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ANT-PLANT INTERACTIONS MEDIATED BY EXTRAFLORAL NECTARIES IN THE BRAZILIAN AMAZON:

A NETWORK APPROACH

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Orientador: Prof. Dr. José Eduardo Lahoz da Silva Ribeiro
Coorientador: Dr. Wesley Dáttilo

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Dedico este trabalho a minha amada
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RESUMO

Algumas espécies de formigas se alimentam de néctar produzido por determinadas espécies de plantas em glândulas conhecidas como nectários extraflorais (NEFs). Ao forragearem nestas glândulas, as formigas acabam protegendo a planta contra ataques herbívoros. Esta interação tem sido bastante estudada, e nos últimos anos tem aumentado o número de trabalhos que utilizam uma abordagem de redes de interações para avaliar esta associação em nível de comunidade. O objetivo deste trabalho foi estudar as interações formiga-planta mediadas por NEFs em uma abordagem de rede. O trabalho foi realizado no Estado do Acre, Amazônia brasileira, mais especificamente em 10 fragmentos florestais cujos tamanhos variaram entre aproximadamente 5 e 3000 há. A partir das amostragens realizadas, foi possível a elaboração de quatro capítulos, os quais abordam diferentes fatores que influenciam na estrutura destas redes, tais como morfologia dos NEFs, categoria de matrizes de dados (binário ou ponderados), sazonalidade das chuvas e modificação do hábitat (isto é, fragmentação florestal). No primeiro capítulo, utilizando-se de imagens de microscopia eletrônica de varredura, foi realizada a descrição morfológica dos NEFs de 68 espécies de plantas. As famílias mais representativas foram Fabaceae, Bignoniaceae e Malpighiaceae. Os NEFs denominados elevados foram os mais frequentes, seguidos dos morfotipos achatados, sem forma, côncavos, ocos e transformados. Esta maior representatividade dos NEFs do tipo elevado evidencia a importância do sistema de defesa de formigas contra a herbivoria nas florestas da Amazônia brasileira, pois estas glândulas são as que produzem maior volume de néctar, sendo consequentemente bastante visitadas por formigas. No segundo capítulo, a estrutura destas redes de interações formiga-planta foi avaliada a partir de diferentes categorias de dados (presença e ausência da interação, frequência de interação e abundância de formigas). O uso de diferentes categorias de dados resultou em diferenças estruturais significativas destas redes de interações, no que diz respeito a descritores de rede como generalidade, vulnerabilidade, aninhamento e modularidade. Estes resultados destacam a necessidade de se expandir a discussão sobre categorias de dados em estudos de interação ecológica a fim de se evitar inferências inapropriadas. No terceiro capítulo, foram detectadas diferenças significativas da estrutura destas redes de interações formiga-planta (isto é, número de interações, diversidade de interações e aninhamento) entre estação de chuvas intensas e de seca. Estes resultados indicam que a sazonalidade das chuvas na Amazônia é um fator determinante na organização estrutural destas redes, sendo essencial se considerar a dinâmica sazonal destas glândulas em estudos similares, a fim de se garantir que a maior parte das possibilidades de interação entre formigas e plantas para uma dada localidade esteja presente nas redes. Finalmente, os resultados do quarto capítulo indicam que a estrutura das redes formigaplanta é relativamente estável à fragmentação florestal. O único descritor de rede afetado foi o aninhamento. Especificamente, foi observado que o aninhamento diminuiu com o aumento da irregularidade da borda. A relativa estabilidade estrutural das redes estudadas está possivelmente relacionada à constância das espécies de formigas nos núcleos centrais de espécies altamente interativas. Em suma, os resultados observados indicam que a abordagem de redes é uma ferramenta adequada para o estudo das interações formiga-planta mediadas por NEFs em nível de comunidade, e alertam para alguns cuidados relacionados à definição da categoria de dados a ser usada e ao período amostral apropriado, considerando a dinâmica sazonal destas glândulas.

Palavras chaves: Categoria de dados. Fragmentação florestal. Floresta amazônica. Interações formiga-planta. Morfologia de nectários. Nectários extraflorais. Sazonalidade das chuvas.

Miranda, Patrícia Nakayama. **Ant-plant interactions mediated by extrafloral nectaries in the Brazilian Amazon: a network approach** 2019. 210 p. PhD Thesis in Biological Sciences - Londrina State University, Londrina, 2019.

ABSTRACT

Some ant species feed on the nectar produced by some plant species in glands called extrafloral nectaries (EFNs). The ants protect the plant against herbivore attack by foraging in these glands. This ant-plant interaction has been studied extensively and in the last few years there has been an increase in the number of studies that use an interaction networks approach to evaluate this association at community level. The aim of this investigation was to study ant-plant interactions mediated by EFNs using a network approach. The work was carried out in the State of Acre, Brazilian Amazon, more specifically in 10 forest fragments ranging in size from approximately 5 to 3000 hectares. From the sampling carried out, it was possible to generate four chapter, which address different factors that influence the structure of these networks, such as EFN morphology, matrix categories (binary or weighted data), rainfall seasonality, and habitat modification (i.e., forest fragmentation). In the first chapter, using scanning electron microscopy images, morphological descriptions of the EFNs of 68 plant species were produced. The most representative families were Fabaceae, Bignoniaceae, and Malpighiaceae. Elevated extrafloral nectaries were the most frequent type found, followed by flattened, formless, pit, hollow, and transformed. The greater frequency of elevated EFNs evidences the importance of the ant defense system against herbivory in the Brazilian Amazon forest, since these glands are the ones that produce greater nectar volume, consequently being more often visited by ants. In the second chapter, the structural organization of these ant-plant interaction networks was evaluated from different data categories (presence and absence of interaction, interaction frequency, and ant abundance). The use of different data categories resulted in significant structural differences among these interaction networks, in relation to network descriptors such as generality, vulnerability, nestedness, and modularity. These results highlight the need to expand the discussion regarding data categories in ecological interaction studies, in order to avoid inappropriate inferences. In the third chapter, significant differences were detected in the structure of the ant-plant interaction networks (i.e., number of interactions, diversity of interactions, and nestedness) between the rainy and dry seasons. These results indicate that rainfall seasonality in the Amazon rainforest is a determining factor in the structural organization of these networks and, therefore, it is essential to consider the seasonal dynamics of these glands in similar studies, to ensure that most of the ant-plant interaction possibilities of a given locality are present in the network. Finally, the results of the fourth chapter indicate that the structure of ant-plant networks is relatively stable in face of forest fragmentation. Nestedness was the only network descriptor affected by fragmentation. Specifically, nestedness decreased with increasing edge irregularity. The relative structural stability of the networks studied is possibly related to the constancy of ant species in the central core of highly interacting species. In summary, the observed results indicate that network approach is an adequate tool to study the ant-plant interactions mediated by EFNs at community level, and alert to some care related to the definition of the data category to be used and to the appropriate sampling period, considering the seasonal dynamics of these glands.

Key words: Amazon rainforest. Ant-plant interactions. Data category. Extrafloral nectaries. Extrafloral nectary morphology. Forest fragmentation. Rainfall seasonality.

SUMÁRIO

GENERAL INTRODUCTION

For years ant-plant interactions have been an important object of study (Cole 1932; Uphof 1942; Bentley 1976; Rosuomek *et al.* 2009; Keller *et al.* 2018) and some studies highlight mutualistic interactions, such as those mediated by extrafloral nectaries (EFNs), due to the benefits generated for both associated organisms (Fagundes *et al.* 2017). In these interactions some ant species protect certain plants against herbivore attack when they feed on a substance (rich in carbohydrates and amino acids) secreted through glands called extrafloral nectaries (EFNs) (Koptur *et al.* 1998; Kwok and Laird 2012; Fagundes *et al.* 2017). Although these ant-plant interactions are considered facultative, due to the low fidelity of ant species when foraging on EFN-bearing plants (Schoereder *et al.* 2010), studies indicate positive and significant impacts of these associations on the survival, growth, and reproduction of ants (Lach *et al.* 2009; Byk and Del-Claro 2011), as well as on plant growth (De La Fuente and Marquis 1999).

Currently, a relatively large diversity of EFN-bearing plant species is recognized, corresponding to approximately 25% of the species total of angiosperms already described (Elias 1983; Koptur 1992), with Fabaceae, Passifloraceae, and Malvaceae, being the richest families (McKey 1989; Marazzi *et al.* 2013; Weber and Keeler 2013). All this species diversity is reflected in the great morphological variation that these glands present (Elias 1983). Some EFNs, for example, because they are non-vascularized and do not present a well-defined external structure (Koptur 1992), tend to produce a small volume of nectar, which makes them unattractive to ants (Díaz-Castelazo *et al.* 2005). In contrast, vascularized EFNs, because they present a well-defined external structure (Koptur 1992) and are relatively larger than non-vascularized glands, secrete a larger volume of nectar (Díaz-Castelazo *et al.* 2005) making them very attractive to ants (Blüthgen *et al.* 2000). Therefore, knowledge of the morphology of these glands is fundamental to better understanding the mechanisms that model these ant-plant interactions. However, although the Brazilian Amazon is widely known for its high floristic diversity (Ribeiro *et al.* 1999; Giulietti *et al.* 2005), no study of the morphological characterization of EFNs has been published for the region until the present moment.

At the community level, ant-plant interactions mediated by EFNs are usually evaluated through a network approach, in which different ant and plant species are depicted as nodes and their interactions as links (Dáttilo *et al.* 2013a). The structure of these networks is evaluated based on network descriptors, such as network size, number of interactions, generality and vulnerability (Bersier *et al.* 2002), network specialization (Blüthgen *et al.* 2006), diversity of interactions (Bersier *et al.* 2002), nestedness (Almeida-Neto *et al.* 2008), and modularity (Guimerà *et al.* 2004), which cover a wide range of possible structures with complementary biological meanings (Del-Claro *et al.* 2016). In general, ant-plant interactions present a nested pattern in which specialist species tend to interact with generalist species (Bascompte and Jordano 2007), which in turn results in a low degree of modularity (Diaz-Castelazo *et al.* 2013), intermediate values of network specialization (Dáttilo 2012; Dáttilo *et al.* 2014a; Schoereder *et al.* 2010), and diversity of interactions (Dáttilo and Dyer 2014; Falcão *et al.* 2015).

To calculate these network descriptors, the information is systematized in matrices in which the row vectors represent plant species, the column vectors represent ant species, and the matrix elements represent ant-plant interactions. These matrices are usually expressed in a binary (1 when an interaction is recorded and 0 when no interaction is recorded) or weighted way (elements indicate the number of times in which a plant species interacted with an ant species over a spatial or temporal gradient) (Dáttilo *et al.* 2014b). Some authors have questioned the use of binary matrices in studies of ecological interactions (Dáttilo *et al.* 2014b) since in these matrices all interactions are considered ecologically equivalent (Almeida-Neto and Ulrich 2011) and consequently interaction strength is not represented. Conversely, in weighted matrices the partner's preference (i.e., resource selection) is taken into account (Blüthgen 2010). In this context, studies that investigate possible variations in the network structure using different matrix categories (binary and weighted data) are fundamental to avoid making inadequate inferences.

Besides paying attention to the data category to be adopted, it is also very important to consider selection of the sample period in studies dealing with ant-plant interactions mediated by EFNs, due mainly to phenological variations of EFN-bearing plants through the year (Falcão *et al.* 2016). Such phenological variations are possibly related to the seasonality of abiotic factors, such as water availability (Rico-Gray *et al.* 2012), with consequent effect on gland productivity (Rico-Gray 1993). EFNs associated with leaves, for example, are productive mainly in young leaves (Rico-Gray 1993), a phenological stage usually observed in the rainy season (Belchior *et al.* 2016). In contrast, EFNs located in reproductive structures reach their production peak during the dry season (Stevenson *et al.* 2008). Therefore, the ant species feeding on these glands tend to change from one plant species to another during the year (Rico-Gray *et al.* 2012), which may result in structural changes in the ant-plant networks (Lange *et al.* 2013; Falcão *et al.* 2016) considering the differences in preference for extrafloral nectar composition among ant species (Dáttilo *et al.* 2014c).

As mentioned above, the structural organization of ant-plant networks mediated by EFNs is influenced by the variation in extrafloral nectar productivity among plant species (Díaz-Castelazo *et al.* 2005) and by the differences in preference for the extrafloral nectar composition among ant species (Dáttilo *et al.* 2014c). In this context, habitat modification, generated, for example, by forest fragmentation, may result in structural changes in the ant-plant networks, since studies indicate changes in the composition of plants (Hill and Curran 2001; Benítez-Malvido and Martínez-Ramos 2003) and ants (Vasconcelos *et al.* 2006; Morini *et al.* 2007; Leal *et al.* 2012) arising from this process. In addition, changes in the vegetation structure arising from forest fragmentation, such as canopy cover reduction near the forest edges (Ewers and Banks-Leite 2013), may also cause structural changes in the ant-plant networks. In general, the amount of nectar secreted by EFN-bearing plants, a determining factor for the attraction of ants to the EFNs (Marazzi *et al.* 2013), varies negatively as a function of canopy cover (Kersch *et al.* 2005; Bixenman *et al.* 2011; Dáttilo *et al.* 2013b). Therefore, edge areas, with lower canopy cover, tend to be associated with ant-plant networks with a greater number of interactions and diversity of interactions (Dáttilo and Dyer 2014). Unfortunately, although forest fragmentation is a sad reality in the Brazilian Amazon (Laurance *et al.* 2001; Laurance *et al.* 2011), to date no study of the effects of this process on the structure of ant-plant networks mediated by EFNs has been carried out for the region.

Based on the above, this thesis aims to study the ant-plant interactions mediated by EFNs in the Brazilian Amazon, using a network approach. For this, we consider different aspects that directly influence these networks, such as EFN morphology, matrix categories (binary and weighted data), rainfall seasonality, and habitat modification (i.e. forest fragmentation). In general, this thesis is structured in four chapters with the following objectives: Chapter 1 presents a brief morphological description of EFNs in Brazilian Amazonian rainforests, in terms of morphotype, size (length and width), and location on the plant body; Chapter 2 evaluates whether the structure of ant-plant networks mediated by EFNs (i.e. generality, vulnerability, nestedness, and modularity) are affected by changes in binary or weighted data; Chapter 3 investigates whether rainfall seasonality, typical of the Brazilian Amazonian rainforest, influences the structure of the ant-plant networks mediated by EFNs (i.e., number of interactions, network size, network specialization, diversity of interactions, and nestedness); and, Chapter 4 investigates the effects of configuration structures of tropical rainforest fragments (i.e., forest fragment area, edge irregularity, and connectivity) and forest structure on the structure of the ant-plant networks mediated by EFNs (i.e., network size, number of interactions, network specialization, diversity of interactions, and nestedness).

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CHAPTER 1

Morphological characterization of extrafloral nectaries in Amazonian plant species

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Abstract

Despite the increase in the number of studies devoted to morphological description of the different morphotypes of extrafloral nectaries, no study has been published for the Brazilian Amazon rainforest. Our aim was to perform a morphological characterization of extrafloral nectaries in the Brazilian Amazonian rainforests, and to contribute to the knowledge about their distribution at genus and family level. We sampled plant species with extrafloral nectaries (EFN-bearing plant species) in 10 forest fragments, situated in the state of Acre, Brazil. We used scanning electron microscopy images to morphologically characterize the extrafloral nectaries of 68 species, distributed in 29 genera and 20 families. We collected the greatest number of species belonging to Fabaceae, followed by Bignoniaceae and Malpighiaceae. Liana was the most representative life form, followed by tree, shrub and herb. Elevated extrafloral nectaries were the most representative morphotype, followed by flattened, formless, pit, hollow and transformed extrafloral nectaries. Our results show a large variation in the distribution of EFN-bearing plant species among different families. In addition, the higher frequency of some morphotypes of extrafloral nectaries possibly affects the interactions between these plants and other organisms, considering the function of these glands to provide food in exchange for protection against herbivory.

Keywords: Amazon plant species; Brazilian Amazon rainforest; EFN-bearing plant species; extrafloral nectaries; morphological characterization

Resumo

Apesar do aumento no número de estudos dedicados à descrição morfológica dos diferentes morfotipos de nectários extraflorais, nenhum estudo foi publicado para a floresta amazônica brasileira. Nosso objetivo foi realizar uma caracterização morfológica de nectários extraflorais em florestas tropicais da Amazônia brasileira e contribuir para o conhecimento sobre sua distribuição em nível de gênero e família. Foram amostradas espécies de plantas com nectários extraflorais em 10 fragmentos florestais, situados no estado do Acre, Brasil. Utilizamos imagens de microscopia eletrônica de varredura para caracterizar morfologicamente os nectários extraflorais de 68 espécies, distribuídos em 29 gêneros e 20 famílias. A família com maior número de espécies foi Fabaceae, seguida de Bignoniaceae e Malpighiaceae. A forma de vida mais representativa foi liana, seguida de árvore, arbusto e erva. O morfotipo mais representativo de nectário extrafloral foi o elevado, seguido de achatado, sem forma, côncavo, oco e transformado. Nossos resultados mostram uma grande variação na distribuição de espécies de plantas com nectários extraflorais entre diferentes famílias. Além disso, a maior frequência de alguns morfotipos bastante produtivos, possivelmente afete as interações entre essas plantas e outros organismos, considerando a função dessas glândulas em fornecer alimentos em troca de proteção contra a herbivoria.

Palavras chaves: espécies de plantas da Amazônia; floresta amazônica brasileira; espécies de plantas com NEFs; nectários extraflorais; caracterização morfológica

Introduction

Among the high diversity of plant species occurring in tropical rainforests (Ribeiro *et al.* 1999; Giulietti *et al.* 2005), some species are known for the presence of secretory glands called extrafloral nectaries that can be distributed in different parts of the plants (EFN-bearing plant species) and are not directly involved in pollination processes (Koptur 1992a). These glands secrete an aqueous solution composed of sugars, amino acids, lipids and other organic compounds (Baker and Baker 1975; 1983; Heil 2011). Sugars constitute the largest proportion of the extrafloral nectar composition, reaching concentrations ranging from 5% to 50% (Koptur 1990). Of the amino acids that compose extrafloral nectar, some are possibly related to the different nutritional requirements of ants or wasps (Koptur 1992a), such as cysteine/cystine, lysine, asparagine and tyrosine (Baker *et al.* 1978). Therefore, due to its nutritional components, mainly sugars and amino acids, extrafloral nectar is used as a food resource by many arthropod groups such as ants, spiders and wasps (Koptur *et al.* 2010; Schoereder *et al.* 2010; Heil 2015; Del-Claro *et al.* 2016). Among these, ants constitute one of the groups relatively well studied in the insect–plant interactions context (Blüthgen *et al.* 2000; Aguirre *et al.* 2013). In exchange for the food provided by the ENF-bearing plant species, some ants can protect their host plants against herbivores (Rico-Gray and Oliveira 2007; Koptur *et al.* 2010), which can increase plant fitness (De La Fuente and Marquis 1999).

The diversity of EFN-bearing plant species is high, corresponding to approximately 25% of the angiosperms (Elias 1983; Koptur 1992a). According to analysis of the systematic distribution of EFN-bearing plant species performed by Weber and Keeler (2013), based on studies published over 135 years, a total of 3,941 plant species, distributed among 745 genera and 108 families, are recognized. Of these, three angiosperm families account for approximately 50% of the total species: Fabaceae, Passifloraceae and Malvaceae (McKey 1989; Marazzi *et al*. 2013; Weber and Keeler 2013). Part of this diversity occurs in temperate zones, but these EFN-bearing plant species are more common in tropical areas (Koptur 1992a). In tropical regions, EFN-bearing plant species may represent from 5% to 33% of the general floristic diversity (Schupp and Feener 1991; Fiala and Linsenmair 1995; Aguirre *et al*. 2013). According to Morellato and Oliveira (1991), in the Brazilian Amazonian rainforests, depending on the vegetation type ("terra firme" forest, successional forest, palm vegetation and savanna), plants with extrafloral nectaries may represent 18% to 53% of the total woody species.

The high diversity of EFN-bearing plant species matches the morphological diversity of these glands, which may or may not be vascularized (Elias 1983; Koptur *et al*. 1998). Nonvascularized extrafloral nectaries do not have a well-defined internal structure (Koptur 1992a). Some of these glands do not even have an external structural specialization (formless nectaries) or are only glandular tissues sunken in tissue of other organs, in depressions as large as or larger than the gland (pit nectaries) (Koptur 1992a; Díaz-Castelazo *et al*. 2005). On the other hand, vascularized extrafloral nectaries have a well-defined external structure (Koptur 1992a), the majority being vascularized by both phloem and xylem (Frey-Wyssling 1955; Durkee 1983) and are also diverse in relation to morphology. For example, the glandular tissue of flattened extrafloral nectaries is closely pressed against underlying tissue (Koptur 1992a). Instead, the glandular tissue of elevated extrafloral nectaries is raised above ground tissue (Koptur 1992a). Differently from these two morphotypes, hollow extrafloral nectaries are cavities in other plant tissues with a narrow channel, slit or pore extending to the surface (Díaz-Castelazo *et al*. 2005). In general, the nectar volume produced by extrafloral nectaries varies significantly among the different morphotypes, with vascularized glands tending to secrete a larger volume than nonvascularized glands (Díaz-Castelazo *et al*. 2005), which in turn tends to directly affect insect–plant interactions (Blüthgen *et al*. 2000). In relation to their location on the plant body, these glands occur in both vegetative and reproductive structures (Elias 1983). On leaves, for example, the extrafloral nectaries occur on the petiole, the rachis of compound leaves, the upper and lower surfaces of the blade, the leaf margin and on stipules (Koptur 1992a). However, extrafloral nectaries located in reproductive structures are also called “extrafloral” because they are not directly involved with pollination (Koptur 1992a). They can occur on buds, calyx, inflorescence axis, flower peduncles and fruits (Rico-Gray and Thien 1989; Rico-Gray 1993; Rico-Gray *et al*. 2004). Thus, based on the studies published up to the present moment, we can conclude that extrafloral nectaries are very diverse in terms of morphology, location, structure and physiology.

Until now, a reasonable number of studies have been devoted to morphological description of extrafloral nectaries (Elias and Gelband 1976; Pascal *et al*. 2000; Blüthgen and Reifenrath 2003; Díaz-Castelazo *et al*. 2005; Aguirre *et al*. 2013; Rodríguez-Morales *et al*. 2016). In Brazil, despite being a megadiverse country (Frehse *et al*. 2016), most studies about the morphological characterization of these glands have been concentrated in the Cerrado region (Oliveira and Leitão-Filho 1987; Machado *et al*. 2008; Melo *et al*. 2010), and no study has been published for the Brazilian Amazon rainforest, a region widely known for its high floristic biodiversity (Ribeiro *et al*. 1999; Giulietti *et al*. 2005). Therefore, our main aim was to perform a brief morphological description of extrafloral nectaries in Brazilian Amazonian rainforest, in terms of morphotype, size (length and width), location on the plant body and life form of the EFN-bearing plant species. In addition, we also aim to contribute to the taxonomic knowledge of these EFN-bearing plant species in relation to their distribution at genus and family level.

Material and methods

Study site

The study was carried out in 10 forest fragments, ranging in size from approximately 5 to 3,000 ha, situated in the west of the state of Acre, Brazilian Amazon (Supplementary material 1). All forest fragments studied have already undergone anthropic actions, mainly the removal of wood. According to the Köppen climate classification (1936), our study area is classified as having a monsoon climate (Peel *et al*. 2007), with an average rainfall of 1,450 mm per year (Macêdo *et al*. 2013) and marked seasonality, with most rain falling between November and March (Acre 2006). The average annual temperature is 24 °C (INMET 2016), with daily variation of around 9 °C (Acre 2006). The region's altitude varies between 110 and 270 m a.s.l. (Acre 2006). The predominant vegetation types are open tropical rainforest (open ombrophilous forest) that can be dominated by bamboo and/or with palm trees, and dense tropical rainforest (dense ombrophilous forest) (Acre 2006).

Field work

We established a plot of 6,250 m^2 (250 × 25 m) in each forest fragment, with a minimum distance of 100 m from the edge, except for the smallest forest fragment, where a plot was placed at approximately 20 m from the edge. In each plot, we performed intensive field surveys in order to locate EFN-bearing plant species. The presence of ants feeding on extrafloral nectaries helped us to locate the EFN-bearing plants within plots. We collected a plant sample from all specimens with extrafloral nectaries detected in the field, for further identification and morphological characterization of these glands through a scanning electron microscopy study (see below). Plants whose extrafloral nectaries were not detected in the field, but which had immobile ants with mouthparts in contact with plant tissues for several minutes, were also collected for later confirmation of the presence of extrafloral nectaries through observations with a stereoscope and a literature review. The fieldwork was carried out from July 2016 to March 2017. During this period, each plot was sampled twice, the first sampling being performed in the dry season, and the second in the rainy season, in order to increase the probability of detecting plants that exhibit extrafloral nectaries active only in one of these seasons.

Plants were identified at species or morphospecies level through identification guides (Ribeiro *et al*. 1999; Pennington *et al*. 2004) and by comparison with specimens deposited at the Herbário do Parque Zoobotânico (HPZ) of the Universidade Federal do Acre, Brazil. Voucher specimens of plants were deposited at the Herbário (FUEL) of the Universidade Estadual de Londrina, Brazil. All taxa were revised by a specialist (Dr. José Eduardo Lahoz da Silva Ribeiro, Universidade Estadual de Londrina); however, as we did not have reproductive structure samples for most of the EFN-bearing plant species, it was not possible to reach species level for all species sampled. Considering our difficulty in identifying the botanical samples, our focus was basically to describe the morphology of some extrafloral nectaries found in the Amazon and their distribution in terms of genera and botanical families.

Morphological studies

We performed morphological characterization of the extrafloral nectaries for 68 EFN-bearing plant species or morphospecies, which represent 48% of the total number of species sampled in the 10 forest fragments (n = 148 EFN-bearing plant species). These 68 species were selected in order to contemplate almost all botanical families sampled, prioritizing the most abundant species and respecting the proportion of the number of species among families, however, some less abundant species were selected, in order to better evaluate the morphological variation of the samples. Therefore, although the selected species constitute only 48% of the total number of species sampled, their abundance represents about 80% of the total abundance of EFN-bearing plant species registered in all forest fragments. The complete list of all species and morphospecies registered in the 10 forest fragments is in Supplementary material 2.

We fixed the extrafloral nectaries in FAA solution (10 ml formaldehyde + 5 ml glacial acetic acid + 50 ml ethanol + 35 ml water) (Kraus and Arduim 1997). After a week in this solution, the extrafloral nectaries were washed with water for 2 h, and then dehydrated with an ethanol solution series (30%, 50%, 70% and 100%). For scanning electron microscopy studies, samples were critical point-dried using CO_2, sputter-coated with gold–palladium and examined using a JEOL JSM 5600LV scanning electron microscope (25 kV) in the Intituto de Ecología, A.C. (México).

For the morphological characterization of the extrafloral nectaries, we followed the nomenclature adapted by Díaz-Castelazo *et al*. (2005), from Elias (1983) and Koptur (1992a; b). In the present study, we classified the morphotypes of extrafloral nectaries into: (i) elevated – vascularized nectaries that have glandular tissues raised above ground tissue; (ii) flattened – vascularized nectaries whose glandular tissues are closely pressed against the fundamental tissue of other organs, such as leaf surfaces; (iii) formless – nonvascularized nectaries that do not present structural specialization, but may be colored in contrast to the background; (iv) pit – nonvascularized nectaries whose glandular tissues are sunken in tissues of others organs, in depressions as large as or larger than the nectary; (v) hollow – vascularized nectaries described as cavities in other plant tissues or organs with a narrow channel extending to the surface; and (vi) transformed – vascularized nectaries originated by the transformation of an organ, including abscission or abortion of an organ, and development of reproductive or vegetative meristems. Finally, we also measured the length and width of each extrafloral nectary analyzed from the scanning electron microscopy images.

Results

We morphologically characterized the extrafloral nectaries of 68 EFN-bearing plant species or morphospecies, distributed in 29 genera and 20 families (Table 1). We could not identify three EFN-bearing plant morphospecies at the genus level. Therefore, for these morphospecies, we left the identification at the family level (Chrysobalanaceae sp., Lecythidaceae sp., Olacaceae sp.) (Table 1). Of the 20 families sampled, Fabaceae was the most representative, with the highest number of EFN-bearing plant species (42.64% of the total plant species, n = 29 plant species), followed by Bignoniaceae

(13.24%, n = 9) and Malpighiaceae (5.88%, n = 4) (Figure 1). Each of the families Chrysobalanaceae, Passifloraceae, Sapindaceae and Euphorbiaceae were represented by three species (4.41%, n = 3) (Figure 1). Convolvulaceae was represented by two species (2.94%), and each of the other 12 remaining botanical families were represented by only one plant species (1.47%, n = 1) (Figure 1). Fabaceae was also the family that presented the highest number of genera (six genera, 20.70% of the total), followed by Euphorbiaceae and Malpighiaceae, both being represented by three genera (10.34% of the total). The other botanical families were represented only by two (6.90% of the total) or one genus (3.45% of the total).

The life form with the greatest representation was liana (52.94% of the total plant species, n = 36 plant species) followed by trees (42.65%, n = 29). Shrubs (2.94%, n = 2) and herbs (1.47%, n = 1) were the life forms with a smaller number of EFN-bearing plant species (Figure 2). In relation to morphotype, we found that elevated extrafloral nectaries were the most representative (57.35% of the total plant species, n = 39 plant species), followed by flattened (26.47%, n = 18), formless (7.35%, n = 5), pit (4.41%, n = 3), hollow (2.94%, n = 2) and transformed (1.47%, n = 1) (Figure 3).

Table 1. Taxonomic distribution of EFN-bearing plant species sampled in 10 forest fragments located in the state of Acre, Brazilian Amazon. Description of the location, type, length and width of the extrafloral nectaries observed.

Family	Plant species	Life form	Site (location)	Type of EFN	Length (mm)	Width (mm)
Bignoniaceae	*Fridericia* sp. 2	Liana	Pseudo-stipules	Flattened	0.231	0.208
	Fridericia sp. 3	Liana	Pseudo-stipules	Flattened	0.238	0.183
	Fridericia sp. 5	Liana	Pseudo-stipules	Flattened	0.211	0.153
	Fridericia sp. 8	Liana	Inter-petiolar region	Flattened	0.166	0.147
	Fridericia sp. 19	Liana	Pseudo-stipules	Flattened	0.719	0.565
	Fridericia sp. 31	Liana	Inter-petiolar region	Flattened	0.178	0.113
	Fridericia sp. 35	Liana	Inter-petiolar region	Flattened	0.152	0.084
	Memora sp. 2	Liana	Pseudo-stipules	Flattened	0.368	0.256
	Memora sp. 3	Liana	Pseudo-stipules and surface of leaflets	Flattened	0.131	0.089
Chrysobalanaceae	*Hirtella racemosa* Lam.	Tree	Young leaves	Flattened	0.467	0.447
	Hirtella sp.	Tree	Young leaves	Flattened	0.217	0.191
	Chrysobalanaceae sp.	Tree	Margin of young leaves	Elevated	0.949	0.439
Convolvulaceae	*Ipomoea philomega* (Vell.) House	Liana	Petiole	Formless	0.037	0.028
	Ipomoea regnellii Meisn.	Liana	Petiole	Formless	0.031	0.029
Costaceae	*Costus scaber* Ruiz & Pav.	Herb	Bracts	Hollow	3.348	0.538
Cucurbitaceae	*Gurania* sp.	Liana	Young leaves	Flattened	1.13	0.635
Euphorbiaceae	*Dalechampia* sp.	Liana	Young leaves	Elevated	2.455	1.188
	Omphalea diandra L.	Liana	Young leaves	Elevated	1.074	0.716
	Aparisthmium cordatum (A. Juss.) Baill.	Tree	Young leaves	Flattened	1.015	0.873
Fabaceae	*Senegalia* sp. 1	Liana	Petiole	Elevated	0.951	0.6

		Rachis of compound leaves		0.433	0.359
Senegalia sp. 2	Liana	Petiole	Elevated	3.251	2.117
		Rachis of compound leaves		0.918	0.583
Senegalia sp. 3	Liana	Petiole	Elevated	2.816	1.004
		Rachis of compound leaves		1.091	1.03
Senegalia sp. 4	Liana	Petiole	Elevated	3.661	0.873
		Rachis of compound leaves		1.282	0.589
Senegalia sp. 7	Liana	Petiole	Elevated	2.599	1.409
		Rachis of compound leaves		0.842	0.762
Senegalia sp. 8	Liana	Petiole	Elevated	1.418	0.585
		Rachis of compound leaves		0.468	0.28
Bauhinia sp. 1	Liana	Petiole	Elevated	4.518	1.219
		Rachis of compound leaves		2.28	1.19
Erythrina sp.	Tree	Rachis of compound leaves	Elevated	0.814	0.64
Inga acreana Harms	Tree	Rachis of compound leaves	Elevated	2.612	2.546
Inga alba (Sw.) Willd.	Tree	Rachis of compound leaves	Elevated	1.769	1.726
Inga calantha Ducke	Tree	Rachis of compound leaves	Elevated	0.817	0.739
Inga capitata Desv.	Tree	Rachis of compound leaves	Elevated	1.668	1.62
Inga chartacea Poepp.	Tree	Rachis of compound leaves	Elevated	1.458	1.203
Inga densiflora Benth.	Tree	Rachis of compound leaves	Elevated	2.715	2.451
Inga edulis Mart.	Tree	Rachis of compound leaves	Elevated	1.379	1.363
Inga heterophylla Willd.	Tree	Rachis of compound leaves	Elevated	0.458	0.425
Inga lateriflora Miq.	Tree	Rachis of compound leaves	Elevated	1.724	1.269
Inga laurina (Sw.) Willd.	Tree	Rachis of compound leaves	Elevated	1.227	1.066
Inga microcoma Harms	Tree	Rachis of compound leaves	Elevated	1.375	1.159
Inga punctata Willd.	Tree	Rachis of compound leaves	Pit	1.151	0.786

	Inga sertulifera DC.	Tree	Rachis of compound leaves	Elevated	1.185	1.075
	Inga suaveolens Ducke	Tree	Rachis of compound leaves	Elevated	1.825	1.729
	Inga tenuistipula Ducke	Tree	Rachis of compound leaves	Elevated	1.509	1.383
	Inga sp. 4	Tree	Rachis of compound leaves	Elevated	1.839	1.257
	Inga sp. 5	Tree	Rachis of compound leaves	Elevated	0.726	0.714
	Senna sp.	Shrub	Rachis of compound leaves	Elevated	1.549	0.41
	Zygia sp. 1	Tree	Petiole	Elevated	1.81	0.801
	Zygia sp. 2	Tree	Petiole	Elevated	1.402	0.464
	Zygia sp. 3	Tree	Petiole	Elevated	3.723	0.729
Lecythidaceae	Lecythidaceae sp.	Tree	Petiole	Hollow	0.865	0.606
Malpighiaceae	*Banisteriopsis* sp. 2	Liana	Young leaves	Flattened	1.211	0.683
	Banisteriopsis sp. 5	Liana	Young leaves	Flattened	0.77	0.457
	Heteropterys sp.	Liana	Young leaves	Flattened	0.555	0.387
	Tetrapterys sp.	Liana	Young leaves	Flattened	0.564	0.314
Malvaceae	*Byttneria benensis* Britton	Liana	Young leaves	Pit	3.313	1.405
Menispermaceae	*Abuta* sp. 1	Liana	Young leaves	Flattened	0.961	0.635
Ochinaceae	*Ouratea* sp.	Tree	Stipules	Pit	1.256	0.339
Olacaceae	Olacaceae sp.	Tree	Leaf axils	Elevated	1.185	0.922
Passifloraceae	*Dilkea* sp.	Liana	Bracts	Formless	0.905	0.606
	Passiflora coccinea Aubl.	Liana	Margin of young leaves	Elevated	1.05	1.03
	Passiflora sp. 3	Liana	Bracts	Formless	1.047	0.661
Rhamnaceae	*Gouania* sp.	Liana	Margin of young leaves	Elevated	0.457	0.277
Rubiaceae	*Palicourea* sp.	Shrub	Ovary	Transformed	0.862	0.875
Sapindaceae	*Paullinia* sp. 1	Liana	Margin of young leaves	Elevated	0.761	0.451
	Paullinia sp. 2	Liana	Margin of young leaves	Elevated	0.572	0.323
	Paullinia sp. 6	Liana	Margin of young leaves	Elevated	0.377	0.314

Solanaceae	*Solanum* sp.	Liana	Bracts	Formless	0.861	0.734
Vitaceae	*Cissus* sp.	Liana	Margin of young leaves	Elevated	0.919	0.819
Vochysiaceae	*Qualea grandiflora* Mart.	Tree	Stems	Elevated	1.3	1.12

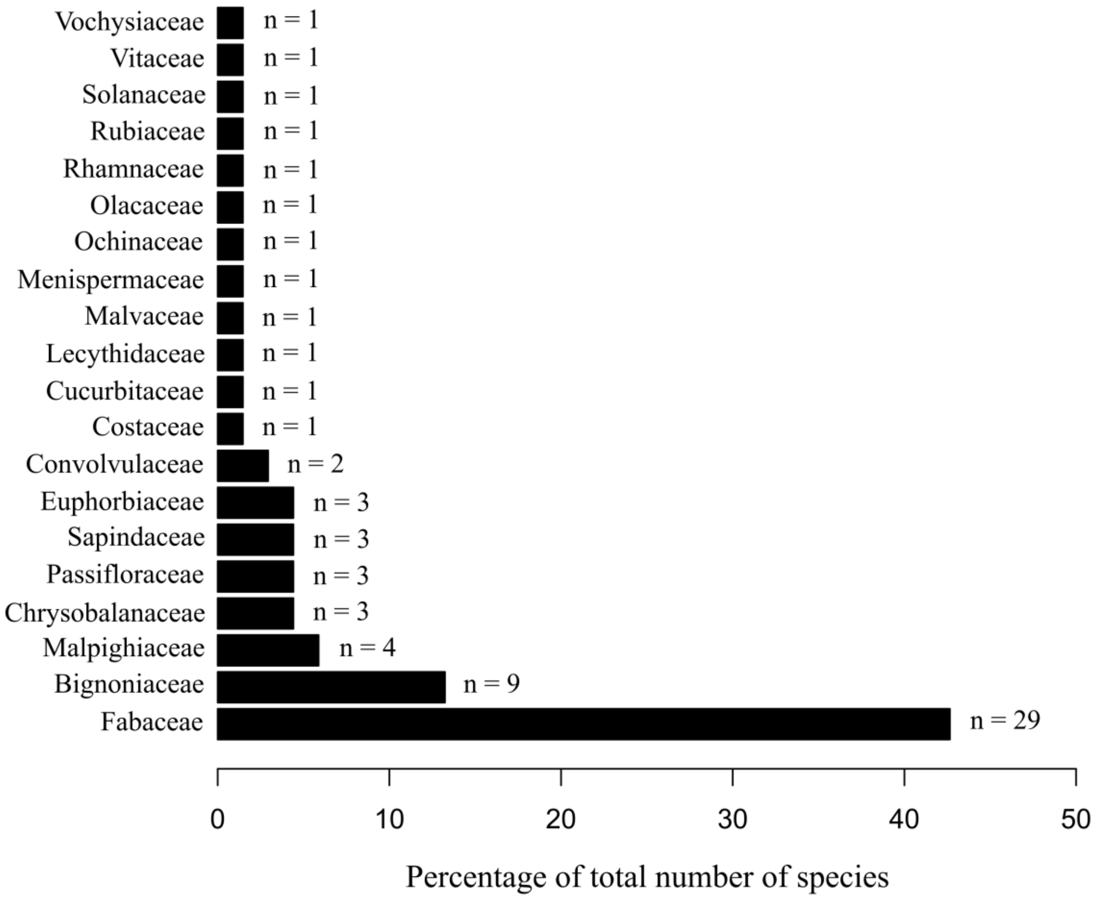

Figure 1. Taxonomical representation of EFN-bearing plant species sampled in 10 forest fragments located in the state of Acre, Brazilian Amazon.

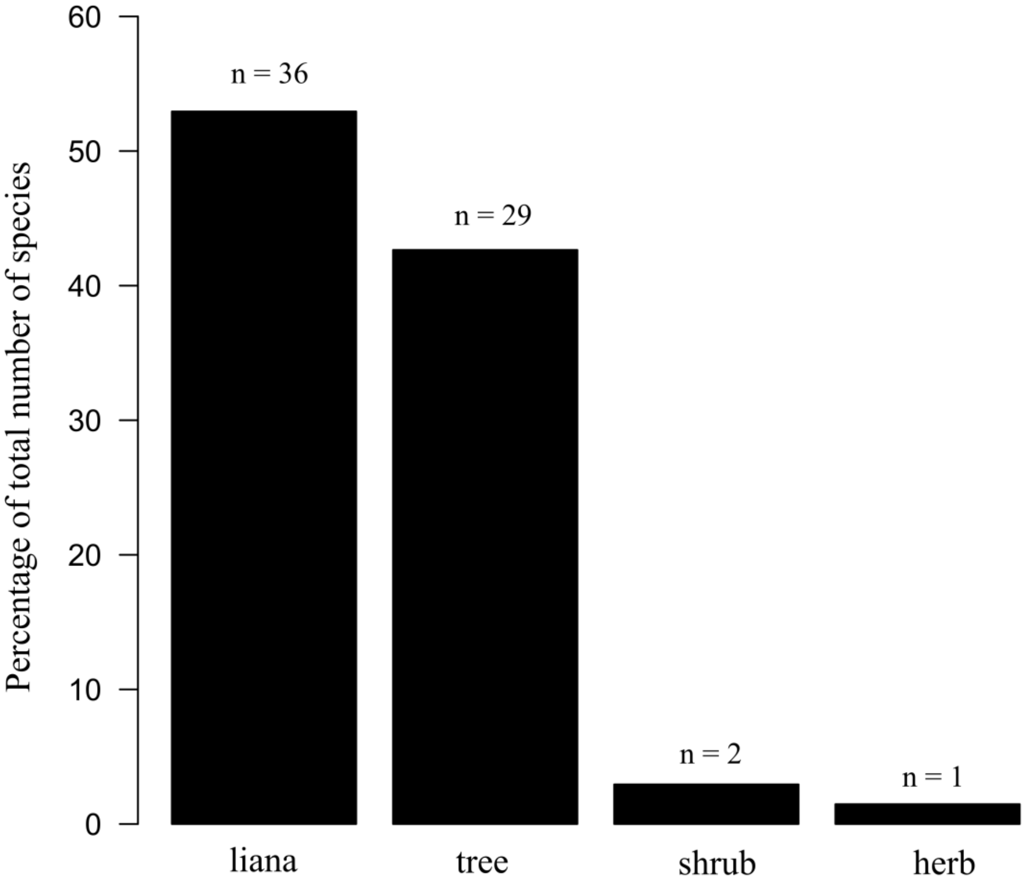

Figure 2. Life form of EFN-bearing plant species sampled in 10 forest fragments located in the state of Acre, Brazilian Amazon.

