VICTOR HUGO DUARTE DA SILVA

TEMPORAL VARIATION MECHANISMS OF PLANT-FLORAL VISITORS' NETWORKS IN A SEASONAL AREA OF CERRADO *STRICTO SENSU*

LAVRAS – MG
2021

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Prof. Dr. Lucas Del Bianco Faria
Orientador
Dr. Matheus Tenório Baumgartner
Coorientador

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Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Silva, Victor Hugo Duarte da.
Temporal variation mechanisms of plant-floralvisitors' networks in a seasonal area of Cerrado *stricto sensu* / Victor Hugo Duarte da Silva. - 2021.
154 p. : il.

Orientador(a): Lucas Del Bianco Faria.
Coorientador(a): Matheus Tenório Baumgartner.
Dissertação (mestrado acadêmico) - Universidade Federal de Lavras, 2021.
Bibliografia.

1. interação planta-inseto. 2. Variação temporal. 3. Ecologia de comunidades. I. Faria, Lucas Del Bianco. II. Baumgartner, Matheus Tenório. III. Título.

VICTOR HUGO DUARTE DA SILVA

TEMPORAL VARIATION MECHANISMS OF PLANT-FLORAL VISITORS' NETWORKS IN A SEASONAL AREA OF CERRADO *STRICTO SENSU*

MECANISMOS DE VARIAÇÃO TEMPORAL DE REDES PLANTA-VISITANTE FLORAL EM UMA ÁREA SAZONAL DE CERRADO *STRICTO SENSU*

Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ecologia Aplicada, área de concentração em Ecologia e monitoramento de ecossistemas sob interferência antrópica, para a obtenção do título de Mestre.

Aprovada em 03 de maio de 2021.

Dr. Paulo Inácio de Knegt López de Prado (USP)

Dra. Yasmine Antonini Itabaiana (UFOP)

Prof. Dr. Lucas Del Bianco Faria
Orientador
Dr. Matheus Tenório Baumgartner
Coorientador

LAVRAS – MG

2021

À minha mãe e minha vó, que sempre foram fonte de luz na minha vida,

Ao meu irmão, por mesmo longe sempre estar perto,

E aos meus amigos, que sempre arrancam os melhores sorrisos do meu rosto,

Dedico.

AGRADECIMENTOS

Primeiramente, gostaria de agradecer a Deus, por me guiar ao longo de todo o meu caminho e por me dar forças para correr atrás dos meus sonhos.

Agradeço à minha mãe, à minha vó e ao meu irmão. Vocês sempre acreditaram em mim e, se estou onde estou hoje, é por vocês serem a minha base, a minha força e o motivo de eu estar aqui. Eu amo vocês!

Agradeço a todos os meus amigos, por todos os momentos incríveis que tivemos até hoje. Vocês são luzes na minha vida. Luzes que eu tive a oportunidade de conhecer andando pela UFLA, dançando no centro de cultura ou nas festas mais aleatórias que Lavras me proporcionou. Continuem brilhando, para mim e para outras pessoas. A amizade de cada um de vocês, fez e faz a vida ser leve e valer a pena. O mundo precisa de mais pessoas assim.

Agradeço em especial à Ana Beatriz Ligo, Ana Carolina Faria Martins, Ana Fávaro, Giovanna de Oliveira dos Reis, Júlia Isnard Moulin Gomes, Jonatas Anselmo Fonda Antonio e ao Kelvi Wilson Evaristo Miranda, por serem sol na minha vida, por me levantarem quando estou caído, por me fazerem rir de coisas idiotas, por escutarem os meus dilemas, por fazerem eu me apegar aos nossos momentos mais simples e por me proporcionarem momentos únicos na vida. Vocês são incríveis e quero vocês para sempre na minha vida.

Agradeço à UFLA, por ter sido meu lar ao longo dos últimos 6 anos. Agradeço a todos os professores, aos colaboradores, as “tias” da limpeza (que sempre me cumprimentam com um sorriso sincero no rosto) e a todos que, de alguma forma, fazem a UFLA ser gigante como é. A minha vitória também é de vocês.

Agradeço ao Programa de Pós-graduação em Ecologia Aplicada da UFLA, especialmente a todos os professores com quem tive o prazer e a oportunidade de aprender e de conversar. Tenham certeza que vocês fizeram um profissional melhor.

Agradeço à Ellen, a nossa “deusa” da Ecologia, que sempre esteve disponível a me ajudar e a tirar todas as minhas dúvidas. Obrigado por ser incrível e única, Ellen.

Agradeço ao Dr. Lucas Del Bianco Faria, por ter sido um orientador incrível. Você me permitiu aprender e crescer com você. Você acreditou em mim, quando nem eu mesmo acreditei. Obrigado por compartilhar sua sabedoria comigo, por me fazer rir nas nossas reuniões, por me ensinar tudo que eu sei de estatística e por ter me feito um profissional melhor.

Agradeço ao Dr. Matheus Tenório Baumgartner, por me coorientar e por me apresentar uma visão diferente da ciência. Suas contribuições foram valiosas durante todo esse tempo.

Agradeço aos meus companheiros do Laboratório de Ecologia e Complexidade (LEC): Tamires, Tiago, Maria, Mariane, Joyce, Gabriela e Bruna. Obrigado por me receberem tão bem, por me ajudarem com tudo que eu precisei no início, por me fazerem rir das coisas mais idiotas possíveis e por cada conversa que tivemos. Vou sentir saudades disso.

E por fim, agradeço o apoio da Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), pois o presente trabalho somente pode ser realizado com apoio da Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), que concedeu a minha bolsa de estudos.

A todos que passaram pela minha vida, o meu muito obrigado. Vocês me fizeram um profissional e uma pessoas melhor, tenham certeza disso!

“Animals will attack if provoked, to protect their family or just to put food on the table, yet man still hasn’t learned these simple basics of life. Somehow I don’t think we are the intelligent species after all.”

Anthony T. Hincks

RESUMO GERAL

Redes de interação variam no tempo e no espaço. Isso acontece devido ao fato de serem influenciadas por mecanismos bióticos e abióticos, que atuam diretamente sobre a probabilidade de interação das espécies. Contudo, assim como a grande maioria dos estudos em ecologia, a nossa percepção sobre as redes de interação é substancialmente influenciada pela amostragem, seja pela variedade de métodos de coleta, ou pela capacidade do esforço amostral em estimar com precisão a organização das interações. Posto isto, esta dissertação está dividida em dois capítulos. No primeiro capítulo, investigamos a influência de mecanismos bióticos (abundância de visitantes florais e número de flores) e abióticos (temperatura e precipitação) sobre métricas de redes de interação planta-visitante floral em ambientes de Cerrado, ao longo de uma série temporal de doze meses. No segundo capítulo, utilizando as mesmas redes, nos preocupamos em avaliar como o esforço amostral influencia as estimativas de métricas das redes em diferentes estações do ano (primavera, verão, outono e inverno). Para o capítulo um, observamos que as redes de interação foram potencialmente mais sensíveis à perda de espécies durante a estação seca (caracterizada por períodos de menor temperatura e precipitação), enquanto que a abundância de visitantes florais aumentou a modularidade e diminuiu a conectância ponderada das redes. O número de flores abertas e aptas a receberem visitantes não apresentou efeito significativo sobre as métricas, sugerindo a abundância de visitantes como efeito limitante das interações. No segundo capítulo, usando curvas de acumulação de espécies e modelos não lineares, observamos que as estações do ano diferem em relação ao esforço amostral necessário para atingir a estabilidade das redes em seis propriedades de redes: número de animais, número de plantas, número de interações, diversidade de Shannon, especialização da rede (H2) e conectância ponderada. Observamos que o mesmo esforço amostral produziu estimativas significativamente diferentes de cada métrica, sendo essas diferenças geralmente explicadas pela riqueza e abundância das espécies obtidas em cada estação. Por fim, nossos resultados reiteram a importância de se estudar padrões de interações e os mecanismos por trás delas, visto que as interações entre espécies são determinantes da biodiversidade e mantêm o funcionamento dos ecossistemas.

Palavras-chave: Estrutura de redes de interação, esforço amostral, variação espaço-temporal, sazonalidade e interação inseto-planta.

GENERAL ABSTRACT

Interaction networks vary in time and space. This happens because they are influenced by biotic and abiotic mechanisms, which act directly on the probability of species interaction. However, like the vast majority of studies in ecology, interaction networks are also influenced by sampling, either by the influence of different sampling methods, or by the low sampling effort. That said, this dissertation is divided into two chapters. In the first chapter, we verified the influence of biotic mechanisms (abundance of floral visitors and number of flowers) and abiotics (temperature and precipitation) on metrics of floral plant-visitor interaction networks over a 12-month time series. In the second chapter, we are concerned with verifying the sampling effort required in each of the four seasons (spring, summer, autumn and winter) and whether that effort differed between seasons. For chapter one, we observed that the dry season (characterized by periods of lower temperature and precipitation) negatively affects the robustness of the networks; while, the abundance of floral visitors increases modularity and decreases the weighted connectance of networks. The number of flowers had no significant effect on the metrics. In the second chapter, using species accumulation curves and non-linear models, we observed that the seasons differ in relation to the sampling effort required to achieve stability in six properties of the network (animals richness, plants richness, interactions richness, Shannon's diversity, network specialization (H2) and weighted connectivity). We observed that the seasons of the year differed in each metric, and these differences are generally explained by the richness and abundance of the species. Finally, our results reiterate the importance of studying the interactions and the mechanisms behind them, since the interaction between species is what forms biodiversity and maintains the functioning of the ecosystem.

Keywords: Interaction networks structure, sampling effort, space-time variation, seasonality.

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PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

Comunidades ecológicas são formadas por conjuntos de espécies que interagem entre si, em um dado espaço e durante um mesmo período de tempo (CARADONNA; WASER, 2020). Essas espécies podem interagir de diferentes maneiras, sendo as mais conhecidas: o mutualismo, o parasitismo, a predação e a competição (BEGON; TOWNSEND; HARPER, 2007). Assim como as espécies, as interações ecológicas variam ao longo do tempo e do espaço, podendo tal variação ocorrer na frequência em que ocorrem ou em suas identidades (quem interage com quem) (TRØJELSGAARD et al., 2015). Tais variações são importantes não somente para a formação da comunidade, mas também para a manutenção da biodiversidade, do funcionamento e da dinâmica dos ecossistemas (ANDRESEN; ARROYO-RODRÍGUEZ; ESCOBAR, 2018).

As mudanças espaciais e temporais nas comunidades biológicas podem ser causadas por diferentes fatores (PERALTA et al., 2020a; PETANIDOU et al., 2018; VÁZQUEZ; CHACOFF; CAGNOLO, 2009). Entre os principais mecanismos causadores de variação espacial em comunidades biológicas, estão a estrutura da paisagem, como relevo e transições entre biomas, e a heterogeneidade ambiental (MOREIRA et al., 2018; MOREIRA; BOSCOLO; VIANA, 2015). Já em relação a variação temporal, os principais mecanismos direcionadores são o clima, a abundância relativa das espécies, a sobreposição fenológica e a correspondência de traços entre as espécies (CLASSEN et al., 2020; PERALTA et al., 2020a; PETANIDOU et al., 2018; VÁZQUEZ et al., 2009). Assim como podem atuar conjuntamente sobre a organização das comunidades em ambas as escalas, espaço e tempo também atuam diretamente na probabilidade de interações das espécies, seja por meio da quantidade de recursos e consumidores disponíveis ou de restrições biológicas que impedem que as espécies interajam (TYLIANAKIS; MORRIS, 2017; VÁZQUEZ et al., 2007).

Uma das maneiras mais usadas para entender como e porque as comunidades biológicas variam são as redes de interação (LANDI et al., 2018). Redes de interação são um retrato do que acontece entre as espécies de uma comunidade biológica na medida em que representam as espécies (nós da rede) e as interações entre elas (links entre os nós) (BASCOMPTE; JORDANO, 2014). Logo, através das redes de interações, seja pela sua forma visual (grafos bipartidos) ou pelas suas propriedades quantitativas (métricas), é possível observar como as interações mudam e as consequências dessas mudanças sobre a estrutura e a complexidade das comunidades ecológicas (BASCOMPTE; JORDANO, 2014; LANDI et al., 2018). Além disso, as métricas que descrevem redes de interação permitem enxergar as diferentes facetas de uma comunidade biológica, uma vez que são capazes de fornecer informações por espécie (e.g., quantidade de links por espécie) e até de toda comunidade (como por exemplo, a capacidade da comunidade de resistir a um distúrbio, ou seja, robustez) (LANDI et al., 2018).

Diferentes mecanismos que atuam sobre a variação na estrutura das redes ecológicas já foram explorados em trabalhos anteriores (CARADONNA; WASER, 2020; DALSGAARD et al., 2017; MORENTE-LÓPEZ et al., 2018; PETANIDOU et al., 2018; SONNE et al., 2020; TAKEMOTO; KANAMARU; FENG, 2014). González-Castro et al. (2012) observaram em redes de interação planta-frugívoro que a sobreposição fenológica e as características específicas das espécies são muito mais importantes para a probabilidade de interações do que a própria abundância das espécies. Semelhante a isso, Peralta et al. (2020) demonstraram que a correspondência entre características e a sobreposição fenológica entre as espécies estão intimamente relacionadas à estabilidade das interações planta-polinizador e, portanto, são determinantes da variação temporal dessas redes. Por outro lado, os resultados de Caradonna et al., (2017) mostram que a sobreposição fenológica e a abundância relativa das espécies são os principais fatores que determinam as interações em redes planta-polinizador. Portanto, a forma de atuação de tais mecanismos não é conservativa e as comunidades tendem a ser afetadas diferentemente por cada mecanismo.

Tendo em vista que não há um consenso entre os fatores das variações temporais em redes ecológicas, esse estudo foi realizado com o intuito de entender como uma comunidade biológica, composta por plantas e visitantes florais, varia ao longo tempo e quais os mecanismos por trás de tal variação. Além disso, nós discutimos sobre a sensibilidade do esforço amostral, considerando as diferentes estações do ano, já que muitas vezes os efeitos da suficiência amostral são negligenciados em estudos com redes de interação (DALSGAARD et al., 2017).

2 REFERENCIAL TEÓRICO

2.1 Interações ecológicas

Estudos anteriores mostraram que a estrutura, a estabilidade e o funcionamento das comunidades ecológicas, além de depender da composição de espécies, também dependem da forma como essas espécies interagem (BARTOMEUS et al., 2016; TYLIANAKIS et al., 2008). Isso acontece, porque as espécies raramente vivem isoladas e a maioria delas interage com outras espécies através de diferentes tipos de interação (BEGON; TOWNSEND; HARPER, 2007; TOWNSEND; BEGON; HARPER, 2008). Entre os principais tipos de interação ecológica estão o parasitismo, a competição, a predação e o mutualismo (HEMBRY; WEBER, 2020), e diferem na maneira com que os participantes da interação são afetados (positiva ou negativamente) (BEGON; TOWNSEND; HARPER, 2007). No parasitismo, por exemplo, o parasita se beneficia da interação, enquanto que o hospedeiro é prejudicado (+ -) (HATCHER; DICK; DUNN, 2006). Nesse caso, dizemos que essa é uma relação ecológica interespecífica desarmônica (HATCHER; DICK; DUNN, 2006). Já no mutualismo, ambas as espécies em interação são beneficiadas (+ +) e, nesse caso, dizemos que essa é uma relação ecológica interespecífica harmônica (BASCOMPTE; JORDANO, 2014).

Além de terem papel fundamental para a conservação de comunidades ecológicas e dos ecossistemas, as interações também podem influenciar na evolução das espécies, já que elas podem impactar processos associados à dinâmica populacional, à especiação e à seleção natural (HEMBRY; WEBER, 2020; ZOGRAFOU et al., 2020). Um exemplo clássico é o estudo de Janzen (1966), onde o autor descreve a coevolução entre acácias e formigas. Durante o processo de coevolução, as acácias desenvolveram espinhos ocos e passaram a produzir néctar nessas estruturas, atraindo as formigas que começaram a nidificar nesses espinhos (JANZEN, 1966).

2.2 Interações mutualísticas

As interações mutualísticas são aquelas em que duas espécies em interação se beneficiam (BASCOMPTE; JORDANO, 2014). Esse tipo de interação está amplamente difundido na natureza e está ligado a processos ecológicos como facilitação e às principais transições evolutivas (CHOMICKI; KIERS; RENNER, 2020; WEST et al., 2015). As interações mutualísticas mais estudadas são a dispersão de sementes por animais herbívoros, a defesa contra herbivoria feita por parte das formigas presentes em plantas hospedeiras e a polinização cruzada realizada por animais, principalmente insetos e aves (BEGON; TOWSEND; HARPER, 2007; BASCOMPTE; JORDANO, 2014; BURKLE; ALARCON, 2014; HEMBRY; WEBER, 2020) .

A dispersão de sementes é fundamental para o ciclo de vida das plantas e consiste no deslocamento das sementes para longe da planta-mãe, podendo ser realizada tanto por fatores ambientais, como o vento e chuva, quanto por animais (GONZÁLEZ-CASTRO; CALVIÑO-CANCELA; NOGALES, 2015). Entre os principais animais dispersores de sementes estão as aves e os morcegos (JORDANO et al., 2006). Esses frugívoros se alimentam de frutas carnosas comendo a polpa e descartando as sementes (ZWOLAK; SIH, 2020). Como consequência disso, as plantas tem suas sementes levadas para longe, diminuindo assim a competição e a exposição a predadores, o que resulta em maiores chances de sobrevivência e colonização de novas áreas (GONZÁLEZ-CASTRO; CALVIÑO-CANCELA; NOGALES, 2015).

Já no caso da interação entre formigas e plantas, as formigas auxiliam na defesa das plantas contra a herbivoria (JANZEN, 1966; ROSUMEK et al., 2009). Nesse caso, as formigas se alimentam e/ou se abrigam em estruturas da planta e afastam os herbívoros que poderiam causar prejuízos à planta hospedeira (JANZEN,1966). Rosumek et al. (2009), em estudo experimental de remoção das formigas, observaram que as plantas apresentaram maiores taxas de herbivoria quando as formigas estavam ausentes. Além disso, os autores observaram que a presença/ausência de formigas influenciava alguns parâmetros do fitness das plantas, entre eles a biomassa, a produção de folhas e a reprodução (ROSUMEK et al., 2009). Portanto, neste caso, acredita-se que as formigas podem estar contribuindo diretamente para a persistência das plantas, seja pela diminuição da herbivoria ou pela influência no fitness.

Por fim, as interações entre plantas e polinizadores é considerada uma das mais importantes, visto que a sobrevivência e o bem-estar do ser humano estão diretamente relacionados a elas (BPBES, 2018). A polinização é um dos principais impulsionadores da produtividade agrícola mundial e a maioria das plantas tropicais dependem, em algum grau, da polinização natural (FOOD AND AGRICULTURE ORGANIZATION, 2018). Os insetos são os principais representantes dos polinizadores, sendo os mais abundantes: abelhas, moscas, borboletas, mariposas, vespas e besouros; além dos polinizadores vertebrados como aves e morcegos (BPBES, 2018). Nesse tipo de interação, o polinizador se alimenta do néctar da planta que, em troca, tem seu pólen transportado de uma planta para outra pelos insetos (NICOLSON; WRIGHT, 2017).

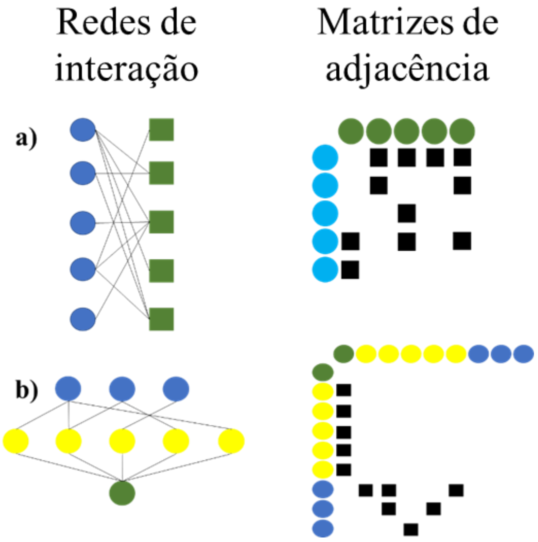
2.3 Interações mutualísticas e redes de interação

Uma vez que os mutualismos presentes na natureza são moduladores-chave da biodiversidade global e desempenham importantes papéis nos processos de diversificação e coexistência das espécies, entender como tais interações ocorrem é essencial (BASTOLLA et al., 2009; CHOMICKI et al., 2019). Uma maneira frequentemente usada para compreender as interações mutualísticas são as redes de interação (BASCOMPTE; JORDANO, 2014).

Redes de interação são baseadas em uma abordagem de teoria de redes complexas onde as espécies que participam do mutualismo são representadas por nós e as interações representada por links entre eles (BASCOMPTE; JORDANO, 2014). Existem dois tipos de redes de interação: redes unipartidas - que mostram apenas um grupo de nós e todos eles podem se conectar entre si, ou seja, um conjunto de espécies em diferentes níveis tróficos que podem interagir com espécies do mesmo e de outros níveis tróficos (Figura 1) - e redes bipartidas - que mostram dois grupos de nós se conectando apenas com nós de outro grupo, ou seja, espécies de um mesmo grupo não interagem entre si (Figura 1) (BASCOMPTE; JORDANO, 2014; DEHLING, 2018).

No caso da interação entre plantas e polinizadores (também chamados de visitantes florais), as interações mutualísticas são comumente representadas através de redes bipartidas (BASCOMPTE; JORDANO, 2014). Elas são construídas a partir de matrizes de adjacência, onde os dados de entrada podem ser do tipo presença/ausência ou frequência de interação (Figura 1) (LANDI et al., 2018). Se a matriz de adjacência for baseada em dados de presença/ausência, é dito que as redes são não-ponderadas. Já se a matriz for baseada em dados de frequência de interação, é dito então que as redes são ponderadas (LANDI et al., 2018).

Figura 2.1 - Esquema representativo de redes de interação (à esquerda) e matrizes de adjacência (à direita).



Legenda: A) Redes de interação bipartida e sua matriz de adjacência. B) Rede de interação unipartida e sua matriz de adjacência. As espécies são representadas pelos círculos coloridos e as interações entre elas pelos links. No caso da matriz de incidência, as interações são representadas pelos quadrados pretos. As cores dos círculos coloridos indicam os diferentes níveis tróficos.

Fonte: (LEWINSOHN et al., 2006).

Além de permitir uma visualização gráfica das interações ecológicas, as redes também fornecem informações quantitativas (métricas) que descrevem as espécies e e possibilitam a obtenção de informações sobre a estrutura, a complexidade e a estabilidade das redes como um todo (BASCOMPTE; JORDANO, 2014; LANDI et al., 2018). Entre as principais métricas de redes de interação mutualística estão o aninhamento, a conectância, a densidade de links, a força de interação e a modularidade (ver Tabela 1 para uma breve de descrição de cada métrica) (BASCOMPTE; JORDANO, 2014; LANDI et al., 2018).

Quadro 1 - Métricas de redes de interação, sua definição e qual característica principal representam.

Métrica	Definição	Característica que representa
Conectância	A proporção de links realizados entre todos os que são possíveis. É baseada em dados de presença/ausência. $C = \frac{\text{número de links realizados}}{\text{riqueza de espécies}^2}$	Complexidade
Densidade de links	O número médio de interações (links) por espécie.	Complexidade
Força de interação	O peso de uma interação dentro da matriz de interação.	Complexidade
Riqueza de espécies	Número total de espécies na rede.	Complexidade
Aninhamento	O grau em que as espécies com poucos links (especialistas) interagem com um subconjunto de parceiros das outras espécies (generalistas).	Arquitetura
Modularidade	A extensão máxima que uma rede pode ser dividida em módulos delimitados por grupos de espécies.	Arquitetura
Robustez	Resistência de uma comunidade a extinções secundárias, seguidas de uma remoção primária de espécies.	Estabilidade
Especialização da rede (H2)	Uma medida a nível de rede que estima o nível médio de especialização entre os pares de espécies.	Complexidade

Shannon’s diversity	A diversidade de Shannon das interações.	Complexidade
Aninhamento ponderado baseado em NODF	Índice para aninhamento baseado no índice NODF, proposto por Almeida-Neto et al., 2008.	Arquitetura
Uniformidade da interação	Uniformidade de Shannon para as interações.	Complexidade
Conectância ponderada	A proporção de links realizados entre todos os que são possíveis (número de links/riqueza de espécies ^{2}) baseada em dados de frequência de interação.	Complexidade
Assimetria da força de interação (ISA)	Explica a assimetria de dependência entre ambos os níveis tróficos.	Complexidade
Assimetria da especialização	Assimetria (nível trófico superior vs. inferior) de especialização base na especialização de Blüthgen (d’). Valores positivos indicam maior especialização do nível trófico superior.	Complexidade

Fonte: Adaptado de Antoniazzi; Dáttilo; Rico-Gray (2018) e Landi et al. (2018).

2.4 Padrões em redes de interação mutualística

Alguns padrões das métricas das redes foram identificados em estudos anteriores (DALSGAARD et al., 2017; JORDANO, 1987; SEBASTIÁN-GONZÁLEZ et al., 2015; TRØJELSGAARD; OLESEN, 2013). Esses padrões são hipotetizados como estando diretamente relacionados à estabilidade da comunidade e ao funcionamento dos ecossistemas (SCHLEUNING; FRÜND; GARCÍA, 2015; THEBAULT; FONTAINE, 2010). O principal padrão encontrado em redes de interação mutualísticas é que elas apresentam baixa conectância e são altamente modulares ou aninhadas, tendo em vista que estas duas últimas métricas são conceitualmente opostas(BASCOMPTE et al., 2003; JORDANO, 1987; OLESEN et al., 2007).

A conectância está diretamente relacionada à riqueza de espécies. Quanto mais espécies compõem uma rede, menor a conectância (OLESEN; JORDANO, 2002). Isso porque a própria conectância é calculada usando a riqueza de espécies e, portanto, redes com mais espécies têm menor probabilidade de interação entre um dado par de espécies(LANDI et al., 2018). Não obstante, estudos anteriores observaram que a riqueza de espécies também aumenta a modularidade e, consequentemente, diminui o aninhamento.

Modularidade e aninhamento também estão diretamente associados a temperatura e precipitação (DALSGAARD et al., 2011, 2013, 2017; TRØJELSGAARD; OLESEN, 2013). Em redes mutualísticas do tipo planta-polinizador, a modularidade e a especialização são positivamente afetadas por maiores níveis de precipitação (DALSGAARD et al., 2011, 2013, 2017). Já em redes mutualísticas do tipo planta-dispersor de sementes, o aninhamento aumenta tanto com a temperatura quanto com a precipitação (RICO-GRAY et al., 2012; SEBASTIÁN-GONZÁLEZ et al., 2015).

Por fim, análises anteriores mostraram que as ligações em redes mutualísticas, são fracas e altamente assimétricas (DÍAZ-CASTELAZO et al., 2010). Esses padrões de rede podem ser explicados por processos ecológicos e de história evolutiva, mas existem poucos dados sobre sua estabilidade espacial e temporal (DÍAZ-CASTELAZO et al., 2010).

2.5 Variação temporal em redes de interação mutualística e os mecanismos responsáveis

Apesar de padrões comuns entre redes de interação, elas apresentam variações ao longo do tempo e do espaço, que podem ser causadas por diferentes fatores (CARADONNA; WASER, 2020). Por exemplo, estudos anteriores mostraram que a variação espacial está diretamente relacionada a fatores ambientais locais, heterogeneidade espacial e mobilidade animal (BURKLE; ALARCON, 2011; MORALES; VÁZQUEZ, 2008), enquanto que a variação temporal está diretamente ligada a abundância das espécies, sobreposição fenológica e correspondência de traços (PERALTA et al., 2020). O clima também influencia a variação espaço-temporal das redes, uma vez que ele influencia o ciclo de vida e a dinâmica populacional das espécies (PETANIDOU et al., 2014, 2018; TYLIANAKIS; MORRIS, 2017).

Entre os principais mecanismos impulsionadores da variação temporal em redes de interação mutualística estão a abundância relativa das espécies, a sobreposição fenológica, a correspondência de traços e o clima (PERALTA et al., 2020a; VÁZQUEZ; CHACOFF; CAGNOLO, 2009). Com exceção do clima, os mecanismos estão diretamente ligados à probabilidade de encontro entre as espécies e podem ser divididos em duas hipóteses: a hipótese da neutralidade e a de links proibidos (OLESEN et al., 2011; SAZATORNIL et al., 2016; TYLIANAKIS; MORRIS, 2017; VÁZQUEZ et al., 2007, 2009).

A importância da abundância relativa das espécies como fator determinante das interações se encaixa na hipótese da neutralidade (VÁZQUEZ et al., 2009; VÁZQUEZ; CHACOFF; CAGNOLO, 2009). Segundo ela, todos os indivíduos são ecologicamente equivalentes e, portanto, potencialmente capazes de interagir entre si sem nenhuma restrição (SAZATORNIL et al., 2016; VÁZQUEZ et al., 2009). Desta maneira, as espécies mais abundantes interagiriam com mais espécies e com maior frequência do que as espécies raras (SAZATORNIL et al., 2016; VÁZQUEZ et al., 2009). Vázquez, Chacoff e Cagnolo (2009), em um estudo com redes do tipo planta-polinizador, observaram que a abundância das espécies e a sobreposição fenológica são suficientes para explicar a estrutura das redes de interação. Ao mesmo tempo, em um estudo realizado com um sistema planta-dispersor de sementes, Laurindo, Gregorin e Tavares (2017) mostraram que a abundância de plantas frutificando influenciava tanto as métricas da rede quanto a abundância do dispersor (morcegos). Portanto, a abundância, de fato, desempenha um importante papel na frequência de interação das espécies (PERALTA et al., 2020a; VÁZQUEZ; CHACOFF; CAGNOLO, 2009).

Contudo, existem algumas incompatibilidades biológicas e temporais que impedem a interação das espécies (OLESEN et al., 2011; SAZATORNIL et al., 2016; TYLIANAKIS; MORRIS, 2017; VÁZQUEZ et al., 2007, 2009). A hipótese de links proibidos se baseia nessas restrições (OLESEN et al., 2011). Segundo a hipótese, por mais que as espécies sejam extremamente abundantes, se ambas são abundantes em diferentes momentos (e.g., estações do ano), ou seja, não se sobrepõem fenologicamente, a probabilidade de interação diminui (OLESEN et al., 2011; SANTAMARIA; RODRÍGUEZ-GIRONÉS, 2007). Um exemplo disso é o estudo de González-Castro et al. (2012), onde os autores sugerem que a sobreposição fenológica entre as espécies seria mais importante do que a abundância na determinação das interações e, com isso, das métricas da rede. Caradonna et al. (2017) também relatam sobre a importância da sobreposição fenológica, mostrando seu papel aliado à abundância relativa das espécies no cálculo das métricas da rede.

Outro aspecto da hipótese de links proibidos é que ela também considera as características das espécies (JORDANO; BASCOMPTE; OLESEN, 2002; VIZENTIN-BUGONI; MARUYAMA; SAZIMA, 2014). Segundo a hipótese, a correspondência de características (traços) entre espécies em interação poderia não somente possibilitar, mas também facilitar interações. Isso acontece porque os traços das espécies podem determinar desde a resposta à condições alteradas até a capacidade de interagir com outras espécies (COUX et al., 2016; DEHLING et al., 2014; EKLÖF et al., 2013). Ou seja, se uma espécie de polinizador obtém maior quantidade de alimento em determinadas flores, pressupõem-se que a frequência aumentada de visitação se dá por algum favorecimento. Portanto , esse polinizador tende a visitar essas flores mais vezes e assim aumentar a frequência de interação entre as espécies, como em um mutualismo com benefícios aumentados para ambas as espécies (KLUMPERS; STANGE; KLINKHAMER, 2019). O inverso acontece quando uma espécie de polinizador não consegue alcançar o recurso nas flores, isso faria com que eles não interagissem (KLUMPERS; STANGE; KLINKHAMER, 2019). Portanto, a correspondência de características entre polinizadores e plantas também restringe a ocorrência das interações (PERALTA et al., 2020a).

Por fim, o clima também está relacionado a variação temporal das redes de interação mutualística (RICO-GRAY et al., 2012; TYLIANAKIS; MORRIS, 2017). Isso porque temperatura e precipitação podem influenciar tanto as espécies de plantas quanto as de animais (LAURINDO; GREGORIN; TAVARES, 2017; PETANIDOU et al., 2014; RICO-GRAY, 1993; TYLIANAKIS; MORRIS, 2017). No caso das espécies de plantas, os níveis de temperatura e precipitação, além de alterarem a estrutura da comunidade, também podem alterar a fenologia das espécies (HEGLAND et al., 2009; PETANIDOU et al., 2014). Tal influência significa alterações na persistência das espécies e isso implica diretamente nas probabilidades de interação, uma vez que as plantas são fonte de alimento para muitos dos animais (LAURINDO; GREGORIN; TAVARES, 2017). Já no caso das espécies de animais, temperatura e precipitação podem alterar as taxas metabólicas das espécies, podendo assim afetar a frequência de interações (RICO-GRAY, 1993; TYLIANAKIS; MORRIS, 2017).

2.6 Influência da amostragem sobre as redes de interação mutualística

A amostragem pode ser um fator importante ao analisar as redes de interações (JORDANO, 2016; RIVERA-HUTINEL et al., 2012). Sabe-se que a maioria dos estudos em redes de interação são subamostrados (CHACOFF et al., 2012) e isso pode ser em decorrência de um esforço amostral considerado insuficiente, muito embora não haja uma análise que quantifique essa insuficiência(JORDANO, 2016). Entretanto, comparar redes com diferentes graus de incerteza decorrentes da amostragem é uma conduta factível, porém precipitada, podendo resultar em erros nas conclusões, visto que o número de interações registradas e as métricas são altamente propensas a sofrer por vieses da amostragem (CHACOFF et al., 2012; FRÜND; MCCANN; WILLIAMS, 2016; RIVERA-HUTINEL et al., 2012).

A maioria das métricas de redes são influenciadas pela amostragem, principalmente métricas qualitativas, ou seja, com redes não-ponderadas (DALSGAARD et al., 2017; RIVERA-HUTINEL et al., 2012). Uma das métricas mais conhecidas por serem influenciadas pela amostragem é a conectância, que está diretamente associada à adição de novas espécies, diminuindo conforme novas espécies são incluídas (FRÜND; MCCANN; WILLIAMS, 2016; RIVERA-HUTINEL et al., 2012). De maneira semelhante, métricas como a especialização da rede e a diversidade de interações dependem fortemente do esforço amostral e tendem a ser infladas em estudos com amostragem limitada, que acabam estimando as interações baseadas em uma sub-rede contendo apenas as espécies mais abundantes da comunidade (PETANIDOU et al., 2008; RIVERA-HUTINEL et al., 2012).

