2013). Although classic studies on taxonomic diversity are essential, they may be insufficient to capture the interactions occurring in ecosystems, because they usually assume that species are equally distinct regarding their relative influences and responses to ecological relationships (Mouchet et al. 2010). Functional diversity can be defined as the value and range of the functional differences (i.e. trait differences) among species in a community (Tilman et al. 2007). Recent syntheses and empirical studies have highlighted that functional traits predict the effects of global changes on ecosystem services better than species diversity does (Cadotte et al. 2011). However, some studies showed that the different facets of diversity are not necessarily equivalent and may capture different processes of community assembly along gradients (Bernard-Verdier et al. 2013, Janeček et al. 2013). Bird traits have been used to assess the functional response to different kinds of ecosystem change (Vandewalle et al. 2010), and as basis for making predictions about changes in biological and functional diversity in response to land use changes (Hausner et al. 2003).

Functional traits can be grouped into two broad categories (not mutually exclusive): 1) traits that influence a species response to the environment and/or 2) traits that exert effects on ecosystem processes (Lavorel and Garnier 2002). Birds exhibit a diverse range of ecological functions, mainly related to what they eat and how/where they look for food (Sekercioglu 2006). Characteristics such as foraging behaviour or diet are crucial to understanding how an animal may respond to environmental changes and how it impacts ecosystem function (Luck et al. 2013). For example, in frugivorous birds, the capacity to move between spatially discrete habitat patches can determine, on one hand, a species response to declining landscape connectivity and, on the other, its contribution to forest maintenance through seed dispersal (Luck et al. 2012). Morphological traits can be related to ecological traits (Fitzpatrick 1980), and an indicative of species functions in communities. Most morphological traits are considered as effect traits (Luck et al. 2012). For example, in frugivorous birds bill morphology influences the kinds of seeds a species can eat. On the other hand, some morphological traits can be considered both effect and response traits (e.g. body mass, as an effect trait, will impact on the amount and type of food consumed; as a response traits, large-bodied species are more vulnerable to habitat loss in forests). The classification in responses or effect traits will vary according to the selected environmental change or ecosystem service selected. For instance, a trait may be considered a response trait when it changes across an environmental gradient such as from forest to grassland in ecotones. Previous studies evaluated plants (Müller et al. 2007, Carlucci et al. 2012, Brownstein et al. 2013) and mammals (Luza et al. 2015) with a functional approach in ecotones but, to our knowledge, studies using functional diversity of birds in ecotones are scarce.

Niche theory is based on the responses of organisms to environmental conditions and biotic interactions (Weiher and Keddy 1999). In relation to environmental conditions, community assembly involves environmental filters, which lead to a pattern of trait convergence: species colonizing a site with a particular set of environmental conditions will tend to exhibit similarity for certain traits (Keddy 1992, Weiher et al. 1998). Trait convergence may be identified when neighboring sites along an ecological gradient consistently contain species with similar traits and changes in these traits are related to the gradient (Pillar et al. 2009).

Our study was conducted in areas characterized by a natural ecological gradient (mosaics of grassland and different forest formations), enabling us to assess patterns reflecting community assembly processes and the importance of environmental restrictions imposed by ecological filters. The Southern part of Brazil is situated at a transitional zone between tropical vegetation types to the north, and temperate vegetation to the South (Overbeck et al. 2007). Paleopalynological studies have shown that the formation of grassland-forest mosaic in Southern Brazil was strongly influenced by climatic changes during the Quaternary (Behling and Pillar 2007).

Here we investigate in forest-grassland mosaics in Southern Brazil how bird taxonomic diversity and functional diversity vary across the transition between forest and grassland and whether or not functional diversity and taxonomic diversity respond similarly to habitat transition from forest to grassland. We searched for sets of traits that maximized the expression of patterns of trait convergence related to environment changes from forest to grassland. We focused on morphological, dietary, foraging behavioural and foraging substrate traits of birds. We hypothesized that 1) forest and forest edge directly in contact with grassland (henceforth *edge*) have similar species composition and taxonomic diversity due to similarity in vegetation structure compared to grassland; 2) functional diversity is higher in grassland than forest and edges, because of natural environmental heterogeneity; 3) environment changes from forest to grassland act as habitat filtering, leading to trait convergence.

Methods

Study area

We conducted the study in nine areas with forest-grassland mosaics in three physiographic regions in the state of Rio Grande do Sul: *Campos de Cima da Serra*

(CCS), *Campanha* (CA) and *Serra do Sudeste* (SS) (Figure 1). These regions are included in the Atlantic Forest (CCS) and the Pampa (CA, SS) biomes and both are characterized by mosaics of forest and grassland. In the Pampa biome, grasslands cover large continuous areas and forests are mostly restricted to riparian zones, whereas in the Atlantic Forest grasslands are located in the highlands of the South-Brazilian Plateau. The Atlantic Forest biome includes tropical rainforest (Atlantic forest *sensu stricto*), mixed ombrophilous forest (*Araucaria* forest), and seasonal forests (both deciduous and semideciduous) (Oliveira-Filho and Fontes 2000).

The South Brazilian grasslands (known as *Campos Sulinos*) form a natural ecosystem that has characterized this region long before the forest expansion that took place after mid Holocene (Behling and Pillar 2007, Dümig et al. 2008, Behling et al. 2009). Fire and grazing by domestic animals are considered to be the principal factors impeding expansion of forest over grassland vegetation in the past centuries and under current climatic conditions (Pillar and Quadros 1997). Domestic herbivores were present in our sampling areas, and had access to forest patches. In the CCS areas, cattle grazing is less intense, as sampling was conducted in two conservation units, the Tainhas State Park and the Aparados da Serra National Park. In one of the areas in CCS cattle has been excluded since 1994.

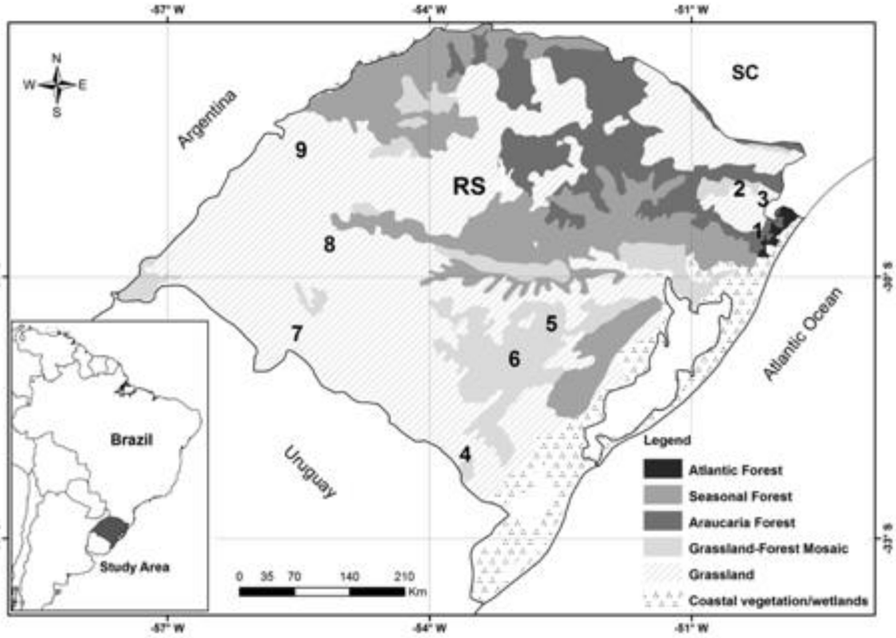

Figure 1. Bottom left: South America, Brazil and Rio Grande do Sul state; map of the dominant vegetation physiognomies of Southern Brazil (according to IBGE, 2004). Numbers are the study areas location in CCS region (1 to 3), SS region (4 to 6) and CA region (7 to 9), and represent the order of temporal sequence of bird sampling.

Bird sampling

For the quantitative survey of the avifauna, we used the point count method (Bibby et al. 1992). We performed point counts in forest interiors, forest edges and grasslands, with three point counts in each environment, nine per area (Appendix Figure 1). Forest point counts were located between 140 to 240 m from the edge. We performed the point counts located in grassland with minimum distance from edge point of 375 m. A total of 81 point counts were surveyed from December 2011 to January 2012, covering the breeding season that is a favourable time for bird sampling. All individuals seen and/or heard were counted, except those that only flew over the area and, consequently, not using the local habitat. Surveys started 15 min after dawn and lasted 3 h. Permanence in each point was 15 min. The order in which point counts were surveyed varied systematically to avoid bias related to time of day.

The fixed radius of point counts differed between environments due to influence of vegetation structure on the probability of bird detection (Emlen 1971, Rodgers 1981). Point counts in forest and at edges had 25 m fixed radius and grassland 100 m radius. Birds are less easily detected with increasing distance from observers, mainly in forests, because of concealment by vegetation and increased sound attenuation due to obstruction (Waide and Narins 1988). Therefore, fixed-radius circular plots of ≤50 m radius were appropriate for sampling in forest and edge environments. In grassland with low woody densities, on the other hand, the detection of most bird individuals occurs outside 25 m radius, probably due to disturbance created by the observer's presence. For the analyses, we first summed the number of recorded birds obtained in the three point counts in each environment per area, resulting in three sampling units in each area: forest, edge and grassland (Appendix Figure 1). After that, we used the relative number of detection counts of a species standardized by the total number of detected birds in a sampling unit, in order to control the effect of sampling detection differences. The resulting values for each species represented a relative frequency value, which provided information about how much that species is using the local resources.

One of our aims is to investigate how bird species traits vary across environmental habitats in transitional areas, from forest to grassland. Since our study area is inserted in a regional context of forest expansion over grasslands, we selected, based on the literature, traits related to habitat use that should be responsive to differences in habitat areas across different environments. Examples of the selected traits and the corresponding process involved are: a) morphological traits related to seed dispersion and pollination, such as bill morphology and b) wing length, which is related to capacity to use open spaces or to manoeuvre through tree canopies, which in turn influences resource use, seed dispersal and migratory status. We also used dietary and behavioural data based on foraging methods and foraging substrate. All these traits are related to resource acquisition and are expected to strongly influence biodiversity–ecosystem function relationships. For details on all sampled traits see Table 1.

We collected fourteen morphological traits from measurements made on specimens of bird collections of the PUCRS Science and Technology Museum (Museu de Ciências e Tecnologia da PUCRS, Porto Alegre) and the Zoobotanical Foundation of Rio Grande do Sul Museum (Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre) (Figure 2). Subsequently, we also included calculated traits: an index of beak curvature using the ratio between culmen arc and culmen, and an index of beak shape using the ratio between beak height and width (Table 1). For each bird species, we measured one to ten specimens, according to quantity and quality of specimens available at museums. For all analysis we considered the average of measured individuals. To identify redundant morphological measurements, we performed a Pearson correlation test between traits and excluded those that were highly correlated (correlation > 0.80). Six traits were highly correlated: culmen *vs.* arc of culmen (0.99); claw of digit III *vs.* arc of its claw (0.81); halux *vs.* claw of halux (0.85). Thus, culmen, claw of halux and claw arc of digit III were excluded. To exclude the influence of differences on bird body sizes on morphological traits, we divided each trait value by the cubic root of the body mass, in our case, species mean body mass. Although all morphological traits are dependent on body mass, the latter was included as a trait in our analyses due its relationship with various ecosystem functions (see Table 1).

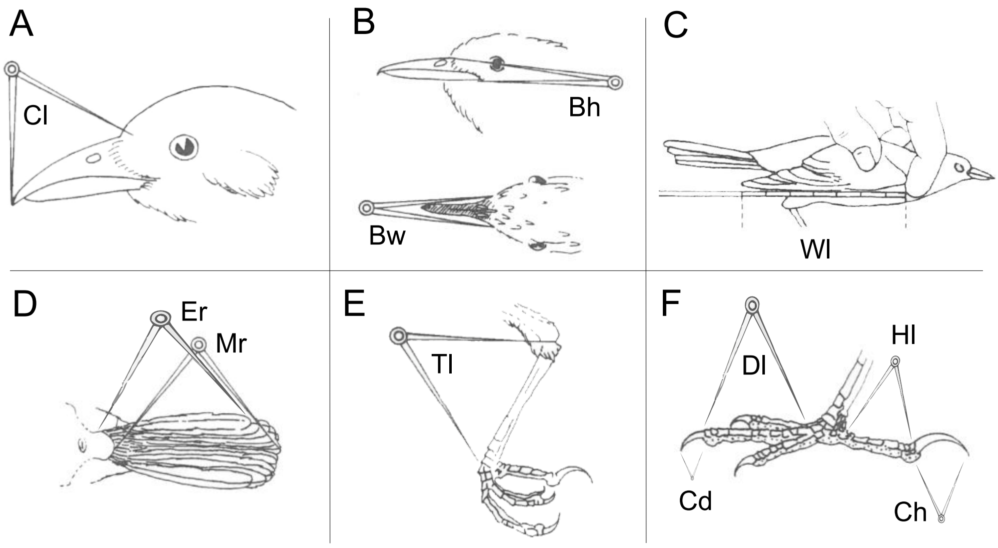

Figure 2. Measures of bird morphological traits. (A) culmen-Cl, (B) beak height-Bh and width-Bw, (C) wing length-Wl, (D) external-Er and mid rectrix-Mr of tail, (E) tarsus length-Tl, (F) digit III-Dl, claw of digit III-Cd, hallux-Hl and claw of hallux-Ch. Adapted from Sick (1997).

We searched the literature for information on dietary and behavioural traits based on foraging methods and foraging substrate (Hidasi-Neto et al. 2013, with some modifications) (Table 1). These categories followed Sick (1997), del Hoyo et al. (1992-2002), and Stotz (1996). Foraging and dietary categories were not mutually exclusive for a given species (e.g. species A can consume invertebrates, fruits and grains).

Table 1: List of morphological, dietary, foraging methods and foraging substrate traits collected from museum bird collections and literature. Table adapted from Luck et al. (2012).

Trait	Description	Functional meaning
Morphological (quantitative)		
Bill morphology		Influences pollination effectiveness, handling of fruit and seeds, and the type and location of food consumed

Trait	**Description**	**Functional meaning**
Exposed culmen (mm)	Bill tip from the point where the tips of the forehead feathers begin to hide the culmen	
Culmen arc (mm)	Length considering the curvature, measured with thread	
Ratio between culmen arc and culmen	Indicative of beak curvature	
Beak height		
Beak width		
Ratio between beak height and width	Indicative of beak shape	Relates to diet and food handling. One of the morphological traits that best predicted foraging behavior of Tyrant-flycatchers (Botero-Delgadillo and Bayly 2012).
Wing length (mm)		Flight capacity
Tarsus length (mm)		Can influence foraging behaviour and hence services such as pest regulation and nutrient cycling
Feet morphology (mm)		Can influence foraging behaviour and small-scale nutrient cycling (e.g. scraping the ground to turnover soil)
Digit III		
Claw of digit III		
Arc of claw of digit III	length considering the curvature, measured with thread	
Halux		
Claw of halux		Indicator of foraging substrate (Feduccia 1993)
Tail (mm)		Can influence foraging behaviour and foraging substrate (Botero-Delgadillo and Bayly 2012)
External rectrix		
Mid rectrix		

Trait	Description	Functional meaning
Mass (g)	the live bird weight	Strongly relates to a range of other traits in birds including metabolic rate, foraging behaviour and home-range size
Dietary traits (presence/absence)		
Vertebrates		
Invertebrates		
Plant vegetative parts	encompass birds that feed leaves, and/or flower, young shoots, roots, bulbs and buds	
Fruits		
Grains		
Nectar		
Behavioural traits based on foraging methods (presence/absence)		Impacts all aspects of resource use by birds. Species with particular foraging behaviour traits may be sensitive to particular environmental changes.
Pursuit	The term usually refers to a technique of sallying out from a perch to attack a food item	
Gleaning	To pick food items from a nearby substrate, including the ground, that can be reached without full extension of legs or neck (Remsen Jr and Robinson 1990)	
Pouncing	Bird drops to ground and takes prey	
Grazing		
Pecking	To drive the bill against the substrate to remove some of the exterior of the substrate (Remsen Jr and Robinson 1990)	
Scavenging	Scavenging is a carnivorous feeding behaviour in which the scavenger feeds on dead animal	

Trait	Description	Functional meaning
Probing	To insert the bill into cracks or holes to capture hidden food (Remsen Jr and Robinson 1990) (e.g. woodpeckers probe trees and hummingbirds probe flowers)	
Foraging substrate (presence/absence)		Dictates where birds will conduct their foraging activities
Water		
Mud		
Ground		
Vegetation		
Air		

Data analysis

In our analyses we searched for differences in bird taxonomic and functional diversity between forest-edge-grassland environments, considering species composition, their relative number of recorded species (relative species frequency), and trait values. Due to the larger sampling area on grassland compared to forest and edges, we used Chao 1 (Chao 1984, Colwell and Coddington 1994) to estimate species richness. This estimator uses the number of registered species in a sample, as well as singletons and doubletons, as in the equation below:

$$S_1 = S_{obs} + a^2/2b,$$

where S_{obs} is the number of species in the sample, a is the number of singletons (i.e., the number of species with only a single occurrence in the sample) and b is the number of doubletons (the number of species with two occurrences in the sample).

Then, we tested for differences in species richness between environmental types considering both observed (number of species registered) and estimated species richness (Chao 1) using ANOVA with permutation (Manly 2007). We restricted all permutations within regions, because our aim was not to test differences between regions, but to compare habitat types. Taxonomic diversity (TD) was estimated using Simpson index, and we used ANOVA with permutation to evaluate differences in TD between forest-edge-grassland habitats, also restricting permutations within regions. We also compared species taxonomic diversity patterns between environments using diversity profile based on Rényi entropy values (Rényi 1961). The different values obtained for Rényi's entropy series correspond to different diversity indexes, according to the value of the scale parameter α (Melo 2008). As the value of parameter α rises, more weight is given to dominant species over rare ones.

Differences in species composition among habitat types were tested through multivariate analysis of variance (MANOVA), using Euclidean distance and with randomization (1000 permutations) following the method described by Pillar and Orloci (1996). Species composition pattern across sampling units (habitat types in each site) was also examined through ordination by principal coordinate analysis (PCoA) (Podani 2000). We used Euclidean distance as the measure of similarity between sampling units.

We adopted the method described in Pillar et al. (2009), Pillar and Duarte (2010) for analysing functional patterns and their correlation to environmental gradient, and to select trait subsets maximizing such correlation. For this, we organized the data in three matrices: 1) the relative species frequency in the communities in matrix **W** of species by sampling units; 2) functional traits describing the bird species in matrix **B** of species by traits; and 3) the ecological gradient of interest, in our case the ordinal environmental variable of habitat types (3: forest, 2: edge, and 1: grassland) in matrix **E,** as a gradient of habitat structure. Community-weighted mean (CWM) traits were computed by matrix multiplication $\mathbf{T} = \mathbf{B}'\mathbf{W}$. Matrix **T** contains the mean of each trait in each community. Then, species pairwise similarities based on traits in **B** were used to define matrix **U** with degrees of belonging of species to fuzzy sets (Pillar and Orlóci 1991). In matrix **U** each species (given by the column in **U**) may simultaneously belong, in functionally terms, to more than one species fuzzy type (the rows of **U**) based on the species trait similarities, with certain degrees of belonging. In other words, a species *a* in matrix **U** could "belong" to species *b* with, say, a 0.5 degree of belonging, according to the functional similarities between *a* and *b*. Values in matrix **U** range from 0 to 1, and the sum of each column is standardized to 1. By matrix multiplication, **X** = **UW** will contain the species composition of the communities after the fuzzy weighting by their trait similarities. Matrix **X** retains more trait information at the community level than community-weighted means do (Pillar et al. 2013), because it keeps the identity of species (weighted by their trait similarities) and can express the variation (divergence) of its traits between communities too.