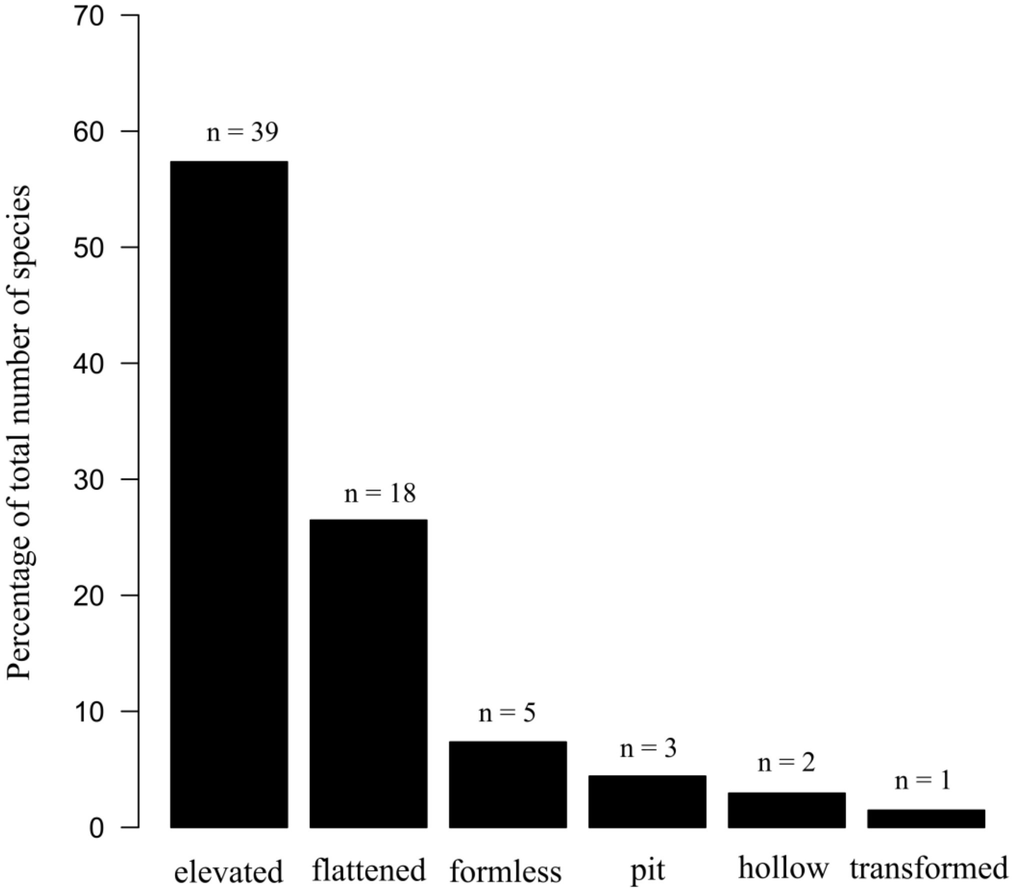

Figure 3. Morphotypes of extrafloral nectaries found in 10 forest fragments located in the state of Acre, Brazilian Amazon, according to Díaz-Castelazo et al. (2005): (i) elevated nectaries – nectaries that have glandular tissue raised above ground tissue; (ii) flattened nectaries – glandular tissue closely pressed against underlying tissue, common on leaf surfaces; (iii) formless nectaries – with no structure specialization, but may be colored in contrast to background; (iv) pit nectaries – glandular tissue sunken in the tissue of other organs, in depressions as large as or larger than the nectary; (v) hollow nectaries – cavities in other organs with a narrow channel extending to the surface; and (vi) transformed nectaries – nectaries originated by the transformation of an organ.

Extrafloral nectary morphology

Bignoniaceae

Within the Bignoniaceae family we evaluated nine EFN-bearing plant species representing two genera, *Fridericia* and *Memora*. All species of this family evaluated are lianas and present the same morphotype of extrafloral nectaries (flattened). In some species, as in *Fridericia* sp. 31, extrafloral nectaries are located on the inter-petiolar regions, mainly of young branches (Figure 4, A1 and A2), and in other species, as in *Fridericia* sp. 2, the extrafloral nectaries are located on the pseudo-stipules, which are more evident in the inter-petiolar regions of young branches (Figure 4, B1 and B2). *Memora* sp. 3, besides having the extrafloral nectaries located on the pseudo-stipules, also presents these glandular structures on the surface of leaflets (Supplementary material 3, G). In general, these flattened extrafloral nectaries were small, with an average length of 0.27 ± 0.18 mm and width of 0.20 ± 0.15 mm (n = 9) (mean ± SD). *Fridericia* sp. 19 (Supplementary material 3, C) was the plant species that presented the largest extrafloral nectary (0.72 mm in length and 0.57 in width) (Table 1).

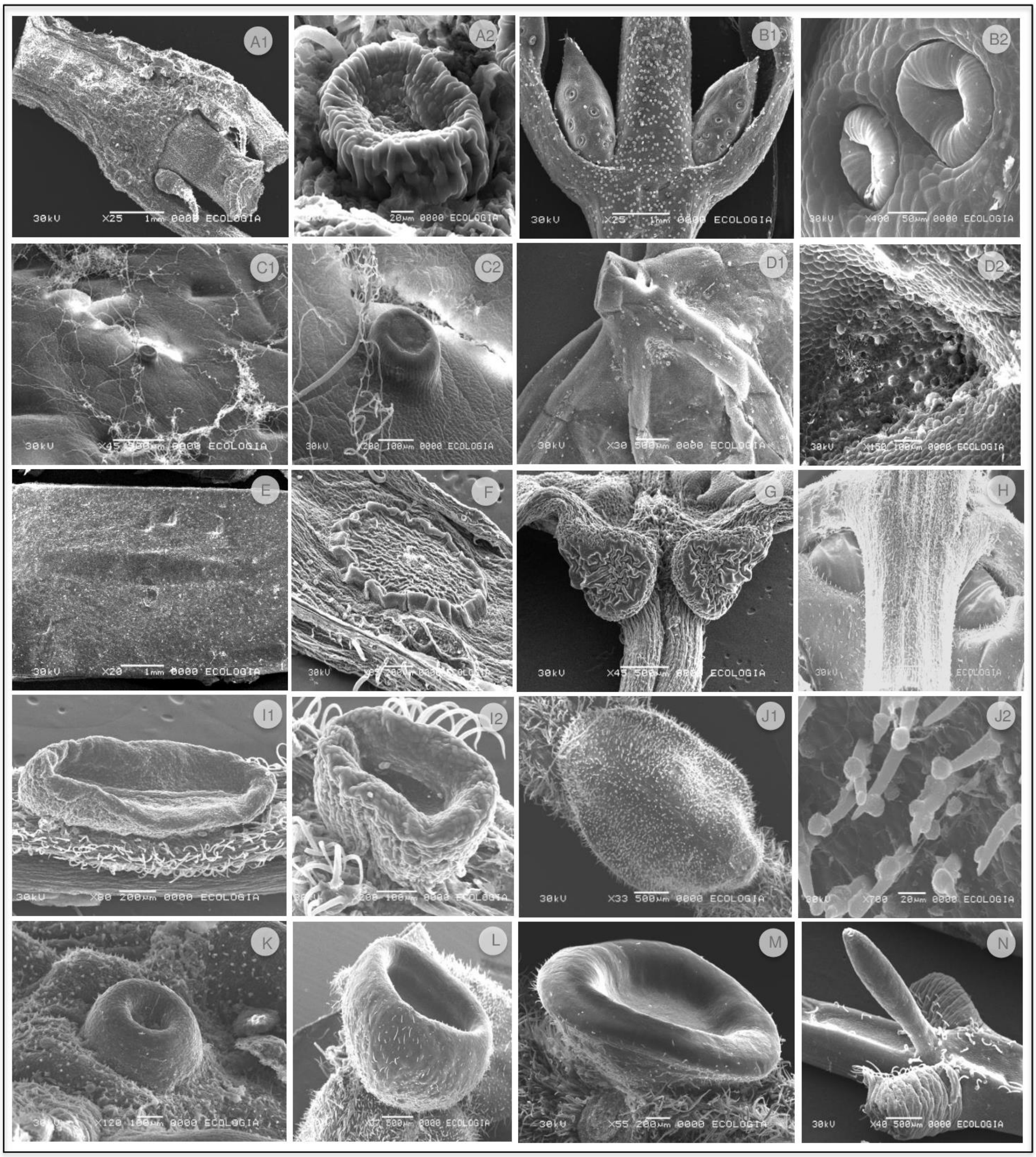

Figure 4. Flattened extrafloral nectaries in *Fridericia* sp. 31 (A1 and A2); flattened extrafloral nectaries in *Fridericia* sp. 2 (B1 and B2); flattened extrafloral nectary in *Hirtella* sp. (C1 and C2); formless extrafloral nectary in *Ipomoea regnellii* (D1 and D2); hollow extrafloral nectary in *Costus scaber* (E); flattened extrafloral nectary in *Gurania* sp. (F); elevated extrafloral nectaries in *Omphalea diandra* (G); flattened extrafloral nectaries in *Aparisthmium cordatum* (H); elevated extrafloral nectary in *Senegalia* sp. 8 located on the

petiole (I1) and on the rachis of compound leaves between a pair of petiolules (I2); elevated extrafloral nectary with glandular trichomes (peltate trichomes) in *Senegalia* sp. 2 (J1 and J2); elevated extrafloral nectary in *Inga heterophylla* (K); elevated extrafloral nectary in *Inga acreana* (L); elevated extrafloral nectary in *Inga densiflora* (M); elevated extrafloral nectary (stipitate nectaries) in *Senna* sp. (N).

Chrysobalanaceae

The Chrysobalanaceae family was represented by three EFN-bearing plant species, two trees species belonging to the genus *Hirtella*, and a third tree species that we could not identify at the genus level (Chrysobalanaceae sp.). The two species evaluated of the genus *Hirtella*, *Hirtella* sp. (Figure 4, C1 and C2) and *Hirtella racemosa* Lam. (Supplementary material 3, H), have flattened extrafloral nectaries situated on the adaxial side of young leaves, more specifically at the apex and base of young leaves. Differently, the species Chrysobalanaceae sp. presents flattened extrafloral nectaries situated on the margin of young leaves (Supplementary material 3, I). These flattened extrafloral nectaries presented an average length of 0.55 ± 0.30 mm and width of 0.36 ± 0.12 mm (n = 3) (mean ± SD).

Convolvulaceae

The two EFN-bearing plant species belonging to the Convolvulaceae family were *Ipomoea philomega* (Vell.) House (Supplementary material 3, J) and *Ipomoea regnellii* Meisn. (Figure 4, D1 and D2). Both species are lianas and have formless extrafloral nectaries, with two secretory fields of these formless glands located on opposite sides of the petiole, near its junction with the leaf blade (Figure 4, D1 and D2). In these secretory fields there are small holes with an average length of 0.034 ± 0.004 mm and width of 0.028 ± 0.001 mm (n = 2) (mean ± SD), where extrafloral nectar is probably released.

Costaceae

The Costaceae family was represented by only one EFN-bearing plant species (*Costus scaber* Ruiz & Pav.). *C. scaber* was the only herb species sampled by us and presents hollow extrafloral nectaries located on the central part of the bracts that cover the flowers (Figure 4, E). The measured hollow gland presented a length of 3.35 mm and width of 0.54 mm (Table 1).

Cucurbitaceae

The only species belonging to the Cucurbitaceae family was a liana of the genus *Gurania* (*Gurania* sp.), that presents flattened extrafloral nectaries concentrated on the basal region of the leaves (Figure 4, F). The flattened extrafloral nectary measured by us presented a length of 1.13 mm and width of 0.64 mm (Table 1).

Euphorbiaceae

Within the Euphorbiaceae family we evaluated three EFN-bearing plant species representing three genera, two liana species, *Dalechampia* sp. (Supplementary material 3, K) and *Omphalea diandra* L. (Figure 4, G), and a tree species, *Aparisthmium cordatum* A. Juss. (Figure 4, H). The species *Dalechampia* sp. and *O. diandra* have one pair of elevated extrafloral nectaries situated at the base of the leaf blade, near its junction with the petiole (Supplementary material 3, K and Figure 4, G). These elevated extrafloral nectaries presented a medium size, with an average length of 1.76 ± 0.98 mm and width of 0.95 ± 0.33 mm (n = 2) (mean ± SD). The species *A. cordatum* presents flattened extrafloral nectaries at the base of the leaf blades, more specifically on the axils of the main and secondary veins (Figure 4, H). The measured flattened gland presented a length of 1.02 mm and width of 0.87 mm (Table 1).

Fabaceae

Fabaceae was the most representative family, with the highest number of EFN-bearing plant species (20 plant species) and genera (six genera). The genera belonging to the Fabaceae family were *Senegalia*, *Bauhinia*, *Erythrina*, *Inga*, *Senna* and *Zygia*. The genus *Inga* was the most representative, with the highest number of species (17 species), followed by *Senegalia* (six species). All species belonging to the genus *Senegalia* and also the only species analyzed of the genus *Bauhinia* (*Bauhinia* sp. 1) are lianas and have elevated extrafloral nectaries located on the petiole and on the rachis of compound leaves between pairs of petiolules, as in *Senegalia* sp. 8 (Figure 4, I1 and I2). In all species of *Senegalia* and in *Bauhinia* sp. 1, extrafloral nectaries located on the petiole (Figure 4, I1) were larger than extrafloral nectaries located on the rachis of compound leaves between pairs of petiolules (Figure 4, I2). The first ones presented an average length of 2.75 ± 1.24 mm and width of 0.54 ± 1.12 mm (n = 7) (mean ± SD), and the second ones presented an average length of 0.63 ± 1.05 mm and width of 0.33 ± 0.69 mm (n = 7). Among the species of the genus *Senegalia*, *Senegalia* sp. 2 is the only one that presents glandular trichomes on the extrafloral nectaries (Figure 4, J1 and J2). The only species of the genus *Erythrina* (*Erythrina* sp.) is a tree species and presents pairs of elevated extrafloral nectaries situated on the rachis of compound leaves, near the petiolule (Supplementary material 3, Q). The measured elevated gland presented a length of 0.81 mm and width of 0.64 mm (Table 1). All species of the genus *Inga* sampled by us are trees, and almost all species have elevated extrafloral nectaries located on the rachis of compound leaves, more specifically between opposite leaflets, as in *Inga heterophylla* Willd. (Figure 4, K), *I. acreana* Harms (Figure 4, L) and *I. densiflora* Benth. (Figure 4, M). In general, these extrafloral nectaries presented a medium size, with an average length of 1.75 ± 0.60 mm and width of 1.35 ± 0.57 mm (n = 16) (mean ± SD). Only *I. punctata* Willd. has pit extrafloral nectaries located on the rachis of compound leaves between opposite leaflets (Supplementary material 4, F). The pit extrafloral nectaries of this species measured by us presented a length of 1.15 mm and width of 0.77 mm (Table 1). The shrub species *Senna* sp. was the only one that present elevated extrafloral nectaries of the morphotype “stipitate” located on the rachis of compound leaves between opposite leaflets (Figure 4, N). The three species of the genus *Zygia* evaluated are trees and have elevated extrafloral nectaries located on the petiole (Supplementary material 4, L, M and N). These elevated extrafloral nectaries were relatively large, with an average length of 2.31 ± 1.24 mm and width of 0.67 ± 0.18 mm (n = 3) (mean ± SD).

Lecythidaceae

We could not identify at genus level the only EFN-bearing plant species belonging to the Lecythidaceae family. Lecythidaceae sp. has hollow extrafloral nectaries located on the leaf petiole (Figure 5, A), and the gland measured by us presented a length of 0.87 mm and width of 0.61 mm (Table 1).

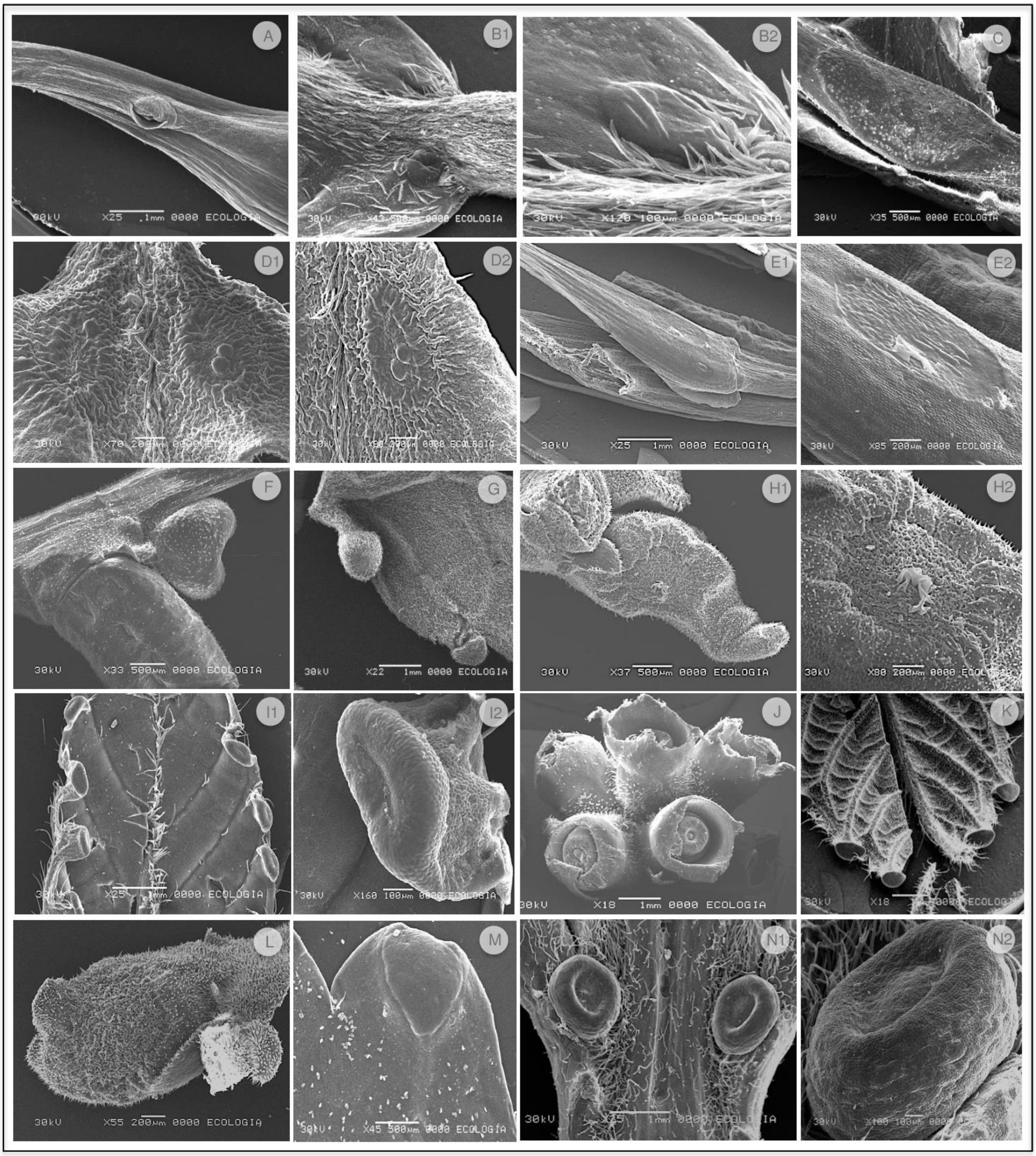

Figure 5. Hollow extrafloral nectary in Lecythidaceae sp. (A); flattened extrafloral nectaries in *Heteropterys* sp. (B1 and B2); pit extrafloral nectary in *Byttneria benensis* (C); flattened extrafloral nectaries in *Abuta* sp. 1 (D1 and D2); pit extrafloral nectary in *Ouratea* sp. (E1 and E2); elevated extrafloral nectary in Olacaceae sp. (F); elevated extrafloral nectaries in *Passiflora coccinea* (G); formless extrafloral nectary in *Dilkea* sp. (H1 and H2); elevated extrafloral nectaries in *Gouania* sp. (I1 and I2); transformed extrafloral nectaries in

Palicourea sp. (J); elevated extrafloral nectaries in *Paullinia* sp. 1 (K); formless extrafloral nectary in *Solanum* sp. (L); elevated extrafloral nectary in *Cissus* sp. (M); elevated extrafloral nectaries in *Qualea grandiflora* (N1 and N2).

Malpighiaceae

The Malpighiaceae family was represented by four liana species belonging to the genera *Banisteriopsis*, *Heteropterys* and *Tetrapterys*. All species, as in *Heteropterys* sp., have one pair of flattened extrafloral nectaries located on the abaxial leaf surface, more specifically on the leaf base, which are separated from each other by the primary vein (Figure 5, B1 and B2). In general, these glands presented a medium size, with an average length of 0.78 ± 0.31 mm and width of 0.46 ± 0.16 mm (n = 4) (mean ± SD).

Malvaceae

The only EFN-bearing plant species belonging to the Malvaceae family was *Byttneria benensis*. This species is a liana and has a pit extrafloral nectary situated on the base of the mid rib (Figure 5, C), that measured 3.31 mm in length and 1.41 mm in width (Table 1).

Menispermaceae

The Menispermaceae family was represented by the liana species *Abuta* sp. 1. Similar to the lianas of the Malpighiaceae family, *Abuta* sp. 1 presents one pair of flattened extrafloral nectaries located on the abaxial leaf surface, more specifically on the leaf base, which are separated from each other by the primary vein (Figure 5, D1 and D2). The measured flattened gland presented a length of 0.96 mm and width of 0.64 mm (Table 1).

Ochinaceae

The Ochinaceae family was represented by only one EFN-bearing plant species (*Ouratea* sp.). This is a tree species that has pit extrafloral nectaries situated on the base of stipules (Figure 5, E1 and E2). The pit extrafloral nectary measured by us presented a length of 1.27 mm and width of 0.34 mm (Table 1).

Olacaceae

We could not identify at genus level the only EFN-bearing plant species belonging to the Olacaceae family. Olacaceae sp. is a tree species that has elevated extrafloral nectaries located on the leaf axils (Figure 5, F). The measured elevated gland presented a length of 1.19 mm and width of 0.92 mm (Table 1).

Passifloraceae

The Passifloraceae family was represented by three liana species, distributed in two genera, *Dilkea* and *Passiflora*. We observed elevated extrafloral nectaries on the margin of young leaves of *Passiflora coccinea* Aubl. (Figure 5, G), and the measured gland presented a length of 1.05 mm and width of 1.03 mm (Table 1). We also observed formless extrafloral nectaries on the bracts of *Dilkea* sp. (Figure 5, H1 and H2) and *Passiflora* sp. 3 (Supplementary material 4, R), which presented an average length of 0.98 ± 0.1 mm and width of 0.63 ± 0.04 mm (n = 2) (mean ± SD). For both plant species with formless extrafloral nectaries, the secretory region is yellow, differing from the green color of the bracts.

Rhamnaceae

The only EFN-bearing plant species belonging to the Rhamnaceae family was *Gouania* sp. which is a liana that has elevated extrafloral nectaries situated on the margin of young leaves (Figure 5, I1 and I2). The elevated extrafloral nectary of *Gouania* sp. measured by us presented a length of 0.46 mm and width of 0.28 mm (Table 1).

Rubiaceae

The Rubiaceae family was represented by *Palicourea* sp., a shrub species that has transformed extrafloral nectaries on the ovary (Figure 5, J). The measured ovary presented a length of 0.86 mm and width of 0.88 mm (Table 1).

Sapindaceae

The Sapindaceae family was represented by three liana species belonging to the genus *Paullinia*. All species, as in *Paullinia* sp. 1, have elevated extrafloral nectaries situated on the margin of young leaves (Figure 5, K). In general, these glands were relative small, with an average length of 0.57 ± 0.19 mm and width of 0.36 ± 0.08 mm (n = 3) (mean ± SD).

Solanaceae

The Solanaceae family was represented only by a liana species belonging to the genus *Solanum*. We observed formless extrafloral nectaries on the bracts of *Solanum* sp. (Figure 5, L). The formless gland measured by us presented a length of 0.86 mm and width of 0.73 mm (Table 1). Just like in the bracts of *Dilkea* sp. and *Passiflora* sp. 1 (both species belonging to the Passifloraceae family), the secretory region located on the bracts of *Solanum* sp. is yellow, differing from the green color of the bract.

Vitaceae

The only species belonging to the Vitaceae family was a liana of the genus *Cissus* (*Cissus* sp.), that presents elevated extrafloral nectaries on the margin of young leaves (Figure 5, M). The measured elevated gland presented a length of 0.92 mm and width of 0.82 mm (Table 1).

Vochysiaceae

Finally, the tree species *Qualea grandiflora* Mart. was the only EFN-bearing plant species belonging to the Vochysiaceae family that we evaluated. This species presents elevated extrafloral nectaries along its stems, next to the insertion of leaves (Figure 5, N1 and N2). The measured elevated gland presented a length of 1.30 mm and width of 1.12 mm.

Discussion

In this study, we observed that Fabaceae was the family with greatest representation, accounting for 42.64% of the EFN-bearing plant species total analyzed, followed by Bignoniaceae (13.24%) and Malpighiaceae (5.88%). A greater abundance and diversity of EFN-bearing plant species belonging to the Fabaceae family was also detected in other studies. Machado *et al*. (2008), in a study carried out in the Brazilian Cerrado, found a greater representation of the Fabaceae family, equivalent to 26.3% of the total EFN-bearing plant species recorded. However, only studies carried out in tropical forest have found as high a representation of this family as in our study, equivalent to more than 40% of the total number of species (Morellato and Oliveira 1991; Díaz-Castelazo *et al*. 2005; Aguirre *et al*. 2013). The dominance observed for Fabaceae is possibly related to the evolution of reproductive structures of the species belonging to the former subfamilies Mimosoideae and Caesalpinioideae (Polhill *et al*. 1981). Most of the EFN-bearing plant species of the Fabaceae family are distributed in these two subfamilies (Elias 1983; Judd *et al*. 1999; Melo *et al*. 2010) which present flowers whose reproductive organs are highly exposed and susceptible to environmental aggression (Polhill *et al*. 1981). In this context, the defense system provided by the association with ants (Rico-Gray and Oliveira 2007) may have favored the wide distribution of extrafloral nectaries in Fabaceae, considering that according to Polhill *et al*. (1981), the co-evolution involving plants and animals as a function of defense possibly led to the appearance of these glands. Just like ours, other studies carried out in tropical regions of the American continent also observed a relatively high representation of the Bignoniaceae and Malpighiaceae families (Díaz-Castelazo *et al*. 2005; Machado *et al*. 2008; Aguirre *et al*. 2013; Dáttilo and Dyer 2014), indicating a probable pattern for the tropical region of this continent.

In our study, lianas were the life form with the greatest number of EFN-bearing plant species, followed by trees, shrubs and herbs. Schupp and Feener (1991), sampling EFN-bearing plants in Barro Colorado Island (Panama), also observed a larger number of liana species in their samples. In contrast, Aguirre *et al*. (2013), sampling EFN-bearing plants in the tropical rain forest at Los Tuxtlas Biological Field Station (Mexico), found a greater number of tree species with extrafloral nectaries, when compared to other life forms. The different patterns observed by us and by Aguirre *et al*. (2013) are possibly related to structural differences of the forests studied (e.g. tree and shrub density, canopy cover and litter depth) and to differences in floristic composition. In addition, considering that most lianas are light-loving and tend to positively respond to forest disturbances (Putz 1984; Laurance *et al*. 2001), the greater representation of lianas found in our study could also be related to the conservation status of the forest fragments studied, since they have all already undergone anthropic actions, mainly the removal of wood.

Based on scanning electron microscopy images, we observed that elevated extrafloral nectaries were the most representative, followed by flattened extrafloral nectaries. The highest number of plant species with elevated extrafloral nectaries is directly associated to the dominance of the Fabaceae family, since almost all species of this family presented this morphotype of extrafloral nectary. Machado *et al*. (2008) and Aguirre *et al*. 2013 also found a higher dominance of elevated extrafloral nectaries as a result of the dominance of this family in their samples. In our study, elevated extrafloral nectaries occurred mostly on the leaf rachis, which is the main location of these glands in EFN-bearing plant species of the Fabaceae family (Pascal *et al*. 2000; Marazzi *et al*. 2013). Concerning significant morphological specificities within the elevated morphotype, two species stood out. The first was *Senna* sp. (Fabaceae) that has elevated extrafloral nectaries classified as stipitate, which present the secretory portion of the organ subtended by a non-secretory stalk (Marazzi *et al*. 2013). The second was *Senegalia* sp. 2 (Fabaceae) that has glandular trichomes classified as peltate trichomes on the elevated extrafloral nectaries, which consist of an elongated stalk and a spherical secretory head (Díaz-Castelazo *et al*. 2005). It is important to emphasize that elevated extrafloral nectaries, because they are relatively larger and secrete a larger nectar volume than smaller and nonvascularized glands (Díaz-Castelazo *et al*. 2005), are more frequently visited by ants (Blüthgen *et al*. 2000). Therefore, the large number of EFN-bearing plant species with the elevated morphotype in our study indicates that the ant defense system is an important strategy against herbivory in the Brazilian Amazonian rainforest.

The flattened extrafloral nectary was the morphotype with the second highest number of EFN-bearing plant species, and this result can also be attributed to the relatively great representation of the Bignoniaceae and Malpighiaceae families in our samples, considering that all species sampled which belong to these families presented flattened extrafloral nectaries. Concerning gland location on the plant body, in Malpighiaceae and also in the less representative family Chrysobalanaceae, flattened extrafloral nectaries occurred on the leaf blade, according to the usual pattern observed for the respective gland morphotype (Koptur

1992a). However, for the Bignoniaceae family, these extrafloral nectaries occurred on the pseudo-stipules and in the inter-petiolar region, which is a typical region of secretory gland occurrence in the family (Ribeiro *et al*. 1999). In general, flattened extrafloral nectaries are smaller than elevated extrafloral nectaries, and consequently tend to produce a smaller nectar volume (Díaz-Castelazo *et al*. 2005), which represents a disadvantage in terms of attractive potential. However, flattened extrafloral nectaries usually occur in a large number in the same region (e.g. leaf blade or pseudo-stipules), unlike elevated extrafloral nectaries that usually occur in isolation or in a small number in the same region (Koptur 1992a). According to Elias and Gelband (1976), the occurrence of many extrafloral nectaries in the same region represents an excellent advantage, since damage to one or more glands does not eliminate the attractive potential total of the region.

Formless, pit, hollow and transformed were the morphotypes of extrafloral nectaries with a smaller number of EFN-bearing plant species. Similar results were observed in other localities for formless and pit extrafloral nectaries, such as in a mesophyll vine forest in Australia (Blüthgen and Reifenrath 2003), in a Brazilian Cerrado region (Machado *et al*. 2008) and in a tropical rainforest in Mexico (Aguirre *et al*. 2013). Unlike us, Aguirre *et al*. (2013) sampled a greater number of EFN-bearing plant species with hollow extrafloral nectaries than with flattened ones. This difference is related to the high number of liana species with flattened extrafloral nectaries in our samples, mainly of the Bignoniaceae and Malpighiaceae family. We sampled only two plant species with hollow extrafloral nectaries, one of them being *Costus scaber* (Costaceae), also recorded by Aguirre *et al*. (2013), and recently documented with more morphological and anatomical detail by Rodríguez-Morales *et al*. (2016). Finally, we observed an interesting situation for the only species evaluated with transformed extrafloral nectaries, *Palicourea* sp. (Rubiaceae). In some species of the genus *Palicourea*, after pollination, the corolla falls but the ovary remains with active cells producing nectar, which in turn attracts ants that defend the plants against herbivores (Del-Claro *et al*. 2013). Based on the exposure, we morphologically categorized the nectaries of *Palicourea* sp., that are located on the ovary, as “transformed” extrafloral nectaries, which by definition are nectaries originated by the transformation of an organ (Díaz-Castelazo *et al*. 2005).

Conclusions

In summary, we collected a greater number of EFN-bearing plant species belonging to the Fabaceae family, followed by the Bignoniaceae and Malpighiaceae families, which, in general, corresponds to the results of studies carried out in other localities. Liana was the life form with the highest number of EFN-bearing plant species, followed by trees, shrubs and herbs. We believe that the pattern found is related to the conservation status of the forest fragments studied, since all forest fragments present a significant disturbance level, due mainly to removal of wood, this being a condition that favors liana growth. We also observed that elevated extrafloral nectaries were the most representative morphotype, followed by flattened, formless, pit, hollow and transformed extrafloral nectaries. The dominance of the elevated extrafloral nectaries is directly associated with the dominance of the Fabaceae family, considering that almost all EFN-bearing plant species of this family presented this gland morphotype. Similarly, the relatively greater representation of flattened extrafloral nectaries is also associated with the high number of liana species belonging to the Bignoniaceae and Malpighiaceae families which presented, without exception, this gland morphotype. It is also important to emphasize that elevated and flattened extrafloral nectaries have advantages in relation to the attraction potential of ants, the first ones because they secrete a larger nectar volume due to their larger sizes (Díaz-Castelazo *et al*. 2005), and the latter because they occurred, at least in most of the species sampled, in a greater number in a same region, which in turn ensures the attraction potential of the region even if one or more glands are damaged (Elias and Gelband 1976). Therefore, the high frequency of these two advantageous morphotypes of extrafloral nectaries in our samples evidences the importance of the ant defense system against herbivory in these Brazilian Amazonian rainforests (Elias and Gelband 1976; Blüthgen *et al*. 2000). Finally, our results point to the existence of a high diversity of EFN-bearing plant species in the Brazilian Amazon, which in turn constitutes a large study area to be considered in future research.

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Supplementary material

Supplementary material 1. Location of the 10 forest fragments sampled in the state of Acre, Brazilian Amazon.

Fragment	Site	Geographic coordinates	Area (ha)
1	Senador Guiomard private fragment	10° 3' 59.16" S 67° 59' 20.12" W	5.26
2	Senador Guiomard private fragment	10° 4' 52.34" S 67° 36' 2.64" W	27.82
3	Senador Guiomard private fragment	10° 6' 58.02" S 67° 41' 6.90" W	123.16
4	Forestry School	9° 59' 48.12" S 67° 59' 20.12" W	332.15
5	Projeto de Assentamento Walter Arce	9° 48' 0.46" S 67° 51' 26.95" W	681.05
6	Porto Acre private fragment	9° 36' 28.60" S 67° 34' 6.15" W	1072.34
7	Catuaba Experimental Farm	10° 4' 48.9" S 67° 37' 8.6" W	1282.42
8	Embrapa Acre	10° 2' 17.64" S 67° 40' 54.24" W	1871.17
9	Senador Guiomard private fragment	10° 1' 24.66" S 67° 35' 48.66" W	2894.77
10	Humaitá Reserve	9° 45' 15.2" S 67° 39' 44.9" W	3042.02

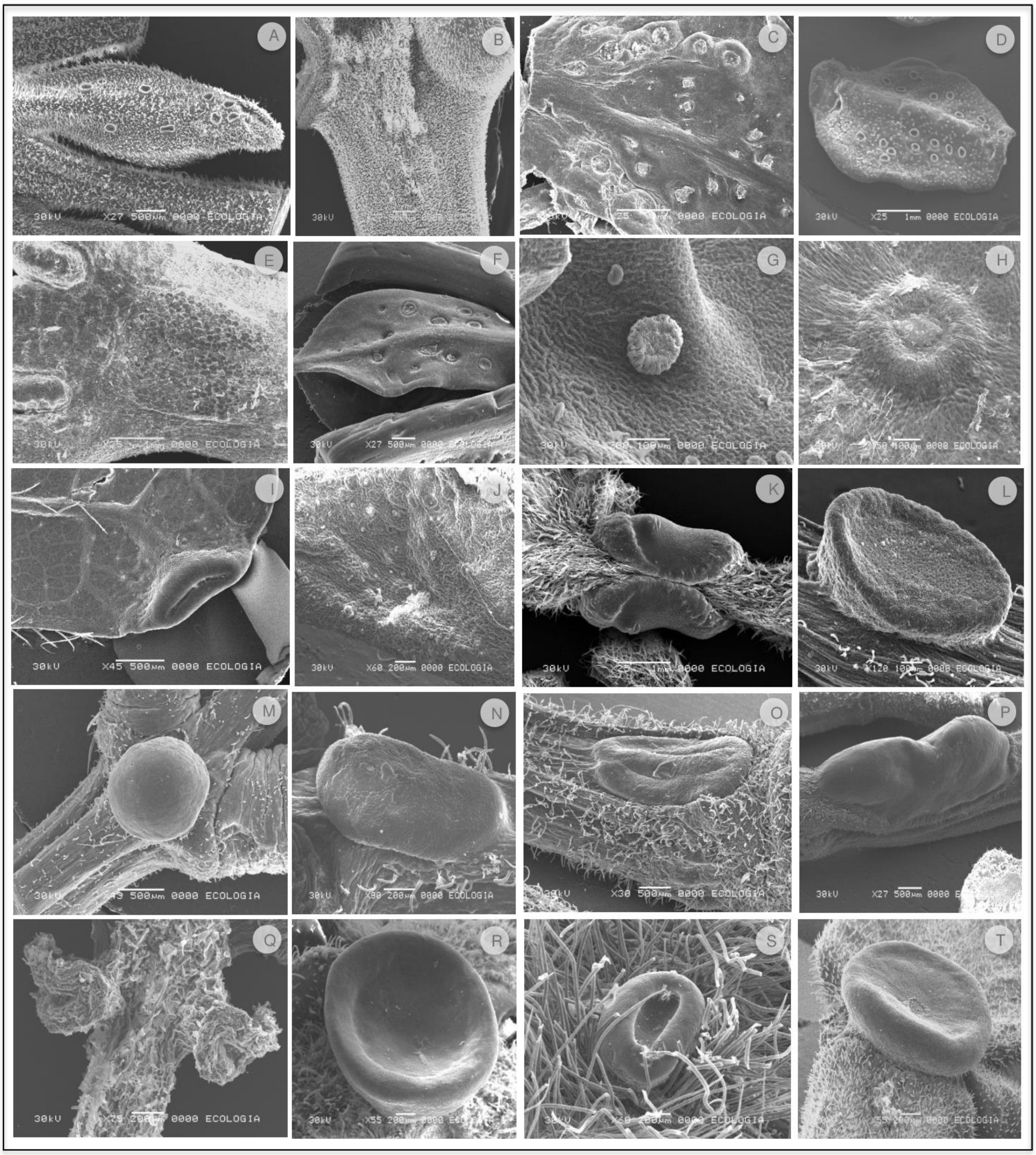
Supplementary material 2. Total abundance of EFN-bearing plant species and morphospecies registered in 10 forest fragments located in the state of Acre, Brazilian Amazon, between June 2016 and February 2017.