Existem duas maneiras de examinar o efeito da amostragem em uma rede de interação: 1) padronização do método de coleta de dados e, 2) manipulação dos dados após a sua coleta (JORDANO, 2016; RIVERA-HUTINEL et al., 2012). Em relação aos métodos de coleta de dados, existem diferentes métodos de amostragem: abordagem fitocêntrica, abordagem zoocêntrica, observação cronometrada e observação por transecto são alguns dos meios utilizados em redes de interação; a escolha do método influencia diretamente os resultados encontrados (BALLANTYNE; BALDOCK; WILLMER, 2015; GIBSON et al., 2011; JORDANO, 2016; RIVERA-HUTINEL et al., 2012). Um exemplo disso é o trabalho feito por Gibson et al. (2011), que compararam os métodos cronometrado e de observação em transecto, evidenciando diferenças significativas no número de interações registradas, assim como na assimetria das redes. De forma semelhante, Bosch et al. (2009), observaram que redes baseadas na abordagem fitocêntrica apresentaram menor número de animais registrados, menor conectância e menor número de espécies especialistas, quando comparadas às redes baseadas na abordagem zoocêntrica. Sendo assim, é importante ter cautela ao escolher o método de amostragem e buscar alternativas para eliminar qualquer possível viés amostral.

Entre as alternativas que podem ser usadas para eliminar potenciais vieses de amostragem, estão o z-score e as curvas de acumulação (CHACOFF et al., 2012; DALSGAARD et al., 2017; SEBASTIÁN-GONZÁLEZ et al., 2015). O z-score (i.e., normalização) representa o quanto uma estrutura observada se desvia da estrutura esperada aleatoriamente (DALSGAARD et al., 2017; SEBASTIÁN-GONZÁLEZ et al., 2015). Essa normalização mostra o quanto os dados variam do acaso, possibilitando a desconsideração dos vieses de amostragem que podem estar ligados ao tamanho da rede. Já as curvas de acumulação consistem no registro de novas espécies (ou novas interações) para uma comunidade, em função do aumento do esforço de amostragem (CHACOFF et al., 2012; GOTELLI; COLWELL, 2011). Dessa forma, por meio das curvas de acumulação, é possível avaliar não somente se o esforço amostral foi adequado, mas também o número esperado de interações e se há a necessidade de um aumento na amostragem, o que proporcionaria uma maior robustez aos dados. Por fim, o uso de ferramentas como o z-score e a curva de acumulação permitem não somente compreender o esforço de amostragem necessário e os efeitos dos tipos de amostragem nas redes de interação e suas métricas, mas também eliminar vieses de amostragem das análises e produzir redes mais fiéis às comunidades biológicas que representam (COSTA et al., 2016; HELENO et al., 2014).

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SEGUNDA PARTE – ARTIGOS

ARTIGO 1 - DRY SEASON AND THE ABUNDANCE OF VISITORS AFFECT PLANT-FLORAL VISITOR NETWORKS OVER TIME

(Artigo redigido e submetido de acordo com as normas do periódico)

Periódico: *Oecologia*

Fator de impacto: 2.654

Dry season and the abundance of visitors affect plant-floral visitor networks over time

Victor Hugo Duarte da Silva[1*], *Matheus Tenório Baumgartner*[2], *Marcel Gustavo Hermes* [3]
Lucas Del Bianco Faria[1]

1- Departamento de Ecologia e Conservação, Instituto de Ciências Naturais, Universidade Federal de Lavras, CEP 37200-900, Lavras, Minas Gerais, Brazil.
2- Laboratory of Insect Systematics and Biology, Department of Biology, Federal University of Lavras, Mailbox 3037, Zip Code 37200-900, Lavras, Minas Gerais, Brazil.

3- Center for Biological Sciences, State University of Maringá, Zip Code 87020-900, Maringá, Paraná, Brazil.

*Author for correspondence: victorfaculufla@gmail.com

ABSTRACT

Interaction networks are topological representations of what happens among species in ecological communities. Similar to all ecological communities, interaction networks likely vary over space and time, but studies assessing the variations in interaction patterns are scarce, especially in the highly diverse tropics. Thus, this study aims to identify the abiotic and biotic factors that influence the dynamics of interactions in plant-floral visitor networks over time. We recorded interactions between plant species and insect visitors in an area of the Cerrado *strictu sensu* every fortnight for 13 consecutive months. Subsequently, we fitted time series models and used Bayesian analysis to assess whether abiotic (temperature and precipitation) and biotic (abundance of floral visitors and open flowers) factors affected the robustness to species loss and other network-level structural metrics. Bayesian analysis showed that the dry season, characterized by periods of lower temperature and precipitation, negatively affected the robustness of ecological networks. This outcome was likely because networks decreased in size during the dry season, making them more vulnerable to the loss of important interactions. In addition, the analyses revealed that the abundance of floral visitors increased modularity and decreased weighted connectance. Finally, the number of flowers did not influence any metric over the time series. The results show that both abiotic and biotic factors influence network dynamics but in different ways over time. Understanding the influence of such factors is fundamental since the complexity and dynamics of ecological communities are the result of such tradeoffs.

Keywords: Bayesian analysis; Climatic seasonality; Ecological communities; Modularity; Mutualistic networks; Relative abundance of species; Network robustness; Temporal variation; Time series.

INTRODUCTION

The study of ecological communities goes far beyond the identification and estimation of relative abundances of species, and it also comprises understanding the whole set of interactions among species (CARADONNA; WASER, 2020). Such interactions play a fundamental role in the formation of ecological communities and in the provision of ecosystem services, such as pollination and seed dispersal (BASCOMPTE; JORDANO, 2007, 2014). Because of the dynamic nature of ecological communities, interactions also change over space and time, increasing the complexity of reliable ecological models (ACEVEDO-QUINTERO; ZAMORA-ABREGO; GARCÍA, 2020; TRØJELSGAARD et al., 2015; ZOGRAFOU et al., 2020). In this context, understanding the complexity and dynamics of ecological interactions is fundamental because ecosystem services and responses are susceptible to changes in community structure (NOVELLA-FERNANDEZ et al., 2019).

A useful approach to address the complexity of ecological communities is to treat species as participants in interaction networks (KAISER-BUNBURY; BLÜTHGEN, 2015; NOVELLA-FERNANDEZ et al., 2019). This approach allows us not only to describe how species interact but also to understand the structure and functioning of ecosystems and to explore whether there is an underlying stable structure of these interactions (BANZA; BELO; EVANS, 2015; MONTOYA; PIMM; SOLÉ, 2006). Furthermore, since networks may represent the interactions that occur in a given community, this topological approach allows for the observation of variations over time and space and, more interestingly, whether these variations may emerge from alternative mechanisms (BASCOMPTE; JORDANO, 2014).

Different mechanisms are expected to cause spatial and temporal variations in ecological networks. Spatial variation, for example, is usually associated with habitat heterogeneity, which may promote spatial overlap among species, causing differences in the composition and relative abundances of species across space (CARTENSEN et al., 2015; MONTOYA; YALLOP; MEMMOTT, 2015; NEKOLA; WHITE, 1999; TYLIANAKIS; MORRIS, 2017). At the same time, temporal variations are often associated with changes in species richness and composition dictated by both seasonal patterns and the phenology of species, which inevitably affects the likelihood of species interactions (BURKLE; ALARCON, 2011; MORELLATO et al., 2016; OLESEN et al., 2008). In this sense, there are abiotic and biotic factors that are expected to influence the probability of species interacting at a given spatial or temporal scale (CLASSEN et al., 2020; HOISS; KRAUSS; STEFFAN-DEWENTER, 2015; PERALTA et al., 2020b; VÁZQUEZ et al., 2007, 2009). Consequently, these factors are responsible for the variations within interaction networks (CLASSEN et al., 2020; HOISS; KRAUSS; STEFFAN-DEWENTER, 2015; PERALTA et al., 2020b; VÁZQUEZ et al., 2007, 2009).

Among the abiotic factors, temperature and precipitation are those more intimately linked to climate, which is one of the major determinants of the spatial distribution of both plant and insect species and therefore how they interact (CASE; TAPER, 2000; CLASSEN et al., 2020; PETANIDOU et al., 2018).

For instance, variations in temperature have been directly associated with a high diversity of species in mutualistic networks (TAKEMOTO; KANAMARU; FENG, 2014). In addition, it negatively influences the nestedness and connectance of networks (TAKEMOTO; KANAMARU; FENG, 2014; WELTI; JOERN, 2015). Precipitation leads to more modular structures, acting directly on network topology (TRØJELSGAARD; OLESEN, 2013).

Among biotic factors, the phenological overlap, the correspondence of traits and the abundance of species (of plants and floral visitors) are the main mechanisms that generate the variation in the interaction networks (PERALTA et al., 2020b; VÁZQUEZ; CHACOFF; CAGNOLO, 2009). These mechanisms act directly on the probability that a pair of species will interact and can be explored as a *tradeoff* between two hypotheses. First, the neutrality hypothesis defends that the probability of interaction among two species increases with the relative abundances of the participants, assuming no biological constraints among them (VÁZQUEZ et al., 2007). Second, some potential interactions among abundant participants are prevented because of the mismatch between the biological traits of species, known as the forbidden links hypothesis (VÁZQUEZ; CHACOFF; CAGNOLO, 2009). Although these two hypotheses may seem controversial, we assume that they both apply in most ecological networks, and this study will focus on the neutrality hypothesis, assuming that highly abundant species are more likely to interact than less abundant (i.e., rare) species (VÁZQUEZ et al., 2007, 2009). This hypothesis is based on the assumption that there are no restrictions between species, that is, “everyone is able to interact with everyone” (STANG; KLINKHAMER; MEIJDEN, 2007).

Finally, the influence of these abiotic and biotic factors on the interactions among species is a frequent concern in ecological studies, mainly because these factors are determinants of the spatiotemporal variations in networks at different scales (PERALTA et al., 2020b; PETANIDOU et al., 2018; SOUZA et al., 2018; TRØJELSGAARD et al., 2015; VÁZQUEZ et al., 2009). In an attempt to fill the existing knowledge gap on how these mechanisms affect the temporal dynamics of interactions, we developed this study to identify which abiotic and biotic factors act on plant-floral visitor networks over time. For this, we used a 13-month time series of mutualistic networks to answer the following question: Does the dry season (the period with lower temperatures and less rain), the abundance of floral visitors, and the number of flowers affect the structure of floral plant-visitor networks over time? We expected the dry season to negatively affect the network's metrics due to the lower availability of floral resources (ESCOBEDO-KENEFIC et al., 2020; RABELING et al., 2019; SOUZA et al., 2018). In addition, we expected the abundance of floral visitors and the number of flowers to positively influence the networks, since a greater number of flowers, in addition to representing a greater amount and diversity of resources, also represents a greater abundance of visitors and a greater probability of species interacting (neutrality hypothesis) (ESCOBEDO-KENEFIC et al., 2020; FISHER et al., 2017; VÁZQUEZ et al., 2007).

MATERIAL AND METHODS

Study area

The study was conducted at the Biological Reserve of the UniLavras - Boqueirão (hereafter, RBUB) in southern Minas Gerais, Brazil (Fig. 1). The RBUB occupies a total area of 159 ha, with altitudes varying between 1100 and 1250 m (PIRES; POMPEU; SOUZA-SILVA, 2012). The average annual precipitation is 1411 mm, and the average annual temperature ranges from 19°C to 25°C, with very scattered rainfall throughout the year but concentrated in the summer, with 66.77% of the annual rainfall occurring between November and February (PIRES; POMPEU; SOUZA-SILVA, 2012). Winter spans from June to September and is characterized by a remarkable water deficit, with average precipitation ranging from 10 mm to 30 mm (PIRES; POMPEU; SOUZA-SILVA, 2012). The vegetation of the RBUB is characterized by typical phytophysiognomies from the Cerrado *stricto sensu*, altitude fields, Rupestrian fields, gallery forest, and patches of *Brachiaria* sp (Poaceae) pastures (PIRES; POMPEU; SOUZA-SILVA, 2012).

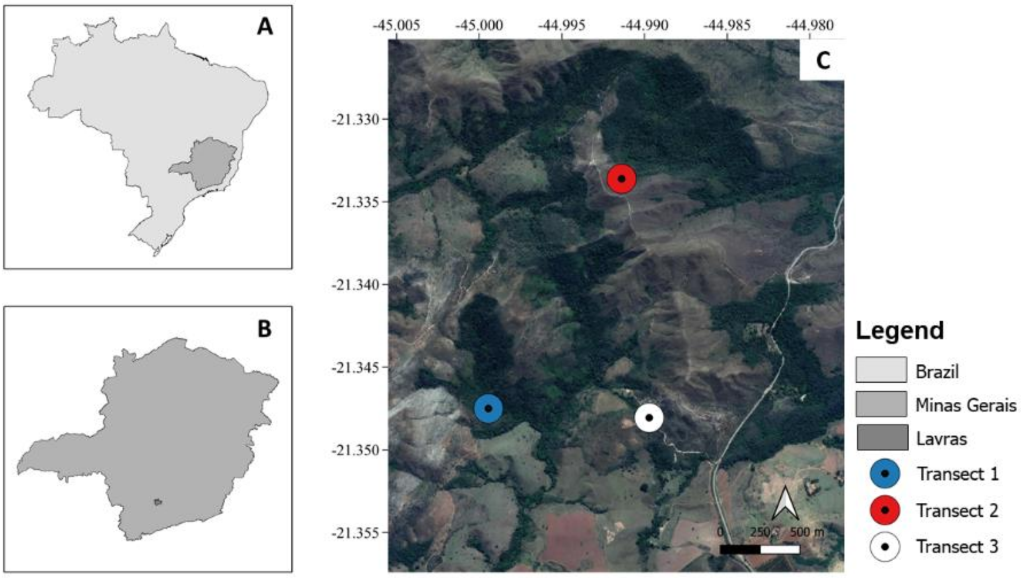


Fig. 1- Location of the study area. A- Map of Brazil with emphasis on the state of Minas Gerais. B- Map of Minas Gerais with emphasis on the city of Lavras. C- Map of the study area highlighting the transects used.

Data collection

We collected data on the interacting plant and insect species every fortnight, starting in early July 2015 and ending in late July 2016. Collections were performed along three 200 × 50 m transects: T1 (21°20'53" S/44°59'23" W), T2 (21°21'01" S/44°59'29" W), and T3 (21°20'51" S/44°59'58" W) (Fig. 1).

We divided each transect into 10 plots of 40 × 25 m. Each plot was numbered from ‘1’ to ‘10’. Before each collection, plots were drawn to define the sampling sequence. The draw was carried out only for the plots that were at the beginning and at the end of the transects (plots 1, 2, 9, and 10). For example, if plot ‘1’ was drawn, the collection at that given fortnight initiated from left to right (1, up to plot 10) until all plots were surveyed. Conversely, when the number ‘10’ was drawn, the collection started in the reverse order. By carrying out this randomization procedure on the direction of each collection, we had all plots sampled in all periods.

Within each plot, floral visitors were collected near the opening of flowers between 7:30 am and 4:30 pm, twice during each period (morning and afternoon). Within each transect, the collector moved along the previously selected plots, capturing the insects that were visiting the flowering plants. The route was performed twice during each collection (morning and afternoon). For each flowering plant individual, the collector's residence time was 10 minutes. We considered a realized interaction only when the insect was in physical contact with the flower's reproductive structure, thus suggesting that the visitor could be a potential pollinator. Hovering insects were not considered interaction partners. In the present study, the term “floral visitors” was adopted for all registered insects, as the qualification in terms of its effectiveness as a pollinator was not assessed (ALVES-DOS-SANTOS et al., 2016). As an estimation of the abundance of each plant, we counted the number of flowers. For species with reduced flower abundance, all flowers were counted. For species with flowers arranged in inflorescences, we estimated the number of flowers based on the number of inflorescences. We multiplied the average number of open flowers per inflorescence, multiplied by the average number of inflorescences per branch, and finally by the average number of branches per plant (VOSGUERITCHIAN, 2010).

All collected insect specimens were deposited in the Entomological Collection of the Federal University of Lavras (CEUFLA). To determine the potential sources of the resources used by flower visitors, parts from those plants whose flowers were found in the insects were sampled. The plant material was deposited in the Herbarium of the Federal University of Lavras (ESAL-UFLA). The identification of plants and insects was carried out using specialized keys and with the aid of specialists.

Data analysis

First, because some fortnight samplings resulted in oversimplified interaction records (e.g., only two or three insect species), we grouped data for each month. Then, we classified the sampling months into dry (April 2015 to September 2015 and June 2016) and wet (October 2015 to March 2016) seasons based on temperature and precipitation data (ALVARES et al., 2013). For this categorization, we extracted the monthly climatic (temperature and precipitation) data for the period between July 2015 and July 2016 from the WorldClim database (WORLDCLIM, 2020) based on the geographical coordinates of each transect. The maximum and minimum temperature values corresponded to the monthly averages of the daily temperature records. The precipitation data corresponded to the monthly totals observed in the sampling period. Subsequently, we performed a Kruskal-Wallis test to verify whether the seasons differed significantly in temperature and precipitation because the data did not follow a normal distribution.

Next, we compiled all species and their respective interactions from the three transects by month (July 2015 to July 2016), building one interaction matrix for each month (13 plant-floral visitor networks). The three transects were grouped because most visitor species were common among transects, likely because of their ability to fly long distances and the relative simplification of the networks. Indeed, the classification by month improved our ability to test other questions about seasonality as a factor influencing the temporal dynamics of plant-insect interactions.

Third, we estimated the following nine metrics for each network: network specialization (H2), interaction evenness, interaction strength asymmetry (ISA), linkage density, modularity, Shannon's diversity, specialization asymmetry (SA), weighted connectance, and weighted nestedness based on NODF. With the exception of modularity, the metrics were generated using the *networklevel* function. Modularity was generated through the *computeModules* function. Then, we compared the observed values of each metric with null distributions from 1000 randomized interaction networks based on the Patefield model (PATEFIELD, 1981), which assumes that the degree of species (marginal sums) is fixed but the connectance is variable (DORMANN et al., 2009). We standardized all metrics based on Z-score transformations to eliminate any possible bias of comparing networks with different architectures. The Z-score represents how much an observed structure deviates from the expected structure at random (DALSGAARD et al., 2017; SEBASTIÁN-GONZÁLEZ et al., 2015), as follows:

$$Z = \frac{x_{obs} - \mu_{null}}{\sigma_{null}}$$

where x_{obs} is the observed value for each metric, and μ_{null} and σ_{null} are the mean and standard deviation of the null distributions, respectively. In addition, we estimated the robustness to secondary extinctions (R) for all 13 monthly plant-floral visitor networks, assuming random deletion of species with 1000 replicates for each one. All functions used in these steps are presented in the *bipartite* package in the R software (DORMANN; GRUBER; FRÜND, 2008).

To explore the temporal trends in the structure of each network, we performed time series analysis looking for stationary or nonstationary dynamics by adjusting autoregressive integrated moving average models (ARIMA (p, d, q)). These models integrate both autoregressive and moving average estimators to describe the temporal trends of a time series based on different coefficients: p (the number of time delays), d (the number of differential transformations required for achieving the stationary state), and q (the delay of the error component in the model). ARIMA models also assume that the time series may have a mean value that is significantly different from zero. We assessed the best ARIMA model for every network metric using the *auto.arima* function from the *forecast* package (HYNDMAN; KHANDAKAR, 2008), which performs a parsimonious search among possible models within a range of order restrictions based on Akaike's information criteria corrected for small samples (AICc). We explored trends in time series residuals through autocorrelation plotting (ACF) and tested the pairwise dependence between each sampling month using the Box-Ljung test, with adjusted degrees of freedom as $\textit{estimated lag} > p + q$ (FOKIANOS; PITSILLOU, 2017). We also evaluated the dispersion around the mean for each time series using the coefficient of variation, which is a standardized dispersion measure.

Before testing our hypotheses, we performed Spearman's rank correlations among the nine estimated network metrics and robustness to secondary extinction and discarded those that were highly correlated ($|\rho| \geq 0.80$) for simplification of further analyses; for more details, please see the Supplementary material (Fig. S1). The Bayesian statistical framework provides unique advantages in terms of interpretability of the estimated parameters, flexibility to adjust complex models, and the possibility of explicitly testing hypotheses (KORNER-NIEVERGELT et al., 2015; MCELREATH, 2015; MUTH; ORAVECZ; GABRY, 2018). To simulate and analyze our Bayesian models, we used the *rstanarm* package (GABRY; GOODRICH, 2016, 2017), which is a Stan-based toolkit (STAN DEVELOPMENT TEAM, 2020) that mimics the high-level syntax of linear models built based on R functions (e.g., lm, glm, lmer, and glmer (BATES et al., 2015)). Stan was developed to be numerically efficient and reliable and able to fit both simple and highly complex models (CARPENTER et al., 2017; HOFFMAN; GELMAN, 2014; STAN DEVELOPMENT TEAM, 2020).

Those uncorrelated metrics that were used as response variables in our fitted models were interaction strength asymmetry (ISA), modularity, specialization asymmetry (SA), weighted connectance, weighted nestedness based on NODF, and robustness to secondary extinctions, whereas the explanatory variables were the dry season, abundance of floral visitors, and number of flowers, which were all treated as fixed factors. The regression models followed the Gaussian distribution and were adjusted using the function *stan_glm*–, i.e., time series analyses showed no dependence between each pair of adjacent months (Table 01). As we had three explanatory variables, we initially performed model selection based on the value of the widely applicable information criterion (WAIC) and assumed weak informative priors, providing numerical stabilization, avoiding excessive adjustments, and allowing for extreme values (GELMAN et al., 2014; MUTH; ORAVECZ; GABRY, 2018; STAN DEVELOPMENT TEAM, 2020). Most commonly used in Bayesian numerical simulations, the Markov Chain Monte Carlo (MCMC) is an iterative routine used for very large samples consisting of many consecutive drawings to project the posterior distribution of each parameter. We used the Hamiltonian Monte Carlo algorithm, which is the default in the *rstanarm* package (GABRY; GOODRICH, 2016, 2017) and provides an appropriate estimation of uncertainty for the adjusted models. We defined models to run 10 independent chains with 5000 steps and 500 burn-in samples, resulting in 45,000 draws for each model. We verified the performance of our approach based on the convergence of models ($\hat{R}$), the effective posterior sample size (ESS), and the Monte Carlo standard error (MCSE) (MUTH; ORAVECZ; GABRY, 2018).

Finally, we explicitly tested the hypotheses that the explanatory variables (dry season, abundance of floral visitors, and number of blooming flowers) affect the nine network metrics and robustness using the Bayes factor (GOODMAN, 1999a, 1999b). The Bayes factor index, also called the likelihood ratio or relative betting odds, is an alternative to frequentist hypothesis testing and can improve statistical summaries and knowledge, leading to a more intuitive interpretation of the results (GOODMAN, 1999a, 1999b). Using the *bayesfactor_restricted* function from the *bayestestR* package (MAKOWSKI et al., 2019; MAKOWSKI; BEN-SHACHAR; LÜDECKE, 2019), we tested model restrictions on its parameters (our hypotheses) considering unrestricted models. We also compared whether the influence of one predictor prevailed over the others based on the slope parameters (i.e., the strength of the influence of each predictor variable) by calculating the proportion of ties in relation to the user-specified condition (i.e., our hypotheses; Muth et al. 2018).

RESULTS

The months from the dry season presented an average temperature of 11.89 ± 2.77°C (mean ± standard deviation) and an average precipitation of 38.10 ± 37.12 mm. Conversely, the wet season presented an average temperature of 17.20 ± 0.62°C and an average precipitation of 210.40 ± 64.69 mm (CHI-SQUARED STATISTICS $X^2 = 9.0$; P = 0.0027) (Fig. 2; Table S1).

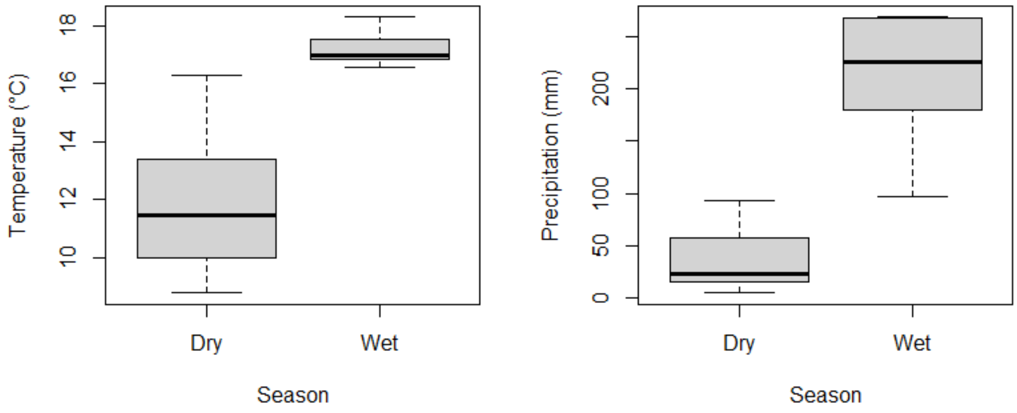


Fig. 2 - Box-plots of the average temperature and precipitation in each season. The dry season is composed of the months: July 2015, August 2015, September 2015, April 2016, May 2016, June 2016, and July 2016. The rainy season is composed of the months: October 2015, November 2015, December 2015, January 2016, February 2016, and March 2016. The black bar in the boxplot represents the median of each season and the dashed bars represent the upper and lower limits of each station.

The bipartite networks presented here are the result of a sampling effort that collected and identified 11,905 insects belonging to 346 species of floral visitors and 111 species of flowering plants (Fig. 3; Table S2; Table S3). The floral visitors belonged to six orders: Hymenoptera, Coleoptera, Diptera, Lepidoptera, Hemiptera, and Neuroptera. Hymenoptera and Diptera were the most abundant orders, with 197 and 66 species, respectively. Among the floral visitors, only *Apis mellifera* was an exotic species. The plant species belonged to 32 families, with Asteraceae and Malvaceae being the richest families, with 37 and 10 species, respectively. Among them, only *Brachiaria* sp. was considered exotic.

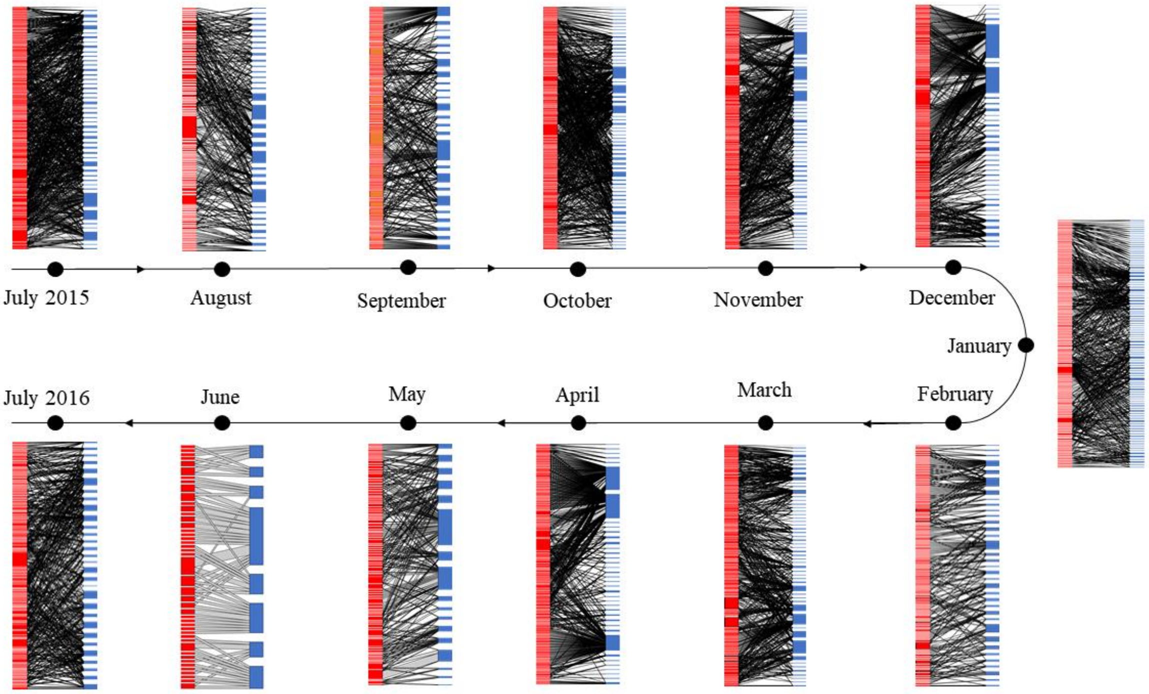


Fig. 3 - Bipartite networks for each sampling month between July 2015 and July 2016 of plant-floral visitor interactions from the UniLavras – Boqueirão Biological Reserve (RBUB). The horizontal bars represent plant species (blue bars) and floral visitors (red bars). The connection between species is the record of interaction between plant species and floral visitors.

Network specialization (H2), interaction evenness, linkage density, modularity, Shannon's diversity, specialization asymmetry (SA), and weighted connectance showed statistically significant differences between the observed values and those values from the null distributions (Figs. S2- A, B, D, E, F, G e H; see the Supplementary material). For all networks, the observed values of network specialization and modularity were significantly greater than the null distribution, whereas the values of interaction evenness, linkage density, Shannon diversity, and weighted connectance were significantly lower than the null distribution.

The interaction strength asymmetry (ISA), specialization asymmetry (SA), and weighted NODF showed months when the observed values of the metrics and the value expected by the null model did not differ significantly (Figs. S2- C, G, I; see the Supplementary material). The ISA showed significant differences in all months, except in January (P = 0.44) (Fig. S2 - C: see the Supplementary material). The weighted NODF showed significant differences in all months, except in February and June (P = 0.16 and P = 0.26, respectively) (Fig. S2 - I: see the Supplementary material). Specialization asymmetry also did not present significant differences in December, May and June (P = 0.27, P = 0.19, and P = 0.30, respectively) (Fig. S2 - G: see the Supplementary material).

The time series analysis of all ten metrics (nine metrics plus robustness) demonstrated non-trended and stationary dynamics over the months; that is, the metrics did not increase or decrease significantly during our sampling period. The selected ARIMA models had both p and q parameters with low or zero order, highlighting the prevalence of white noise dynamics with only weak or no dependence between consecutive months (Table 01; see also Fig. S3 in the Supplementary material for plotting autocorrelations).

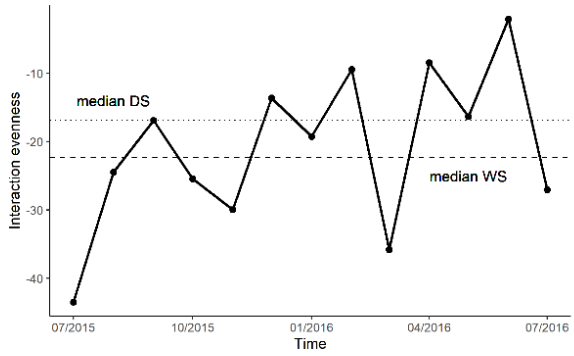
Regarding the variation in the metrics across the sampling months, the interaction evenness, linkage density, Shannon diversity, weighted connectance, and weighted NODF were more variable in June than in July 2015 (Fig. 4). The interaction strength asymmetry showed its maximum value in March and its minimum value in January (Fig. 4). Robustness had its maximum value in January and minimum value in July 2016 (Fig. 4). Specialization asymmetry had its maximum value in July 2016 and minimum value in October (Fig. 4). Finally, the network specialization and modularity had their maximum values in July 2015 and minimum values in June and February, respectively (Fig. 4).

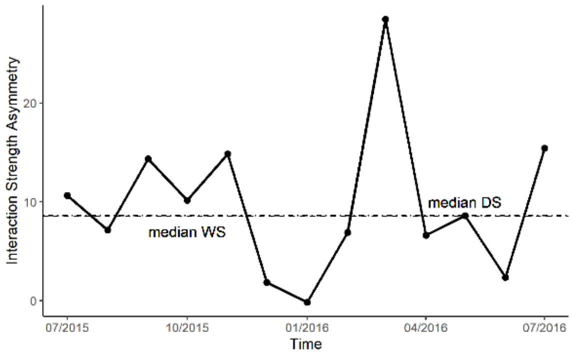
Most of the maximum values for all metrics were recorded in the dry season (80%), which is also when most of the minimum values occurred (70%) (Fig. 4). Modularity, specialization asymmetry, and robustness presented maximum and minimum values in different seasons (Fig. 4). Network specialization (H2), interaction evenness, linkage density, Shannon’s diversity, weighted connectance, and weighted NODF presented the two extreme values in the dry season, in contrast to the interaction strength asymmetry, which presented the two extreme values in the wet season (Fig. 4).

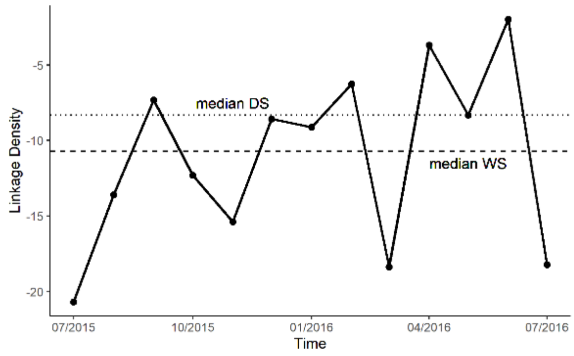
Some specific metrics (network specialization (H2), interaction evenness, linkage density, Shannon’s diversity, and weighted NODF) showed a high correlation ($|\rho| > 0.80$) (Fig. S1). Thus, we selected interaction strength asymmetry, modularity, specialization asymmetry, weighted connectance and robustness for model fitting. Models for interaction strength asymmetry, modularity, and weighted connectance performed better with the abundance of floral visitors as the only explanatory variable (WAIC = 91.68, WAIC = 94.63, WAIC= 74.60, respectively) (Table S4). Likewise, the best model with robustness as the response variable was that with the dry season as the only predictor variable (WAIC = -36.96), and specialization asymmetry was better described by the model with the abundance of floral visitors and the number of flowers as predictors (WAIC = 107.50) (Table S4).

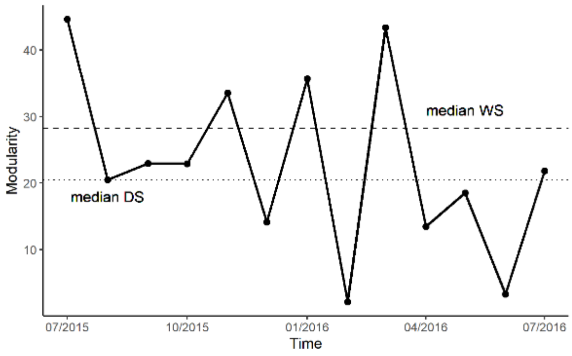
Table 01. Autoregressive integrated moving average (ARIMA) models for all nine network metrics and network robustness. CV is the coefficient of variation (CV = standard deviation / mean). The two best ARIMA models are presented for each metric selected by the lowest second order Akaike Information Criterion (AICc). The p-values of the Box-Ljung test in pairs in the residuals of the time series are also shown.