Our interest was to examine the distribution of traits across the forest-edge-grassland environments to possibly identify community assembly processes related to environmental filtering. By using matrix correlation (Mantel), we evaluate the correlation between community distances based on matrices **T** (trait means) and **E** (ecological gradient). A strong correlation ρ(**TE**) indicates the factors directly or indirectly represented in **E** are involved in ecological filtering of species that, at least for the traits considered in the analysis, consistently produce trait-convergence assembly patterns (TCAP) along the gradient (Pillar et al. 2009, Pillar and Duarte 2010). Optimal trait subsets were searched that maximized the correlation ρ(**TE**) using an algorithm analogous to Pillar and Sosinski Jr. (2003). The significance of the correlation ρ(**TE**) was tested against a null model. The selected trait subset was used in all following analyses. Matrix **X** was submitted to ordination by principal coordinates analysis (PCoA) using Euclidean distances between sampling units. Matrix **T** was projected on the ordination diagram based on the correlations between community-weighted mean traits and the ordination axes. With the selected trait subset, we used ANOVA with permutation to evaluate differences in each community-weighted mean trait (matrix **T**) between forest, edge and grassland habitats.

Functional diversity (FD) was estimated using Rao's quadratic entropy index (Rao 1982), which was computed using the relative species frequency in the communities (matrix **W**) and the species dissimilarities based on optimal traits. The FD calculated with Rao's index generalizes Simpson's index of species diversity because if all species have completely different traits, FD values will be equivalent to Simpson diversity (Pavoine et al. 2004, Ricotta 2005). We used ANOVA with permutation to evaluate differences in FD between forest, edge and grassland habitats.

We used software SYNCSA (v 2.9.0) for functional analyses and MULTIV (v 3.1.7. beta) for all other analyses, both available at http://ecoqua.ecologia.ufrgs.br.

Results

We recorded 137 bird species, distributed in 40 families (Appendix Table 1). Tyrannidae was the most representative family in species richness, and *Elaenia mesoleuca* and *E. parvirostris*, both belonging to this family, were the most commonly recorded species. From all species, only 21 occurred across the three environments in the forest-edge-grassland, and the grassland encompassed the higher number of exclusive species (49; 19 in forest and 13 in edge).

Species richness did not differ significantly (restricting permutations within regions) between forest, edge and grassland using observed or estimated species richness (P=0.10 and 0.72, respectively). Therefore, any differences that might have resulted from comparing data obtained by different sampling radius were minimal and did not affect species richness or diversity estimations. Considering the Rényi entropy index, grasslands showed higher species richness (Figure 3; parameter α at zero), and forest and edge showed similar values. With increasing value of parameter α, forest areas were less influenced by dominance, since the curve remained more constant in comparison with edge and grassland areas. Considering richness alone (parameter $a=0$), Shannon's or Simpson's index (parameter α=1 and 2, respectively), the results would be different due to the influence of dominant species in the edge and grassland. However, when we compared areas using Simpson's index, there were significant differences in TD between forest and edge (P=0.01), but not between forest and grassland (P=0.27) and between edge and grassland (P=0.35).

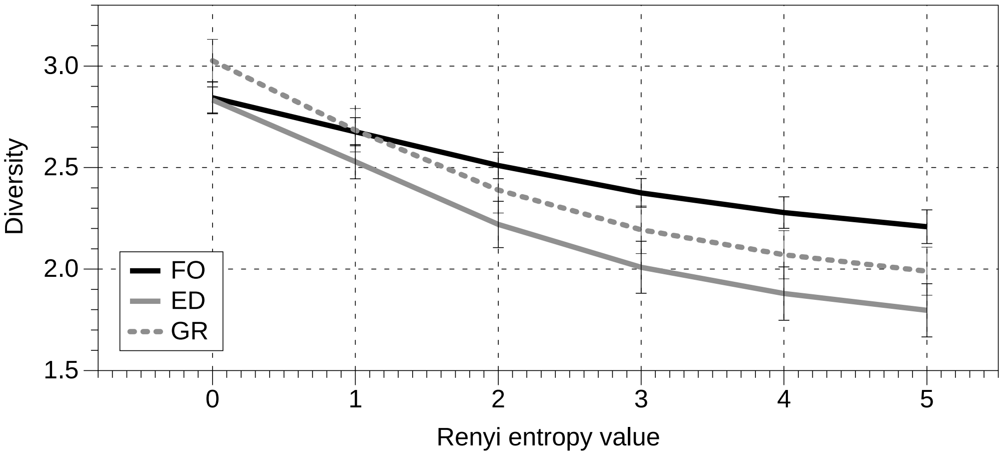

Figure 3. Diversity profiles for forest (FO), edge (ED) and grassland (GR) using the Rényi entropy index.

Multivariate analysis of variance (MANOVA) revealed significant differences in species composition between forest, grassland and edge (P=0.001 between forest and edge; P=0.002 for both, forest and edge compared to grassland). PCoA ordination (Figure 4) showed evidence of segregation between grasslands and the other habitats along axis 2. Besides the significant differences revealed by MANOVA, on the ordination scatter diagram some edge sampling units showed intermediate composition in comparison to forest and grassland. Since the SS region is characterized by a more pronounced mosaic than CCS region, we expected that forest and edge would be more similar regarding species composition, but its edge sampling units were different from forest sampling in species composition. We projected on the ordination diagram the bird species with the highest scores for the first 2 axes, and some species were more associated with grassland, as *Xolmis irupero* (species dependent of grassland), *Thamnophilus ruficapillus*, *Zonotrichia capensis*, *Tyrannus melancholicus* and *Pitangus sulphuratus*. The last three species were also recorded in edge environments, but they were more common in grasslands, foraging in trees and shrubs. The same was observed for *Elaenia mesoleuca* and *E. parvirostris*, two species recorded in all habitat types, but mostly associated with forest and edge, respectively, on the scatter diagram.

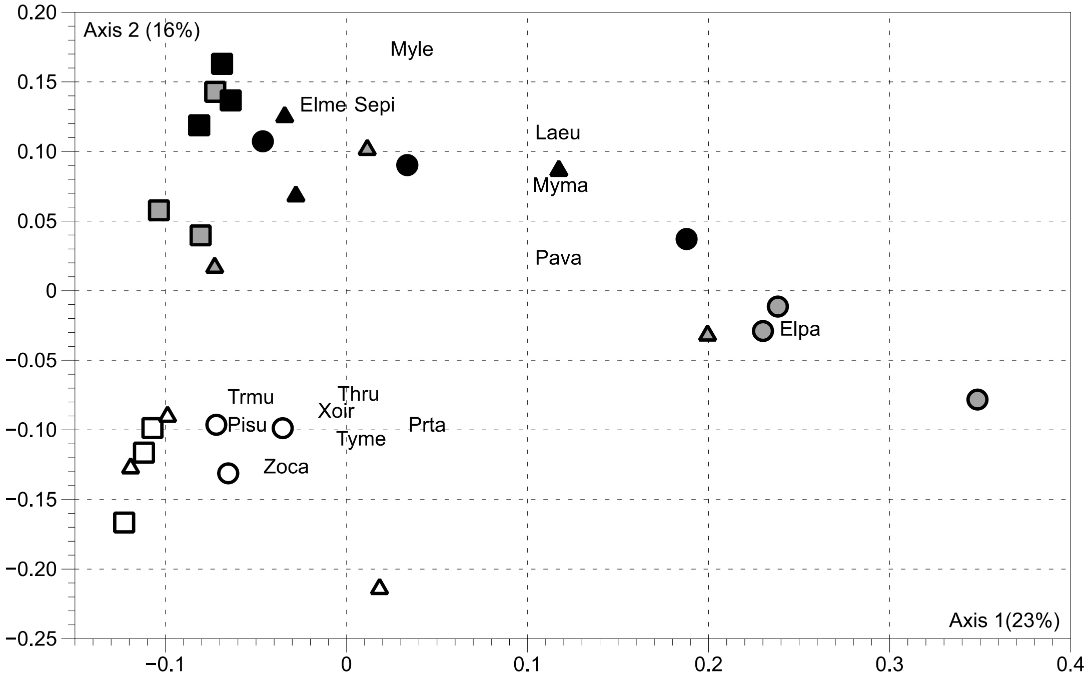

Figure 4. Ordination of sampling units described by avifauna species composition across forest (black symbols), forest edges (gray symbols) and grassland (white symbols) habitat types, in CCS region (squares), SS region (circles), and CA region (triangle), represented by the first two ordination axes (PCoA). Species with highest scores values in the first 2 axis are shown. Species labels: Elme- *Elaenia mesoleuca*, Elpa- *Elaenia parvirostris*, Laeu- *Lathrotriccus euleri*, Myma- *Myiodynastes maculatus*, Myle- *Myiothlypis leucoblephara*, Pava- *Pachyramphus validus*, Pisu- *Pitangus sulphuratus*, Prta- *Progne tapera*, Sepi- *Setophaga pitiayumi*, Thru- *Thamnophilus ruficapillus*, Trmu- *Troglodytes musculus*, Tyme- *Tyrannus melancholicus*, Xoir- *Xolmis irupero*, Zoca- *Zonotrichia capensis*.

Among all traits, seven maximized TCAP related to the environmental gradient (ordinal environmental variable of habitat types) from forest to grassland (ρ(**TE**)=0.52; P=0.002): beak curvature, claw of digit three, mid rectrix, diet grains and invertebrate, gleaning foraging method and vegetation foraging substrate. The ordination diagram in Figure 5 is based on bird composition of communities after fuzzy weighting by their trait similarities (matrix **X**), with CWM traits (matrix **T**) projected on the diagram based on their correlations with the ordination axes. The diagram showed segregation between forest/edge and grassland sampling units along the first axis. On the second axis, there is a gradient from forest to edge and a differentiation in relation to trait mean values: sampling units at the lower right side of the scatter diagram showed birds with higher mean values of beak curvature, and at the upper right side birds with higher mean values of mid rectrix and a higher proportion of gleaning foraging methods. Birds that have grains in their diet were more associated with grassland. We highlight that all morphological traits were standardized by the body mass (divided each trait value by the cubic root of the body mass), and when we refer to higher means in trait values they are proportionately higher according to the species mean body mass.

We found significant differences in CWM traits for beak curvature between forest, edge and grassland habitats (P=0.03 between forest and edge; P<0.005 both forest and grassland and between edge and grassland) (Appendix Figure 2). The traits mid rectrix, diet grains, gleaning foraging method and vegetation foraging substrate presented significant differences between forest and edge compared to grassland (P>0.1 between forest and edge; *P*<0.005 for both between forest and grassland and between edge and grassland). The traits diet invertebrate and claw of digit three did not differ significantly among habitats types (P>0.05).

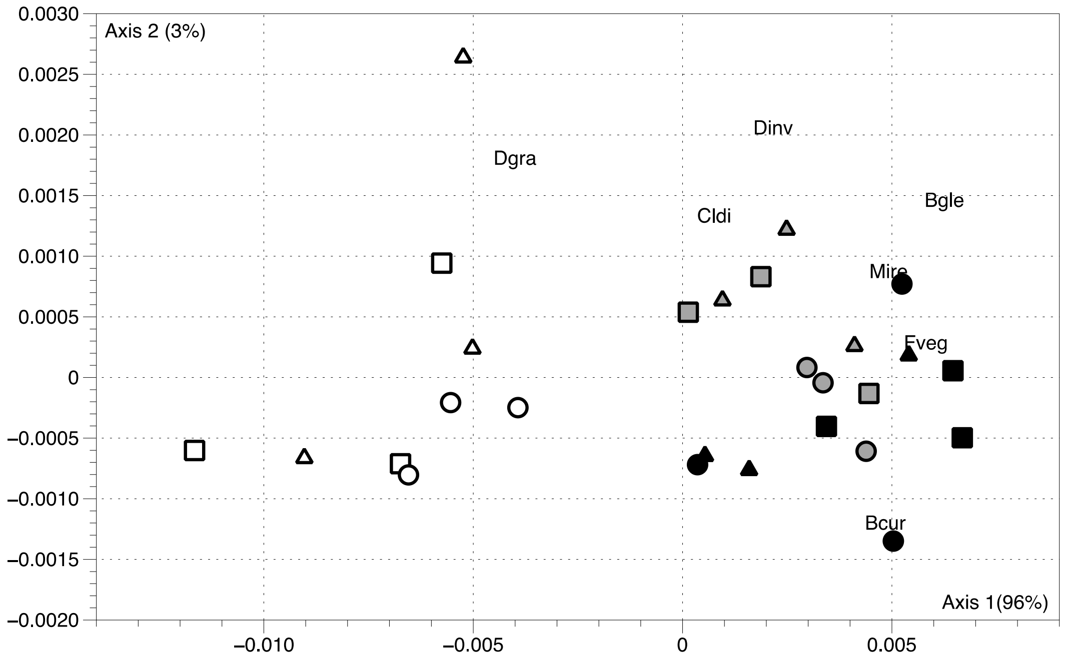

Figure 5. PCoA based on fuzzy-weighted composition of birds on 27 communities (matrix **X**) from Southern Brazil. The community-weighted mean traits (matrix **T**) were projected on the diagram based on their correlations with the ordination axes. Labels for traits: Cldi- claw of digit III, Bcur- beak curvature, Mire- mid rectrix, Dinv- diet invertebrate, Dgra- diet grans, Bgle- gleaning foraging method, and Fveg- vegetation foraging substrate. Sampling units are color-coded according to habitat type: forest (black symbols), forest edge (gray symbols), and grassland (white symbols). Different symbols indicate different sampling regions: CCS (squares), SS (circles) and CA (triangle).

Functional diversity differed significantly between forest and grassland (P=0.0003) and between edge and grassland (P=0.0002), but not between forest and edge (P=0.31). Grasslands presented the highest value of FD, whereas forests showed the highest variation (Figure 6).

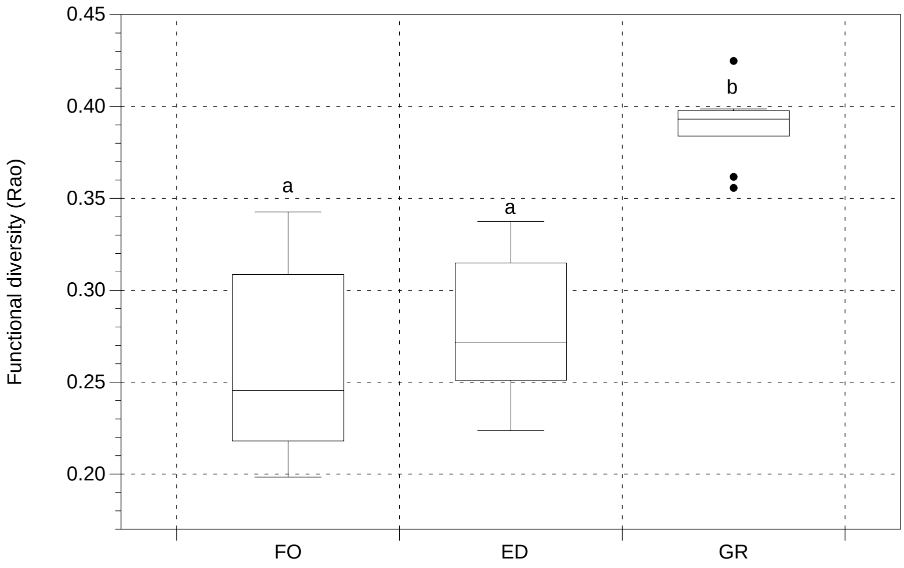

Figure 6. Functional diversity (Rao's quadratic entropy index) on forest (FO), edge (ED) and grassland (GR), based on composition of bird species in 27 communities (matrix **W**) from Southern Brazil. Different letters indicate significant differences between forest, edge and grassland.

Discussion

We reported differences on species composition, different response between taxonomic and functional diversity across the environments, and a trait convergence related to ordinal environmental variable of habitat types as environmental filters.

Bird composition differed between forest-edge-grassland in our study. Baker et al. (2007) found species composition at forest edges in grassland/forest ecotones to be intermediate between the two adjacent communities. In our case, some edge sampling units seemed to have intermediate composition between forest and grassland (Figure 4). We recorded 13 species only in the edge environment, including *Lanio cucullatus*, *Nemosia pileata*, *Polioptila dumicola*, *Serpophaga subcristata* and *Synallaxis frontalis*, species that usually occur in forest edges. Among the 19 species recorded only in forest, few are characteristic of forest interiors, such as *Lanio melanops*, *Hemitriccus obsoletus* and *Sclerurus scansor*. This is expected, since our areas are mosaics of forests and grasslands, characterized in most areas by forest patches rather than continuous forest. The grassland encompassed the highest number of exclusive species (49), some of which are grassland-dependent (i.e. *Xolmis dominicanus* and *Emberizoides ypiranganus*) (Bencke 2009). From the 21 species recorded across the three habitat types, 15 have fruits on their diet. Bird disperses are a key factor in the initial phase of nucleation of trees over grassland, and isolated tree individuals inside the grassland matrix can serve as perching structures for frugivore birds (Duarte et al. 2006). Frugivorous bird species registered across the three habitat types, especially those with high frequency of occurrence, such as *Elaenia parvirostris* and *E. mesoleuca*, probably play an important role in the expansion of forest over grassland.

Regarding taxonomic diversity, only forest and edge differed significantly. Using richness only, forest-edge-grassland habitats did not differ. Because forest had more equability than edge (as depicted in the diversity profile; Figure 3), they differed significantly when using alpha-values > 2. Despite this difference, edges showed the lowest taxonomic diversity. Studies with birds in ecotonal areas with different types of boundaries can be found, from abrupt to gradient borders of the vegetation types. As a consequence, different results regarding patterns of bird species richness, composition and taxonomic diversity in ecotonal areas can be found in the literature. Baker et al. (2007), working in sharp boundaries between woody and heath plant communities, found that bird density and species richness in edges were similar to the adjacent woody habitat, and both were higher than heath habitat. Lloyd et al. (2012) found differences in bird community parameters between tree-line vegetation, cloud forests and *puna* grasslands in the Peruvian Andes, with changes in bird species composition across all three vegetation communities, and richness and diversity varying according to seasons. The tree line vegetation presented distinctive vegetation from the adjacent cloud forest and *puna* grassland.

Functional diversity was significantly different between grassland and forest, and between grassland and edges. In our study areas, grasslands ranged from almost completely grass-dominated landscapes to grasslands with scattered trees and large shrub species. This natural environmental heterogeneity might have contributed to the increased bird functional diversity in grassland habitats based on the selected traits. Barbaro et al. (2014) found higher bird functional richness at forest edges than interiors in New Zealand and lower functional richness at edges in France. Similarly to TD, FD is apparently site-specific, and probably dependent on local environmental variables, specific features of the adjacent ecosystem, as well as traits selected to calculate FD.