Family	Plant species	Abundance	Morphological description
Anonaceae	Anonaceae sp1	1	
Araceae	*Philodendron* sp.1	1	
Bignoniaceae	*Fridericia* sp. 1	1	
	Fridericia sp. 2	20	Used in this study
	Fridericia sp. 3	7	Used in this study
	Fridericia sp. 4	2	
	Fridericia sp. 5	10	Used in this study
	Fridericia sp. 6	5	
	Fridericia sp. 7	4	
	Fridericia sp. 8	30	Used in this study
	Fridericia sp. 9	4	
	Fridericia sp. 10	1	
	Fridericia sp. 11	3	
	Fridericia sp. 12	3	
	Fridericia sp. 13	6	
	Fridericia sp. 14	2	
	Fridericia sp. 15	2	
	Fridericia sp. 16	4	
	Fridericia sp. 17	2	
	Fridericia sp. 18	1	
	Fridericia sp. 19	8	Used in this study
	Fridericia sp. 20	2	
	Fridericia sp. 21	3	
	Fridericia sp. 22	1	
	Fridericia sp. 23	1	
	Fridericia sp. 24	2	
	Fridericia sp. 25	1	
	Fridericia sp. 26	4	
	Fridericia sp. 27	1	
	Fridericia sp. 28	1	
	Fridericia sp. 29	1	
	Fridericia sp. 30	1	
	Fridericia sp. 31	3	Used in this study
	Fridericia sp. 32	3	

	Fridericia sp. 33	1	
	Fridericia sp. 34	1	
	Fridericia sp. 35	6	Used in this study
	Memora sp. 1	1	
	Memora sp. 2	6	Used in this study
	Memora sp. 3	1	Used in this study
Chrysobalanaceae	*Hirtella racemosa* Lam.	16	Used in this study
	Hirtella sp.	2	Used in this study
	Chrysobalanaceae sp.	1	Used in this study
Combretaceae	*Buchenavia* sp.1	1	
Convolvulaceae	*Ipomoea philomega* (Vell.) House	1	Used in this study
	Ipomoea regnellii Meisn.	1	Used in this study
Costaceae	*Costus scaber* Ruiz & Pav.	6	Used in this study
Cucurbitaceae	Cucurbitaceae sp.	1	
	Gurania sp.	2	Used in this study
Euphorbiaceae	*Acalypha* sp.	4	
	Aparisthmium cordatum (A. Juss.) Baill.	10	Used in this study
	Dalechampia sp.	1	Used in this study
	Omphalea diandra L.	6	Used in this study
Fabaceae	*Senegalia* sp. 1	16	Used in this study
	Senegalia sp. 2	41	Used in this study
	Senegalia sp. 3	21	Used in this study
	Senegalia sp. 4	16	Used in this study
	Senegalia sp. 5	1	
	Senegalia sp. 6	1	
	Senegalia sp. 7	7	Used in this study
	Senegalia sp. 8	15	Used in this study
	Senegalia sp. 9	1	
	Bauhinia sp. 1	99	Used in this study
	Bauhinia sp. 2	1	
	Bauhinia sp. 3	1	
	Bauhinia sp. 4	3	
	Bauhinia sp. 5	1	
	Bauhinia sp. 6	2	
	Centrosema sp. 1	3	
	Centrosema sp. 2	2	
	Centrosema sp. 3	2	
	Erythrina sp.	3	Used in this study
	Fabaceae sp.	4	
	Inga acreana Harms	2	Used in this study
	Inga alba (Sw.) Willd.	6	Used in this study
	Inga calantha Ducke	2	Used in this study
	Inga capitata Desv.	4	Used in this study

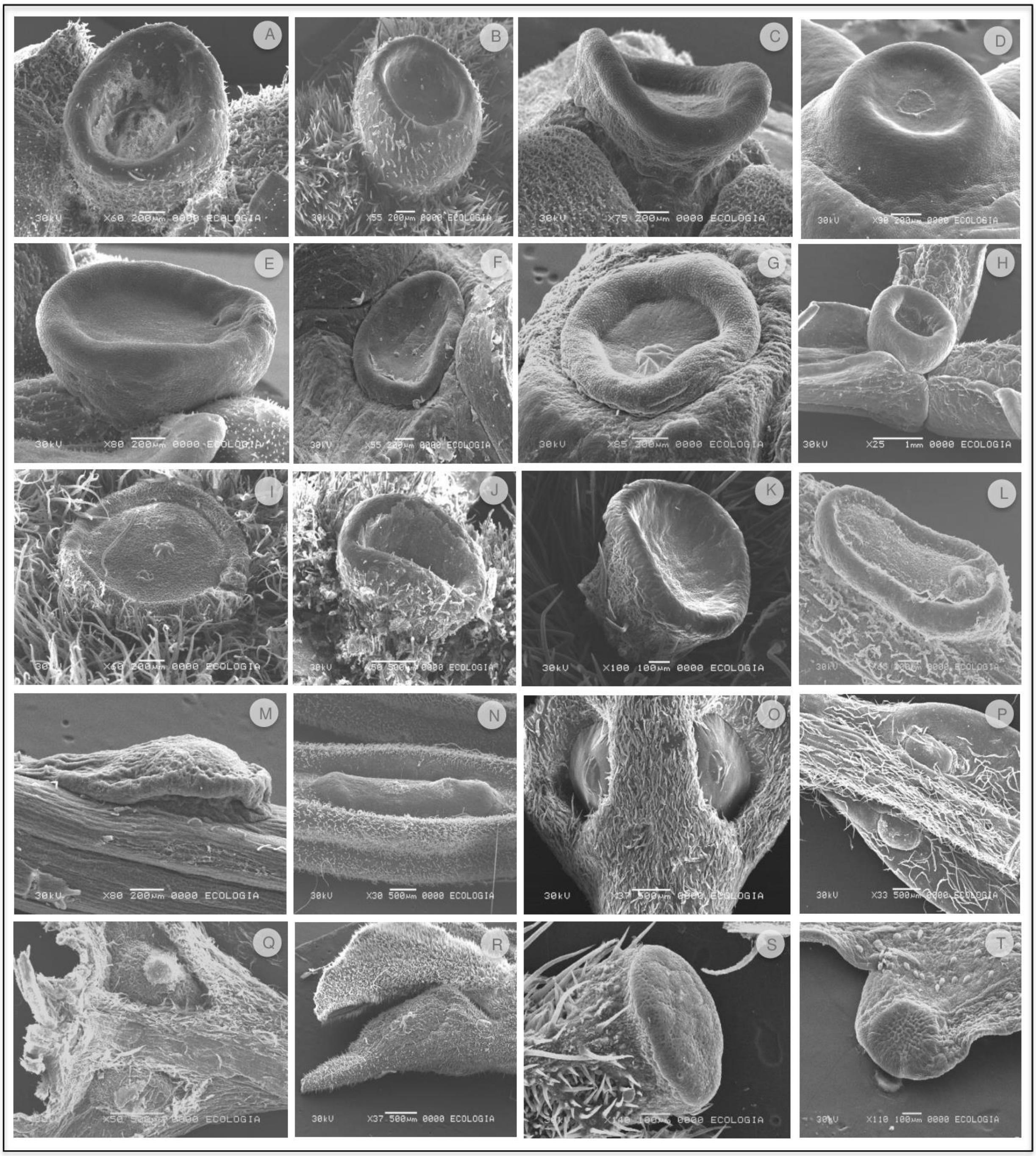
	Inga chartacea Poepp.	3	Used in this study
	Inga densiflora Benth.	6	Used in this study
	Inga edulis Mart.	6	Used in this study
	Inga heterophylla Willd.	1	Used in this study
	Inga lateriflora Miq.	10	Used in this study
	Inga laurina (Sw.) Willd.	18	Used in this study
	Inga microcoma Harms	1	Used in this study
	Inga punctata Willd.	6	Used in this study
	Inga sertulifera DC.	8	Used in this study
	Inga suaveolens Ducke	1	Used in this study
	Inga tenuistipula Ducke	7	Used in this study
	Inga sp. 1	1	
	Inga sp. 2	2	
	Inga sp. 3	3	
	Inga sp. 4	5	Used in this study
	Inga sp. 5	15	Used in this study
	Inga sp. 6	1	
	Inga sp. 7	3	
	Inga sp. 9	3	
	Inga sp. 10	2	
	Inga sp. 12	2	
	Inga sp. 13	2	
	Inga sp. 14	3	
	Inga sp. 15	1	
	Inga sp. 16	3	
	Inga sp. 18	1	
	Inga sp. 20	1	
	Senna sp.	1	Used in this study
	Zygia sp. 1	6	Used in this study
	Zygia sp. 2	3	Used in this study
	Zygia sp. 3	1	Used in this study
	Zygia sp. 4	1	
Lecythidaceae	*Gustavia augusta* L.	4	
	Lecythidaceae sp.	3	Used in this study
Loganiaceae	*Strychnos panurensis* Sprague & Sandwith	2	
Malpighiaceae	*Banisteriopsis* sp. 1	4	
	Banisteriopsis sp. 2	95	Used in this study
	Banisteriopsis sp. 3	4	
	Banisteriopsis sp. 4	2	
	Banisteriopsis sp. 5	10	Used in this study
	Banisteriopsis sp. 6	3	
	Banisteriopsis sp. 7	1	
	Banisteriopsis sp. 8	1	

	Heteropterys sp.	3	Used in this study
	Tetrapteryx sp.	7	Used in this study
Malvaceae	*Byttneria benensis* Britton	6	Used in this study
Menispermaceae	*Abuta* sp. 1	1	Used in this study
	Abuta sp. 2	1	
Ochinaceae	*Ouratea* sp.	5	Used in this study
Olacaceae	*Olacaceae* sp.	7	Used in this study
Passifloraceae	*Dilkea* sp.	1	Used in this study
	Passiflora coccinea Aubl.	3	Used in this study
	Passiflora sp. 1	1	
	Passiflora sp. 2	1	
	Passiflora sp. 3	1	Used in this study
	Passiflora sp. 4	1	
Polygonaceae	*Polygonaceae* sp.1	21	
	Polygonaceae sp.2	1	
Rhamnaceae	*Gouania* sp.	1	Used in this study
Rubiaceae	*Tocoyena* sp.	1	
	Palicourea sp.	26	Used in this study
Sapindaceae	*Paullinia* sp. 1	3	Used in this study
	Paullinia sp. 2	6	Used in this study
	Paullinia sp. 3	3	
	Paullinia sp. 5	1	
	Paullinia sp. 6	1	Used in this study
	Serjania clematidea Triana & Plach.	4	
Solanaceae	*Solanum* sp.	1	Used in this study
Vitaceae	*Cissus* sp.	1	Used in this study
Vochysiaceae	*Qualea grandiflora* Mart.	4	Used in this study
	Volchysia sp.	1	



Supplementary material 3. Flattened extrafloral nectaries in *Fridericia* sp. 5 (A); flattened extrafloral nectaries in *Fridericia* sp. 8 (B); flattened extrafloral nectaries in *Fridericia* sp. 19 (C); flattened extrafloral nectaries in *Fridericia* sp. 3 (D); flattened extrafloral nectaries in *Fridericia* sp. 35 (E); flattened extrafloral nectaries in *Memora* sp. 2 (F); flattened extrafloral nectaries in *Memora* sp. 3 (G); flattened extrafloral nectary in *Hirtella racemosa* (H); flattened extrafloral nectary in Chrysobalanaceae sp. (I); formless extrafloral nectaries in

Ipomoea philomega (J); elevated extrafloral nectaries in *Dalechampia* sp. (K); elevated extrafloral nectary in *Senegalia* sp. 1 (L); elevated extrafloral nectary in *Senegalia* sp. 3 (M); elevated extrafloral nectary in *Senegalia* sp. 4 (N); elevated extrafloral nectary in *Senegalia* sp. 7 (O); elevated extrafloral nectary in *Bauhinia* sp. 1 (P); elevated extrafloral nectaries in *Erythrina* sp. (Q); elevated extrafloral nectary in *Inga alba* (R); elevated extrafloral nectary in *Inga calantha* (S); elevated extrafloral nectary in *Inga capitata* (T).



Supplementary material 4. Elevated extrafloral nectary in *Inga chartacea* (A); elevated extrafloral nectary in *Inga edulis* (B); elevated extrafloral nectary in *Inga lateriflora* (C); elevated extrafloral nectary in *Inga laurina* (D); elevated extrafloral nectary in *Inga microcoma* (E); pit extrafloral nectary in *Inga punctata* (F); elevated extrafloral nectary in *Inga sertulifera* (G); elevated extrafloral nectary in *Inga suaveolens* (H); elevated extrafloral nectary in *Inga tenuistipula* (I); elevated extrafloral nectary in *Inga* sp. 4 (J); elevated extrafloral nectary in *Inga* sp. 5 (K); elevated extrafloral nectary in *Zygia* sp. 1 (L); elevated extrafloral nectary in *Zygia* sp. 2 (M); elevated extrafloral nectary in *Zygia* sp. 3 (N); flattened extrafloral nectaries in *Banisteriopsis* sp. 2 (O); flattened extrafloral nectaries in *Banisteriopsis* sp. 5 (P); flattened extrafloral nectaries in *Tetrapterys* sp. (Q); formless extrafloral nectary in *Passiflora* sp. 3 (R); elevated extrafloral nectary in *Paullinia* sp. 2 (S); elevated extrafloral nectary in *Paullinia* sp. 6 (T).

CHAPTER 2

The dilemma of binary or weighted data in interaction networks

Manuscript approved in the journal *Ecological Complexity* (manuscript in general appendix 1) https://www.elsevier.com/journals/ecological-complexity/1476-945x/guide-for-authors (guide for authors)

ABSTRACT

Despite the increasing number of studies dealing with interaction networks in the last few years, there is still a lack of knowledge about how their structural organization are affected by changes in binary or weighted data. To fill this gap, we collected ants foraging on plants with extrafloral nectaries in 10 sites within the Brazilian Amazon to evaluate if the generality, vulnerability, nestedness, and modularity observed in these ant-plant networks could be affected by changes in data categories. Specifically, we used three matrices built by different data categories: i) binary data (i.e., presence or absence of an interaction between a plant and an ant species); ii) frequency data (i.e., number of times in which a plant species interacted with an ant species); and iii) abundance data (i.e., number of workers of an ant species recorded foraging on a plant species). In general, when analyzing different matrix categories, we observed changes in the structure organization of the studied ant-plant interaction networks. Surprisingly, however, at the species level, both categories of weighted data (i.e., frequency and abundance data) seem to be equally appropriate for describing the role of ant species. Our results highlight the need to expand the discussion about data categories in ecological interaction studies to understand how different data categories may lead to different ecological interpretations.

Keywords: ant-plant relationship, ecological network, extrafloral nectaries, matrix categories, qualitative data, quantitative data

Introduction

In recent years the number of studies dealing with interaction networks has increased, where species are depicted as nodes and their interactions by links (Dáttilo and Rico-Gray, 2018). Such a complex network approach provides important information about structural organization and the functionality of species interactions (Vázquez et al., 2009), and has provided us with a more integrative framework of ecological and evolutionary processes at a community level (Raimundo et al., 2018). The structural organization of these interaction networks is defined through different network descriptors (Antoniazzi et al., 2018). The biological information needed to calculate these network descriptors is systematized in interaction matrices in which the row vectors represent species from one trophic group (e.g., plants) and the column vectors represent species from another trophic group (e.g., animals). Often, these matrices are expressed in a binary (1 when an interaction is recorded and 0 when no interaction is recorded) or weighted way, the latter considering both frequency data (i.e., number of times in which a plant species interacted with an animal species over a spatial or temporal gradient, for instance) or abundance data (i.e., number of individuals of an animal species recorded interacting with a plant species). In binary matrices, all interactions are considered ecologically equivalent (Almeida-Neto and Ulrich, 2011) since the strength of the interaction is not represented. On the contrary, weighted entries in an interaction matrix take into account different factors such as partner's preference (i.e., resource selection) and abundance (Blüthgen, 2010), which makes them more biologically realistic since the intensity of such interactions is recorded (Dáttilo and Rico-Gray, 2018). It is thus necessary to investigate how different data types affect the properties of species interaction matrices, since the amount of theoretical and empirical information available regarding the use of binary or weighted data in studies of interaction networks is still limited.

According to Corso et al. (2015), the nestedness metric used with binary matrices (Nestedness Based on Overlap and Decreasing Fill - NODF) (Almeida et al., 2008) is a good predictor of the network descriptor used with weighted matrices (Weighted Nestedness Metric Based on Overlap and Decreasing Fill - WNODF) (Almeida-Neto and Ulrich, 2011), since there are positive correlations between the nestedness values calculated from these two matrix categories. On the contrary, some authors question the use of binary matrices because of the probable influence of the interaction frequency on the structural organization of interaction networks (Almeida-Neto and Ulrich, 2011; Bascompte et al., 2006; Blüthgen, 2010; Dáttilo et al., 2014a; Del-Claro et al. 2018; Staniczenko et al., 2013). For instance, Dáttilo et al. (2014a) found lower nestedness values in weighted ant-plant networks compared to binary networks. However, the dataset compiled by the authors involved matrices with both frequency and abundance data, which could lead to biased conclusions on the structure of weighted ant-plant interaction networks. In this context, it is worth noting the importance of adopting relevant matrix criteria that consider the biology of the interacting organisms. Studies involving social insects mention that the individual is represented by the colony (including all members such as queens, males, workers, and soldiers) (Anderson and McShea, 2001). Therefore, researchers sampling ant species in a locality usually use frequency/occurrence data (i.e., number of times a species is captured independently in the samples) rather than abundance data (i.e., total number of workers captured in all samples) (Greenslade and Thompson, 1981; Greenslade and Halliday, 1983; Underwood and Fischer, 2006). The choice of this criterion, to the detriment of absolute abundance, is mainly justified by the social behavior of these organisms, which results in an extreme spatial clumping of ant workers within samples (Andersen, 1991; Longino et al., 2002). In other words, the amount of specimens in a sample does not absolutely reflect the distribution and quantity of nests (i.e., individuals) in the environment.

Despite the many discussions about weighted data in studies of ant communities, as described above, there is still a lack of knowledge about how the use of different matrices (i.e., binary, frequency, or abundance) should affect the structure and ecological interpretation of interaction networks involving ants and plants. Of the different types of ecological interactions between ants and plants (Mayer et al., 2014; Nelsen et al., 2018; Rico-Gray and Oliveira, 2007), ant-plant interactions mediated by extrafloral nectaries are the most studied using an interaction networks approach (Dáttilo and Rico-Gray, 2018; Del-Claro et al., 2016). In this interaction, ants protect host plants against herbivore attacks (Koptur et al., 1998). In exchange for protection, host plants secrete a substance rich in carbohydrates and amino acids from plant glands called extrafloral nectaries, which serves as a food source for ants (Fagundes et al., 2017; Kwok and Laird, 2012). In a network approach, numerous studies have shown that this ant-plant interaction networks mediated by extrafloral nectaries usually present a non-random asymmetric pattern (hereafter called “nestedness”) (Guimarães et al., 2006), in which specialist species tend to interact with generalist species (Bascompte and Jordano, 2007). Besides that, these ant-plant networks also exhibit low modularity and, consequently, a high overlap among interacting species (Díaz-Castelazo et al., 2013). In other words, these ant-plant networks are not structured in subgroups of ant species (i.e., modules) strongly associated with a particular set of plant species (Díaz-Castelazo et al., 2013).

Taking the ant-plant interactions mediated by extrafloral nectaries as the object of study, we investigated whether the structural organization (i.e., generality, vulnerability, nesting and modularity) observed in these interaction networks could be affected by changes in binary or weighted data. For this, we used empirical ant-plant networks obtained through a standardized sampling protocol, whose ant-plant interactions data were systematized in three different matrix categories: i) binary data (i.e., presence or absence of an interaction between a plant and an ant species); ii) frequency data (i.e., number of times in which a plant species interacted with an ant species within the sampling site); and iii) abundance data (i.e., number of workers of an ant species recorded foraging on a plant species). Moreover, we also tested if the centrality (i.e., a measure of the structural importance of a species within the network) of ant species within each network was different when analyzed with different matrix categories. Our findings will represent a valuable tool for future studies dealing with ant-plant interaction networks since, by using a standardized sampling protocol, we show how the structure of ant-plant networks varies according to changes in binary or weighted data.

Material and methods

Study area

This study was carried out in 10 sites situated in the west of the State of Acre, Brazilian Amazon (Appendix S1). According to Köppen's climate classification (1936), the climate in our study area is classified as monsoon climate (Peel et al., 2007), with an average rainfall of 1,450 mm per year (Macêdo et al., 2013) and a marked seasonality, with most rainfall falling between November and March (Acre, 2006). Average annual temperature is 24.5ºC (INMET, 2016), with a daily variation of around ± 7.5ºC (Acre, 2006). Elevation varies between 110 and 270 m.a.s.l. (Acre, 2006). The predominant vegetation types are open tropical rainforest (Open Ombrophilous Forest), which can be dominated by bamboo and/or palm trees, and dense tropical rainforest (Dense Ombrophilous Forest) (Acre, 2006).

Sampling design

At each site, a plot of 6,250 m^2 (250 x 25 m) was established, located at least 100 m from the edge of the forest, except for the smaller site, where a plot was placed approximately

20 m from the edge due the reduced size of this forest fragment (5.26 ha). Each plot was intensively sampled, where we recorded all interactions between ants and plants with extrafloral nectaries at a height accessible for the collector. Although the relationship between plant size and ant abundance feeding on extrafloral nectaries is not necessarily a rule (Dáttilo et al., 2014b), since the ant attraction by plants is mainly influenced by the quality and quantity of the extrafloral nectar secreted (Díaz-Castelazo et al., 2005; Fagundes et al., 2017), we opted to control plant size. Therefore, we only sampled plants located in the understory and belonging to the same size group (ranging from 0.5 to 3 m) (Dáttilo and Dyer, 2014), regardless of their habits. All plants with extrafloral nectaries belonging to this size group were carefully observed for five minutes to record the occurrence of at least one ant worker. When an interaction was confirmed, all ants present, together with a botanical sample, were collected for further identification. Those plant species whose extrafloral nectaries were not detected in the field, but which had immobile ants with mouth-parts in contact with plant tissues for several minutes (Falcão et al., 2016), were also collected, as well as the respective ants. Subsequently, we confirmed the presence of extrafloral nectaries through a literature review and observations under a stereoscope. Ants were manually collected with the aid of an entomological umbrella to record those ants that drop from the plant at the slightest sign of disturbance (Dáttilo and Dyer, 2014). Considering the phenological and structural variation in ant-plant networks throughout the year (Falcão et al., 2016; Lange et al., 2013), we performed two samplings in each site between June 2016 and February 2017. All samplings were carried out from 9:00 A.M. to 3:00 P.M.

Data analysis

For the analysis, we combined the data from the two sampling events performed in each site to ensure that most of the ant-plant interaction possibilities of each site were represented in the networks. Initially, to verify whether we sampled enough ant and plant species and interactions to describe the ant-plant networks of each site studied, we generated accumulation curves with the numbers of ant and plant species and the number of distinct pairwise interactions as a function of the number of plants sampled (Falcão et al., 2016). For all accumulation curves, we used the non-parametric bootstrapping estimator with 1,000 iterations (Gotelli and Colwell, 2001) to estimate the expected numbers of plant and ant species and interactions (Falcão et al., 2016).

To describe ant-plant interaction patterns in each site, we used interaction matrices comprised of the total number of (A) ants and (P) plants (i.e., $A \times P$ matrix), where plant species are in rows and ant species are in columns (Dormann et al., 2017). Using the same sampling, we built three different matrix categories W_{ij}: 1) binary data (where an element W_{ij} of such a matrix is 1 if plant species i and ant species j interact and zero otherwise); 2) frequency data (where W_{ij} = number of times which the plant species i interacted with the ant species j inside the sampling site); and 3) abundance data (where W_{ij} = total number of workers of the ant species j recorded foraging on the plant species i inside the sampling site). See Figure 1 for the differences in the ant-plant networks generated from different matrix categories (abundance, frequency, and binary matrices).

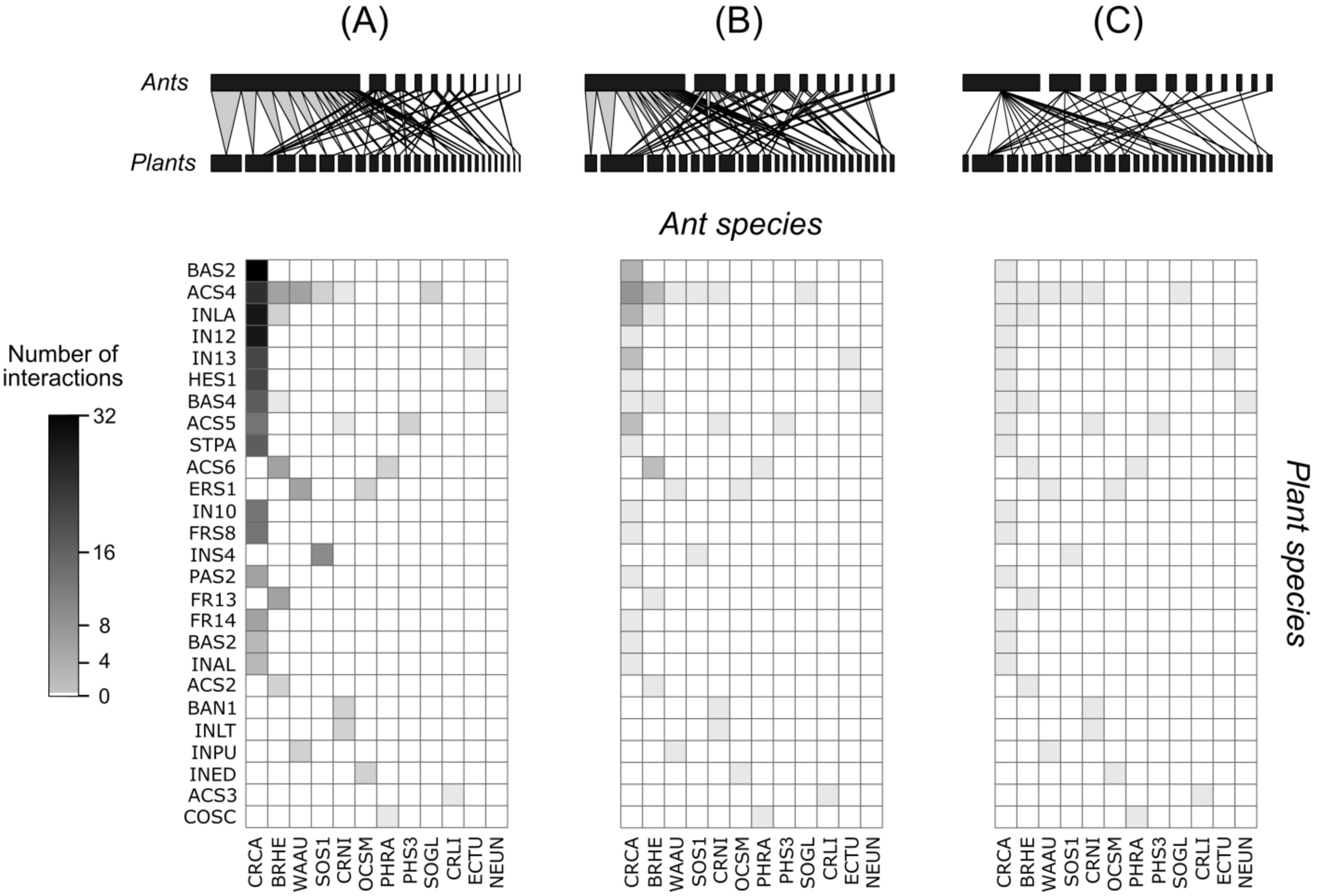


Fig. 1. Ant-plant interaction matrices generated from different data categories: (A) abundance, (B) frequency, and (C) binary data at the Forestry School (site 4), located in the state of Acre, Brazilian Amazon. Above each matrix, its bipartite network is presented. Darker cells represent a higher number of interactions. The number of interactions in each matrix category represents: i) abundance data - the number of workers of an ant species recorded foraging on a plant species; ii) frequency data - the number of times in which a plant species interacted with an ant species; and iii) binary data - the presence or absence of an interaction between a plant and an ant species. In bipartite networks, a box size represents the marginal total number of links made by an ant species or received by a plant species, and the width of the edges is proportional to the total number of interactions recorded between each pair-wise combination of ant species and plant species. Species codes are shown in Appendix S2. The species in the bipartite networks are in the same order as in the matrix representation.

To calculate the ant-plant network structure from different matrix categories, we estimated the following descriptors for each network: generality, vulnerability, nestedness, and modularity. We choose these network descriptors because they can be calculated from qualitative and quantitative data (Almeida-Neto et al., 2008; Almeida-Neto and Ulrich, 2011; Bersier et al., 2002; Dormann and Strauss, 2014; Guimerà et al., 2004). Besides that, they are often used in studies dealing with ant-plant networks and cover a wide range of possible structures with complementary biological interpretations (Del-Claro et al., 2016, 2018).

We estimated ant generality (*G*) and plant vulnerability (*V*) with binary data, based on Bersier et al. (2002), as the mean number of plants per ant and the mean number of ants per plant, respectively. Based on Bascompte and Jordano (2013), adapted from Bersier et al. (2002), we calculated the quantitative versions of generality and vulnerability as follows:

$$G_{qw} = \sum_{j=1}^{n_1} \frac{n_{1j}}{m} 2^{H_j}$$

$$V_{qw} = \sum_{i=1}^{n_2} \frac{n_{2i}}{m} 2^{H_i}$$

In these equations, m is the total number of interactions in the matrix, n_1 is the total number of (*A*) ants, n_2 is the total number of (*P*) plants, n_{1j} is the number of interactions of a given i ant, n_{2i} is the number of interactions of a given j plant. H_j and H_i are the Shannon entropy. We choose these network descriptors (generality and vulnerability) because they express the diversity in effective number of species, in this case effective number of interactions (Jost, 2006). We estimated the degree of nestedness in our networks with binary data, using the *NODF*-metric method (Nestedness Based on Overlap and Decreasing Fill) (Almeida-Neto et al., 2008) with the ANINHADO software (Guimarães and Guimarães, 2006). For weighted networks, we calculated nestedness using the *WNODF*-metric method (Weighted Nestedness Metric Based on Overlap and Decreasing Fill) (Almeida-Neto and Ulrich, 2011). The values of both nestedness indices range from 0 (non-nested) to 100 (perfectly nested). We used the modularity index (*M*) based on Simulated Annealing (Guimerà et al., 2004) to calculate the modularity for networks with binary data, using the software MODULAR (Marquitti et al., 2014). The QuanBiMo (*Q*) algorithm (Dormann and Strauss, 2014) was used to test the modularity of weighted networks (abundance and frequency data). This algorithm calculates the modularity for weighted networks using the Likelihood and Simulated Anneling-Monte Carlo approach (Dormann and Strauss, 2014). Both modularity indices (*M* and *Q*) range from 0 (no subgroups) to 1 (totally separated subgroups). Generality, vulnerability, *WNODF*, and *Q* algorithm were calculated with the "*bipartite*" package (Dormann et al., 2017) available in R 3.2.3 (R Core Team, 2016).

To standardize the differences in connectance and heterogeneity of interactions between networks, which could affect the binary and weighted values of nestedness and modularity, we calculated the Z scores of these network descriptors to allow cross-network comparisons. The *Z*-transformed score is defined as follows: $Z = [x - \mu] / \sigma$, where x is the observed index value, μ is the mean of the values from simulated matrices, and σ is the standard deviation of the values from simulated matrices (Almeida-Neto et al., 2008). The simulated matrices used to calculate μ and σ were generated by different null models for binary and weight networks. We used different null models because the algorithms that are used to randomize binary matrices are not useful for randomizing weighted matrices. For instance, when a network contains only 1s the weighted null model will identify that the observed network as identical to the null model. This is because the null model is going to reflect the fact that the network contains little or no information. For binary networks, we generated 1,000 networks according to the Null Model II available in the ANINHADO software, in which the probability of an interaction occurring is relative to the observed number of interactions of both plant and ant species (Bascompte et al., 2003). For weighted networks, we used two different null models, of which one was more restrictive than the other. For the most restrictive null model, we generated 1,000 networks using the *r2dtable* algorithm (Patefield, 1981), which holds the marginal totals constant (i.e., observed row and column totals) whilst allowing connectance to vary. For the less restrictive null model, we generated 1,000 networks using the *vaznull* algorithm (Vázquez et al., 2007), which restricts only the connectance that is the same as that in the original network, being relatively more similar to the null model adopted for the binary matrices. We used these two different null models for weighted matrices to verify that the possible differences between network descriptors calculated from binary and weighted matrices are not only artifacts of the differences between metrics and null models. We estimated the significance of the network descriptors calculated from binary data using P-values (based on the null models) and from weighted data using Z scores (values ≥ 2) (Dormann and Strauss, 2014). Both statistical tests (P-values and Z scores) are based on null modeling, and used to verify the null hypothesis that the observed results differ from that expected by chance. The main difference is the correction of the observed values of nestedness (WNODF) and modularity (Q) calculated with weighted data and standardized by the calculation of the Z scores values. This correction is performed due to the dependence of these network descriptors (WNODF and Q) on factors such as network size (i.e., number of species), number of links and total number of interactions observed (Dormann et al., 2009). Using the Z score values, we can also verify the magnitude of the difference between the null model and the observed network descriptor. According to Dorman and Strauss (2014), a Z score value of 7 (e.g., for modularity) means that the observed modularity is 7 standard deviations higher than would be expected from random networks. Values above 2 are considered significantly modular. The use of P values does not consider the magnitude of the difference, but since binary networks contain less information than weighted networks, its use is not generalized with the use of Z scores to control the effect of interaction strength in these kind of matrices.

Subsequently, we investigated possible variations in the values of the network descriptors between different matrix categories (binary data, abundance data, and frequency data), using linear mixed-effects models. We considered the values of each network descriptor as a response variable and the different matrix categories as fixed factor. The identity of each site was included in the models as a random factor. Model significance was tested by using Wald χ^2 tests. Subsequently, contrast tests were performed to verify the pairwise differences between the three matrix categories used. We also performed residual analyses and tested for overdispersion (Crawley, 2012) to evaluate model prediction. Linear mixed-effects models were created with the "*lme4*" package (Bates et al., 2017), Wald χ^2 tests were performed with the "*car*" package (Fox et al., 2016), contrasts tests with the "*phia"* package (Rosario-Martinez et al., 2015), and residual analysis of linear mixed-effects models with the "*DHARMa"* package (Hartig, 2017) available in R 3.2.3 (R Core Team, 2016).

We also performed a sensitivity analysis to help evaluate the effects of different weighted data (frequency and abundance) on the detection of nestedness and modularity. We analyzed the behavior of these network descriptors under successive link weight thresholds (cut-off values) used to define a binary matrix (Cantor et al., 2017). To perform comparisons between the different weighted matrix categories, we first standardized the link weight distribution of all weighted networks (10 networks with frequency data and 10 networks with abundance data, relative to the 10 sampling sites) by dividing the link weights by the maximum weight of each system ($a_{ij} \in [0,1]$). Subsequently, for each sampling site, we defined 10 binary matrices from the respective original frequency matrix, one for each of the 10 weight cut-offs defined at 0.1 intervals. The same procedure was performed for the abundance matrix of each sampling site. For example, for a cut-off value of 0.2, all links with a weight below this limit were excluded ($a_{i\,j} < 0.2 \rightarrow a_{ij} = 0$) or otherwise maintained ($a_{ij} \geq 0.2 \rightarrow ai_j = 1$). According to Cantor et al. (2017), a system may be considered nested or modular if the properties are present, regardless of the value used as a threshold to build binary networks, despite link weight heterogeneity.

Finally, we also calculated two measures to describe the role of ants (i.e., species level network descriptors) in the networks: individual species specialization (*d’*) and species strength (*ss*). The first measure (*d’*) describes the degree of interaction specialization at the species level by the deviation of the actual interaction frequencies from a null model, which assumes that all partners are used in proportion to their availability (Blüthgen et al., 2006). The second measure (*ss*) is defined as the sum of dependencies of each species and aims to quantify the relevance of a species across all its partners (Bascompte et al., 2006). Both measures were calculated with abundance and frequency matrices, using the “*bipartite*” package (Dormann et al., 2017) available in R 3.2.3 (R Core Team, 2016). To evaluate whether the role of ant species in the networks varies between abundance and frequency data, we performed Pearson´s correlation test between *d’* calculated from the abundance matrix and *d’* calculated from the frequency matrix. The same procedure was adopted for species strength and for the absolute values of abundance and frequency of each species. Specifically, in the case of a positive relationship between both abundance and frequency data, it is because, in general, using different types of weighted data does not change the observed role of a species in the network.

Results

We recorded a total of 56 ant species (or morphospecies) belonging to 19 genera and seven subfamilies (Appendix S3). The subfamily Myrmicinae comprised 48.21% of the total ant species (n = 27 ant species), followed by Formicinae (26.80%, n = 15) and Dolichoderinae (12.50%, n = 7). In terms of plants, we recorded a total of 148 plant species (or morphospecies) distributed in 44 genera and 25 families (Appendix S4). The family Fabaceae comprised 37.84% of the total plant species (n = 56 plant species), followed by Bignoniaceae (25.68%, n = 38) and Malpighiaceae (6.80%, n = 10). According to the species and interaction accumulation curves performed, we collected an average (mean ± SD) of 84.30 ± 0.02% of the ant species (n = 18.60 ± 3.60 species of the 22.04 ± 4.04 species estimated) and 80.60 ± 0.01% of the plant species (n = 31.90 ± 4.53 species of the 39.61 ± 5.81 species estimated). For interactions, we observed 76.23 ± 0.01% of the interactions between ants and plants with extrafloral nectaries (n = 63.70 ± 11.06 interactions of the 83.57 ± 14.44 interactions estimated), indicating that we sampled sufficient ant and plant species as well as interactions to adequately describe the ant-plant networks of each sampling site.

The generality values (*G*) differed when analyzed with different matrix categories (binary and weighted data) (Linear mixed-effects models: $\chi^2 = 14.647$, d.f. = 2, $P < 0.001$). However, when we performed contrast tests, we detected significant differences of this descriptor only between binary (generality = 8.83 ± 2.74, mean ± SD) and frequency matrices (generality = 7.24 ± 1.78) and between binary and abundance matrices (generality = 6.71 ± 2.42), but not between frequency and abundance matrices (contrast test: binary-frequency $\chi^2 = 9.4187$, d.f. = 1, $P < 0.001$; binary-abundance $\chi^2 = 12.353$, d.f. = 1, $P < 0.001$; frequency-abundance $\chi^2 = 0.1987$, d.f. = 1, $P = 0.656$) (Table 1 and Figure 2). We also found differences in the vulnerability values (*V*) between different matrix categories (linear mixed-effects models: $\chi^2 = 24.028$, d.f. = 2, $P < 0.001$); in this case, the vulnerability values calculated with abundance data were significantly lower (vulnerability = 2.93 ± 1.17) than those calculated with frequency data (vulnerability = 3.65 ± 0.23) and binary data (vulnerability = 3.76 ± 1.25). However, we did not find differences between the vulnerability values calculated with frequency and binary data (contrast test: binary-frequency $\chi^2 = 0.3457$, d.f. = 1, $P = 0.557$; binary-abundance $\chi^2 = 20.3264$, d.f. = 1, $P < 0.001$; frequency-abundance $\chi^2 = 15.3704$, d.f. = 1, $P < 0.001$) (Table 1 and Fig. 2). It is important to note that for both generality and vulnerability, the results obtained with binary and weighted data were not regular. That is, there are some values of generality and vulnerability calculated with binary data greater than those established with weighted data (abundance and frequency data) (Table 1), however there are also values calculated with binary data less than the respective values calculated with weighted data.

When evaluating the non-random patterns of ant-plant interactions of each network, we observed that 80% (n = 8) of the networks with binary data were significantly nested, while no network with weighted data (abundance and frequency) exhibited a nested pattern of ant-plant interactions, both when we used a more restrictive null model to calculate the Z score values (*r2dtable* algorithm) (Patefield, 1981) and when we used a less restrictive null model (*vaznull* algorithm) (Vázquez et al., 2007) (Table 1). In addition, we observed differences between the Z score values of nestedness calculated from different matrix categories; this pattern was detected using a more restrictive null model for weighted data (linear mixed-effects models: $\chi^2 = 381.44$, d.f. = 2, $P < 0.001$) and a less restrictive null model (linear mixed-effects models: $\chi^2 = 160.7$, d.f. = 2, $P < 0.001$). In these two cases, we observed higher Z score values of nestedness for binary matrices (Z score$_{\text{nestedness (null model II)}}$ = 26.84 ± 7.27, mean ± SD), followed by frequency (Z score$_{\text{nestedness }(\textit{r2dtable})}$ = -0.05 ± 0.92; Z score$_{\text{nestedness }(\textit{vaznull})}$ = 0.23 ± 0.86) and abundance matrices (Z score$_{\text{nestedness }(\textit{r2dtable})}$ = -5.18 ± 1.00; Z score$_{\text{nestedness }(\textit{vaznull})}$ = -2.15 ± 0.87) (contrast test: (1) Z score$_{\text{nestedness }(\textit{r2dtable})}$ binary-frequency $\chi^2 = 56.584$, d.f. = 1, $P < 0.001$; binary-abundance $\chi^2 = 375.203$, d.f. = 1, $P < 0.001$; frequency-abundance $\chi^2 = 140.374$, d.f. = 1, $P < 0.001$ and (2) Z score$_{\text{nestedness }(\textit{vaznull})}$ binary-frequency $\chi^2 = 49.318$, d.f. = 1, $P < 0.001$; binary-abundance $\chi^2 = 160.057$, d.f. = 1, $P < 0.001$; frequency-abundance $\chi^2 = 31.682$, d.f. = 1, $P < 0.001$) (Table 1 and Fig. 2). In all site studied, the Z score values calculated with binary data were higher than the values calculated with weighted data.

For modularity, we observed that all networks and nine networks with abundance data presented significant modularity when we used the more restrictive and the less restrictive null model to calculate the Z score values, respectively (Table 1). On the other hand, all networks with binary and frequency data (both null models) were non-modular (Table 1). We found significant differences between Z score values of modularity calculated from different matrix categories; this pattern was detected using the more restrictive null model for weighted data (linear mixed-effects models: $\chi^2 = 379.92$, d.f. = 2, $P < 0.001$) and the less restrictive null model (linear mixed-effects models: $\chi^2 = 104.36$, d.f. = 2, $P < 0.001$). In both cases, we did not detect significant differences of this descriptor between frequency (Z $\text{score}_{\text{modularity (2dtable)}}$ = 0.18 ± 1.01; Z $\text{score}_{\text{modularity }(\textit{vaznull})}$ = -1.16 ± 1.37) and binary matrices (Z $\text{score}_{\text{modularity (null model II)}}$ = -0.27 ± 0.53), only between each of these matrix categories and the abundance matrices (Z $\text{score}_{\text{modularity }(\textit{r2dtable})}$ = 15.60 ± 3.95; Z $\text{score}_{\text{modularity }(\textit{vaznull})}$ = 4.73 ± 2.26) (contrast test: (1) Z $\text{score}_{\text{modularity }(\textit{r2dtable})}$ binary-frequency $\chi^2 = 0.229$, d.f. = 1, $P = 0.633$; binary-abundance $\chi^2 = 292.894$, d.f. = 1, $P < 0.001$; frequency-abundance $\chi^2 = 276.760$, d.f. = 1, $P < 0.001$ and (2) Z $\text{score}_{\text{modularity }(\textit{vaznull})}$ binary-frequency $\chi^2 = 3.656$, d.f. = 1, $P = 0.056$; binary-abundance $\chi^2 = 59.824$, d.f. = 1, $P < 0.001$; frequency-abundance $\chi^2 = 93.056$, d.f. = 1, $P < 0.001$) (Table 1 and Fig. 2). In all site studied, the Z score values of modularity calculated with abundance data were higher than the values calculated with frequency and binary data.

Table 1. Nestedness, modularity, generality, and vulnerability of ant-plant networks, calculated from matrices with binary and weighted data (abundance and frequency) sampled in 10 sites located in the state of Acre, Brazil. We used the null model II to calculate the Z score values of nestedness and modularity with binary data. For the weighted data, we used two different null models based on the *r2dtable* and *vaznull* algorithm.

Matrix category	**Network Descriptor**	**Null model**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**	**Site 7**	**Site 8**	**Site 9**	**Site 10**
Abundance	WNODF		23.229	23.465	17.662	12.809	6.665	18.301	8.141	9.453	10.262	18.26
	Z score$_{\text{nestedness}}$	*r2dtable* algorithm	-5.802	-4.644	-5.603	-6.291	-6.05	-3.096	-4.433	-5.05	-6.163	-4.616
	Z score$_{\text{nestedness}}$	*vaznull* algorithm	-3.075	-2.833	-1.992	-2.623	-2.845	-0.406	-2.291	-0.935	-2.579	-1.891
	Q		0.220*	0.230*	0.317*	0.324*	0.540*	0.406*	0.584*	0.630*	0.485*	0.359*
	Z score$_{\text{modularity}}$	*r2dtable* algorithm	12.687	10.509	10.398	12.819	17.377	17.978	18.664	22.82	16.401	16.34
	Z score$_{\text{modularity}}$	*vaznull* algorithm	7.565	6.007	2.37	6.272	1.421	6.5	4.06	2.62	3.161	7.363
	Generality		10.471	7.999	9.204	9.699	5.947	5.261	3.542	5.134	4.912	4.912
	Vulnerability		2.374	2.512	2.622	1.601	2.625	5.918	3.038	2.545	2.394	3.632
Frequency	WNODF		19.856	22.735	14.188	10.038	2.734	14.228	4.573	7.251	7.855	10.368
	Z score$_{\text{nestedness}}$	*r2dtable* algorithm	0.324	-0.356	0.414	-0.087	0.108	1.178	-1.03	1.334	-1.286	-1.116
	Z score$_{\text{nestedness}}$	*vaznull* algorithm	0.401	0.266	0.014	0.502	0.071	1.794	-0.719	1.332	-0.313	-1.024
	Q		0.273	0.29	0.333	0.474	0.549	0.387	0.521	0.558	0.494	0.375
	Z score$_{\text{modularity}}$	*r2dtable* algorithm	-1.859	0.186	-0.549	0.323	-0.106	-0.374	0.813	1.803	1.225	0.31
	Z score$_{\text{modularity}}$	*vaznull* algorithm	-3.115	1.712	-2.622	-0.805	-1.694	-2.044	-1.1	0.053	-0.834	-1.147
	Generality		9.156	8.14	9.869	7.987	6.681	5.63	5.075	6.652	8.566	4.623
	Vulnerability		3.419	3.435	2.864	2.218	2.851	7.416	3.599	2.912	2.684	5.116
Binary	NODF		36.700*	37.320*	32.290*	22.78	16.4	27.570*	19.050*	20.150*	26.520*	29.600*
	Z score$_{\text{nestedness}}$	null model II	5.075	4.591	4.135	1.305	1.703	4.649	2.026	2.017	3.143	3.365

M		0.479	0.485	0.498	0.585	0.548	0.504	0.568	0.586	0.523	0.501
Z score$_{\text{modularity}}$	null model II	-0.761	-0.577	-0.878	0.502	-0.54	-0.391	0.037	0.73	-0.541	-0.243
Generality		11.849	10.015	12.967	7.769	11.849	6.358	5.373	6.467	9.233	6.387
Vulnerability		4.068	4.224	2.541	2.282	4.068	6.432	3.576	2.967	2.7	4.742

*Significant descriptor value (95% CI).