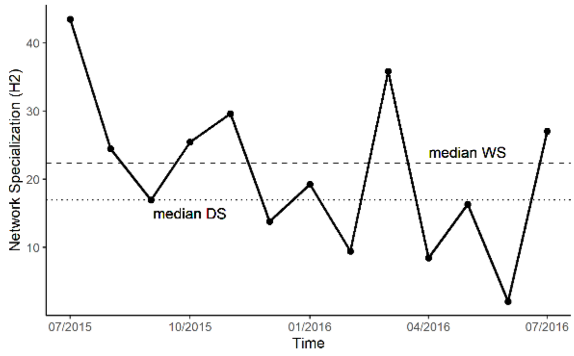
Network Metric	**CV**	**ARIMA(*p*,*d*,*q*) model (first two lowest-AICc models)**	**AICc**	**Box-Ljung**
Network Specialization	0,55	(0,0,0) non-zero mean	104.73	$p = 0.49$
		(1,0,0) zero mean	107.65	
Interaction Evenness	-0,55	(0,0,0) non-zero mean	104.79	$p = 0.49$
		(1,0,0) zero mean	107.71	
Interaction Strength Asymmetry	0.76	(0,0,0) non-zero mean	93.39	$p = 0.61$
		(1,0,0) zero mean	96.65	
Linkage Density	-0.53	(0,0,0) non-zero mean	87.12	$p = 0.38$
		(0,0,1) non-zero mean	89.44	
Modularity	0.58	(1,0,0) non-zero mean	108.33	$p = 0.41$
		(0,0,0) non-zero mean	108.61	
Shannon Diversity	-0.55	(0,0,0) non-zero mean	104.74	$p = 0.49$
		(1,0,0) non-zero mean	107.66	
Specialization asymmetry	12.83	(0,0,0) zero mean	104.03	$p = 0.99$
		(0,0,0) non-zero mean	106.78	
Weighted NODF	-0.63	(0,0,0) non-zero mean	75.33	$p = 0.78$
		(0,0,1) non-zero mean	78.68	
Weighted Connectance	-0.53	(0,0,0) non-zero mean	87.12	$p = 0.38$
		(0,0,1) non-zero mean	89.44	
Robustness	0.22	(1,0,0) zero mean	-33.61	$p = 0.59$
		(1,0,0) non-zero mean	-33.15	

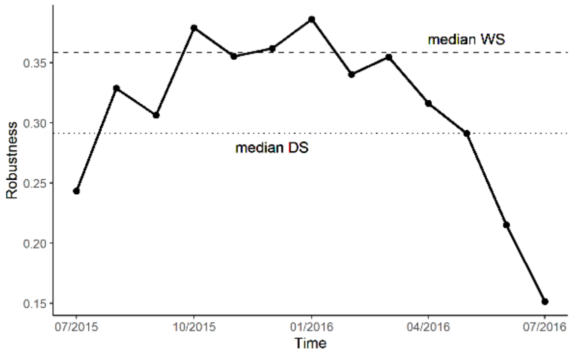

Interaction evenness
-10
-20
-30
-40
median DS
median WS
07/2015
10/2015
01/2016
04/2016
07/2016
Time

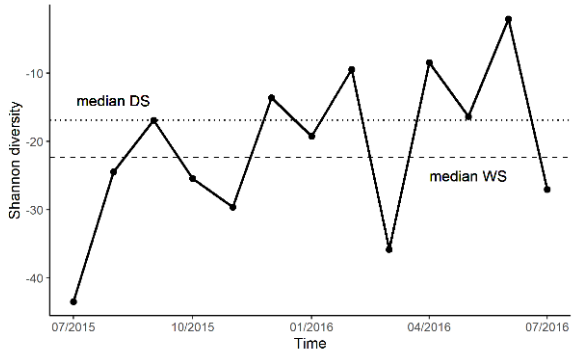

Interaction Strength Asymmetry
20
10
0
median DS
median WS
07/2015
10/2015
01/2016
04/2016
07/2016
Time

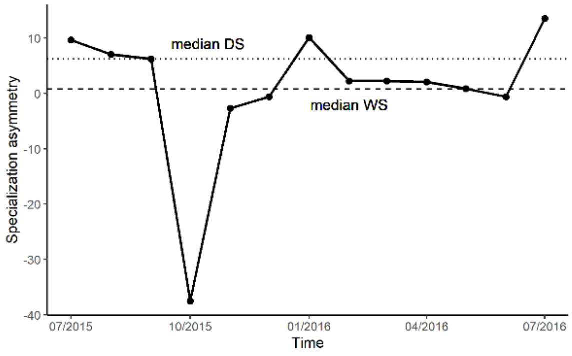

Linkage Density
-5
-10
-15
-20
median DS
median WS
07/2015
10/2015
01/2016
04/2016
07/2016
Time


Modularity
40
30
20
10
median WS
median DS
07/2015
10/2015
01/2016
04/2016
07/2016
Time


Network Specialization (H2)
40
30
20
10
median WS
median DS
07/2015
10/2015
01/2016
04/2016
07/2016
Time


Robustness
0.35
0.30
0.25
0.20
0.15
median WS
median DS
07/2015
10/2015
01/2016
04/2016
07/2016
Time


Shannon diversity
-10
-20
-30
-40
median DS
median WS
07/2015
10/2015
01/2016
04/2016
07/2016
Time


Specialization asymmetry
10
0
-10
-20
-30
-40
median DS
median WS
07/2015
10/2015
01/2016
04/2016
07/2016
Time

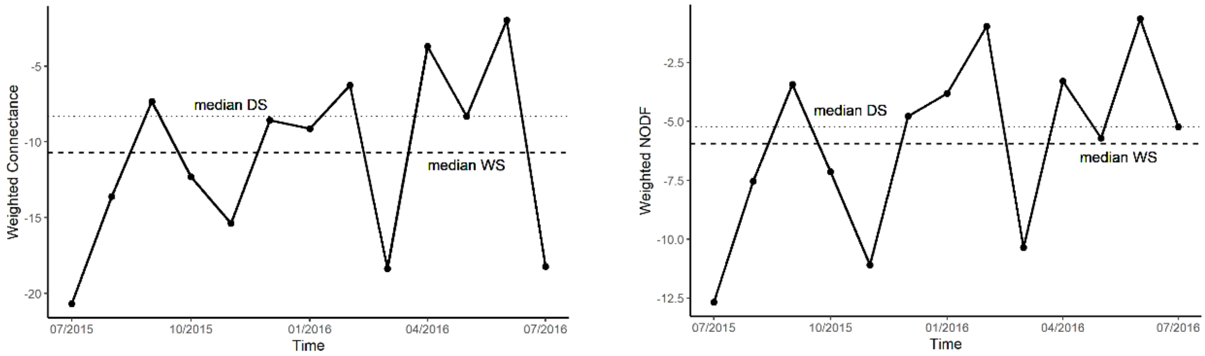


Fig. 4 - Time series for ten network metrics and robustness of the network. The dashed line is the median of the wet season (WS), while the dotted line is the median of the dry season (DS).

The diagnostics of the MCMC simulations are shown in Table 02. After evaluating the convergence criterion ($\widehat{R} < 1.1$), the effective posterior sample size (ESS > 1000), and the Monte Carlo standard error (0 < MCSE < standard deviation), we confirmed the quality of the draws for all models (MUTH; ORAVECZ; GABRY, 2018) (Table 02).

The estimated mean of the posterior distribution of model parameters showed that the abundance of floral visitors had a positive influence on modularity; that is, the higher abundance of floral visitors apparently increased the probability of species interacting with a more restricted cluster of partners (Table 02; Fig. 5). For weighted connectance, the estimated mean of the posterior distribution showed that the abundance of floral visitors had a negative influence on the metric (Table 02; Fig. 5). In addition, the uncertainty of the posterior distribution, also called the credible interval (CI - 95%), for the slopes of modularity and weighted connectance remained at positive and negative values, respectively. The estimated mean of the posterior distributions indicated that the abundance of floral visitors and the number of flowers had no effect on the asymmetry of the specialization and on the interaction strength (Table 02; Fig. 5). Finally, for robustness, the mean of the posterior distribution demonstrated that the dry season had a negative influence; that is, networks were more prone to secondary extinctions in the dry season than in the wet season (Table 02; Fig. 5). In addition, the posterior distribution uncertainty (CI - 95%) showed that all values remained negative for robustness.

As each metric has its own scale, we must avoid comparing them directly with each other. Consequently, all metric comparisons were plausible only within their own models. For example, according to its mean posterior value under the hypothesis-driven Bayesian approach (Table 02), the modularity of networks would be 0.02 times higher when the number of floral visitors increases. Accordingly, the weighted connectance is expected to be 0.008 times lower when flower visitors are scarcer, whereas the network robustness is expected to be 0.095 times lower in the dry season than the expectation in rainy periods (Table 02).

Finally, by testing the hypotheses based on the Bayes factor (BF), we observed that there was a higher probability of modularity being positively affected by the abundance of floral visitors (BF = 1.9990) (Table 03). Weighted connectance was negatively affected by the abundance of floral visitors (BF = 1.9970) (Table 03). Modularity and weighted connectance represented a probability of additional ties greater than 0.95 (Table 03); in this case, the values were 1.00 and 0.99, respectively, indicating that a large proportion of the subsequent draws had higher values when the abundance of floral visitors was also high (Table 03). When comparing the restricted with unrestricted approaches, the tested hypotheses also demonstrated a greater probability of robustness being negatively affected by the dry season (BF = 1.9980) (Table 03). In addition, robustness presented a probability of subsequent draws of 0.99, revealing that the subsequent draws had higher values in the dry season (Table 03).

Table 02: Model diagnosis and statistical summary of the posterior distributions of each of the estimated parameters of the Bayesian regression models. Mean and SD are the mean and standard deviation for each parameter. ESS (effective posterior sample size), $\widehat{R}$ (convergence assessment) and MCSE (Monte Carlo standard error) are indicators to ensure model performance. 2.5% and 97.5% are lower and upper limits of the credible interval (CI) of the posterior distirbution for each model parameter. When the CI (2.5%) and CI (97.5%) values overlap 0 (zero), it means that there was no significant effect of the parameter on that metric.

Models	Parameters	Mean	SD	$\widehat{R}$	ESS	MCSE	CI (2.5%)	CI (97.5%)
Interaction Strength Asymmetry	Intercept	4.430	3.8500	1	31502	0.0231738400	-3.710	12.600
	Abundance of floral visitors	0.006	0.0037	1	32980	0.0000215605	-0.002	0.014
	Error SD	7.170	1.6900	1	25463	0.0000000000	4.970	11.500
Modularity	Intercept	5.050	4.5400	1	29078	0.0286255500	-4.610	14.900
	Abundance of floral visitors	0.020	0.0044	1	28383	0.0000278300	0.011	0.029
	Error SD	8.390	2.0500	1	23353	0.0000000000	5.780	13.700
Specialization Asymmetry	Intercept	7.660	6.5700	1	35226	0.0371220000	-6.320	21.200
	Abundance of floral visitors	-0.019	0.0102	1	20368	0.0000761391	-0.041	0.003
	Number of Flowers	0.000	0.0001	1	19964	0.0000005652	-0.0000369	0.000283
Weighted Connectance	Intercept	-3.490	2.1200	1	32325	0.0125456600	-7.990	0.974
	Abundance of floral visitors	-0.008	0.0020	1	32770	0.0000119628	-0.013	-0.004
	Error SD	3.870	0.9610	1	25852	0.0000000000	2.650	6.380
Robustness	Intercept	0.361	0.0205	1	30149	0.0001271332	0.317	0.405
	Dry season	-0.095	0.0280	1	28995	0.0001744495	-0.153	- 0.036
	Error SD	0.051	0.0125	1	24627	0.0000000000	0.035	0.083

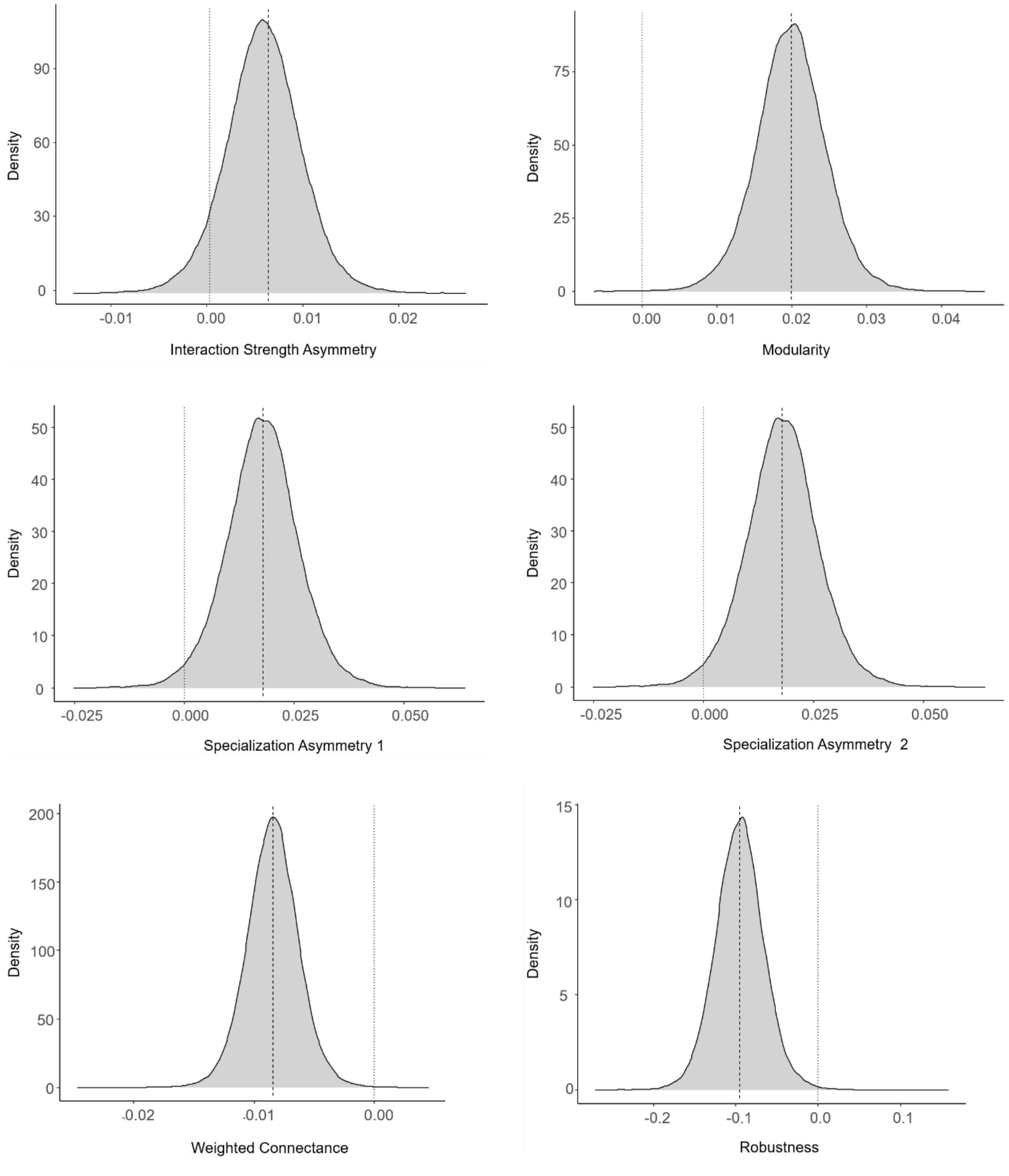


Fig. 5 - Posterior distributions of slope parameters for five network metrics. The dashed line shows the mean of the posteriors; the dotted line shows zero. As the best specialization asymmetry model consists of two predictive variables, posterior distributions were drawn for each parameter, separately: specialization asymmetry 1 (posterior distribution of the slope parameter for the abundance of floral visitors) and specialization asymmetry 2 (posterior distribution of the slope parameter for the number of flowers).

Table 03. Testing the hypotheses that the given predictor variables significantly affect the metrics of the floral visitor network, evaluated using the Bayes Factor (BF) and the probability of the estimated slope for each predictor being different from zero.

Metric	Hypothesis	BF	Probability
Interaction Strength Asymmetry	Abundance of flower visitors > 0	1.87	0.94
Modularity	Abundance of flower visitors > 0	2.00	1.00
Specialization Asymmetry	Abundance of flower visitors > 0 Number of flowers > 0	0.42	0.08
Weighted Connectance	Abundance of flower visitors < 0	2.00	1.00
Robustness	Dry season < 0	2.00	1.00

2.7 DISCUSSION

Here, we aimed to understand the influence that the dry season (period of less rain and lower temperatures), the abundance of floral visitors, and the number of flowers have on metrics of the plant-floral visitor interaction networks over a whole seasonal cycle using empirical time series data collected over 13 months. First, we observed that the time series analysis of all ten metrics (nine metrics and robustness to secondary extinction) that demonstrated non-trending and stationary dynamics over the months. Subsequently, we found that the dry season negatively affected the robustness of networks along the time series. The abundance of floral visitors positively influenced modularity and negatively affected weighted connectance. Finally, the estimated number of flowers had no effect on the metrics of each network.

Time series analyses demonstrated non-trend and stationary dynamics over the months, i.e., typical white noise (HALLEY; KUNIN, 1999). We confirmed the prevalence of white noise dynamics with only weak or no dependence between consecutive months. Previous studies have reported that short time series tend to have white noise, while long time series tend to have red noise(GILLJAM et al., 2019; PIMM; REDFEARN, 1988). Pimm and Redfearn (1988), in one of the most classic studies on population variation, reported that longer time series tended to show red noise, while shorter time series tended to show white noise. At the same time, Gilljam et al. (2019) showed that time series, which assess environmental variables measured annually, are best described by white or faintly red noise. Studies such as these support our results and allow us to assume that the variation in our time series was not the result of the influence of time but rather the influence of other mechanisms that act on them. Furthermore, the lack of knowledge on ecological network metrics and time series in the literature, at least to us, highlighted the necessity to investigate how long-term effects affect network structure and function and the existence of cycles or periodicity.

The dry season showed lower temperatures and average precipitation than the wet season. This pattern was already expected, as the study area is classified as having a wet subtropical climate (Cwa) by the Kopen classification (DANTAS; CARVALHO; FERREIRA, 2007; DE SÁ JÚNIOR et al., 2012; MARTINS et al., 2018). The Cwa group characterizes regions with well-defined dry and wet seasons, with increased temperatures and precipitation values in the wet season (BECK et al., 2018).

This contrast between seasons likely affects interaction networks from the moment that they influence the probability of interaction among a given pair of species (ESCOBEDO-KENEFIC et al., 2020; POVEDA-CORONEL; RIAÑO-JIMÉNEZ; CURE, 2018; SOUZA et al., 2018). In our study, only the robustness of the networks was affected by seasonal variations, and the dry season had a negative impact on the robustness of the networks. This result was likely because communities presented fewer species during the drought period, representing a smaller number of potential interaction links and a greater vulnerability to extinction, since the chance of losing an important partner was inflated when there were fewer species in the local pool (RABELING et al., 2019; SOUZA et al., 2018). This decrease in the size of the community was because many plants had flowering strategies associated with the transition from the dry to the wet season (MORELLATO et al., 2013, 2016; OLIVEIRA, 2008). In addition, most of the plants in the study area are herbs and shrubs, such as *Lantana camara* (shrubs), *Erythroxylum suberosum* (shrubs), *Borreria verticillata* (herbs), and *Elephantopus mollis* (herbs). These types of plants perform poorly in terms of absorbing water at great depths; thus, they often suffer from water stress and may not flower in the dry season (JACKSON et al., 1999; RABELING et al., 2019).

Although few studies report this seasonal variation in the robustness of the community, studies such as those by Souza et al. (2018) and Rabeling et al. (2019) support our result. The former authors studied Cerrado areas (phytophysiognomy Dirty Field) and reported a less robust community during the dry season, which was similarly argued to be a consequence of simplified communities. Similarly, Souza et al. (2018), in a study conducted in areas of the Cerrado, Chaco, and Pantanal, reported a decrease in the interactions richness between plants and pollinators during the dry season, which was mainly triggered by the shortage of viable open flowers. Therefore, it is notable that the abiotic factors that characterize the dry and rainy seasons influence networks over time by acting directly on resource availability and the probability of interaction among species.

The abundance of floral visitors positively influenced modularity and negatively influenced weighted connectance. However, we have not identified studies that relate modularity to the abundance of floral visitors. Previous studies show only that modularity decreases with increasing latitude and increases with higher levels of precipitation (TRØJELSGAARD; OLESEN, 2013; VIZENTIN-BUGONI et al., 2018) but without relating these patterns with the abundance or foraging strategy of floral visitors. Trøjelsgaard and Olesen (2013), for example, in a global study of pollination networks, reported a linear increase in modularity with precipitation and a linear decrease in modularity with latitude. Such patterns can be observed by comparing modularity in tropical and temperate regions, with tropical regions being more modular than temperate regions (VIZENTIN-BUGONI et al., 2018).

In addition, modularity is directly influenced by species richness, being greater when there is a greater diversity of species (SCHWARZ et al., 2020; VIZENTIN-BUGONI et al., 2018). Martín González et al. (2015), in a macroecological study with plant-pollinator interaction networks across the Americas, found that species richness had a positive relationship with complementary specialization and modularity. A similar pattern was reported by Petanidou et al. (2018) and Schwarz et al. 2020. However, Petanidou et al. (2018) also found that the climate affected modularity directly and indirectly through the influence that it exerts on species richness. Particularly, the prediction of such direct and indirect influences of species richness on modularity may apply in our work, since we grouped all individuals without distinguishing them by species. However, species-rich networks also had inflated abundances, which was the prevalent mechanism behind the influence of richness on modularity. Thus, we believe that grouping plots and transects in our approach still reveals that modularity is likely driven by species richness and not by the inflated abundance of floral visitors.

Analogous to modularity, weighted connectance was also influenced by the abundance of floral visitors. Again, we have not identified studies that relate abundance to this metric. A possible explanation for our findings would be the use of the general abundance of floral visitors per month. In other words, abundance is formed by all individuals of all species that occurred during a given month. Therefore, it seems intuitive that those months with increased species richness may have presented a greater abundance of floral visitors, even if the number of collected individuals was not proportionally high, due to the abundance having been treated as the sum of all individuals without distinction per species.

Connectance is sensitive to the size of the network, that is, it decreases in species-rich networks (JORDANO, 1987b; OLESEN; JORDANO, 2002; VIZENTIN-BUGONI et al., 2018). This sparsity emerges because connectance is the proportion of realized links among all possible links on a network (LANDI et al., 2018). For an interaction network, a greater number of species present in the network (increased richness) could mean an increased number of nodes and thereby a lower probability of isolated species (BASCOMPTE; JORDANO, 2014). However, such an increased possibility of forming links actually coincides with decreased connectance in empirical networks since many interactions remain only as potential interactions (JORDANO, 1987b). Intuitively, we could say that networks with a greater abundance of individuals from many species on both levels (plants and floral visitors) would have lower connectance, assuming the neutrality hypothesis where species are ideally identical and have a higher chance of interacting (VÁZQUEZ et al., 2007). Therefore, more abundant species could interact with nearly all species, further increasing both the size and the connectance of networks. However, it is worth remembering that although the abundance of individuals is an important contributor to the interaction between a given pair of species, there are biological restrictions that prevent species from interacting. These constraints, theoretically assumed under the "forbidden links hypothesis", require making some interactions only potential (VÁZQUEZ; CHACOFF; CAGNOLO, 2009). Thus, the fact that the tradeoff between the neutrality and the forbidden links hypotheses produces sparse species-rich empirical networks suggests that biological constraints must prevail over the probabilistic assignment of interactions among species in nature. Our results reinforced this view when weighted connectance was influenced by the abundance of floral visitors.

Previous studies have already reported decreased connectance with increased species richness (LARA-ROMERO et al., 2019; OLESEN; JORDANO, 2002; PETANIDOU et al., 2018; SCHWARZ et al., 2020; VIZENTIN-BUGONI et al., 2018). For example, Schwarz et al. (2020) reported that species richness directly affected connectance, the generality of plants, and the generality of pollinators. Similarly, Olesen and Jordano (2002), in a study involving five regions of the globe (tropical, arctic, temperate, alpine, and Mediterranean), observed that connectance decreased with the increase in the number of species in all five regions.

Finally, contrary to our expectation, the number of flowers had no effect on network metrics. The number of flowers was expected to positively influence network metrics, as well as plant species richness and the abundance of floral visitors, which offers feedback to the pollinator community (EBELING et al., 2008; HEGLAND; BOEKE, 2006; POTTS et al., 2003). However, previous studies have shown that abundance alone does not explain variations in ecological communities (MORENTE-LÓPEZ et al., 2018; PERALTA et al., 2020b; VÁZQUEZ; CHACOFF; CAGNOLO, 2009). Such variations would result directly from the abundance of species and the restrictions imposed by the correspondence of traits, phenological overlap and phylogenetic relationships between species (MORENTE-LÓPEZ et al., 2018; PERALTA et al., 2020b; VÁZQUEZ; CHACOFF; CAGNOLO, 2009). Therefore, a next step is to focus on the abundance of species while considering the phenological overlap and the correspondence of functional traits among interacting partners, as well as the evolutionary history among involved species.

Interaction networks are often treated as static. However, this is a mistake, as networks vary over time and space. With that in mind, we seek to understand which biotic and abiotic factors influence network metrics over a time series. We observed that the dry season (lower levels of temperature and precipitation) affects only the robustness of the networks, which was explained by the decrease in the size of the community. In addition, we found that the abundance of floral visitors positively influenced modularity and negatively influenced weighted connectance. The number of flowers did not influence any network metrics. Our results demonstrate that biotic and abiotic factors influence interaction networks in different ways over time. Understanding how these and other mechanisms influence interaction networks is fundamental information for the conservation of ecological communities, since it can help us understand how and why these communities vary.

DECLARATIONS

Acknowledgements: We thank Dr. Mariana Esteves Mansanares and Dr. Daniel Quedes Domingos for having identified the plant species. We also thank Dr. Mírian Nunes Morales for identifying the syrphids and Dr. Fernando Amaral da Silveira for identifying the bees. Finally, we would like to thank the Research Support Foundation of the State of Minas Gerais (FAPEMIG) for the first author's master's scholarship. MGH is a Brazilian National Council for Scientific and Technological Development fellow (CNPq 304102/2018-0) and thanks FAPEMIG (APQ-02784-17). Faria, L.D.B. thanks the Minas Gerais Research Foundation (FAPEMIG - APQ-02700-17) for financial support, and he is a Brazilian National Council for Scientific and Technological Development fellow (CNPq 306196/2018-2). This work was carried out with support from the Minas Gerais Research Support Foundation (FAPEMIG).

Author Contributions: MGH and LDBF conceived and designed the experiments. MGH performed the experiments. VHDS, MTB, LDBF analyzed the data. VHDS, MTB, MGH, LDBF wrote the manuscript.

Code availability: The codes used during the current study are available from the corresponding author upon request.

Conflict of interest: The authors declare no conflict of interest.

Consent to participate: Not applicable.

Consent for publication: Not applicable.

Data availability: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval: Not applicable.

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SUPPLEMENTARY MATERIAL

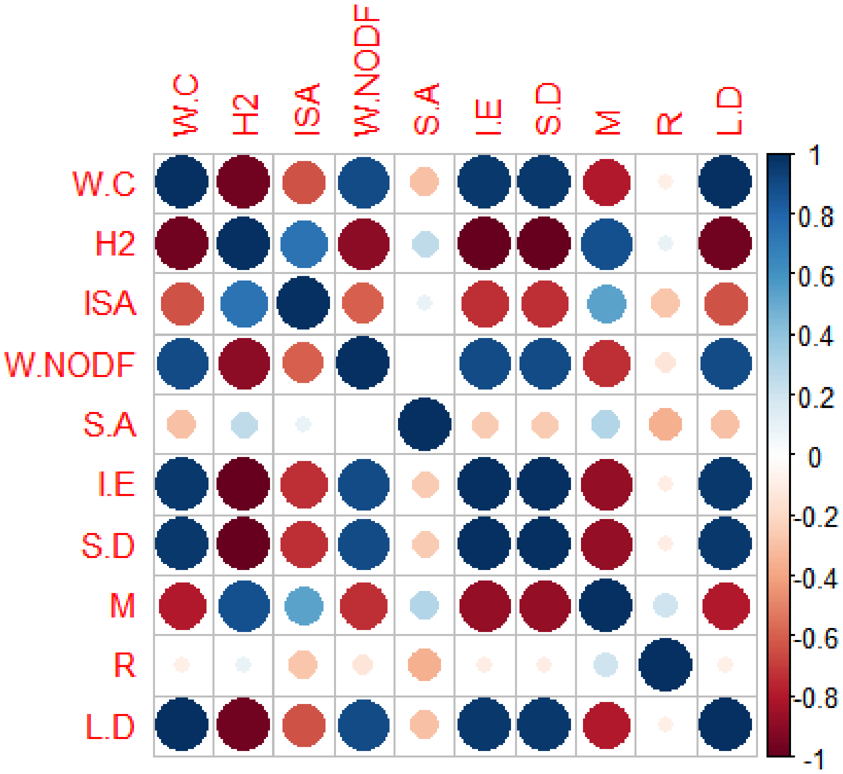
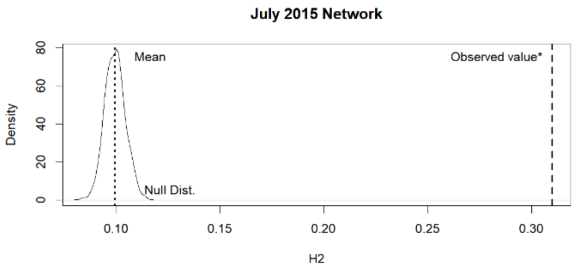
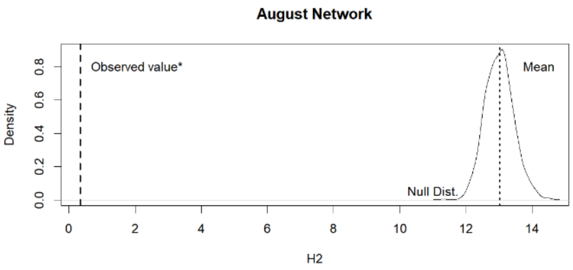


Fig. S1. Spearman's correlation of metrics and robustness.; H2 = network specialization; I.E = interaction evenness; ISA = interaction strength asymmetry;; L.D = linkage density; S.D = Shannon diversity; S.A = specialization asymmetry; M = modularity;; W.C = weighted connectance; W.NODF = weighted NODF; R = robustness.