Forest and forest edges did not differ significantly in FD and probably present high functional redundancy, with species having similar functional traits (Petchey et al. 2007). The small differences in vegetation structure (mainly in mosaic areas) between forest and edges, which are much more evident in comparison to grasslands, probably influenced the similarity in FD between these two habitats. Despite the importance of disturbance regimes (grazing and fire) for grassland conservation, they may have the opposite effect in forest environments: the presence of cattle leads to the simplification of understory vegetation structure (Dufour-Dror 2007, Michels et al. 2012). Considering that bird abundance and species composition vary in response to different degrees of changes in vegetation structure (Karr and Freemark 1983), the simplification of vegetation structure probably affected the FD of avifauna, promoting increased similarity between forest and edge habitats in our study.

Grasslands encompassed the highest FD and the number of exclusive species in comparison to forest and edge. Available information indicates that faunal assemblages in grass-dominated systems are diverse and distinct (Bond and Parr 2010). Grasslands in Southern Brazil contain high plant diversity (Boldrini et al. 2009) and many animal species, including extinction-threatened birds (Bencke 2009), making the grassland and transition zones important for bird communities (Fontana et al. 2009). Moreover, the conservation of these grassland ecosystems has been neglected. Large areas have been converted into agricultural areas and exotic tree plantations and only 0.33% of Southern Brazilian grasslands are protected by conservation units (Overbeck et al. 2007). Besides, the natural expansion of forests over grassland in conservation units where grazing and fire have been suppressed (Pillar and Vélez 2010) induces fragmentation of grassland-dominated landscapes by itself. Grazing and fire seem to keep the forest expansion process at bay (especially in the Atlantic Rainforest biome), preserving the grassland landscape (Overbeck et al. 2005, 2007). This is a relevant issue regarding conservation efforts in Brazil, since the use of grazing animals and fire inside conservation units is legally forbidden in many cases (Pillar and Vélez 2010). Therefore, one question arises that could and should be addressed in future studies: what would be the effects of the suppression of grazing and fire on the taxonomic and functional diversity of bird communities in grassland and forests?

Effects of environmental variables as ecological filters lead to trait-convergence assembly patterns (TCAP). Our results indicate that differences in environmental habitat structure from forest to grassland were important for the assembly of the bird communities in ecotonal areas. Using CWM traits we were able to explore TCAP related to the gradient (Pillar et al. 2009). A higher proportion of birds that forage on vegetation, with gleaning foraging behaviour and with higher mean values of mid rectrix occurred on forests and forest edges. Our results are consistent with Botero-Delgadillo and Bayly (2012) who found that tail feathers in tyrant-flycatchers is a characteristic of birds that prey on the underside of leaves, indicating a likely relation between gleaning foraging behavior with mid rectrix feather. Most birds have invertebrates on their diet, for this reason the mean value of diet invertebrate was not significantly different between forest-edge-grassland. However, this trait probably influenced the convergence in both sides of the gradient, as well as claw of the third digit. Mean values of beak curvature differed between all environments, and higher mean values were in forest. As in the ordination diagram it is located in the opposite side of invertebrate diet and gleaning foraging behaviour, it had an inverse relation with those traits. The only trait that had higher proportion on grasslands was diet based on grains. Many birds that feed on grains in our study make extensive use of grassland habitats, but can also use other natural habitats not sampled for this study, such as wetlands, shrublands and row-crop habitats. Besides this, they can be locally sensitive to grassland loss, especially those species that depend on non-substitutable resources available both in grasslands and other natural habitats (Azpiroz et al. 2012).

Conclusion

Bird taxonomic (TD) and functional diversity (FD) responded differently to environmental change from forest to grassland in forest-grassland mosaics in Southern Brazil. TD differed between forest and forest edges, but not between forest and edge compared to grassland. Both forest and edge differed from grassland in FD, and grassland encompassed the higher FD. The taxonomic and functional approach employed here was very useful to infer that these two facets of diversity may capture different aspects of community assembly along forest-grassland transitional areas. The composition of species also differed between forest-edge-grassland, which can be an evidence of species turnover according to habitat characteristics. Trait-convergence assembly patterns (TCAP) indicated niche mechanisms underlying assembly of bird communities, and differences in environmental variables across forest-edge-grassland habitats are acting as ecological filters.

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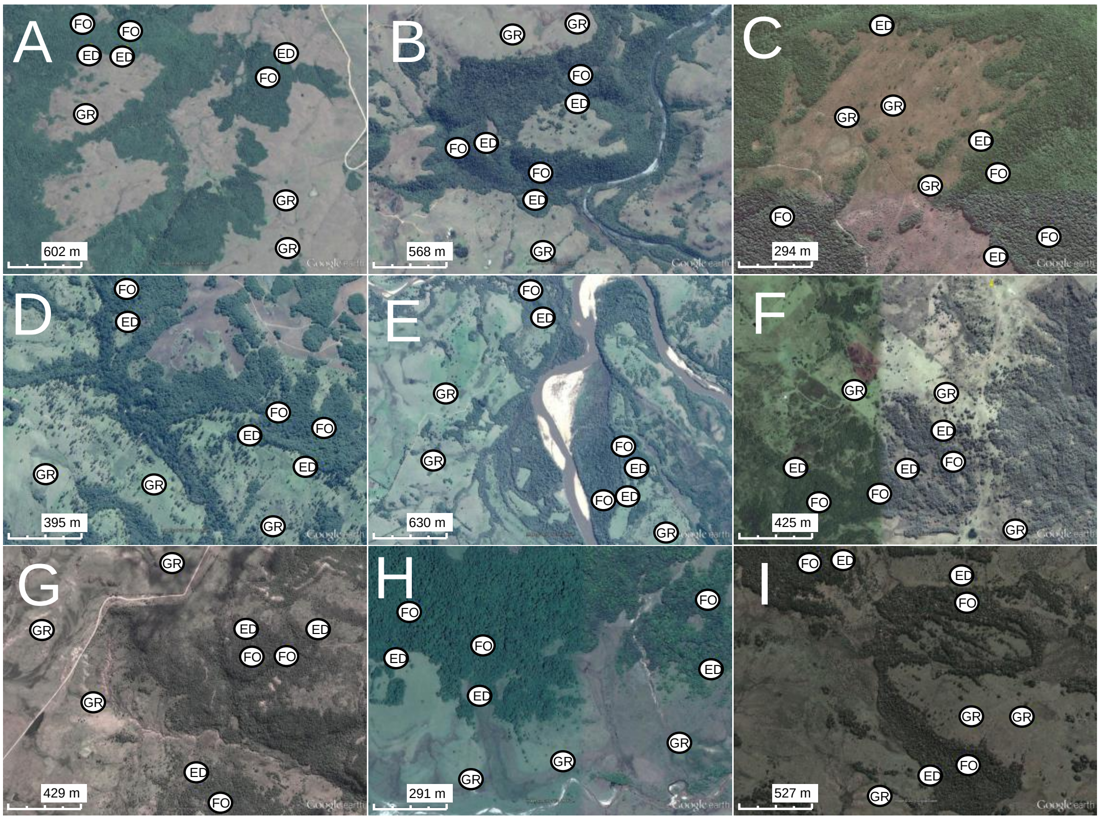
APPENDIX



Appendix Figure 1: Point counts on forest (FO), forest edge (ED) and grassland (GR) habitats in nine study areas: letter A to C correspond to CCS region; D to F SS; and G to I CA region.

Appendix Table 1. Species registered in the listening points in forest, forest edges and grassland habitats. Scientific nomenclature is in accordance to the rules established by the Brazilian committee of ornithological records (Comitê Brasileiro de Registros Ornitológicos 2014).

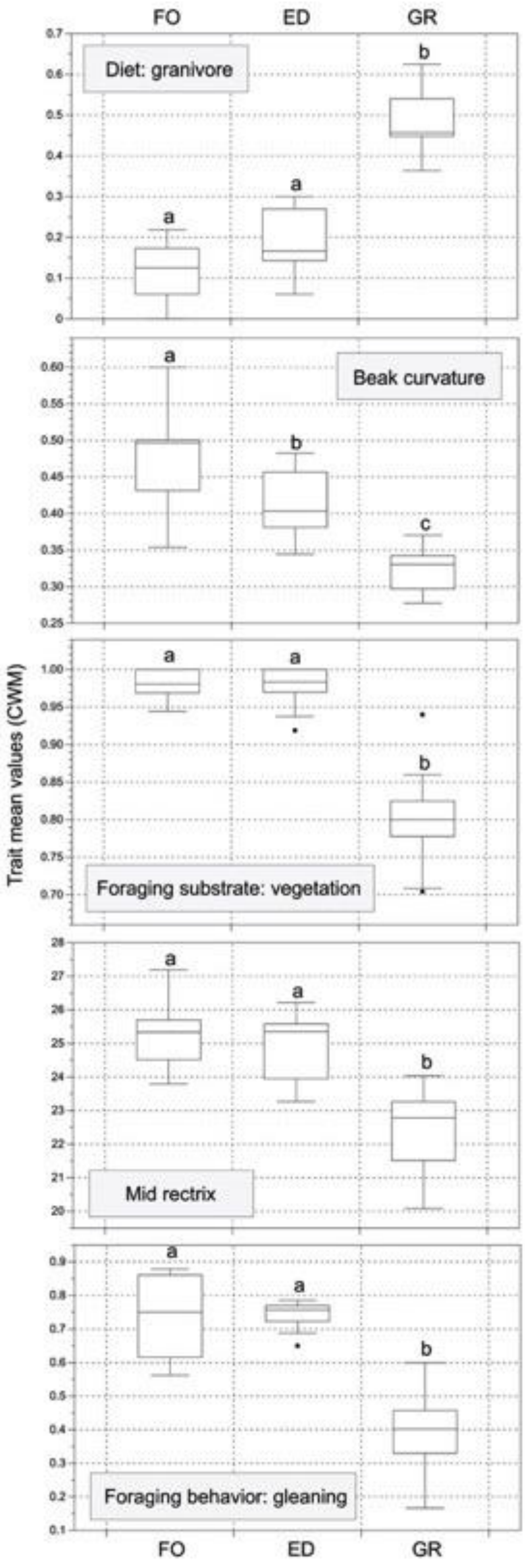
Family and Species	**Forest**	**Edge**	**Grassland**
Tinamiformes Huxley, 1872			
Tinamidae Gray, 1840			
Crypturellus obsoletus (Temminck, 1815)		X	
Rhynchotus rufescens (Temminck, 1815)			X
Nothura maculosa (Temminck, 1815)			X
Anatidae Leach, 1820			
Amazonetta brasiliensis (Gmelin, 1789)			X
Galliformes Linnaeus, 1758			
Cracidae Rafinesque, 1815			
Penelope obscura (Temminck, 1815)	X		
Pelecaniformes Sharpe, 1891			
Ardeidae Leach, 1820			
Bubulcus ibis (Linnaeus, 1758)			X
Threskiornithidae Poche, 1904			
Phimosus infuscatus (Lichtenstein, 1823)			X
Cathartiformes Seebohm, 1890			
Cathartidae Lafresnaye, 1839			
Coragyps atratus (Bechstein, 1793)			X
Accipitriformes Bonaparte, 1831			
Accipitridae Vigors, 1824			
Rupornis magnirostris (Gmelin, 1788)	X		
Geranoaetus albicaudatus (Vieillot, 1816)			X
Falconiformes Bonaparte, 1831			
Falconidae Leach, 1820			
Milvago chimachima (Vieillot, 1816)			X
Milvago chimango (Vieillot, 1816)		X	
Falco sparverius (Linnaeus, 1758)			X
Gruiformes Bonaparte, 1854			
Rallidae Rafinesque, 1815			
Aramides saracura (Spix, 1825)			X
Charadriiformes Huxley, 1867			
Charadrii Huxley, 1867			
Charadriidae Leach, 1820			
Vanellus chilensis (Molina, 1782)			X
Columbiformes Latham, 1790			
Columbidae Leach, 1820			
Patagioenas picazuro (Temminck, 1813)	X	X	X
Zenaida auriculata (Des Murs, 1847)			X

Family and Species	**Forest**	**Edge**	**Grassland**
Leptotila verreauxi (Bonaparte, 1855)	X		X
Leptotila rufaxilla (Richard & Bernard, 1792)	X	X	
Psittaciformes Wagler, 1830			
Psittacidae Rafinesque, 1815			
Pyrrhura frontalis (Vieillot, 1817)	X		X
Myiopsitta monachus (Boddaert, 1783)			X
Pionus maximiliani (Kuhl, 1820)		X	
Cuculiformes Wagler, 1830			
Cuculidae Leach, 1820			
Guira guira (Gmelin, 1788)			X
Strigiformes Wagler, 1830			
Strigidae Leach, 1820			
Glaucidium brasilianum (Gmelin, 1788)	X		
Apodiformes Peters, 1940			
Trochilidae Vigors, 1825			
Stephanoxis lalandi (Vieillot, 1818)	X		
Chlorostilbon lucidus (Shaw, 1812)		X	
Hylocharis chrysura (Shaw, 1812)		X	
Leucochloris albicollis (Vieillot, 1818)		X	X
Trogoniformes A. O. U., 1886			
Trogonidae Lesson, 1828			
Trogon surrucura (Vieillot, 1817)	X	X	
Piciformes Meyer & Wolf, 1810			
Picidae Leach, 1820			
Veniliornis spilogaster (Wagler, 1827)	X	X	
Colaptes melanochloros (Gmelin, 1788)			X
Colaptes campestris (Vieillot, 1818)			X
Passeriformes Linnaeus, 1758			
Tyranni Wetmore & Miller, 1926			
Thamnophilida Patterson, 1987			
Thamnophilus ruficapillus (Vieillot, 1816)			X
Thamnophilus caerulescens (Vieillot, 1816)	X	X	X
Drymophila malura (Temminck, 1825)	X		
Conopophagidae Sclater & Salvin, 1873			
Conopophaga lineata (Wied, 1831)	X		
Furnarioidea Gray, 1840			
Scleruridae Swainson, 1827			
Sclerurus scansor (Ménétriès, 1835)	X		
Dendrocolaptidae Gray, 1840			
Sittasomus griseicapillus (Vieillot, 1818)	X	X	
Lepidocolaptes falcinellus (Cabanis & Heine, 1859)	X	X	X
Dendrocolaptes platyrostris Spix, 1825	X		
Furnariidae Gray, 1840			
Furnarius rufus (Gmelin, 1788)			X
Heliobletus contaminatus (Berlepsch, 1885)		X	
Syndactyla rufosuperciliata (Lafresnaye, 1832)	X	X	X
Leptasthenura striolata (Pelzeln, 1856)	X	X	

Family and Species	Forest	Edge	Grassland
Leptasthenura setaria (Temminck, 1824)		X	
Phacellodomus striaticollis (d'Orbigny & Lafresnaye, 1838)			X
Anumbius annumbi (Vieillot, 1817)			X
Schoeniophylax phryganophilus (Vieillot, 1817)			X
Synallaxis ruficapilla (Vieillot, 1819)			X
Synallaxis cinerascens (Temminck, 1823)	X		
Synallaxis frontalis (Pelzeln, 1859)		X	
Synallaxis spixi (Sclater, 1856)		X	X
Cranioleuca obsoleta (Reichenbach, 1853)	X	X	X
Tyrannida Wetmore & Miller, 1926			
Pipridae Rafinesque, 1815			
Chiroxiphia caudata (Shaw & Nodder, 1793)	X		
Pachyramphus polychopterus (Vieillot, 1818)	X	X	
Pachyramphus validus (Lichtenstein, 1823)	X	X	
Tyrannoidea Vigors, 1825			
Platyrinchus mystaceus (Vieillot, 1818)	X		
Leptopogon amaurocephalus (Tschudi, 1846)	X		
Phylloscartes ventralis (Temminck, 1824)	X	X	X
Tolmomyias sulphurescens (Spix, 1825)	X	X	
Poecilotriccus plumbeiceps (Lafresnaye, 1846)	X		
Hemitriccus obsoletus (Miranda-Ribeiro, 1906)	X		
Tyrannidae Vigors, 1825			
Camptostoma obsoletum (Temminck, 1824)		X	X
Elaenia parvirostris (Pelzeln, 1868)	X	X	X
Elaenia mesoleuca (Deppe, 1830)	X	X	X
Elaenia obscura (d'Orbigny & Lafresnaye, 1837)	X	X	X
Phyllomyias virescens (Temminck, 1824)	X		
Phyllomyias fasciatus (Thunberg, 1822)	X	X	
Serpophaga subcristata (Vieillot, 1817)		X	
Myiarchus swainsoni (Cabanis & Heine, 1859)		X	X
Pitangus sulphuratus (Linnaeus, 1766)			X
Machetornis rixosa (Vieillot, 1819)			X
Myiodynastes maculatus (Statius Muller, 1776)	X	X	X
Megarynchus pitangua (Linnaeus, 1766)			
Tyrannus melancholicus Vieillot, 1819		X	X
Tyrannus savana (Vieillot, 1808)		X	X
Empidonomus varius (Vieillot, 1818)			X
Myiophobus fasciatus (Statius Muller, 1776)	X	X	X
Lathrotriccus euleri (Cabanis, 1868)	X		X
Knipolegus cyanirostris (Vieillot, 1818)	X		
Knipolegus lophotes (Boie, 1828)			X
Satrapa icterophrys (Vieillot, 1818)			X
Legatus leucophaius (Vieillot, 1818)		X	X
Xolmis irupero (Vieillot, 1823)			X
Xolmis dominicanus (Vieillot, 1823)			X

Family and Species	Forest	Edge	Grassland
Passeri Linnaeus, 1758			
Corvida Wagler 1830			
Vireonidae Swainson, 1837			
Cyclarhis gujanensis (Gmelin, 1789)	X	X	X
Vireo chivi (Linnaeus, 1766)	X	X	X
Corvidae Leach, 1820			
Cyanocorax caeruleus (Vieillot, 1818)		X	X
Cyanocorax chrysops (Vieillot, 1818)	X		
Passerida Linnaeus, 1758			
Hirundinidae Rafinesque, 1815			
Pygochelidon cyanoleuca (Vieillot, 1817)			X
Progne tapera (Vieillot, 1817)		X	X
Tachycineta leucorrhoa (Vieillot, 1817)			X
Troglodytidae Swainson, 1831			
Troglodytes musculus (Naumann, 1823)		X	X
Polioptilidae Baird, 1858			
Polioptila dumicola (Vieillot, 1817)		X	
Turdidae Rafinesque, 1815			
Turdus flavipes (Vieillot, 1818)	X	X	X
Turdus rufiventris (Vieillot, 1818)	X	X	X
Turdus amaurochalinus (Cabanis, 1850)	X	X	X
Turdus subalaris (Seebohm, 1887)	X	X	
Turdus albicollis (Vieillot, 1818)	X	X	
Mimidae Bonaparte, 1853			
Mimus saturninus (Lichtenstein, 1823)			X
Motacillidae Horsfield, 1821			
Anthus hellmayri (Hartert, 1909)			X
Thraupidae Cabanis, 1847			
Saltator similis (d'Orbigny & Lafresnaye, 1837)	X		X
Saltator aurantiirostris (Vieillot, 1817)		X	X
Nemosia pileata (Boddaert, 1783)		X	
Pyrrhocoma ruficeps (Strickland, 1844)	X		
Lanio cucullatus (Statius Muller, 1776)		X	
Lanio melanops (Vieillot, 1818)	X		
Tangara sayaca (Linnaeus, 1766)	X	X	X
Tangara preciosa (Cabanis, 1850)	X	X	
Stephanophorus diadematus (Temminck, 1823)	X	X	X
Paroaria coronata (Miller, 1776)			X
Pipraeidea melanonota (Vieillot, 1819)	X	X	
Pipraeidea bonariensis (Gmelin, 1789)			X
Emberizidae Vigors, 1825			
Zonotrichia capensis (Statius Muller, 1776)		X	X
Ammodramus humeralis (Bosc, 1792)			X
Haplospiza unicolor (Cabanis, 1851)		X	
Donacospiza albifrons (Vieillot, 1817)			X
Poospiza nigrorufa (d'Orbigny & Lafresnaye,		X	

