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DYNAMICS OF INSECT-PLANT INTERACTIONS: THE ROLE OF BIOTIC AND ABIOTIC PARAMETERS ALTERING INSECT COMMUNITY RELATIONSHIPS

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Orientador: Prof. Dr. Ricardo Ribeiro De Castro Solar
Co-orientador: Prof. Dr. Marcílio Fagundes
Co-orientador: Prof. Dr. Geraldo Wilson Fernandes

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Letícia Fernanda Ramos Leite

No dia 17 de dezembro de 2021, às 14:00 horas, por vídeo conferência, teve lugar a defesa de tese de doutorado no Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre, de autoria do(a) doutorando(a) Letícia Fernanda Ramos Leite, orientando do Professor Ricardo Ribeiro De Castro Solar , intitulada: **"Dynamics of insect-plant interactions: the role of abiotic and biotic parameters altering insect community relationships"**. Abrindo a sessão, o(a) Presidente(a) da Comissão, Doutor(a) Ricardo Ribeiro de Castro Solar, após dar a conhecer aos presentes o teor das normas regulamentares do trabalho final, passou a palavra para o(a) candidato(a) para apresentação de seu trabalho. Estiveram presentes a Banca Examinadora composta pelos Doutores: Laura Leal (USP), Inara Roberta Leal (UFPE), Tatiana Garabini Cornelissen (UFMG), Walter Santos de Araújo (UNIMONTES) e demais convidados. Seguiu-se a arguição pelos examinadores, com a respectiva defesa do(a) candidato(a). Após a arguição, apenas os senhores examinadores permaneceram no recinto para avaliação e deliberação acerca do resultado final, sendo a decisão da banca pela:

(X) Aprovação da tese, com eventuais correções mínimas e entrega de versão final pelo orientador diretamente à Secretaria do Programa, no prazo máximo de 30 dias;
( ) Reavaliação da tese com avaliação pelos membros da banca do documento revisado, sem nova defesa, no prazo máximo de 30 dias, sob possibilidade de reprovação;
( ) Reformulação da tese com indicação de nova defesa em data estabelecida a critério do Colegiado em observância às Normas Gerais da Pós-graduação na UFMG a ao Regimento do PPG-ECMVS;
( ) Reprovação

A banca indica esta tese aos Prêmios CAPES e UFMG de teses? ( ) SIM (X) NÃO

Nada mais havendo a tratar, o Presidente da Comissão encerrou a reunião e lavrou a presente ata, que será assinada por todos os membros participantes da Comissão Examinadora.

Belo Horizonte, 17 de dezembro de 2021.

Assinaturas dos Membros da Banca Examinadora

Documento assinado eletronicamente por **Inara Roberta Leal**, **Usuário Externo**, em 23/12/2021, às 09:00, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

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Dedico esta Tese à minha mamãe,
Maria Odete Ramos Leite

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Resumo

As interações envolvendo insetos e plantas são moldadas por variações abióticas e bióticas que alteram as dinâmicas e estrutura destas comunidades. As alterações antrópicas estão impactando estas interações com perda na diversidade de insetos, desta forma, estudos que visam compreender a dinâmica destas interações são cruciais. Nós avaliamos como *Copaifera langsdorffii* (Fabaceae), uma planta adaptada a diferentes pressões ambientais, responde a suplementação nutricional e hídrica e como as comunidades de insetos são afetadas. Além disso, também avaliamos como a presença de nectários extraflorais (NEFs) ao longo de todo o ciclo fenológico da planta modifica as comunidades de insetos. Procuramos responder às seguintes questões: (1.) A suplementação de nutrientes e água afeta a esclerofilia, a área foliar e a produção de frutos em *C. langsdorffii*?; e (2.) As taxas de herbivoria, diversidade de insetos galhadores e padrões de co-ocorrência de galhas nas comunidades são afetados pela suplementação de nutrientes e água e NEFs artificiais? (3.) A estabilidade das interações inseto/planta é afetada por fatores bióticos e abióticos?; (4.) A disponibilidade constante de exsudato açucarado (NEF) reduz a frequência de interações herbívoro-planta? O experimento foi realizado em um ecossistema rupestre de topo de uma montanha chamado Canga (afloramento de pedra de ferro), onde aplicámos tratamentos de suplementação em 80 indivíduos vegetais divididos nos seguintes tratamentos de campo: (T1 = Fertilizante, T2 = NEF simulação (Tubos de Eppendorf com uma solução de açúcar 20%), T3 = Fertilizante + NEF simulação, T4 = água borrifada, T5 = NEF Controle (microtubo com água), e T6 = Controle. Observamos menor esclerofilia e maior área foliar nos indivíduos de plantas que foram suplementados com nutrientes e água. As taxas de herbivoria foram menores e a abundância de formigas foi maior nas plantas com EFNs artificiais disponíveis. Embora não tenhamos observado variações na riqueza e abundância de insetos galhadores, os padrões de coocorrência de galhas variaram com a disponibilidade de recursos (espaço de nidificação) na planta. As redes de todos os tratamentos mostraram estabilidade (robustez) e especialização, no entanto, a rede do tratamento com fertilizante foi menos especializadas. Os insetos de vida livre apresentaram baixa especialização no tratamento com adição de fertilizante e as formigas foram menos especializadas no tratamento com tubos com água. A proporção de ocorrências das guildas dos insetos de vida livre variou entre os tratamentos, o tratamento com fertilizante teve uma maior proporção de insetos de vida livre e uma relação negativa com formigas oportunistas. Por outro lado, o tratamento com adição de NEFs tiveram menor relação com insetos de vida livre e relação positiva com algumas guildas de formigas. Este estudo ilustra como as variações abióticas e bióticas modificam a ocorrência e as interações das comunidades de insetos.