July 2015 Network
Mean
Observed value*
Null Dist.
Density
0
20
40
60
80
0.10
0.15
0.20
0.25
0.30
H2


August Network
Observed value*
Mean
Null Dist.
Density
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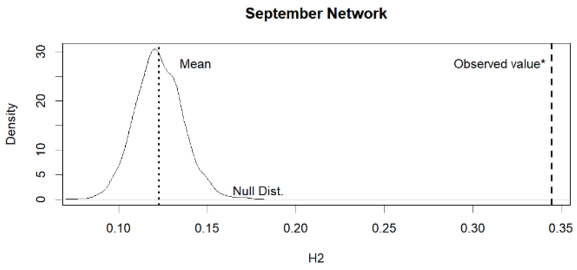
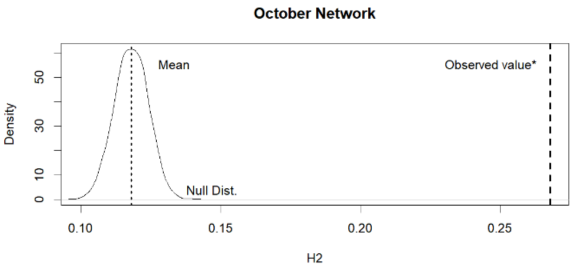
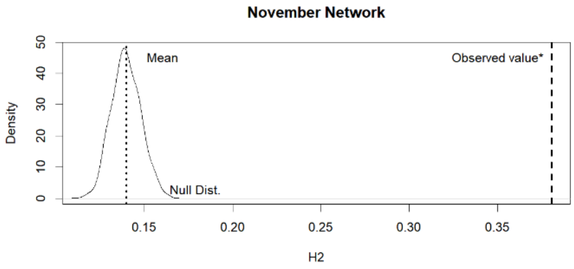
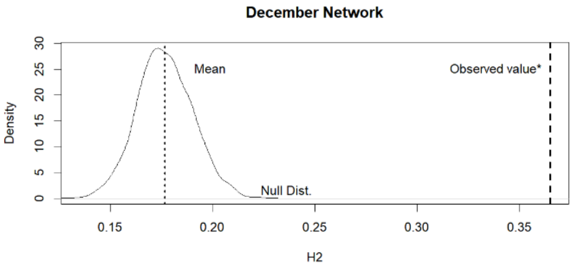
0.2
0.4
0.6
0.8
0
2
4
6
8
10
12
14
H2


September Network
Mean
Observed value*
Null Dist.
Density
0
5
10
20
30
0.10
0.15
0.20
0.25
0.30
0.35
H2


October Network
Mean
Observed value*
Null Dist.
Density
0
10
30
50
0.10
0.15
0.20
0.25
H2


November Network
Mean
Observed value*
Null Dist.
Density
0
10
20
30
40
50
0.15
0.20
0.25
0.30
0.35
H2


December Network
Mean
Observed value*
Null Dist.
Density
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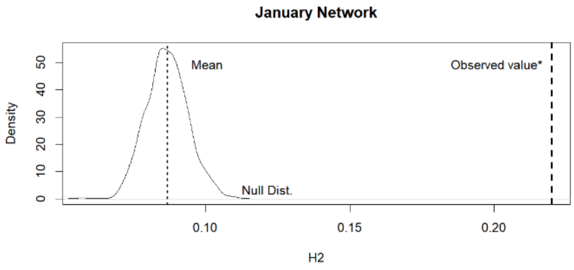
5
10
15
20
25
30
0.15
0.20
0.25
0.30
0.35
H2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Mean
Observed value*
Null Dist.
Density
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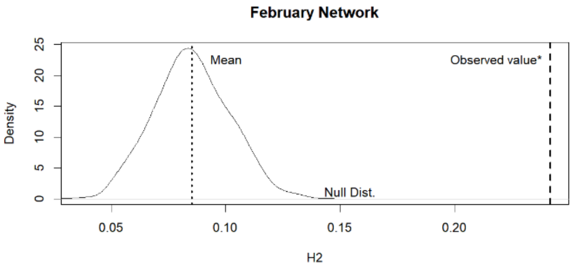
10
20
30
40
50
0.10
0.15
0.20
H2


February Network
Mean
Observed value*
Null Dist.
Density
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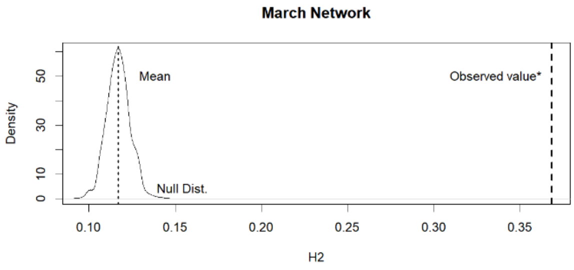
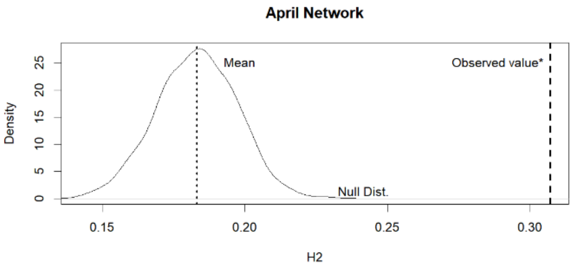
5
10
15
20
25
0.05
0.10
0.15
0.20
H2


March Network
Mean
Observed value*
Null Dist.
Density
0
10
30
50
0.10
0.15
0.20
0.25
0.30
0.35
H2


April Network
Mean
Observed value*
Null Dist.
Density
0
5
10
15
20
25
0.15
0.20
0.25
0.30
H2

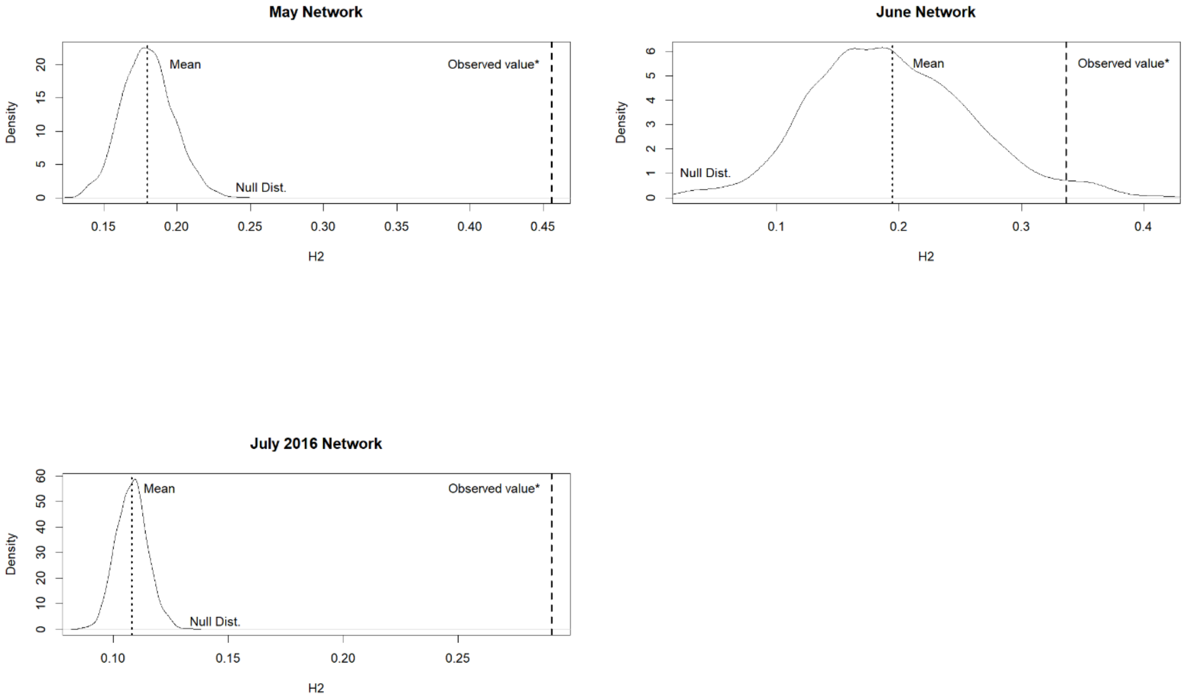
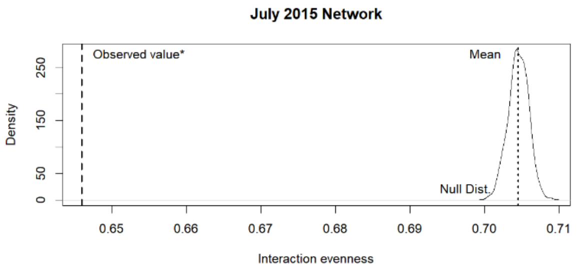


Fig. S2 - A. Graph of null distribution density (solid line) for each month, average of null distribution(vertical dotted line) and observed value (vertical dashed line) for network specialization (H2).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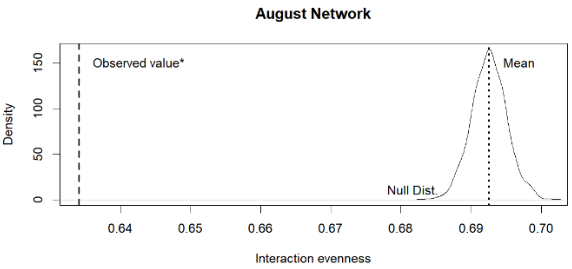
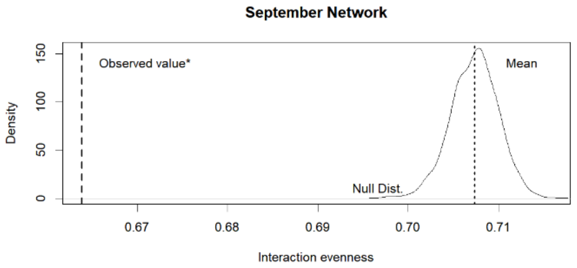
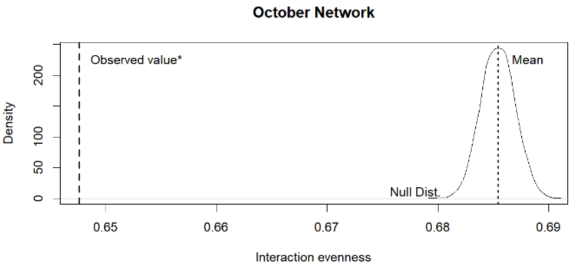

August Network


September Network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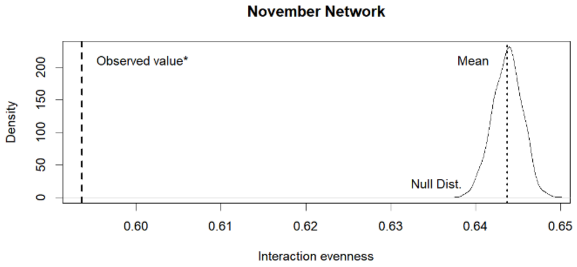
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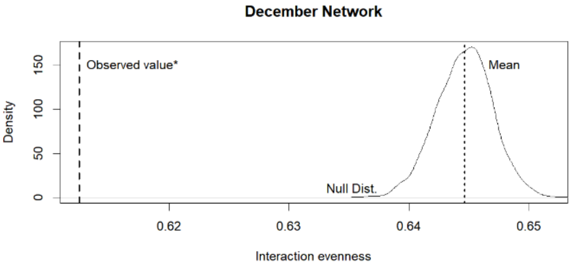
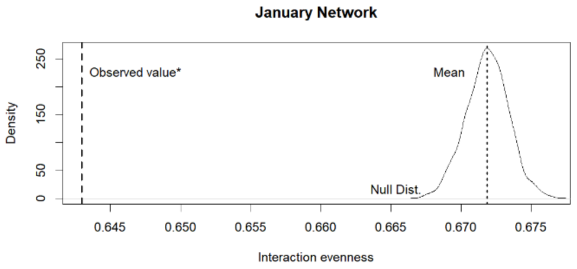

December Network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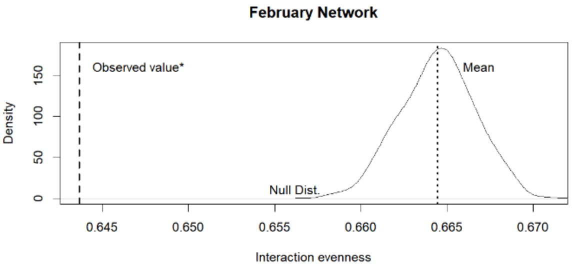
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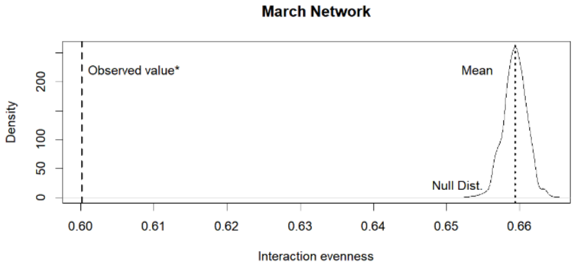
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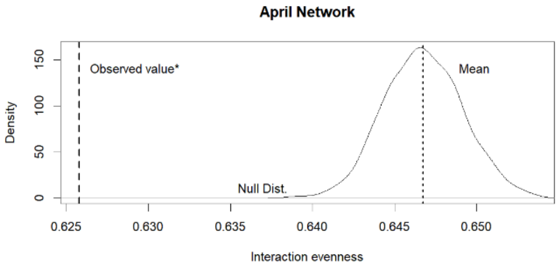
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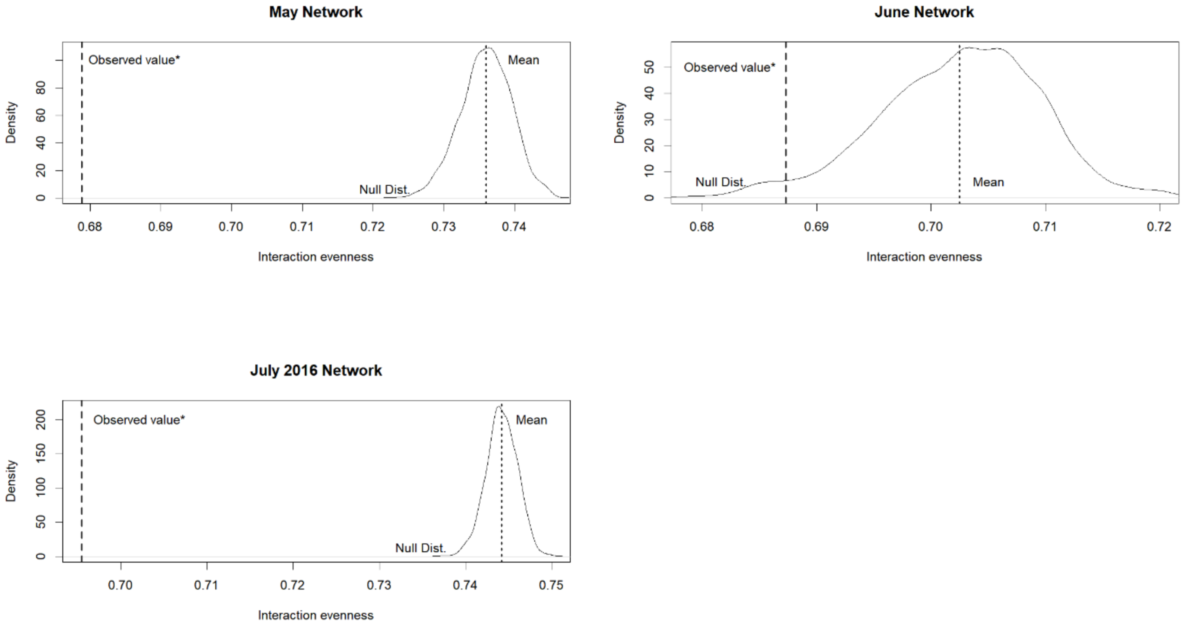
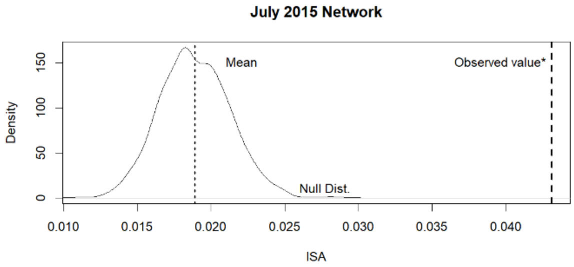
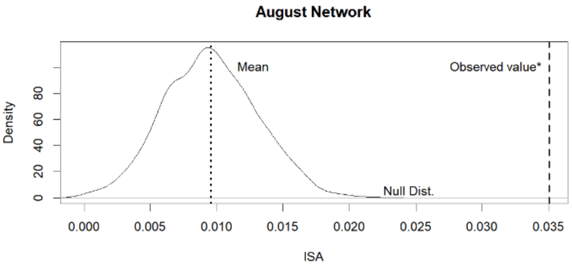
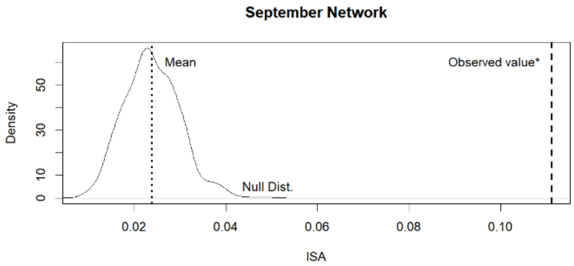


Fig. S2 - B. Graph of null distribution density (solid line) for each month, average of null distribution (vertical dotted line) and observed value (vertical dashed line) for interaction evenness (I.E).


July 2015 Network
Density
0
50
100
150
Mean
Observed value*
Null Dist.
0.010
0.015
0.020
0.025
0.030
0.035
0.040
ISA


August Network
Density
0
20
40
60
80
Mean
Observed value*
Null Dist.
0.000
0.005
0.010
0.015
0.020
0.025
0.030
0.035
ISA


September Network
Density
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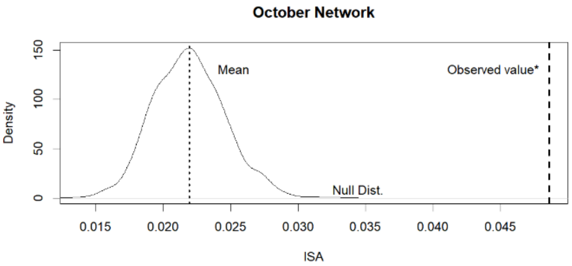
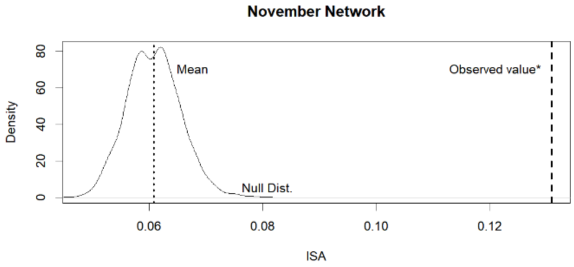
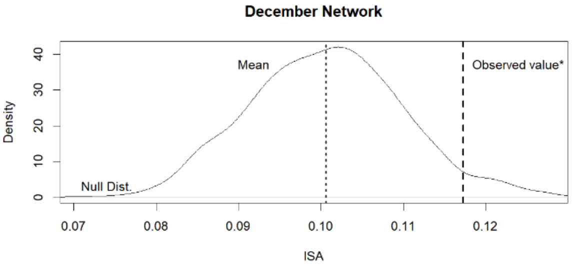
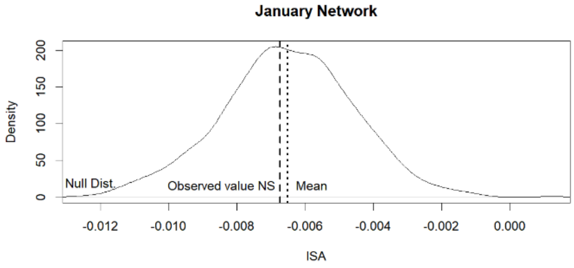
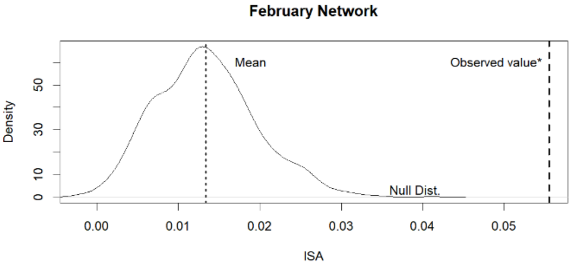
30
50
Mean
Observed value*
Null Dist.
0.02
0.04
0.06
0.08
0.10
ISA

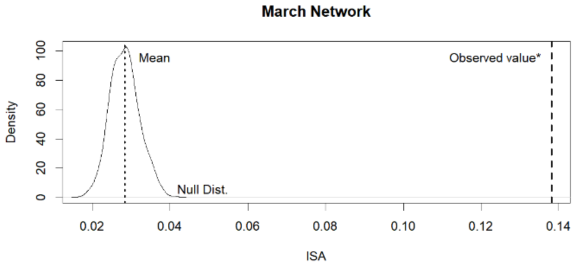

October Network
Density
0
50
100
150
Mean
Observed value*
Null Dist.
0.015
0.020
0.025
0.030
0.035
0.040
0.045
ISA

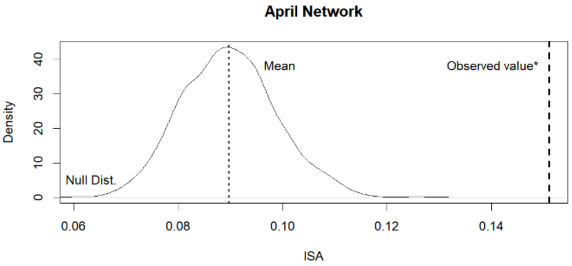

November Network
Density
0
20
40
60
80
Mean
Observed value*
Null Dist.
0.06
0.08
0.10
0.12
ISA


December Network
Density
0
10
20
30
40
Mean
Observed value*
Null Dist.
0.07
0.08
0.09
0.10
0.11
0.12
ISA


January Network
Density
0
50
100
150
200
Null Dist.
Observed value NS
Mean
-0.012
-0.010
-0.008
-0.006
-0.004
-0.002
0.000
ISA


February Network
Density
0
10
30
50
Mean
Observed value*
Null Dist.
0.00
0.01
0.02
0.03
0.04
0.05
ISA


March Network
Density
0
20
40
60
80
100
Mean
Observed value*
Null Dist.
0.02
0.04
0.06
0.08
0.10
0.12
0.14
ISA


April Network
Density
0
10
20
30
40
Mean
Observed value*
Null Dist.
0.06
0.08
0.10
0.12
0.14
ISA

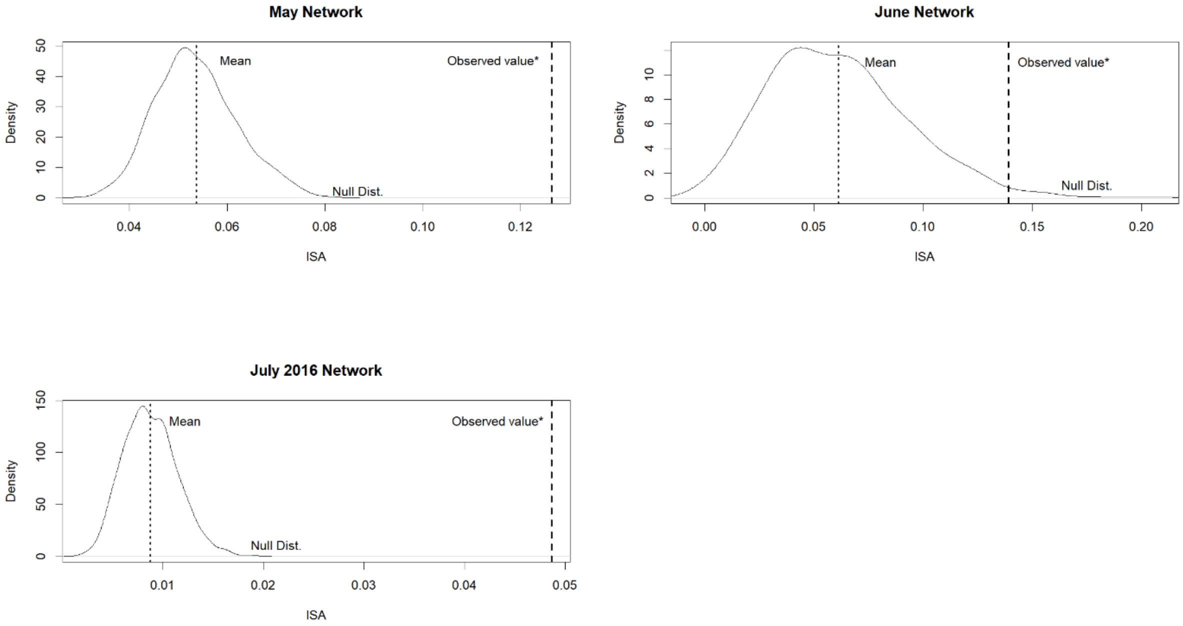
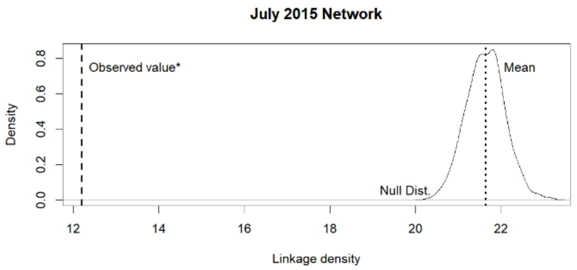
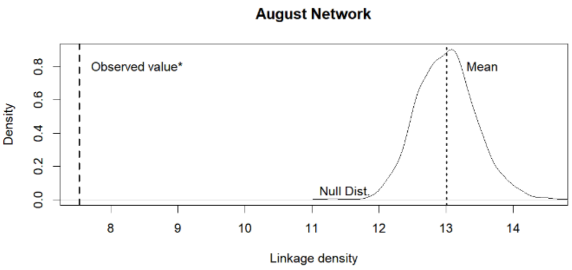
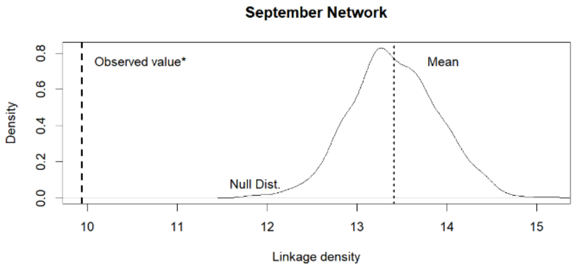
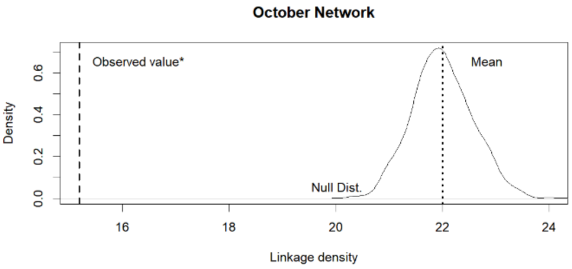


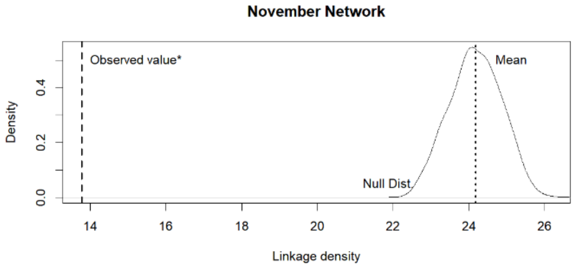
Fig. S2 - C. Graph of null distribution density (solid line) for each month, average of null distribution (vertical dotted line) and observed value (vertical dashed line) for interaction strength asymmetry (ISA).

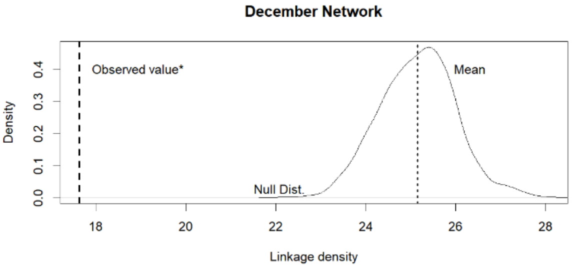

July 2015 Network
Observed value*
Mean
Null Dist.
Density
0.0
0.2
0.4
0.6
0.8
12
14
16
18
20
22
Linkage density

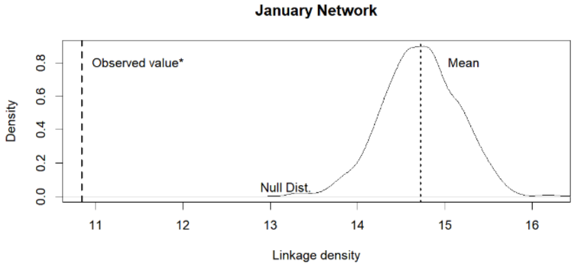

August Network
Observed value*
Mean
Null Dist.
Density
0.0
0.2
0.4
0.6
0.8
8
9
10
11
12
13
14
Linkage density

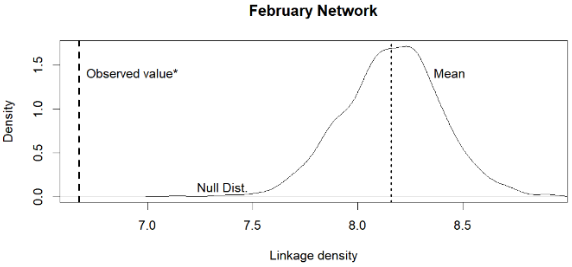

September Network
Observed value*
Mean
Null Dist.
Density
0.0
0.2
0.4
0.6
0.8
10
11
12
13
14
15
Linkage density

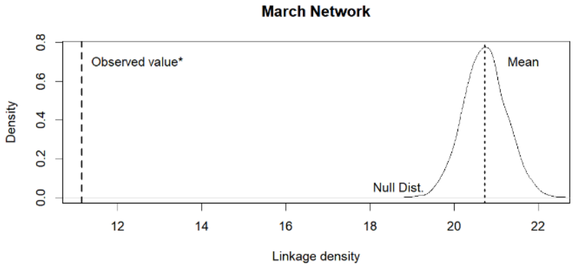

October Network
Observed value*
Mean
Null Dist.
Density
0.0
0.2
0.4
0.6
16
18
20
22
24
Linkage density

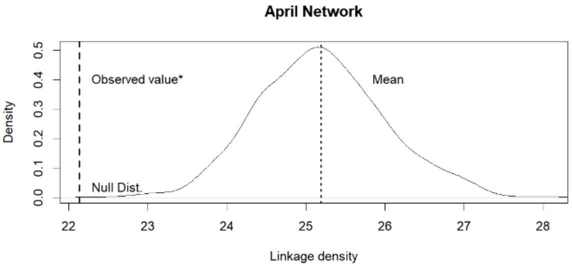

November Network
Observed value*
Mean
Null Dist.
Density
0.0
0.2
0.4
14
16
18
20
22
24
26
Linkage density


December Network
Observed value*
Mean
Null Dist.
Density
0.0
0.1
0.2
0.3
0.4
18
20
22
24
26
28
Linkage density


January Network
Observed value*
Mean
Null Dist.
Density
0.0
0.2
0.4
0.6
0.8
11
12
13
14
15
16
Linkage density


February Network
Observed value*
Mean
Null Dist.
Density
0.0
0.5
1.0
1.5
7.0
7.5
8.0
8.5
Linkage density


March Network
Observed value*
Mean
Null Dist.
Density
0.0
0.2
0.4
0.6
0.8
12
14
16
18
20
22
Linkage density


April Network
Observed value*
Mean
Null Dist.
Density
0.0
0.1
0.2
0.3
0.4
0.5
22
23
24
25
26
27
28
Linkage density

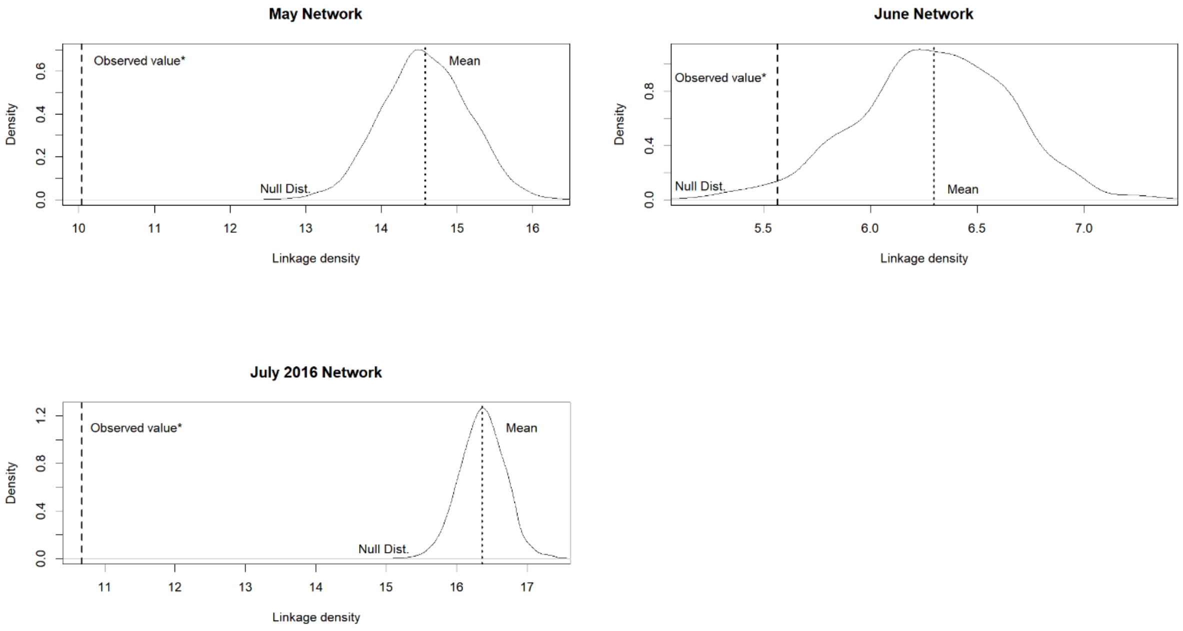
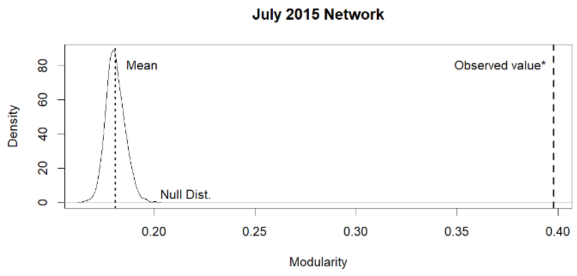
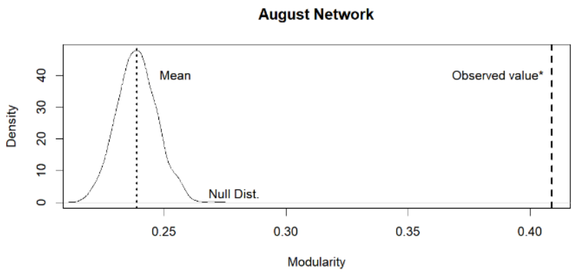
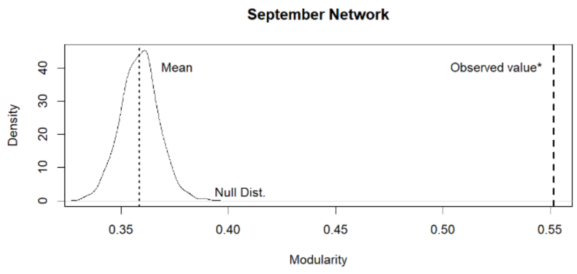
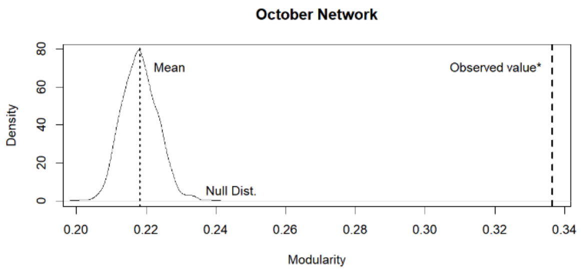


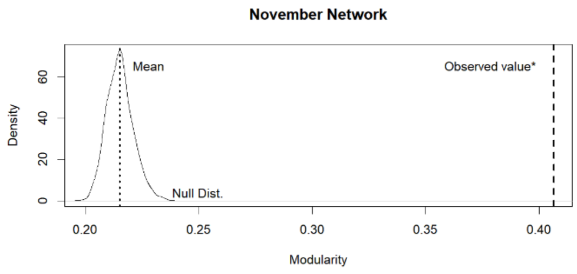
Fig. S2 - D. Graph of null distribution density (solid line) for each month, average of null distribution (vertical dotted line) and observed value (vertical dashed line) for linkage density (L.D).