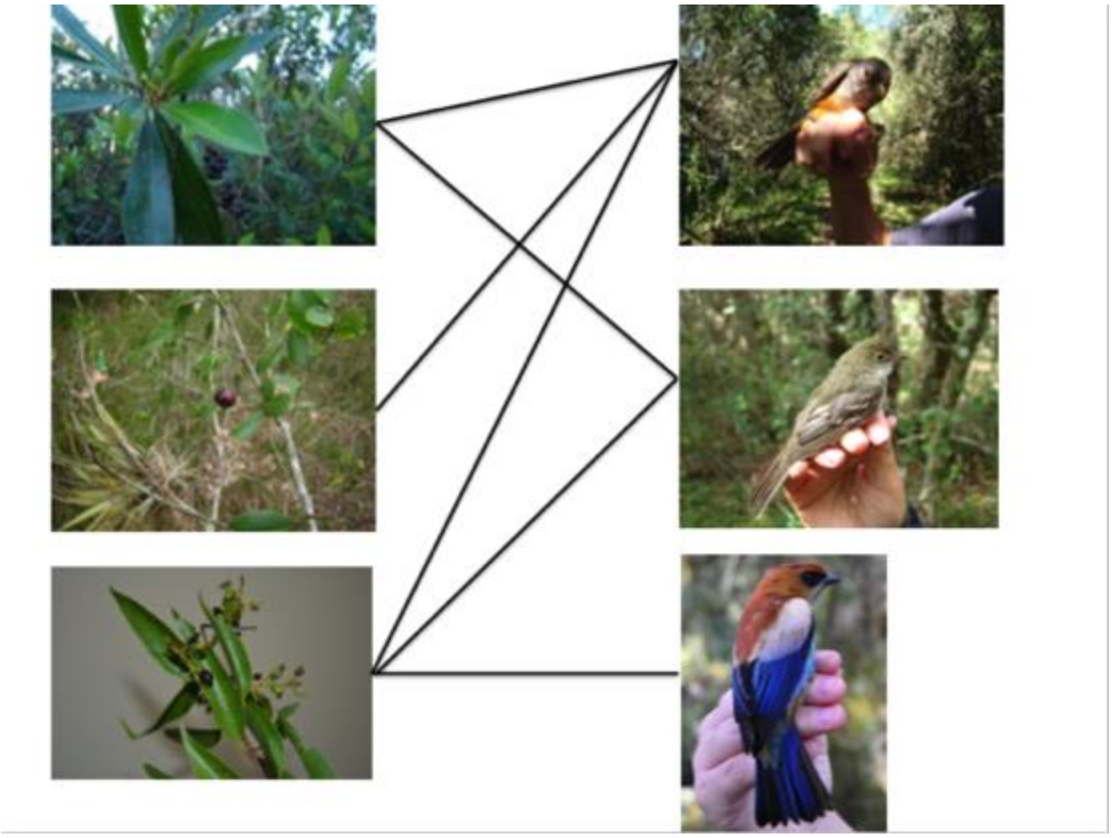
Family and Species	**Forest**	**Edge**	**Grassland**
1837)			
Poospiza cabanisi (Bonaparte, 1850)	X	X	
Sicalis flaveola (Linnaeus, 1766)			X
Sicalis luteola (Sparrman, 1789)		X	X
Emberizoides herbicola (Vieillot, 1817)			X
Emberizoides ypiranganus (Ihering & Ihering, 1907)			X
Embernagra platensis (Gmelin, 1789)			X
Sporophila caerulescens (Vieillot, 1823)			X
Cardinalidae Ridgway, 1901			
Cyanoloxia brissonii (Lichtenstein, 1823)			X
Parulidae Wetmore, Friedmann, Lincoln, Miller, Peters, van Rossem, Van Tyne & Zimmer 1947			
Setophaga pitiayumi (Vieillot, 1817)	X	X	X
Geothlypis aequinoctialis (Gmelin, 1789)			X
Basileuterus culicivorus (Deppe, 1830)	X	X	
Myiothlypis leucoblephara (Vieillot, 1817)	X	X	
Icteridae Vigors, 1825			
Cacicus chrysopterus (Vigors, 1825)	X	X	
Pseudoleistes guirahuro (Vieillot, 1819)			X
Agelaioides badius (Vieillot, 1819)			X
Molothrus bonariensis (Gmelin, 1789)			X
Fringillidae Leach, 1820			
Sporagra magellanica (Vieillot, 1805)	X	X	X
Euphonia chlorotica (Linnaeus, 1766)	X	X	



Appendix Figure 2: Traits that maximized the correlation between functional community patterns and the habitat types, in forest (FO), forest edges (ED), and grassland (GR) habitats. Different letters indicate significant differences between forest, edges and grassland.

CHAPTER 2

STRUCTURE OF SEED-DISPERSAL NETWORKS BETWEEN BIRDS AND PLANTS



This article will be submitted to the journal OIKOS

Structure of seed-dispersal networks between birds and plants

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Abstract:

Mutualistic plant–animal interactions are vital for ecosystem functioning as they play a central role in plant reproduction (i.e. dispersal and pollination). Understanding the structure and dynamics of mutualistic interactions is essential for biodiversity studies and allows the investigation of ecological and evolutionary mechanisms. Our goal was to investigate the structure of seed-dispersal networks between plants and birds. For this purpose, we analysed 19 seed dispersal networks between plants and birds, of which two were described in the current study and 17 obtained from literature. We described the generalized mutualistic network patterns such as nestedness, connectance, modularity and degree distribution. We sampled two of these networks in areas of forest-grassland mosaic in Southern Brazil. The most important birds as disperser that we found in the two collected networks were generalists. Those birds were found in every environment type of our study areas (forest, forest edge and grassland) and they probably play an important role in the process of forest expansion into grasslands. Additionally, we found a nested structure in almost every network studied, suggesting that interactions among plants and birds show some robustness to random species extinctions. Most of our datasets were not modular, which might indicate that seed dispersal has low interaction specificity, but our result is not consistent enough to assume a pattern (only 10 from 19 seed dispersal networks were not modular). The lack of modularity could also be an effect of network size, since most of the datasets used were small networks (less than 100 species). The truncated power law fit was prevalent in most networks, suggesting the role of constraints (forbidden links) in potential interactions shaping seed dispersal mutualistic networks. Our findings suggest the existence of an apparent common assembly process occurring in seed dispersal mutualistic networks, regardless the differences in sampling methodology or continents where the networks were sampled.

Keywords: frugivory, interactions, mutualistic networks, Southern Brazil.

Introduction

Mutualistic interactions between plants and animals are vital for ecosystem functioning as they play a central role in plant reproduction through seed dispersal and pollination mechanisms (Wright 2002), and the life histories of animals by influencing community dynamics and diversity (Jordano et al. 2003). Most tropical plant species depend on animals to disperse their seeds (Howe and Smallwood 1982), and seed dispersal by vertebrates is crucial. In areas with forest-grassland mosaics, bird-dispersers are especially important for the process of forest expansion as they contribute to the initial phase of nucleation of trees as diaspore carriers from forest to grassland (Duarte et al. 2006).

Network theory facilitates the understanding of the structure and dynamics of mutualistic interactions, as well as the ecosystem impact of the loss of interactions and species extinctions (Bascompte and Jordano 2007). Understanding network structure is essential for studying biodiversity and allows the investigation of ecological and evolutionary mechanisms. However, coevolutionary processes can be expected to diverge between mutualistic and antagonistic networks, and consequently, their structural patterns are expected to be different as well (Lewinsohn et al. 2006). Thus, the dynamic and stability of networks constrain the existing structure of mutualistic and trophic networks toward opposite patterns (Thébault and Fontaine 2010). In the past, more studies have dealt with comparison of mutualistic networks than with antagonistic networks (Miranda et al. 2013) with pollination being the most investigated network type so far.

The most basic structure described in ecological networks is connectance (Dunne et al. 2002), which is the proportion of links observed relative to the possible amount of links that could exist in a network. Mutualistic networks are often nested (Bascompte et al. 2003), creating a pattern where specialist species (species with few interactions) interact with other species that interact with both specialists and generalists (species with many interactions). Nested and connected networks may be more resistant to species loss because species with few interactions are probably connected to generalist species which tend to be more tolerant to environmental stress (Bastolla et al. 2009).

Connectivity can also be characterized by the distribution that describes the probability of finding a species within a certain number of interactions which is called “degree distributions” (Jordano et al. 2003). The statistical properties of degree distributions have important consequences for the interpretation of the processes underlying ecological networks (Otto et al. 2007). Complex networks often vary in their structure, having a frequency distribution of vertex connectivity (i.e., number of links per species) that decays as a scale-free (power-law), broad-scale (i.e., truncated power-law distributions) or faster-decaying functions (i.e., exponential) (Amaral et al. 2000). Networks that fit the power law distribution are described as heterogeneous, in which the bulk of the nodes have a few links, but a few nodes are more connected than randomly expected. Truncated power law means that as the number of interactions reaches an average value, the probability of finding more connected species drops faster than expected in a power-law. In contrast, in exponential fits, the probability of a node having a number of links larger than the average drops very fast (Bascompte and Jordano 2007). Jordano et al. (2003) explored the organization of mutualistic networks and found that are often characterized by a few super-generalists species, while the majority of species establish just a few interactions.

Some mutualistic networks are also modular, i.e., subsets of species interact more frequently among themselves than with other species in the community. Modularity is assumed to increase stability in pollination networks, because cascading disturbances are expected to spread more slowly through a modular than a non-modular structure (Olesen et al. 2007). For seed dispersal networks, the coexistence of modular networks has been also reported (Mello et al. 2011, Vidal et al. 2014), nevertheless modularity has been little investigated in seed dispersal networks. On the other hand, mutualistic networks have been shown to be less modular than antagonistic networks, while modularity seems to decrease their persistence when compared to trophic networks (Thébault and Fontaine 2010).

Our goal is to investigate the structure of seed-dispersal networks between plants and birds, using the metrics of nestedness, modularity, connectance and degree distribution. We utilized collected data from two seed dispersal networks in Southern Brazil and another 17 seed dispersal networks obtained from the literature (including one network also sampled in Southern Brazil) to describe mutualistic network patterns.

Methods

Study area of collected networks

Study areas were located in two physiographic regions of the state of Rio Grande do Sul, Southern Brazil, *Campos de Cima da Serra* (CCS) and *Serra do Sudeste* (SS) (Figure 1). CCS is located in the Atlantic Forest biome, whereas SS is located in the Pampa biome. Each area is composed of mosaics of vegetation that include grassland, forest and other less representative vegetation types. In the Pampa biome, grasslands cover large continuous areas and forests are mostly restricted to riparian zones, whereas in Atlantic Forest grasslands are located in the highlands of the South-Brazilian Plateau. The CCS is located between 450 m and 900 m above sea level, and SS is between 100 m and 350 m. We sampled two areas each in the CCS and SS regions.

Fire and grazing by domestic animals are considered to be the principal factors impeding expansion of forest over grassland vegetation in the past centuries and under current climatic conditions (Pillar and Quadros 1997). Management of the study areas included cattle ranching occurring in both grasslands and forest patches, with grazing pressure less intense in CCS than SS, since the sampling in CCS occurred inside the two nature reserves: Tainhas State Park and the Aparados da Serra National Park.

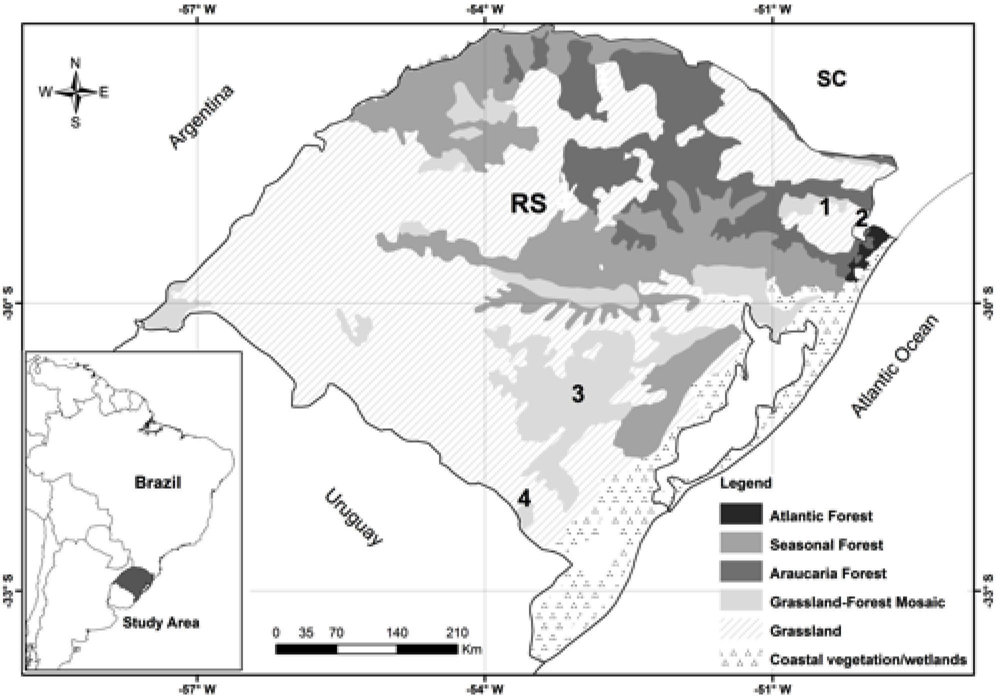

Figure 1. Rio Grande do Sul state with South America and Brazil insert; map of the dominant vegetation physiognomies of Southern Brazil (IBGE 2004). Numbers represent the study locations in CCS (1 and 2) and SS (3 and 4) areas.

Bird sampling and seed collection

We captured birds placing eight mist nets in forest interiors (with ≥ 100 m distance from edges in three areas and ≥ 50 m for one area) and another eight in forest-grassland edges. We captured, identified, banded, and maintained birds in restraining bags for at least 20 minutes to collect faeces during three mornings (weather permitted) in all four seasons between August 2012 and May 2013. In total, we sampled 215 hrs in each environment (forest and forest edge). If we multiply the total hours by eight mist nets, we sampled in each environment 1,720 hrs.

Seeds found in fecal samples were identified to species, when possible, to build an interaction matrix between birds and the plant that they consumed, considering the number of interaction events (the number of times that a specific bird species consumed a specific plant species). To facilitate the process of seed identification, fruiting plants were also collected in the study area in the same period of bird sampling. These collected seeds were compared to local herbaria for identification.

In the same areas that birds were captured, we also recorded the abundance of bird species through point counts in forest environments and forest edges (three point counts in each environment) (Casas and Pillar, unpub. data, Chapter 1). All individuals seen and/or heard were accounted for, except those that only flew over the area.

Plant species composition data were obtained by another study carried out at the same areas (Gliesch-Silva 2015). Each local unit (two per area) consisted in 140 x 70 m, divided into two plots of 70 x 70 m located in the forest and grassland areas right at the forest edge with evidence of forest expansion, i.e., with the presence of woody individuals over grassland. Fifteen circular sub-plots of 100 m^2 were randomly distributed and sampled, where all woody plant individuals with DBH≥5 cm (DBH -

diameter at breast height) were measured. At the forest edge the woody individuals measured were with DGH≥5 cm (DGH - diameter at ground height). In our study, plant and bird abundance was used to construct the graph 3D where nodes were proportional to abundance of species.

Datasets

To compare the structure between our collected networks with other seed-dispersal networks, we analysed 14 published and three unpublished datasets (quantitative and qualitative, i.e., only representing the presence or absence of a given interaction), including one other seed dispersal networks collected in Southern Brazil (Table 1). Two of those datasets were obtained from the Interaction Web Database (http://www.nceas.ucsb.edu/interactionweb/), and 14 from the web of life (http://www.web-of-life.es/).

Table 1: Description of the 19 plant-frugivore networks. Information about dataset source, habitat type, location, data type, methodology approach and the number of species of birds and plants are given.

Code	Dataset	Habitat type	Location	Data type	Methodology	Bird	Plant
BA	Baird (1980)	Oak-hickory, red maple and floodplain forest	Princeton, Mercer, New Jersey, USA	Number of times a specific bird species was observed eating a specific plant fruit	Transects	21	7
CA	Carlo et al. (2003)	Subtropical secondary forests	Frontón, Puerto Rico	Number of interactions observed on a plant	Focal bird species	15	21
CO			Cordillera, Puerto Rico			13	25
CG			Caguana, Puerto Rico			16	25
CI			Cialitos, Puerto Rico			20	34

Code	Dataset	Habitat type	Location	Data type	Methodology	Bird	Plant
Ca1	Casas et al. (unpub. data)	Atlantic Forest biome (forest/grassland mosaics - CCS region)	Rio Grande do Sul state, Brazil	Number of times a specific bird species consumed a specific plant species	Mist net	6	10
Ca2		Pampa biome (grassland/forest mosaics – SS region)				15	28
FR	Frost (1980)	Coastal dune forest	Mtunzini, South Africa	Quantitative	----	10	16
GE	Galetti and Pizo (1996)	Forest fragment (Santa Genebra Reserve)	Sao Paulo state, Brazil	Number of feeding bouts (bird species and a flock)	Transects	29	35
HA	Hamann and Curio (1999)	Tropical rainforest (Island Negros)	Central Philippine Islands	Binary data (foraging observed or not observed)	Foraging birds observation	19	36
HR	Jordano (1985)	Saltmarsh	Sevilla, Spain	Density of interactions observations	Transects	17	16
NG	Jordano (unp. Data)	----	Sierra de Cazorla, SE Spain	Binary data	----	28	18
NR		----	Cazorla, SE Spain.	Quantitative	----	33	25
MK	Mack and Wright (1996)	Tropical Forest (primary and secondary)	Chimbu Province, Papua New Guinea	Binary data (presence or absence of interaction bird-plant)	Foraging birds observation and mist net	32	29
AD	Scherer et al. (2007)	Atlantic Forest (Restinga forest)	Rio Grande do Sul state, Brazil	Number of interactions observed and seeds in birds' faeces.	Transects and mist net	18	11
SC	Schleuning et al. (2011)	Primary and secondary tropical rainforest	Africa, Kenya	Number of fruit-eating individuals on a plant species	Focal plants species	83	32
WS	Silva et al. (2002b)	Atlantic Forest (mosaic of primary and secondary forest)	Sao Paulo state, Brazil	Binary data	Transects, focal plant and mist net	86	207
SO	Sorensen (1981)	Temperate woodland	Near Oxford, Wytham Estate, Britain	Number of seeds consumed by birds	Transects	14	11

Code	Dataset	Habitat type	Location	Data type	Methodology	Bird	Plant
MT	Wheelwright et al. (1984)	Montane Forest (area surrounding by forest, woodlots and pastures)	Monteverde, Costa Rica	Binary data	Transects, mist net, and focal plant and bird species	40	169

Data analysis

For the 19 seed dispersal networks, we explored nestedness, connectance, modularity, degree of distributions and the medium degree for plants and animal (see below). All analyses of network metrics (except for modularity) were performed with *bipartite* package (Dormann et al. 2008) using the R platform (R Core Team 2013).