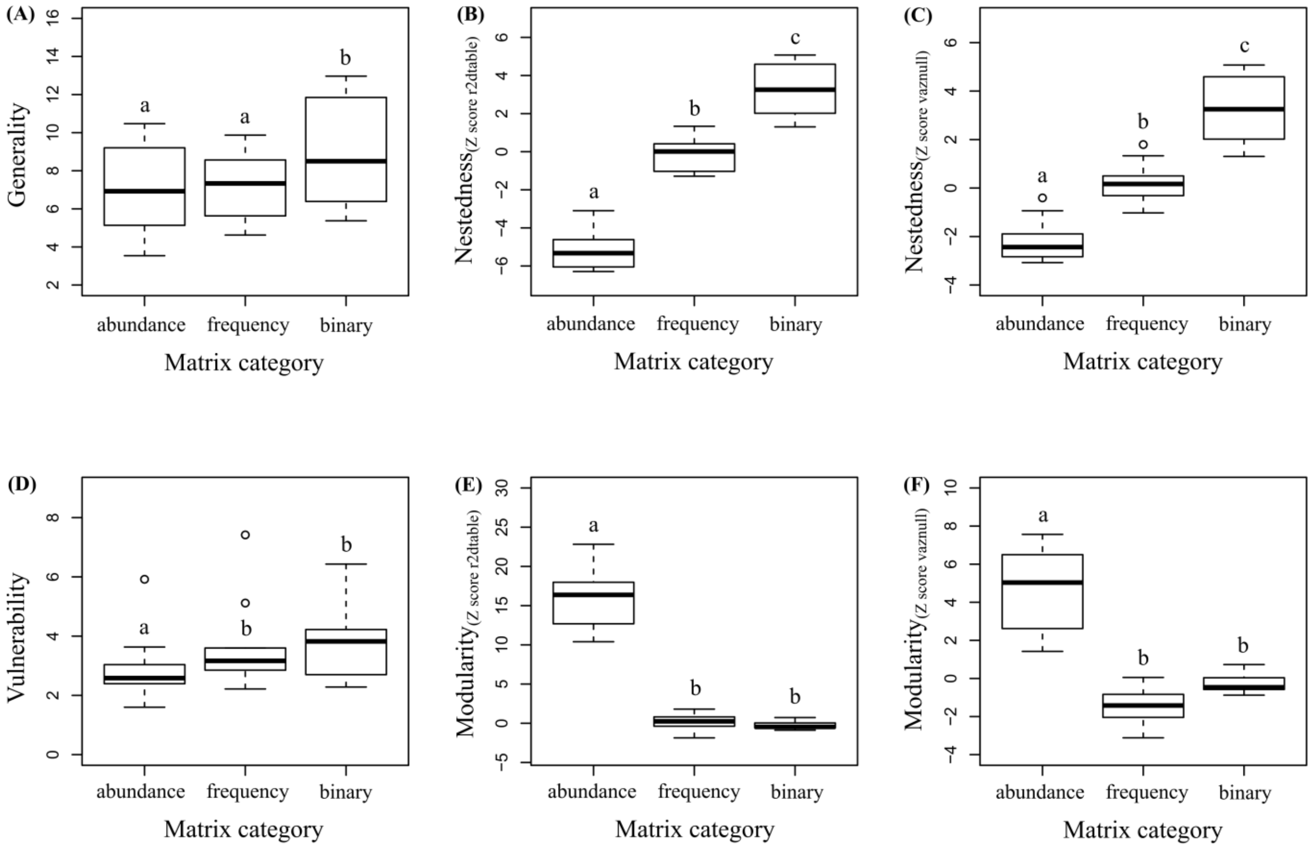
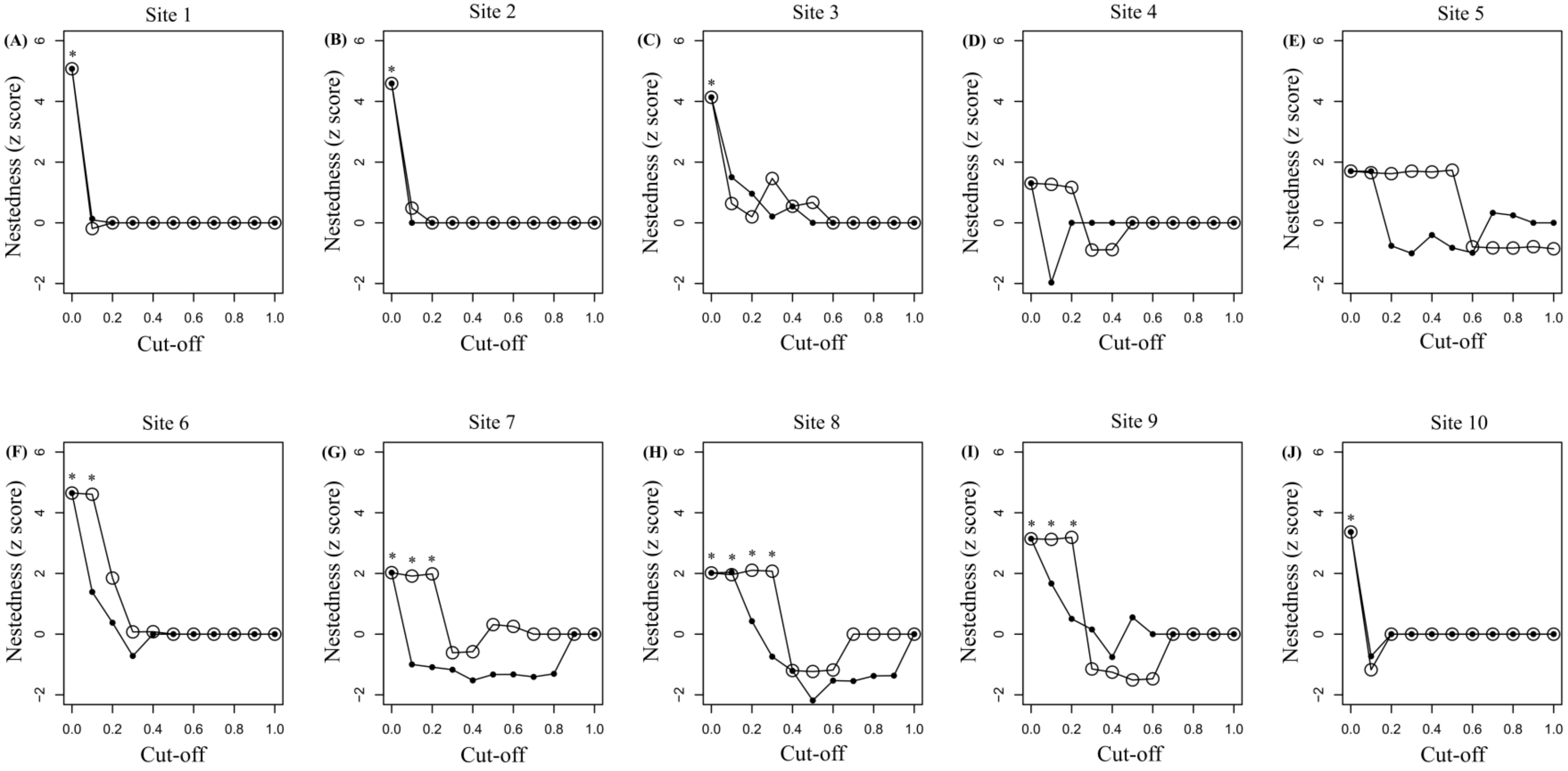


Fig. 2. Values of the four network descriptors (generality, vulnerability, nestedness, and modularity) calculated from three different matrix categories (binary, frequency, and abundance). To calculate the Z score values of nestedness and modularity with binary data, we used the null model II, while for weighted data, we used two different null models: one more restrictive model (*r2dtable* algorithm) (B and E) and one less restrictive model (*vaznull* algorithm) (C and F). The lines represent the first and fourth quartiles, the box represents the second and third quartile, and the line within the box represents the median for each category. The different letters above the boxes represent statistical differences between the categories, calculated using the contrast test.

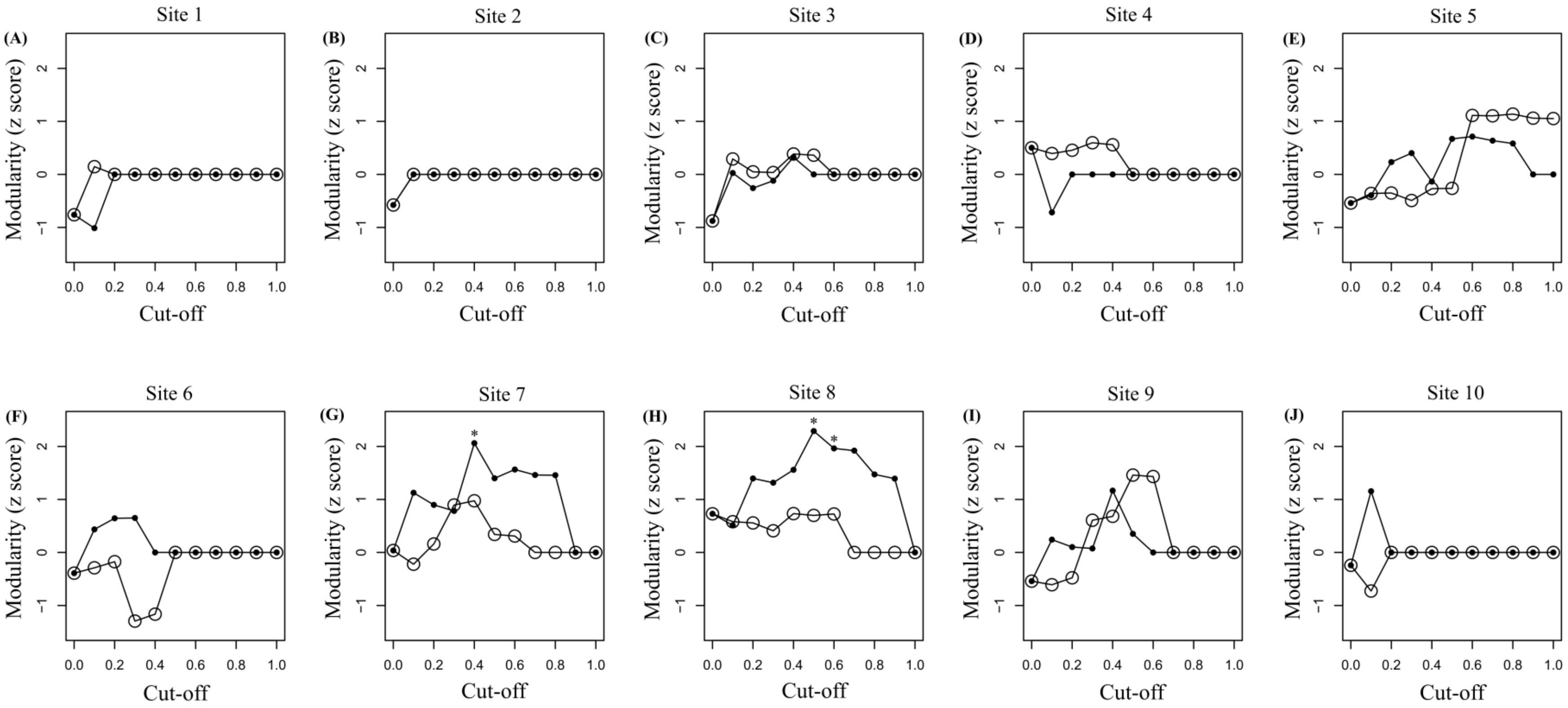
In general, we observed that nestedness and modularity of binary networks generated after applying successive cut-offs in the original weighted networks were in a relatively similar range for abundance and frequency matrices, with only slight differences (Figs. 3 and 4). In most sampling sites, regardless of the used weighted matrix category, the Z score values of nestedness decreased considerably in the first cut-offs (0.1 to 0.4), while in higher cut-offs (above 0.5), these values tended to approach 0 (Fig. 3). More precisely, we observed that in 90 and 60% of the sampling sites, the binary networks derived from abundance and frequency matrices, respectively, were no longer significantly nested from the cut-off level 0.1 (Fig. 3). For modularity, unlike the results found for nestedness, we observed that regardless of the weighted matrix category used, the Z score values tended to increase between cut-offs of 0.1 to 0.7 (Fig. 4), albeit with few significant values. Only 20% of the sampling sites presented significant values of modularity in cut-offs below 0.7 applied in abundance matrices (Fig. 4). For the frequency matrices, no sites presented any significant values of modularity for the cut-offs applied (Fig. 4).

Additionally, we observed that in 90% of our ant-plant networks (n = 9), the individual species specialization (*d'*) values calculated from the abundance matrices were positively correlated with the values calculated from the frequency matrices (Pearson´s correlation: r (mean ± SD) = 0.90 ± 0.13; range of r = 0.094; all *P*-values < 0.001; range of *P*-values = 0). For only one of the 10 networks, we did not detect this correlation (Pearson´s correlation: r = 0.539; *P*-value = 0.071). For all ant-plant networks, the species strength (*ss*) values calculated from the abundance matrices were also positively correlated with the values calculated from the respective frequency matrices (Pearson´s correlation: r (mean ± SD) = 0.99 ± 0.005; range of r = 0.012; all *P*-values < 0.001; range of *P*-values = 0). The same pattern was found for absolute values of abundance and frequency of each species (Pearson`s correlation (mean ± SD): r = 0.92 ± 0.08; range of r = 0.224; all *P*-values < 0.001; range of *P*-values = 0).



*Significant descriptor value (95% CI)

Figure 3 - Nestedness values (Z scores) calculated from binary matrices obtained through different cut-off levels (0 to 1) applied in weighted matrices with frequency (open circles) and abundance data (filled circles). Each graph (A - J) is relative to 1 of the 10 sites sampled.



*Significant descriptor value (95% CI).

Figure 4 - Modularity values (Z scores) calculated from binary matrices obtained through different cut-off levels (0 to 1) applied in weighted matrices with frequency (open circles) and abundance data (filled circles). Each graph (A - J) is relative to 1 of the 10 sites sampled.

Discussion

Using a standardized sampling protocol in different sites within the Brazilian Amazon, we observed that different matrix categories (binary or weighted data) result in the different structural organization of ant-plant interaction networks. This finding suggests that some network descriptors usually employed in studies of ecological interactions (e.g., generality, vulnerability, nestedness and modularity) are quite sensitive to data category. Surprisingly, at the species level, both categories of weighted data (i.e., frequency and abundance data) seem to be equally appropriate for describing the role of ant species within the networks. It is therefore necessary to adopt relevant matrix criteria based on the research question and on the biology of the interacting organisms in order to avoid inappropriate inferences.

We observed higher generality values (average number of plants per ant) calculated with binary data than with weighted data, and the values calculated with frequency and abundance data did not differ from each other. Binary versions of generality and vulnerability are strongly influenced by differences in sample size and sample effort, making the respective weighted versions much more robust against these sampling differences (Banasek-Richter et al., 2004; Tylianakis et al. 2007). In addition, in binary matrices, the same weight is attributed to both common and rare species (Bersier et al., 2002), and therefore, differences in partner availability are not taken into account (Blüthgen et al., 2006). The absence of significant differences between the generality values calculated from frequency and abundance matrices is, possibly, due to the fact that in our networks, the most frequent ant species were also species that collectively recruit on plants with extrafloral nectaries (*Crematogaster carinata* Mayr, 1878, *Crematogaster brasiliensis* Mayr, 1862, and *Crematogaster limata* Fr. Smith, 1858). This means that, although the number of individuals of each ant species and the total interactions differed among weighted matrices, the ratio between these factors remained similar. That is, the weight of each ant species is maintained in both weighted matrices. However, we cannot state that in ant-plant networks mediated by extrafloral nectaries, the most frequent ant species are necessarily species that recruit collectively. For this reason, we suggest that greater attention is required when using abundance data in similar studies dealing with ant-plant interaction networks. The frequency matrices adequately represent ant availability through the number of interactions that these organisms establish with plants. These matrices also adequately represent plant availability, since its elements also represent plant abundance in the networks (Tylianakis et al., 2007).

For vulnerability (average number of ants per plant), we did not detect differences between the values calculated from binary and weighted matrices with frequency data. For the same reasons described above for generality, we do not encourage the use of binary matrices. That is, binary matrices are sensitive to variations in sample size and sample effort (Tylianakis et al., 2007) and do not consider differences in the partners' availability (Blüthgen et al., 2006). Regarding the weighted matrices, we observed higher values calculated with frequency data than with abundance data. As previously mentioned, the frequency matrix elements adequately represent the plant abundance in the networks (Plowman et al., 2017; Tylianakis et al., 2007). On the contrary, in our abundance matrices, the elements represent the abundance of ants (i.e., number of workers recorded) feeding on plants with extrafloral nectaries. Consequently, when we calculated the vulnerability from our abundance matrices, we mistakenly attributed to plant species the abundance of ant species, among which many present collective recruitment. In other words, plant abundance values from our abundance matrices are higher than the actual values observed in frequency matrices, resulting in lower values of vulnerability, since this descriptor represents the proportion of interactions per plant.

The Z score values of nestedness varied greatly among different matrix categories. Specifically, we found higher values for binary matrices, followed by both weighted matrices, which did not present any significantly nested network, regardless of the null model used with the weighted data. Note that when being calculated for both binary or weighted matrices, nestedness must be in agreement with two basic principles: (1) decreasing marginal total (differences of the marginal totals among columns and/or among rows) and (2) overlapping of pairs (overlap of the presences in the less-filled columns and rows with the presences in the more-filled columns and rows, respectively) (Almeida-Neto et al., 2008; Almeida-Neto et al., 2011). However, only in a weighted approach, the individual differences of interactions are considered (Almeida-Neto et al., 2011), for example, in relation to information about species preferences (Blüthgen, 2010). A previous study found a reduction in nestedness values calculated from weighted matrices when compared to binary matrices (Dáttilo et al., 2014a). In addition, the authors observed that most networks are no longer significantly nested when generated from weighted matrices, as opposed to the significantly nested patterns found for networks generated from binary matrices. These results corroborate the hypothesis that, due to nectar-composition preferences among ants, ant species with high interaction frequencies do not necessarily have more links in binary matrices (Dáttilo et al., 2014c). That is, an ant species with a preference for the nectar composition of a specific abundant plant species might present a high interaction frequency in a weighted matrix and a low number of links in a binary matrix due to the low or absent interaction with other plant species. In contrast, an ant species that does not have any preference for specific nectar composition will tend to interact with several plant species, although less frequently with each one, possibly resulting in a lower interaction frequency in a weighted matrix and a relatively high number of links in a binary matrix. Therefore, ant species with lower interaction frequencies are not necessarily subsets of ant species with higher interaction frequencies, which generates the non-nested structure in weighted networks (Dáttilo et al., 2014a). Based on the above, we encourage the use of weighted data for nestedness, mainly frequency data, due to the valuable information about species preferences contained in these matrices (Blüthgen, 2010). However, based on Corso et al. (2015), we do not rule out the use of binary matrices. These authors detected a high correlation between WNODF and NODF values calculated from 69 different ecological networks, encompassing multiple types of interactions (pollination, frugivory, herbivory, and ant-plant interactions). Therefore, despite of the intrinsic differences between the two metrics (i.e., WNODF is always smaller than NODF), the high correlation between them indicates that NODF is a good predictor for WNODF.

As observed for nestedness, we detected similar results for modularity, regardless of the null model used with the weighted data. Interestingly, we found no differences between the Z score values of modularity for binary and frequency matrices, and both Z score averages presented extremely low values. Moreover, none of these networks was significantly modular. These findings corroborate previous studies which also detected a non-modular structure of ant-plant networks mediated by extrafloral nectaries (Dáttilo, 2012; Dáttilo et al., 2014a; Díaz-Castelazo, 2013), most likely because of the low fidelity of generalist ant species when foraging on plants with extrafloral nectaries (Rico-Gray et al., 1998; Schoereder et al., 2010). The high modularity values detected for abundance matrices, however, as well as the significance found for all networks, clearly demonstrates a distortion in the structural organization of this ant-plant interaction, generated by ant species with collective recruitment. Such an increase in modularity through the use of abundance matrices indicates a greater network specialization, in turn related to the variation in ant abundance across different plant species. The number of interactions within the modules constituted by ant species with collective recruitment was considerably higher in abundance matrices than in frequency matrices. This might result in a greater contrast in relation to the number of interactions among modules and, consequently, in higher modularity values. Finally, it is important to emphasize that for both nestedness and modularity, we observed that the nature of the data can lead to similar results, both when we used a more restrictive null model with weighted data (*r2dtable* algorithm) (Patefield, 1981) and when we used a less restrictive null model (*vaznull* algorithm) (Vázquez et al., 2007) more similar to the null model used with binary data. This indicates that the detected results are not just artifacts of the differences between metrics and null models; they represent significant structural differences of the ant-plant networks, generated by the different data categories considered in the present study.

In general, we observed that nestedness and modularity of binary networks, generated after applying successive cut-offs in the original weighted networks, were in a similar range for abundance and frequency matrices, albeit with slight differences. For nestedness, regardless of the weighted matrix category used, the Z score values decreased considerably in the first cut-offs (0.1 to 0.4). This reduction is possibly related to the large amount of rare species (both ant and plant species) present in our matrices. Similar to what Cantor et al. (2017) detected for interaction networks of different biological systems, the nested structure of our networks depends on all links, but especially the weak links that glue peripheral elements to the network. For modularity, unlike the results found for nestedness, we observed that regardless of the weighted matrix category used, the Z score values tended to increase between cut-offs of 0.1 to 0.7. In marine predation, network modularity also increases towards higher cut-off values (Tinker et al., 2012). This increase reflects clusters of individuals specializing on alternative suites of resources for their core diets (Tinker et al. 2012). In this way, we postulate that, in our study, the increase of modularity in higher cut-off values reflects clusters of ant species with a clear preference for a specific set of plants with extrafloral nectaries, with these plant species being their core sources.

It is important to note that the cut-off analysis used in this study enabled a more detailed evaluation of the behavior of different weighted data (abundance and frequency) in the structural organization of these ant-plant networks, as a complement to the comparative analysis between different data categories (binary and weighted data). As previously discussed, the comparative analysis between different data categories for nestedness showed that in general, the use of weighted matrices reduces the Z score values compared to binary matrices. Moreover, the cut-off analysis showed that among the weighted data, the frequency data tends to better capture the typical nested structure of these ant-plant interactions. We observed that 90% of the binary matrices derived from abundance data lost the nested structure at the first cut-off (0.1). Conversely, in binary matrices derived from frequency data, this nested structure was maintained at a higher cut-off (0.2). The loss of the nested structure in binary matrices derived from abundance matrices already at the first cut-off is related to the fact that in these binary matrices, ant species that do not present collective recruitment tend to be quickly excluded. In abundance matrices, these ant species that do not present collective recruitment are considered rare, since their abundances are significantly lower than the abundance of ant species with collective recruitment. Conversely, in frequency matrices, ant species that do not present collective recruitment, but present a high number of interactions, tend to remain in the analysis in the first and also in the second cut-offs (0.2), allowing maintenance of the typical nested structure of these ant-plant interaction networks mediated by extrafloral nectaries. The cut-off analysis, as well as the comparative analysis between different data categories, detected a distortion in the structural organization of networks generated with abundance data (i.e., highly modular networks). In the cut-off analysis, only binary matrices generated from abundance matrices (20% of sampling sites) presented some significant values of modularity at higher cut-offs. This result does not match the non-modular structure frequently observed for these ant-plant interactions mediated by extrafloral nectaries (Dáttilo, 2012; Dáttilo et al., 2014a; Díaz-Castelazo, 2013).

Finally, when investigating the role of each ant species in the networks in terms of the degree of individual species specialization (Blüthgen et al., 2006) and species strength (i.e., its importance from the perspective of the partner set) (Bascompte et al., 2006), we observed strong correlations between the results calculated from different categories of weighted matrices. More specifically, for all networks, we found a positive correlation between the values of these measures calculated with abundance and frequency data as well as for the absolute values of ant abundance and interaction frequency. Most likely, this pattern is related to the fact that most ant species with high interaction frequency values in our networks are also numerically dominant species (Baccaro et al., 2012; Parr, 2008) that collectively recruit on plants with extrafloral nectaries (Nicolis and Deneubourg, 1999). Summing up, the definition of the weighted matrix category to be adopted (frequency or abundance matrix) does not seem to be important at the species level, at least for the two measures we used to describe the role of ant species in ant-plant networks mediated by extrafloral nectaries (individual species specialization and species strength).

Our findings suggest some specific recommendations for similar studies dealing with ant-plant interaction networks, and some general lessons that can be extended beyond these mutualistic interactions, due the similarities in structural organization (e.g., pollination or seed dispersal) (Bascompte and Jordano, 2007). In terms of specific recommendations for analog studies, the frequency data for both generality and vulnerability seems to be the most adequate. This is because frequency data best represents ant availability, through the number of interactions that these organisms are able to establish with plants, and plant availability, since the elements of these matrices also represent plant abundance in the networks (Tylianakis et al., 2007). For nestedness, we recommend the WNODF indices, because a weighted approach takes into account the individual differences of interactions, such as the preferences for specific nectar-composition. However, we do not rule out the use of binary matrices due to the high correlation between WNODF and NODF values, as detected by Corso et al. (2015). For modularity, we suggest the use of binary or weighted matrices with frequency data, as the abundance data completely distorted the structural organization of this ant-plant interaction. Surprisingly, at the species level, both categories of weighted data (i.e., frequency and abundance data) seemed to be equally appropriate for describing the role of ant species when considering the “role” of each species, its degree of individual species specialization, and its strength in the network. In a broader way, the sensitivity of the network descriptors evaluated in relation to the different data categories observed in our study, warn about the importance of defining appropriate criteria that take into account the research question and the biology of interacting organisms, to define the data category to be used. These criteria may help researchers to avoid inappropriate inferences about the structural organization of ecological networks. Finally, we still suggest that other studies about interaction networks working with different organisms should further evaluate these methodological details frequently ignored by researchers.

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Supplementary material

Appendix S1 – Location of the sites sampled in the municipalities of Rio Branco, Senador Guiomard, Porto Acre and Bujari, state of Acre, Brazil.

Site	Coordinates	area (ha)
Senador Guiomard private area	10°30'59.16"S and 67°34'51.90"W	5.26
Senador Guiomard private area	10°40'52.34"S and 67°36'20.64"W	27.82
Senador Guiomard private area	10°60'58.02"S and 67°41'60.90"W	123.16
Forestry School	09°59'48.12"S and 67°59'20.12"W	332.15
Projeto de Assentamento Walter Arce	09°48'00.46"S and 67°51'26.95"W	681.05
Porto Acre private area	09°36'28.60"S and 67°34'60.15"W	1072.34
Catuaba Experimental Farm	10°04'48.90"S and 67°37'08.60"W	1282.42
Embrapa Acre	10°20'17.64"S and 67°40'54.24"W	1871.17
Senador Guiomard private area	10°10'24.66"S and 67°35'48.66"W	2894.77
Humaitá Reserve	09°45'15.20"S and 67°39'44.90"W	3042.02

Appendix S2 - Codes of the ant species belonging to the ant-plant networks generated from the data sampled in the site 4 (Forestry School) located in the state of Acre, Brazil.

Plant species	**CODE**	**Ant species**	**CODE**
Acacia sp1	ACS1	*Crematogaster carinata* Mayr, 1862	CRCA
Acacia sp2	ACS2	*Brachymyrmex heeri* Forel, 1874	BRHE
Acacia sp3	ACS3	*Crematogaster nigropilosa* Mayr, 1870	CRNI
Acacia sp4	ACS4	*Wasmannia auropunctata* (Roger, 1863)	WAAU
Acacia sp5	ACS5	*Solenopsis* sp.1	SOS1
Acacia sp6	ACS6	*Pheidole radoszkowskii* Mayr, 1884	PHRA
Banisteriopsis sp2	BAS2	*Ochetomyrmex semipolitus* Mayr, 1878	OCSM
Bauhinia sp1	BAN1	*Solenopsis globularia* (Smith, 1858)	SOGL
Bauhinia sp4	BAS4	*Pheidole* (gr. *Fallax*) sp.3	PHS3
Costus scaber Ruiz & Pav.	COSC	*Neoponera unidentada* Mayr, 1862	NEUN
Erythrina sp1	ERS1	*Ectatomma tuberculatum* (Oliver, 1792)	ECTU
Fridericia sp8	FRS8	*Crematogaster limata* Fr. Smith, 1858	CRLI
Fridericia sp13	FR13		
Fridericia sp14	FR14		
Heisteria sp1	HES1		
Inga alba (Sw.) Willd.	INAL		
Inga edulis Mart.	INED		
Inga lateriflora Miq.	INLT		
Inga laurina (Sw.) Willd.	INLA		
Inga punctata Willd.	INPU		
Inga sp10	IN10		
Inga sp12	IN12		
Inga sp13	IN13		
Inga sp4	INS4		
Paullinia sp2	PAS2		
Strychnos panurensis Sprague & Sandwith	STPA		

Appendix S3 – Total frequency and abundance of ant species present in ant-plant networks sampled in 10 sites located in the state of Acre, Brazilian Amazon, between June 2016 and February 2017.

Subfamily	Ant species	Total frequency	Total abundance
Dolichoderinae	*Azteca chartifex*	**1**	**2**
	Azteca sp.1	**23**	**95**
	Dolichoderus attelaboides (Fabricius, 1775)	**26**	**45**
	Dolichoderus bispinosus (Olivier, 1792)	**11**	**25**
	Dolichoderus debilis Emery, 1890	**16**	**50**
	Dolichoderus quadridenticulatus (Roger, 1862)	**1**	**1**
	Dolichoderus septemspinosus Emery, 1894	**4**	**12**
Dorylinae	*Eciton mexicanum* Roger, 1863	**1**	**1**
Ectatomminae	*Ectatomma tuberculatum* (Oliver, 1792)	**123**	**172**
	Gnamptogenys moelleri (Forel, 1912)	**2**	**3**
	Gnamptogenys sulcata (Smith, 1858)	**1**	**2**
Formicinae	*Brachymyrmex heeri* Forel, 1874	**15**	**34**
	Camponotus bidens Mayr, 1870	**1**	**1**
	Camponotus cingulatus Mayr, 1862	**3**	**6**
	Camponotus depressus Mayr, 1866	**3**	**5**
	Camponotus femoratus (Fabricius, 1804)	**1**	**1**
	Camponotus godmani Forel, 1899	**1**	**1**
	Camponotus latangulus Roger, 1863	**33**	**55**
	Camponotus nidulans (Fr. Smith, 1860)	**12**	**21**
	Camponotus prox. Novogranadensis	**3**	**4**
	Camponotus punctulatus andigenus Emery, 1903	**4**	**8**
	Camponotus sexguttatus (Fabricius, 1793)	**1**	**1**
	Camponotus sp.1	**3**	**9**

	Gigantiops destructor (Fabricius, 1804)	**2**	**3**
	Nylanderia guatemalensis (Forel, 1885)	**1**	**3**
Myrmicinae	*Cephalotes atratus* (Linnaeus, 1758)	**1**	**2**
	Cephalotes marginatus (Fabricius, 1804)	**1**	**2**
	Cephalotes pinelii (Guérin-Méneville, 1844)	**1**	**1**
	Cephalotus opacus Santschi, 1920	**2**	**2**
	Crematogaster brasiliensis Mayr, 1878	**182**	**785**
	Crematogaster carinata Mayr, 1862	**178**	**825**
	Crematogaster curvispinosa Mayr, 1862	**2**	**6**
	Crematogaster erecta Mayr, 1866	**1**	**2**
	Crematogaster flavosensitiva Longino, 2003	**10**	**46**
	Crematogaster limata Fr. Smith, 1858	**78**	**201**
	Crematogaster longispina Emery, 1890	**14**	**48**
	Crematogaster nigropilosa Mayr, 1870	**8**	**19**
	Crematogaster sp.1	**2**	**5**
	Megalomyrmex balzani Emery, 1894	**2**	**2**
	Ochetomyrmex semipolitus Mayr, 1878	**38**	**134**
	Pheidole (gr. *Fallax*) sp.1	**3**	**5**
	Pheidole (gr. *Fallax*) sp.2	**2**	**11**
	Pheidole (gr. Fallax) sp.3	**5**	**10**
	Pheidole (gr. *Fallax*) sp.4	**6**	**24**
	Pheidole (gr. *Flavens*) sp.1	**1**	**2**
	Pheidole radoszkowskii Mayr, 1884	**9**	**17**
	Solenopsis globularia (Smith, 1858)	**2**	**3**
	Solenopsis sp.1	**9**	**34**
	Solenopsis sp.3	**9**	**24**
	Wasmannia auropunctata (Roger, 1863)	**34**	**146**
Ponerinae	*Neoponera carinulata* (Roger, 1861)	**2**	**2**
	Neoponera unidentada Mayr, 1862	**7**	**8**

	Odontomachus haematodus (Linnaeus, 1758)	**10**	**13**
	Odontomachus hastatus (Fabricius, 1804)	**1**	**1**
Pseudomyrmecinae	*Pseudomyrmex oculatus* (Smith, 1855)	**4**	**4**
	Pseudomyrmex tenuis (Fabricius, 1804)	**14**	**15**

Appendix 4 – Frequency of plant species present in the ant-plant networks sampled in sites located in the state of Acre, Brazilian Amazon, between June 2016 and February 2017

Family	Plant species	Total frequency
Anonaceae	Anonaceae sp.1	**1**
Araceae	*Philodendron* sp.1	**1**
Bignoniaceae	*Fridericia* sp.1	**1**
	Fridericia sp.2	**20**
	Fridericia sp.3	**7**
	Fridericia sp.4	**2**
	Fridericia sp.5	**10**
	Fridericia sp.6	**5**
	Fridericia sp.7	**4**
	Fridericia sp.8	**30**
	Fridericia sp.9	**4**
	Fridericia sp.10	**1**
	Fridericia sp.11	**3**
	Fridericia sp.12	**3**
	Fridericia sp.13	**6**
	Fridericia sp.14	**2**
	Fridericia sp.15	**2**
	Fridericia sp.16	**4**
	Fridericia sp.17	**2**
	Fridericia sp.18	**1**
	Fridericia sp.19	**8**
	Fridericia sp.20	**2**

	Fridericia sp.21	**3**
	Fridericia sp.22	**1**
	Fridericia sp.23	**1**
	Fridericia sp.24	**2**
	Fridericia sp.25	**1**
	Fridericia sp.26	**4**
	Fridericia sp.27	**1**
	Fridericia sp.28	**1**
	Fridericia sp.29	**1**
	Fridericia sp.30	**1**
	Fridericia sp.31	**3**
	Fridericia sp.32	**3**
	Fridericia sp.33	**1**
	Fridericia sp.34	**1**
	Fridericia sp.35	**6**
	Memora sp.1	**1**
	Memora sp.2	**6**
	Memora sp.3	**1**
Chrysobalanaceae	*Hirtella racemosa* Lam.	**16**
	Hirtella sp.1	**2**
	Hirtella sp.2	**1**
Combretaceae	*Buchenavia* sp.1	**1**
Convolvulaceae	*Ipomoea philomega* (Vell.) House	**1**
	Ipomoea regnellii Meisn.	**1**
Costaceae	*Costus scaber* Ruiz & Pav.	**6**
Cucurbitaceae	Cucurbitaceae sp.1	**1**
	Gurania sp.1	**2**
Euphorbiaceae	*Acalypha* sp.1	**4**
	Aparisthmium cordatum (A. Juss.) Baill.	**10**

	Dalechampia sp.1	**1**
	Omphalea diandra L.	**6**
Fabaceae	*Senegalia* sp.1	**16**
	Senegalia sp.2	**41**
	Senegalia sp.3	**21**
	Senegalia sp.4	**16**
	Senegalia sp.5	**1**
	Senegalia sp.6	**1**
	Senegalia sp.7	**7**
	*Senegalia*sp.8	**15**
	Senegalia sp.9	**1**
	Bauhinia sp.1	**99**
	Bauhinia sp.2	**1**
	Bauhinia sp.3	**1**
	Bauhinia sp.4	**3**
	Bauhinia sp.5	**1**
	Bauhinia sp.6	**2**
	Centrosema sp.1	**3**
	Centrosema sp.2	**2**
	Centrosema sp.3	**2**
	Erythrina sp.1	**3**
	Fabaceae sp.1	**4**
	Inga acreana Harms	**2**
	Inga alba (Sw.) Willd.	**6**
	Inga calantha Ducke	**2**
	Inga capitata Desv.	**4**
	Inga chartacea Poepp.	**3**
	Inga densiflora Benth.	**6**
	Inga edulis Mart.	**6**

Inga heterophylla Willd.	**1**
Inga lateriflora Miq.	**10**
Inga laurina (Sw.) Willd.	**18**
Inga microcoma Harms	**1**
Inga punctata Willd.	**6**
Inga sertulifera DC.	**8**
Inga suaveolens Ducke	**1**
Inga tenuistipula Ducke	**7**
Inga sp.1	**1**
Inga sp.2	**2**
Inga sp.3	**3**
Inga sp.4	**5**
Inga sp.5	**15**
Inga sp.6	**1**
Inga sp.7	**3**
Inga sp.9	**3**
Inga sp.10	**2**
Inga sp.12	**2**
Inga sp.13	**2**
Inga sp.14	**3**
Inga sp.15	**1**
Inga sp.16	**3**
Inga sp.18	**1**
Inga sp.20	**1**
Senna sp.1	**1**
Zygia sp.1	**6**
Zygia sp.2	**3**
Zygia sp.3	**1**
Zygia sp.4	**1**

Lecythidaceae	*Gustavia augusta* L.	**4**
	Lecythidaceae sp.1	**3**
Loganiaceae	*Strychnos panurensis* Sprague & Sandwith	**2**
Malpighiaceae	*Banisteriopsis* sp.1	**4**
	Banisteriopsis sp.2	**95**
	Banisteriopsis sp.3	**4**
	Banisteriopsis sp.4	**2**
	Banisteriopsis sp.5	**10**
	Banisteriopsis sp.6	**3**
	Banisteriopsis sp.7	**1**
	Banisteriopsis sp.8	**1**
	Heteropterys sp1	**3**
	Tetrapterys sp.1	**7**
Malvaceae	*Byttneria benensis* Britton	**6**
Menispermaceae	*Abuta* sp.1	**1**
	Abuta sp.2	**1**
Ochinaceae	*Ouratea* sp.2	**5**
Olacaceae	*Heisteria* sp.1	**7**
Passifloraceae	*Dilkea* sp.1	**1**
	Passiflora coccinea Aublet.	**3**
	Passiflora sp.1	**1**
	Passiflora sp.2	**1**
	Passiflora sp.3	**1**
	Passiflora sp.4	**1**
Polygonaceae	*Polygonaceae* sp.1	**119**
	Polygonaceae sp.2	**1**
Rhamnaceae	*Gouania frangulifolia* Radlk.	**1**
Rubiaceae	*Tocoyena* sp.1	**1**
	Palicourea sp.1	**26**

Sapindaceae	*Paullinia* sp.1	**3**
	Paullinia sp.2	**6**
	Paullinia sp.3	**3**
	Paullinia sp.5	**1**
	Paullinia sp.6	**1**
	Serjania clematidea Triana & Plach.	**4**
Solanaceae	*Solanum* sp.1	**1**
Vitaceae	*Cissus* sp.1	**1**
Volchysiaceae	*Qualea grandiflora* Mart.	**4**
	Volchysia sp.1	**1**

CHAPTER 3

The rainfall seasonality of Brazilian Amazon tropical forests and its effects on the organization of ant-plant interaction networks

Manuscript under review in the journal *Neotropical Entomology*.

https://www.springer.com/life+sciences/entomology/journal/13744 (guide for authors)

ABSTRACT

Several studies have shown the effects of temporal variation on ecological interactions such as those involving ants and plants bearing extrafloral nectaries (EFN), however little is known about how the climate shapes these ant-plant interactions. We specifically investigated whether rainfall seasonality in tropical rainforests influences the organization of ant-plant interaction networks mediated by extrafloral nectaries, in terms of the number of interactions, network size, network specialization, diversity of interactions, and nestedness (using both binary and weighted data). We collected ants foraging on EFN-bearing plants in ten forest fragments situated in the west of the state of Acre, in the Brazilian Amazon. These ant-plant interactions were recorded in both dry and rainy seasons, resulting in a total of 20 ant-plant networks (n=10 networks sampled in the dry season and n=10 in the rainy season). We observed that network size and network specialization were not affected by rainfall seasonality, however we found that the diversity of ant-plant interactions was higher in the rainy season, while the number of ant-plant interactions and nestedness was higher in the dry season. We also observed that the central core of highly interacting ant species tends to be more stable than the central core of highly interacting plant species between dry and rainy seasons.

In short, our results indicate that rainfall seasonality is a determining factor shaping the organization of ant-plant interaction networks in tropical rainforests.

Keywords: Formicidae, EFN-bearing plants, interactions, networks, rainfall seasonality

Introduction

Extrafloral nectaries are plant glandular structures that secrete a liquid rich in carbohydrates and amino acids (González-Teuber & Heil 2009), which is quite attractive to numerous ant species (Fagundes *et al* 2017). In exchange for the food provided by the plant, the ants can protect their host plants against herbivores (Rico-Gray & Oliveira 2007). These secretory structures may or may not be vascularized (Elias 1983), and are usually located in leaves, petioles, young stems, stipules and reproductive structures (e.g. buds, calyx, inflorescence axis, flower peduncle and fruit) (Rico-Gray 1993). According to an analysis of the systematic distribution of EFN-bearing plant species performed by Weber and Keeler (2013), based on studies published over 135 years, a total of 3,941 plant species distributed among 745 genera and 108 families are recognized. The extrafloral nectar is an excellent food resource for ants, and may have a significant positive impact on their survival, growth and reproduction (Lach *et al* 2009, Byk & Del-Claro 2011), since carbohydrates are the primary fuel for workers in foraging and defense activities, and indirectly benefit the other castes (Davidson 1998).

At the community level, these interactions between ants and EFN-bearing plants can usually be evaluated using a network approach, in which different ant and plant species are depicted as nodes and their interactions as links (Dáttilo *et al* 2013a, Dáttilo *et al* 2014a). The topological structure (i.e. organization) of these ant-plant interaction networks is defined from the distribution of links between partner species (Bascompte *et al* 2003). In general, these ant-plant networks exhibit a low degree of specialization (Dáttilo 2012), and a nested pattern (Díaz-Castelazo *et al* 2013), in which species with few interactions tend to interact with highly interactive species, and interactions between species with few interactions rarely occur (Guimarães *et al* 2006, Guimarães & Guimarães 2006). Biologically, network specialization describes how species restrict their interactions from those randomly expected based on a partner's availability (Blüthgen *et al* 2006), and nestedness describes the organization of niche overlap within interacting assemblages (Blüthgen 2010).

Interactions between ants and EFN-bearing plants are also considered facultative (Del-Claro 2004) due to the low fidelity of ant species when foraging on EFN-bearing plants (Rico-Gray *et al* 1998, Schoereder *et al* 2010). This low fidelity between partners may result in the structural variation of these networks in space (Chanam *et al* 2014) and in time (Rico-Gray *et al* 2012, Lange *et al* 2013, Falcão *et al* 2016). Some studies dealing with ant-plant networks mediated by extrafloral nectaries, performed in dry environments, have observed variations in the magnitude of network descriptors such as nestedness, network specialization (Lange *et al* 2013) and the diversity of ant-plant interactions (Falcão *et al* 2016) throughout the year. In dry environments, these annual structural variations of the ant-plant networks are possibly related to the seasonality of abiotic factors, such as water availability (Rico-Gray *et al*. 2012), due to the direct influence of this factor on plant species phenology (Lange *et al*. 2013) and ant activity (Rico-Gray 1993). For example, young leaves tend to secrete more extrafloral nectar than old leaves, as a plant defense strategy (Millán-Cañongo *et al* 2014), and are therefore more visited by ants (Dáttilo *et al* 2015). In dry environments, the phenological stage of young leaves is mainly observed in the rainy season (Rico-Gray 1993), and moreover, in these dry environments, higher ant activity is associated with higher precipitation and temperature, which may lead to an increase in the number of interactions (Rico-Gray 1993).