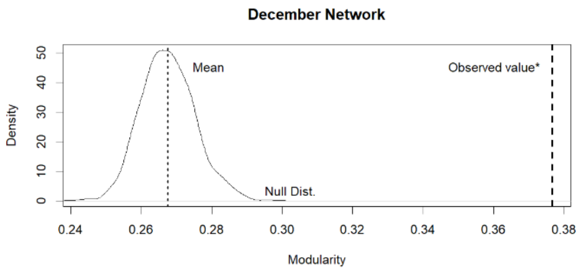

July 2015 Network
Density
0
20
40
60
80
Mean
Observed value*
Null Dist.
0.20
0.25
0.30
0.35
0.40
Modularity

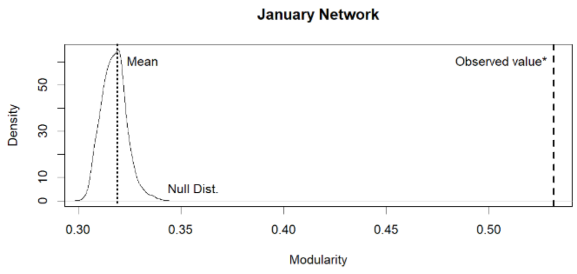

August Network
Density
0
10
20
30
40
Mean
Observed value*
Null Dist.
0.25
0.30
0.35
0.40
Modularity

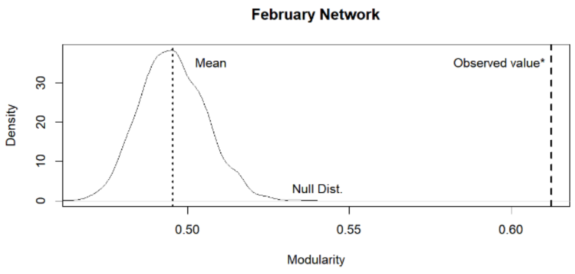

September Network
Density
0
10
20
30
40
Mean
Observed value*
Null Dist.
0.35
0.40
0.45
0.50
0.55
Modularity

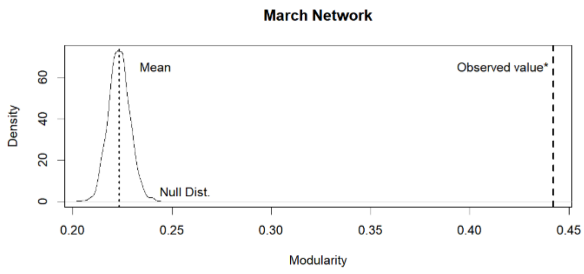

October Network
Density
0
20
40
60
80
Mean
Observed value*
Null Dist.
0.20
0.22
0.24
0.26
0.28
0.30
0.32
0.34
Modularity

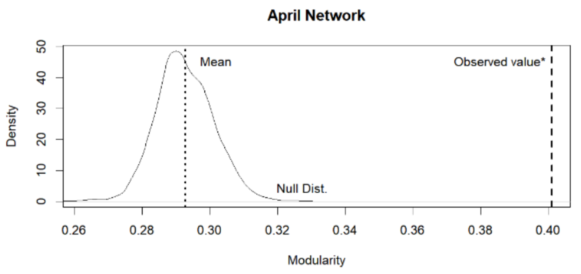

November Network
Density
0
20
40
60
Mean
Observed value*
Null Dist.
0.20
0.25
0.30
0.35
0.40
Modularity


December Network
Density
0
10
20
30
40
50
Mean
Observed value*
Null Dist.
0.24
0.26
0.28
0.30
0.32
0.34
0.36
0.38
Modularity


January Network
Density
0
10
30
50
Mean
Observed value*
Null Dist.
0.30
0.35
0.40
0.45
0.50
Modularity


February Network
Density
0
10
20
30
Mean
Observed value*
Null Dist.
0.50
0.55
0.60
Modularity


March Network
Density
0
20
40
60
Mean
Observed value*
Null Dist.
0.20
0.25
0.30
0.35
0.40
0.45
Modularity


April Network
Density
0
10
20
30
40
50
Mean
Observed value*
Null Dist.
0.26
0.28
0.30
0.32
0.34
0.36
0.38
0.40
Modularity

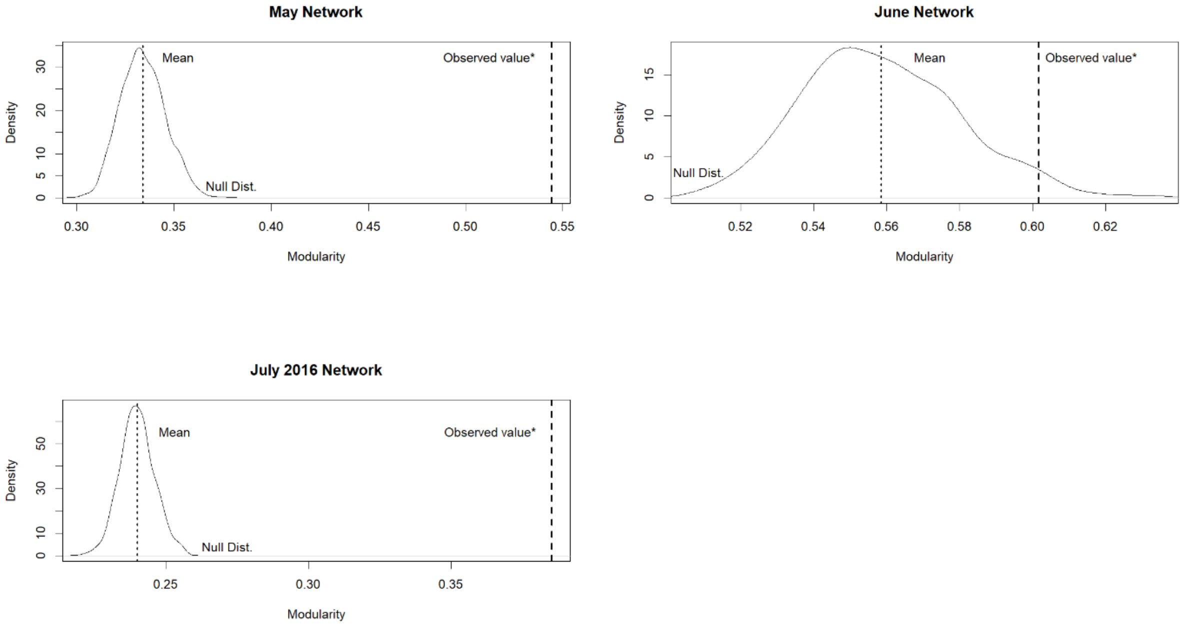
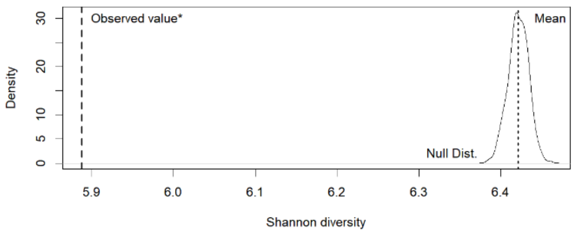
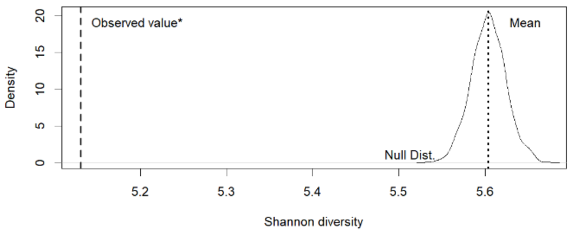
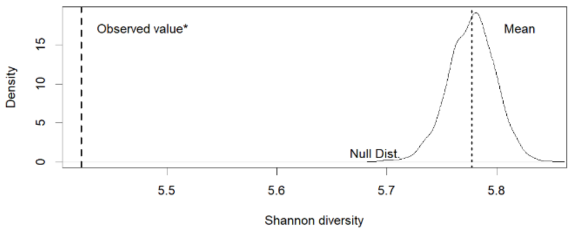
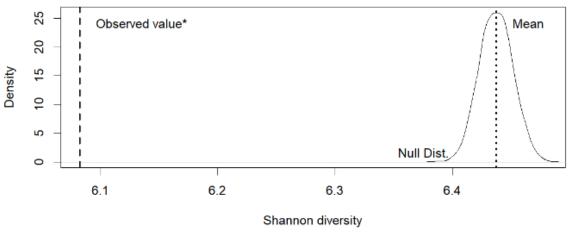


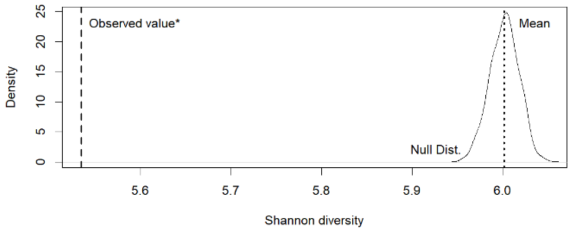
Fig. S2 - E. Graph of null distribution density (solid line) for each month, average of null distribution (vertical dotted line) and observed value (vertical dashed line) for modularity (M).

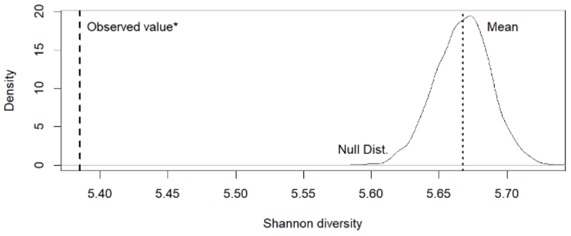

July 2015 Network

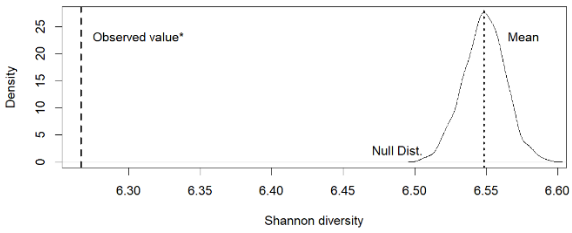

August Network

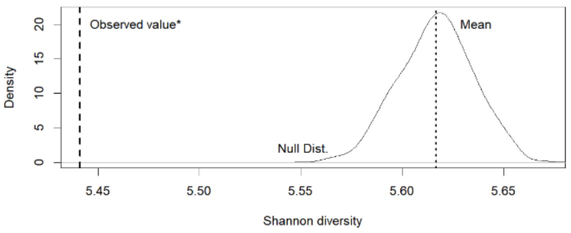

September Network

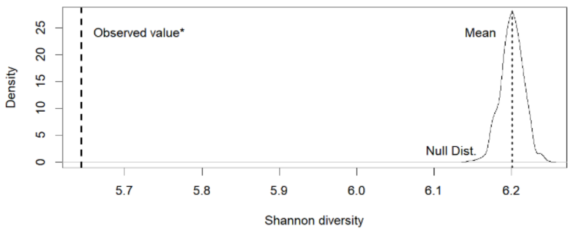

October Network

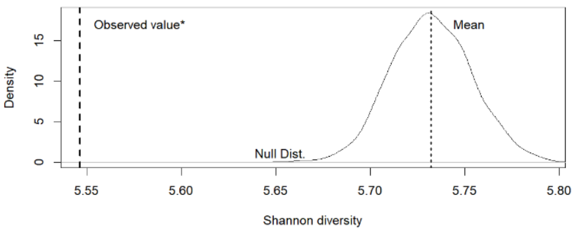

November Network


December Network


January Network


February Network


March Network


April Network

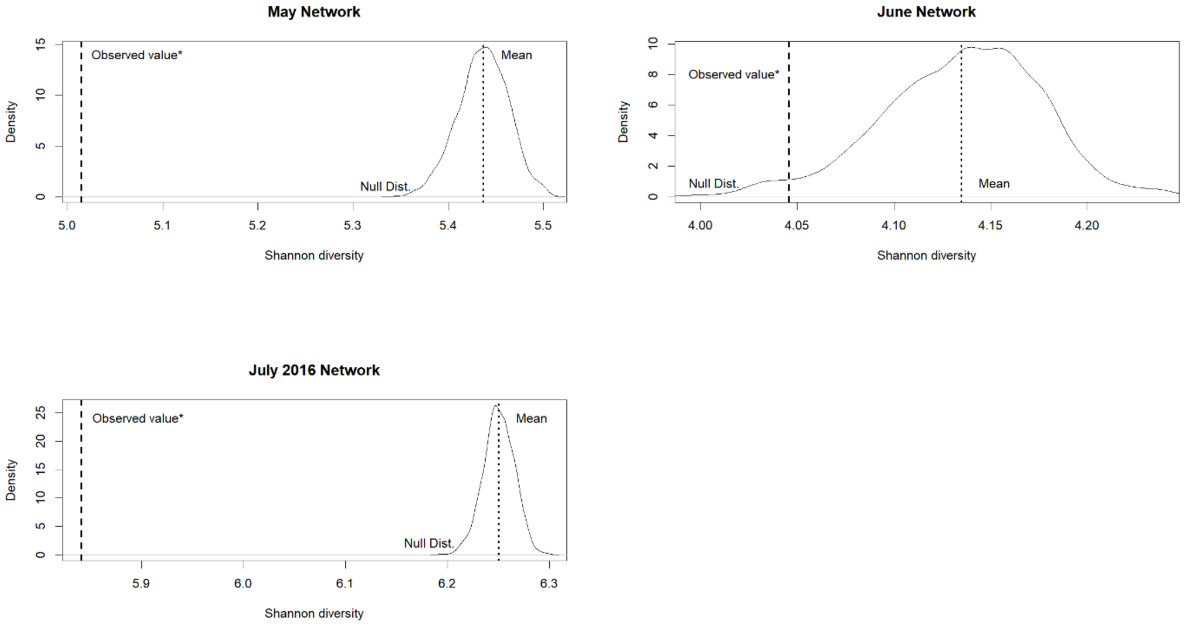
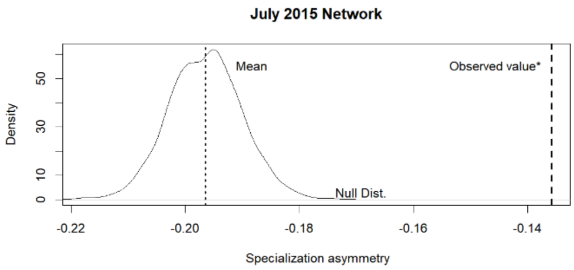
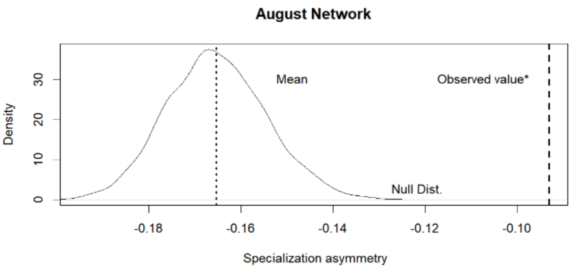
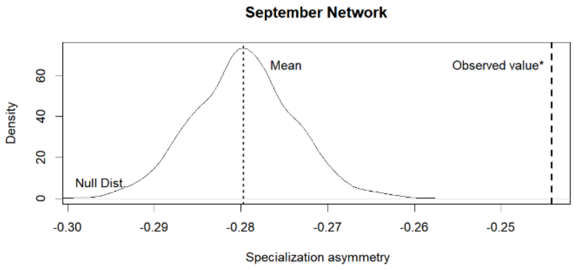
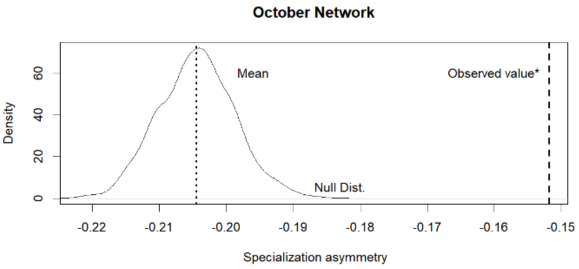


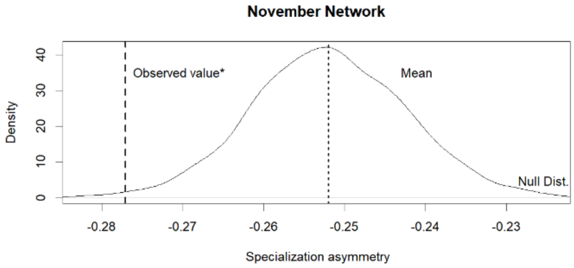
Fig. S2 - F. Graph of null distribution density (solid line) for each month, average of null distribution (vertical dotted line) and observed value (vertical dashed line) for Shannon diversity (S.D).

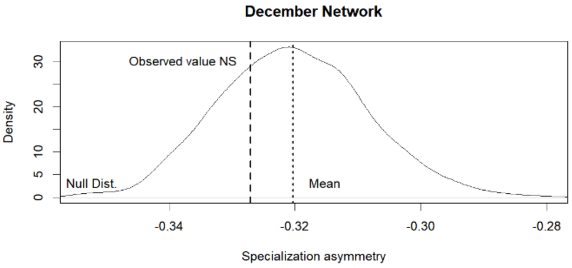

July 2015 Network
Density
0
10
30
50
Mean
Observed value*
Null Dist.
-0.22
-0.20
-0.18
-0.16
-0.14
Specialization asymmetry

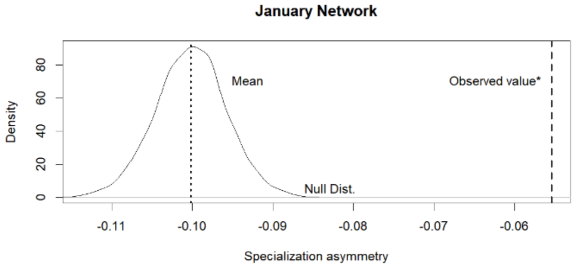

August Network
Density
0
10
20
30
Mean
Observed value*
Null Dist.
-0.18
-0.16
-0.14
-0.12
-0.10
Specialization asymmetry

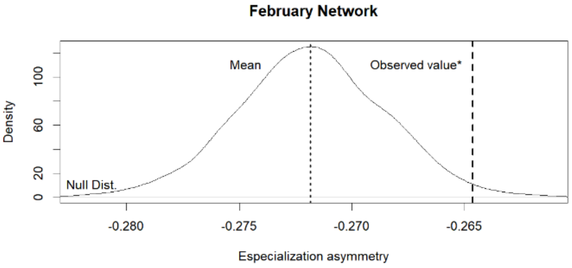

September Network
Density
0
20
40
60
Mean
Observed value*
Null Dist.
-0.30
-0.29
-0.28
-0.27
-0.26
-0.25
Specialization asymmetry

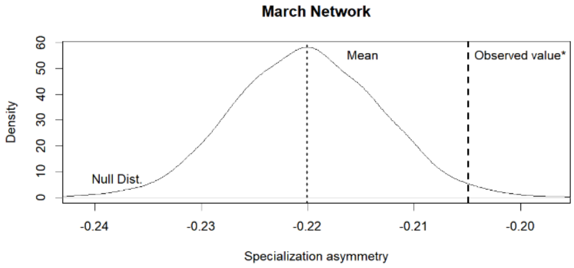

October Network
Density
0
20
40
60
Mean
Observed value*
Null Dist.
-0.22
-0.21
-0.20
-0.19
-0.18
-0.17
-0.16
-0.15
Specialization asymmetry

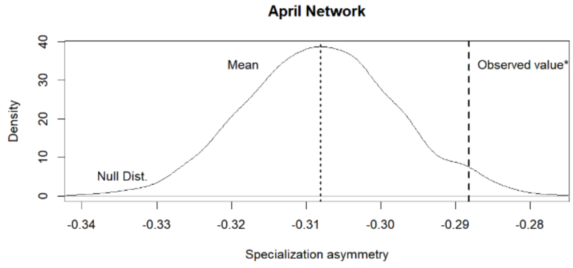

November Network
Density
0
10
20
30
40
Observed value*
Mean
Null Dist.
-0.28
-0.27
-0.26
-0.25
-0.24
-0.23
Specialization asymmetry


December Network
Density
0
5
10
20
30
Observed value NS
Null Dist.
Mean
-0.34
-0.32
-0.30
-0.28
Specialization asymmetry


January Network
Density
0
20
40
60
80
Mean
Observed value*
Null Dist.
-0.11
-0.10
-0.09
-0.08
-0.07
-0.06
Specialization asymmetry


February Network
Density
0
20
60
100
Mean
Observed value*
Null Dist.
-0.280
-0.275
-0.270
-0.265
Especialization asymmetry


March Network
Density
0
10
20
30
40
50
60
Mean
Observed value*
Null Dist.
-0.24
-0.23
-0.22
-0.21
-0.20
Specialization asymmetry


April Network
Density
0
10
20
30
40
Mean
Observed value*
Null Dist.
-0.34
-0.33
-0.32
-0.31
-0.30
-0.29
-0.28
Specialization asymmetry

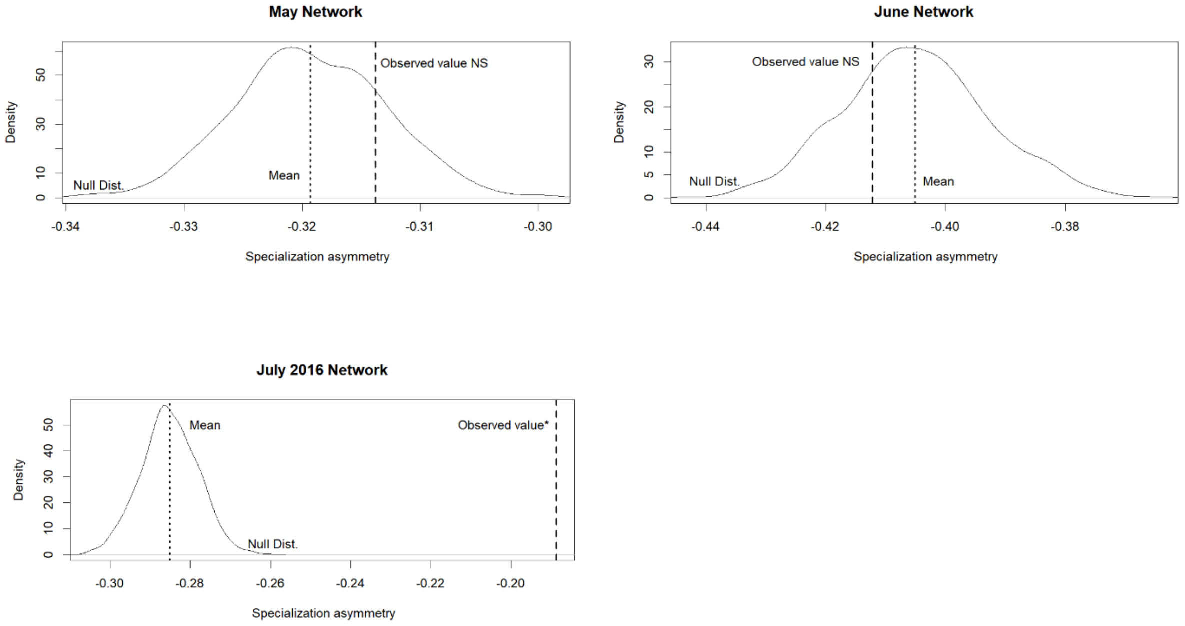
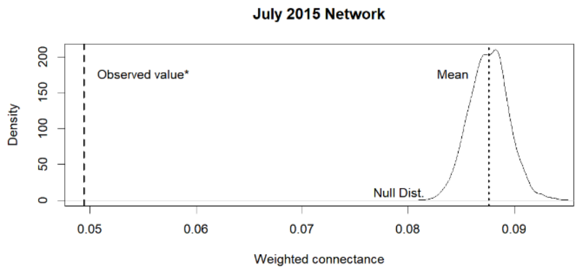
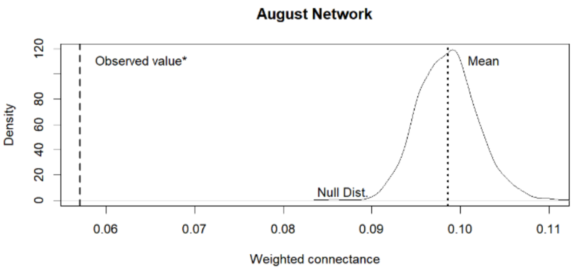
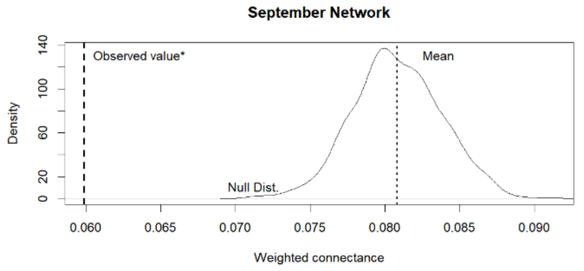
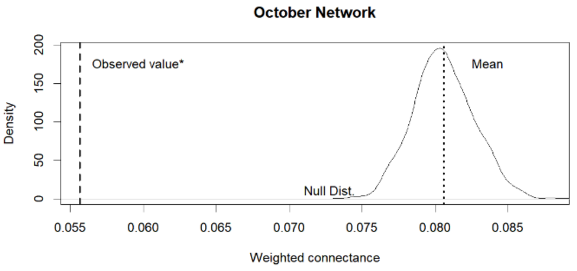


Fig. S2 - G. Graph of null distribution density (solid line) for each month, average of null distribution (vertical dotted line) and observed value (vertical dashed line) for specialization asymmetry (S.A).


July 2015 Network


August Network


September Network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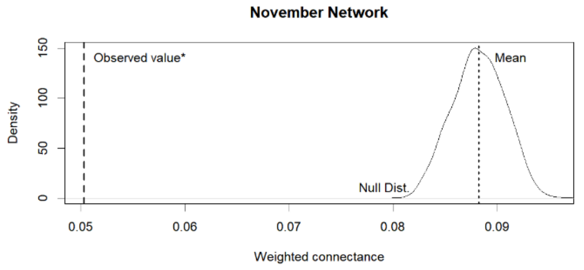
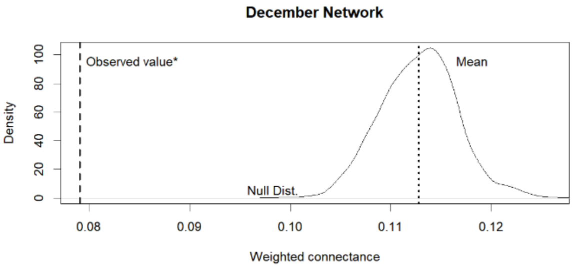
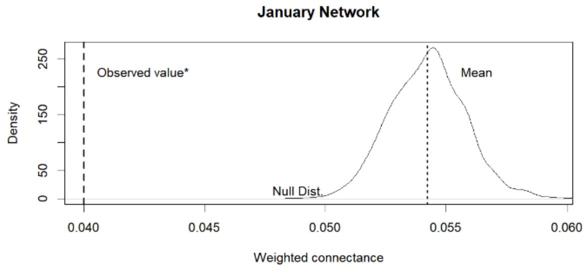
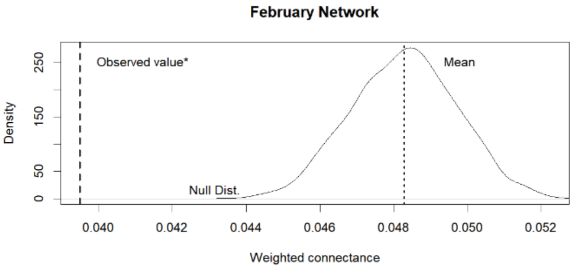
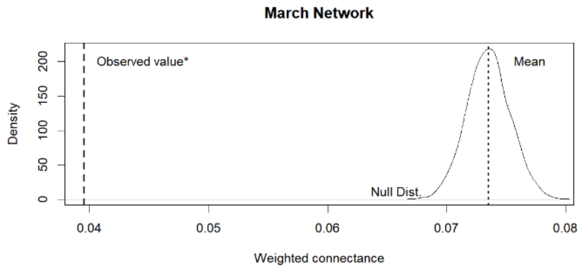
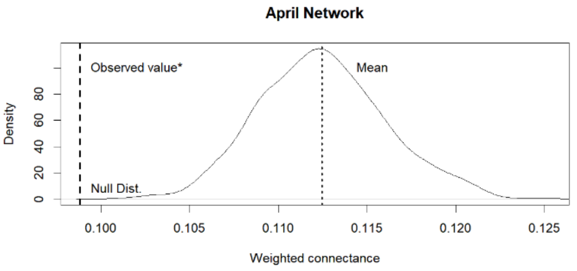

November Network


December Network


January Network


February Network


March Network


April Network

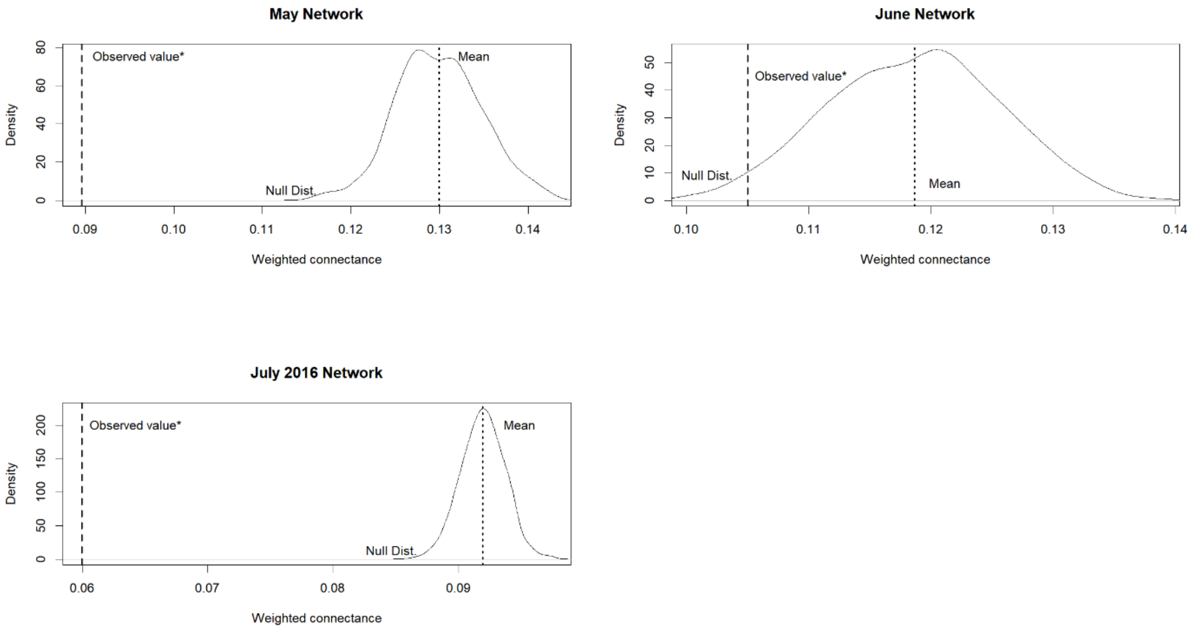
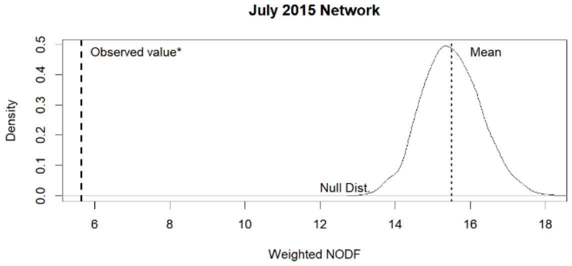
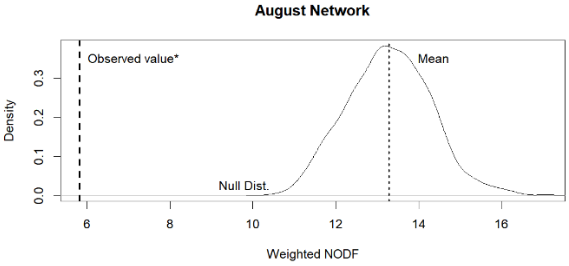


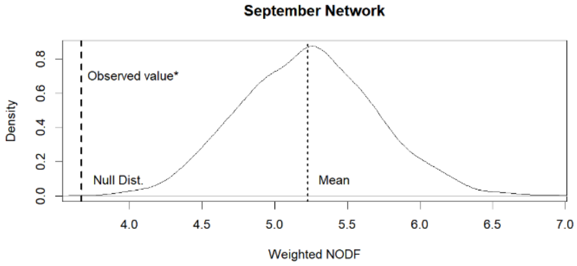
Fig. S2 - H. Graph of null distribution density (solid line) for each month, average of null distribution(vertical dotted line) and observed value (vertical dashed line) for weighted connectance (W.C).



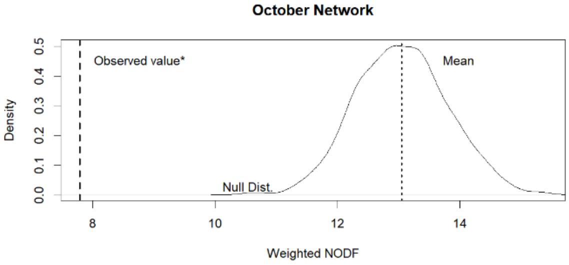
July 2015 Network



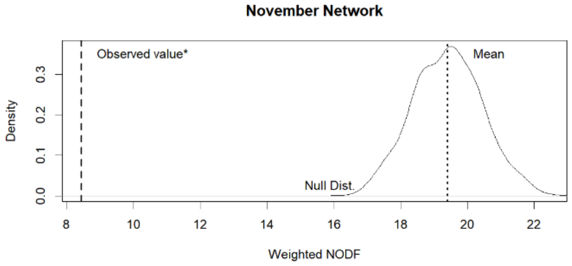
August Network



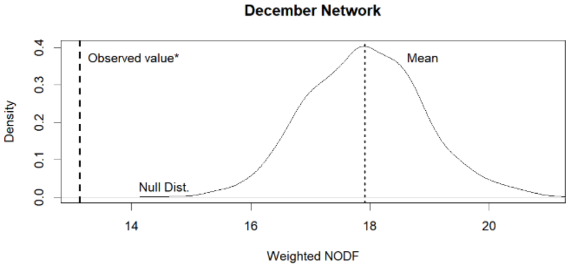
September Network



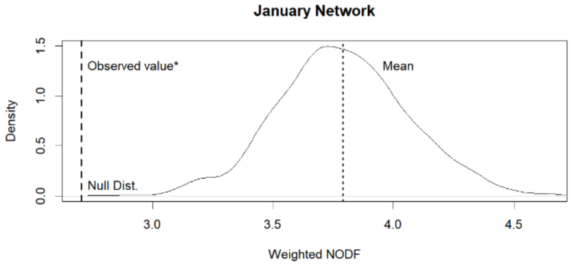
October Network



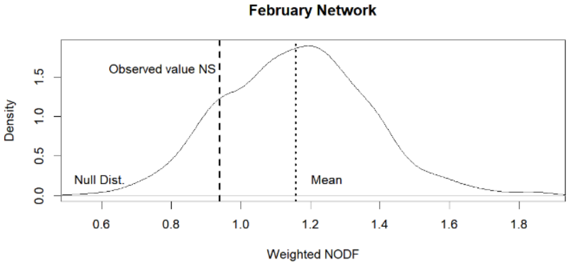
November Network



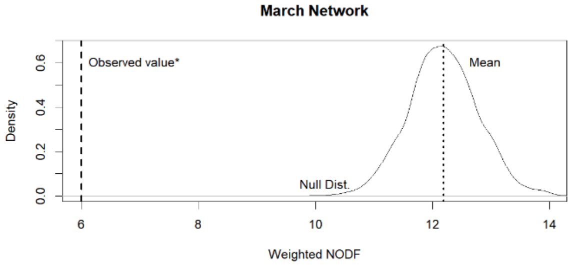
December Network



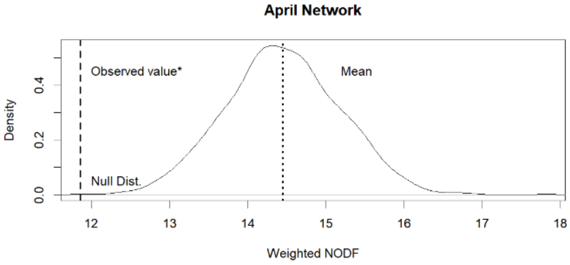
January Network



February Network



March Network



April Network

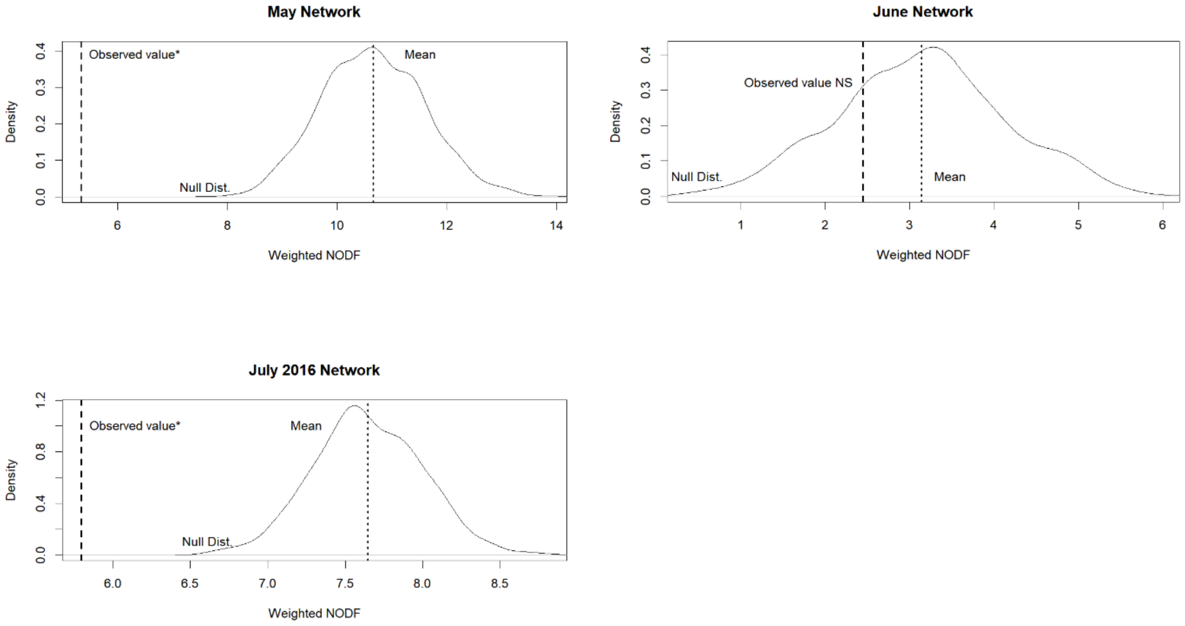
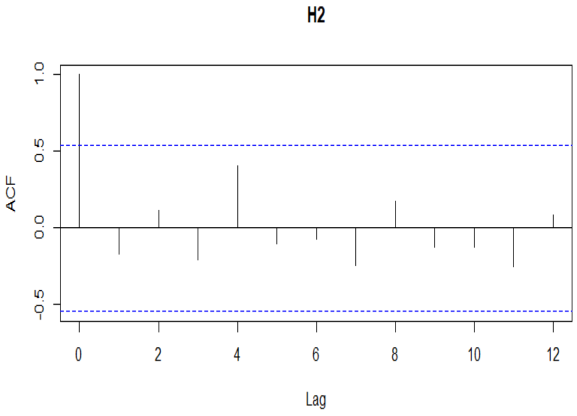
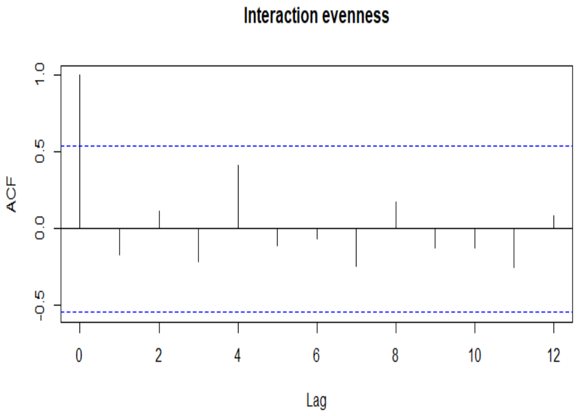
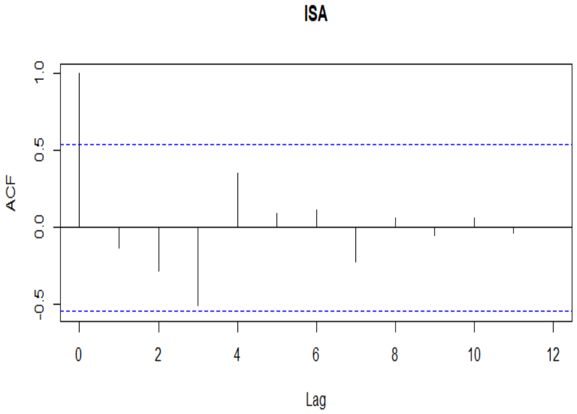
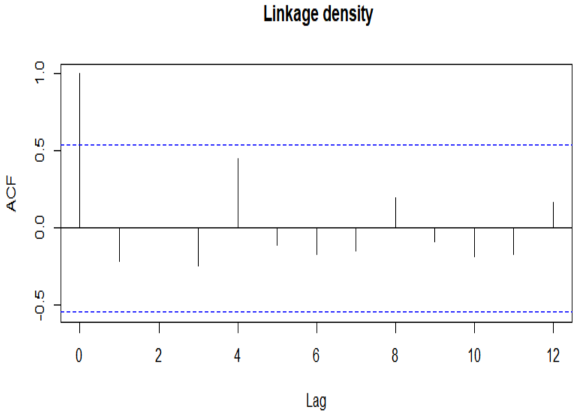


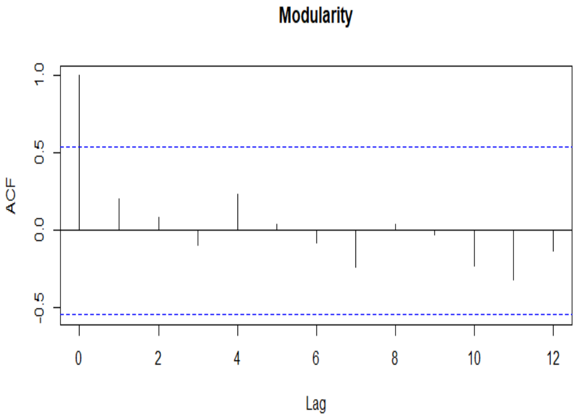
Fig. S2 - I. Graph of null distribution density (solid line) for each month, average of null distribution (vertical dotted line) and observed value (vertical dashed line) for weighted NODF (W. NODF).