Nested networks are characterized by a core of highly connected species (generalists) that interact mainly with each other, and a group of specialist species that interact mainly with the generalist species (Bascompte et al. 2003). We assessed nestedness by computing the metric NODF (Almeida-Neto et al. 2008), which corrects biases resulting from matrix fill and matrix dimensions. NODF values of 0 indicate non-nestedness and 100 perfect nesting. We used *nested* function in *bipartite* package.

Connectance (C) is the proportion of links observed in a network relative to the possible amount of links (Dunne et al. 2002), with values from 0 to 1. For bipartite networks it is calculated as $C=L/(I \times J)$, were L is the number of realized links; I and J are the number of species of each party in bipartite networks, e.g., plants and animals. The species degree is the number of different species that a certain species interacts with. We also calculated the medium degree for plants and birds for each network.

Modularity is characterized by the existence of groups of nodes (species), that interact more among each other than with other groups (modules) in a network

(Guimera and Amaral 2005). It ranges between 0 (random network with no modules) to 1 (maximum modularity). We calculated modularity with the MODULAR program (Marquitti et al. 2014). To calculate nestedness and modularity significance we used the model 2 (Bascompte et al. 2003) as null model, which generates networks in which the probability of two species to interact depends on the number of interactions of both species in the real network.

Degree distribution can be described as the probability of finding a species with a certain number of interactions (Jordano et al. 2003). We examine the cumulative distributions $P(k)$ of the number of interactions per species, k, fitting three different models: exponential, $P(k) \sim \exp(-\Upsilon k)$; power-law, $P(k) \sim k^{-\Upsilon}$; and truncated power-law, $P(k) \sim k^{-\Upsilon} \exp(-k/k_X)$, where Υ is the fitted constant (degree exponent) and k_X is the truncation value. The crucial step for this analysis is how often an exponential, power-law or truncated power-law provides the best fit to the data. We calculated the degree distributions with the *degreedist* function in *bipartite* package and for the species of each trophic level separately. The Akaike Information Criterion (AIC) was used to evaluate which model provided the best fit.

For the networks that we sampled the contribution of each bird species in relation to each plant species from which it consumed resources was assessed, using the importance index (I). The importance index was calculated as in the equation below:

$Ij = \Sigma[(Cij/Ti)/S]$,

where i is the plant species, j is the bird species, Ti is the total number of birds species feeding on the plant species i, S is the total number of plants species included in the sample, Cij corresponded to the binary data, with 1 if bird species j consumes the fruits of plant species i, and 0 if it does not. The same importance index was used to evaluate the importance of each plant species on the diet of bird dispersers, where *i* is the bird species and *j* the plant species. This index varies from 0 to 1, and tends to 1 when a bird disperser (or plant species) has many interactions in the community or has a large number of exclusive interactions (Murray et al. 2000, Silva et al. 2002).

Results

Datasets

The 19 seed dispersal networks we analysed (including the two sampled for this study) ranged in size from 16 to 293 species and had different sampling methodologies (transects, focal plants and/or mist net) and sampling intensities. Connectance ranged from 0.05 to 0.69 and the medium degree of both birds and plants ranged from 1.40 to 16.65. Almost all networks exhibited a significantly nested structure (Table 2). Also, ours results pointed towards a relationship between connectance and network size. We tested, through Spearman rank correlation analysis, the relationship between connectance and network size and found a significant inverse relationship (r_S=-0.75, P<0.005, n=19). Nine networks were significantly modular, and we found a prevalence of truncated power-law model (Table 2, Figure 2).

Table 2: Descriptors of the 19 plant-frugivore networks: NODF- nested overlap and decreasing fill; CON- connectance; MOD- modularity; Pl D. and An D.- plant and bird degree distributions respectively; K.M Pl and K.M An– Medium Degree of both, plants and birds.

Code	Size	NODF	CON	MOD	Pl D.	An D.	K.M Pl	K.M An
Ca1	16	14.39^{NS}	0.23	0.55^{NS}	**NF**	**NF**	2.33	1.40
SO	25	45.92***	0.3	0.32^{NS}	TR	**NF**	4.18	3.29

FR	26	74.57***	0.69	0.12**	TR	PL/TR	6.88	11.00
BA	28	50.98*	0.34	0.31^{NS}	TR	EX/TR	7.14	2.38
AD	29	49.82*	0.25	0.34^{NS}	PL-TR	EX/TR	2.83	4.63
HR	33	78.76*	0.44	0.22*	EX-TR	EX/TR	7.56	7.12
CA	36	34.17*	0.16	0.47*	PL-TR	EX	2.43	3.40
C0	38	29.69*	0.34	0.50*	TR	TR	1.96	3.77
Ca2	43	27.31*	0.12	0.53^{NS}	PL-TR	NF	2.04	3.50
CG	41	44.7*	0.17	0.39^{NS}	EX-TR	EX/TR	2.72	4.25
NG	46	61.52*	0.26	0.27^{NS}	EX-TR	EX/TR	7.17	4.61
CI	54	43.38*	0.14	0.38^{NS}	**NF**	EX/TR	2.79	4.75
HA	55	37.37NS	0.29	0.37*	TR	TR	5.47	10.37
NR	58	58.98*	0.18	0.31**	TR	TR	6.00	4.55
MK	61	11.21*	0.07	0.65*	PL-TR-EX	PL/TR	2.28	2.06
GE	64	35.49*	0.14	0.37*	EX/TR	TR	4.17	5.03
SE	121	34.58*	0.14	0.31^{NS}	TR	TR	12.70	4.76
MT	209	32.87*	0.1	0.40*	TR	TR	3.94	16.65
WS	293	17.06*	0.05	0.34^{NS}	TR	TR	5.42	10.19

*, $P<0.005$; **, $P<0.05$, ***,$P=0.05$; NS, not significant. Degree of distributions: TR, truncated power-law; EX, exponential; PL, Power-law; NF, No Fit (no model fitted the distributions of links per species well).

Networks sampled in the current study

From 480 bird individuals sampled in our two networks, 112 individuals presented seeds in their faeces, distributed in 21 species of passerine birds and 38 bird-dispersed plants of woody and non-woody plants species (16 not identified) (Figure 3). Mainly in Ca2 network, bird and plant species that had more links (i.e., higher “degree”) were also the more abundant species (Figure 3). We analysed this relationship between plant and bird abundance and degree using Spearman’s rank correlation. The abundance and degree of plants and birds were significantly correlated for Ca2 network (birds: $r_S=0.53$, $P=0.04$, $n=15$; plants: $r_S=0.41$, $P=0.02$, $n=28$), but not for Ca1 network (birds: $r_S=0.44$, $P=0.37$, $n=6$; plants: $r_S=0.04$, $P=0.90$, $n=10$).

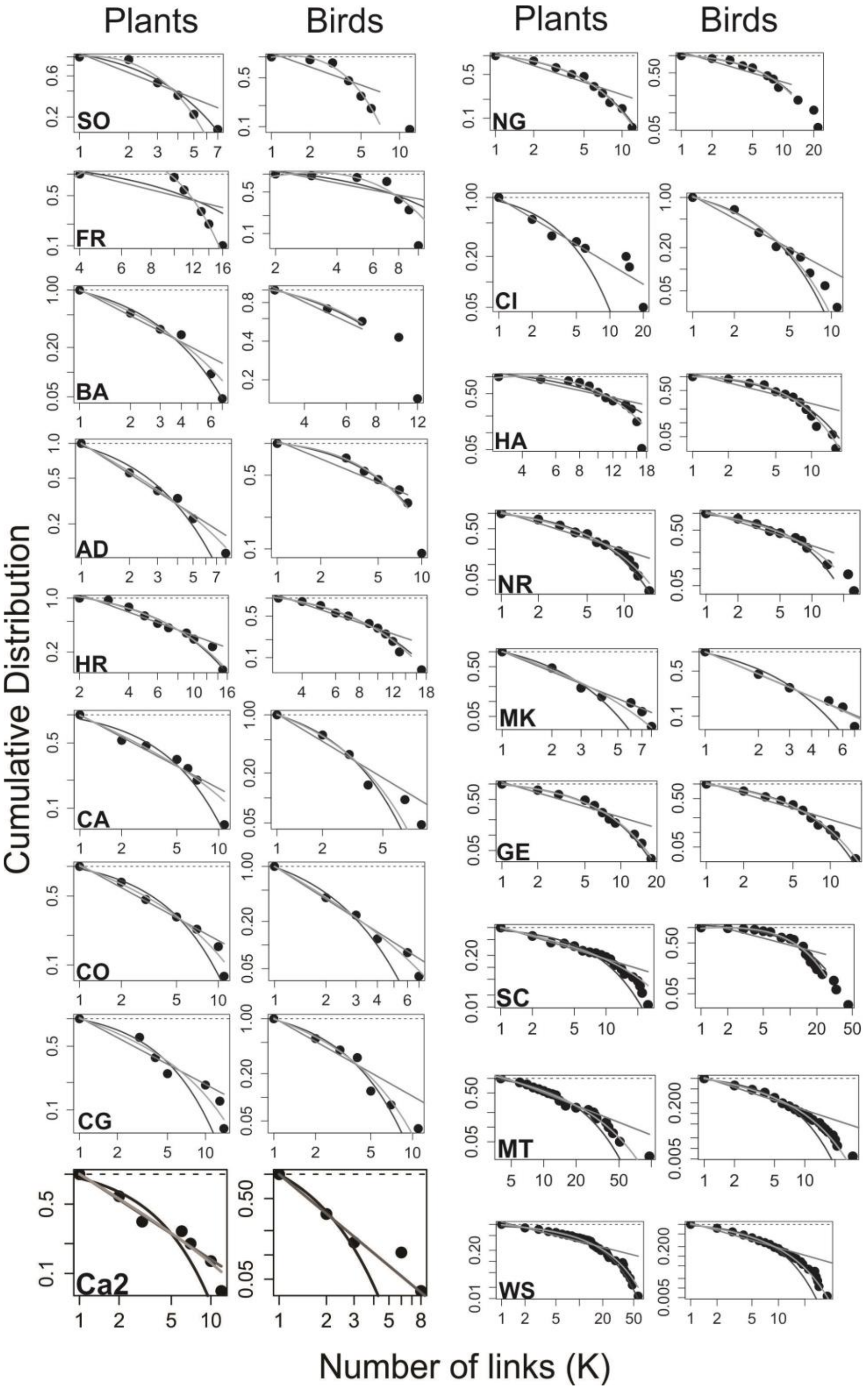

Figure 2: Cumulative distribution of connectivity (number of links per species, k, or degree) for 18 seed dispersal interaction networks. Panels show the cumulative distributions of species with 1, 2, 3, ..., k links (dots), exponential fits (light gray), power-law fits (gray lines) and truncated power-law fits (black lines). See network codes in Table 1. We do not show network Ca1 because no model fitted the distributions of links per species.

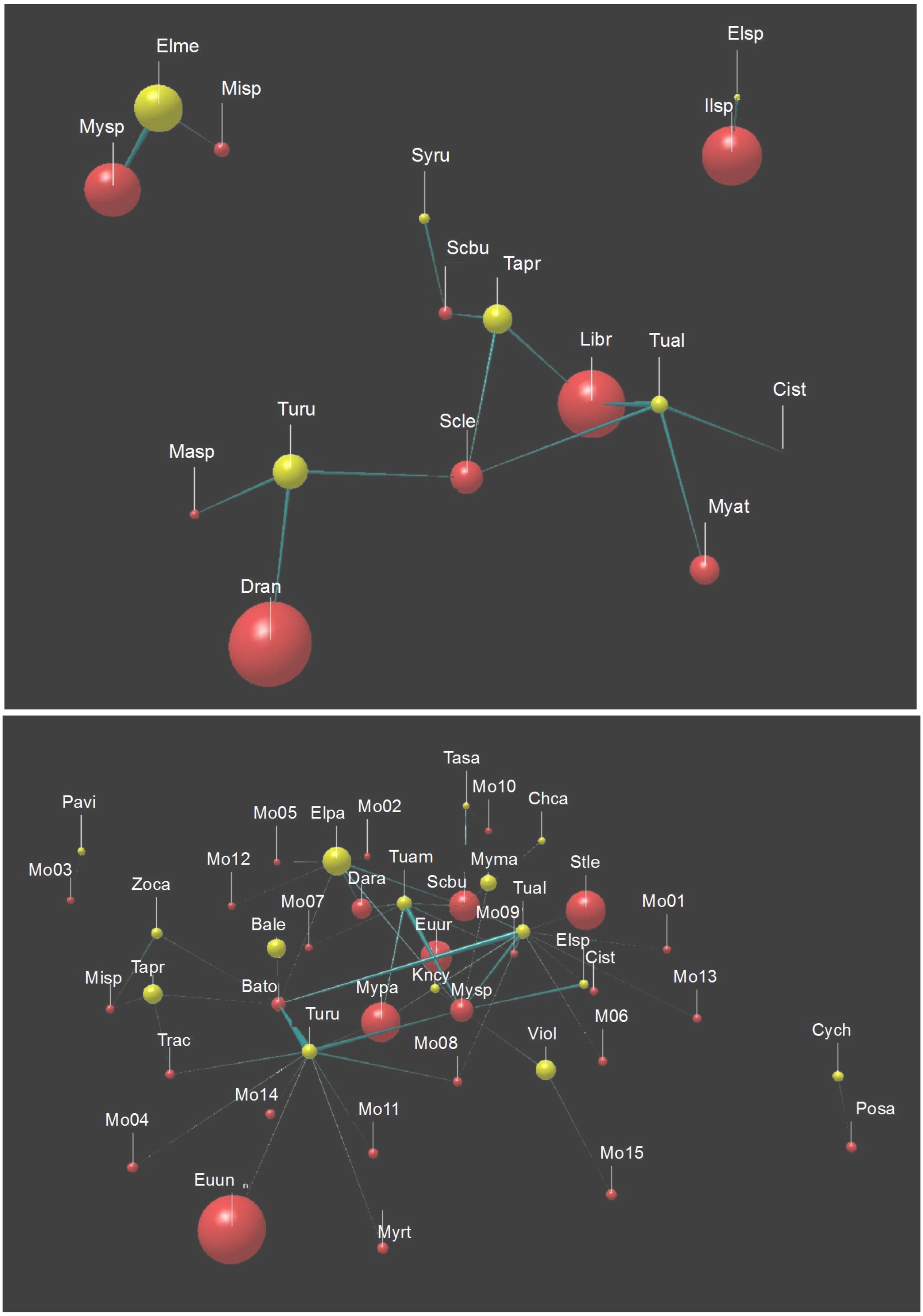

Figure 3: Seed dispersal networks sampled in Rio Grande do Sul state, Brazil (Ca1 and Ca2 respectively). Node size is proportional to abundance of species and link width is proportional to the number of interaction events. Red nodes depict plant species, whereas yellow nodes are bird species. Bird species lables: Bale- *Basileuterus leucoblepharus*, Chca- *Chiroxiphia caudata*, Cych- *Cyanocorax chrysops*, Elpa- *Elaenia parvirostris*, Elme- *Elaenia mesoleuca*, Elsp- *Elaenia* sp., Kncy- *Knipolegus cyanirostris*,

Myma- *Myiodinastes maculatus*, Pavi- *Pachyramphus viridis*, Syru- *Syndactyla rufosuperciliata*, Tapr- *Tangara preciosa*, Tasa- *Tangara sayaca*, Tual- *Turdus albicollis*, Tuam- *Turdus amaurochalinus*, Turu- *Turdus rufiventris*, Viol- *Vireo olivaceus*, Zoca- *Zonotrichia capensis*. Plant species lables: Bato- *Banara tomentosa*, Cist- *Cissus striata*, Dara- *Daphnopsis racemosa*, Dran- *Drimys angustifolia*, Euun- *Eugenia* cf. *uniflora*, Euur- *Eugenia uruguaiensis*, Ilsp- *Ilex* sp., Libr- *Lithraea brasiliensis*, Masp- *Maytenus* sp., Misp- *Miconia* sp., Mypa- *Myrcia palustris*, Myat- *Myrrhinium atropurpureum*, Mysp- *Myrsine* sp., Myrt- *Myrtaceae*, Posa- *Pouteria salicifolia*, Scle- *Schinus lentiscifolius*, Scbu- *Scutia buxifolia*, Stle- *Stirax leprosus*, Trac- *Tripodanthus acutifolius*, Mo- morph, plant species where the seeds could not be identified. Image produced with FoodWeb3D, written by R.J. Williams and provided by the Pacific Ecoinformatics and Computational Ecology Lab (http://www.foodwebs.org).

The two bird species with higher importance index were the same for Ca1 and Ca2 network: *Turdus albicollis* and *T. rufiventris* (Figure 4 and Figure 5), that together represented 50% of total from importance index. *Elaenia* flycatchers were placed in the third position in both networks. The most common species of *Elaenia* changed according to the physiography region, with *Elaenia mesoleuca* in CCS region and *E. parvirostris* in SS region, but in both, *Elaenia* flycatchers were the most abundant. The important index of plant species as suppliers of fruit resources differed between the two networks: in Ca1 the most important species were *Scutia buxifolia* and *Ilex* sp., and in Ca2 *Myrsine* sp. and *Scutia buxifolia*. In both networks, the two plant species represented 38% of the total importance index. *Myrsine* sp. appeared in Ca1 in fifth place. These plants species also differed in abundance in the phytosociological sampling between regions. *Scutia buxifolia* was the third most abundant plant species in SS region (Ca2 network), while in CCS region (Ca1 network) only three individuals were recorded in all local units sampled. In CCS *Myrsine* sp. was the second most abundant plant species and in SS region the sixth.

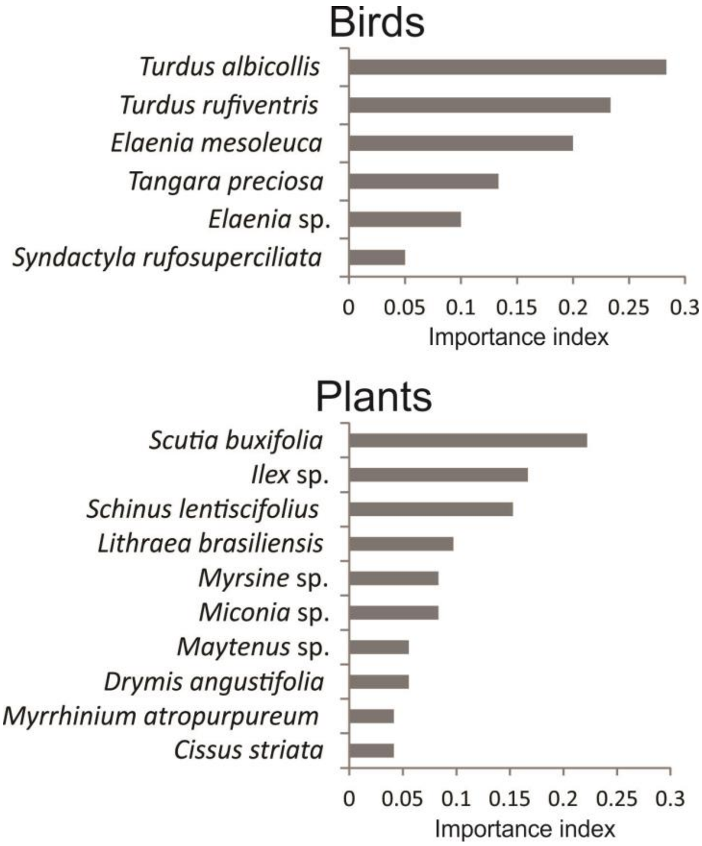

Figure 4: Importance index (I) of the Ca1 network for the bird species as dispersers and for plant species suppliers of fruit resources for birds. Network sampled in Rio Grande do Sul state, *Campos de Cima da Serra* region, Brazil.