To date, there has been no study of the annual structural variation of these ant-plant networks mediated by extrafloral nectaries in wet environments, such as the Brazilian Amazonian rainforests. In general, this region presents marked rainfall seasonality, characterized by a period of intense and frequent rainfall and a dry period (Liebmann & Marengo 2001). In this context, considering the influence of rainfall seasonality on the biological cycle of plants (Brando *et al* 2006) and on ant-plant interactions (Wirth & Leal 2001) in tropical forests, it is likely that the structure of these networks also varies between seasons. In these forests, for example, the flowering peak at the community level occurs mainly in the dry season (Alencar *et al* 1979, van Dulmen 2001, Brando *et al* 2006), and therefore extrafloral nectaries located on these reproductive structures tend to have higher productivity in this season. Studies have also shown an increase in the photosynthetic capacity of trees during the dry season (Huete *et al* 2006). This increase, in turn, tends to increase the productivity of extrafloral nectaries, due to the positive relationship between light incidence and extrafloral nectar production (Jones & Koptur 2015), and consequently increase the foraging activity of ants on these glands. Wirth and Leal (2001), observed that tropical rainfall events tend to reduce the defense of EFN-bearing plants by ants. In this light, understanding how the rainfall seasonality in wet environments influences the structure of ant-plant networks mediated by extrafloral nectaries, will allow a broader view of the dynamics of these communities in different environments. This is because, during the dry season in wet environments, water remains available for plants in deep soil layers (Nepstad et al 1994), and consequently, the air humidity can be relatively maintained through a plant’s evapotranspiration (da Rocha et al 2004), as opposed to what happens in typically dry environments.

The state of Acre, located in the extreme southwest of the Brazilian Amazon, presents two well-defined rainfall seasons (Alvares *et al* 2014), denominated by the Köppen climate classification (1936), as a monsoon climate (Am), mainly in the west of the state. The dry season, with reduced average monthly precipitation, is concentrated in June, July and August. The rainy season occurs from September to May, but the highest averages of monthly precipitation are concentrated between December and February (Acre 2006, Duarte *et al* 2005). The temperature in the region remains high throughout the year, with a maximum ranging between 30 and 33°C (Duarte *et al* 2005), thus, facilitating the evaluation of rainfall effects in non-experimental studies, since we can assume that temperature is a relatively controlled factor. Due to the two well-defined rainfall seasons and the relative stability in temperatures throughout the year, the state of Acre can be considered an adequate environment in which to investigate whether rainfall seasonality in tropical forests influences the structure of ant-plant networks mediated by extrafloral nectaries. We postulated that during the dry season in tropical forests, the increase in the extrafloral nectaries production will increase the number of interactions, network size and diversity of interactions. Consequently, ants will overlap many plants, as this is a very facultative and opportunistic interaction, which in turn will decrease network specialization and increase nestedness. We also investigated whether the generalist core of ant-plant networks remains stable between seasons, in order to verify whether phenological variations in EFN-bearing plants can affect the assemblage of ants that feed on extrafloral nectaries and consequently the structure of these ant-plant networks.

Material and methods

Study area

Fieldwork was carried out in ten forest fragments, ranging in size from approximately 5 to 3,000 hectares (Supplementary Material 1), located in the west of the state of Acre, in the Brazilian Amazon, and more specifically in the municipalities of Bujari, Porto Acre, Rio Branco and Senador Guiomard. According to the Köppen climate classification (1936), the region is classified as a monsoon climate (Am), with an average rainfall of 1,450 mm per year and two well defined seasons, a rainy season between September and May, with the highest average rainfall from December to February (approximately 43% of the total rainfall per year), and a dry season from June to August (approximately 6% of the total rainfall per year) (Macêdo *et al* 2013). The average annual temperature in the region is 24.5ºC, with daily variation of around ± 7.5ºC (Acre 2006). The region's altitude varies between 110 and 270 m a.s.l. (Acre 2006). The predominant vegetation type is tropical open rainforest (i.e. open ombrophilous forest) which may be dominated by native species of bamboo and/or with species of palm trees and dense tropical rainforest (i.e. dense ombrophilous forest) (Acre 2006).

Sampling design

We established a plot of 6,250 m^2 (250 x 25 m) in each forest fragment, at least 100 meters from the edge. In the smaller forest fragment, we established the plot approximately 20 meters from the edge, due to its reduced size (5.26 ha). It is important to note that we selected only forest fragments surrounded by pasture, in order to control the influence of the matrix composition on the focal fragments (Prevedello & Vieira 2010). We performed two samplings per plot, one in the dry season, from June to

August 2016, and the other in the period of highest rainfall in the region, from December 2016 to February 2017.

We intensively sampled each plot during both seasons by walking slowly inside the plots, looking for EFN-bearing plants interacting with ants, with a height accessible to the collector (ranging from 0.5 to 3m) (Dáttilo & Dyer 2014). When we found an EFN-bearing plant with no ants foraging on its glands, we observed this plant for five minutes. If no ant came to forage on its glands during this period of time, we discarded it from our sampling. We took care to keep a distance of at least 10 meters between EFN-bearing plants sampled, in order to minimize the possibility of collecting the same ant colony foraging on different plants (Dáttilo *et al* 2013b). When interaction was confirmed, we collected all the ants present and a botanical sample for further identification. Plants whose extrafloral nectaries were not detected in the field, but which had immobile ants, with mouthparts in contact with plant tissues for several minutes (Falcão *et al* 2016), were also collected, along with the respective ants, for later confirmation of the presence of extrafloral nectaries through observations via stereoscope and using literature reviews. We collected the ants manually with the aid of an entomological umbrella, in order to record ants that dropped from the plant at the slightest sign of disturbance (Dáttilo & Dyer 2014). The samplings were always carried out from 9:00 a.m. to 3:00 p.m.

We identified the ants by species or morphospecies through identification keys and other taxonomic tools (Wilson 2003, Fernández 2003, Bolton *et al* 2006, Bolton 2018), and by comparison with specimens deposited at the Laboratório de Mirmecologia of the Centro de Pesquisas do Cacau, Brazil (CPDC Collection), in which the voucher specimens were deposited. We identified the plants at species or morphospecies level using identification guides (Ribeiro *et al* 1999, Pennington *et al*

2004) and by comparison with specimens deposited at the Herbário do Parque Zoobotânico (HPZ) of the Universidade Federal do Acre, Brazil. Voucher specimens of plants were also deposited at the Herbário (FUEL) of the Universidade Estadual de Londrina, Brazil.

Network descriptors

We examined the ant-plant structure in the ten samplings of forest fragments using an ecological network approach. We systematized the data for each forest fragment in each season, into a quantitative matrix with elements (a*ij*) representing the number of times the plant species *i* interacted with the ant species *j* inside the plot (Bascompte *et al*. 2003). We therefore considered a total of 20 ant-plant networks (n=10 networks sampled in the dry season and n=10 in the rainy season).

We used five network descriptors calculated from a quantitative matrix to characterize the patterns of ant-plant interactions in the forest fragments (network size, number of interactions, network specialization, diversity of interactions and nestedness). These binary-weighted descriptors are the ones most often used in studies dealing with ant-plant networks and cover a wide range of possible structures with complementary biological meanings (Del-Claro *et al* 2016).

We calculated network size by multiplying the number of plant species by the number of ant species. The number of interactions comprises the total interactions observed in the network, considering all the ant and plant species involved (Dáttilo *et al* 2014b). We calculated the diversity of interactions (*DI*) using an index based on the Shannon-Weiner entropy index, which ranges from zero to infinity (Bersier *et al* 2002). This index assumes that interactions are randomly sampled from an infinitely large community and measures the mean uncertainty of collecting new interactions

(Magurran 2004). We estimated specialization using the H_2' index, which describes how species restrict their interactions from those randomly expected based on partner availability (Blüthgen *et al* 2006). In this index, the extreme generalization of an ecological network is H_2' =0 and the extreme specialization is H_2' =1. We calculated nestedness, which evaluates whether selective ant species only visit a subset of plant individuals visited by the generalist ant species (Dáttilo *et al* 2014b), using two indices, the NODF-metric for binary nestedness (Nestedness Based on Overlap and Decreasing Fill) (Almeida-Neto *et al* 2008) and the WNODF-metric for quantitative nestedness (Weighted Nestedness Metric Based on Overlap and Decreasing Fill) (Almeida-Neto & Ulrich 2011). We calculated the NODF-metric with the ANINHADO software (Guimarães & Guimarães 2006) using binary matrices generated from the quantitative matrices described above. We estimated the NODF significance using p-values based on Null Model II (1,000 randomizations), for which the probability of an interaction occurring is proportional to the observed number of interactions of both plant and ant species (Bascompte *et al* 2003). All interactions are considered ecologically equivalent in binary nestedness, and individual differences in interactions are not taken into account (Almeida-Neto & Ulrich 2011). We calculated the WNODF-metric from quantitative matrices and estimated the WNODF significance using p-values based on null model (1,000 randomizations), generated according to the *r2dtable* algorithm (Patefield 1981), which holds the marginal totals constant (i.e. observed row and column totals), whilst allowing connectance to vary. Unlike binary nestedness, quantitative nestedness considers the differences of interactions, and is a value weighted by abundance ranking (Almeida-Neto & Ulrich 2011). The values of both nestedness indices ranged from 0 (non-nested) to 100 (perfectly nested). Network specialization, diversity of interaction and WNODF were calculated using the bipartite package

(Dormann *et al* 2017) in the R software, version 3.2.3 (R Development Core Team 2016).

We also evaluated the core and peripheral species components of the networks, which were defined according Dáttilo *et al* (2013a) as: $G_c = (k_i - k_{mean})/\sigma_k$, where k_i = mean number of links for a given plant/ant species, k_{mean} = mean number of links for all plant/ant species in the network, and σ_k = standard deviation of the number of links for plant/ant species. When $Gc > 1$, the species presents a large number of interactions in comparison to other species of the same trophic level, and is considered the species constituting the generalist core. When $Gc < 1$, the species presents a lower number of interactions in comparison to other species of the same trophic level, and is considered a species on the periphery of networks.

Data analysis

We use the generalized linear mixed model (GLMM) (Bolker *et al* 2009) and linear mixed-effects model (LMM) (Crawley 2012) to test whether the descriptors of ant-plant networks differ between rainy and dry seasons. We considered each ant-plant network descriptor as a response variable and the season (dry and rainy) as an explanatory variable. We also included the identity of each forest fragment as random effects in the models to account for variance associated with random site effects. We tested the models' significance via analysis of variance between the evaluated model and the null model (intercept and random effect only). We also performed residual analysis and tests for overdispersion (Crawley 2012) to verify the adequacy of the models' prediction. Due to the overdispersion of residues, which occurs when the deviance of the response is greater than that expected by the chosen distribution (Hinde & Demétrio 1998), we examined the network size through a GLMM with negative binomial error distribution. We performed a GLMM with Poisson error distribution for the number of interactions, as we did not detect overdispersion of the data. We performed an LMM with Gaussian error distribution for network specialization, diversity of interactions and nestedness (binary and quantitative matrix). GLMM and LMM were created with the '*lme4*' package (Bates *et al* 2017), the analysis of variance between each model and the null model were determined with the '*vegan*' package (Oksanen *et al* 2017), and the residual analysis of GLMM and LMM with the '*DHARMa*' package (Florian 2017) in R 3.2.3 (R Development Core Team 2016).

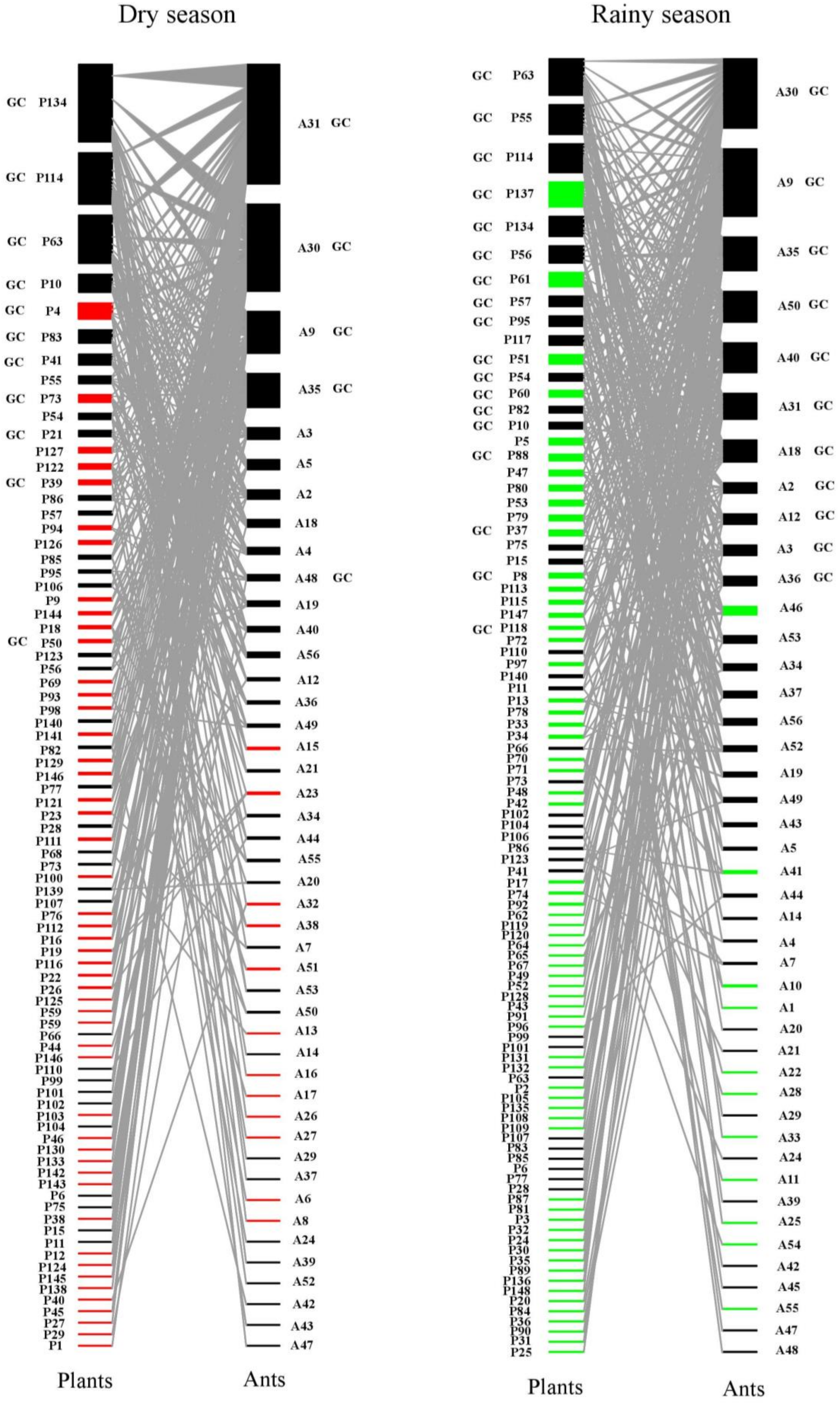
Results

We recorded a total of 56 ant species, distributed in 19 genera and seven subfamilies (Supplementary material 2), and a total of 148 plant species, distributed in 44 genera and 25 families (Supplementary material 3). The subfamilies with the highest ant species richness were Myrmicinae (48.2% of the total species, n=27 species), Formicinae (26.8%, n=15) and Dolichoderinae (12.5%, n=7), and the families with highest plant species richness were Fabaceae (37.8%, n=58), Bignoniaceae (25.7%, n=38) and Malpighiaceae (6.8%, n=10). The most frequent ant species in the ant-plant networks were *Crematogaster brasiliensis* Mayr, 1878 (Myrmicinae, 19.6% of the total number of interactions, n=182 interactions), *Crematogaster carinata* Mayr, 1862 (Myrmicinae, 19.1%, n=178) and *Ectatomma tuberculatum* (Oliver, 1792) (Ectatomminae, 13.2%, n=123), and the most frequent plant species were Polygonaceae sp.1 (Polygonaceae, 12.8%, n=119), *Bauhinia* sp.1 (Fabaceae, 10.7%, n=99) and *Banisteriopsis* sp.2 (Bignoniaceae, 10.2%, n=95).

The ant species richness in the ant-plant networks was similar in both seasons. In the rainy season, we recorded 44 species, distributed in 18 genera and six subfamilies, and in the dry season, 45 species, distributed in 17 genera and seven subfamilies. The subfamilies with the highest ant species richness were the same for both seasons, Myrmicinae (rainy: 47.7% on the total species, n=21 species; dry: 44.4%, n=20), followed by Formicinae (rainy: 20.5%, n=9; dry: 26.7%, n=12) and Dolichoderinae (rainy: 13.6%, n=6; dry: 13.3%, n=6). The networks sampled in the rainy season had greater plant species richness, with 97 species distributed in 29 genera and 16 families, than those sampled in the dry season, with 83 species distributed in 28 genera and 18 families. In the rainy season, the families with the highest plant species richness were Fabaceae (45.4%, 44 species), Bignoniaceae (21.7%, n=21) and Malpighiaceae (7.2%, n=7) and in the dry season were Fabaceae (38.6%, n=32), Bignoniaceae (26.5%, n=22) and Sapindaceae (7.2%, n=6). The most frequent ant species in the ant-plant networks sampled in the rainy season were *C. brasiliensis* (Myrmicinae, 16.9% of the total number of interactions, n=72 interactions), *E. tuberculatum* (Ectatomminae, 16.5%, n=70) and *Crematogaster limata* Fr. Smith, 1858 (Myrmicinae, 8.2%, n=35), and in the dry season were *C. carinata* (Myrmicinae: 29.9%, n=151), *C. brasiliensis* (Myrmicinae, 21.8%, n=110) and *E. tuberculatum* (Ectatomminae, 10.5%, n=53). For plants, the most frequent species in rainy season were *Bauhinia* sp.1 (Fabaceae, 8.9%, n=38), *Acacia* sp.2 (Fabaceae, 7.3%, n=31) and *Banisteriopsis* sp.2 (Malpighiaceae, 7.1%, n=30) and in the dry season were Polygonaceae sp.1 (Polygonaceae, 19.4%, n=98), *Banisteriopsis* sp.2 (Malpighiaceae, 12.8%, n=65) and *Bauhinia* sp.1 (Fabaceae, 12.1%, n=61).

The generalist core of ant species in the ant-plant networks tends to be more stable than the generalist core of plant species between the rainy and dry seasons (Fig 1). One to three ant species comprised the generalist core of ant-plant networks sampled in the rainy and dry season. Eleven ant species were present in the generalist cores, taking into account all networks of the rainy season, and the most frequent species were

C. brasiliensis (present in the generalist core in 60% of the network of rainy season, n=6) and *E. tuberculatum* (60%, n=6) (Table 1). Five ant species were present in the generalist cores of ant-plant networks sampled in the dry season, and the most frequent species were *C. carinata* (60%, n=6) followed by *C. brasiliensis* (50%, n=5) (Table 1). One to six and one to three plant species made up the generalist cores of ant-plant networks sampled in the rainy and dry seasons, respectively. Nineteen plant species were present in the generalist cores of networks sampled in the rainy season, and the most frequent species were *Bahinia* sp.1 (40%, n=4) and *Palicourea* sp.1 (Rubiaceae, 30%, n=3) (Table 1). Finally, only 11 plant species were present in the generalist cores of ant-plant networks sampled in the dry season, with the most frequent species being Polygonaceae sp.1 (50%, n=5) followed by *Bauhinia* sp.1 (40%, n=4) (Table 1).


Dry season
GC P134
GC P114
GC P63
GC P10
GC P4
GC P83
GC P41
P55
GC P73
P54
GC P21
P127
P122
GC P39
P86
P57
P94
P126
P85
P95
P106
P9
P144
P18
GC P50
P123
P56
P69
P93
P98
P140
P141
P82
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A47
Plants
Ants
Rainy season
GC P63
GC P55
GC P114
GC P137
GC P134
GC P56
GC P61
GC P57
GC P95
P117
GC P51
GC P54
GC P60
GC P82
GC P10
P5
GC P88
P47
P80
P53
P79
GC P37
P75
P15
GC P8
P113
P115
P147
GC P118
P72
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A47
A48
Plants
Ants

Fig 1 Ant-plant networks sampled during the dry and rainy season in 10 fragments located in the Brazilian Amazon. P=plant species; A=ant species and GC=generalist core species in at least one fragment. Links indicate interactions between ants and plants mediated by extrafloral nectaries. Red bars represent plant and ant species sampled only in the dry season, green bars represent plant and ant species sampled only in the rainy season and black bars represent plant and ant species sampled in both seasons. The codes for the ant and plant species are in Supplementary material 2 and 3, respectively.

Table 1 - Frequency of ant and plant species recorded in generalist core of ant-plant networks sampled during the rainy and dry season, in 10 tropical forest fragments located in the state of Acre, Brazilian Amazon, between June 2016 and February 2017.

Generalist core of plant species	Frequency (%)		Generalist core of ant species	Frequency (%)	
	Rainy season	Dry season		Rainy season	Dry season
Bauhinia sp.1	40% (n=4)	40% (n=4)	*Crematogaster brasiliensis*	60% (n=6)	50% (n=5)
Palicourea sp.1	30% (n=3)	0% (n=0)	*Ectatomma tuberculatum*	60% (n=6)	30% (n=3)
Polygonaceae sp.1	20% (n=2)	50% (n=5)	*Crematogaster carinata*	20% (n=2)	60% (n=6)
Banisteriopsis sp.2	20% (n=2)	20% (n=2)	*Crematogaster limata*	20% (n=2)	20% (n=2)
Senegalia sp.2	20% (n=2)	0% (n=0)	*Ochetomyrmex semipolitus*	20% (n=2)	0% (n=0)
Senegalia sp.3	20% (n=2)	0% (n=0)	*Brachymyrmex heeri*	10% (n=1)	0% (n=0)
Senegalia sp.8	20% (n=2)	0% (n=0)	*Camponotus latangulus*	10% (n=1)	0% (n=0)
Fridericia sp.8	10% (n=1)	10% (n=1)	*Crematogaster longispina*	10% (n=1)	0% (n=0)
Senegalia sp.1	10% (n=1)	0% (n=0)	*Crematogaster nigropilosa*	10% (n=1)	0% (n=0)
Senegalia sp.4	10% (n=1)	0% (n=0)	*Dolichoderus attelaboides*	10% (n=1)	0% (n=0)
Senegalia sp.7	10% (n=1)	0% (n=0)	*Wasmania auropuctata*	10% (n=1)	0% (n=0)
Aparisthmium cordatum	10% (n=1)	0% (n=0)	*Solenopsis* sp.1	0% (n=0)	10% (n=1)
Banisteriopsis sp.6	10% (n=1)	0% (n=0)			
Fridericia sp.35	10% (n=1)	0% (n=0)			
Fridericia sp.6	10% (n=1)	0% (n=0)			
Inga lateriflora	10% (n=1)	0% (n=0)			
Inga tenuistipula	10% (n=1)	0% (n=0)			
Inga sp.5	10% (n=1)	0% (n=0)			
Zygia sp.1	10% (n=1)	0% (n=0)			
Hirtella racemosa	0% (n=0)	20% (n=2)			
Acalypha sp.1	0% (n=0)	10% (n=1)			
Fridericia sp.2	0% (n=0)	10% (n=1)			
Fridericia sp.5	0% (n=0)	10% (n=1)			
Fridericia sp.19	0% (n=0)	10% (n=1)			
Inga laurina	0% (n=0)	10% (n=1)			
Memora sp.2	0% (n=0)	10% (n=1)			

We observed that network size (Mean ± SD) was not affected by the seasons (rainy season, 269.2 ± 77.1; dry season, 186.9 ± 92.2; ANOVA: χ^2=3.409, d.f.=1, P=0.065), however the number of ant-plant interactions was lower in the rainy season than in the dry season (rainy, 42.5 ± 9. 9 and dry, 50.4 ± 19.5; ANOVA: χ^2=6.726, d.f.=1, P=0.01) (Fig 2). We did not find any significant differences in network specialization between seasons (rainy, 0.2 ± 0.1 and dry, 0.2 ± 0.1; ANOVA: χ^2=0.570, d.f.=1, P=0.450) (Fig 2). We detected greater values for the diversity of interactions in the rainy season than in the dry season (rainy, 3.5 ± 0.2 and dry, 3.2 ± 0.3; ANOVA: χ^2=9.001, d.f.=1, $P < 0.01$) (Fig 2).

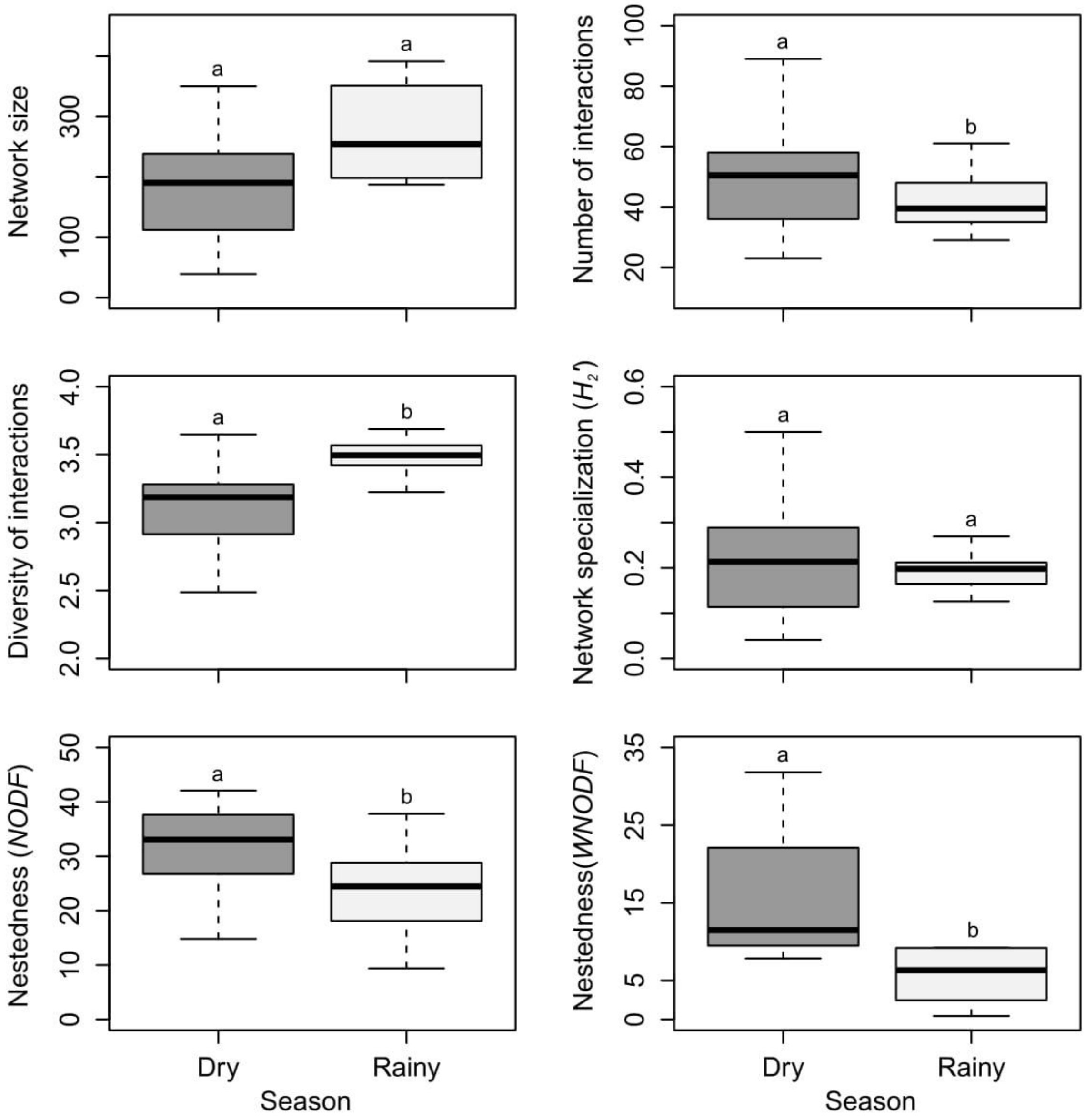

Fig 2 Boxplot of the ant-plant network descriptors (network size, number of interaction, diversity of interaction, network specialization, binary and quantitative nestedness) calculated from samplings performed in the rainy and dry season, in 10 tropical

forest fragments located in the state of Acre, Brazilian Amazon, between June 2016 and February 2017. The letters (‘a’ and ‘b’) are related to the results of the statistical analyses carried out. The same letters (‘a’ and ‘a’) on the same graph indicate the absence of a significant difference for the respective metric between the rainy and dry seasons, and different letters (‘a’ and ‘b’) indicate significant differences.

Forty percent (n=4) and 30% (n=3) of the ant-plant networks of the rainy and dry season, respectively, presented a significant value for NODF. No rainy season ant-plant network presented a significant value for WNODF, and only 10% of the ant-plant networks sampled in the dry season presented a significant value for WNODF, however we observed that ant-plant networks sampled in the rainy season presented lower values for NODF (rainy, 23.6 ± 9.4 and dry, 31.2 ± 9.0; ANOVA: χ^2=4.498, d.f.=1, P=0.034) and WNODF (rainy, 6.7 ± 5.7 and dry, 15.5 ± 8.6; ANOVA: χ^2=8.151, d.f.=1, P< 0.01) than ant-plant networks sampled in the dry season (Fig 2).

Discussion

Our evaluation of the ant-plant networks mediated by extrafloral nectaries in tropical forest fragments demonstrated that networks sampled in the dry season differed structurally from networks sampled in the rainy season, in relation to four of the six network descriptors evaluated. We also observed that the generalist core of the ant species in the ant-plant networks tended to be more stable than the generalist core of the plant species between the rainy and dry seasons. Our results thus suggest that the rainfall seasonality in tropical forests directly influences the structuring of ant-plant interaction networks mediated by extrafloral nectaries.

We noted that although the number of EFN-bearing plant species was relatively higher in the rainy season (97 species) compared to the dry season (83 species), network size did not differ between periods, mainly due to the similar number of ant species sampled (i.e. 44 and 45 ant species sampled in the rainy and dry season, respectively). As many plant species have extrafloral nectaries located on leaves (Koptur 1992), the highest number of EFN-bearing plant species in the ant-plant networks sampled in the rainy season is, possibly, related to the lower leaf-fall rates observed during this season in tropical forests (Frankie *et al* 1974, Alencar *et al* 1979). The ant-plant interactions sampled in the dry season include plant species with extrafloral nectaries located on reproductive structures, since the peak flowering in tropical forests occurs mainly at this season (Alencar *et al* 1979, van Dulmen 2001, Brando *et al* 2006). On the other hand, the ant-plant interactions sampled in the rainy season count on larger number of plant species with glands located on leaves. The similar number of ant species between seasons, responsible for the stability of the network size, is related to the low fidelity between partners, characteristic of this ant-plant interaction mediated by extrafloral nectaries (Rico-Gray *et al* 1998, Schoereder *et al* 2010). That is, the ant species that forage in these glands, possibly use this resource in both seasons, however, they tend to change from one plant species to another throughout the year (Rico-Gray *et al* 2012), due to the phenological variation of these EFN-bearing plants (Lange *et al* 2013, Falcão *et al* 2016).

In agreement with our hypothesis, we detected a greater number of interactions in the dry season. Schoereder *et al* (2010), studied ant-plant interactions in dry environments, and observed higher ant abundance in EFN-bearing plants during the dry season than during the rainy season. For the authors, this seasonal difference in ant abundance is related to differences in the availability of other resources throughout the year, with extrafloral nectaries being more important to ants during the dry season. Although our work was carried out in a completely different environment from that studied by Shoereder *et al* (2010), this same logic can be applied to our results, especially considering that in tropical forests, during the rainy season, there is also a greater availability of other resources used by ants, such as seeds with elaiosomes (Griz & Machado 2001) and fruits (Araujo 1970, Alencar *et al* 1979). Moreover, as mentioned in our hypothesis, the increase in the photosynthetic capacity of plants in tropical forests during the dry season (Huete *et al* 2006) tends to increase the productivity of extrafloral nectaries, due to the positive relationship between light incidence and extrafloral nectar production (Jones & Koptur 2015), and consequently, the foraging activity by ants on these glands. Finally, there is evidence that the nectar produced under conditions of low water availability in the soil is more concentrated in sugars and amino acids, making the EFN-bearing plants even more attractive for ants in the dry season (Blüthgen & Fiedler 2004).

We observed a greater diversity of interactions in ant-plant networks sampled in the rainy season than in the dry season. The diversity of interactions is based on the Shannon entropy index, which considers the number of species interacting and the equitability in the distribution of links strength (Bersier *et al* 2002). In this context, the greatest diversity of interactions detected in the rainy season is related to the greater number of EFN-bearing plant species sampled during this period. Furthermore, as there are more available resources for ants in this season, other than extrafloral nectar (Araújo 1970, Alencar *et al* 1979, Griz & Machado 2001), the distribution of the interactions between ants and EFN-bearing plants tends to be more equitable, since dominant ant species do not necessarily need to dominate this resource. In the networks sampled in the rainy season, for example, the most common ant species (*C. brasiliensis*) was responsible for only 16.9% of the total interactions observed (72 interactions of the 426 total interactions), whereas in the networks sampled in the dry season, the most frequent ant species (*C. carinata*) was responsible for 29.9% of the total interactions observed (151 interactions of the 505 total interactions). In other words, the most common ant species sampled in the dry season was twice as dominant in the networks than the most frequent ant species sampled in the rainy season, which is in agreement with the lower diversity of interactions observed in the dry season.

Differently from what we expected in our hypothesis, we did not observe any differences in the network specialization values between seasons. The ant-plant networks mediated by extrafloral nectaries are shaped by a few generalist species, which are essential for the maintenance of the network stability, and by multiple species that are linked to one or a few species (Díaz-Castelazo *et al* 2010, Mello *et al* 2011). In our study, the four most generalist ant species (*C. brasiliensis*, *E. tuberculatum*, *C. carinata* and *C. limata*) were the same in both seasons, but the generalist core of plant species was not as stable as the generalist core of ant species. Possibly, this lower stability is associated with the phenological variations of plant species over time (Santos *et al* 2014), with some plant species producing extrafloral nectar in the rainy season and others in the dry season. We suggest that the low fidelity of ants when foraging on EFN-bearing plants (Rico-Gray *et al* 1998), previously mentioned, is one of the main factors responsible for maintaining the low level of network specialization (Lange & Del-Claro 2014) between seasons.

In contrast to other studies (Rico-Gray *et al* 2012, Lange *et al* 2013), we observed higher nestedness values (binary and quantitative) in the dry season. The divergence of our results is possibly related to climatic differences. Both Rico-Gray *et al* (2012) and Lange *et al* (2013) worked in relatively “dry” environments (costal environments in Mexico and the Brazilian *cerrado*, respectively), while our study was conducted in the Amazon rainforest, a region that is characterized by intense and daily rainfall during the rainy season (Acre 2006). In dry environments, rainfall events seem to favor these ant-plant interactions mediated by extrafloral nectaries, due, for example, to the positive relationship between ant activity and precipitation (Rico-Gray 1993). Instead, in wet environments such as the Amazonian rainforests, the pattern of intense and daily rainfall may reduce the attractiveness of extrafloral nectaries for ants during the rainy season, due to their constant washing. Wirth and Leal (2001), have observed that rainfall events tend to reduce the defense of EFN-bearing plants by ants in tropical forests. As previously mentioned, the demand for extrafloral nectar may be lower during the rainy season in these wet environments, since the availability of other resources used by ants is higher in this period (Araújo 1970, Alencar *et al* 1979, Griz & Machado 2001). The lower nestedness values observed in the rainy season thus suggest a less heterogeneous niche overlap of the species in these ant-plant networks, which in turn is related to the lower number of interactions observed in this season.

We detected greater stability in the generalist core of ant species over time when compared to the generalist core of plant species. This greater variation in the generalist core of plant species is mainly due to the influence of the seasonality of abiotic factors, such as rainfall, on EFN-bearing plant species (Thompson 2009), possibly resulting in a production/availability of extrafloral nectar differentiated between plant species throughout the year, as discussed above. Of the 19 plant species belonging to the generalist core of networks sampled in the rainy season, only four (21%) were also plant species belonging to the generalist core of networks sampled in the dry season. Similarly, of the 11 plant species belonging to the generalist core of networks sampled in the dry season, only four (36%) were also plant species belonging to the generalist core of networks sampled in the rainy season, which in turn suggests a high seasonal variation in the extrafloral nectar production among the EFN-bearing plant species sampled. As a result of this seasonality in the production of extrafloral nectaries, ant species that forage on these glands change from one plant species to another during the year (Rico-Gray *et al* 2012), thus allowing greater stability in the generalist core of ant species over time. The same pattern (the greater stability of the generalist core of ant species than of plant species) was observed by Lange *et al* (2013) in dry environments, which indicates that, regardless of how seasonal rainfall acts on plant phenology throughout the year in different environments, the generalist core of ant species always remains stable.

In summary, our results suggest that rainfall seasonality in tropical forests affects the ant-plant networks structure in terms of the number and diversity of interactions and nestedness, mainly due to possible differences in the quality and availability of extrafloral nectar between both seasons (Blüthgen & Fiedler 2004, Schoereder *et al* 2010). In general, in these wet environments, the dry season seems to favor ant-plant interactions mediated by extrafloral nectaries in terms of the number of interactions and nestedness (binary and quantitative). The highest values for number of interactions and nestedness observed in the dry season is possibly a consequence of a reduction in the availability of other resources used by ants, and the greater attractiveness that extrafloral nectar produced under conditions of low water availability (i.e. more concentrated in sugars and amino acids) should exert on these organisms, however the ant-plant networks sampled in the rainy season presented higher values for diversity of interactions, due to the higher number of plant species with productive extrafloral nectaries observed in this season. The pattern is possibly associated with the phenological variation in these structures; since extrafloral nectaries located in leaves tend to be more common in the rainy season due to the lower leaf-fall rates, characteristic of this period. Finally, the generalist core of plant species was less stable between periods than the generalist core of ant species, also due to this phenological variation in the extrafloral nectaries. We conclude that the rainfall seasonality in tropical forests is a determinant factor in the structure of ant-plant networks mediated by extrafloral nectaries. For the real characterization of these ant-plant networks in ecological studies, it is essential to consider the seasonal dynamics of these structures, in order to ensure that most of the ant-plant interaction possibilities for a given locality are present in the networks.

Author Contribution Statement PNM, JL and WD performed the experiment, PNM and IB undertook the fieldwork, JELSR and IB identified the plants, JHCD identified the ants, PNM and WD analyzed the data, PNM wrote the first draft of the manuscript, all authors contributed to the interpretation of the data and approved the final draft of the paper.

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Supplementary material

Appendix S1 – Location of the forest fragments sampled in the municipalities of Rio Branco, Senador Guiomard, Porto Acre and Bujari, Acre, Brazil.

Fragment	Site	Geographic coordinates	area (ha)
1	Senador Guiomard private fragment	10° 3'59.16"S and 67°59'20.12"W	5.26
2	Senador Guiomard private fragment	10° 4'52.34"S and 67°36'2.64"W	27.82
3	Senador Guiomard private fragment	10°6'58.02"S and 67°41'6.90"W	123.16
4	Forestry School	9°59'48.12"S and 67°59'20.12"W	332.15
5	Projeto de Assentamento Walter Arce	9°48'0.46"S and 67°51'26.95"W	681.05
6	Porto Acre private fragment	9°36'28.60"S and 67°34'6.15"W	1072.34
7	Catuaba Experimental Farm	10°04'48.9"S and 67°37'08.6"W	1282.42
8	Embrapa Acre	10°2'17.64"S and 67°40'54.24"W	1871.17
9	Senador Guiomard private fragment	10° 1'24.66"S and 67°35'48.66"W	2894.77
10	Humaitá Reserve	09°45'15.2"S and 67°39'44.9"W	3042.02

Appendix S2 - List of ant species present in the ant-plant networks sampled in 10 tropical forest fragments located in the state of Acre, Brazilian Amazon, between June 2016 and February 2017. The network codes refer to Figure 1.

Subfamily	Ant species	Ant species frequency		Network codes
		rainy season	dry season	
Dolichoderinae	*Azteca chartiflex*	1	0	A1
	Azteca sp.1	11	12	A2
	Dolichoderus attelaboides (Fabricius, 1775)	11	15	A3
	Dolichoderus bispinosus (Olivier, 1792)	2	9	A4
	Dolichoderus debilis Emery, 1890	3	13	A5
	Dolichoderus quadridenticulatus (Roger, 1862)	0	1	A6
	Dolichoderus septemspinosus Emery, 1894	2	2	A7
Ecitoninae	*Eciton mexicanum* Roger, 1863	0	1	A8
Ectatomminae	*Ectatomma tuberculatum* (Oliver, 1792)	70	53	A9
	Gnamptogenys moelleri (Forel, 1912)	2	0	A10
	Gnamptogenys sulcata (Smith, 1858)	1	0	A11
Formicinae	*Brachymyrmex heeri* Forel, 1874	11	4	A12
	Camponotus bidens Mayr, 1870	0	1	A13
	Camponotus cingulatus Mayr, 1862	2	1	A14
	Camponotus depressus Mayr, 1866	0	3	A15
	Camponotus femoratus (Fabricius, 1804)	0	1	A16
	Camponotus godmani Forel, 1899	0	1	A17
	Camponotus latangulus Roger, 1863	23	10	A18
	Camponotus nidulans (Fr. Smith, 1860)	5	7	A19
	Camponotus prox. Novagranadensis	1	2	A20
	Camponotus punctulatus andigenus Emery, 1903	1	3	A21
	Camponotus sexguttatus (Fabricius, 1793)	1	0	A22
	Camponotus sp.1	0	3	A23
	Gigantiops destructor (Fabricius, 1804)	1	1	A24
	Nylanderia guatemalensis (Forel, 1885)	1	0	A25
Myrmicinae	*Cephalotes atratus* (Linnaeus, 1758)	0	1	A26
	Cephalotes marginatus (Fabricius, 1804)	0	1	A27
	Cephalotes pinelii (Guérin-Méneville, 1844)	1	0	A28
	Cephalotus opacus Santschi, 1920	1	1	A29
	Crematogaster brasiliensis Mayr, 1878	72	110	A30
	Crematogaster carinata Mayr, 1862	27	151	A31
	Crematogaster curvispinosa Mayr, 1862	0	2	A32
	Crematogaster erecta Mayr, 1866	1	0	A33
	Crematogaster flavosensitiva Longino, 2003	7	3	A34
	Crematogaster limata Fr. Smith, 1858	35	43	A35
	Crematogaster longispina Emery, 1890	10	4	A36
	Crematogaster nigropilosa Mayr, 1870	7	1	A37
	Crematogaster sp.1	0	2	A38
	Megalomyrmex balzani Emery, 1894	1	1	A39
	Ochetomyrmex semipolitus Mayr, 1878	31	7	A40
	Pheidole (gr. *Fallax*) sp.1	3	0	A41
	Pheidole (gr. *Fallax*) sp.2	1	1	A42

	Pheidole (gr. Fallax) sp.3	4	1	A43
	Pheidole (gr. *Fallax*) sp.4	3	3	A44
	Pheidole (gr. *Flavens*) sp.1	1	0	A45
	Pheidole radoszkowskii Mayr, 1884	9	0	A46
	Solenopsis globularia (Smith, 1858)	1	1	A47
	Solenopsis sp.1	1	8	A48
	Solenopsis sp.3	5	4	A49
	Wasmannia auropunctata (Roger, 1863)	32	2	A50
Ponerinae	*Neoponera carinulata* (Roger, 1861)	0	2	A51
	Neoponera unidentada Mayr, 1862	6	1	A52
	Odontomachus haematodus (Linnaeus, 1758)	8	2	A53
	Odontomachus hastatus (Fabricius, 1804)	1	0	A54
Pseudomirmicinae	*Pseudomyrmex oculatus* (Smith, 1855)	1	3	A55
	Pseudomyrmex tenuis (Fabricius, 1804)	7	7	A56

Appendix S3 - List of plant species present in the ant-plant networks sampled in 10 tropical forest fragments located in the state of Acre, Brazilian Amazon, between June 2016 and February 2017. The network codes refer to Figure 1.