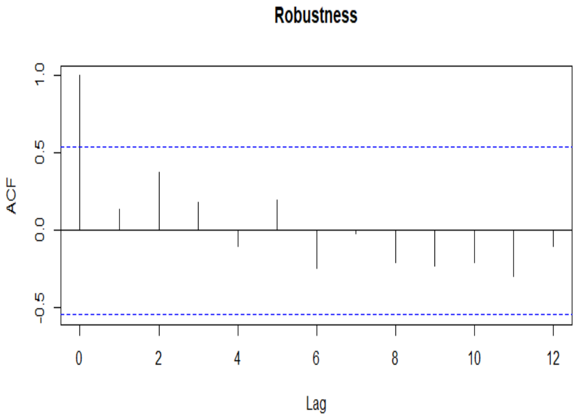

H2


Interaction evenness


ISA


Linkage density


Modularity


Robustness

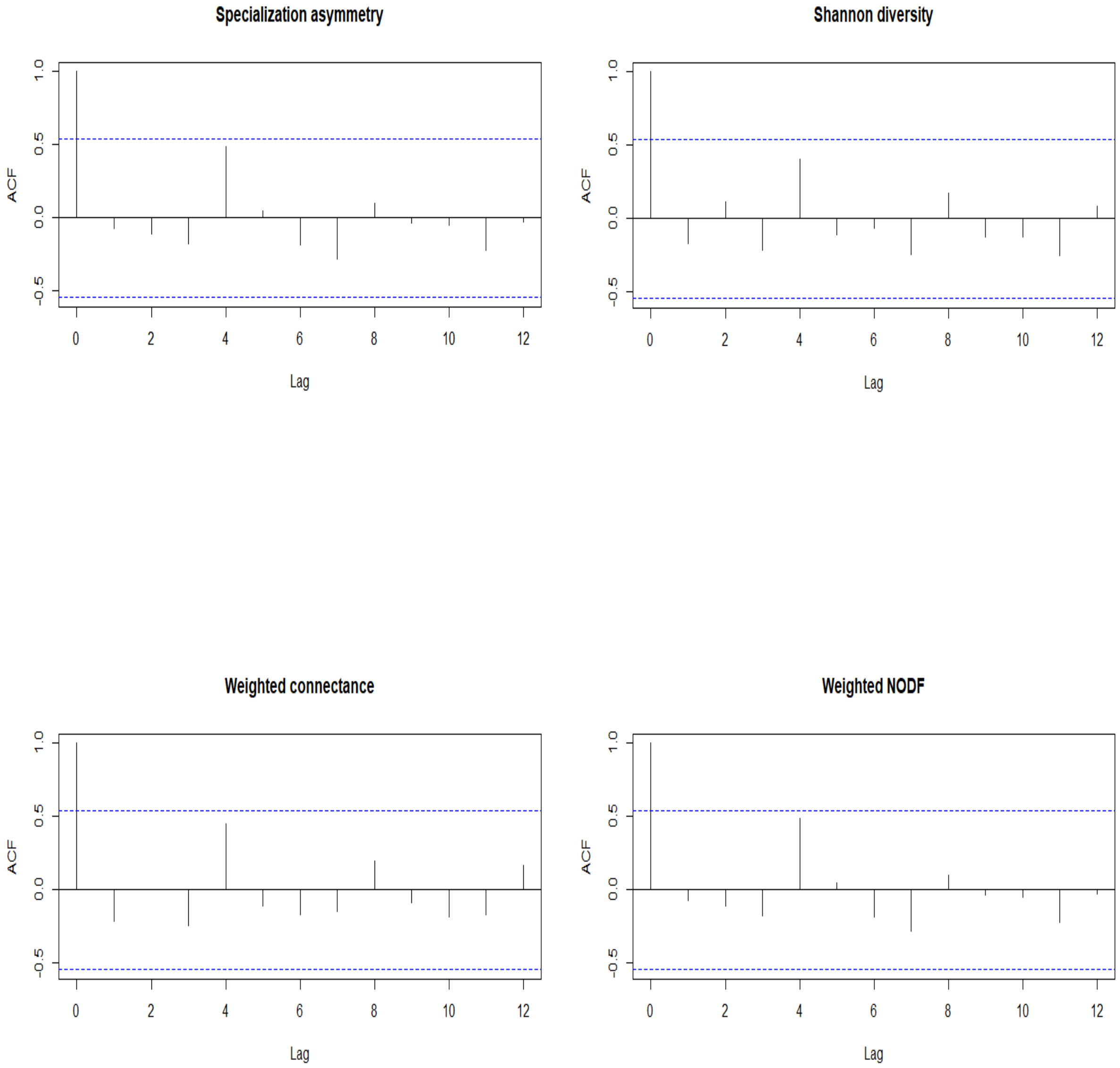


Fig. S3. Autocorrelation (ACF) in ARIMA models of residual time series of network metrics showing no delay or white noise effect - that is, no dependence between consecutive metrics across sampling months.

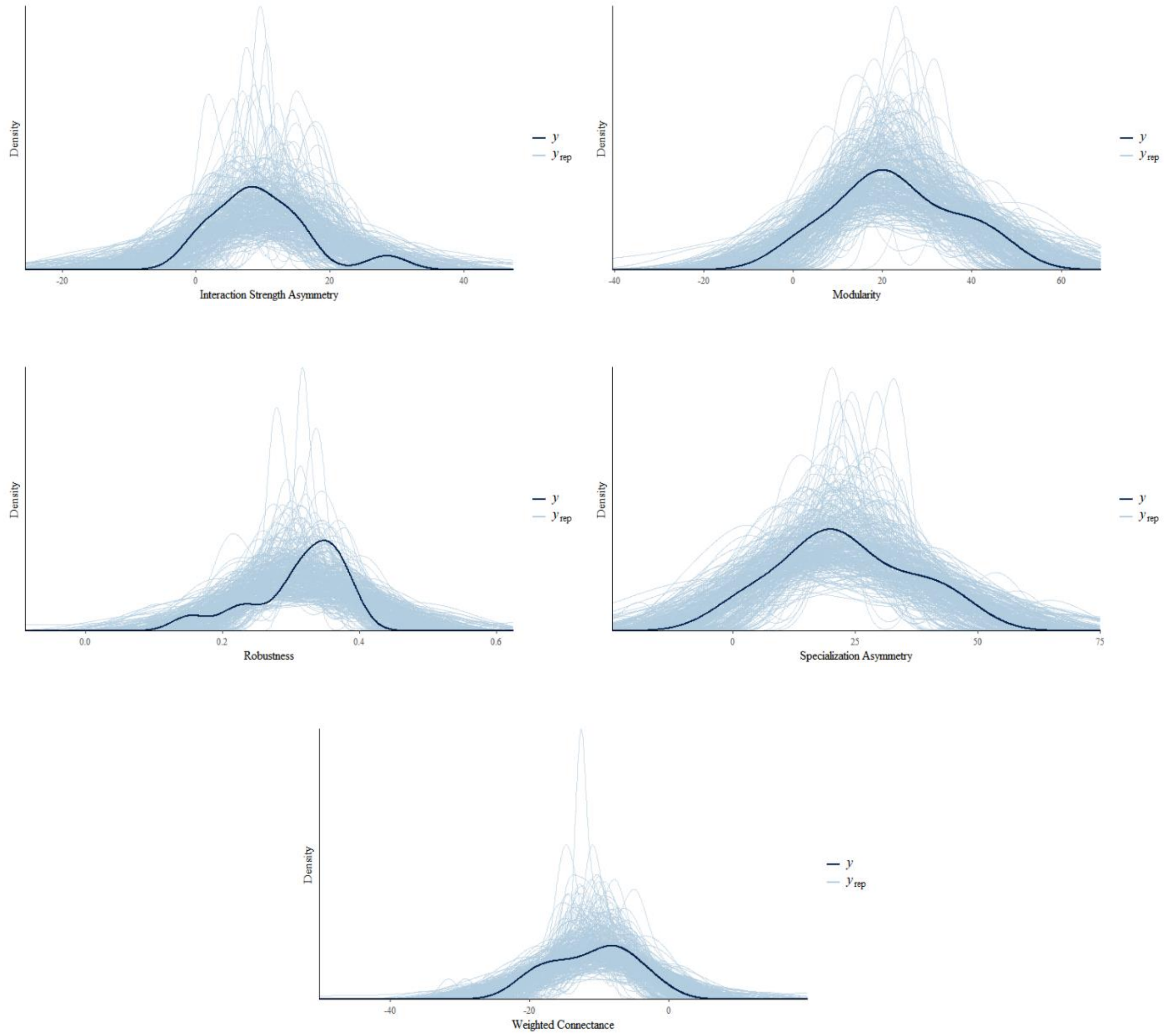


Fig. S4. Graphical posterior predictive verification comparing (y:smoothed core density) the observed distribution of the metrics to 300 simulated data sets of the posterior predictive distribution (yrep: light blue lines).

Table S1. Kruskal-Wallis test for temperature and precipitation between seasons (dry and wet).

	Kruskal-Wallis			**Dunn test**
	Chi-squared	**DF**	**P value**	**P value**
Temperature-Season	9	1	0.0027	0,0027
Precipitation-Season	9	1	0.0027	0,0027

Table S2. List of flower-visiting species recorded in the Cerrado area of the UniLavras-Boqueirão Biological Reserve (RBUB), Lavras, Minas Gerais, Brazil.

Visitor species
Acroceridae sp.
Actinote canutia (Hopffer, 1874)
Actinote pellenea (Geyer, 1832)
Adelpha syma (Godart, 1824)
Aeria olena (Weymer, 1875)
Agelaia multipicta (Haliday, 1836)
Allograpta exotica (Wiedemann, 1830)
Allograpta hastata (Fluke, 1942)
Allograpta neotropica (Curran, 1936)
Alphamenes campanulatus (Fabricius, 1804)
Anthocoridae sp.
Apidae sp.1
Apidae sp.2
Apidae sp.3
Apidae sp.4
Apidae sp.5
Apidae sp.6
Apidae sp.7
Apidae sp.8
Apidae sp.9
Apidae sp.10
Apidae sp.11

Apidae sp.12

Apidae sp.13

Apidae sp.14

Apidae sp.15

Apidae sp.16

Apidae sp.17

Apidae sp.18

Apidae sp.19

Apidae sp.20

Apis mellifera (Linnaeus, 1758)

Argentinomya migrans

Argentinomyia sp.

Ascia monuste (Latreille, 1764)

Asilidae sp.1

Asilidae sp.2

Augochlora sp.1

Augochlora sp.2

Augochlora sp.3

Augochlora sp.4

Augochlora sp.5

Augochlora sp.6

Augochlora sp.7

Bembicini sp.

Biblis hyperia (Cramer, 1779)

Bicyrtes sp.

Bombus atratus (Franklin, 1913)

Bombus morio (Swederus, 1787)

Bombyliidae sp.1

Bombyliidae sp.2

Bombyliidae sp.3

Brachygastra lecheguana (Latreille, 1824)

Brachymenes dyscherus (de Saussure, 1852)

Braconidae sp.1

Braconidae sp.2

Braconidae sp.3

Calliphoridae sp.1

Calliphoridae sp.2

Calliphoridae sp.3

Calliphoridae sp.4

Calliphoridae sp.5

Carabidae sp.

Centris aenea (Lepeletier, 1841)

Centris sp.1

Centris sp.2

Centris sp.3

Centris sp.4

Centris tarsata (Smith, 1874)

Cephalotrigona capitata (Smith, 1854)

Ceratina sp.1

Ceratina sp.2

Ceratina sp.3

Ceratina sp.4

Ceratina sp.5

Chalcididae sp.

Chioides catillus (Cramer, 1779)

Chrysomelidae sp.

Chrysopidae sp.

Clypearia augustior (Ducke, 1906)

Coccinellidae sp.1

Coccinellidae sp.2

Coccinellidae sp.3

Coelioxys sp.

Coleoptera sp.1

Conopidae sp.

Coreidae sp.

Curculionidae sp.

Cynipidae sp.

Danaus gilippus (Cramer, 1775)

Dialictus sp.

Dione juno juno (Cramer, 1779)

Diptera sp.1

Diptera sp.2

Dircenna dero (Hübner, 1823)

Dryadula phaetusa (Linnaeus, 1758)

Elateridae sp.

Epicharis sp.1

Epicharis sp.2

Epicharis sp.3

Epicharis sp.4

Epicharis sp.5

Episcada hymenaea (Prittwitz, 1865)

Eucerini sp.1

Eucerini sp.2

Eucharitidae sp.

Eufriesea violacea (Blanchard, 1840)

Euglossa sp.

Eulaema nigrita (Lepeletier, 1841)

Eulophidae sp.

Exaerete smaragdina (Guérin, 1844)

Exomalopsis analis (Spinola, 1853)

Exomalopsis auropilosa (Spinola, 1853)

Exomalopsis sp.

Formicidae sp.1

Formicidae sp.2

Formicidae sp.3

Formicidae sp.4

Halictidae sp.1

Halictidae sp.2

Halictidae sp.3

Halictidae sp.4

Halictidae sp.5

Haplothrips gowdeyi (Franklin, 1908)

Heliconius erato (Linnaeus, 1758)

Hymenoptera sp.1

Hymenoptera sp.2

Hymenoptera sp.3

Hymenoptera sp.4

Hypalastoroides brasiliensis (de Saussure, 1856)

Hypancistrocerus advena (de Saussure, 1855)

Hypancistrocerus dentiformis (Fox, 1902)

Hypancistrocerus sp.

Hypanthidium sp.1

Hypanthidium sp.2

Hypodynerus arechavaletae (Brèthes, 1903)

Ichneumonidae sp.1

Ichneumonidae sp.2

Ichneumonidae sp.3

Ichneumonidae sp.4

Ichneumonidae sp.5

Ichneumonidae sp.6

Ichneumonidae sp.7

Ichneumonidae sp.8

Ichneumonidae sp.9

Ichneumonidae sp.10

Ipsiura sp.

Isodontia costipennis (Spinola 1851)
Lagriidae sp.
Lampyridae sp.
Lycorea halia (Hübner, 1816)
Mechanitis lysimnia (Fabricius, 1793)
Megachile nigripennis (Spinola. 1841)
Megachile sp.1
Megachile sp.2
Megachile sp.3
Megachile sp.4
Megachilidae sp.1
Megachilidae sp.2
Megachilidae sp.3
Megachilidae sp.4
Melipona bicolor (Lepeletier, 1836)
Melipona marginata marginata (Lepeletier, 1836)
Melipona quadrifasciata anthidioides (Lepeletier, 1836)
Melipona quinquefasciata (Lepeletier, 1836)
Mesembrinellidae sp.
Mesocheira bicolor (Fabricius, 1804)
Mesocheira sp.
Mesoplia sp.
Midas sp.
Minixi brasilianum (de Saussure, 1875)
Minixi tricoloratum (Zavattari, 1911)

Mischocyttarus cassununga (R. Von. Ihering, 1903)

Mischocyttarus sp.1

Mischocyttarus sp.2

Mischocyttarus sp.3

Montezumia infernalis (Spinola, 1851)

Montezumia nigriceps (Spinola, 1841)

Montezumia pelagica (de Saussure, 1852)

Montezumia petiolata (de Saussure 1855)

Multilidae sp.

Muscidae sp.1

Muscidae sp.2

Muscidae sp.3

Muscidae sp.4

Mydidae sp.

Neochrysis sp.

Neocorynura sp.

Ocyptamus sp.

Omicron gondwanianum (Giordani Soika, 1978)

Omicron opifex (Brèthes, 1909)

Omicron paranymphus (Zavattari, 1912)

Omicron sp.1

Omicron sp.2

Omicron sp.3

Omicron spegazzinii (Brèthes, 1905)

Omicron tuberculatum (Fox, 1899)

Ornidia major (Curran, 1930)

Ornidia obesa (Fabricius, 1775)

Ortilia ithra (Kirby, 1900)

Oxaea flavescens (Klug, 1807)

Oxytrigona tataira tataira (Smith, 1863)

Pachodynerus argentinus (de Saussure, 1870)

Pachodynerus grandis (Willink & Roig-Alsina, 1998)

Pachodynerus guadulpensis (de Saussure, 1853)

Pachodynerus nasidens (Latreille, 1812)

Pachodynerus sp.

Pachymenes olympicus (Zavattari, 1912)

Pachymenes picturatus (Fox, 1899)

Pachymenes sericeus (de Saussure, 1852)

Palpada fasciculata (Curran, 1938)

Palpada precipua (Williston, 1888)

Palpada vinetorum (Fabricius, 1798)

Parachartergus fraternus (Gribodo, 1892)

Parancistrocerus sp.

Paratetrapedia sp.1

Paratetrapedia sp.2

Paratrigona lineata (Lepeletier, 1836)

Paratrigona subnuda (Moure, 1947)

Parides agavus (Drury, 1782)

Partamona helleri (Friese, 1900)

Penapodium sp.

Pentatomidae sp.

Pepsis sp.1

Pepsis sp.2

Phoebis sennae (Linnaeus, 1758)

Pirhosigma limpidum (Giordani Soika, 1978)

Pirhosigma superficiale (Fox, 1899)

Platygastridae sp.

Podium sp.

Polistes actaeon (Haliday, 1836)

Polistes billardieri (Fabricius, 1804)

Polistes cinerascens (de Saussure, 1854)

Polistes ferreri (de Saussure, 1853)

Polistes geminatus (Fox, 1898)

Polistes simillimus (Zikan, 1951)

Polistes subsericeus (de Saussure, 1854)

Polistes versicolor (Olivier, 1792)

Polybia bifasciata (de Saussure, 1854)

Polybia fastidiosuscula (de Saussure, 1854)

Polybia ignobilis (Haliday, 1836)

Polybia jurinei (de Saussure, 1854)

Polybia minarum (Ducke, 1906)

Polybia occidentalis (Olivier, 1971)

Polybia platycephala (Richards, 1978)

Polybia punctata (du Buysson, 1907)

Polybia sericea (Oliver, 1796)

Pompilidae sp.1

Pompilidae sp.2

Pompilidae sp.3

Pompilidae sp.4

Pompilidae sp.5

Pompilidae sp.6

Pompilidae sp.7

Protonectarina sylveirae (de Saussure, 1854)

Protopolybia sedula (de Saussure, 1854)

Pseudaugochlora sp.1

Pseudaugochlora sp.2

Pseudaugochlora sp.3

Pseudaugochlora sp.4

Pseudaugochlora sp.5

Pseudaugochlora sp.6

Pseudaugochlora sp.7

Pseudaugochlora sp.8

Pseudaugochlora sp.9

Pseudodoros clavatus (Fabricius, 1794)

Pseudopolybia vespiceps (Ducke, 1907)

Pteromalidae sp.

Pyrisitia nise (Cramer, 1775)

Quichuana sp.

Rhingia nigra (Macquart, 1846)

Salpingogaster sp.

Sarcophagidae sp.1

Sarcophagidae sp.2

Sarcophagidae sp.3

Sarcophagidae sp.4

Sarcophagidae sp.5

Sarcophagidae sp.6

Sarcophagidae sp.7

Sarcophagidae sp.8

Scaptotrigona tubiba (Smith, 1863)

Scaptotrigona xanthotricha (Moure, 1950)

Sceliphron sp.

Schwarziana quadripunctata (Lepeletier, 1836)

Scoliidae sp.1

Scoliidae sp.2

Scoliidae sp.3

Scoliidae sp.4

Sphecidae sp.1

Sphecidae sp.2

Sphecidae sp.3

Sphecidae sp.4

Sphecidae sp.5

Sphecidae sp.6

Sphecidae sp.7

Sphecidae sp.8

Sphecidae sp.9

Sphecidae sp.10

Sphecidae sp.11

Sphecidae sp.12

Sphecidae sp.13

Sphecidae sp.14

Sphecidae sp.15

Stenodynerus sp.

Stenonartonia apicipennis Fox, 1902

Stratiomyidae sp.

Synoeca cyanea (Fabricius, 1775)

Tachinidae sp.1

Tachinidae sp.2

Tachinidae sp.3

Tachinidae sp.4

Tachinidae sp.5

Tachinidae sp.6

Tachinidae sp.7

Tachinidae sp.8

Tachinidae sp.9

Tegosa sp.

Tephritidae sp.1

Tephritidae sp.2

Tetragona clavipes (Fabricius, 1804)

Tetragonisca angustula (Latreille, 1811)

Tetrapedia sp.1

Tetrapedia sp.2

Tiphiidae sp.1

Tiphiidae sp.2

Tiphiidae sp.3

Toxomerus dispar (Fabricius, 1794)

Toxomerus elisa (Hull, 1951)

Toxomerus sp.

Trigona hyalinata (Lepeletier, 1836)

Trigona sp.

Trigona spinipes (Fabricius, 1793)

Trimeria americana (de Saussure, 1853)

Trimeria howardi (Bertoni, 1911)

Trypoxylon sp.1

Trypoxylon sp.2

Urbanus sp.

Xenophanes tryxus (Cramer, 1780)

Xylocopa sp.1

Xylocopa sp.2

Xylocopa sp.3

Xylocopa sp.4

Xylocopa subcyanea (Perez, 1901)

Zeta argillaceum (Linnaeus, 1758)

Zethus brasiliensis (de Saussure, 1852)

Table S3. Plant species visited in the Cerrado area of the Unilavras-Boqueirão Biological Reserve, Minas Gerais, Brazil.

Family	**Species**
Acanthaceae	*Justicia riparia* Kameyama
Amaranthaceae	*Althernanthera tenella* Colla
Amaranthaceae	(Not identified)
Apiaceae	*Eryngium lacustre* Pohl ex Urb.
Apocynaceae	*Mandevilla emarginata* (Vell.) C.Ezcurra
Apocynaceae	*Prestonia erecta* (Malme) J.F.Morales
Aquifoliaceae	*Ilex* sp.
Asteraceae	(Not identified)
Asteraceae	*Ageratum fastigiatum* (Gardner) R.M.King & H. Rob.
Asteraceae	*Aldama robusta* (Gardner) E.E.Schill. & Panero
Asteraceae	*Aldama robusta* (Gardner) E.E.Schill. & Panero
Asteraceae	*Aspilia riedelli* Baker
Asteraceae	*Baccharis brevifolia* DC.
Asteraceae	*Baccharis* cf. *crispa* Spreng.
Asteraceae	*Baccharis helychrysoides* DC.
Asteraceae	*Baccharis retusa* DC.
Asteraceae	*Baccharis* sp.
Asteraceae	*Baccharis tarchonanthoides* Baker
Asteraceae	*Bidens segetum Mart.* ex colla
Asteraceae	*Chresta scapigera* (Less.) Gardner
Asteraceae	*Chromolaena squalida* (DC.) R.M.King & H.Rob.
Asteraceae	*Chromolaena squalida* (DC.) R.M.King & H.Rob.

Asteraceae	*Elephantopus mollis* Kunth.
Asteraceae	*Elephantopus palustris* Gardner
Asteraceae	*Gochnatia barrosii* Cabrera
Asteraceae	*Grazielia dimorpholepis* (Baker) R.M.King & H.Rob.
Asteraceae	*Grazielia intermedia* (DC.) R.M.King & H.Rob.
Asteraceae	*Heterocondylus alatus* (Vell.) R.M.King & H.Rob.
Asteraceae	*Lessingianthus brevipetiolatus* (Sch.Bip. ex Baker) H.Rob.
Asteraceae	*Lessingianthus lacunosus* (Mart. ex DC.) H.Rob.
Asteraceae	*Lessingianthus* sp.1
Asteraceae	*Lessingianthus* sp.2
Asteraceae	*Lucilia lycopodioides* (Less.) S.E.Freire
Asteraceae	*Mikania glauca* Mart.
Asteraceae	*Mikania sessifolia* DC.
Asteraceae	*Richterago radiata* (Vell.) Roque
Asteraceae	*Stenocephalum megapotamicum* (Spreng.) Sch.Bip.
Asteraceae	*Stomatanthes dyctiophyllus* (DC.) H. Rob.
Asteraceae	*Tilesia baccata* (L.f.) Pruski
Asteraceae	*Trichogonia villoza* Sch.Bip. ex Baker
Asteraceae	*Trix* sp.
Asteraceae	*Vernonanthura phosphorica* (Vell.) H.Rob.
Asteraceae	*Vernonia* sp.
Bignoniaceae	*Adenocalymma bracteatum* (Cham.) DC.
Bignoniaceae	*Fridericia formosa* (Bureau) L.G.Lohmann
Bignoniaceae	*Pyrostegia venusta* (Ker Gawl.) Miers
Bignoniaceae	*Zeyheria montana* Mart.

Calophyllaceae	*Kielmeyera corymbosa* Mart. & Zucc
Convolvulaceae	*Evolvulus sericeus* Sw.
Convolvulaceae	*Merremia tomentosa* Hallier
Convolvulaceae	*Merremia umbellata* (L.) Hallier f.
Dilleniaceae	*Davilla rugosa* Poir.
Erythroxylaceae	*Erythroxylum suberosum* A.St.-Hil.
Erythroxylaceae	*Erythroxylum tortuosum* Mart.
Euphorbiaceae	*Croton antisyphiliticus* Mart.
Euphorbiaceae	*Croton lundianus* (Didr.) Müll.Arg.
Fabacaceae	*Stryphnodendron adstringens* (Mart.) Coville
Fabaceae	*Chamaecrista cathartica (Mart.)* H.S.Irwin & Barneby
Fabaceae	*Desmodium barbatum (L.) Benth*
Fabaceae	*Desmodium* sp.
Fabaceae	*Desmodium tortuosum (Sw.) DC.*
Fabaceae	*Neonotonia wightii* (Graham ex Wight & Arn.) J.A.Lackey
Fabaceae	*Senna macranthera (Collad.) H.S.Irwin & Barneby*
Fabaceae	*Stylosanthes viscosa* (L.) Sw.
Juncaceae	*Juncuns* sp.
Lamiaceae	*Eriope macrostachya* Mart. ex Benth.
Lamiaceae	*Hyptidendron canum* (Pohl ex Benth.) Harley
Lamiaceae	*Hyptis marrubioides* Epling
Lamiaceae	*Hyptis radicans* (Pohl) Harley & J.F.B. Pastore
Lamiaceae	*Hyptis suaveolens* (L.) Poit.
Lythraceae	*Cuphea* sp.
Malpighiaceae	*Byrsonima intermedia A. Juss.*

Malpighiaceae	*Byrsonima verbascifolia* (L.) DC.
Malpighiaceae	*Heteropterys umbellata A. Juss.*
Malpighiaceae	Malpighiaceae sp.1
Malpighiaceae	Malpighiaceae sp.2
Malpighiaceae	*Peixotoa tomentosa* A. Juss.
Malvaceae	*Pavonia* sp.1
Malvaceae	*Pavonia* sp.2
Malvaceae	*Peltaea polymorpha* (A. St.-Hil.) Krapov. & Cristóbal
Malvaceae	*Sida glaziovii* K. Schum.
Malvaceae	*Sida rhombifolia* L.
Malvaceae	*Triumfetta* sp.1
Malvaceae	*Triumfetta* sp.2
Malvaceae	*Waltheria indica* L.
Melastomataceae	*Miconia ligustroides* (DC.) Naudin
Melastomataceae	*Pleroma granulosum* (Desr.) D. Don
Melastomataceae	*Trembleya phlogiformis* DC.
Musaceae	*Musa paradisiaca* L.
Myrtaceae	*Campomanesia pubescens* (DC.) O.Berg
Myrtaceae	*Eugenia bimarginata* DC.
Myrtaceae	*Psidium grandifolium* Mart. ex DC.
Ochnaceae	*Ouratea spectabilis* (Mart. ex Engl.) Engl.
Orobanchaceae	*Buchnera lavandulacea* Cham. & Schltdl.
Oxalidaceae	*Oxalis hirsutissima* Mart. & Zucc.
Poaceae	*Brachiaria* sp.
Poaceae	*Echinolaena inflexa* (Poir.) Chase

Poaceae	*Megathyrsus maximus* (Jacq.) B.K.Simon & Jacobs
Polygalaceae	*Polygala longicaulis* Kunth
Polygonaceae	*Asemeia violacea* (Aubl.) J.F.B.Pastore & J.R.Abbott
Polygonaceae	*Polygala poaya* Mart.
Rhamnaceae	*Gouania latifolia* Reissek
Rubiaceae	*Borreria verticillata* (L.) G.Mey.
Rubiaceae	*Declieuxia cordigera* Mart. & Zucc. ex Schult. & Schult.f.
Solanaceae	*Cestrum corymbosum* Schltdl.
Solanaceae	*Solanum lycocarpum* A. St.-Hil.
Solanaceae	*Solanum subumbellatum* Vell.
Styracaceae	*Styrax camporum* Pohl
Verbenaceae	*Lantana camara* L.
Verbenaceae	*Lippia lupulina* Cham.

Table S4. Selection of the best models with the explanatory variables: dry season, abundance of floral visitors, and number of flowers. The best models (in bold) are those with the lowest widely applicable information criteria (WAIC) value.

Metric	Model	Parameter			
		WAIC	R2	R2 adjusted	RMSE
Interaction Strength Asymmetry	**Interaction Strength Asymmetry ~ Abundance of floral visitors**	**91.68**	**0.18**	**-0.08**	**6.41**
	Interaction Strength Asymmetry ~ Number of flowers	93.14	0.12	-0.35	6.48
	Interaction Strength Asymmetry ~ Abundance of floral visitors + Number of flowers	93.74	0.22	-0.30	6.41
	Interaction Strength Asymmetry ~ Dry season + Abundance of floral visitors	94.15	0.23	-0.38	6.38
	Interaction Strength Asymmetry ~ Dry season + Number of flowers	95.18	0.18	-0.57	6.67
	Interaction Strength Asymmetry ~ Dry season	95.18	0.04	-0.40	7.17
	Interaction Strength Asymmetry ~ Dry season + Abundance of floral visitors + Number of flowers	96.09	0.26	-0.76	6.38
Modularity	**Modularity ~ Abundance of floral visitors**	**94.63**	**0.64**	**0.56**	**7.36**
	Modularity ~ Season + Abundance of floral visitors	96.49	0.64	0.51	7.21
	Modularity ~ Abundance of floral visitors + Number of flowers	97.03	0.63	0.44	7.32

	Modularity ~ Dry season + Abundance of floral visitors + Number of flowers	99.00	0.63	0.36	7.21
	Modularity ~ Number of flowers	100.70	0.44	0.32	9.24
	Modularity ~ Dry season + Number of flowers	102.36	0.47	0.22	9.04
	Modularity ~ Dry season	109.13	0.05	-0.35	12.71
Specialization Asymmetry	**Specialization Asymmetry ~ Abundance of floral visitors + Number of flowers**	**107.5**	**0.27**	**-0.4**	**10.18**
	Specialization Asymmetry ~ Dry season	108.03	0.14	-0.12	10.98
	Specialization Asymmetry ~ Dry season + Abundance of floral visitors + Number of flowers	108.79	0.32	-0.47	10.00
	Specialization Asymmetry ~ Dry season + Number of flowers	109.24	0.20	-0.13	10.92
	Specialization Asymmetry ~ Dry season + Abundance of floral visitors	109.62	0.20	-0.33	10.90
	Specialization Asymmetry ~ Abundance of floral visitors	110.04	0.07	-0.41	11.67
	Specialization Asymmetry ~ Number of flowers	110.43	0.04	-0.25	12.00
Weighted Connectance	**Weighted Connectance ~ Abundance of floral visitors**	**74.60**	**0.59**	**0.54**	**3.40**
	Weighted Connectance ~ Dry season + Abundance of floral visitors	75.57	0.62	0.52	3.23
	Weighted Connectance ~ Dry season + Abundance of floral visitors + Number of flowers	77.12	0.65	0.32	3.02

	Weighted Connectance ~ Abundance of floral visitors + Number of flowers	77.34	0.59	0.30	3.36
	Weighted Connectance ~ Number of flowers	83.10	0.31	0.11	4.58
	Weighted Connectance ~ Dry season + Number of flowers	85.06	0.33	-0.03	4.57
	Weighted Connectance ~ Dry season	87.53	0.04	-0.36	5.62
Robusteness	**Robustness ~ Dry season**	**-36.96**	**0.49**	**0.39**	**0.04**
	Robustness ~ Dry season + Abundance of floral visitors	-35.27	0.49	0.32	0.04
	Robustness ~ Dry season + Number of flowers	-34.98	0.49	0.29	0.04
	Robustness ~ Dry season + Abundance of floral visitors + Number of flowers	-32.92	0.49	0.14	0.04
	Robustness ~ Abundance of floral visitors	-27.55	0.06	-0.37	0.06
	Robustness ~ Number of flowers	-27.04	0.04	-0.50	0.07
	Robustness ~ Abundance of floral visitors + Number of flowers	-26.19	0.15	-0.50	0.06

ARTIGO 2 – PLANT-FLORAL VISITORS NETWORKS AND SAMPLING EFFORT: HOW SEASONS AFFECT SAMPLING COMPLETENESS IN CERRADO MUTUALISTIC NETWORKS?