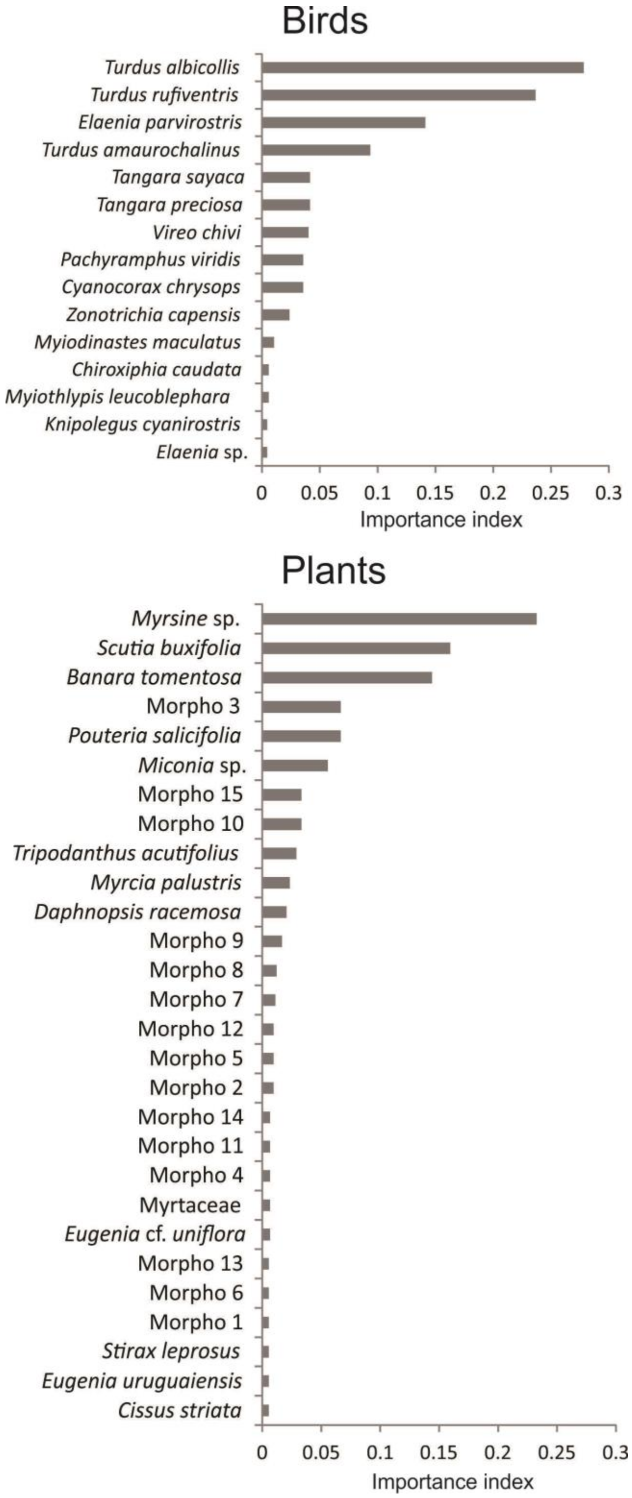

Figure 5: Importance index (I) of the Ca2 network for the bird species as dispersers and for plant species suppliers of fruit resources for birds. Network sampled in Rio Grande do Sul state, *Serra do Sudeste* region, Brazil. Morph = plant species where the seeds could not be identified.

The Ca1 network was smaller than Ca2, with size of 16 species. It showed 14 out of the 60 possible interactions and had connectance C=0.23. This smallest network was not significantly nested (Table 2) and we also could only poorly estimate the degree distribution of this network due to the small sample size. The Ca2 network showed 52 out of the 420 possible interactions and had connectance of C =0.12. It exhibited a significantly nested structure fitting a truncated power-law model for plants, although no model fitted the distributions of links per bird species (Figure 2). Neither of the networks were modular (Table 2).

Discussion

We analysed the structural properties of two seed dispersal networks we collected in Southern Brazil and another 17 networks obtained from the literature. We reported that seed dispersal mutualistic networks showed a common assembly pattern regarding nestedness, connectance, and degree distributions.

There is a debate on the implications of nestedness for the stability and persistence of species within networks, with studies concluding that nestedness promotes stability (Bastolla et al. 2009, Thébault and Fontaine 2010) or imperils species persistence (Allesina and Tang 2011). However, as found in our and previous studies (Bascompte et al. 2003, Thébault and Fontaine 2010), mutualistic networks are highly nested and this cohesive pattern likely provides alternative routes for system responses to perturbations (Bascompte et al. 2003). The asymmetrical pattern in nested networks, with specialist species interacting only with generalists, can provide pathways for rare species to persist (Jordano 1987). The smallest network that we studied (Ca1) was not significantly nested, and both Ca1 and Ca2, were not modular. However, a relationship between network size and nestedness and modularity has been reported (Olesen et al. 2007). Bascompte et al. (2003) found, for plant-frugivore and plant-pollinator networks, that with sizes above 28 and 50 species respectively, all networks were significantly nested. In our datasets, only two networks were not significant nested, including one with more than 28 species (HA network with size 55 and Ca1 network with 16 species). Since most of our seed dispersal networks comprise more than 28 species, we cannot suggest an influence of network size on nestedness.

We found that connectance and network size were negatively correlated in most of the analysed networks. Our results seem to fit the general pattern reported for seed dispersal networks (Mello et al. 2011). However, the results for connectance, as well as those found for nestedness, have to be interpreted with caution due to the influence of sampling bias in these metrics (Rivera-Hutinel et al. 2012). The small size of the Ca1 network probably was due to the limitation of the method, where the local topographic conditions and taller trees resulted in few interactions registered by the mist nets capture method. However, the mist net interaction has the advantage in relation to the focal plant method because we registered in fact the birds that are dispersing plants species through their faeces. In areas such as where Ca1 network was sampled, maybe a combination of mist net in more than one forest strata could probably find more interactions.

The existence of a modular structure has been reported for other seed dispersal networks (Mello et al. 2011, Vidal et al. 2014) and for pollination networks (Olesen et al. 2007). The mutualistic modules hypothesis (Jordano 1987) proposes that phylogenetically related species tend to form subgroups that play similar ecological roles. Conversely, Donatti et al. (2011) showed that modularity can emerge by a combination of trait convergence of phylogenetically unrelated species. Therefore, mutualistic networks have been shown to be less modular than antagonistic networks, such as herbivory, because modularity is expected to increase with link specificity (Lewinsohn et al. 2006), and seed-dispersal networks are characterized by low interaction specificity. In addition, modularity seems to decrease the persistence of a mutualistic network, and nestedness increases its resilience (Thébault and Fontaine 2010). However, we found that only 10 from 19 seed dispersal networks were not modular (almost half of our dataset), and we assumed that this result is not consistent enough to assume it is a pattern. Another possible explanation is the influence of network size on modularity. For pollination networks, Olesen et al. (2007) found that all networks larger than 150 species were modular, whereas networks with less than 50 species were never modular. In our datasets, only two networks were larger than 150 (only one presented a significant modularity). However, we do not know if for seed dispersal networks this minimal network size to infer a pattern would hold true. Connectance can also influence modularity, because if it increases with small network size, the size of the core of links between generalized species also increases, which may reduce the level of modularity (Olesen et al. 2007).

We found a prevalence of truncated power law fits in our datasets, similar to the Jordano et al. (2003), which verified the degree distributions to explore the organization of pollination and seed dispersal mutualistic networks. The power law fit is described by a large number of species with few interactions that coexist with a relatively small number of super-generalists. The degree of nodes (species) is important, because each time a new node is introduced, it tends to interact with the most-connected nodes, leading to a kind of “rich-gets-richer process” (Bascompte and Jordano 2007). If this is the only process of link addition building the network, generalized power-law distributions of interaction frequencies would be expected (Jordano et al. 2003). Filtering imposed by the biological properties of plant–animal mutualisms (constraints) limits the growth of these interaction networks due to the existence of forbidden links, and confers them a truncated power law fit (broad-scale behavior) (Jordano et al. 2003). A pairwise interaction that is impossible to occur, for example, owing to phenological or size mismatch, is an example of forbidden link in seed dispersal network. However, it has been proposed that the degree distribution can be generated by a random interaction model (Vázquez 2005).

Bird species with higher importance index in the sampled networks (Ca1 and Ca2) were *Turdus albicollis*, *T. rufiventris*, *Elaenia mesoleuca* and *E. parvirostris*. This result was similar to those showed by Scherer et al. (2007), a seed dispersal network also collected in Southern Brazil. These bird species occurred in all environmental types in our study areas (forest, forest edge and grassland) (Casas and Pillar, unpub. data, Chapter 1), and are probably important in the expansion process of forest over grasslands in these mosaic areas. These species probably contribute for the resilience to species loss of the network, since species with few interactions and generally more sensitive, are usually connected to generalist species, providing robustness to the network (Bascompte and Jordano 2007, Thébault and Fontaine 2010). The most important plant species considering fruit resources were *Myrsine* sp., *Scutia buxifolia* and *Ilex* sp. These plants have small seeds, thus birds with wide range of beak size can eat them, which may contribute to the importance of these plants on network organization. The identity of the species in the core of a nested network, with the potential to drive the coevolution of the whole network, can change geographically. However, results in different local assemblages of mutualists and part of network structure could be explained by properties at the landscape level

(Bascompte and Jordano 2007).

In seed dispersal networks the relative abundance of species is correlated with their degree (Jordano 1987), suggesting that abundance affects the occurrence and frequency of interactions, because abundant species may interact more frequently and with more species than rare species (Vázquez et al. 2009). Only in the Ca2 network we found correlation between the abundance of species and the number of links (degree). However, the most important species in our two networks, considering the importance index, were not necessarily the most abundant ones. Previous studies found that other factors are important in determining the organization of interacting assemblages, such as phenological overlapping and morphological matches (Stang et al. 2007, Vizentin-Bugoni et al. 2014). The core of the studies that tested the importance of abundance and forbidden links in mutualistic networks was with pollination networks, and similar studies with seed dispersal networks are currently lacking.

Conclusions

In conclusion, our findings point out that seed dispersal mutualistic networks apparently show a common assembly pattern regardless of the differences in sampling methodology or continents that these networks were sampled. The nested pattern found in our dataset can suggest that interactions among plants and birds show some robustness to random species extinctions. Most of datasets were not modular, which suggests that seed dispersal has low interaction specificity, but our result is not consistent enough to assume a pattern. However, most of datasets were small networks (less than 100 species), and the lack of modularity could be an effect of network size. The truncated power law fit was prevalent in most networks, possibly due to constraints (forbidden links) in potential interactions in shaping these mutualistic networks.

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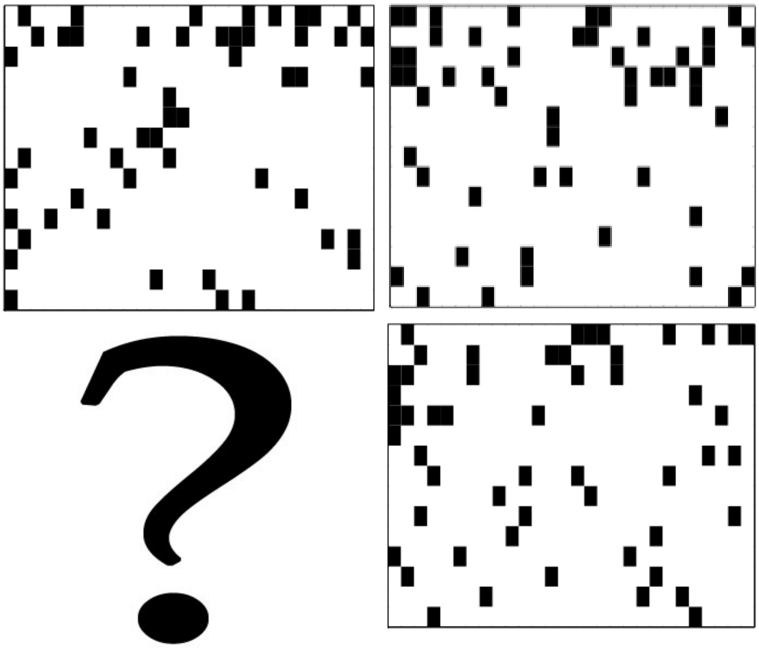
CHAPTER 3

ASSESSING SAMPLING SUFFICIENCY OF NETWORK METRICS USING BOOTSTRAP



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Assessing sampling sufficiency of network metrics using bootstrap

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Abstract:
Sampling the full diversity of interactions in an ecological community is a highly intensive effort. Recent studies have demonstrated that many network metrics are sensitive to both sampling effort and network size. Here, we develop a statistical framework that aims to assess sampling sufficiency for some of the most widely used metrics in network ecology, namely connectance, nestedness (NODF- nested overlap and decreasing fill) and modularity (using the QuaBiMo algorithm), based on bootstrap methods. Our framework is a resampling technique that can generate confidence intervals for each network metric with increasing sample size (i.e., the number of sampled interaction events, or number of sampled individuals), which can be used to evaluate sampling sufficiency. The sample is considered sufficient when the confidence limits reach stability or lie within an acceptable level of precision for the aims of the study. We illustrate our framework with data from three quantitative networks of plant and frugivorous birds, varying in size from 16 to 115 species, and 17 to 2,745 interactions. The results indicate that, for the same dataset, sampling sufficiency may be reached at different sample sizes depending on the metric of interest. The bootstrap confidence limits reached stability, thus the samples were sufficient for two networks analysed for connectance, nestedness, and modularity. The sample size of the smallest network was not sufficient for any of the metrics, for its confidence limit values were wide and unstable with increasing sample size. The bootstrap method is useful to empirical ecologists to indicate the minimum number of interactions necessary to reach sampling sufficiency for a specific network metric. It is also useful to compare sampling techniques of networks in their capacity to reach sampling sufficiency. Our method is general enough to be applied to different types of metrics and networks.

Keywords: confidence intervals, ecological networks, bootstrap resampling, sample size.

Introduction

Network studies have grown extraordinarily in the past few decades, and currently they have mainly focused on the analyses of network structure and robustness (Bascompte and Jordano 2007, Miranda et al. 2013). Understanding the patterns of interaction networks may allow researchers to have a better understanding of ecological and evolutionary mechanisms responsible for these patterns.

The most commonly used metrics to describe the structure of plant–animal networks are connectance, nestedness, modularity, and robustness (Dormann et al. 2009, Miranda et al. 2013). The most basic structure described in ecological networks is connectance (Dunne et al. 2002), which is the proportion of links observed in a network relative to the possible number of links. Most mutualistic networks are nested, where specialist species interact with subsets of the species interacting with generalists (Bascompte et al. 2003). A highly connected and nested architecture promotes community stability in mutualistic networks, but in trophic networks this stability is enhanced with compartmented (modular) and weakly connected architectures (Thébault and Fontaine 2010). A modular pattern, that is, subsets of species interacting more frequently among themselves than with other species in the community, sometimes prevents the spread of perturbations across the network (Stouffer and Bascompte 2011).

Nevertheless, most network metrics are sensitive to sampling effort and network size (Dormann et al. 2009). Studying pollination networks, Olesen et al. (2007) found a relationship between the size of the network and nestedness and modularity. In general, if network size in seed dispersal increases, connectance decreases (Mello et al. 2011). Bascompte et al. (2003) also verified, for plant-frugivore and plant-pollinator networks, that above a size of 50 species, all networks were significantly nested. Consequently, studies having low sampling effort need to be interpreted with caution (Rivera-Hutinel et al. 2012). However, sampling the full diversity of interactions is a highly intensive effort, and ecologists have now come to realize that most networks published to date may be under-sampled. Chacoff et al. (2012) found that, despite a large sampling effort, their pollination network was under-sampled, as they detected less than 60% of the potential interactions. Relating to seed dispersal mutualistic networks, because most of the published data sets consist on small networks, they may also be insufficiently sampled.

To evaluate the accuracy of the sampling procedure in networks, the approach used is manipulating data after sampling. In this regard, the common analyses applied in the literature for mutualistic networks are rarefaction and accumulation curve analyses (Nielsen and Bascompte 2007, Chacoff et al. 2012, Rivera-Hutinel et al. 2012). For instance, Martinez et al. (1999) evaluated the relationships between food-web properties and richness among taxonomic webs and trophic webs using Monte Carlo simulations and confidence intervals.

Although a robust and well-designed sampling procedure is essential for the quality of data, the optimality of sample size and/or intensity effort depends on the objectives of the study (Orloci and Pillar 1989). Probably sampling most of species or interactions in a given area is not necessary to detect a specific pattern in a network. Similarly, the number of interactions needed to reach sampling sufficiency will be different according to the network metrics and the different types of taxonomic groups within mutualistic and antagonistic networks.

Here, we developed a statistical framework that aims to assess sampling sufficiency for some of the most used metrics in network ecology based on bootstrap resampling. The bootstrap method (Efron 1979, Efron and Tibshirani 1993) is based on the idea that the distribution of observed values in a sample is the best indicator of their distribution in the sampling universe from which the sample was taken. Our framework is very similar to that of Martinez et al. (1999), though our resampling is made according to the bootstrap method with replacement, ultimately mimicking the resampling of the sampling universe.

In this paper, we applied bootstrap resampling to evaluate sampling sufficiency for nestedness, modularity, and connectance metrics, using as test cases three quantitative mutualistic networks widely ranging in size. Using the bootstrap method to assess sampling sufficiency for network metrics, we attempted to answer the following question: how many interaction events or number of individuals will need to be sampled in order to reach stability for a given network and network metric?

Methods

Bootstrap resampling technique

We adapted the method of bootstrap resampling from Pillar (1998) to assess sampling sufficiency for network metrics. Our framework is a resampling technique that can generate confidence intervals for each network metric with increasing sample size (i.e., the number of interaction events sampled), which can be used to evaluate sampling sufficiency (Manly 1992, Pillar 1998). The observed values in a sample are taken as “a pseudo sampling universe”, the best available representation of the actual sampling universe from which the sample was taken: each sample obtained by resampling the sample with replacement is a “bootstrap sample”. The algorithm was the following:

1) Randomly selects a bootstrap sample of n_k interaction events with replacement from the observed sample (pseudo sampling universe) with n interaction events;

2) Computes the network metric of interest (θ_k) for the bootstrap sample and stores the resulting value;

3) Repeats steps 1 and 2 a large number of times (say 1,000 times);

4) Sorts the values of θ_k from the smallest to the largest value. Based on this ordering, delimits the confidence limits for a given specified probability α. For example, with 1,000 times and a probability α of 0.05, the lower confidence limit at a given sample size will be the value of θ_k at the 25th position and the upper limit will be the value of θ_k at the 976th position.

5) Repeats steps 1, 2, 3, and 4 for a new bootstrap sample size $n_k + \delta$, where δ is an increase in sample size, repeating the process up to sample size of n interaction events.

We also applied the same algorithm considering as sampling units, instead of the interaction events, the captured individuals based on which interaction events were observed.

Resampling data according to the bootstrap method will create a frequency distribution for the network metric of interest in samples with increasing size, mimicking the resampling of the sampling universe. The sample is considered sufficient within the range of sample sizes evaluated when the confidence limits reach stability or lie within an acceptable level of precision for the objectives of the study (Pillar 1998).