		Plant species frequency		Network
Family	Plant species	Rain season	Dry season	codes
Anonaceae	Anonaceae sp1	0	1	P1
Araceae	*Philodendron* sp.1	1	0	P2
Bignoniaceae	*Fridericia* sp.1	1	0	P3
	Fridericia sp.2	0	20	P4
	Fridericia sp.3	7	0	P5
	Fridericia sp.4	1	1	P6
	Fridericia sp.5	0	10	P7
	Fridericia sp.6	5	0	P8
	Fridericia sp.7	0	4	P9
	Fridericia sp.8	7	23	P10
	Fridericia sp.9	3	1	P11
	Fridericia sp.10	0	1	P12
	Fridericia sp.11	3	0	P13
	Fridericia sp.12	0	3	P14
	Fridericia sp.13	5	1	P15
	Fridericia sp.14	0	2	P16
	Fridericia sp.15	2	0	P17
	Fridericia sp.16	0	4	P18
	Fridericia sp.17	0	2	P19
	Fridericia sp.18	1	0	P20
	Fridericia sp.19	0	8	P21
	Fridericia sp.20	0	2	P22
	Fridericia sp.21	0	3	P23
	Fridericia sp.22	1	0	P24
	Fridericia sp.23	1	0	P25
	Fridericia sp.24	0	2	P26
	Fridericia sp.25	0	1	P27
	Fridericia sp.26	1	3	P28
	Fridericia sp.27	0	1	P29
	Fridericia sp.28	1	0	P30
	Fridericia sp.29	1	0	P31
	Fridericia sp.30	1	0	P32
	Fridericia sp.31	3	0	P33
	Fridericia sp.32	3	0	P34
	Fridericia sp.33	1	0	P35
	Fridericia sp.34	1	0	P36

	Fridericia sp.35	6	0	P37
	Memora sp.1	0	1	P38
	Memora sp.2	0	6	P39
	Memora sp.3	0	1	P40
Chrysobalanaceae	*Hirtella racemosa* Lam.	2	14	P41
	Hirtella sp.1	2	0	P42
	Hirtella sp.2	1	0	P43
Combretaceae	*Buchenavia* sp.1	0	1	P44
Convolvulaceae	*Ipomoea philomega* (Vell.) House	1	0	P45
	Ipomoea regnellii Meisn.	1	0	P46
Costaceae	*Costus scaber* Ruiz & Pav.	6	0	P47
Cucurbitaceae	*Gurania* sp.1	2	0	P48
	Cucurbitaceae sp.1	1	0	P49
Euphorbiaceae	*Acalypha* sp.1	0	4	P50
	Aparisthmium cordatum (A. Juss.) Baill.	10	0	P51
	Dalechampia sp.1	1	0	P52
	Omphalea diandra L.	6	0	P53
Fabaceae	*Senegalia* sp.1	8	8	P54
	Senegalia sp.2	31	10	P55
	Senegalia sp.3	18	3	P56
	Senegalia sp.4	11	5	P57
	Senegalia sp.5	0	1	P58
	Senegalia sp.6	0	1	P59
	Senegalia sp.7	7	0	P60
	Senegalia sp.8	15	0	P61
	Senegalia sp.9	1	0	P62
	Bauhinia sp.1	38	61	P63
	Bauhinia sp.2	1	0	P64
	Bauhinia sp.3	1	0	P65
	Bauhinia sp.4	2	1	P66
	Bauhinia sp.5	1	0	P67
	Bauhinia sp.6	0	2	P68
	Centrosema sp.1	0	3	P69
	Centrosema sp.2	2	0	P70
	Centrosema sp.3	2	0	P71
	Erythrina sp.1	3	0	P72
	Fabaceae sp.1	2	2	P73
	Inga acreana Harms	2	0	P74
	Inga alba (Sw.) Willd.	5	1	P75
	Inga calantha Ducke	0	2	P76
	Inga capitata Desv.	1	3	P77
	Inga chartacea Poepp.	3	0	P78
	Inga densiflora Benth.	6	0	P79

	Inga edulis Mart.	6	0	P80
	Inga heterophylla Willd.	1	0	P81
	Inga lateriflora Miq.	7	3	P82
	Inga laurina (Sw.) Willd.	1	17	P83
	Inga microcoma Harms	1	0	P84
	Inga punctata Willd.	1	5	P85
	Inga sertulifera DC.	2	6	P86
	Inga suaveolens Ducke	1	0	P87
	Inga tenuistipula Ducke	7	0	P88
	Inga sp.1	1	0	P89
	Inga sp.2	2	0	P90
	Inga sp.3	0	3	P91
	Inga sp.4	0	5	P92
	Inga sp.5	11	4	P93
	Inga sp.6	1	0	P94
	Inga sp.7	3	0	P95
	Inga sp.9	0	3	P96
	Inga sp.10	1	1	P97
	Inga sp.12	0	2	P98
	Inga sp.13	1	1	P99
	Inga sp.14	2	1	P100
	Inga sp.15	0	1	P101
	Inga sp.16	2	1	P102
	Inga sp.18	0	1	P103
	Inga sp.20	0	1	P104
	Senna sp.1	1	0	P105
	Zygia sp.1	2	4	P106
	Zygia sp.2	1	2	P107
	Zygia sp.3	1	0	P108
	Zygia sp.4	1	0	P109
Lecythidaceae	*Gustavia augusta* L.	3	1	P110
	Lecythidaceae sp.1	0	3	P111
Loganiaceae	*Strychnos panurensis* Sprague & Sandwith	0	2	P112
Malpighiaceae	*Banisteriopsis* sp.1	4	0	P113
	Banisteriopsis sp.2	30	65	P114
	Banisteriopsis sp.3	4	0	P115
	Banisteriopsis sp.4	0	2	P116
	Banisteriopsis sp.5	10	0	P117
	Banisteriopsis sp.6	3	0	P118
	Banisteriopsis sp.7	1	0	P119
	Banisteriopsis sp.8	1	0	P120
	Heteropterys sp1	0	3	P121
	Tetrapterys sp.1	0	7	P122

Malvaceae	*Byttneria benensis* Britton	2	4	P123
Menispermaceae	*Abuta* sp.1	0	1	P124
	Abuta sp.2	0	1	P125
Ochinaceae	*Ouratea* sp.2	0	5	P126
Olacaceae	*Heisteria* sp.1	0	7	P127
Passifloraceae	*Dilkea* sp.1	1	0	P128
	Passiflora coccinea Aublet.	0	3	P129
	Passiflora sp.1	0	1	P130
	Passiflora sp.2	1	0	P131
	Passiflora sp.3	1	0	P132
	Passiflora sp.4	0	1	P133
Polygonaceae	*Polygonaceae* sp.1	21	98	P134
	Polygonaceae sp.2	1	0	P135
Rhamnaceae	*Gouania frangulifolia* Radlk.	1	0	P136
Rubiaceae	*Palicourea* sp.1	26	0	P137
	Tocoyena sp.1	0	1	P138
Sapindaceae	*Paullinia* sp.1	1	2	P139
	Paullinia sp.2	3	3	P140
	Paullinia sp.3	0	3	P141
	Paullinia sp.5	0	1	P142
	Paullinia sp.6	0	1	P143
	Serjania clematidea Triana & Plach.	0	4	P144
Solanaceae	*Solanum* sp.1	0	1	P145
Vitaceae	*Cissus* sp.1	0	1	P146
Volchysiaceae	*Qualea grandiflora* Mart.	4	0	P147
	Vochysia sp.1	1	0	P148

CHAPTER 4

Effects of forest fragmentation on ant-plant mutualistic networks mediated by extrafloral nectaries in the Brazilian Amazon

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(guide for authors)

ABSTRACT

Rainforest fragmentation drastically affects biodiversity and species composition, mainly due to habitat loss. Several studies have already shown the effects of forest fragmentation on plant and ant communities. To date, however, there is limited empirical knowledge of how forest fragmentation affects ant-plant interaction in networks. We investigated the effects of the configuration of rainforest fragments on the structure of ant-plant interaction networks mediated by extrafloral nectaries (EFNs). We carried out this study in ten forest fragments, ranging in size from approximately 5 to 3,000 ha, located in the Brazilian Amazon. In each fragment we established a plot of 6,250 m^2, in which all ant-plant interactions were recorded, and calculated the following network descriptors: number of interactions, network size, network specialization, diversity of interactions, and nestedness. We used four explanatory variables to investigate the effects of forest fragmentation on these network descriptors: three metrics of the configuration of fragments (i.e., fragment area, edge irregularity, and connectivity) and the forest structure within each fragment, represented by canopy cover. In general, we found that only nestedness was affected by the configuration of the forest fragments. Specifically, nestedness decreased with increasing edge irregularity. The relative structural stability of the networks sampled in forest fragments with different configurations is possibly related to the observed constancy of ant species in the central core of highly interacting species. Our results are compatible with other studies highlighting the structural stability of these facultative ant-plant networks mediated by EFNs in different spatial and temporal gradients. In short, our study shows that the structure of ant-plant interaction networks is relatively stable in the face of forest fragmentation, however the few structural changes detected should be understood as a warning, mainly because the functionality of this protective mutualism (i.e., food secretions in exchange for protection against herbivory) remains unknown.

Keywords: community ecology; ecological networks; landscape ecology; plant-animal interactions; tropical rainforest.

Introduction

Rainforest fragmentation has been a prominent topic of discussion between members of the scientific community for years (Skole and Tucker 1993; Laurance et al. 2001a; Vedovato et al. 2016). In general, historic forest fragmentation has occurred due to the subdivision of large areas of continuous forest generating an altered landscape composed of forest fragments with different configurations in terms of fragment area, edge irregularity, and connectivity (Klingbeil and Willig 2009). This process is usually accompanied by habitat loss which involves the outright removal of habitat patches (Bartlett et al. 2016). Habitat loss thus makes forest fragmentation one of the main drivers of biodiversity loss (Newbold et al. 2015). Productive activities in the Brazilian Amazon, such as logging, cattle ranching, and small-scale farming are the main types of land use responsible for this process of landscape change (Laurance et al. 2001a; Laurance et al. 2011).

This landscape configuration may lead to changes in plant and animal communities (Fischer and Lindenmayer 2007; Laurance et al. 2011). For instance, forest area reduction may directly affect the rate of local species losses, with small forest fragments tending to lose species more quickly (Stouffer et al. 2008), due to their inability to support viable populations of certain groups (Didham et al. 1998). Connectivity reduction between forest fragments and/or continuous forests also tends to negatively affect the maintenance of some populations, since it changes resource availability spatially and, in consequence, the foraging behavior or pollination success, for example, of these populations (Haddad et al. 1999; Martensen et al. 2008; Hadley and Betts 2011). Edge effect, generated by an abrupt transition between two adjacent ecosystems (Murcia 1995), is another important factor in the ecological processes of forest fragmentation (Laurance et al. 2011). In general, changes in biotic and abiotic conditions at the forest edge (Laurance 1991) may result in elevated rates of tree mortality and tree damage (Laurance et al. 1998), and abundance may be reduced near the forest edge for some animal species, such as insects (Fowler et al. 1993; Carvalho and Vasconcelos 1999) and birds (Laurance 2004). Proximity to the forest edge may result in an increase in the populations of some generalist and tolerant species (Bailey et al. 2014; Terraube et al. 2016).

Ants are frequently studied in forest fragments (Vasconcelos et al. 2006; Leal et al. 2012). In general, the population density, number and diversity of ant species decline with reductions in the area of forest fragments (Bruhl et al. 2003; Vasconcelos et al. 2006; Hill et al. 2011), and the number of ant species tends to decrease toward the fragment edge (Carvalho and Vasconcelos 1999; Brandão et al. 2011). Habitat loss and fragmentation may also change ant species composition (Vasconcelos et al. 2006; Leal et al. 2012), mainly due to local extinctions, or generalist or even invasive species colonization (i.e., biotic homogenization) (Carvalho and Vasconcelos 1999; Bruhl et al. 2003; Holwaya and Suarez 2006; Solar et al. 2015).

Many plant species, mainly in the tropics, secrete a liquid rich in carbohydrates and amino acids through specialized and non-floral glands called as a whole extrafloral nectaries (EFNs), which attracts different ant species (Koptur et al. 1998; Marques et al. 2015). In exchange for the food provided by the plant, some ants can protect their host plants against herbivores (Rico-Gray and Oliveira 2007). At the community level, these mutualistic associations are usually evaluated using a network approach, in which different ant and plant species are depicted as nodes and their interactions as links (Dáttilo et al. 2013a). This network approach has provided important information about the organization of these ant-plant interactions (Lange et al. 2013; Dáttilo et al. 2013a, b; Dáttilo et al. 2014a). For instance, some studies have recently shown that ant-plant networks are highly nested, indicating species with few links interact with a subset of interactive species with several interactions (Del-Claro et al. 2018). Biologically, nestedness describes the organization of the niche breadth of an interactive community, in which more nested networks tend to have the highest niche overlap (Blüthgen 2010). On the other hand, despite a growth in knowledge about the structure of ant-plant networks and the effects of habitat loss and forest fragmentation on the ant and plant communities, little is known about how these two anthropogenic processes affect ant-plant networks mediated by EFNs (but see Braschler et al. 2005; Falcão et al. 2015).

Changes in vegetation structure due to forest fragmentation, such as canopy cover reduction near the forest edge (Ewers and Banks-Leite 2013), may lead to structural changes of these ant-plant networks. For instance, Dáttilo and Dyer (2014) found an increase in the diversity of interactions in ant-plant networks mediated by EFNs due to canopy cover reduction. The number of interactions may also be affected by canopy cover reduction, since the quality and quantity of nectar secreted, which are the determinants of the attraction of ants to the plants (Marazzi et al. 2013), are directly related to the amount of canopy cover (Dáttilo et al. 2013b). Changes in the configuration of forest fragments, such as forest area reduction (Klingbeil and Willig 2009), may also affect the structure of these ant-plant networks. Sugiura (2010), in a study conducted on Japanese islands, showed that the connectance and nestedness values of ant-plant interaction networks mediated by EFNs tend to increase with decreasing island size. These results, although based on subtropical forest islands (Government of Japan 2010) that present an impenetrable matrix for ants and plants, at least suggested that researchers investigate the effect of rainforest fragmentation on these ant-plant networks.

The aim of this study was therefore to investigate the effects of configurations of rainforest fragments on the structure of ant-plant interaction networks mediated by EFNs in the Brazilian Amazon. We based our research questions on hypotheses related to the configuration of forest fragments and the forest structure within the fragments, here represented by canopy cover. Specifically, we postulated that larger, more connected, and less irregular forest fragments with higher canopy cover would present: (i) higher network size, since well-preserved forest fragments tend to support higher number species (Ahuatzin et al. 2019); (ii) lower number of interactions, because extrafloral nectaries are more active in higher light environments (e.g., edge and forest gaps) (Radhika et al. 2010); (iii) higher network specialization, since only a few dominant ant species could monopolize the scarce resources available (Dáttilo et al. 2014b); (iv) lower diversity of interactions, due to the lower number of species and interactions in these sites (Dáttilo and Dyer 2014); and (v) lower nestedness, due to the lower overlap of pairwise interactions (Dáttilo et al. 2013b).

Materials and methods

Study area

This study involved ten forest fragments, ranging in size from approximately 5 to 3,000 ha (Fig. 1 and Supplementary Material 1), situated in the west of the state of Acre, in the Brazilian Amazon. All forest fragments studied were created in the 1980s due to human activity. Most of the primary type of vegetation was converted to croplands and pasture during this period (Acre 2011). Due to the effect of the matrix composition on the focal fragments, we only selected forest fragments surrounded by pasture (Prevedello and Vieira 2010; Prugh et al. 2008). According to the Köppen (1936) climate classification, the region is classified as monsoon climate (Am), with an average rainfall of 1,450 mm per year (Macêdo et al. 2013) and marked seasonality, with most rainfall between November and March (Acre 2006). The average annual temperature is 24 ºC (INMET 2016) with daily variation around 9 ºC (Acre 2006). The region varies between 110 and 270 m.a.s.l. (Acre 2006). The predominant vegetation type is open tropical rainforest (i.e., open ombrophilous forest) dominated by native species of bamboo and/or with species of palm trees (Acre 2006).

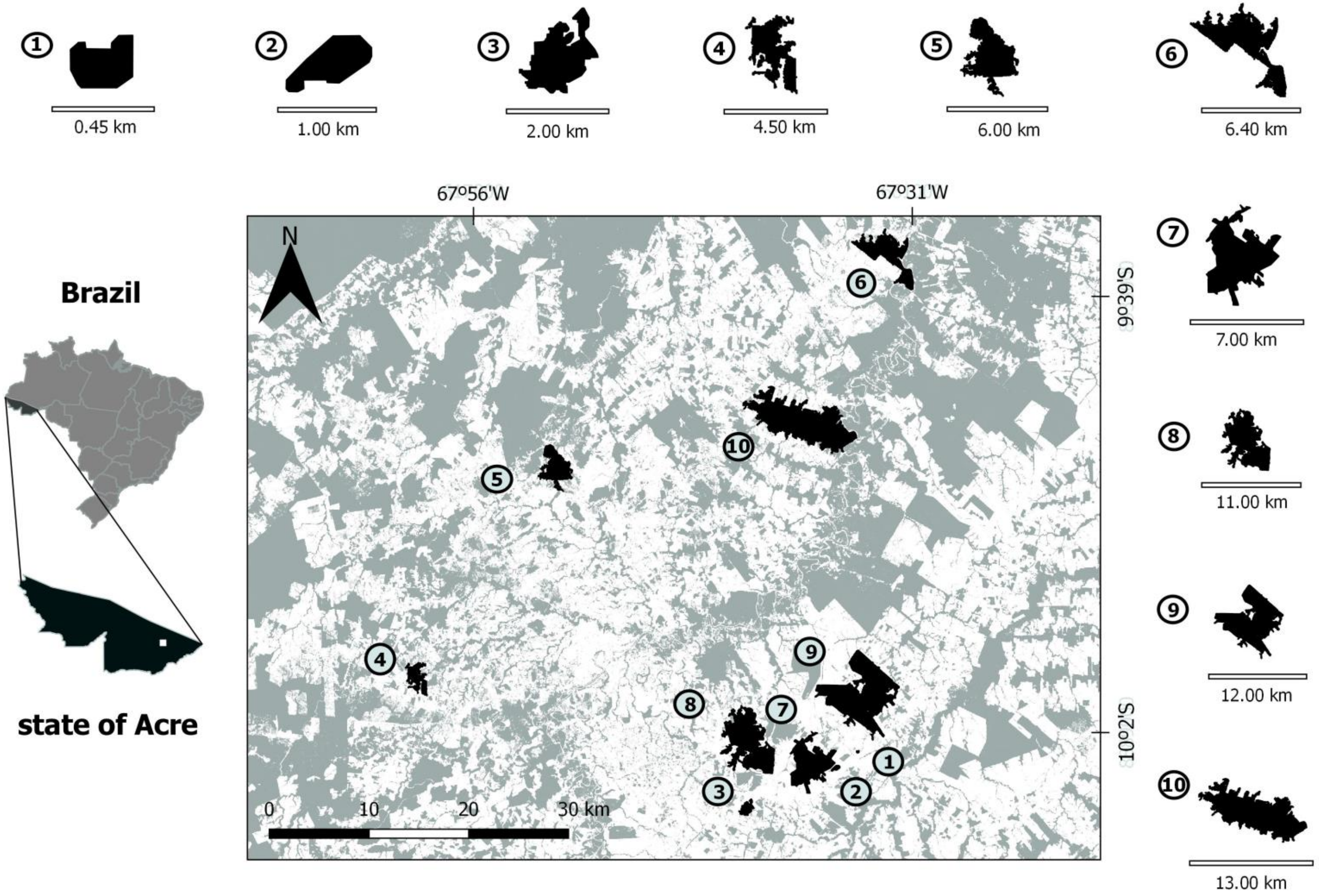

Fig. 1 Map of the study area showing all forest fragments sampled.

Sampling ant-plant interactions

We established a plot of 6,250 m^2 (250 x 25 m) in each forest fragment, at a minimum distance of 100 meters from the edge, except for the smallest forest fragment, which had a plot that began approximately 20 meters from the edge. We recorded all interactions between ants and EFN-bearing plants with a height accessible to the collector (ranging from 0.5 to 3m) between 09:00 am and 03:00 pm for each plot (Dáttilo and Dyer 2014). All EFN-bearing plants were observed for five minutes. When the interaction was confirmed, we collected all foraging ants and a plant sample for further identification (see below). Plants in which we did not detect the presence of EFNs, but which had immobile ants with mouthparts in contact with plant tissues for several minutes, were also collected for later confirmation of the presence of EFNs through stereoscope observations and literature review. Ants were manually collected with the aid of an entomological umbrella (Bestelmeyer et al. 2000) so as to record those that drop from the plant at the slightest sign of disturbance (Dáttilo and Dyer 2014). Considering the temporal variation in ant-plant networks mediated by EFNs (i.e., phenological variation) (Lange et al. 2013; Falcão et al. 2016), we sampled each plot twice (June 2016 and February 2017).

Ants were identified by species or morphospecies using identification keys (Fernández 2003; Wilson 2003; Bolton et al. 2006) and by comparison with specimens deposited at the Laboratório de Mirmecologia of the Centro de Pesquisas do Cacau, Brazil (CPDC Collection), where voucher specimens were deposited. Plants were identified at species or morphospecies level using identification guides (Ribeiro et al. 1999; Pennington et al. 2004) and by comparison with specimens deposited at the Herbário do Parque Zoobotânico (HPZ) of the Universidade Federal do Acre, Brazil. Voucher specimens of plants were deposited at the Herbário (FUEL) of the Universidade Estadual de Londrina, Brazil.

Canopy cover

In order to measure the canopy cover in the plots where the ant-plant interactions were sampled, we established a 250 m long transect within each plot. We installed this transect in the longitudinal direction of the plot, and maintained the same distance from the sides (12.5 m). In each transect, we installed ten subplots of 100 m^2 (10 x 10 m) at intervals of 15 m. The first and last 100 m^2 subplots were installed 7.5 m from the beginning and end of transects, respectively. We used the canopy cover because this variable can affect the light incidence into the forest understory and, therefore stimulate the production and secretion of EFNs (Dáttilo et al. 2013b). We estimated canopy cover using a spherical convex densiometer. The measurements were taken at the center and at the four edges of the 100 m^2 subplots. The mean of these five measurements was used to calculate the average canopy cover per sampling point. We used the average of all sampling points to characterize each fragment for canopy cover.

Landscape descriptors

We used LANDSAT8 satellite images (path/row: 002/067) with a 30 m resolution, acquired on 07/23/2016, in the QGIS Software 2.18.10 (QGis Development Team 2016) to describe the configuration of the forest fragments. The images were classified into two categories: forested areas (primary and secondary forests) and non-forested areas (water bodies, built areas, and pastures). We described the configuration of each forest fragment through metrics: forest fragment area (ha), edge irregularity index, and connectivity index. The forest fragment area was calculated based on its polygon shape. Patton's diversity index (DI) (Patton 1975) was adopted as the edge irregularity index, which evaluates the forest fragment regularity, defined as $DI = P/2(\sqrt{\pi A})$, where P = perimeter (m) and A = Area (m^2). To measure the isolation and fragmentation degree around the forest focal fragments, we used the index of connectivity named proximity (PROX) (Gustafson and Parker 1992), calculated by the following equation: $PROX = \sum_{s=1}^{n} \frac{a_{ijs}}{h_{ijs}^2}$

In this equation, a_{ijs} represents the area (m^2) of fragment *ijs* within specified neighborhood (m) of the focal fragment; and h_{ijs} represents the distance (m) between fragment *ijs* and the focal fragment based on fragment edge-to-edge distance. The connectivity index is dimensionless and considered the inverse, since the isolation decreases with its increase (Bender et al. 2003). We define buffers of 500 m around the focal fragment, established from the respective edge, to define the neighboring fragments considered for the calculation of the index. We used this buffer size for two main reasons: i) this distance explains the greater variation in the patterns of richness and diversity of ant species at the community level (Spiesman and Cumming 2008); and ii) it is equivalent to the greater observed distance of an ant gyne’s flight (Hölldobler and Wilson 1990). All forest fragment metrics were calculated using QGIS Software 2.18.10 (QGis Development Team 2016).

Ant-plant interaction networks

Initially, we combined the data of the two samples per plot on different dates, in order to maximize the number of EFN-bearing plants species in our samples, considering the annual variation in the extrafloral nectar production by different plant species (Falcão et al. 2016). To verify whether our sampling was adequate to describe the ant-plant networks of each plot studied, we then generated accumulation curves with the number of ant and plant species and the number of distinct pairwise of interactions, as a function of the number of plants sampled (Falcão et al. 2016). We used the non-parametric bootstrapping estimator with 1000 replicates (Gotelli and Colwell 2001) for all accumulation curves, to estimate the expected number of plant and ant species and interactions for each network.

The ant-plant interaction patterns were examined using an ecological network approach. The data of each plot was organized in a quantitative matrix in which the elements (aij) represented the frequency (number of times) that plant species *i* interacted with ant species *j* inside the plot (Bascompte et al. 2003). To evaluate the constancy of the central core of highly interacting species among the different forest fragments sampled, we categorized ant and plant species following the formula proposed by Dáttilo et al. (2013a): $G_c = (k_i - k_{mean})/\sigma_k$, where k_i = mean number of links for a given plant/ant species, k_{mean} = mean number of links for all plant/ant species in the network, and σ_k = standard deviation of the number of links for plant/ant species. When $Gc \geq 1$, the species presents a large number of interactions in relation to other species of the same trophic level, as a species constituting the generalist core. When $Gc < 1$, the species presents a lower number of interactions in relation to other species of the same trophic level, such as species constituting the periphery of networks.

We used three network descriptors established from a quantitative matrix (number of interactions, network specialization, and diversity of interactions), and two calculated from a binary matrix (network size and nestedness), to describe the patterns of ant-plant interactions in the fragment. In frequency matrices, as mentioned above, the elements represent the number of times in which a plant species interacted with an ant species within the plot, whereas in binary matrices the elements (1 or 0) represent the presence or absence of an interaction between a plant and an ant species within the plot. These binary-weighted descriptors are the most commonly used in studies dealing with ant-plant networks, and they cover a wide range of possible structures with complementary biological meanings (Del-Claro et al. 2016). Network size was calculated by multiplying the number of plant species by the number of ant species. The number of interactions represents the total interactions observed in the network, considering all ant and plant species. The diversity of interactions was calculated using an index (H') based on the Shannon's diversity index, which ranges from zero to infinity (Bersier et al. 2002). We estimated specialization using the H_2' index, which describes how species restrict their interactions from those randomly expected based on a partner's availability (Blüthgen et al. 2006). In this index, the low specialization of an ecological network represents values close to $H_2' = 0$, and total specialization is $H_2' = 1$. Nestedness, which evaluates whether selective ant species only visit a subset of plant individuals visited by the generalist ant species (Dáttilo et al. 2014c), was calculated using the *NODF*-metric (Nestedness Based on Overlap and Decreasing Fill) (Almeida-Neto et al. 2008), and ANINHADO software (Guimarães and Guimarães 2006), using binary matrices. The *NODF* significance was estimated using p-values based on the Null Model II (1000 randomizations), in which the probability of an interaction occurring is proportional to the observed number of interactions of both plant and ant species (Bascompte et al. 2003). These index values range from 0 (non-nested) to 100 (perfectly nested). The network specialization and diversity of interaction were calculated using the bipartite package (Dormann et al. 2017) in R 3.2.3 (R Core Team 2016).

Data analysis

To evaluate the effect of rainforest fragmentation on ant-plant networks, we related each network descriptor (network size, number of interactions, network specialization - H_2', diversity of interactions - H' and, nestedness - *NODF*) to the explanatory metrics of the configuration of forest fragments (fragment area, border index, and proximity index), and to canopy cover. Initially, we verified multicolinearity among all explanatory variables (fragment area, border index, proximity index, and canopy cover) using the variance inflation factor (VIF) value (Dormann et al. 2013). The VIF indicates the degree to which each explanatory variable is explained by another exploratory variable in the model. VIF values greater than 10 indicate high multicolinearity. As none of our exploratory variables showed collinearity between them, we included all four in the model selection. We used generalized linear models for all response variables (i.e., network descriptors). We used a negative binomial distribution for number of interactions and network size, due to an overdispersion of residues from our data, which occurs when the deviance of the response is greater than expected by the chosen distribution (Hinde and Demétrio 1998). We used a Gaussian distribution for network specialization - H_2', diversity of interaction - H', and nestedness - *NODF*. For all response variables, we ranked the candidate models using Akaike's information criterion corrected for small samples (AICc) (Akaike 1974), which considers the fit quality and the numbers of parameters included in the model. We adopted balanced model sets (i.e., all explanatory variables were present in the same number of models) without considering the interaction effects between them. We included a null model against a constant value in order to represent the absence of an effect. We estimated the Akaike Information Criterion corrected for small samples (AICc) (Akaike 1974), the ΔAICc, which is the difference between the AICc of each model against the best model (the lowest AICc), and the relative weight (wAIC), which represents the likelihood of a given model being the best among a set of concurrent models (Johnson and Omland 2004). We considered that models with ΔAICc < 2.0 and wAIC > 0.1 were plausible to explain the observed patterns (Burnham and Anderson 2002). When the null model was among the best selected models, we only considered the models that presented ΔAICc values smaller than the values of the null model and wAIC values higher than the values of the null model. Model selections were made according to Akaike's Information Criterion (AIC) using the MuMIn package (Bartoń 2016) and the model diagnostics were run with the RT4Bio package (Reis Jr. et al. 2013) in R 3.2.3 (R Core Team 2016).

Results

We observed 930 pairwise interactions between ants and EFN-bearing plants on the ten plots studied. We recorded a total of 56 ant species, distributed in 19 genera and seven subfamilies (Supplementary Material 2). The subfamily Myrmicinae comprised 48.21% of the total ant species (n= 27 ant species), followed by Formicinae (26.80%, n=15) and Dolichoderinae (12.50%, n= 7). Myrmicinae was also the subfamily that showed higher interaction frequency (64.62% of the total interactions, n= 601 interactions), followed by Ectatomminae (13.55%, n= 126) and Formicinae (8.90%, n= 83). We recorded a total of 148 plant species, distributed in 44 genera and 25 families, where liana was the plant habit with the highest number of species (60.14% of the plant species, n = 89 species) followed by trees (34.46%, n = 51) (Supplementary Material 3). The Fabaceae family comprised 37.84% of the total plant species (n= 56 plant species), followed by Bignoniaceae (25.68%, n= 38) and Malpighiaceae (6.80%, n= 10). Fabaceae was also the family that had a higher interaction frequency (40.97% of the total interaction, n= 381), followed by Bignoniaceae (16.70%, n= 155) and Malpighiaceae (14.00%, n= 130). The generalist core of ant species in the ant-plant networks tends to be more constant than the generalist core of plant species, between forest fragments. We observed that the generalist core of ant-plant networks ranged from one to five ant species, and from two to five plant species per forest fragment. Seven ant species were present in the generalist cores, taking into account all ten ant-plant networks evaluated, and the most common species was *Crematogaster brasiliensis* (present in the generalist core in 70% of the networks, n= 7 forest fragments), followed by *Crematogaster carinata* (60%, n= 6) and *Ectatomma tuberculatum* (50%, n= 5) (Table 1). Twenty-one plant species were present in the generalist cores of the ant-plant networks evaluated, and the most common species were *Bauhinia* sp.1 (90%, n= 9) followed by Polygonaceae sp.1 (50%, n= 5) and *Senegalia* sp.2 (50%, n= 5) (Table 1).

Table 1 Frequency of ant and plant species in generalist core of ant-plant networks sampled in 10 rainforest fragments located in the state of Acre, Brazilian Amazon, between June 2016 and February 2017, n= number of fragments where the species was part of the core of the network.

Generalist core plant species	Occurrence frequency	Generalist core ant species	Frequency
Bauhinia sp.1	90% (n= 9)	*Crematogaster brasiliensis*	70% (n= 7)
Polygonaceae sp1	50% (n= 5)	*Crematogaster carinata*	60% (n= 6)
Senegalia sp.2	50% (n= 5)	*Ectatomma tuberculatum*	50% (n= 5)
Fridericia sp.8	40% (n= 4)	*Crematogaster limata*	40% (n= 4)
Hirtella racemosa	40% (n= 4)	*Dolichoderus attelaboides*	10% (n= 1)
Banisteriopsis sp.2	40% (n= 4)	*Wasmannia auropunctata*	10% (n= 1)
Inga sp.5	40% (n= 4)	*Ochetomyrmex semipolitus*	10% (n= 1)
Palicourea sp.1	40% (n= 4)		
Senegalia sp.3	30% (n= 3)		
Fridericia sp.19	20% (n= 2)		
Memora sp.2	20% (n= 2)		
Zygia sp.1	20% (n= 2)		
Fridericia sp.6	20% (n= 2)		
Senegalia sp.8	20% (n= 2)		
Inga punctata	20% (n= 2)		
Aparisthmium cordatum	10% (n= 1)		
Fridericia sp.2	10% (n= 1)		
Inga sp.16	10% (n= 1)		
Senegalia sp.4	10% (n= 1)		
Bauhinia sp.4	10% (n= 1)		
Senegalia sp.7	10% (n= 1)		

According to the species and interactions accumulation curves estimated, we collected an average (Mean ± SD) of 84.30 ± 0.02% of the ant species (n=18.60 ± 3.60 species of the 22.04 ± 4.04 species estimated) and 80.60 ± 0.01% of the plant species (n= 31.90 ± 4.53 species of the 39.61 ± 5.81 species estimated). We observed 76.23 ± 0.01% of the interactions between ants and EFN-bearing plants (n= 63.70 ± 11.06 interactions of the 83.57 ± 14.44 interactions estimated), indicating that we had sampled enough ant and plant species and interactions to describe the ant-plant networks of each plot.

We describe the values of the network descriptors (network-size, number of interactions, network specialization - H_2', diversity of interactions - H', and nestedness - *NODF*) for each plot in Supplementary Material 4. Eighty percent (n= 8 fragments) of the ant-plant networks presented a significant value for *NODF*, when compared to null models (Supplementary Material 4).

The edge irregularity index was the best explanatory variable for nestedness (Table 2). Nestedness tended to be lower in plots established in forest fragments with a higher value of edge irregularity index (Fig. 2). For the other network descriptors, the null model was the best selected model (Table 2), and therefore, the other selected models were not considered. The results of all candidate models for each ant-plant network descriptor are in Supplementary Material 5.

Table 2 Summary of plausible models fitted to explain each ant-network descriptor in response to landscape structure metrics and forest structure (canopy cover) at 10 rainforest fragments located in the state of Acre, Brazilian Amazon. ΔAICc, df, and wAICc indicate the difference in corrected Akaike values, degrees of freedom of the model, and Akaike weights, respectively. The values in bold represent the best model selected for each of the network descriptors evaluated.

Response variable	Model	ΔAICc	d.f.	wAIC	Slope symbol
Number of interactions	**Null model**	**0**	**2**	**0.39**	
	Edge irregularity index	0.75	3	0.27	-
Network size	**Null model**	**0**	**2**	**0.59**	
Network specialization	**Null Model**	**0**	**2**	**0.51**	
	Fragment area	1.63	3	0.23	+
Diversity of interaction	**Null model**	**0**	**2**	**0.52**	
NODF	**Edge irregularity index**	**0**	**3**	**0.44**	-
	Null model	1.2	2	0.24	

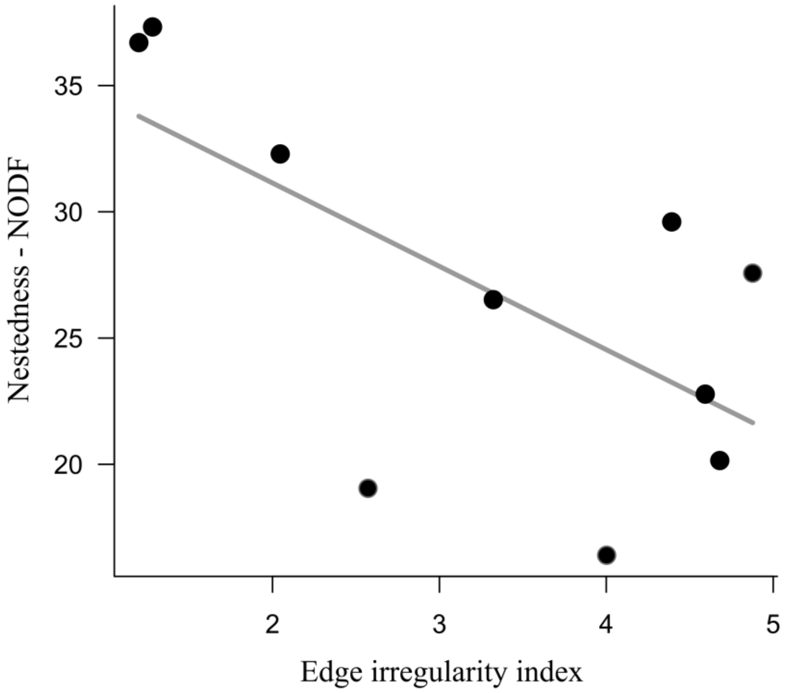

Fig. 2 Effects of the edge irregularity index on the nestedness (*NODF*), based on samples taken in 10 rainforest fragments located in the state of Acre, Brazilian Amazon.

Discussion

In general, we observed, contrary to our hypotheses, that the structure of ant-plant interaction networks mediated by EFNs remained stable in forest fragments with different configurations, in terms of fragment area, edge irregularity and connectivity. Of the five network descriptors we used (number of interactions, network size, network specialization, diversity of interactions, and nestedness), only nestedness was affected by a metric of the configuration of fragments. Specifically, we observed that nestedness decreased with increasing edge irregularity. We also did not find evidence that canopy cover shapes the interaction networks, suggesting that the self-organization of the networks is independent of local characteristics of canopy openness in our area of study.

The generalist core tended to be more constant among the sampled plots as regards ants rather than plants. Of the seven ant species observed in the generalist cores, four (57%) were core species in more than 40% of the networks. Of these four ant species, three are competitively superior due to their numerical dominance (*C. brasiliensis*, *C. carinata* and *Crematogaster limata*) (Parr 2008; Baccaro et al. 2012) and one due to aggressive displacement of competitors (*E. tuberculatum*) (Hossaert-Mckey et al. 2001; Bächtold and Alves-Silva 2013). This competitive nature is a feature of ant species of the generalist core (Dáttilo et al. 2014b), and is possibly one of the main factors responsible for the relative constancy of ant species in the studied networks. In addition, most of the ant species of our generalist cores belonged to the functional group Generalist Myrmicinae (Silvestre et al. 2003; Silva and Brandão 2010), and are highly tolerant of habitat modification (Hoffmann and Andersen 2003), which allows them to a competitive advantage, even in forest fragments with a high disturbance level. Of the 21 plant species observed in the generalist cores, 12 (57%) were classified as in the central core of highly interacting species in less than 20% of the networks. This lowest constancy in the generalist core of plant species is possibly related to changes in plant species composition due to forest fragmentation, since the reduction in forest fragment areas tends to increase the mortality rate of trees, especially near the forest edge (Laurance 1991; Ferreira and Laurance 1997), favoring fast-growing pioneer trees and liana growth (Laurance et al. 2001b; Laurance et al. 2006; Santo-Silva et al. 2016). The plant species turnover in South American tropical forests is also relatively high, with forests 60 km distant from each other (i.e., the maximum distance between our forest fragments), sharing only some 35% of species (Condit et al. 2002).

When evaluating the effects of forest fragmentation on the structure of ant-plant networks, we found, in general, a relative structural stability in the face of perturbation. This relative stability is possibly related to the low fidelity of ant species when foraging on EFN-bearing plants (Rico-Gray et al. 1998, Schoereder et al. 2010), and to the constancy of ant species in the generalist cores observed in our study, as previously discussed. In fact, the ant-plant networks mediated by EFNs are shaped by a few generalist species, which are essential for the maintenance of network stability (Díaz-Castelazo et al. 2010; Mello et al. 2011). Dáttilo et al. (2013a) observed that the structure of ant-plant networks mediated by EFNs remains stable throughout space, as does the generalist core of species. Their results indicated that the nonrandom pattern organization of these interacting assemblages does not change in function with variations in local and landscape environmental factors. Other studies have also detected the structural stability of these ant-plant networks mediated by EFNs over time (i.e., 20 years) (Díaz-Castelazo et al. 2013), between day and night periods (Dáttilo et al. 2014a), and after tropical hurricanes (Sánchez-Galván et al. 2012). More recently, Fagundes et al. (2018) also observed the limited effects of fire disturbances on the structure of ant-plant interaction networks mediated by EFNs. In our study, only nestedness was negatively related to the edge irregularity index. This finding is contrary to our initial hypothesis, in which we expected to find a positive relationship between nestedness and edge irregularity. It therefore seems that nestedness (a nonrandom pattern emerging from ant-plant interactions) is more vulnerable to edge irregularity than fragment area or connectivity. At forest edges, ecological communities present a narrow subset of pioneer and exotic plants (Santos et al. 2010), thus impoverishing plant assemblages, which could collapse ant-plant networks, as previously demonstrated by Pinho et al. (2017).We know that the structure of mutualistic networks allows a greater robustness to perturbation, a higher number of coexisting species, and a higher rate of return to initial equilibrium (Bascompte and Jordano 2013). Our results therefore provide more empirical evidence that ant-plant networks mediated by EFNs are also robust to habitat fragmentation and loss, at least in the spatial context of our landscapes; however, the thresholds of this robustness remain unknown. Corro et al. (2019) observed landscape effects on network specialization and on the diversity of interaction of ant-plant networks, but the authors used co-occurrence networks. It is also important to note that the forest fragments studied have a relatively low level of isolation, which may contribute to the structural stability of the networks studied.

We have provided some important insights into the effects of rainforest fragmentation on the structure of ant-plant interactions mediated by EFNs. In an interaction networks context, the ant species of the generalist core seem to be little affected by forest fragmentation compared to the plant species of the generalist core. Our results regarding the network descriptors indicate that, in general, the structure of ant-plant networks mediated by EFNs remains relatively stable in the face of the forest fragmentation, possibly due to the constancy of ant species in the generalist cores, however, the decrease in nestedness with increasing edge irregularity represents a warning, possibly related to an impoverishment of the plant assemblage on fragment edges, and the consequent collapse of the nonrandom pattern of these ant-plant networks. It is important to clarify that we limited our sampling to three meters high, and we recognize the potentially serious implications of this sampling limitation on our results. We thus suggest that future studies evaluate how ant-plant interactions are structured along a vertical stratification gradient within the forest and to test their vulnerability to fragmentation and habitat loss. In short, this study makes a valuable contribution to biodiversity and conservation, mainly because we show the vulnerability and robustness of tropical species-rich habitats to forest fragmentation.