(Artigo escrito de acordo com as normas da NBR 6022 da ABNT. Material a ser submetido para publicação em periódico)

Plant-floral visitor networks and sampling effort: how seasons affect sampling completeness in Cerrado mutualistic networks?

Victor Hugo Duarte da Silva[1*], *Matheus Tenório Baumgartner* [3], *Marcel Gustavo Hermes* [2], *Lucas Del Bianco Faria*[1]

1- Departamento de Ecologia e Conservação, Instituto de Ciências Naturais, Universidade Federal de Lavras, Mailbox 3037, CEP 37200-900, Lavras, Minas Gerais, Brazil.
2- Laboratory of Systematics and Insect Biology, Department of Biology, Federal University of Lavras, Mailbox 3037, Zip Code 37200 -000, Lavras, Minas Gerais, Brazil.
3- Center for Biological Sciences, State University of Maringá, Zip Code 87020-900, Maringá, Paraná, Brazil.

*E-mail: victorfaculufla@gmail.com

ABSTRACT

Dealing with sampling is a constant challenge in ecology, especially in networks, where most of the studies already published suffer from under-sampling. Different sampling methods are used in interaction networks, ranging from continuous observations over time to observations at peak flowering of the plants (spring). However, species richness and abundance varies over time. Therefore, it is necessary to exercise caution when carrying out studies that cover only the peak of flowering of the plants, as important information may be being lost. With that in mind, we seek to identify what sampling effort would be necessary in each of the seasons (fall, winter, spring, and summer) and whether they differed in terms of the effort required. For that, we use accumulation curves based on abundance data, using Chao 1 as an estimator. In addition, we build nonlinear models to help us understand if the seasons vary and what is the number of fortnights needed to reach 100% of sampling in six network properties. Initially, we observed that our sampling effort for animals and plants, over the seasons, was sufficient to record most species in the study area. As for the interactions, the sampling effort showed low values, indicating that an increase in the sampling effort would increase the interactions richness recorded over the seasons. Such results can directly be the availability (richness and abundance) of animals and floral resources, which varies over the seasons, as well as the climatic conditions. In addition, we observed that animals richness, interactions richness, plants richness and Shannon diversity showed an growth behavior. Whereas, network specialization and weighted connectance showed an exponential decay pattern. Finally, we observed that for each metric, the number of fortnights required to achieve 100% sampling varied between seasons, with a few fortnights requiring a large number of sampling fortnights.

Keywords: Sampling completeness, Species richness, Interaction networks.

INTRODUCTION

We know that interaction networks are not static and their structure is prone to vary over time and space (CARADONNA; WASER, 2020; DUPONT; OLESEN, 2012; SCHWARZ et al., 2020). Such variations in network structure places how and why species interact among the most complex and dynamic features of biodiversity, perhaps more complex than species diversity itself (DORMANN; FRÜND; SCHAEFER, 2017; GARCÍA-CALLEJAS; MOLOWNY-HORAS; ARAÚJO, 2018). Temporal variations in interaction networks have thus been a constant concern within theoretical research because of the different mechanisms that may produce it, such as, for example, relative species abundance, phenological overlap, trait match, and phylogenetic structures (PERALTA et al., 2020a; VÁZQUEZ; CHACOFF; CAGNOLO, 2009).

Although these are theoretically independent mechanisms, they are directly linked with the probability of interaction between two species. For instance, previous studies have reported the probabilistic consequences of greater species abundance and/or richness, which increases the chances of finding links between two species (BASCOMPTE; JORDANO, 2014; LAURINDO; GREGORIN; TAVARES, 2017; VÁZQUEZ et al., 2007; VÁZQUEZ; CHACOFF; CAGNOLO, 2009). Derived from the neutral theory, the simplifying assumption that all species are ecologically equivalent within communities increases the interaction probability between any pair of species, as long as they have highly abundant populations (VÁZQUEZ et al., 2009; HUBBELL, 2001). However, there are striking biological restrictions that decrease the odds for some interspecific interactions, especially trait matching and phenological overlap (BASCOMPTE; JORDANO, 2014; PERALTA et al., 2020a; STANG; KLINKHAMER; MEIJDEN, 2007). Considering trait matching, for example, in trophic relationships, the interaction with a given prey species requires the predator to have the ability of identifying and acquiring prey successfully, otherwise the interaction is forbidden (OLESEN et al., 2011). Nevertheless, no matter how specialized an insect is in identifying the flower of its mutualistic partner species if they never meet in space or time, suggesting that phenological overlap is a requisite for determining interspecific interactions (Bascompte et a., 2014; Peralta et al., 2020a). These two contrasting assumptions provide a robust theoretical baseline to investigate the probability of species interaction and the mechanisms underlying variations in interaction networks.

However, while interactions among species may result from any combination of probabilistic and phenological drivers, our perception of network structures also suffer from sampling bias, whether related to contrasting methods of estimating species diversity and their interactions or different sampling efforts (NIELSEN AND BASCOMPTE, 2007; GIBSON et al., 2011; JORDANO, 2016; RIVERA-HUTINEL et al., 2012). Sampling is a constant challenge in nearly all areas within ecology, thus would not vanish from the study of biotic interactions because most communities and therefore networks are subsampled (CHACOFF et al., 2012; COSTA et al., 2016). Interaction networks are only a slice of the complexity that is inherent to ecosystems and even extensive sampling designs with exhaustive data collection tends to produce only modest estimations of biodiversity (CHACOFF et al., 2012; FRÜND; MCCANN; WILLIAMS, 2016). In addition, the complexity of species interactions prevents the specification of sampling protocols that are robust enough to deal with different types of interaction networks, which makes it difficult to compare and combine networks for integrative approaches.

Previous studies comparing different sampling methods and efforts have shown that not only the network structure that do suffer from sampling effects, but the metrics are much likely influenced as well (BANAŠEK-RICHTER; CATTIN; BERSIER, 2004; GIBSON et al., 2011; RIVERA-HUTINEL et al., 2012). An example of this is the study by Bosch et al. (2009), which compared the phytocentric against the zoocentric approach (pollen load carried by pollinators). These authors reported that networks estimated based on sampling efforts emphasizing the pollen load carried on the pollinators' body surface (i.e., zoocentric approach) showed increased connectance, interactions richness, and nestedness than phytocentric networks. Conversely, Gibson et al. (2011) reported that interaction networks estimated from multiple times and transects do not differ in terms of nestedness and connectance, but deviate when comparing the record of rare interactions and the network asymmetry, with time-based networks showing the highest record of rare interactions and less web asymmetry than transect-based networks.

Although many studies have already dealt with the influence of sampling on the construction of ecological networks, only a few were concerned with trying to understand what sampling effort would be necessary to achieve the most reliable estimation of real-world networks (CHACOFF et al., 2012; COSTA et al., 2016; FRÜND; MCCANN; WILLIAMS, 2016; VIZENTIN-BUGONI et al., 2016). A pioneering study in this scope was the work by Chacoff et al. (2012), where the authors explored plant-pollinator interactions and found that an intensive sampling effort was only able to report 55% of extant interactions. Therefore, understanding the effects of sampling efforts on networks and their metrics is essential to minimize sampling biases within the analyzes and to produce more realistic ecological networks (COSTA et al., 2016; HELENO et al., 2014).

While insufficient sampling efforts tend to underestimate species diversity and thereby ecological interactions, excessive sampling efforts frequently result in unnecessary work and excessive expenses. Therefore, knowing that an adequate sampling effort is essential to get as close as possible to an accurate description of communities, and that seasonal variation is the most pervasive source of temporal gradients in natural environments, we seek to identify what sampling effort would be necessary in each of the four seasons (fall, winter, spring, and summer) and whether they differ in terms of the required sampling effort. For this proposal, we seek to answer the following questions: (i) Do seasons differ in relation to the necessary sampling effort? (ii) Can we estimate the sampling effort required in each season? We hypothesize that the seasons differ in the required sampling effort and that seasons with higher numbers of flowers and floral visitors would require a greater sampling effort than seasons that have a lower number of flowers and floral visitors.

MATERIAL AND METHODS

Study area

This study was conducted at the Reserva Biológica of the UniLavras - Boqueirão (RBUB), located south in the Minas Gerais State, Brazil (Figure 1). The RBUB has an area of 159 ha and the altitude varies between 1100 and 1250 m (PIRES; POMPEU; SOUZA-SILVA, 2012). The average annual temperature ranges between 19°C and 25°C and the average annual precipitation is 1411 mm, with 66.77% of the annual rainfall occurring between November and February (PIRES; POMPEU; SOUZA-SILVA, 2012). Winter spans from June to September and is characterized by a remarkable water deficit, with average precipitation ranging from 10 mm to 30 mm (PIRES; POMPEU; SOUZA-SILVA, 2012). The vegetation of the RBUB is characterized by typical phytophysiognomies from the Cerrado *stricto sensu*, altitude Fields, rupestrian fields, gallery forest, and discrete patches of *Brachiaria* sp (Poaceae) pastures (PIRES; POMPEU; SOUZA-SILVA, 2012).

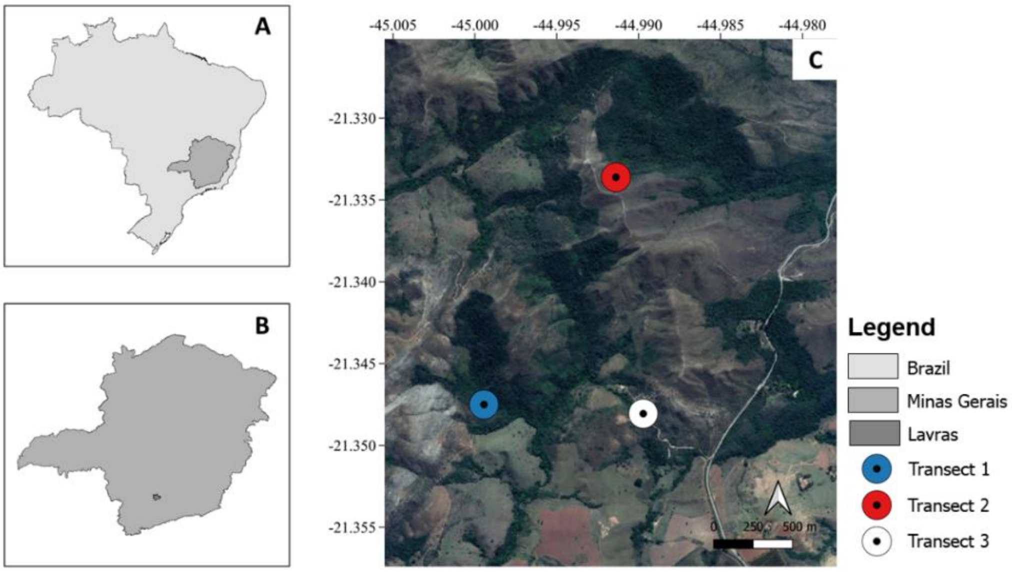


Figure 1- Location of the study area. A- Map of Brazil with emphasis on the Minas Gerais state. B- Map of Minas Gerais with emphasis on the Lavras City. C- Map of the study area highlighting the sampling transects.

Data collection

We collected data on the interacting plant and insect species every fortnight, starting in early July 2015 and ending in late July 2016. Collections were performed in three 200 x 50 m transects: T1 (21º 20' 53” S / 44º 59' 23” W), T2 (21º 21' 01” S / 44º 59' 29” W), and T3 (21º 20' 51” S / 44º 59' 58” W) (Figure 1). We divided transects into 10 plots of 40 x 25 m, each labeled from ‘1’ to ‘10’ (Figure 2). Before each collection, one plot among plots 1, 2, 9, and 10 was randomly drawn to define the sampling sequence (Figure 2). For example, if plot ‘1’ was drawn, the collection at that given fortnight was carried out from plot 1 to plot 10 (Figure 2). Conversely, when the number ‘10’ was drawn, the collection started in the reverse order (Figure 2). By carrying out this randomization procedure on the direction of each collection, we had all plots sampled at different periods of time, throughout each day of sampling.

Within each plot, floral visitors were collected near the open flowers between 7:30 am and 4:30 pm, twice during each period (morning and afternoon). Within each transect, the collector moved along the previously selected order, capturing the insects that were visiting the flowering plants. In each flowering plant individual, the collector's residence time was 10 minutes (Figure 2). We considered as a realized interaction only when the insect was in physical contact with the flower's reproductive structure, thus suggesting that the visitor was a potential pollinator. Hovering insects were not considered as interacting partners. In the present study, the term “floral visitors” was adopted for all registered insects, as the qualification in terms of its effectiveness as a pollinator was only presumed (ALVES-DOS-SANTOS et al., 2016). As an estimation of the abundance of each plant, we counted the number of flowers. For species with reduced flower abundance, all flowers were counted. For species with flowers arranged in inflorescences, we estimated the number of flowers based on the number of inflorescences. We then multiplied the average number of open flowers per inflorescence, multiplied by the average number of inflorescences per branch, and finally by the average number of branches per plant (VOSGUERITCHIAN, 2010).

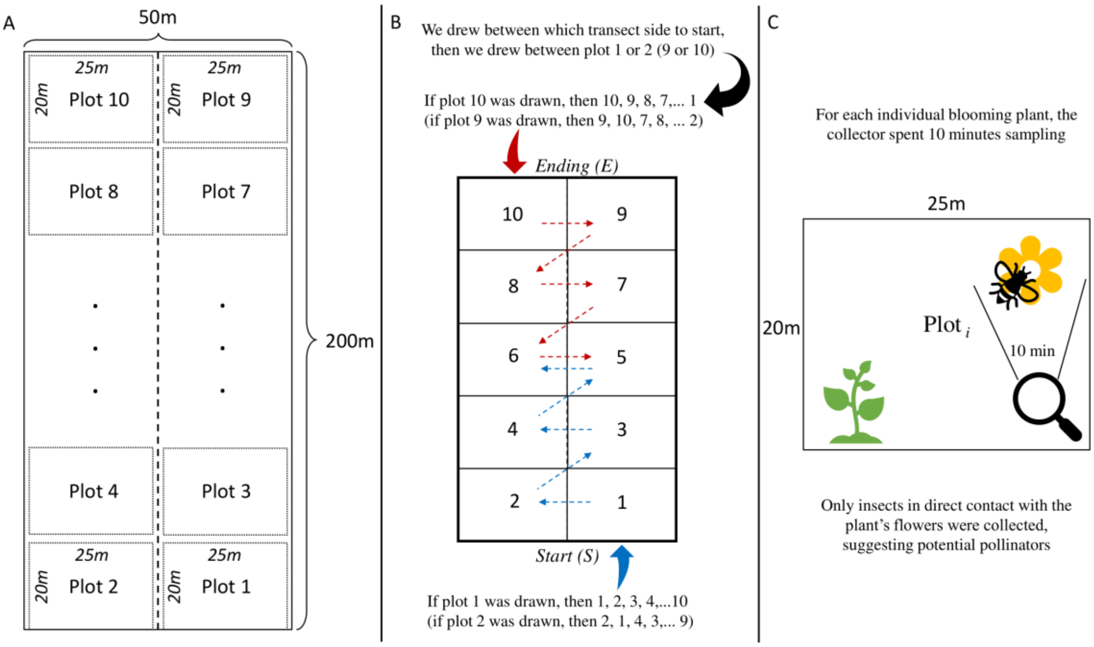


Figure 2- Scheme representative of data collection. A- Arrangement of plots in each transect. B- Data collection order, depending on the draw previously carried out. Blue arrows represent the order of data collection when 1 or 2 were drawn. Red arrows represent the order of data collection when 9 or 10 were drawn. C- Plot of data collection and explanation of collection of floral visitors.

We deposited all insect collected during the study in the Entomological Collection of the Federal University of Lavras (CEUFLA). To determine the potential resources used by each flower visitor, we also collected parts from those plants where the insects were sampled. We deposited all plant specimens and parts in the Herbarium of the Federal University of Lavras (ESAL-UFLA). The identification of plants and insects were carried out using specialized keys and with the aid of specialists.

Data analysis

We initially addressed the integrity of our sampling protocol in estimating the abundance of plants and floral visitors, and their interactions for all seasons, in a similar approach to that reported in Chacoff et al. (2012) and Falcão, Dáttilo, Rico-Gray (2016). Using the *inext* package, we generated accumulation curves for the plants richness, number of floral visitors, and interactions richness in each season (CHAO; MA; HSIEH, 2016). As our data was in the format of integer numbers representing the frequency of interactions among species, so the accumulation curves could be drawn based on individual-level abundances, we used the *Chao 1* estimator, which is suitable for this type of data (COLWELL et al., 2012; GOTELLI; COLWELL, 2011). We also used the function *estimateR* from the *vegan* package to calculate sampling completeness (OKSANEN et al., 2019). Sampling completeness ($\hat{S}$) is measured as the percentage of observed richness in relation to the expected richness estimated by the *Chao 1* and represents the percentage of expected richness that is achieved by the current sampling effort (COSTA et al., 2016), calculated as:

$$\hat{S} = \frac{S_{obs}}{S_{Chao1}} \cdot 100$$

From all the data on the frequency of interactions between plants and floral visitors, we built one bipartite weighted network for each fortnight, totaling 25 plant-floral visitors networks. We then grouped these networks into four groups according to the season that they corresponded. The next step was to combine these fortnight networks as contiguous sampling surveys and check the progress in achieving sampling completeness. We did these combinations using two approaches: adjacent and at random (DÁTTILO et al., 2019; VIZENTIN-BUGONI et al., 2016). The adjacent approach combined networks representing consecutive surveys, whereas the random procedure combined non-adjacent surveys (DÁTTILO et al., 2019). This latter type of aggregation can be considered as a null model to test the hypothesis that the grouped distribution of plants and floral visitors would influence network metrics (DÁTTILO et al., 2019). In practice, we evaluated the effective sampling completeness using different sampling efforts represented by pooled networks, considering different combinations of fortnights from one up to the maximum number of networks in each season.

For each of these pooled networks under different approaches (adjacent and random), we obtained the animals richness, interactions richness, plants richness, network specialisation (H2), Shannon diversity, and weighted connectance. We choose these metrics because they are complementary and represent most of the characteristics of network structure and specialization, but are also prone to suffer from variations in sampling efforts at some degree (COSTA et al., 2016; RIVERA-HUTINEL et al., 2012). These metrics were obtained using the *networklevel* function from the *bipartite* package (DORMANN; GRUBER; FRÜND, 2008).

To visualize the effect of combining networks and thereby increasing the sampling effort on network metrics, we drew boxplot graphs with the metrics values for each season over the pooled networks (WICKHAM, 2016). With these graphs, we could infer whether networks metrics might reveal patterns once we increased sampling effort. Nevertheless, only visual inspections of graphs were not sufficient to numerically compare how sampling completeness varied among seasons. Consequently, we extended this approach and fitted models to the relationships between metrics and progressive sampling efforts so we would have estimated parameters to help us investigating the difference in sampling completeness across seasons. As metrics are philosophically different in terms of which network feature that they represent, our approach required different fitted models. All graphs suggested nonlinear relationships along with different sampling efforts (i.e., pooled networks), so we fitted independent nonlinear models using the *nls* function of the *stats* package (R CORE TEAM, 2021), depending on the response variable (i.e., network metric). Nonlinear models encompass a wide variety of functions and have the advantages of parsimony (few parameters to be estimated), easy interpretability, and robust predictions (compared with, for example, polynomial models) (ARCHONTOULIS; MIGUEZ, 2015). However, for nonlinear models to work fine, it is necessary to have a specific base function (ARCHONTOULIS; MIGUEZ, 2015; ONOFRI, 2019).

The animals richness, plants richness, interactions richness, and Shannon diversity followed an increasing but saturating growth pattern, soused the Von Bertalanffy growth equation (ESSINGTON; KITCHELL; WALTERS, 2001), adapted to deal with sampling effort instead of body size, defined as:

$$M_s = M_\infty \cdot (1 - e^{-Ks})$$

where M_s is the network-level metric value obtained with sampling effort *s*; M_∞ is the asymptotic metric value, expected when sampling completeness is fully achieved; and *K* is the growth coefficient describing the rate at which the metric value varies with increasing sampling effort. Considering that higher values of *K* translates into faster stabilization of the curves, we can assume that this is the most important parameter determining sampling completeness, so we used it to compare seasons. Except for the model for the interactions richness, which was log-transformed to homogenize residuals, we built all other models using the original metric values. Finally, confidence intervals for model parameters were estimated using 999 bootstraps.

For weighted connectance and network specialisation (H2), we built nonlinear exponential decay models using the *SSaSymp* functions from the *stats* package (R CORE TEAM, 2021), also adapted to deal with sampling effort, which is described as:

$$M_s = M_\infty + M_\infty e^{-(\log a)s}$$

where M_s is the network-level metric value obtained with sampling effort s; M_∞ is the asymptotic metric value, expected when sampling completeness is fully achieved; and $\log a$ is the log-scaled decay rate, which was used to compare metrics among seasons because higher values of $\log a$ means faster stabilizing curves. For exponential decay models, the number of bootstraps varied between 830 and 999 runs, depending on the season and the metric.

By using nonlinear models based on the Von Bertalanffy equation (for nonlinear growth models) and the *SSaSymp* function (for nonlinear decay models), we were able to estimate what would be the number of fortnights required to achieve sampling completeness in each of the different sampling seasons depending on each of the targeted network metrics.

Finally, we built generalized mixed linear models to verify the hypotheses that the seasons differ in animals and plants richnes. For this, we group fall and winter in the dry season and spring and summer in the wet season. Next, we build generalized linear mixed models, using species richness as an answer variable and station as an explanatory variable. The random factor was the fortnights and the distribution used was Poison. This analysis was done in order to give more robustness to our data and also our discussion.

RESULTS

We collected 111 species of plants and 346 species of floral visitors during this study. The floral visitors belonged to six different orders (Hymenoptera, Coleoptera, Diptera, Lepidoptera, Hemiptera, and Neuroptera), with Hymenoptera and Diptera being the most diverse, with 197 and 66 species, respectively. Plant species belonged to 32 families, with Asteraceae and Malvaceae being the richest families, with 37 and 10 species, respectively (see Pires et al., 2021 (In press) for more information).

We explored the sampling completeness in each season for the animals richness, plants, and interactions. Regarding the sampling completeness of the animals richness (floral visitors), we recorded 84.59% of the animal richness in the fall, 87.72% in winter, 93.89% in the spring, and 95.77% in the summer (Table 1) (Figure 3-A). For the plants richness, we recorded 100% of the plant richness in all four seasons (Table 1) (Figure 3-B). Finally, for the interactions richness, we recorded 60.19% of the interactions richness in the fall, 55.54% in the winter, 63.91% in the spring and 57.68% in the summer (Table 1) (Figure 3-C).

Table 1 – Observed, estimated (by Chao1) and calculated sampling completeness for the animals richness, interactions richness and, plants richness in each season. Observed value is the value of the richness observed by combining all surveys from the same season. Sampling completeness is percentage of the true value that is achieved in each season.

Metrics	**Season**	**Observed**	**Estimated (Chao 1)**	**S.E. (Chao 1)**	**Sampling completeness**
Animals richness	Fall	250	295.56	14.5	84.59
Animals richness	Winter	227	258.78	11.94	87.72
Animals richness	Spring	292	311	8.13	93.89
Animals richness	Summer	276	288.2	5.78	95.77
Plants richness	Fall	35	35	0.16	100
Plants richness	Winter	22	22	0	100
Plants richness	Spring	58	58	0	100
Plants richness	Summer	54	54	0	100
Interactions richness	Fall	581	965.2	55.71	60.19
Interactions richness	Winter	817	1471.1	85.95	55.54
Interactions richness	Spring	1332	2084.15	75.4	63.91
Interactions richness	Summer	1362	2361.5	94.82	57.68

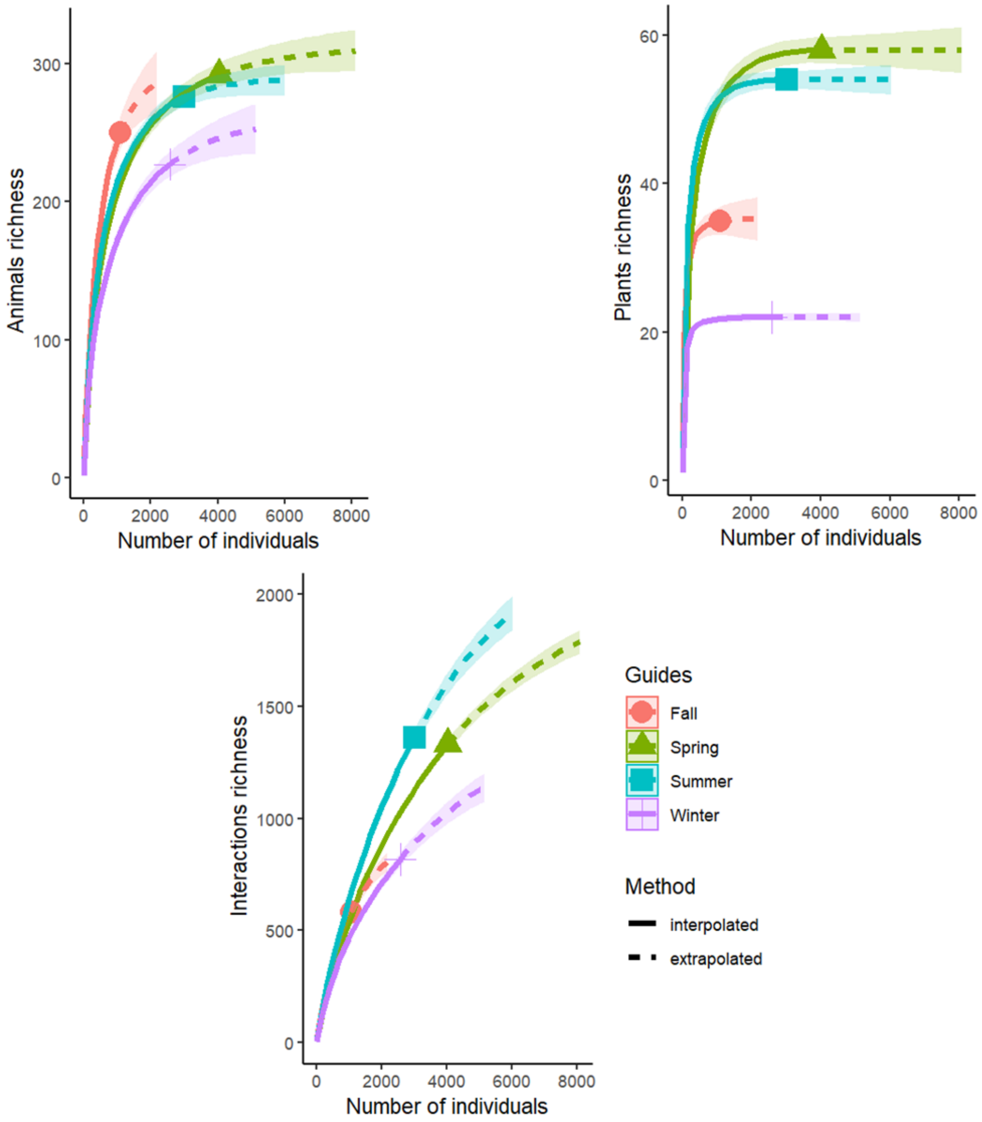


Figure 3 - Accumulation curves for the animals richness (A), plants richness (B) e interactions richness (C), for each season, based on the *Chao 1* estimator.

The estimated metrics for the pooled networks varied in different ways throughout the seasons. The animals richness showed an growth pattern in both random and adjacent networks (Figure 4). However, when we compared the *K* parameter (growth rate) among seasons, we found that the priority of sampling completeness differed according to the type network pooling combination. In the adjacent networks, winter was the one with the highest *K* value, followed by spring, summer, and fall (Table 2). In the random networks, spring was the season that presented the highest *K* value, followed by summer, winter and fall (Table 2). In these networks, collections performed during winter or spring achieves sampling completeness with the shortest sampling effort, considering the number of collected animals (Figure 4).

Table 2 - Estimated parameters of the Von Bertalanffy equation for the animals richness. The parameters were estimated for the four seasons and for the different types of network combination (adjacent and random). M_∞ is the asymptotic value of Y (the value at which the curve stabilizes), *K* is the growth rate of the curve and M_0 is the value of M at time 0.

Season	Parameters					
	Adjacent			Random		
	M_∞	K	M_0	M_∞	K	M_0
Fall	321.9872	0.2966	-0.0384	342.2613	0.2563	-0.1341
Winter	266.7473	0.3943	-0.1811	262.5262	0.3642	-0.3145
Spring	311.2869	0.3862	-0.3398	304.1096	0.3871	-0.4019
Summer	291.8373	0.3682	-0.2819	284.6182	0.3863	-0.2983

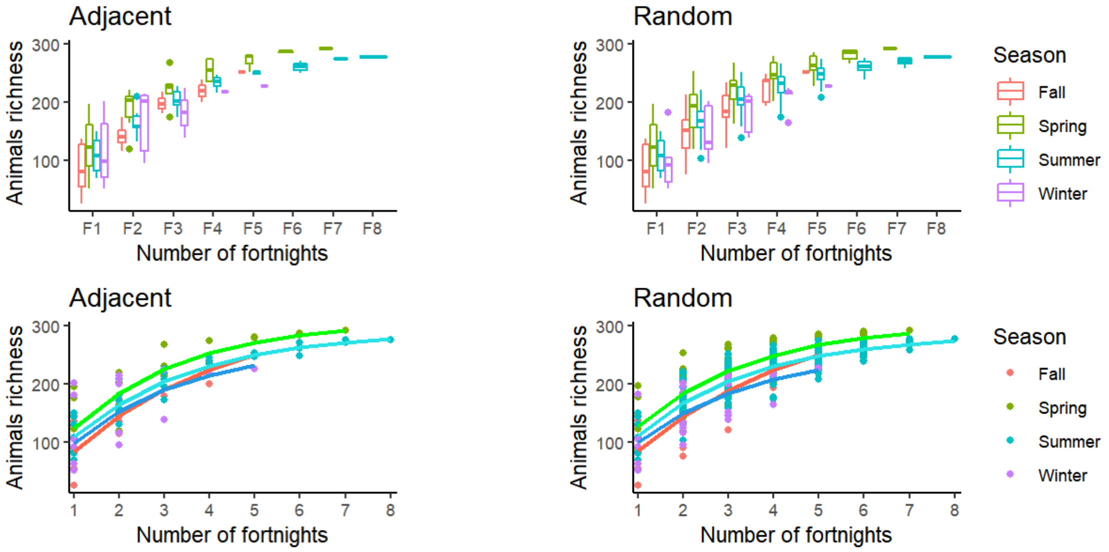


Figure 4 - Fitted curves of the Von Bertalanffy model for the animals richness, in each season. On top, boxplots with the values of animals richness for each season of the year are shown. The horizontal bar in the boxes represents the median animals richness in each season and the dashed bars represent the upper and lower limits of each season, respectively. Through the boxplots it is possible to observe the curve pattern presented by the metric. In the bottom, the adjustment curves of the Von Bertalanffy model for the animals richness, in each season, are shown. The points represent the values of the combined metrics in each of the fortnights. The lines represent the average value of the metrics in each season. The adjacent combination of networks is shown on the left. The random combination of networks is shown on the right.

Similar to the animals richness, the interactions richness also showed growth pattern in both adjacent and random networks (Figure 5). However, when we compare the value of K among seasons, we found that the order between the seasons has been changed according to the type of combination. In the adjacent networks, fall was the one with the highest *K* value, followed by spring, winter, and summer (Table 3). In the random networks, winter was the season that presented the highest *K* value, followed by fall, spring, and summer (Table 3). In this case, in the adjacent networks, fall was the season that achieved sampling completeness in terms of the interactions richness faster (Figure 5).

Table 3 - Estimated parameters of the Von Bertalanffy equation for the interactions richness. The parameters were estimated for the four seasons and for the different network combination types (adjacent and random). $M\infty$ is the asymptotic value of Y (the value at which the curve stabilizes), K is the growth rate of the curve and M_0 is the value of metric at time 0.