The method described here has been implemented in R (R Core Team 2013), using the package Bipartite (Dormann et al. 2008) to calculate the network metrics.

The bootstrap function and a script with an example are available as supplementary material.

Network metrics

We assessed sampling sufficiency for three of the most used network metrics:

1) Connectance (C), which is the proportion of links observed in a network relative to the possible number of links (Dunne et al. 2002), with values from 0 to 1. For bipartite networks it is calculated as $C=L/(I \times J)$, were L is the number of realized links; I and J are the number of species of each part, e.g., plants and animals. Connectance only distinguishes whether links are present or absent (unweighted, binary links), and the information about interaction frequencies is lost.

2) Nestedness is characterized by a core of highly connected species (generalists) that interact mainly with each other, and a group of specialist species that interact mainly with the generalist species (Bascompte et al. 2003). We used NODF (nested overlap and decreasing fill) algorithm proposed by Almeida-Neto et al. (2008), which corrects biases resulting from matrix fill and matrix dimensions. Similar to connectance, information about interaction frequencies is lost. NODF ranges from 0, (non-nestedness) to 100 (perfect nesting).

3) Modularity is characterized by the degree to which there are groups of nodes (species) that interact more among each other than with other groups (modules) in a network (Guimera and Amaral 2005). We assessed modules using the QuaBiMo algorithm that computes modules in quantitative bipartite networks, based on a hierarchical representation of species link weights and optimal allocation to modules (Dormann and Strauss 2013). It ranges between 0 (random network with no modules) to 1 (maximum modularity).

For nestedness and modularity metrics, we did not look for a P value with null models, since our aim was to evaluate the stability and precision of the metric value with increasing sample size.

Examples from Mutualistic Networks

We illustrate our framework with data from three quantitative networks of plant and frugivorous birds (Table 1). Network size in each dataset varied from 16 to 115 species and 17 to 2,745 interactions events. In two networks (Casas et al. 1 and 2, unpub. data, Chapter 2), the birds were captured with mist-nets, and then placed into fabric bags for 20 minutes to collect their faeces from bags. The seeds found in faecal samples were identified to the species level, when possible, to build an interaction matrix between birds and the plant that they consumed, with the number of interaction events (the number of times a specific bird species was captured with seeds of a specific plant species found in the faeces).

For another test case we used the plat-frugivorous birds network described by Schleuning et al. (2011), which was built based on the observation of focal plants, comprising primary and secondary forests and various vegetation strata. We used this entire network to have an example of a large quantitative network. To record bird species feeding on each focal plant species, frugivorous bird visits were recorded at each plant individual. The interaction frequency was defined as the number of fruit-eating individuals on a plant species independent of fruit handling.

To obtain the bootstrap sample (algorithm step 1), for Casas 2, we started with n_k = 10 interaction events with replacement, and we repeated the resampling 100 times (algorithm's step 3). We then increased sample size by five interaction events (δ), and the process was repeated with $n_k + \delta$ up to the maximum number of n events.

We started with $n_k = 7$ and used $\delta = 1$ for the smallest network Casas 1 (with only 17 interactions events), and $n_k = 30$ and $\delta = 50$ for the largest network Schleuning et al. (2011) (2,745 interactions events).

In a second analysis, we used as sample size the actual number of birds captured in our own datasets (Casas 1 and Casas 2). Our aim here was to investigate how many birds are necessary to reach sufficiency for each network metrics.

Table 1. Description of the three plant-frugivore networks datasets used with the bootstrap resampling technique.

Dataset	Habitat type	Location	Data type	Methodology	Bird	Plant
Casas et al. (unpub. data)	Atlantic Forest biome (forest-grassland mosaics)	Rio Grande do Sul state, Brazil	Number I_{jk} of captures of bird species *j* with seeds of plant species *k* in the faeces	Mist net	6	10
	Pampa biome (grassland-forest mosaics)				15	28
Schleuning et al. (2011)	Primary and secondary tropical rainforest	Africa, Kenya	Number of fruit-eating individuals on a plant species	Focal plant species	83	32

Results

The bootstrap confidence limits reached stability for two networks analysed for connectance, nestedness, and for modularity, therefore, samples were considered sufficient for these metrics (Figure 1). The smallest network (Casas 1) did not present sufficiency for any of the analysed metrics, since its confidence limit values were wide and unstable with increasing sample size up to 17 interaction events. For modularity of the Casas 1 network, e.g., the median with the maximum interactions events (17) was expected in 95% of the cases to lie between 0.46 and 0.76, differing by 0.30. This difference is too wide to be indicative of sampling sufficiency compared to the other networks (see detailed values of the Figure 1 in Supplementary Material Table 1).

The median value of connectance generated by the bootstrap for the second smallest network (Casas 2) reached stability with sample sizes larger than about 90 interaction events, but the nested median became stable with sample sizes larger than about 30 events (Figure 1). This means that increasing the sample size beyond 30 interaction events did not add new information that could affect the precision of the bootstrap estimation of nestedness. For the largest network (Schleuning et al. 2001), the stability of connectance and nestedness were reached, respectively, with sample sizes larger than about 2,300 and 800 interaction events. When we considered as a separated network only the data collected in secondary forest areas in Schleuning et al. (2011) (with 568 interaction events), we found different results: the stability of the metrics connectance and nestedness were reached with sample sizes larger than 60 interaction events (see Supplementary Material Figure 1). For modularity, the results were similar between the two networks from Schleuning et al. (2011).

Examples with number of bird individuals collected as sample size

Using number of individuals as sample size, we found similar results comparing with sample size of number of interactions (Figure 2). Again, the smallest network (Casas 1) did not present sufficiency for any of the metrics analysed, since its confidence limit values were wide and unstable with increasing sample size up to 14 captured individuals. The Casas 2 network was considered sufficient for all analysed metrics, as the bootstrap confidence limits reached stability (Figure 2; detailed values of confidence limits, median, and observed metric values of Figure 2 is available in Supplementary Material Table 1).

Results based on number of individual consumers were very similar compared to interaction events because we captured a bird individual, in most cases, with only one plant species in its faeces. Consequently, the matrix using the number of events and the number of individuals captured in our data were very similar (see Supplementary Material Table 2 for Casas 1 matrix with number of events as sample size, and Table 3 for Casas 1 with number of birds captured as sample size).

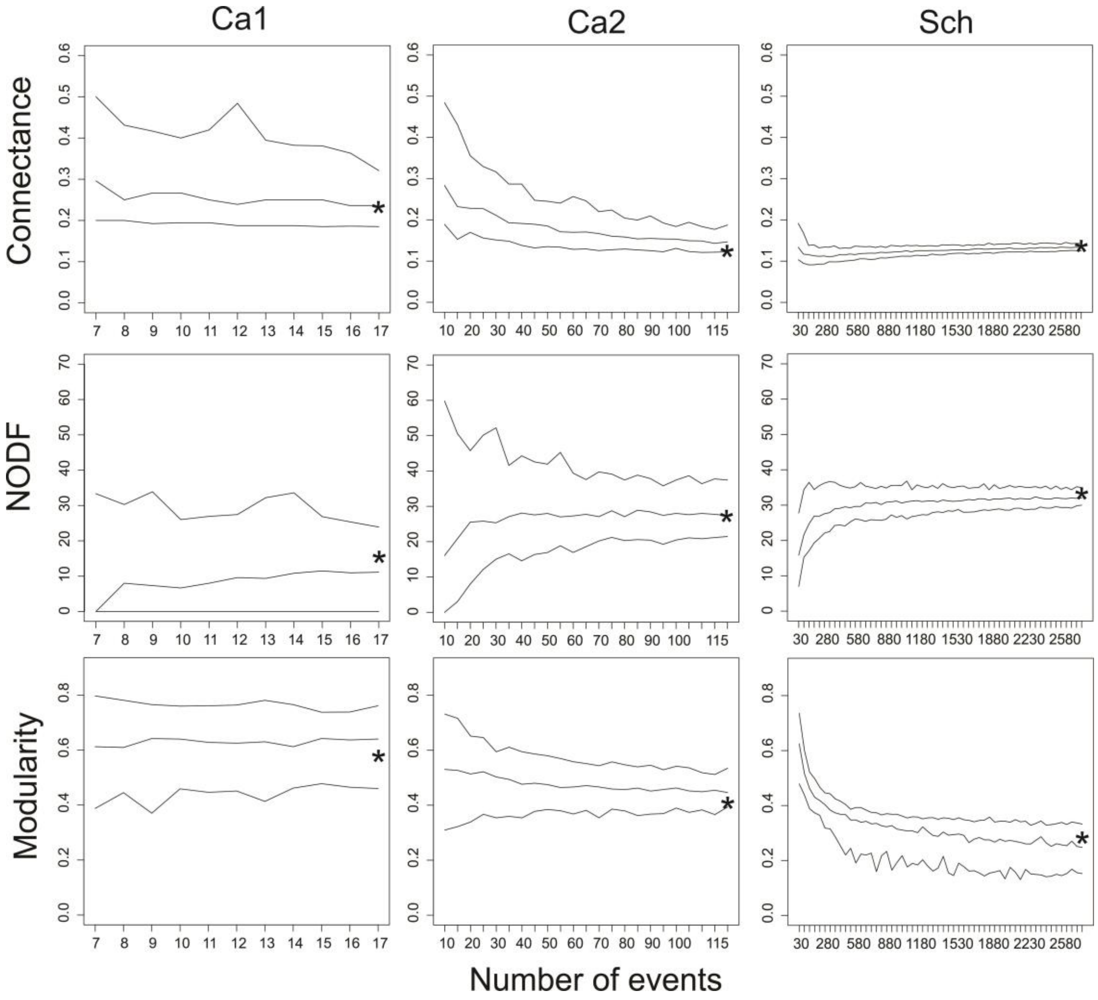

Figure 1. Observed value (star), median and confidence limits of Connectance, Nestedness (NODF) and Modularity metrics obtained by resampling with replacement method using three quantitative mutualistic networks (plants and frugivore birds) and number of interaction events as sample size. The 95% confidence intervals were set based on 100 resampling interactions at each sample size. See table 1 for detailed information of networks.

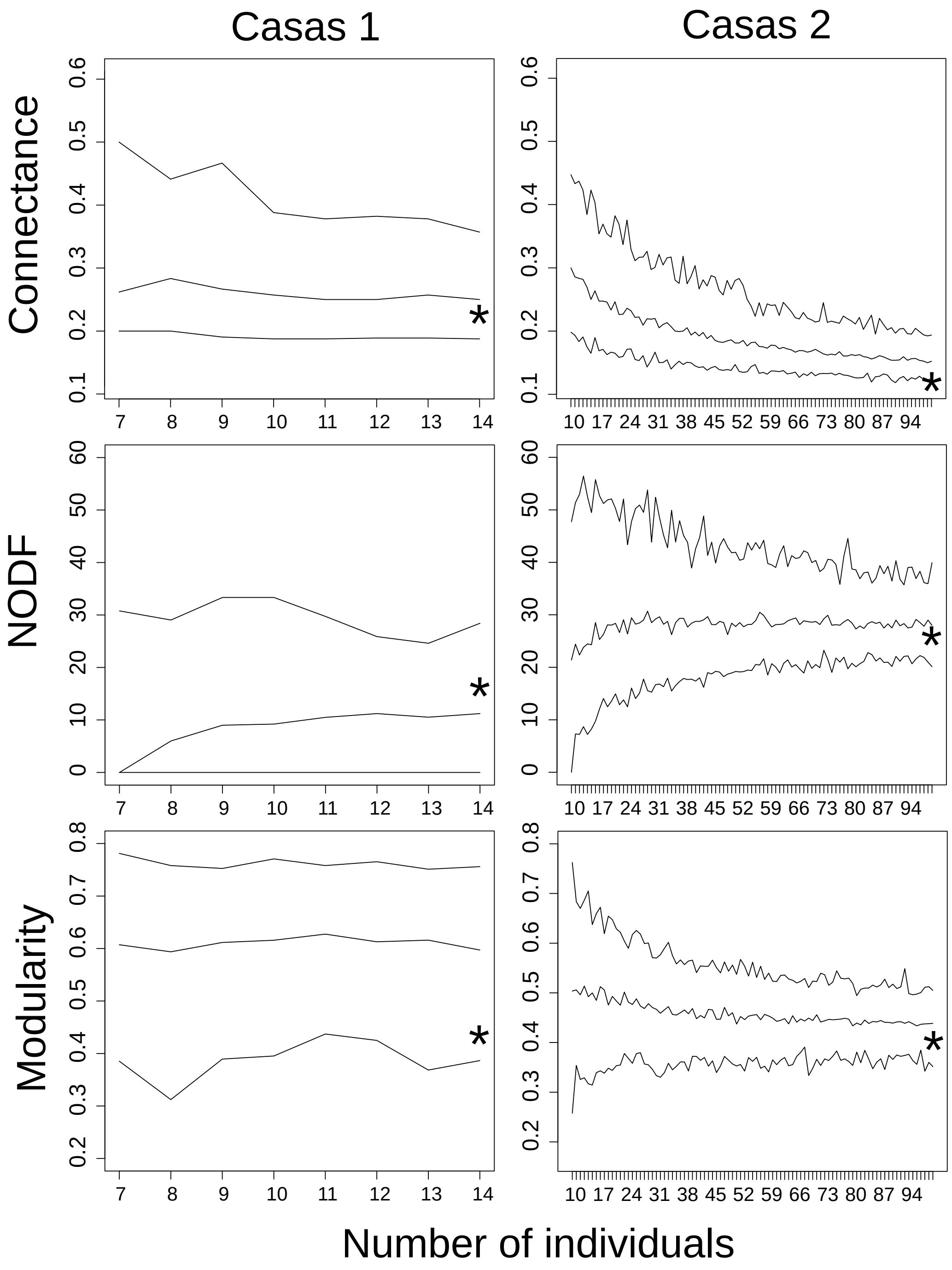

Figure 2. Observed value (star), median and confidence limits of Connectance, Nestedness (NODF) and Modularity metrics obtained by resampling with replacement method using two quantitative mutualistic networks (plants and frugivore birds) and number of bird individuals captured as sample size. The 95% confidence intervals are set based on 100 resampling interactions at each sample size. See table 1 for detailed information of seed-dispersal networks (bird and plant) Casas 1 and Casas 2.

Discussion

Our results suggest an important point: sampling sufficiency can be reached at different sample sizes for the same dataset depending on the metric of interest. Nielsen and Bascompte (2007), analysing the sensitivity of connectance and nestedness metrics to variation in sampling effort, also suggested that sampling intensity does not affect all network metrics in the same way, and that nestedness tends to stabilize rapidly with increasing sampling effort. Some median values of metrics generated by the bootstrap reached stability with less than 100 interaction events, meaning that sampling more interaction events probably would have not significantly affected the estimation of this network pattern.

However, it has been pointed out that studies of interactions should come from a robust and well-designed sampling procedure, mainly due to the influence of limited sampling effort in network properties (Dormann et al. 2009, Vázquez et al. 2009, Chacoff et al. 2012). In our results, even though the bootstrap confidence limits for some network metrics reached stability in networks with less than 50 species, the range of confidence limits for the largest network (Schleuning et al. 2011), with 115 species, was much smaller compared to the other two networks and, consequently, it is considered a more precise sample. Because the study of Schleuning et al. (2011) comprised primary and secondary forests and various vegetation strata, the interactions of this network are heterogeneous (high interaction diversity) and, consequently, the stability for these metrics was reached only with a larger sampling effort compared to the other networks.

With the bootstrap method we are looking for the effect of sampling bias on network metrics. In a different way, previous studies used analysis of fieldwork sampling techniques to investigate the extent to which conclusions are influenced by the way samples are collected. For example, Gibson et al. (2011) analysed the potential bias in network metrics when using time-based observations or transects in plant-pollinator networks, with rarefaction analysis and null models approach. Analogously, the bootstrap method can be used to compare two methodologies in terms of sampling sufficiency. In seed dispersal networks between plants and birds, e.g., the sampling hours or number of observed plant individuals as sample size (through transect or focal-plant methodologies) can be compared with the number of bird individuals captured (with mist net) that need to be sampled in order to reach stability for each network metric.

We assessed sampling sufficiency with the bootstrap method defining sample size as the number of interaction events, and with three mutualistic networks that differed regarding the sampling techniques that were applied to gather the data. Using interaction events, we lose information of the individual interactions, and have only the species interactions, with more dependence between observational units. Independence between sampling units is often an important assumption in data analysis, and the accuracy of the bootstrap method may be affected by lack of independence (Efron and Tibshirani 1993). We used the number of bird individuals captured only with the networks collected by ourselves (Casas et al., unpub. data, Chapter 2). Despite the advantage in using bird or plant individuals observed as sample sizes, the data available for most networks in literature and online databanks unfortunately only allow the extraction of interactions events.

Our method to assess the stability and the precision of the estimate of a specific network metric is general enough to be applied to different types of metrics and networks. However, the type of network metric has to provide a single value at the end of the analysis. For example, modularity involved an optimization method, but we could use it because it gives a modularity *Q* value (Dormann and Strauss 2013). Further, since the aim is to evaluate sampling sufficiency of network metrics, the network must be a quantitative one because data with the frequency of interactions are necessary for the resampling of sampling units (interaction events or captured individuals in the test cases). Our method cannot be used for small datasets with less than 10 species considering both trophic levels and less than 7 interaction events. Also, we stress that the bootstrap resampling does not add species and interactions in the network; it only resamples the data with replacement, mimicking the resampling of the presumed sampling universe represented by the observed sample.

The bootstrap method can be useful to empirical ecologists, since it shows the minimum number of interaction events (or other defined sampling units) necessary to reach sampling sufficiency for a specific network metric, or allows comparing sampling protocols in terms of effort to reach sampling sufficiency. The concerns on the effect of sampling effort on network metrics in mutualistic (Nielsen and Bascompte 2007, Dorado et al. 2011, Chacoff et al. 2012) and food webs (Goldwasser and Roughgarden 1997, Martinez et al. 1999) have grown in the last few years. We believe that our method is a significant contribution to assess sampling sufficiency in network ecology.

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SUPPLEMENTARY MATERIAL

Supplementary material Table 1: Extension of Figure 1 and 2, in the main text of the paper, and Supplementary Material Figure 1, with details of observed metric values, median and confidence intervals obtained by resampling with replacement method. The values are the 95% confidence intervals (CI) based on 100 resampling interactions only with the maximum number of interaction events for each seed dispersal network.