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Supplementary material

Appendix S1 Location of the forest fragments sampled in State of Acre, Brazilian Amazon.

Fragment	Site	Geographic coordinates	Area (ha)
1	Senador Guiomard private fragment	10° 3'59.16"S and 67°59'20.12"W	5.26
2	Senador Guiomard private fragment	10° 4'52.34"S and 67°36'2.64"W	27.82
3	Senador Guiomard private fragment	10°6'58.02"S and 67°41'6.90"W	123.16
4	Forestry School	9°59'48.12"S and 67°59'20.12"W	332.15
5	Projeto de Assentamento Walter Arce	9°48'0.46"S and 67°51'26.95"W	681.05
6	Porto Acre private fragment	9°36'28.60"S and 67°34'6.15"W	1072.34
7	Catuaba Experimental Farm	10°04'48.9"S and 67°37'08.6"W	1282.42
8	Embrapa Acre	10°2'17.64"S and 67°40'54.24"W	1871.17
9	Senador Guiomard private fragment	10° 1'24.66"S and 67°35'48.66"W	2894.77
10	Humaitá Reserve	09°45'15.2"S and 67°39'44.9"W	3042.02

Appendix S2 Ant species present in the ant-plant networks sampled in 10 rainforest fragments located in the state of Acre, Brazilian Amazon, between June 2016 and February 2017.

Subfamily	Ant species	Frequency										
		F7	F8	F4	F3	F10	F6	F1	F2	F9	F5	Total
Dolichoderinae	*Azteca chartiflex*	0	0	0	0	0	0	0	1	0	0	**1**
	Azteca sp.1	5	2	0	0	0	6	0	6	1	3	**23**
	Dolichoderus attelaboides (Fabricius, 1775)	2	1	0	1	4	5	0	5	0	8	**26**
	Dolichoderus bispinosus (Olivier, 1792)	0	0	0	0	0	8	0	1	0	2	**11**
	Dolichoderus debilis Emery, 1890	0	0	0	0	0	6	10	0	0	0	**16**
	Dolichoderus quadridenticulatus (Roger, 1862)	0	0	0	0	1	0	0	0	0	0	**1**
	Dolichoderus septemspinosus Emery, 1894	1	0	0	0	0	1	0	2	0	0	**4**
Ecitoninae	*Eciton mexicanum* Roger, 1863	0	0	0	0	0	0	0	1	0	0	**1**
Ectatomminae	*Ectatomma tuberculatum* (Oliver, 1792)	14	21	1	28	2	0	13	9	24	11	**123**
	Gnamptogenys moelleri (Forel, 1912)	0	0	0	0	0	0	0	0	1	1	**2**
	Gnamptogenys sulcata (Smith, 1858)	0	0	0	0	0	0	0	0	0	1	**1**
Formicinae	*Brachymyrmex heeri* Forel, 1874	2	0	8	0	0	0	1	1	2	1	**15**
	Camponotus bidens Mayr, 1870	0	0	0	0	0	1	0	0	0	0	**1**
	Camponotus cingulatus Mayr, 1862	0	0	0	1	0	0	2	0	0	0	**3**
	Camponotus depressus Mayr, 1866	0	0	0	0	1	1	0	0	0	1	**3**
	Camponotus femoratus (Fabricius, 1804)	0	0	0	0	0	1	0	0	0	0	**1**
	Camponotus godmani Forel, 1899	0	0	0	0	0	0	0	1	0	0	**1**
	Camponotus latangulus Roger, 1863	4	3	0	1	0	10	2	11	2	0	**33**
	Camponotus nidulans (Fr. Smith, 1860)	0	1	0	0	2	5	0	0	2	2	**12**
	Camponotus prox. Novogranadensis	3	0	0	0	0	0	0	0	0	0	**3**
	Camponotus punctulatus andigenus Emery, 1903	1	0	0	0	0	1	0	0	1	1	**4**
	Camponotus sexguttatus (Fabricius, 1793)	0	1	0	0	0	0	0	0	0	0	**1**
	Camponotus sp.1	0	0	0	0	0	0	0	0	2	1	**3**

	Gigantiops destructor (Fabricius, 1804)	0	0	0	1	0	0	0	0	0	1	**2**
	Nylanderia guatemalensis (Forel, 1885)	0	0	0	0	0	1	0	0	0	0	**1**
Myrmicinae	*Cephalotes atratus* (Linnaeus, 1758)	0	1	0	0	0	0	0	0	0	0	**1**
	Cephalotes marginatus (Fabricius, 1804)	0	0	0	0	0	0	1	0	0	0	**1**
	Cephalotes pinelii (Guérin-Méneville, 1844)	1	0	0	0	0	0	0	0	0	0	**1**
	Cephalotus opacus Santschi, 1920	0	0	0	0	2	0	0	0	0	0	**2**
	Crematogaster brasiliensis Mayr, 1878	10	2	0	24	27	23	0	69	19	8	**182**
	Crematogaster carinata Mayr, 1862	0	5	26	40	16	12	69	1	0	9	**178**
	Crematogaster curvispinosa Mayr, 1862	0	0	0	0	0	2	0	0	0	0	**2**
	Crematogaster erecta Mayr, 1866	0	0	0	0	0	1	0	0	0	0	**1**
	Crematogaster flavosensitiva Longino, 2003	0	3	0	0	5	2	0	0	0	0	**10**
	Crematogaster limata Fr. Smith, 1858	14	8	1	0	2	16	1	8	12	16	**78**
	Crematogaster longispina Emery, 1890	2	0	0	1	2	0	9	0	0	0	**14**
	Crematogaster nigropilosa Mayr, 1870	1	0	4	1	1	0	0	1	0	0	**8**
	Crematogaster sp.1	0	0	0	0	2	0	0	0	0	0	**2**
	Megalomyrmex balzani Emery, 1894	0	0	0	0	2	0	0	0	0	0	**2**
	Ochetomyrmex semipolitus Mayr, 1878	2	7	2	2	9	1	7	1	2	5	**38**
	Pheidole (gr. *Fallax*) sp.1	1	0	0	1	0	0	0	0	1	0	**3**
	Pheidole (gr. *Fallax*) sp.2	2	0	0	0	0	0	0	0	0	0	**2**
	Pheidole (gr. Fallax) sp.3	0	0	1	0	2	0	1	1	0	0	**5**
	Pheidole (gr. *Fallax*) sp.4	0	1	0	0	1	0	1	2	0	1	**6**
	Pheidole (gr. *Flavens*) sp.1	0	1	0	0	0	0	0	0	0	0	**1**
	Pheidole radoszkowskii Mayr, 1884	0	0	2	1	1	0	1	0	1	3	**9**
	Solenopsis globularia (Smith, 1858)	0	0	1	0	0	0	1	0	0	0	**2**
	Solenopsis sp.1	0	6	2	0	0	0	0	1	0	0	**9**
	Solenopsis sp.3	1	3	0	0	0	0	1	0	4	0	**9**
	Wasmannia auropunctata (Roger, 1863)	2	5	3	2	8	3	3	0	3	5	**34**
Ponerinae	*Neoponera carinulata* (Roger, 1861)	0	0	0	0	2	0	0	0	0	0	**2**
	Neoponera unidentada Mayr, 1862	2	0	1	0	2	2	0	0	0	0	**7**

	Odontomachus haematodus (Linnaeus, 1758)	1	0	0	0	0	7	2	0	0	0	**10**
	Odontomachus hastatus (Fabricius, 1804)	0	0	0	0	0	0	0	0	0	1	**1**
Pseudomyrmecinae	*Pseudomyrmex oculatus* (Smith, 1855)	0	0	0	0	0	2	0	1	1	0	**4**
	Pseudomyrmex tenuis (Fabricius, 1804)	3	1	0	2	0	1	3	2	1	1	**14**

Appendix S3 Plant species present in the ant-plant networks sampled in 10 rainforest fragments located in the state of Acre, Brazilian Amazon, between June 2016 and February 2017. Habitat: T = tree; L = liana; H = herb; ? = undefined.

Family	Plant species	Frequency										Total	Habitat
		F1	F2	F3	F4	F5	F6	F7	F8	F9	F10		
Anonaceae	Anonaceae sp.1	0	0	0	0	1	0	0	0	0	0	**1**	T
Araceae	*Philodendron* sp.1	0	0	0	0	0	0	0	0	0	1	**1**	L
Bignoniaceae	*Fridericia* sp.1	0	0	0	0	0	0	1	0	0	0	**1**	L
	Fridericia sp.2	0	8	7	0	0	0	3	0	2	0	**20**	L
	Fridericia sp.3	3	0	0	0	0	0	0	4	0	0	**7**	L
	Fridericia sp.4	0	0	0	0	1	0	0	0	0	1	**2**	L
	Fridericia sp.5	0	0	1	0	2	1	0	4	0	2	**10**	L
	Fridericia sp.6	0	0	0	0	0	0	0	5	0	0	**5**	L
	Fridericia sp.7	0	0	0	0	0	0	1	0	3	0	**4**	L
	Fridericia sp.8	10	6	0	1	1	11	0	1	0	0	**30**	L
	Fridericia sp.9	1	0	0	0	1	0	0	0	2	0	**4**	L
	Fridericia sp.10	1	0	0	0	0	0	0	0	0	0	**1**	L
	Fridericia sp.11	2	0	0	0	0	1	0	0	0	0	**3**	L
	Fridericia sp.12	1	0	0	0	0	0	0	0	2	0	**3**	L
	Fridericia sp.13	1	1	2	1	0	0	0	0	1	0	**6**	L
	Fridericia sp.14	2	0	0	0	0	0	0	0	0	0	**2**	L
	Fridericia sp.15	1	0	0	0	0	1	0	0	0	0	**2**	L
	Fridericia sp.16	0	3	0	0	0	0	0	0	1	0	**4**	L
	Fridericia sp.17	0	0	0	0	0	1	0	0	1	0	**2**	L
	Fridericia sp.18	0	0	0	0	0	1	0	0	0	0	**1**	L
	Fridericia sp.19	0	0	1	0	7	0	0	0	0	0	**8**	L
	Fridericia sp.20	0	0	2	0	0	0	0	0	0	0	**2**	L
	Fridericia sp.21	0	1	0	0	0	0	0	2	0	0	**3**	L

	Fridericia sp.22	0	0	0	1	0	0	0	0	0	0	**1**	L
	Fridericia sp.23	0	0	0	0	1	0	0	0	0	0	**1**	L
	Fridericia sp.24	0	0	0	0	2	0	0	0	0	0	**2**	L
	Fridericia sp.25	0	0	0	0	0	0	0	1	0	0	**1**	L
	Fridericia sp.26	0	0	1	0	0	0	0	3	0	0	**4**	L
	Fridericia sp.27	0	0	0	0	0	0	0	1	0	0	**1**	L
	Fridericia sp.28	0	0	1	0	0	0	0	0	0	0	**1**	L
	Fridericia sp.29	0	0	0	0	1	0	0	0	0	0	**1**	L
	Fridericia sp.30	0	0	0	0	0	0	0	0	0	1	**1**	L
	Fridericia sp.31	0	0	0	0	1	0	0	0	2	0	**3**	L
	Fridericia sp.32	0	0	0	0	0	0	0	2	1	0	**3**	L
	Fridericia sp.33	0	1	0	0	0	0	0	0	0	0	**1**	L
	Fridericia sp.34	0	0	0	0	1	0	0	0	0	0	**1**	L
	Fridericia sp.35	0	1	0	0	3	0	0	1	1	0	**6**	L
	Memora sp.1	1	0	0	0	0	0	0	0	0	0	**1**	L
	Memora sp.2	0	1	0	0	5	0	0	0	0	0	**6**	L
	Memora sp.3	0	0	0	0	0	1	0	0	0	0	**1**	L
Chrysobalanaceae	*Hirtella racemosa* Lam.	0	0	0	0	9	1	0	6	0	0	**16**	T
	Hirtella sp.1	0	0	0	0	0	0	0	1	0	1	**2**	T
	Hirtella sp.2	0	0	0	0	0	0	0	0	1	0	**1**	T
Combretaceae	*Buchenavia* sp.1	1	0	0	0	0	0	0	0	0	0	**1**	T
Convolvulaceae	*Ipomoea philomega* (Vell.) House	0	0	0	0	0	0	0	0	1	0	**1**	L
	Ipomoea regnellii Meisn.	0	0	0	0	1	0	0	0	0	0	**1**	L
Costaceae	*Costus scaber* Ruiz & Pav.	0	1	0	1	0	0	1	1	0	2	**6**	H
Cucurbitaceae	Cucurbitaceae sp.1	0	0	1	0	0	0	0	0	0	0	**1**	L
	Gurania sp.1	0	0	1	0	0	1	0	0	0	0	**2**	L
Euphorbiaceae	*Acalypha* sp.1	0	0	0	0	0	0	0	0	4	0	**4**	?
	Aparisthmium cordatum (A. Juss.) Baill.	0	10	0	0	0	0	0	0	0	0	**10**	T

	Dalechampia sp.1	1	0	0	0	0	0	0	0	0	0	**1**	L
	Omphalea diandra L.	0	0	0	0	0	2	2	0	0	2	**6**	L
Fabaceae	*Senegalia* sp.1	3	0	1	1	3	0	3	1	1	3	**16**	L
	Senegalia sp.2	7	4	2	1	0	6	9	3	8	1	**41**	L
	Senegalia sp.3	3	7	0	1	2	1	1	0	6	0	**21**	L
	Senegalia sp.4	1	0	1	11	0	0	3	0	0	0	**16**	L
	Senegalia sp.5	0	0	0	0	0	0	0	0	1	0	**1**	L
	Senegalia sp.6	0	0	0	0	1	0	0	0	0	0	**1**	L
	Senegalia sp.7	2	0	0	4	0	0	1	0	0	0	**7**	L
	*Senegalia*sp.8	1	0	1	3	1	0	3	1	5	0	**15**	L
	Senegalia sp.9	0	1	0	0	0	0	0	0	0	0	**1**	L
	Bauhinia sp.1	33	15	16	0	0	9	17	2	7	0	**99**	L
	Bauhinia sp.2	0	0	0	1	0	0	0	0	0	0	**1**	L
	Bauhinia sp.3	0	0	0	0	0	1	0	0	0	0	**1**	L
	Bauhinia sp.4	0	0	0	3	0	0	0	0	0	0	**3**	L
	Bauhinia sp.5	0	0	0	0	0	0	0	1	0	0	**1**	L
	Bauhinia sp.6	0	0	0	0	0	2	0	0	0	0	**2**	L
	Centrosema sp.1	0	0	0	0	0	0	3	0	0	0	**3**	L
	Centrosema sp.2	0	0	0	0	0	0	2	0	0	0	**2**	L
	Centrosema sp.3	0	0	2	0	0	0	0	0	0	0	**2**	L
	Erythrina sp.1	0	0	1	2	0	0	0	0	0	0	**3**	T
	Fabaceae sp.1	0	1	3	0	0	0	0	0	0	0	**4**	?
	Inga acreana Harms	0	0	0	0	0	1	0	0	0	1	**2**	T
	Inga alba (Sw.) Willd.	0	1	3	1	1	0	0	0	0	0	**6**	T
	Inga calantha Ducke	0	0	0	0	1	0	0	0	0	1	**2**	T
	Inga capitata Desv.	1	1	0	0	0	2	0	0	0	0	**4**	T
	Inga chartacea Poepp.	0	0	1	0	0	0	0	0	0	2	**3**	T
	Inga densiflora Benth.	0	1	2	0	0	2	0	1	0	0	**6**	T
	Inga edulis Mart.	2	0	1	1	0	0	1	0	1	0	**6**	T

Inga heterophylla Willd.	1	0	0	0	0	0	0	0	0	0	**1**	T
Inga lateriflora Miq.	1	0	2	1	2	0	3	0	1	0	**10**	T
Inga laurina (Sw.) Willd.	6	2	2	4	0	0	0	0	0	4	**18**	T
Inga microcoma Harms	0	0	0	0	1	0	0	0	0	0	**1**	T
Inga punctata Willd.	0	0	0	1	0	0	0	0	0	5	**6**	T
Inga sertulifera DC.	0	0	2	0	3	3	0	0	0	0	**8**	T
Inga suaveolens Ducke	1	0	0	0	0	0	0	0	0	0	**1**	T
Inga tenuistipula Ducke	0	0	0	0	2	0	1	1	0	3	**7**	T
Inga sp.1	0	0	0	0	0	1	0	0	0	0	**1**	T
Inga sp.2	0	0	0	0	0	0	0	0	2	0	**2**	T
Inga sp.3	0	0	0	0	0	2	1	0	0	0	**3**	T
Inga sp.4	1	0	0	1	0	0	0	1	0	2	**5**	T
Inga sp.5	0	0	0	0	1	1	1	6	0	6	**15**	T
Inga sp.6	0	0	0	0	0	0	0	0	0	1	**1**	T
Inga sp.7	0	0	2	0	0	1	0	0	0	0	**3**	T
Inga sp.9	1	0	0	0	0	0	0	0	2	0	**3**	T
Inga sp.10	0	0	0	1	0	0	0	1	0	0	**2**	T
Inga sp.12	0	0	0	2	0	0	0	0	0	0	**2**	T
Inga sp.13	0	0	0	2	0	0	0	0	0	0	**2**	T
Inga sp.14	0	0	1	0	0	0	1	0	1	0	**3**	T
Inga sp.15	0	1	0	0	0	0	0	0	0	0	**1**	T
Inga sp.16	0	0	3	0	0	0	0	0	0	0	**3**	T
Inga sp.18	0	0	0	0	0	1	0	0	0	0	**1**	T
Inga sp.20	0	0	0	0	0	1	0	0	0	0	**1**	T
Senna sp.1	0	0	0	0	1	0	0	0	0	0	**1**	T
Zygia sp.1	0	0	1	0	4	0	0	0	1	0	**6**	T
Zygia sp.2	0	3	0	0	0	0	0	0	0	0	**3**	T
Zygia sp.3	0	1	0	0	0	0	0	0	0	0	**1**	T
Zygia sp.4	0	1	0	0	0	0	0	0	0	0	**1**	T

Lecythidaceae	*Gustavia augusta* L.	0	0	0	0	2	0	0	0	2	0	**4**	T
	Lecythidaceae sp.1	0	0	0	0	3	0	0	0	0	0	**3**	T
Loganiaceae	*Strychnos panurensis* Sprague & Sandwith	0	0	0	1	1	0	0	0	0	0	**2**	L
Malpighiaceae	*Banisteriopsis* sp.1	0	0	0	0	0	4	0	0	0	0	**4**	L
	Banisteriopsis sp.2	2	0	0	3	0	44	3	2	0	41	**95**	L
	Banisteriopsis sp.3	1	1	0	0	0	1	1	0	0	0	**4**	L
	Banisteriopsis sp.4	0	1	1	0	0	0	0	0	0	0	**2**	L
	Banisteriopsis sp.5	0	9	1	0	0	0	0	0	0	0	**10**	L
	Banisteriopsis sp.6	0	0	0	0	3	0	0	0	0	0	**3**	L
	Banisteriopsis sp.7	0	0	0	0	1	0	0	0	0	0	**1**	L
	Banisteriopsis sp.8	0	0	0	0	0	0	0	0	0	1	**1**	L
	Heteropterys sp1	0	1	0	0	2	0	0	0	0	0	**3**	L
	Tetrapterys sp.1	1	0	0	0	0	0	2	0	0	4	**7**	?
Malvaceae	*Byttneria benensis* Britton	0	0	0	0	0	5	0	0	1	0	**6**	L
Menispermaceae	*Abuta* sp.1	0	0	0	0	0	0	0	0	1	0	**1**	L
	Abuta sp.2	0	0	0	0	0	0	0	1	0	0	**1**	L
Ochinaceae	*Ouratea* sp.2	0	0	0	0	3	1	0	1	0	0	**5**	T
Olacaceae	*Heisteria* sp.1	0	0	0	2	3	0	0	1	0	1	**7**	?
Passifloraceae	*Dilkea* sp.1	0	0	1	0	0	0	0	0	0	0	**1**	L
	Passiflora coccinea Aublet.	1	0	2	0	0	0	0	0	0	0	**3**	L
	Passiflora sp.1	1	0	0	0	0	0	0	0	0	0	**1**	L
	Passiflora sp.2	0	0	0	0	0	0	1	0	0	0	**1**	L
	Passiflora sp.3	0	0	0	0	0	1	0	0	0	0	**1**	L
	Passiflora sp.4	0	0	0	0	0	0	0	1	0	0	**1**	L
Polygonaceae	*Polygonaceae* sp.1	26	38	31	0	2	1	2	8	11	0	**119**	?
	Polygonaceae sp.2	0	0	0	0	0	0	1	0	0	0	**1**	T
Rhamnaceae	*Gouania frangulifolia* Radlk.	0	0	0	0	0	0	0	0	1	0	**1**	L
Rubiaceae	*Tocoyena* sp.1	0	0	1	0	0	0	0	0	0	0	**1**	T

	Palicourea sp.1	0	3	2	0	1	5	2	6	2	5	**26**	T
Sapindaceae	*Paullinia* sp.1	0	0	0	0	0	0	2	1	0	0	**3**	L
	Paullinia sp.2	3	0	0	1	1	0	0	0	0	1	**6**	L
	Paullinia sp.3	3	0	0	0	0	0	0	0	0	0	**3**	L
	Paullinia sp.5	1	0	0	0	0	0	0	0	0	0	**1**	L
	Paullinia sp.6	0	0	0	0	0	0	0	0	1	0	**1**	L
	Serjania clematidea Triana & Plach.	0	0	0	0	0	0	0	0	2	2	**4**	L
Solanaceae	*Solanum* sp.1	0	0	1	0	0	0	0	0	0	0	**1**	?
Vitaceae	*Cissus* sp.1	0	0	0	0	0	0	0	1	0	0	**1**	L
Volchysiaceae	*Qualea grandiflora* Mart.	0	0	2	0	0	0	2	0	0	0	**4**	T
	Volchysia sp.1	0	0	0	0	0	1	0	0	0	0	**1**	T

Appendix S4. Network size, number of interaction, network specialization, diversity of interaction and nestedness (NODF) of the ant-plant networks sampled in 10 rainforest fragments located in the state of Acre, Brazilian Amazon, between June 2016 and February 2017.

Fragment	Network size	Number of interactions	Network specialization	Diversity of interaction	NODF
1	648	128	0.15	3.81	36.70*
2	580	125	0.19	3.70	37.32*
3	518	106	0.17	3.75	32.29*
4	312	52	0.26	3.54	22.78
5	798	81	0.15	4.24	16.40
6	816	118	0.15	4.21	27.57*
7	609	74	0.21	3.96	19.05*
8	576	72	0.20	4.04	20.15*
9	561	79	0.27	3.97	26.52*
10	525	94	0.21	3.78	29.60*
Mean±SD	594.2 ±136.28	82.90±33.08	0.20±0.04	3.90±0.23	26.84±7.27

*Significant descriptor value (95% CI).

Appendix S5. Model selection analysis to explain the five ant-network descriptors in response to landscape structure metrics and forest structure at 10 rainforest fragments located in the state of Acre, Brazilian Amazon. The ΔAICc, df, and wAICc indicate the difference in corrected Akaike values, degrees of freedom of the model, and Akaike weights, respectively. Ed= Edge irregularity index; Co= Connectivity index; Ar= Fragment area; Cc= Canopy cover. The values in bold represent the models selected for each of the network descriptors evaluated.

Response variable	Model	ΔAICc	d.f.	wAIC	Slope symbol
Number of interaction	**null model**	**0**	**2**	**0.396**	
	Ed	**0.75**	**3**	**0.273**	**-**
	Co	2.91	3	0.093	-
	Cc	3.02	3	0.087	-
	Ar	3.04	3	0.087	-
	Ed+Co	6.45	4	0.016	-Ed+Co
	Ed+Cc	6.55	4	0.015	-Ed-Cc
	Ar+Ed	6.72	4	0.014	-Ar-Ed
	Cc+Co	8.12	4	0.007	-Cc-Co
	Ar+Cc	8.33	4	0.006	-Ar-Cc
	Ar+Co	8.51	4	0.006	-Ar-Co
	Ed+Cc+Co	15.31	5	0.000	-Ed-Cc+Co
	Ar+Ed+Co	15.4	5	0.000	-Ar-Ed+Co
	Ar+Ed+Cc	15.54	5	0.000	-Ar-Ed-Cc
	Ar+Cc+Co	16.91	5	0.000	-Ar-Cc-Co
	Ar+Ed+Cc+Co	30.28	6	0.000	-Ar-Ed-Cc+Co
Network size	**null model**	**0**	**2**	**0.597**	
	Cc	2.75	3	0.151	-

	Co	4.2	3	0.073	+
	Ar	4.27	3	0.070	-
	Ed	4.28	3	0.070	+
	Cc+Co	8.33	4	0.009	-Cc+Co
	Ed+Cc	8.42	4	0.009	Ed-Cc
	Ar+Cc	8.66	4	0.008	Ar-Cc
	Ed+Co	10.07	4	0.004	-Ed+Co
	Ar+Co	10.12	4	0.004	-Ar+Co
	Ar+Ed	10.26	4	0.004	-Ar+Ed
	Ed+Cc+Co	17.32	5	0.000	Ed-Cc+Co
	Ar+Cc+Co	17.33	5	0.000	Ar-Cc+Co
	Ar+Ed+Cc	17.42	5	0.000	Ar+Ed-Cc
	Ar+Ed+Co	19.03	5	0.000	-Ar-Ed+Co
	Ar+Ed+Cc+Co	32.32	6	0.000	-Ar+Ed-Cc+Co
Network specialization	**null model**	**0**	**2**	**0.514**	
	Ar	**1.63**	**3**	**0.228**	**+**
	Ed	3.88	3	0.074	+
	Co	3.91	3	0.073	+
	Cc	4.16	3	0.064	+
	Ar+Ed	7.59	4	0.012	Ar-Ed
	Ar+Co	7.59	4	0.012	Ar-Co
	Ar+Cc	7.63	4	0.011	Ar-Cc
	Ed+Co	9.85	4	0.004	Ed+Co
	Cc+Co	9.87	4	0.004	Cc+Co
	Ed+Cc	9.87	4	0.004	Ed+Cc
	Ar+Ed+Co	16.59	5	0.000	Ar-Ed-Co
	Ar+Ed+Cc	16.59	5	0.000	Ar-Ed+Cc
	Ar+Cc+Co	16.59	5	0.000	Ar+Cc-Co
	Ed+Cc+Co	18.83	5	0.000	Ed+Cc+Co

	Ar+Ed+Cc+Co	31.58	6	0.000	Ar-Ed+Cc-Co
Diversity of interaction	**null model**	**0.00**	**2**	**0.521**	
	Ed	2.81	3	0.127	+
	Co	2.9	3	0.122	+
	Ar	3.59	3	0.086	+
	Cc	3.73	3	0.081	-
	Ed+Cc	6.32	4	0.022	Ed-Cc
	Cc+Co	7.42	4	0.013	-Cc+Co
	Ar+Cc	8.54	4	0.007	Ar-Cc
	Ed+Co	8.69	4	0.007	Ed+Co
	Ar+Ed	8.74	4	0.007	Ar+Ed
	Ar+Co	8.79	4	0.006	Ar+Co
	Ar+Ed+Cc	15.19	5	0.000	Ar+Ed-Cc
	Ed+Cc+Co	15.29	5	0.000	Ed-Cc+Co
	Ar+Cc+Co	16.15	5	0.000	Ar-Cc+Co
	Ar+Ed+Co	17.63	5	0.000	Ar+Ed+Co
	Ar+Ed+Cc+Co	30.17	6	0.000	Ar+Ed-Cc+Co
NODF	**Ed**	**0.00**	**3**	**0.449**	**-**
	null model	**1.20**	**2**	**0.247**	
	Co	2.38	3	0.137	-
	Ar	4.60	3	0.045	-
	Cc	5.09	3	0.035	-
	Ed+Cc	5.84	4	0.024	-Ed+Cc
	Ar+Ed	5.96	4	0.023	Ar-Ed
	Ed+Co	6.00	4	0.022	-Ed+Co
	Cc+Co	8.34	4	0.007	-Cc-Co
	Ar+Co	8.35	4	0.007	-Ar-Co
	Ar+Cc	10.42	4	0.002	-Ar-Cc
	Ar+Ed+Co	14.80	5	0.000	Ar-Ed+Co

Ed+Cc+Co	14.83	5	0.000	-Ed+Cc+Co
Ar+Ed+Co	14.96	5	0.000	Ar-Ed+Co
Ar+Cc+Co	17.32	5	0.000	-Ar-Cc-Co
Ar+Ed+Cc+Co	29.80	6	0.000	Ar-Ed+Cc+Co

GENERAL FINAL CONSIDERATIONS

The results obtained in this study indicate that a network approach allows an efficient evaluation of ant-plant interactions mediated by extrafloral nectaries at community level, however, some care should be taken to avoid inappropriate inferences. The results point to the need to establish adequate criteria for the definition of the data category to be used, which consider mainly the biology of the interacting organisms and the research question. Another important factor to be considered in these studies dealing with ant-plant interactions mediated by extrafloral nectaries is the sampling period, due to the effects of rainfall seasonality on the extrafloral nectaries of different plant species. Therefore, in order to guarantee that most of the interaction possibilities of a locality are present in the netwoks, the sampling period should consider the phenological variation of these glands, directly related to the rainfall seasonality.

GENERAL APPENDIX

General appendix 1 – Manuscript “The dilemma of binary or weighted data in interaction networks” in the journal Ecological Complexity” published in the journal Ecological Complexity.

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Original Research Article

The dilemma of binary or weighted data in interaction networks

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ABSTRACT

Despite the increasing number of studies dealing with interaction networks in the last few years, there is still a lack of knowledge about how their structural organization are affected by changes in binary or weighted data. To fill this gap, we collected ants foraging on plants with extrafloral nectaries in 10 sites within the Brazilian Amazon to evaluate if the generality, vulnerability, nestedness, and modularity observed in these ant-plant networks could be affected by changes in data categories. Specifically, we used three matrices built by different data categories: (i) binary data (i.e., presence or absence of an interaction between a plant and an ant species); (ii) frequency data (i.e., number of times in which a plant species interacted with an ant species); and (iii) abundance data (i.e., number of workers of an ant species recorded foraging on a plant species). In general, when analyzing different matrix categories, we observed changes in the structural organization of the studied ant-plant interaction networks. Surprisingly, however, at the species level, both categories of weighted data (i.e., frequency and abundance data) seem to be equally appropriate for describing the role of ant species. Our results highlight the need to expand the discussion about data categories in ecological interaction studies to understand how different data categories may lead to different ecological interpretations.

1. Introduction

In recent years the number of studies dealing with interaction networks has increased, where species are depicted as nodes and their interactions by links (Dáttilo and Rico-Gray, 2018). Such complex network approach provides important information about structural organization and the functionality of species interactions (Vázquez et al., 2009), and a more integrative framework of ecological and evolutionary processes at a community level (Raimundo et al., 2018). The structural organization of these interaction networks is defined through different network descriptors (Antoniazzi et al., 2018). The biological information needed to calculate these network descriptors is systematized in interaction matrices in which the row vectors represent species from one trophic group (e.g., plants) and the column vectors represent species from another trophic group (e.g., animals). Often, these matrices are expressed in a binary (1 when an interaction is recorded and 0 otherwise) or weighted way, the latter considering both frequency (i.e., number of times in which a plant species interacted with an animal species over a spatial or temporal gradient, for instance) or abundance data (i.e., number of individuals of an animal species recorded interacting with a plant species). In binary matrices, all interactions are considered ecologically equivalent (Almeida-Neto and Ulrich, 2011) since the strength of the interaction is not represented. On the contrary, weighted entries in an interaction matrix take into account different factors such as partner's preference (i.e., resource selection) and abundance (Blüthgen, 2010), which makes them more biologically realistic since the intensity of such interactions is recorded (Dáttilo and Rico-Gray, 2018). It is thus necessary to investigate how different data types affect the properties of species interaction matrices, since the amount of theoretical and empirical information available regarding the use of binary or weighted data in studies of interaction networks is still limited.

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According to Corso et al. (2015), the nestedness metric used with binary matrices (Nestedness Based on Overlap and Decreasing Fill - NODF) (Almeida-Neto et al., 2008) is a good predictor of the network descriptor used with weighted matrices (Weighted Nestedness Metric Based on Overlap and Decreasing Fill - WNODF) (Almeida-Neto and Ulrich, 2011), since there are positive correlations between the nestedness values calculated from these two matrix categories. On the contrary, some authors have questioned the use of binary matrices because of the probable influence of the interaction frequency on the structural organization of interaction networks (Almeida-Neto and Ulrich, 2011; Bascompte et al., 2006; Blüthgen, 2010; Dáttilo et al., 2014a; Del-Claro et al. 2018; Staniczenko et al., 2013). For instance, Dáttilo et al. (2014a) found lower nestedness values in weighted ant-plant networks compared to binary networks. However, the dataset compiled by the authors involved matrices with both frequency and abundance data, which could lead to biased conclusions on the structure of weighted ant-plant interaction networks. In this context, it is worth noting the importance of adopting relevant matrix criteria that consider the biology of the interacting organisms. Studies involving social insects mention that the individual is represented by the colony (including all members such as queens, males, workers, and soldiers) (Anderson and McShea, 2001). Therefore, researchers sampling ant species in a locality usually use frequency/occurrence data (i.e., number of times a species is captured independently in the samples) rather than abundance data (i.e., total number of workers captured in all samples) (Greenslade and Thompson, 1981; Greenslade and Halliday, 1983; Underwood and Fischer, 2006). The choice of this criterion, to the detriment of absolute abundance, is mainly justified by the social behavior of these organisms, which results in an extreme spatial clumping of ant workers within samples (Andersen, 1991; Longino et al., 2002). In other words, the amount of specimens in a sample does not absolutely reflect the distribution and quantity of nests (i.e., individuals) in the environment.

Despite the many discussions about weighted data in studies of ant communities, as described above, there is still a lack of knowledge about how the use of different matrices (i.e., binary, frequency, or abundance) should affect the structure and ecological interpretation of interaction networks involving ants and plants. Of the different types of ecological interactions between ants and plants (Mayer et al., 2014; Nelsen et al., 2018; Rico-Gray and Oliveira, 2007), ant-plant interactions mediated by extrafloral nectaries are the most studied using an interaction networks approach (Dáttilo and Rico-Gray, 2018; Del-Claro et al., 2016). In this interaction, ants protect host plants against herbivore attacks (Koptur et al., 1998). In exchange for protection, host plants secrete a substance rich in carbohydrates and amino acids from plant glands called extrafloral nectaries, which serves as a food source for ants (Fagundes et al., 2017; Kwok and Laird, 2012). In a network approach, numerous studies have shown that this ant-plant interaction networks mediated by extrafloral nectaries usually present a non-random asymmetric pattern (hereafter called “nestedness”) (Guimarães et al., 2006), in which specialist species tend to interact with generalist species (Bascompte and Jordano, 2007). Besides that, these ant-plant networks also exhibit low modularity and, consequently, a high overlap among interacting species (Díaz-Castelazo et al., 2013). In other words, these ant-plant networks are not structured in subgroups of ant species (i.e., modules) strongly associated with a particular set of plant species (Díaz-Castelazo et al., 2013).

Taking the ant-plant interactions mediated by extrafloral nectaries as the object of study, we investigated whether the structural organization (i.e., generality, vulnerability, nestedness and modularity) observed in these interaction networks could be affected by changes in binary or weighted data. For this, we used empirical ant-plant networks obtained through a standardized sampling protocol, whose ant-plant interactions data were systematized in three different matrix categories: (i) binary data (i.e., presence or absence of an interaction between a plant and an ant species); (ii) frequency data (i.e., number of times in which a plant species interacted with an ant species within the sampling site); and (iii) abundance data (i.e., number of workers of an ant species recorded foraging on a plant species). Moreover, we also tested if the centrality (i.e., a measure of the importance of a species within the network) of ant species within each network was different when analyzed with different matrix categories. Our findings will represent a valuable tool for future studies dealing with ant-plant interaction networks since, by using a standardized sampling protocol, we show how the structure of ant-plant networks varies according to changes in binary or weighted data.

2. Material and methods

2.1. Study area

This study was carried out in 10 sites situated in the west of the State of Acre, Brazilian Amazon (Appendix S1). According to Köppen's (1936) climate classification, the climate in our study area is classified as monsoon climate (Peel et al., 2007), with an average rainfall of 1450 mm per year (Macêdo et al., 2013) and a marked seasonality, with most rainfall falling between November and March (Acre, 2006). Average annual temperature is 24.5 °C (INMET, 2016), with a daily variation of around ± 7.5 °C (Acre, 2006). Elevation varies between 110 and 270 m.a.s.l. (Acre, 2006). The predominant vegetation types are open tropical rainforest (Open Ombrophilous Forest), which can be dominated by bamboo and/or palm trees, and dense tropical rainforest (Dense Ombrophilous Forest) (Acre, 2006).

2.2. Sampling design

At each site, a plot of 6250 m^2 (250 × 25 m) was established, located at least 100 m from the edge of the forest, except for the smaller site, where a plot was placed approximately 20 m from the edge due the reduced size of this forest fragment (5.26 ha). Each plot was intensively sampled, where we recorded all interactions between ants and plants with extrafloral nectaries at a height accessible for the collector. Although the relationship between plant size and ant abundance feeding on extrafloral nectaries is not necessarily a rule (Dáttilo et al., 2014b), since the ant attraction by plants is mainly influenced by the quality and quantity of the extrafloral nectar secreted (Díaz-Castelazo et al., 2005; Fagundes et al., 2017), we opted to control plant size. Therefore, we only sampled plants located in the understory and belonging to the same size group (ranging from 0.5 to 3 m) (Dáttilo and Dyer, 2014), regardless of their habits. All plants with extrafloral nectaries belonging to this size group were carefully observed for five minutes to record the occurrence of at least one ant worker. When an interaction was confirmed, all ants present, together with a botanical sample, were collected for further identification. Those plant species whose extrafloral nectaries were not detected in the field, but which had immobile ants with mouth-parts in contact with plant tissues for several minutes (Falcão et al., 2016), were also collected, as well as the respective ants. Subsequently, we confirmed the presence of extrafloral nectaries through a literature review and observations under a stereoscope. Ants were manually collected with the aid of an entomological umbrella to record those ants that drop from the plant at the slightest sign of disturbance (Dáttilo and Dyer, 2014). Considering the phenological and structural variation in ant-plant networks throughout the year (Falcão et al., 2016; Lange et al., 2013), we performed two samplings in each site between June 2016 and February 2017. All samplings were carried out from 9:00 A.M. to 3:00 P.M.

2.3. Data analysis

For the analysis, we combined the data from the two sampling events performed in each site to ensure that most of the ant-plant interaction possibilities of each site were represented in the networks.

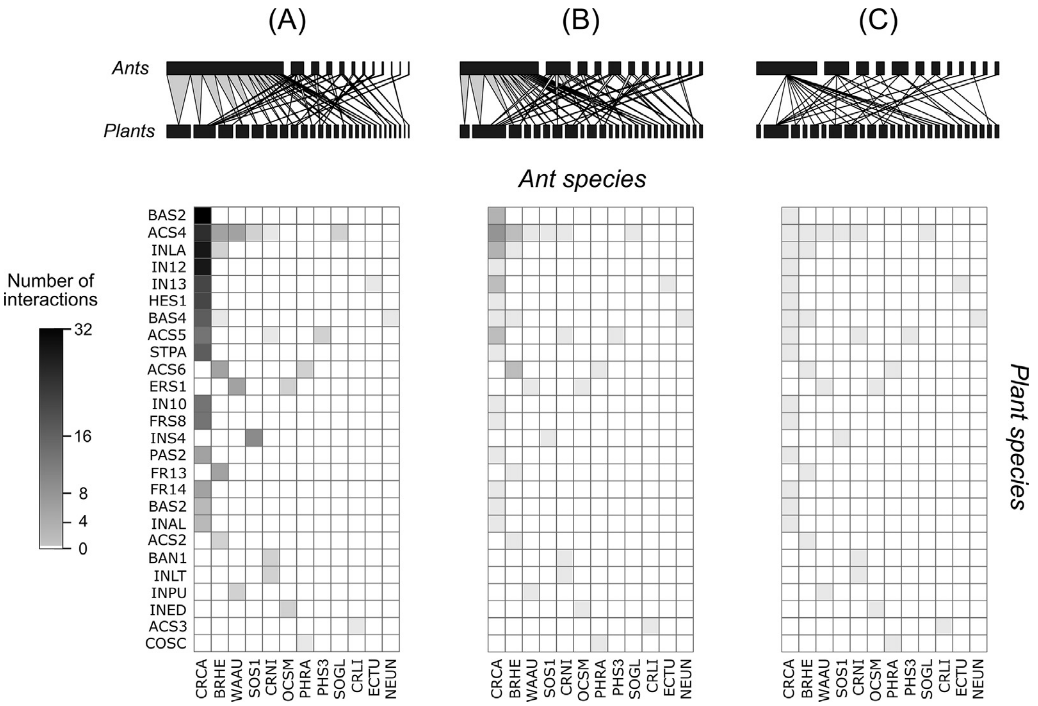

Fig. 1. Ant-plant interaction matrices generated from different data categories: (A) abundance, (B) frequency, and (C) binary data at the Forestry School (site 4), located in the state of Acre, Brazilian Amazon. Above each matrix, its bipartite network is presented. Darker cells represent a higher number of interactions. The number of interactions in each matrix category represents: (i) abundance data – the number of workers of an ant species recorded foraging on a plant species; (ii) frequency data - the number of times in which a plant species interacted with an ant species; and (iii) binary data – the presence or absence of an interaction between a plant and an ant species. In bipartite networks, a box size represents the marginal total number of links made by an ant species or received by a plant species, and the width of the edges is proportional to the total number of interactions recorded between each pair-wise combination of ant species and plant species. Species codes are shown in Appendix S2. The species in the bipartite networks are in the same order as in the matrix representation.

Initially, to verify whether we sampled enough ant and plant species and interactions to describe the ant-plant networks of each site studied, we generated accumulation curves with the numbers of ant and plant species and the number of distinct pairwise interactions as a function of the number of plants sampled (Falcão et al., 2016). For all accumulation curves, we used the non-parametric bootstrapping estimator with 1000 iterations (Gotelli and Colwell, 2001) to estimate the expected numbers of plant and ant species and interactions (Falcão et al., 2016).

To describe ant-plant interaction patterns in each site, we used interaction matrices comprised of the total number of (A) ants and (P) plants (i.e., A × P matrix), where plant species are in rows and ant species are in columns (Dormann et al., 2017). Using the same sampling, we built three different matrix categories W_{ij}: (1) binary data (where an element W_{ij} of such a matrix is 1 if plant species i and ant species j interact and zero otherwise); (2) frequency data (where W_{ij} = number of times which the plant species i interacted with the ant species j inside the sampling site); and (3) abundance data (where W_{ij} = total number of workers of the ant species j recorded foraging on the plant species i inside the sampling site). See Fig. 1 for the differences in the ant-plant networks generated from different matrix categories (abundance, frequency, and binary matrices).