Season	Parameters					
	Adjacent			Random		
	$M\infty$	K	M_0	$M\infty$	K	M_0
Fall	7.1318	0.5750	-1.1798	7.2103	0.5106	-1.4092
Winter	8.5151	0.3506	-2.3578	8.0343	0.5409	-1.4514
Spring	8.3816	0.4257	-1.9803	8.3894	0.4136	-2.0831
Summer	8.1034	0.3347	-2.6885	8.0772	0.3594	-2.4973

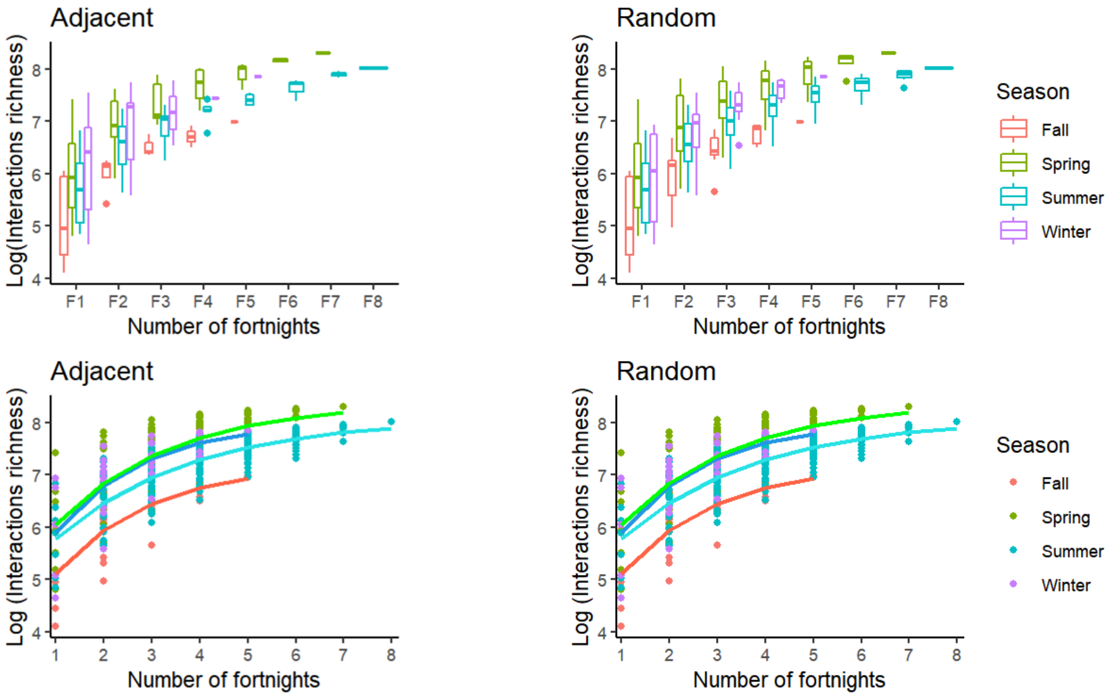


Figure 5 - Adjustment curves of the Von Bertalanffy model for the interactions richness, in each season. For this metric, the log of the interactions richness was used to obtain a more homogeneous distribution. In the top, the boxplots with the values of interactions richness for each season of the year are shown. The horizontal bar in the boxplots represents the median of interactions richness in each season and the dashed bars represent the upper and lower limits of each season. Through the boxplots it is possible to observe the curve pattern presented by the metric. In the bottom, the adjustment curves of the Von Bertalanffy model for the interactions richness, in each season, are shown. The points represent the values of the combined metrics in each of the fortnights. The lines represent the average value of the metrics in each season. The adjacent combination of networks is shown on the left. The random combination of networks is shown on the right.

The plants richness also showed growth pattern in both adjacent and random networks (Figure 6). Comparing the value of *K* among seasons, we found that the order between the seasons changed according to the combination type of the networks. In the adjacent networks, winter was the season with the highest *K* value, followed by fall, spring, and summer (Table 4). In the random networks, winter was the season that presented the highest *K* value, followed by summer, spring and fall (Table 4). In this case, in both adjacent and random networks, winter was the season that achieved the fastest sampling completeness considering the plants richness (Figure 6).

Table 4 - Estimated parameters of the Von Bertalanffy equation for the plants richness. The parameters were estimated for the four seasons and for the different types of network combination (adjacent and random). M_∞ is the asymptotic value of Y (the value at which the curve stabilizes), K is the growth rate of the curve and M_0 is the value of metric at time 0.

Season	**Parameters**					
	Adjacent			**Random**		
	M_∞	**K**	**M_0**	**M_∞**	**K**	**M_0**
Fall	53.3684	0.2232	0.2332	78.9167	0.1182	0.0259
Winter	26.2919	0.4251	0.2773	30.4651	0.2561	-0.0190
Spring	83.3013	0.1632	-0.0625	88.0910	0.1480	-0.1126
Summer	143.1852	0.0516	-0.8847	71.6600	0.1664	-0.1809

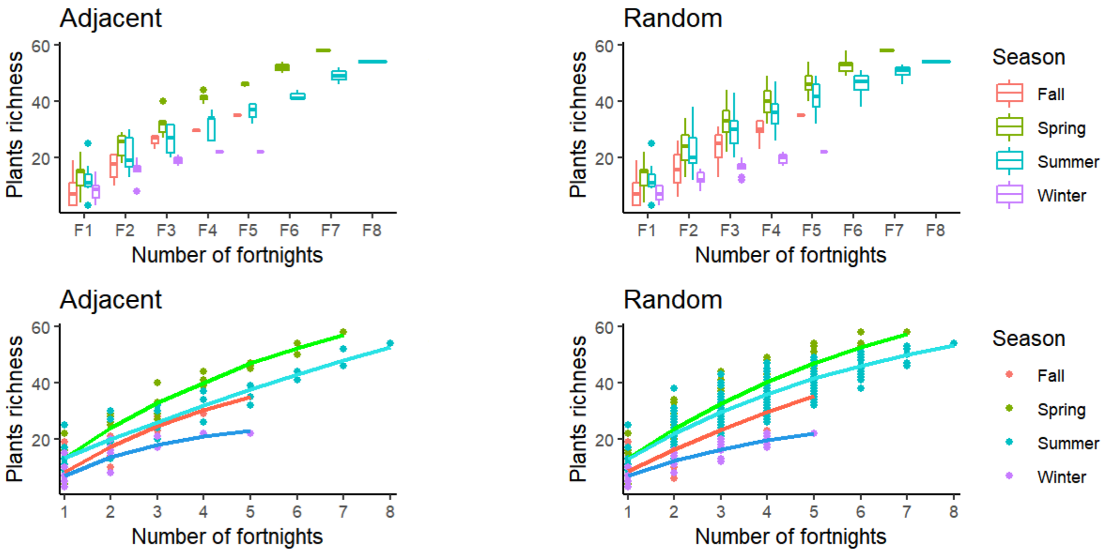


Figure 6 - Adjustment curves of the Von Bertalanffy model for the plants richness, in each season. At the top are the boxplots with the plants richness for each season. The horizontal bar in the boxplots represents the median of the plants richness in each season and the dashed bars represent the upper and lower limits of each season. Through the boxplots it is possible to observe the curve pattern presented by the metric. At the bottom are the adjustment curves of the Von Bertalanffy model for the plants richness, in each season. The points represent the values of the combined metrics in each of the fortnights. The lines represent the average value of the metrics in each season. The adjacent combination of networks is shown on the left. The random combination of networks is shown on the right.

Shannon diversity also showed an growth pattern in adjacent and random networks (Figure 7). However, considering the *K* value among seasons, we found that the order between seasons did not change according to the combination type of network. In both networks (adjacent and random), winter was the season with the highest *K* value, followed by fall, spring, and summer (Table 5). Thus, Shannon diversity requires sampling efforts to reach completeness that are lower than other seasons (Figure 7).

Table 5 - Estimated parameters of the Von Bertalanffy equation for Shannon diversity. The parameters were estimated for the four seasons and for the different types of network combination (adjacent and random). M_∞ is the asymptotic value of Y (the value at which the curve stabilizes), K is the growth rate of the curve and M_0 is the value of metric at time 0.

Season	**Parameters**					
	Adjacent			**Random**		
	M_∞	**K**	**M_0**	**M_∞**	**K**	**M_0**
Fall	6.1469	0.5405	-1.1916	6.1934	0.5139	-1.2775
Winter	5.5879	0.8501	-0.7057	5.5667	0.5739	-1.5589
Spring	5.9327	0.4420	-2.2014	5.8414	0.4729	-2.1348
Summer	6.2272	0.3781	-2.6819	6.2030	0.3923	-2.6204

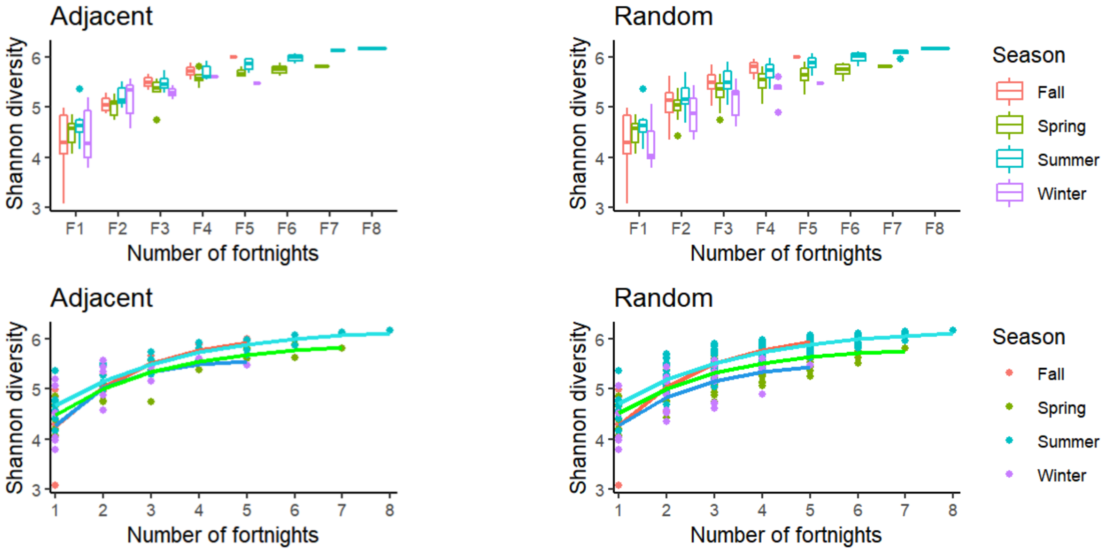


Figure 7 - Adjustment curves of the Von Bertalanffy model for the Shannon diversity, in each season. At the top, boxplots with the values of Shannon diversity for each season of the year are shown. The horizontal bar in the boxplots represents the median of Shannon diversity in each season and the dashed bars represent the upper and lower limits of each season. Through the boxplots it is possible to observe the curve pattern presented by the metric. At the bottom, the adjustment curves of the Von Bertalanffy model for the Shannon diversity, in each season, are shown. The points represent the values of the combined metrics in each of the fortnights. The lines represent the average value of the metrics in each season. The adjacent combination of networks is shown on the left. The random combination of networks is shown on the right.

The network specialization showed an exponential decay pattern in both random and adjacent networks (Figure 8). However, when comparing the value of log(α) among seasons, we found that the order between seasons changed according to the combination type (Table 6). In the adjacent networks, winter was the one with the highest *log α* value, followed by fall, spring and summer (Table 6). In the random networks, fall was the season that presented the highest value of *log α*, followed by spring, winter and summer (Table 6). In this case, in the adjacent networks, winter is the season to achieve the stability of the specialization network faster (H2) (Figure 8). Whereas, in random networks, fall is the season that reaches stability the fastest (Figure 8).

Table 6 - Estimated parameters of the *SSaSymp* function for the specialization of the network (H2). The parameters were estimated for the four seasons and for the different types of network combination (adjacent and random). M_f is the value of metric at the maximum time, M_0 is the value of metric at time 0 and *Log α* is the logarithm of the decay rate.

Season	**Parameters**					
	Adjacent			**Random**		
	$\mathbf{M_f}$	$\mathbf{M_0}$	**Log α**	$\mathbf{M_f}$	$\mathbf{M_0}$	**Log α**
Fall	0.3573	0.9762	-0.1406	0.3640	0.8581	-0.3922
Winter	0.3732	0.7714	-0.0487	0.3513	0.6311	-0.7590
Spring	0.3453	0.6864	-0.3515	0.3329	0.6545	-0.5547
Summer	0.3483	0.5113	-1.5118	0.3694	0.5319	-1.0154

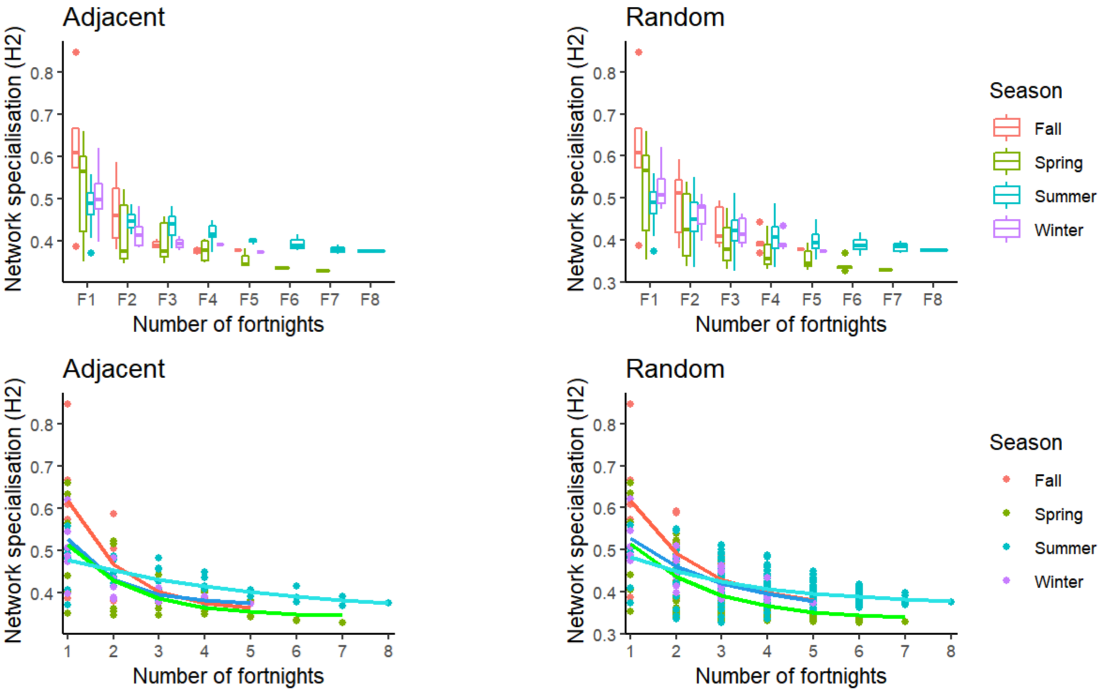


Figure 8 - Adjustment curves of the exponential decay model, based on the *SSaSymp* function, for the network specialization (H2), in each season. At the top, the boxplots with the values of network specialization (H2) for each season of the year are shown. The horizontal bar in the boxplots represents the median of network specialization (H2) in each season and the dashed bars represent the upper and lower limits of each season. Through the boxplots it is possible to observe the curve pattern presented by the metric. At the bottom, the adjustment curves of the the exponential decay model for the network specialization (H2), are shown, in each season. The points represent the values of the combined metrics in each of the fortnights. The lines represent the average value of the metrics in each season. The adjacent combination of networks is shown on the left. The random combination of networks is shown on the right.

Likewise, weighted connectance also showed an exponential decay pattern in both random and adjacent networks (Figure 9). However, when comparing the log (alpha) value between seasons, we observed that the order between seasons did not change according to the combination type (Table 7). In both types of network combination (adjacent and random), winter was the one with the highest log value (alpha), followed by summer, spring and fall (Table 7). Therefore, in both types of networks, winter is the season to achieve weighted connectivity stability faster (Figure 9).

Table 7 - Estimated parameters of the *SSaSymp* function for weighted connectance. The parameters were estimated for the four seasons and for the different types of network combination (adjacent and random). M_f is the value of metric at the maximum time, M_0 is the value of metric at time 0 and *Log α* is the logarithm of the decay rate.

Season	**Parameters**					
	Adjacent			**Random**		
	M_f	**M_0**	**Log α**	**M_f**	**M_0**	**Log α**
Fall	0.0549	0.3084	-0.3416	0.0580	0.3134	-0.2909
Winter	0.0713	0.2626	0.2939	0.0729	0.2401	0.2173
Spring	0.0595	0.1657	-0.2870	0.0628	0.1654	-0.2254
Summer	0.0511	0.2006	-0.0692	0.0513	0.2182	0.0528

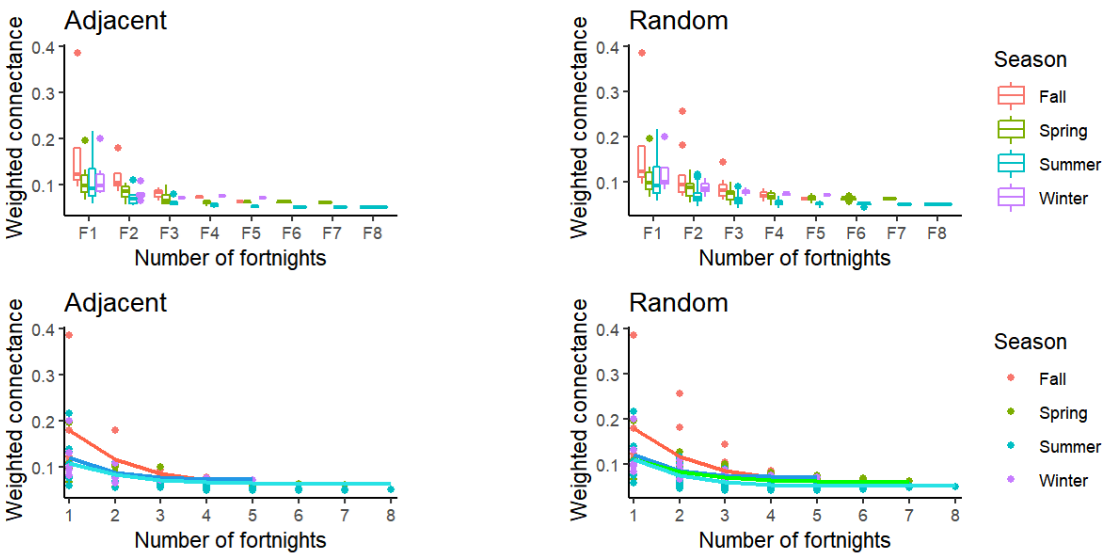


Figure 9 - Adjustment curves of the exponential decay model, based on the *SSaSymp* function, for the weighted connectance, in each season. At the top, the boxplots with the values of weighted connectance for each season of the year are shown. The horizontal bar in the boxplots represents the median of weighted connectance in each season and the dashed bars represent the upper and lower limits of each season. Through the boxplots it is possible to observe the curve pattern presented by the metric. At the bottom, the adjustment curves of the the exponential decay model for the weighted connectance, in each season are shown. The points represent the values of the combined metrics in each of the fortnights. The

lines represent the average value of the metrics in each season. The adjacent combination of networks is shown on the left. The random combination of networks is shown on the right.

Finally, when calculating how many surveys would be necessary to reach 100% of the metric values (represented here as $M\infty$), we found that the different metrics presented quite contrasting sampling efforts to achieve sampling completeness (Table 8). In the adjacent networks, to achieve 100% of the sampling of the animals richness, winter was the season that would need the largest number of sampling fortnights, while spring was the season that would need the least sampling fortnights (Table 8). In the random networks, fall was the one with the highest number of mandatory fortnights, while spring and independent summer the least number of fortnights (Table 8).

For the interactions richness, it was observed that to achieve 100% of sampling in adjacent networks, spring, summer and winter would need to be sampled the same number of fortnights and that fall would need fewer sampling fortnights (Table 8). In the random networks, the summer was the one with the highest number of fortnights required, while the winter had the lowest number of fortnights required (Table 8).

For the plants richness, we observed that in order to reach 100% of the sampling in the adjacent networks, summer is the one that would need the largest number of sampling fortnights and winter is the one that would need the least number of sampling fortnights (Table 8). In the case of random networks, fall is the one that would need the largest number of sampling fortnights, while winter is the one that would need the least number of sampling fortnights (Table 8).

For Shannon's diversity, we observed that in order to reach 100% of sampling in adjacent networks, it would be necessary to sample the same number of fortnights in spring and summer, while fewer fortnights of sampling needed for fall and winter (Table 8). In the case of random networks, summer is the one that needs the largest number of sampling fortnights, while winter is the one that needs the least number of sampling fortnights (Table 8).

For the specialization of the network (H2), we observed that to reach 100% of the sampling in the adjacent networks, winter would need the largest number of sampling fortnights, while summer would need the smallest number of sampling fortnights (Table 9). In the case of random networks, summer is the one that needs the largest number of sampling fortnights and spring is the one that needs the least number of sampling fortnights (Table 9).

For weighted connectance, we observed that to reach 100% of the sampling, the summer would need the largest number of sampling fortnights and the spring would need the smallest number of sampling fortnights (Table 9). In the case of random networks, summer is the one that needs the largest number of sampling fortnights and fall is the one that needs the least number of sampling fortnights (Table 9).

Finally, when grouping fall and winter in the dry station and, spring and summer in the wet station, we observed that the plant richness differs significantly between the dry and wet stations, with the wet station having greater plant richness than the dry station (p = 0.0269). In addition, we observed a tendency for the dry and wet stations to differ in relation to the animals richness. The p-value presented a marginally significant value, which implies a tendency for the two stations to diverge, even with a low significance (p = 0.055) (Figure 10).

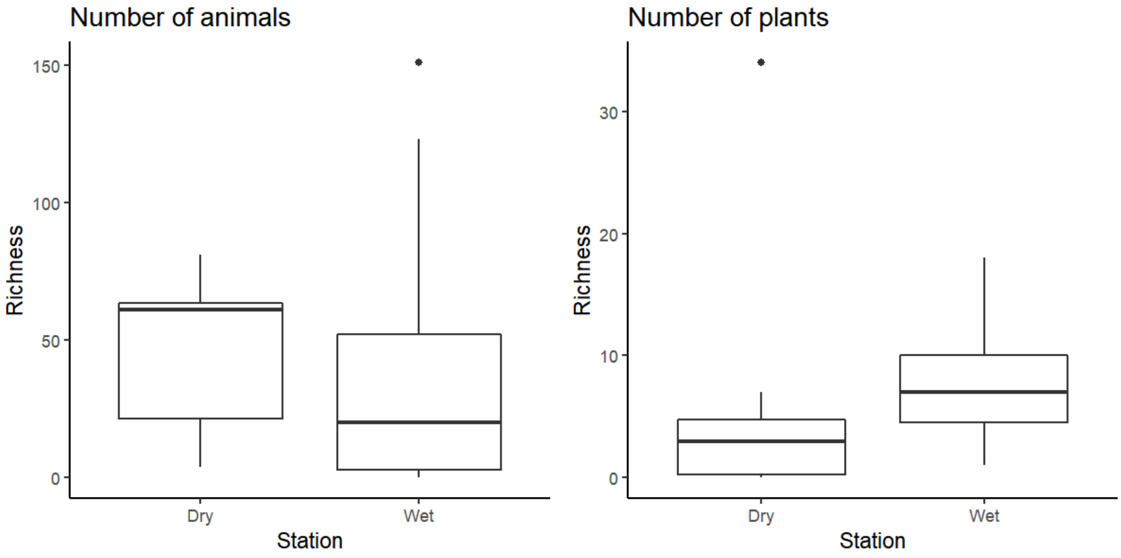


Figure 10 - Boxplots of the richness of species of animals and plants in each station. The black bar in the boxplot represents the median of each season and the dashed bars represent the upper and lower limits of each season.

Table 8 - Estimated sampling effort in terms of number of fortnights required to reach sampling completeness (100% of the value of each metric). 100% of the sampling can also be interpreted as the value at which the metric stabilizes and no longer changes. *M*∞ is asymptotic value of Y (the value at which the curve stabilizes). Therefore, we calculate the number of fortnights required to reach the value of *M*∞, which would indicate that 100% of the sample has been reached. *K* value is the growth rate of the curve.

Metrics	**Adjacent networks**				**Random networks**			
	Parameters			**Fortnights to reach the M∞**	**Parameters**			**Fortnights to reach the M∞**
	Season	**K value**	**M∞**		**Season**	**K value**	**M∞**	
Animals richness	Fall	0.2966	321.9872	50	Fall	0.2563	342.2613	62
Animals richness	Winter	0.3943	266.7473	60	Winter	0.3642	262.5262	44
Animals richness	Spring	0.3862	311.2869	40	Spring	0.3871	304.1096	40
Animals richness	Summer	0.3682	291.8373	45	Summer	0.3863	284.6182	40
Interactions richness	Fall	0.5750	7.1318	30	Fall	0.5106	7.2103	32
Interactions richness	Winter	0.3506	8.5151	45	Winter	0.5409	8.0343	30
Interactions richness	Spring	0.4257	8.3816	45	Spring	0.4136	8.3894	39
Interactions richness	Summer	0.3347	8.1034	45	Summer	0.3594	8.0772	45
Plants richness	Fall	0.2232	53.3684	73	Fall	0.1182	78.9167	120
Plants richness	Winter	0.4251	26.2919	37	Winter	0.2561	30.4651	60
Plants richness	Spring	0.1632	83.3013	100	Spring	0.1480	88.0910	110
Plants richness	Summer	0.0516	143.1852	280	Summer	0.1664	71.6600	80
Shannon diversity	Fall	0.5405	6.1469	30	Fall	0.5139	6.1934	50
Shannon diversity	Winter	0.8501	5.5879	30	Winter	0.5739	5.5667	40
Shannon diversity	Spring	0.4420	5.9327	35	Spring	0.4729	5.8414	60
Shannon diversity	Summer	0.3781	6.2272	45	Summer	0.3923	6.2030	80

Tabela 9 - Estimated number of fortnights required to reach 100% of metrics sampling. 100% of the sampling can also be interpreted as the value at which the metric stabilizes and no longer changes. M_f is the value of Y at the end time. Therefore, we calculate the number of fortnights required to reach the M_f value, which would indicate that 100% of the sample has been reached. *Log α* is the logarithm of the rate of decay of the curve.

Metrics	**Adjacent networks**				**Random networks**			
	Parameters			**Fortnights to reach the M_f**	**Parameters**			**Fortnights to reach the M_f**
	Season	**Log α**	**M_f**		**Season**	**Log α**	**M_f**	
Network specialisation (H2)	Fall	-0.1406	0.3573	140	Fall	-0.3922	0.3640	34
Network speciliasation (H2)	Winter	-0.0487	0.3732	210	Winter	-0.7590	0.3513	25
Network specialisation (H2)	Spring	-0.3515	0.3453	90	Spring	-0.5547	0.3329	18
Network specialisation (H2)	Summer	-1.5118	0.3483	10	Summer	-1.0154	0.3694	60
Weighted connectance	Fall	-0.3416	0.0549	26	Fall	-0.2909	0.0580	24
Weighted connectance	Winter	0.2939	0.0713	26	Winter	0.2173	0.0729	28
Weighted connectance	Spring	-0.2870	0.0595	25	Spring	-0.2254	0.0628	28
Weighted connectance	Summer	-0.0692	0.0511	90	Summer	0.0528	0.0513	98

DISCUSSION

Here we seek to identify the sampling effort in each of the seasons and whether they differ in terms of sampling effort. For this, we use accumulation curves for the animals richness, interactions and plants. In addition, we use growth and decay models to identify the behavior of some network metrics and the sampling effort required to achieve stability. Initially, we observed that the sampling effort for animals and plants was sufficient to record most species in the study area. While, for the interactions, the sampling effort showed low values, indicating that an increase in the sampling effort would increase the interactions richness recorded over the seasons.

In addition, we observed that animals richness, interactions richness, plants richness and Shannon diversity exhibited growth behavior and, therefore, fit the model of Von Bertalanffy. Whereas, the network specialisation and weighted connectance presented an decay pattern and, therefore, fit into the exponential decay model based on the SSaSymp function. Finally, we observed that for each metric, the number of fortnights required to achieve 100% sampling varied widely between seasons.

Regarding the sampling effort, we observed that for the animals richness and plants richness, the sampling effort was sufficient to register more than 80% of animal species and 100% of the plant species that bloomed over the four seasons. For the animals richness, fall was the season with the lowest sampling sufficiency and summer was the season with the highest sampling sufficiency. This may be the result of a greater diversity and abundance of species in the wet season (spring and summer), which makes species registration easier (POVEDA-CORONEL; RIAÑO-JIMÉNEZ; CURE, 2018; SAMNEGÅRD et al., 2015; SOUZA et al., 2018). Consequently, it would be necessary to carry out a greater sampling effort in seasons such as fall and winter, as in these seasons the species are less abundant and this makes it difficult to register them (ESCOBEDO-KENEFIC et al., 2020; VASCONCELLOS et al., 2010).

Previous studies report that the variation in species richness and abundance may be a result of both the influence of climatic variables and the availability of resources (POVEDA-CORONEL; RIAÑO-JIMÉNEZ; CURE, 2018; SAMNEGÅRD et al., 2015; SOUZA et al., 2018). Escobedo-Kenefic et al. (2020), in a study with a community of floral visitors, observed that the richness and abundance of floral visitors are greater in the wet season and that this is directly associated with the availability of floral resources in each season. Similarly, Abrahamczyk et al. (2011), in a study with three different groups of pollinators, observed that the richness and abundance of pollinators responded not only to climatic conditions, but also to the availability of resources. Therefore, we can say that the animals richness found in each season varies not only due to climatic conditions, but also due to the availability of floral resources in each season. For the plants richness that bloomed, the sampling effort in the four seasons was 100%. This might be explained by the fact that our transects are fixed. That is, at each sampling, we visited the same areas. This added to the fact that our collections were throughout the year, may have made it easier to sample all plants. Studies like Jordano (2016), highlight the importance of carefully choosing the sampling method, due to the fact that it may influence our observations.

For the interactions richness, the sampling effort was sufficient to record on average only 60% of the interactions in the seasons. Winter was the season with the lowest sampling sufficiency and spring was the season with the highest sampling sufficiency. This can be related to different factors such as: variation in species richness and abundance, variation in animal foraging activity and forbiden links (ESCOBEDO-KENEFIC et al., 2020; JORDANO, 2016; LASMAR et al., 2021). Despite the low sample sufficiency for the interactions richness , our results corroborate with previous studies, such as Chacoff et al. (2012) and Falcão et al. (2016), who in addition to presenting results similar to ours for the interactions richness , also carried out an intensive sampling. Chacoff et al. (2012), e.g., in a study with plant-floral visitors interactions, during the peak of flowering of the plants over 4 years, they managed to register 80.4% of the species of floral visitors and only 55% of the interactions between the species. Similar to that, Falcão et al. (2016) in a study with plant-ant interaction over 12 months, they managed to register an average of 90% of plant and ants species, and 63% of interactions between species. In other words, our findings are not an exception and highlight the need to carry out major sampling efforts to increase the number of records, mainly of interactions (CHACOFF et al., 2012; GOTELLI; COLWELL, 2011).

Regarding the models, for the metrics that fit the Van Bertallanfy model – i.e., animals richness, interactions richness, plants richness, and Shannon diversity – we observed that the seasons differ in the curves' stabilization time is more evident when observing the value of K. The higher the value of K, the faster the metric stabilizes (ESSINGTON; KITCHELL; WALTERS, 2001). We credit that the difference between seasons is related to the difference in species richness (i.e., plants and floral visitors), similarly to the animals richness, interactions and plants in the accumulation curves. Specifically for Shannon diversity, it was expected that it would respond to species richness in the interaction networks, since the higher species richness, the higher the possibility of new interactions occurring and, consequently, the higher the diversity of network links (BERSIER; BANASEK-RICHTER; CATTIN, 2002; BLÜTHGEN; MENZEL; BLÜTHGEN, 2006).

Similarly, for the metrics that fit into the exponential decay model – i.e., network specialization and weighted connectance - we observed that the seasons also differed in the sampling effort and that this was directly linked to the value of log (α). The higher the value of the log (α), the faster the metric tended to stabilize. Such differences between seasons can again be explained by the richness of species (JORDANO, 1987; OLESEN; JORDANO, 2002). Weighted connectance, for example, decreases as new species are included in networks (JORDANO, 1987; OLESEN; JORDANO, 2002; RIVERA-HUTINEL et al., 2012). At the same time, the augmentation in the richness of plant species is associated with the increasing richness of floral visitors, which would lead them to specialize in certain plants species (ESCOBEDO-KENEFIC et al., 2020; FONTAINE; COLLIN; DAJOZ, 2008; SCHLEUNING et al., 2012). Consequently, seasons that present higher species richness would present higher specialization in relation to seasons with lower species richness (BENDER et al., 2017; MAGRACH et al., 2017).

When comparing adjacent and random networks, we observed that all metrics, but Shannon diversity, differed regarding the season stabilization order. The method of sampling events organization, which was different between adjacent - i.e., accumulative - and randomly drawn fortnights, is affecting it. Datillo et al. (2019) pointed out that the order of the samples through space scales influences the estimates. However, they pointed out that the order of the sampling events did not influence the network metrics. Indeed, Datillo et al. (2019) assumed randomly drawn assembly as a null model for testing the hypothesis that the spatial scales influence the metrics of the network. Here, our sampling events are not related to space but time. Then, we are expanding the hypothesis of the influence of spatial sampling scales to time scales affecting sampling effort. As a result, we observed that time scales affect the sampling efforts directly. Further, the sequence of sampling events has importance on the final outcomes regarding saturation of the parameters to be estimated by means of the difference between adjacent and random sampling events. Furthermore, seasons also mediate the final outcomes, and sampling events should be taken with caution in terms of seasonality and saturation.

Finally, the Von Bertalanffy model is employed to evaluate body size of fish at different ages and the exponential decay model is used to describe physical phenomens, such as chemical reactions, decreased radioactivity and decreased heat temperature (ARCHONTOULIS; MIGUEZ, 2015; ESSINGTON; KITCHELL; WALTERS, 2001; WATSON, 2018). However, because they are nonlinear models, they can be applied to different biological systems, including species and interactions (ARCHONTOULIS; MIGUEZ, 2015; ESSINGTON; KITCHELL; WALTERS, 2001; WATSON, 2018). Nonlinear models encompass a wide variety of functions and advantages, but should be used and interpreted with caution (ARCHONTOULIS; MIGUEZ, 2015). That said, the models we employed here can be used as alternative tools to verify not only the sampling effort, but also the intensity of effort is needed to achieve stability regarding species and network metrics.

Here we identify that the seasons differ from each other in terms of the applied sampling effort. Such differences were influenced by the variation in species richness and abundance. Thus, depending on the season in which a study is carried out, it will be necessary to carry out a greater or lesser sampling effort. The models used, Von Bertalanffy Equation and Exponential Decay Model based on the *SSaSymp* function, proved to be suitable for the study of stability in floral plant-visitor networks. Since the estimated metrics of the adjacent and random networks presented different behaviors throughout the seasons, validating the applicability of the models. However, sampling methods directly influence interaction networks. Therefore, it is important to be cautious when choosing sampling methods based on time and paying attention to sample sufficiency, since it helps us to be aware of the effects of sampling on the data collected. In addition, the evaluation of the sampling effort allows studies with greater robustness and that are closer to the “real” networks that occur in nature. However, research on the temporal dynamics of plant-floral visitors networks and the sampling effects are poorly performed, especially in the tropics. Thus, further studies for interaction networks in tropical environments are important due to the great diversity of active species during the year.

ACKNOWLEDGEMENTS

We immensely thank those involved in identifying the species: Dr. Mariana Esteves Mansanares and Dr. Daniel Quedes Domingos (plants), Dr. Mírian Nunes Morales (syrphids), and Dr. Fernando Amaral da Silveira (bees). We would like to thank the Foundation for Research Support from the State of Minas Gerais (FAPEMIG) for the master's scholarship granted to VHDS. MGH is a Brazilian National Council for Scientific and Technological

Development fellow (CNPq 304102/2018-0) and also thanks FAPEMIG (APQ-02784-17). LDBF thanks FAPEMIG (APQ-02700-17) for financial support to fieldwork, and he is a Brazilian National Council for Scientific and Technological Development fellow (CNPq 306196/2018-2). This work was carried out with support from the Minas Gerais Research Support Foundation (FAPEMIG).

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