FIGURE 1

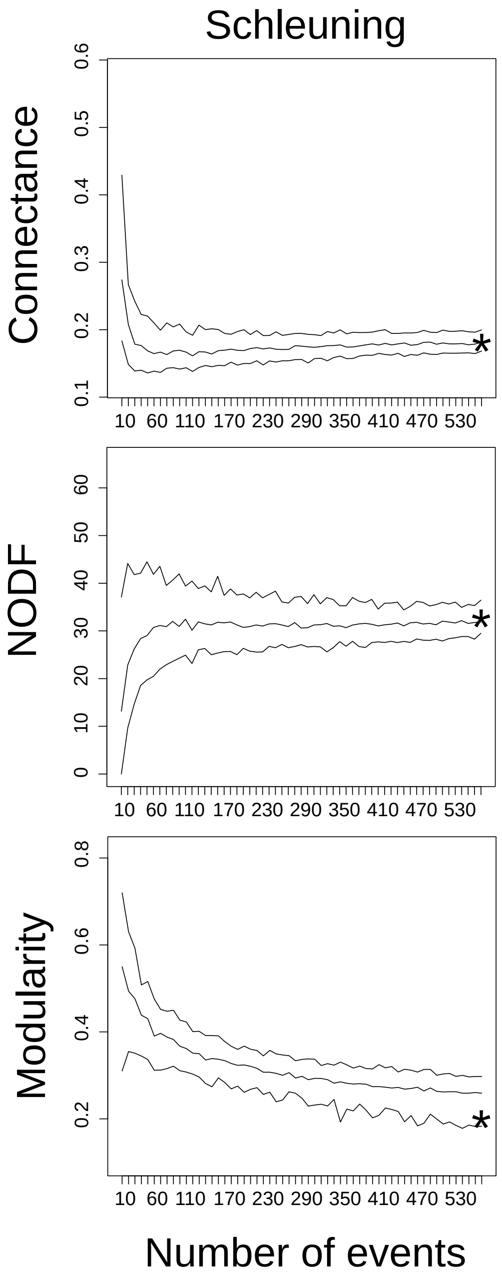
Network	Metric	Observed value	Median	Upper CI	Lower CI	Variation of the CI
Casas 2	Connectance	0,12	0,15	0,19	0,12	0,06
	NODF	27,32	27,76	37,52	20,91	16,61
	Modularity	0,42	0,45	0,53	0,39	0,14
Casas 1	Connectance	0,23	0,24	0,32	0,19	0,14
	NODF	15,83	11,11	23,91	0,00	23,91
	Modularity	0,60	0,64	0,76	0,46	0,30
Schleuning	Connectance	0,14	0,13	0,14	0,13	0,02
	NODF	33,15	32,04	34,98	30,01	4,97
	Modularity	0,23	0,25	0,33	0,15	0,18

FIGURE 2

Network	Metric	Observed value	Median	Upper CI	Lower CI	Variation of the CI
Casas 2	Connectance	0,12	0,15	0,19	0,13	0,07
	NODF	27,32	27,99	39,94	20,14	19,80
	Modularity	0,42	0,44	0,50	0,35	0,15
Casas 1	Connectance	0,23	0,25	0,36	0,19	0,17
	NODF	15,83	11,20	28,43	0,00	28,43
	Modularity	0,60	0,60	0,76	0,39	0,37

SUPPLEMENTARY MATERIAL FIGURE 1

Network	Metric	Observed value	Median	Upper CI	Lower CI	Variation of the CI
Schleuning	Connectance	0,1857765	0,18143	0,19963	0,16823	0,031405
	NODF	33,32811	31,8785	36,445	29,5073	6,93772
	Modularity	0,204762	0,259	0,2973	0,18295	0,1143461



Supplementary material Figure 1: Observed value (star), median and confidence limits of Connectance, Nestedness (NODF) and Modularity metrics obtained by resampling with replacement method using Schleuning et al. (2011) mutualistic network with the data collected only in a secondary forest, and number of interaction events as sample size. The 95% confidence intervals are set based on 100 resampling interactions at each sample size.

Supplementary material Table 2: Example of a matrix (network Casas 1) with interactions events and its dismember matrix used to resampling interactions with replacement according to the bootstrap method. Row represents the code of bird species and columms the plant species.

	Cist	Dran	Ilsp	Libr	Masp	Misp	Myat	Mysp	Scle	Scbu	TOTAL
Elme	0	0	0	0	0	1	0	3	0	0	4
Elsp	0	0	1	0	0	0	0	0	0	0	1
Syru	0	0	0	0	0	0	0	0	0	1	1
Tapr	0	0	0	1	0	0	0	0	1	1	3
Tual	1	0	0	2	0	0	1	0	1	0	5
Turu	0	1	0	0	1	0	0	0	1	0	3
	1	1	1	3	1	1	1	3	3	2	**17**

DISMEMBER MATRIX

Birds	**Plants**
Elme	Misp
Elme	Mysp
Elme	Mysp
Elme	Mysp
Elsp	Ilsp
Syru	Scbu
Tapr	Libr
Tapr	Scle
Tapr	Scbu
Tual	Cist
Tual	Libr
Tual	Libr
Tual	Myat
Tual	Scle
Turu	Dran
Turu	Masp
Turu	Scle

Supplementary material Table 3: Example of a matrix (network Casas 1) used with bootstrap method with the bird individuals captured as sample size. Row represents the code of bird species and columms the plant species.

	Cist	Dran	Ilsp	Libr	Masp	Misp	Myat	Mysp	Scle	Scbu	TOTAL
Elme1	0	0	0	0	0	1	0	0	0	0	1
Elme2	0	0	0	0	0	0	0	1	0	0	1
Elme3	0	0	0	0	0	0	0	1	0	0	1
Elme4	0	0	0	0	0	0	0	1	0	0	1
Elsp1	0	0	1	0	0	0	0	0	0	0	1
Syru1	0	0	0	0	0	0	0	0	0	1	1
Tapr1	0	0	0	1	0	0	0	0	1	0	2
Tapr2	0	0	0	0	0	0	0	0	0	1	1
Tual1	1	0	0	0	0	0	0	0	0	0	1
Tual2	0	0	0	1	0	0	1	0	0	0	2
Tual3	0	0	0	1	0	0	0	0	1	0	2
Turu1	0	1	0	0	0	0	0	0	0	0	1
Turu2	0	0	0	0	1	0	0	0	0	0	1
Turu3	0	0	0	0	0	0	0	0	1	0	1
	1	1	1	3	1	1	1	3	3	2	**17**

Supplementary material Bootstrap function (boot.net)

```
## Packages

require(SYNCSA) # for function progressbar
require(picante) # for function matrix2sample
require(bipartite)

## Description

# The boot.net function assesses sampling sufficiency for network
 metrics, based on bootstrap methods with replacement. It generates
 confidence intervals for each network metric with increasing sample
 size. Here we used as examples connectance, NODF and modularity
 metrics.

## Usage

# boot.net(web, n.min = 30, by = 10, runs = 1000, method = c("nodf",
 "connectance", "modularity"), progressbar = TRUE)

## Arguments

# web = interaction matrix
# n.min = minimum number of interaction for bootstrap method with
 replacement
# by = number of the sample size that will be increased in each step
 of the bootstrap method
# runs = number of times that the step will be repeated
# method = "nodf","connectance" or "modularity"
# progressbar = logic argument (TRUE OR FALSE) to show or not the
 progress bar of the function. Not compatible with RStudio

## Value

# a dataframe with the metrics values, where each column is a sample
 size (n.min, by, and number maximum of interactions) and row is the
 result for each bootstrap sample (runs)

boot.net<-function(web,n.min = 30, by=10, runs = 1000,
 method=c("nodf","connectance","modularity"),progressbar = TRUE){
 if(length(method) > 1){
     stop("\n Only one argument is accepted in method \n")
 }
 if(!(method=="nodf"|method=="connectance"|method=="modularity")){
     stop("\n Invalid method \n")
 }
 n.interaction<-sum(web)
 if(n.min>n.interaction){
     stop("n.min greater than number of interactions")
 }
 if(!by%%1==0){
     stop("by must be an integer")
 }
 if(by>n.interaction){
     stop("by greater than number of interactions")
 }
 n.link<-sum(ifelse(web>0,1,0))
 n.row<-dim(web)[1]
 n.col<-dim(web)[2]
 web.1<-as.matrix(matrix2sample(web))
```

```
web.2<-matrix(NA,n.interaction,2)
k=0
for(i in 1:n.link){
    for(j in 1:as.numeric(web.1[i,2])){
        k=k+1
        web.2[k,]<-c(web.1[i,c(1,3)])
    }
}
sample.seq<-seq(n.min,n.interaction,by)
if(!length(which(sample.seq==n.interaction))>0){
    sample.seq<-c(sample.seq,n.interaction)
}
RES<-matrix(NA,runs,length(sample.seq))
colnames(RES)<-paste("sample.size.",sample.seq,sep="")
k=0
l=0
nt=length(sample.seq)*runs
for(n in sample.seq){
    k=k+1
    i=0
    while(i<runs){
        i=i+1
        l=l+1
        web.boot<-matrix(0,n.row,n.col)
        colnames(web.boot)<-colnames(web)
        rownames(web.boot)<-rownames(web)
        sampled<-web.2[sample(1:n.interaction,n,replace=TRUE),]
        for(j in 1:n){
            row.boot<-which(rownames(web.boot)==sampled[j,1])
            col.boot<-which(colnames(web.boot)==sampled[j,2])
            web.boot[row.boot,col.boot]<-
web.boot[row.boot,col.boot]+1
        }
        web.boot<-
web.boot[!rowSums(web.boot)==0,!colSums(web.boot)==0, drop=FALSE]
        if(method=="nodf"){
            RES[i,k]<-nestednodf(web.boot, order = TRUE, weighted =
FALSE)$statistic[3]
        }
        if(method=="connectance"){
            RES[i,k]<-networklevel(web.boot,"connectance")
        }
        if(method=="modularity"){
            log <- capture.output(mod<-computeModules(web.boot,
steps=1E6))
            if(!is.null(mod)){
                RES[i,k]<-mod@likelihood
            }else{
                i=i-1
                l=l-1
            }
        }
        if(progressbar){
            ProgressBAR(l, nt, style = 3)
        }
    }
}
return(RES)
}
```

Supplementary material Table 4: Example of a matrix **(**network Casas 2) used in the script bellow (object “SS.txt”) of bootstrap method. Row represents the code of bird species and columms the plant species.

	Bato	**Cist**	**Dara**	**Euun**	**Euur**	**Misp**	**Mypa**	**Mysp**	**Myrt**	**Posa**	**Scbu**	**Stle**	**Trac**	**Mo01**	**Mo02**	**Mo03**	**Mo04**	**Mo05**	**Mo06**	**Mo07**	**Mo08**	**Mo09**	**Mo10**	**Mo11**	**Mo12**	**Mo13**	**Mo14**	**Mo15**
Bale	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chca	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyca	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elpa	1	0	4	0	0	0	0	3	0	0	4	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0
Elsp	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kncy	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myma	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pavi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Tapr	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tasa	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Tual	11	1	0	0	1	0	2	6	0	0	2	1	0	1	0	0	0	0	1	0	1	1	0	0	0	1	0	0
Tuam	0	0	2	0	0	0	4	11	0	0	2	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0
Turu	14	0	0	1	0	0	1	6	1	0	0	0	2	0	0	0	1	0	0	0	3	0	0	1	0	0	1	0
Viol	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Zoca	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Supplementary material Bootstrap script (Example)

```
# load packages, sources and files

require(SYNCSA)
require(picante)
require(bipartite)

source("boot_net.r")

webSS<-read.table("SS.txt",header=T)
webSS

# To calculate the observed metric value (e.g., NODF)

Obs_nodf<-nestednodf(webSS, order = TRUE, weighted =
 FALSE)$statistic[3]
Obs_nodf

# To calculate the network metric under assessment for the bootstrap
 samples with replacement method

Res_SS_nodf<-boot.net(webSS,n.min=10,by=5,runs=100,
 method="nodf",progressbar=TRUE)
Res_SS_nodf

# To calculate the median and confidence limits (lower and upper
 confidence intervals) of the bootstrap samples

SS_boot_median<-apply(Res_SS_nodf,2,median)
SS_boot_median
SS_boot_quantile_lower<-apply(Res_SS_nodf,2,quantile,probs=0.025)
SS_boot_quantile_lower
SS_boot_quantile_upper<-apply(Res_SS_nodf,2,quantile,probs=0.975)
SS_boot_quantile_upper

# To extract the sample size for the plot

sample.seq<-as.numeric(substr(colnames(Res_SS_nodf),13,100))
sample.seq

# Plot

plot(SS_boot_median,type="l",xaxt =
 "n",ylim=c(0,100),ylab="NODF",xlab="Number of events",las=1) # Draw
 median
points(SS_boot_quantile_lower,type="l") # Draw lower quantile
points(SS_boot_quantile_upper,type="l") # Draw lower quantile
axis(side = 1, at = 1:length(sample.seq), label = c(sample.seq)) #
 Add axis values and labels
points(length(sample.seq),Obs_nodf,pch="*",cex=3) # Add point for the
observed nodf
```

CONSIDERAÇÕES FINAIS

Esta tese abordou 1) a diversidade taxonômica e funcional de aves, e os padrões de organização de espécies de aves em comunidades refletindo convergência de atributos (TCAP: *Trait Convergence Assembly Patterns*) em transições floresta-campo, 2) a estrutura de redes de interação ave-planta, e 3) a avaliação de suficiência amostral de métricas de rede utilizando o método de reamostragem com reposição *bootstrap*. As principais conclusões de cada capítulo serão apresentadas a seguir.

A diversidade taxonômica diferiu significativamente somente entre floresta e borda de floresta, enquanto que floresta e borda diferiram do campo em relação à diversidade funcional. As áreas de campo apresentaram maior diversidade funcional. Tais resultados possibilitam inferir que estas duas facetas da diversidade podem capturar diferentes processos de organização de comunidades ao longo de mosaicos floresta-campo. No presente estudo, as unidades amostrais em campo variaram de áreas quase que completamente dominadas por uma paisagem campestre a campos com árvores esparsas e grandes espécies arbustivas. Essa heterogeneidade de ambientes provavelmente contribuiu para uma maior diversidade funcional de aves no campo, considerando os atributos selecionados.

Apesar da importância dos regimes de manejo (pastejo e fogo) para a conservação dos campos no Sul do Brasil, estes fatores podem ter um efeito de simplificação na estrutura da vegetação em sub-bosques florestais. A floresta e a borda não diferiram quanto à diversidade funcional, provavelmente devido à semelhança na estrutura da vegetação entre estes dois ambientes quando comparados com o campo. Apesar disto, a composição de espécies de aves diferiu entre todos os ambientes, o que pode evidenciar uma substituição de espécies ao longo da floresta-borda-campo. O padrão de organização a partir da convergência de atributos (TCAP) indicou que mecanismos de nicho atuam na organização da comunidade de aves, e que variáveis ambientais indicadas pelas mudanças na estrutura do habitat ao longo da transição floresta-campo estão agindo como filtros ecológicos.

Duas redes de interação ave-planta foram coletadas no Rio Grande do Sul na presente tese. Outras 14 redes de interação ave-planta foram pesquisadas na literatura (uma também coletada no Rio Grande do Sul) e três redes não são publicadas. Em relação aos padrões de redes de interação, o presente estudo concluiu que redes de dispersão de sementes entre ave-planta aparentemente apresentam um processo comum de organização, mesmo havendo diferenças na metodologia, intensidade de amostragem e continentes onde as redes foram coletadas. O padrão de aninhamento encontrado na maioria das 19 redes de interação pode sugerir que interações entre aves e plantas são robustas à extinção aleatória de espécies. A ausência de modularidade na maioria das redes pode indicar que redes de dispersão de sementes entre ave-planta possuem pouca interação específica. No entanto, 10 das 19 redes analisadas não foram modulares, e esta pequena diferença não é consistente o suficiente para assumir um padrão. Por outro lado, o tamanho pequeno da maioria das redes (menos de 100 espécies) pode ter influenciado no resultado obtido para a modularidade, devido à sensibilidade desta métrica ao tamanho da rede de interação.

Propriedades biológicas em mutualismo entre animal e planta (restrições) podem agir como filtros e limitar o crescimento da rede de interação devido à existência de ligações proibidas. Um par de interação que se torna impossível de ocorrer devido à incompatibilidade fenológica ou em relação ao tamanho do bico e do fruto, são exemplos de links proibidos em redes de dispersão de sementes. A predominância da lei de potência truncada (*truncated power law*) em relação à distribuição do grau provavelmente ocorreu devido a ligações proibidas em interações potenciais, estruturando estas redes mutualísticas entre ave-planta.

As espécies de aves com maior índice de importância nas duas redes descritas nesta tese foram *Turdus albicollis*, *T. rufiventris*, *Elaenia parvirostris* e *E. mesoleuca*. Como são consideradas generalistas, que ocorrem tanto na floresta, quanto na borda e nos campos (dados dos pontos de escuta do primeiro capítulo), estas espécies provavelmente têm maior importância no processo de expansão natural da floresta sobre o campo. Além disto, tais espécies possivelmente contribuem para a resistência da rede à perda de espécies, pois as espécies de plantas com poucas interações, geralmente mais sensíveis, estariam conectadas às aves generalistas, propiciando assim robustez à rede de interações. As espécies de plantas mais importantes como fonte de frutos foram *Myrsine* sp., *Scutia buxifolia* e *Ilex* sp.. Estas plantas possuem sementes pequenas, permitindo que aves com uma ampla gama de tamanho de bico possam consumi-los, o que provavelmente contribuiu para a importância destas plantas na organização das redes.

O método proposto para avaliar suficiência amostral em métricas de rede, utilizando o método de reamostragem com reposição *boostrap*, pode ser usado com diferentes tipos de métricas de rede, e a rede de interação a ser usada precisa ser quantitativa. Tal método foi testado com as métricas aninhamento, conectância e modularidade. Suficiência amostral pode ser alcançada com diferentes tamanhos amostrais para o mesmo conjunto de dados, dependendo da métrica de rede de interesse. Como tamanho amostral foi utilizado o número de eventos de interação. Em alguns casos, a mediana da métrica gerada pelo *bootstrap* atingiu estabilidade com um número amostral menor que 100 eventos. Isto significa que amostrando mais eventos provavelmente não alteraria a precisão da estimativa deste padrão da rede de interação.

O método também pode ser utilizado para comparar duas metodologias, utilizando como tamanho amostral o número de aves capturadas (por exemplo, através de redes de neblina) ou horas de esforço amostral (transecções ou planta focal) necessários a serem amostrados para atingir suficiência amostral para cada métrica de rede. O método de reamostragem com reposição *bootstrap* pode ser útil para ecólogos empíricos, pois mostra o número mínimo de eventos de interação (ou outra definição utilizada para unidade amostral) necessário para atingir suficiência amostral para uma métrica de rede específica. O método entretanto não pode ser utilizado para redes muito pequenas (com menos de 10 espécies considerando ambos níveis tróficos, e com menos de sete eventos de interação). Tendo em vista que a maioria das métricas de redes são sensíveis ao tamanho amostral, a preocupação com este efeito tanto em redes mutualísticas quanto tróficas, vem crescendo nos últimos anos nos estudos de rede. O método proposto nesta tese é uma importante contribuição neste tópico.

Retomando a pergunta feita na introdução geral para a rede pequena coletada (com 16 espécies): "será que esta rede não é aninhada ou o não aninhamento foi devido ao reduzido tamanho amostral?". Através do método proposto, foi possível responder que esta rede não foi suficientemente amostrada para nenhuma das métricas investigadas, ao contrário da segunda rede, com 43 espécies, que apresentou suficiência amostral para todas as métricas, pois os limites de confiança gerados pelo *bootstrap* alcançaram estabilidade.

O presente estudo inseriu-se no projeto SISBIOTA ("Biodiversidade dos campos e dos ecótonos campo-floresta no Sul do Brasil: bases ecológicas para sua conservação e uso sustentável"), abrangendo nove municípios no Rio Grande do Sul.

Além de contribuir para o levantamento da biodiversidade de aves nessa região, este estudo também contribuiu para um banco de dados de atributos funcionais de aves. Como o projeto SISBIOTA abrangeu diferentes grupos biológicos de fauna e flora amostrados nas mesmas unidades amostrais, este banco de dados permitirá que novas abordagens e hipóteses sejam testadas, aprimorando a identificação de padrões taxonômicos, funcionais e filogenéticos de organização de espécies em comunidades biológicas características dos campos sulinos e ecossistemas florestais associados.