To calculate the ant-plant network structure from different matrix categories, we estimated the following descriptors for each network: generality, vulnerability, nestedness, and modularity. We choose these network descriptors because they can be calculated from qualitative and quantitative data (Almeida-Neto et al., 2008; Almeida-Neto and Ulrich, 2011; Bersier et al., 2002; Dormann and Strauss, 2014; Guimerà et al., 2004). Besides that, they are often used in studies dealing with ant-plant networks and cover a wide range of possible structures with complementary biological interpretations (Del-Claro et al., 2016, 2018).

We estimated ant generality (G) and plant vulnerability (V) with binary data, based on Bersier et al. (2002), as the mean number of plants per ant and the mean number of ants per plant, respectively. Based on Bascompte and Jordano (2013), adapted from Bersier et al. (2002), we calculated the quantitative versions of generality and vulnerability as follows:

$$G_{qw} = \sum_{j=1}^{n_1} \frac{n_{1\,j}}{m} 2^{H_j}$$

$$V_{qw} = \sum_{i=1}^{n_2} \frac{n_{2\,i}}{m} 2^{H_i}$$

In these equations, m is the total number of interactions in the matrix, n_1 is the total number of (A) ants, n_2 is the total number of (P) plants, n_{1j} is the number of interactions of a given i ant, n_{2i} is the number of interactions of a given j plant. H_j and H_i are the Shannon entropy. We choose these network descriptors (generality and vulnerability) because they express the diversity in effective number of species, in this case effective number of interactions (Jost, 2006). We estimated the degree of nestedness in our networks with binary data, using the *NODF*-metric method (Nestedness Based on Overlap and Decreasing Fill) (Almeida-Neto et al., 2008) with the ANINHADO software (Guimarães and Guimarães, 2006). For weighted networks, we calculated nestedness using the *WNODF*-metric method (Weighted Nestedness Metric Based on Overlap and Decreasing Fill) (Almeida-Neto and Ulrich, 2011). The values of both nestedness indices range from 0 (non-nested) to 100 (perfectly nested). We used the modularity index (M) based on Simulated Annealing (Guimerà et al., 2004) to calculate the modularity for networks with binary data, using the software MODULAR (Marquitti et al., 2014). The QuanBiMo (Q) algorithm (Dormann and Strauss, 2014) was used to test the modularity of weighted networks (abundance and frequency data). This algorithm calculates the modularity for weighted networks using the Likelihood and Simulated Anneling-Monte Carlo approach (Dormann and Strauss, 2014). Both modularity indices (M and Q) range from 0 (no subgroups) to 1 (totally separated subgroups). Generality, vulnerability, *WNODF*, and Q algorithm were calculated with the "*bipartite*" package (Dormann et al., 2017) available in R 3.2.3 (R Core Team, 2016).

To standardize the differences in connectance and heterogeneity of interactions between networks, which could affect the binary and weighted values of nestedness and modularity, we calculated the Z scores of these network descriptors to allow cross-network comparisons. The Z-transformed score is defined as follows: $Z = [x - \mu] / \sigma$, where x

is the observed index value, μ is the mean of the values from simulated matrices, and σ is the standard deviation of the values from simulated matrices (Almeida-Neto et al., 2008). The simulated matrices used to calculate μ and σ were generated by different null models for binary and weight networks. We used different null models because the algorithms that are used to randomize binary matrices are not useful for randomizing weighted matrices. For instance, when a network contains only 1 s the weighted null model will identify that the observed network as identical to the null model. This is because the null model is going to reflect the fact that the network contains little or no information. For binary networks, we generated 1000 networks according to the Null Model II available in the ANINHADO software, in which the probability of an interaction occurring is relative to the observed number of interactions of both plant and ant species (Bascompte et al., 2003). For weighted networks, we used two different null models, of which one was more restrictive than the other. For the most restrictive null model, we generated 1000 networks using the *r2dtable* algorithm (Patefield, 1981), which holds the marginal totals constant (i.e., observed row and column totals) whilst allowing connectance to vary. For the less restrictive null model, we generated 1000 networks using the *vaznull* algorithm (Vázquez et al., 2007), which restricts only the connectance that is the same as that in the original network, being relatively more similar to the null model adopted for the binary matrices. We used these two different null models for weighted matrices to verify that the possible differences between network descriptors calculated from binary and weighted matrices are not only artifacts of the differences between metrics and null models. We estimated the significance of the network descriptors calculated from binary data using *P*-values (based on the null models) and from weighted data using *Z* scores (values $\geq$ 2) (Dormann and Strauss, 2014). Both statistical tests (*P*-values and *Z* scores) are based on null modeling, and used to verify the null hypothesis that the observed results differ from that expected by chance. The main difference is the correction of the observed values of nestedness (WNODF) and modularity (*Q*) calculated with weighted data and standardized by the calculation of the *Z* scores values. This correction is performed due to the dependence of these network descriptors (WNODF and *Q*) on factors such as network size (i.e., number of species), number of links and total number of interactions observed (Dormann et al., 2009). Using the *Z* score values, we can also verify the magnitude of the difference between the null model and the observed network descriptor. According to Dorman and Strauss (2014), a *Z* score value of 7 (e.g., for modularity) means that the observed modularity is 7 standard deviations higher than would be expected from random networks. Values above 2 are considered significantly modular. The use of *P* values does not consider the magnitude of the difference, but since binary networks contain less information than weighted networks, its use is not generalized with the use of *Z* scores to control the effect of interaction strength in these kind of matrices.

Subsequently, we investigated possible variations in the values of the network descriptors between different matrix categories (binary data, abundance data, and frequency data), using linear mixed-effects models. We considered the values of each network descriptor as a response variable and the different matrix categories as fixed factor. The identity of each site was included in the models as a random factor. Model significance was tested by using Wald χ^2 tests. Subsequently, contrast tests were performed to verify the pairwise differences between the three matrix categories used. We also performed residual analyses and tested for overdispersion (Crawley, 2012) to evaluate model prediction. Linear mixed-effects models were created with the "*lme4*" package (Bates et al., 2017), Wald χ^2 tests were performed with the "*car*" package (Fox et al., 2016), contrasts tests with the "*phia*" package (Rosario-Martinez et al., 2015), and residual analysis of linear mixed-effects models with the "*DHARMa*" package (Hartig, 2017) available in R 3.2.3 (R Core Team, 2016).

We also performed a sensitivity analysis to help evaluate the effects of different weighted data (frequency and abundance) on the detection of nestedness and modularity. We analyzed the behavior of these network descriptors under successive link weight thresholds (cut-off values) used to define a binary matrix (Cantor et al., 2017). To perform comparisons between the different weighted matrix categories, we first standardized the link weight distribution of all weighted networks (10 networks with frequency data and 10 networks with abundance data, relative to the 10 sampling sites) by dividing the link weights by the maximum weight of each system ($a_{ij} \in [0, 1]$). Subsequently, for each sampling site, we defined 10 binary matrices from the respective original frequency matrix, one for each of the 10 weight cut-offs defined at 0.1 intervals. The same procedure was performed for the abundance matrix of each sampling site. For example, for a cut-off value of 0.2, all links with a weight below this limit were excluded ($a_{i\,j} < 0.2 \rightarrow a_{ij} = 0$) or otherwise maintained ($a_{ij} \geq 0.2 \rightarrow ai_j = 1$). According to Cantor et al. (2017), a system may be considered nested or modular if the properties are present, regardless of the value used as a threshold to build binary networks, despite link weight heterogeneity.

Finally, we also calculated two measures to describe the role of ants (i.e., species level network descriptors) in the networks: individual species specialization (*d'*) and species strength (*ss*). The first measure (*d'*) describes the degree of interaction specialization at the species level by the deviation of the actual interaction frequencies from a null model, which assumes that all partners are used in proportion to their availability (Blüthgen et al., 2006). The second measure (*ss*) is defined as the sum of dependencies of each species and aims to quantify the relevance of a species across all its partners (Bascompte et al., 2006). Both measures were calculated with abundance and frequency matrices, using the "*bipartite*" package (Dormann et al., 2017) available in R 3.2.3 (R Core Team, 2016). To evaluate whether the role of ant species in the networks varies between abundance and frequency data, we performed Pearson´s correlation test between *d'* calculated from the abundance matrix and *d'* calculated from the frequency matrix. The same procedure was adopted for species strength and for the absolute values of abundance and frequency of each species. Specifically, in the case of a positive relationship between both abundance and frequency data, it is because, in general, using different types of weighted data does not change the observed role of a species in the network.

3. Results

We recorded a total of 56 ant species (or morphospecies) belonging to 19 genera and seven subfamilies (Appendix S3). The subfamily Myrmicinae comprised 48.21% of the total ant species (n = 27 ant species), followed by Formicinae (26.80%, n = 15) and Dolichoderinae (12.50%, n = 7). In terms of plants, we recorded a total of 148 plant species (or morphospecies) distributed in 44 genera and 25 families (Appendix S4). The family Fabaceae comprised 37.84% of the total plant species (n = 56 plant species), followed by Bignoniaceae (25.68%, n = 38) and Malpighiaceae (6.80%, n = 10). According to the species and interaction accumulation curves performed, we collected an average (mean ± SD) of 84.30 ± 0.02% of the ant species (n = 18.60 ± 3.60 species of the 22.04 ± 4.04 species estimated) and 80.60 ± 0.01% of the plant species (n = 31.90 ± 4.53 species of the 39.61 ± 5.81 species estimated). For interactions, we observed 76.23 ± 0.01% of the interactions between ants and plants with extrafloral nectaries (n = 63.70 ± 11.06 interactions of the 83.57 ± 14.44 interactions estimated), indicating that we sampled sufficient ant and plant species as well as interactions to adequately describe the ant-plant networks of each sampling site.

The generality values (*G*) differed when analyzed with different matrix categories (binary and weighted data) (Linear mixed-effects models: χ^2 = 14.647, d.f. = 2, $P < 0.001$). However, when we performed contrast tests, we detected significant differences of this descriptor only between binary (generality = 8.83 ± 2.74, mean ± SD) and frequency matrices (generality = 7.24 ± 1.78) and between binary and abundance matrices (generality = 6.71 ± 2.42), but not

Table 1

Nestedness, modularity, generality, and vulnerability of ant-plant networks, calculated from matrices with binary and weighted data (abundance and frequency) sampled in 10 sites located in the state of Acre, Brazil. We used the null model II to calculate the *Z* score values of nestedness and modularity with binary data. For the weighted data, we used two different null models based on the *r2dtable* and *vaznull* algorithm.

Matrix category	Network descriptor	Null model	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10
Abundance	WNODF		23.229	23.465	17.662	12.809	6.665	18.301	8.141	9.453	10.262	18.26
	Z score$_{\text{nestedness}}$	*r2dtable* algorithm	−5.802	−4.644	−5.603	−6.291	−6.05	−3.096	−4.433	−5.05	−6.163	−4.616
	Z score$_{\text{nestedness}}$	*vaznull* algorithm	−3.075	−2.833	−1.992	−2.623	−2.845	−0.406	−2.291	−0.935	−2.579	−1.891
	Q		0.220*	0.230*	0.317*	0.324*	0.540*	0.406*	0.584*	0.630*	0.485*	0.359*
	Z score$_{\text{modularity}}$	*r2dtable* algorithm	12.687	10.509	10.398	12.819	17.377	17.978	18.664	22.82	16.401	16.34
	Z score$_{\text{modularity}}$	*vaznull* algorithm	7.565	6.007	2.37	6.272	1.421	6.5	4.06	2.62	3.161	7.363
	Generality		10.471	7.999	9.204	9.699	5.947	5.261	3.542	5.134	4.912	4.912
	Vulnerability		2.374	2.512	2.622	1.601	2.625	5.918	3.038	2.545	2.394	3.632
Frequency	WNODF		19.856	22.735	14.188	10.038	2.734	14.228	4.573	7.251	7.855	10.368
	Z score$_{\text{nestedness}}$	*r2dtable* algorithm	0.324	−0.356	0.414	−0.087	0.108	1.178	−1.03	1.334	−1.286	−1.116
	Z score$_{\text{nestedness}}$	*vaznull* algorithm	0.401	0.266	0.014	0.502	0.071	1.794	−0.719	1.332	−0.313	−1.024
	Q		0.273	0.29	0.333	0.474	0.549	0.387	0.521	0.558	0.494	0.375
	Z score$_{\text{modularity}}$	*r2dtable* algorithm	−1.859	0.186	−0.549	0.323	−0.106	−0.374	0.813	1.803	1.225	0.31
	Z score$_{\text{modularity}}$	*vaznull* algorithm	−3.115	1.712	−2.622	−0.805	−1.694	−2.044	−1.1	0.053	−0.834	−1.147
	Generality		9.156	8.14	9.869	7.987	6.681	5.63	5.075	6.652	8.566	4.623
	Vulnerability		3.419	3.435	2.864	2.218	2.851	7.416	3.599	2.912	2.684	5.116
Binary	NODF		36.700*	37.320*	32.290*	22.78	16.4	27.570*	19.050*	20.150*	26.520*	29.600*
	Z score$_{\text{nestedness}}$	null model II	5.075	4.591	4.135	1.305	1.703	4.649	2.026	2.017	3.143	3.365
	M		0.479	0.485	0.498	0.585	0.548	0.504	0.568	0.586	0.523	0.501
	Z score$_{\text{modularity}}$	null model II	−0.761	−0.577	−0.878	0.502	−0.54	−0.391	0.037	0.73	−0.541	−0.243
	Generality		11.849	10.015	12.967	7.769	11.849	6.358	5.373	6.467	9.233	6.387
	Vulnerability		4.068	4.224	2.541	2.282	4.068	6.432	3.576	2.967	2.7	4.742

* Significant descriptor value (95% CI).

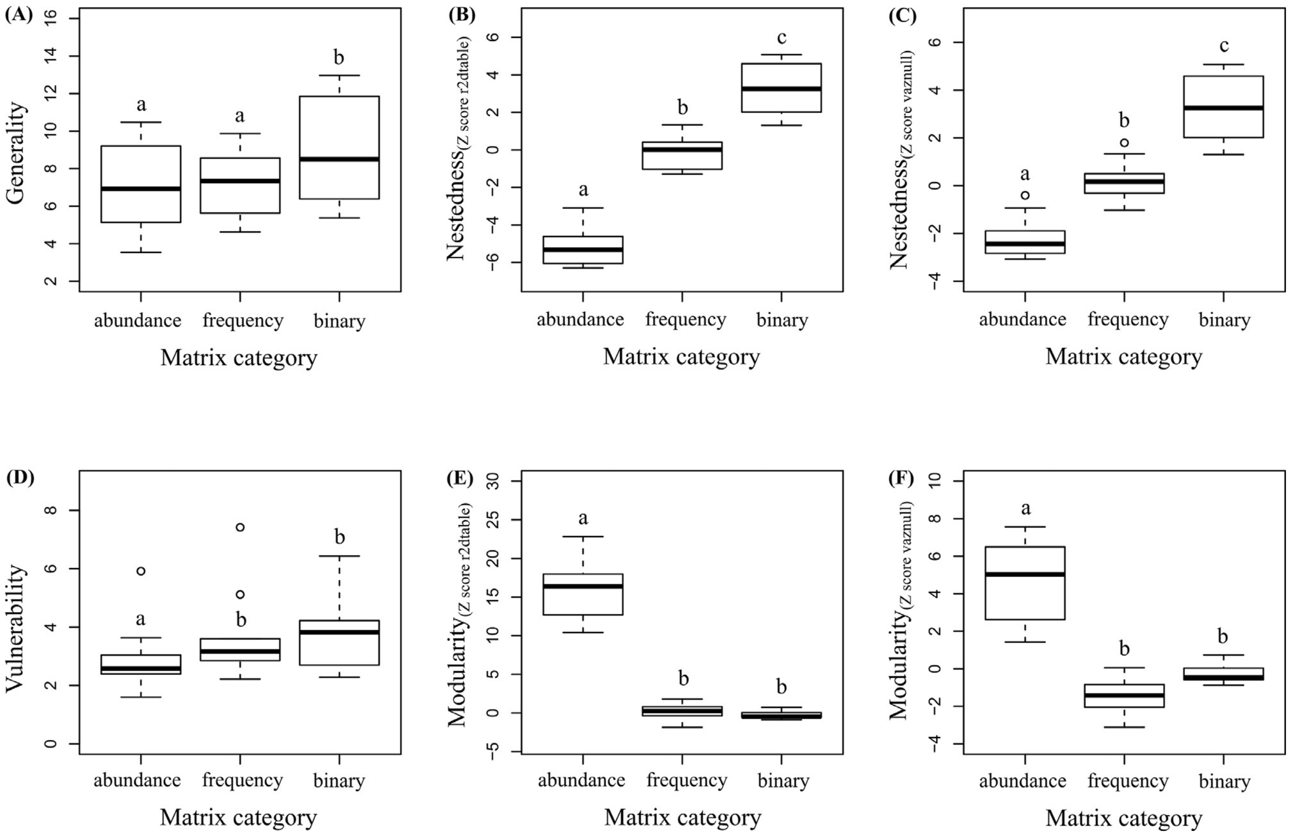

Fig. 2. Values of the four network descriptors (generality, vulnerability, nestedness, and modularity) calculated from three different matrix categories (binary, frequency, and abundance). To calculate the *Z* score values of nestedness and modularity with binary data, we used the null model II, while for weighted data, we used two different null models: one more restrictive model (*r2dtable* algorithm) (B and E) and one less restrictive model (*vaznull* algorithm) (C and F). The lines represent the first and fourth quartiles, the box represents the second and third quartile, and the line within the box represents the median for each category. The different letters above the boxes represent statistical differences between the categories, calculated using the contrast test.

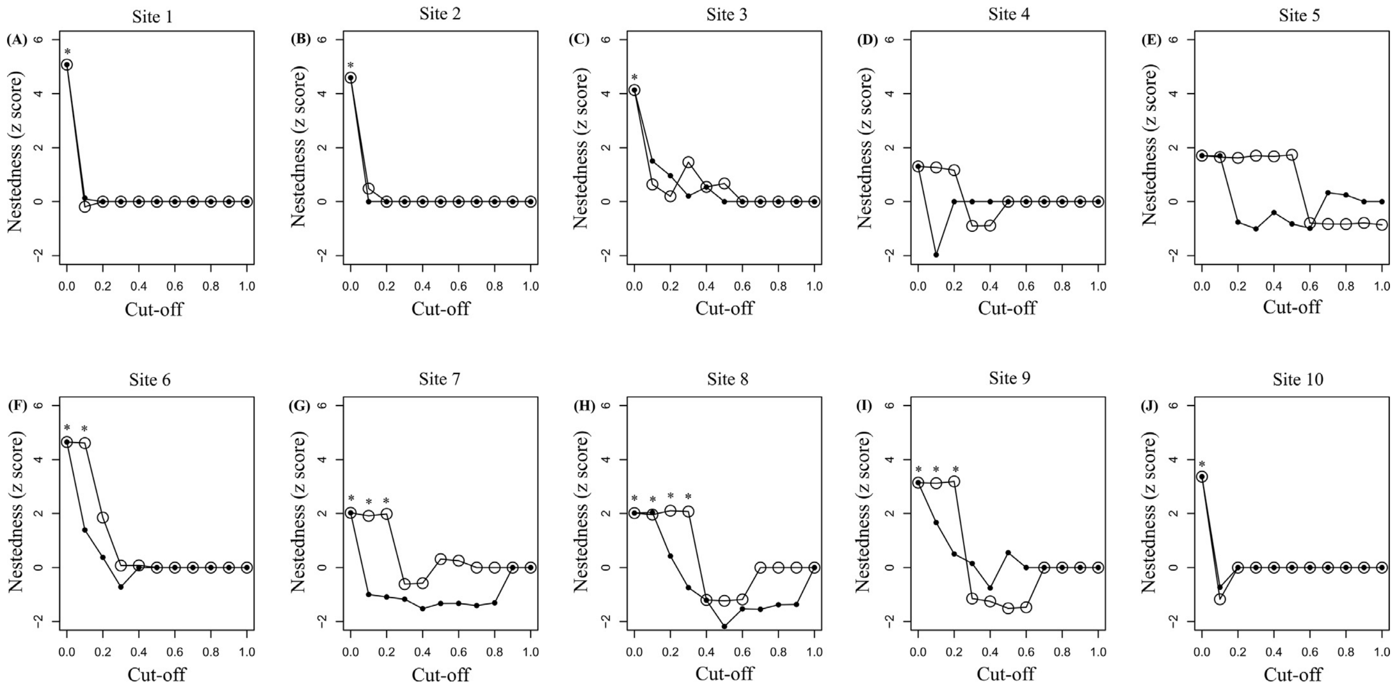

Fig. 3. Nestedness values (Z scores) calculated from binary matrices obtained through different cut-off levels (0–1) applied in weighted matrices with frequency (open circles) and abundance data (filled circles). Each graph (A–J) is relative to 1 of the 10 sites sampled.

between frequency and abundance matrices (contrast test: binary-frequency $\chi^2 = 9.4187$, d.f. = 1, $P < 0.001$; binary-abundance $\chi^2 = 12.353$, d.f. = 1, $P < 0.001$; frequency-abundance $\chi^2 = 0.1987$, d.f. = 1, $P = 0.656$) (Table 1 and Fig. 2). We also found differences in the vulnerability values (V) between different matrix categories (linear mixed-effects models: $\chi^2 = 24.028$, d.f. = 2, $P < 0.001$); in this case, the vulnerability values calculated with abundance data were significantly lower (vulnerability = 2.93 ± 1.17) than those calculated with frequency data (vulnerability = 3.65 ± 0.23) and binary data (vulnerability = 3.76 ± 1.25). However, we did not find differences between the vulnerability values calculated with frequency and binary data (contrast test: binary-frequency $\chi^2 = 0.3457$, d.f. = 1, $P = 0.557$; binary-abundance $\chi^2 = 20.3264$, d.f. = 1, $P < 0.001$; frequency-abundance $\chi^2 = 15.3704$, d.f. = 1, $P < 0.001$) (Table 1 and Fig. 2). It is important to note that for both generality and vulnerability, the results obtained with binary and weighted data were not regular. That is, there are some values of generality and vulnerability calculated with binary data greater than those established with weighted data (abundance and frequency data) (Table 1), however there are also values calculated with binary data less than the respective values calculated with weighted data.

When evaluating the non-random patterns of ant-plant interactions of each network, we observed that 80% ($n = 8$) of the networks with binary data were significantly nested, while no network with weighted data (abundance and frequency) exhibited a nested pattern of ant-plant interactions, both when we used a more restrictive null model to calculate the Z score values (*r2dtable* algorithm) (Patefield, 1981) and when we used a less restrictive null model (*vaznull* algorithm) (Vázquez et al., 2007) (Table 1). In addition, we observed differences between the Z score values of nestedness calculated from different matrix categories; this pattern was detected using a more restrictive null model for weighted data (linear mixed-effects models: $\chi^2 = 381.44$, d.f. = 2, $P < 0.001$) and a less restrictive null model (linear mixed-effects models: $\chi^2 = 160.7$, d.f. = 2, $P < 0.001$). In these two cases, we observed higher Z score values of nestedness for binary matrices (Z score$_{\text{nestedness (null model II)}}$ = 26.84 ± 7.27, mean ± SD), followed by frequency (Z score$_{\text{nestedness }(\textit{r2dtable})}$ = -0.05 ± 0.92; Z score$_{\text{nestedness }(\textit{vaznull})}$ = 0.23 ± 0.86) and abundance matrices (Z score$_{\text{nestedness }(\textit{r2dtable})}$ = −5.18 ± 1.00; Z score$_{\text{nestedness }(\textit{vaznull})}$ = −2.15 ± 0.87) (contrast test: (1) Z score$_{\text{nestedness }(\textit{r2dtable})}$ binary-frequency $\chi^2 = 56.584$, d.f. = 1, $P < 0.001$; binary-abundance $\chi^2 = 375.203$, d.f. = 1, $P < 0.001$; frequency-abundance $\chi^2 = 140.374$, d.f. = 1, $P < 0.001$ and (2) Z score$_{\text{nestedness }(\textit{vaznull})}$ binary-frequency $\chi^2 = 49.318$, d.f. = 1, $P < 0.001$; binary-abundance $\chi^2 = 160.057$, d.f. = 1, $P < 0.001$; frequency-abundance $\chi^2 = 31.682$, d.f. = 1, $P < 0.001$) (Table 1 and Fig. 2). In all site studied, the Z score values calculated with binary data were higher than the values calculated with weighted data.

For modularity, we observed that all networks and nine networks with abundance data presented significant modularity when we used the more restrictive and the less restrictive null model to calculate the Z score values, respectively (Table 1). On the other hand, all networks with binary and frequency data (both null models) were non-modular (Table 1). We found significant differences between Z score values of modularity calculated from different matrix categories; this pattern was detected using the more restrictive null model for weighted data (linear mixed-effects models: $\chi^2 = 379.92$, d.f. = 2, $P < 0.001$) and the less restrictive null model (linear mixed-effects models: $\chi^2 = 104.36$, d.f. = 2, $P < 0.001$). In both cases, we did not detect significant differences of this descriptor between frequency (Z score$_{\text{modularity (2dtable)}}$ = 0.18 ± 1.01; Z score$_{\text{modularity }(\textit{vaznull})}$ = -1.16 ± 1.37) and binary matrices (Z score$_{\text{modularity (null model II)}}$ = -0.27 ± 0.53), only between each of these matrix categories and the abundance matrices (Z score$_{\text{modularity }(\textit{r2dtable})}$ = 15.60 ± 3.95; Z score$_{\text{modularity }(\textit{vaznull})}$ = 4.73 ± 2.26) (contrast test: (1) Z score$_{\text{modularity }(\textit{r2dtable})}$ binary-frequency $\chi^2 = 0.229$, d.f. = 1, $P = 0.633$; binary-abundance $\chi^2 = 292.894$, d.f. = 1, $P < 0.001$; frequency-abundance $\chi^2 = 276.760$, d.f. = 1, $P < 0.001$ and (2) Z score$_{\text{modularity }(\textit{vaznull})}$ binary-frequency $\chi^2 = 3.656$, d.f. = 1, $P = 0.056$; binary-abundance $\chi^2 = 59.824$, d.f. = 1, $P < 0.001$; frequency-abundance $\chi^2 = 93.056$, d.f. = 1, $P < 0.001$) (Table 1 and Fig. 2). In all site studied, the Z score values of modularity calculated with abundance data were higher than the values calculated with frequency and binary data.

In general, we observed that nestedness and modularity of binary networks generated after applying successive cut-offs in the original weighted networks were in a relatively similar range for abundance and

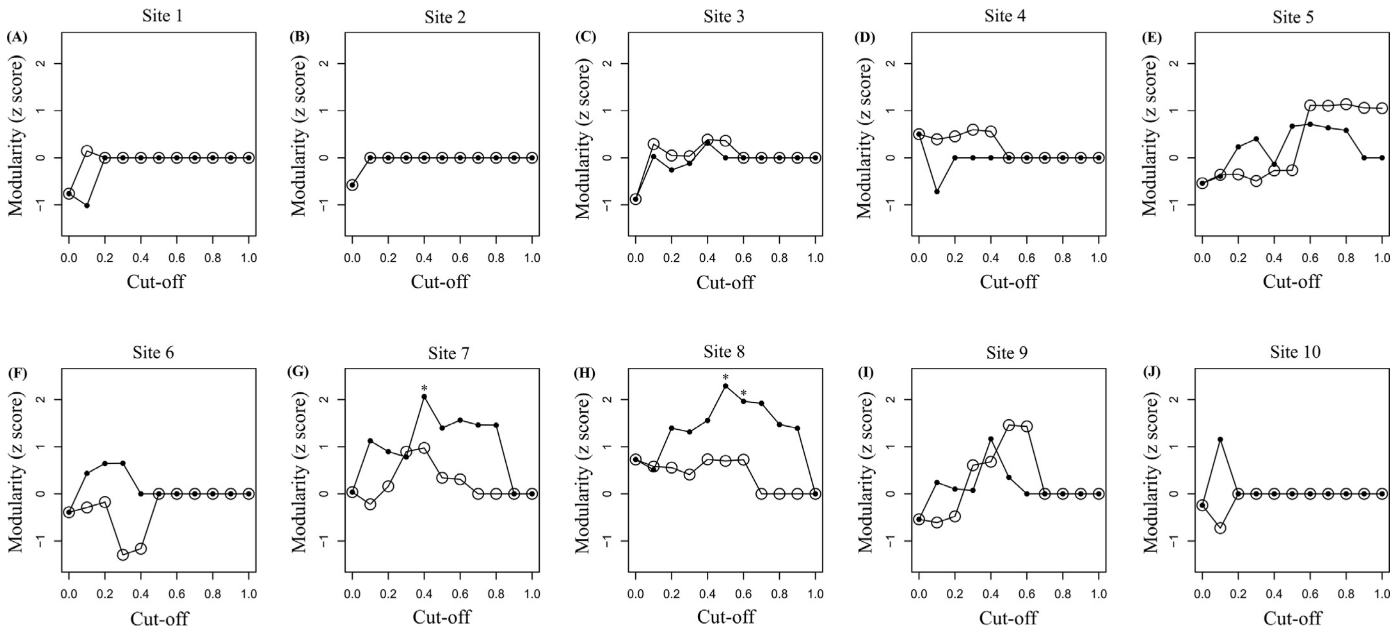

Fig. 4. Modularity values (*Z* scores) calculated from binary matrices obtained through different cut-off levels (0–1) applied in weighted matrices with frequency (open circles) and abundance data (filled circles). Each graph (A–J) is relative to 1 of the 10 sites sampled.

frequency matrices, with only slight differences (Figs. 3 and 4). In most sampling sites, regardless of the used weighted matrix category, the *Z* score values of nestedness decreased considerably in the first cut-offs (0.1–0.4), while in higher cut-offs (above 0.5), these values tended to approach 0 (Fig. 3). More precisely, we observed that in 90% and 60% of the sampling sites, the binary networks derived from abundance and frequency matrices, respectively, were no longer significantly nested from the cut-off level 0.1 (Fig. 3). For modularity, unlike the results found for nestedness, we observed that regardless of the weighted matrix category used, the *Z* score values tended to increase between cut-offs of 0.1–0.7 (Fig. 4), albeit with few significant values. Only 20% of the sampling sites presented significant values of modularity in cut-offs below 0.7 applied in abundance matrices (Fig. 4). For the frequency matrices, no sites presented any significant values of modularity for the cut-offs applied (Fig. 4).

Additionally, we observed that in 90% of our ant-plant networks (n = 9), the individual species specialization (*d'*) values calculated from the abundance matrices were positively correlated with the values calculated from the frequency matrices (Pearson's correlation: *r* (mean ± SD) = 0.90 ± 0.13; range of *r* = 0.094; all *P*-values < 0.001; range of *P*-values = 0). For only one of the 10 networks, we did not detect this correlation (Pearson's correlation: *r* = 0.539; *P*-value = 0.071). For all ant-plant networks, the species strength (*ss*) values calculated from the abundance matrices were also positively correlated with the values calculated from the respective frequency matrices (Pearson's correlation: *r* (mean ± SD) = 0.99 ± 0.005; range of *r* = 0.012; all *P*-values < 0.001; range of *P*-values = 0). The same pattern was found for absolute values of abundance and frequency of each species (Pearson`s correlation (mean ± SD): *r* = 0.92 ± 0.08; range of *r* = 0.224; all *P*-values < 0.001; range of *P*-values = 0).

4. Discussion

Using a standardized sampling protocol in different sites within the Brazilian Amazon, we observed that different matrix categories (binary or weighted data) result in the different structural organization of ant-plant interaction networks. This finding suggests that some network descriptors usually employed in studies of ecological interactions (e.g., generality, vulnerability, nestedness and modularity) are quite sensitive to data category. Surprisingly, at the species level, both categories of weighted data (i.e., frequency and abundance data) seem to be equally appropriate for describing the role of ant species within the networks. It is therefore necessary to adopt relevant matrix criteria based on the research question and on the biology of the interacting organisms in order to avoid inappropriate inferences.

We observed higher generality values (average number of plants per ant) calculated with binary data than with weighted data, and the values calculated with frequency and abundance data did not differ from each other. Binary versions of generality and vulnerability are strongly influenced by differences in sample size and sample effort, making the respective weighted versions much more robust against these sampling differences (Banasek-Richter et al., 2004; Tylianakis et al. 2007). In addition, in binary matrices, the same weight is attributed to both common and rare species (Bersier et al., 2002), and therefore, differences in partner availability are not taken into account (Blüthgen et al., 2006). The absence of significant differences between the generality values calculated from frequency and abundance matrices is, possibly, due to the fact that in our networks, the most frequent ant species were also species that collectively recruit on plants with extrafloral nectaries (*Crematogaster carinata* Mayr, 1878, *Crematogaster brasiliensis* Mayr, 1862, and *Crematogaster limata* Fr. Smith, 1858). This means that, although the number of individuals of each ant species and the total interactions differed among weighted matrices, the ratio between these factors remained similar. That is, the weight of each ant species is maintained in both weighted matrices. However, we cannot state that in ant-plant networks mediated by extrafloral nectaries, the most frequent ant species are necessarily species that recruit collectively. For this reason, we suggest that greater attention is required when using abundance data in similar studies dealing with ant-plant interaction networks. The frequency matrices adequately represent ant availability through the number of interactions that these organisms establish with plants. These matrices also adequately represent plant availability, since its elements also represent plant abundance in the networks (Tylianakis et al., 2007).

For vulnerability (average number of ants per plant), we did not detect differences between the values calculated from binary and weighted matrices with frequency data. For the same reasons described above for generality, we do not encourage the use of binary matrices. That is, binary matrices are sensitive to variations in sample size and sample effort (Tylianakis et al., 2007) and do not consider differences in the partners' availability (Blüthgen et al., 2006). Regarding the weighted matrices, we observed higher values calculated with frequency data than with abundance data. As previously mentioned, the frequency matrix elements adequately represent the plant abundance in the networks (Plowman et al., 2017; Tylianakis et al., 2007). On the contrary, in our abundance matrices, the elements represent the abundance of ants (i.e., number of workers recorded) feeding on plants with extrafloral nectaries. Consequently, when we calculated the vulnerability from our abundance matrices, we mistakenly attributed to plant species the abundance of ant species, among which many present collective recruitment. In other words, plant abundance values from our abundance matrices are higher than the actual values observed in frequency matrices, resulting in lower values of vulnerability, since this descriptor represents the proportion of interactions per plant.

The *Z* score values of nestedness varied greatly among different matrix categories. Specifically, we found higher values for binary matrices, followed by both weighted matrices, which did not present any significantly nested network, regardless of the null model used with the weighted data. Note that when being calculated for both binary or weighted matrices, nestedness must be in agreement with two basic principles: (1) decreasing marginal total (differences of the marginal totals among columns and/or among rows) and (2) overlapping of pairs (overlap of the presences in the less-filled columns and rows with the presences in the more-filled columns and rows, respectively) (Almeida-Neto et al., 2008, 2011). However, only in a weighted approach, the individual differences of interactions are considered (Almeida-Neto et al., 2011), for example, in relation to information about species preferences (Blüthgen, 2010). A previous study found a reduction in nestedness values calculated from weighted matrices when compared to binary matrices (Dáttilo et al., 2014a). In addition, the authors observed that most networks are no longer significantly nested when generated from weighted matrices, as opposed to the significantly nested patterns found for networks generated from binary matrices. These results corroborate the hypothesis that, due to nectar-composition preferences among ants, ant species with high interaction frequencies do not necessarily have more links in binary matrices (Dáttilo et al., 2014c). That is, an ant species with a preference for the nectar composition of a specific abundant plant species might present a high interaction frequency in a weighted matrix and a low number of links in a binary matrix due to the low or absent interaction with other plant species. In contrast, an ant species that does not have any preference for specific nectar composition will tend to interact with several plant species, although less frequently with each one, possibly resulting in a lower interaction frequency in a weighted matrix and a relatively high number of links in a binary matrix. Therefore, ant species with lower interaction frequencies are not necessarily subsets of ant species with higher interaction frequencies, which generates the non-nested structure in weighted networks (Dáttilo et al., 2014a). Based on the above, we encourage the use of weighted data for nestedness, mainly frequency data, due to the valuable information about species preferences contained in these matrices (Blüthgen, 2010). However, based on Corso et al. (2015), we do not rule out the use of binary matrices. These authors detected a high correlation between WNODF and NODF values calculated from 69 different ecological networks, encompassing multiple types of interactions (pollination, frugivory, herbivory, and ant-plant interactions). Therefore, despite of the intrinsic differences between the two metrics (i.e., WNODF is always smaller than NODF), the high correlation between them indicates that NODF is a good predictor for WNODF.

As observed for nestedness, we detected similar results for modularity, regardless of the null model used with the weighted data. Interestingly, we found no differences between the *Z* score values of modularity for binary and frequency matrices, and both *Z* score averages presented extremely low values. Moreover, none of these networks was significantly modular. These findings corroborate previous studies which also detected a non-modular structure of ant-plant networks mediated by extrafloral nectaries (Dáttilo, 2012; Dáttilo et al., 2014a; Díaz-Castelazo, 2013), most likely because of the low fidelity of generalist ant species when foraging on plants with extrafloral nectaries (Rico-Gray et al., 1998; Schoereder et al., 2010). The high modularity values detected for abundance matrices, however, as well as the significance found for all networks, clearly demonstrates a distortion in the structural organization of this ant-plant interaction, generated by ant species with collective recruitment. Such an increase in modularity through the use of abundance matrices indicates a greater network specialization, in turn related to the variation in ant abundance across different plant species. The number of interactions within the modules constituted by ant species with collective recruitment was considerably higher in abundance matrices than in frequency matrices. This might result in a greater contrast in relation to the number of interactions among modules and, consequently, in higher modularity values. Finally, it is important to emphasize that for both nestedness and modularity, we observed that the nature of the data can lead to similar results, both when we used a more restrictive null model with weighted data (*r2dtable* algorithm) (Patefield, 1981) and when we used a less restrictive null model (*vaznull* algorithm) (Vázquez et al., 2007) more similar to the null model used with binary data. This indicates that the detected results are not just artifacts of the differences between metrics and null models; they represent significant structural differences of the ant-plant networks, generated by the different data categories considered in the present study.

In general, we observed that nestedness and modularity of binary networks, generated after applying successive cut-offs in the original weighted networks, were in a similar range for abundance and frequency matrices, albeit with slight differences. For nestedness, regardless of the weighted matrix category used, the *Z* score values decreased considerably in the first cut-offs (0.1–0.4). This reduction is possibly related to the large amount of rare species (both ant and plant species) present in our matrices. Similar to what Cantor et al. (2017) detected for interaction networks of different biological systems, the nested structure of our networks depends on all links, but especially the weak links that glue peripheral elements to the network. For modularity, unlike the results found for nestedness, we observed that regardless of the weighted matrix category used, the *Z* score values tended to increase between cut-offs of 0.1–0.7. In marine predation, network modularity also increases towards higher cut-off values (Tinker et al., 2012). This increase reflects clusters of individuals specializing on alternative suites of resources for their core diets (Tinker et al. 2012). In this way, we postulate that, in our study, the increase of modularity in higher cut-off values reflects clusters of ant species with a clear preference for a specific set of plants with extrafloral nectaries, with these plant species being their core sources.

It is important to note that the cut-off analysis used in this study enabled a more detailed evaluation of the behavior of different weighted data (abundance and frequency) in the structural organization of these ant-plant networks, as a complement to the comparative analysis between different data categories (binary and weighted data). As previously discussed, the comparative analysis between different data categories for nestedness showed that in general, the use of weighted matrices reduces the *Z* score values compared to binary matrices. Moreover, the cut-off analysis showed that among the weighted data, the frequency data tends to better capture the typical nested structure of these ant-plant interactions. We observed that 90% of the binary matrices derived from abundance data lost the nested structure at the first cut-off (0.1). Conversely, in binary matrices derived from frequency data, this nested structure was maintained at a higher cut-off (0.2). The loss of the nested structure in binary matrices derived from abundance matrices already at the first cut-off is related to the fact that in these binary matrices, ant species that do not present collective recruitment tend to be quickly excluded. In abundance matrices, these ant species that do not present collective recruitment are considered rare, since their abundances are significantly lower than the abundance of ant species with collective recruitment. Conversely, in frequency matrices, ant species that do not present collective recruitment, but present a high number of interactions, tend to remain in the analysis in the first and also in the second cut-offs (0.2), allowing maintenance of the typical nested structure of these ant-plant interaction networks mediated by extrafloral nectaries. The cut-off analysis, as well as the comparative analysis between different data categories, detected a distortion in the structural organization of networks generated with abundance data (i.e., highly modular networks). In the cut-off analysis, only binary matrices generated from abundance matrices (20% of sampling sites) presented some significant values of modularity at higher cut-offs. This result does not match the non-modular structure frequently observed for these ant-plant interactions mediated by extrafloral nectaries (Dáttilo, 2012; Dáttilo et al., 2014a; Díaz-Castelazo, 2013).

Finally, when investigating the role of each ant species in the networks in terms of the degree of individual species specialization (Blüthgen et al., 2006) and species strength (i.e., its importance from the perspective of the partner set) (Bascompte et al., 2006), we observed strong correlations between the results calculated from different categories of weighted matrices. More specifically, for all networks, we found a positive correlation between the values of these measures calculated with abundance and frequency data as well as for the absolute values of ant abundance and interaction frequency. Most likely, this pattern is related to the fact that most ant species with high interaction frequency values in our networks are also numerically dominant species (Baccaro et al., 2012; Parr, 2008) that collectively recruit on plants with extrafloral nectaries (Nicolis and Deneubourg, 1999). Summing up, the definition of the weighted matrix category to be adopted (frequency or abundance matrix) does not seem to be important at the species level, at least for the two measures we used to describe the role of ant species in ant-plant networks mediated by extrafloral nectaries (individual species specialization and species strength).

Our findings suggest some specific recommendations for similar studies dealing with ant-plant interaction networks, and some general lessons that can be extended beyond these mutualistic interactions, due the similarities in structural organization (e.g., pollination or seed dispersal) (Bascompte and Jordano, 2007). In terms of specific recommendations for analog studies, the frequency data for both generality and vulnerability seems to be the most adequate. This is because frequency data best represents ant availability, through the number of interactions that these organisms are able to establish with plants, and plant availability, since the elements of these matrices also represent plant abundance in the networks (Tylianakis et al., 2007). For nestedness, we recommend the WNODF indices, because a weighted approach takes into account the individual differences of interactions, such as the preferences for specific nectar-composition. However, we do not rule out the use of binary matrices due to the high correlation between WNODF and NODF values, as detected by Corso et al. (2015). For modularity, we suggest the use of binary or weighted matrices with frequency data, as the abundance data completely distorted the structural organization of this ant-plant interaction. Surprisingly, at the species level, both categories of weighted data (i.e., frequency and abundance data) seemed to be equally appropriate for describing the role of ant species when considering the “role” of each species, its degree of individual species specialization, and its strength in the network. In a broader way, the sensitivity of the network descriptors evaluated in relation to the different data categories observed in our study, warn about the importance of defining appropriate criteria that take into account the research question and the biology of interacting organisms, to define the data category to be used. These criteria may help researchers to avoid inappropriate inferences about the structural organization of ecological networks. Finally, we still suggest that other studies about interaction networks working with different organisms should further evaluate these methodological details frequently ignored by researchers.

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Supplementary materials

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