Palavras-chave: Abiótico; Biótico; Suplemento de nutrientes; NEF; Formigas; Bioestimulante; Co-ocorrência; Interação inseto planta; Guildas de insetos; Redes de interações.

Abstract

Interactions involving insects and plants are shaped by abiotic and biotic variations that alter the dynamics and structure of these communities. Anthropogenic changes are impacting these interactions with loss in insect diversity, thus studies that aim to understand the dynamics of these interactions are crucial. We assess how *Copaifera langsdorffii* (Fabaceae), a plant adapted to different environmental stresses, responds to nutrient and water supplementation and how insect communities are affected. In addition, we also evaluate how the presence of extrafloral nectaries (EFNs) throughout the phenological cycle of the plant modifies insect communities. We seek to answer the following questions: (1.) Does nutrient and water supplementation affect sclerophyll, leaf area, and fruit production in *C. langsdorffii*?; and (2.) Are herbivory rates, galling insect diversity, and patterns of gall co-occurrence in communities affected by nutrient and water supplementation and artificial EFNs? (3.) Is the stability of insect-plant interactions affected by biotic and abiotic factors?; (4.) Does the constant availability of sugar exudate (EFN) reduce the frequency of herbivore-plant interactions? The experiment was conducted in a rupestrian mountaintop ecosystem called Canga (ironstone outcrop), where we applied supplementation treatments to 80 plant individuals divided into the following field treatments: (T1 = Fertilizer, T2 = EFN simulation (Eppendorf tubes with a 20% sugar solution), T3 = Fertilizer + EFN simulation, T4 = water spray, T5 = EFN Control (microtube with water), and T6 = Control. We observed lower sclerophyll and greater leaf area in individuals from plants that were supplemented with nutrients and water. Herbivory rates were lower and ant abundance was higher in plants with artificial EFNs available. Although we did not observe variations in the richness and abundance of galling insects, patterns of galling co-occurrence varied with the availability of resources (nesting space) on the plant. The networks of all treatments showed stability (robustness) and specialization, however, the network of the fertilizer treatment was less specialized. Free-living insects showed low specialization in the treatment with added fertilizer and ants were less specialized in the treatment with tubes with water. The proportion of occurrences of the free-living insect guilds varied among the treatments, the treatment with fertilizer had a higher proportion of free-living insects and a negative relationship with opportunistic ants. On the other hand, the treatment with added EFNs had a lower relationship with free-living insects and a positive relationship with some ant guilds. This study illustrates how abiotic and biotic variations modify the occurrence and interactions of insect communities.

Keywords: Abiotic; Biotic; Nutrient supplementation; EFN; Ants; Biostimulant; Co-occurrence; Insect-plant interaction; Insect guilds; Interaction networks.

Summary

Introdução geral

As interações envolvendo insetos e plantas correspondem a mais de 40% da biodiversidade global (Price, 2002) e são responsáveis por mantêm o fluxo ascendente de energia para níveis tróficos superiores (Futuyma & Agrawal, 2009). As plantas, como produtores primários, fornecem uma elevada diversidade de recursos para os vastos organismos que as exploram, no entanto, existem restrições que dificultam o consumo destes recursos. Durante longas décadas muitos estudos têm procurado compreender os mecanismos responsáveis pela co-evolução destas interações, onde as plantas desenvolvem mecanismos de defesa (Coley & Barone, 1996; Hanley et al., 2007; Janzen, 1970) e os insetos contornam estas defesas (Cárdenas et al., 2015; Coley, 1987; Herms & Mattson, 1992).

As interações insetos planta são de considerável relevância para a compreensão da dinâmica da comunidade e das funções do ecossistema. Mudanças na composição de espécies e qualidade individual das plantas podem alterar as comunidades de herbívoros com efeitos em cascatas nas interações com predadores, parasitoides, até mutualismo como a polinização (Biesmeijer et al., 2006; Novotny & Basset, 2005). Por exemplo, insetos possuem hábitos alimentares distintos e utilizam diferentes partes da planta como recurso, consequentemente desenvolvem graus de especialização que norteiam a classificação em guildas tróficas (Lewinsohn et al., 2005; Novotny et al., 2010), com grupos altamente específicos com suas plantas hospedeiras a outros com hábitos mais generalistas (Novotny et al., 2010). O grau de especialização está relacionada aos fatores ascendentes de disponibilidade de recursos, como o a qualidade da planta hospedeira, que, entre outros fatores, é influenciada por mudanças sazonais na disponibilidade de água e nutrientes (Barbour et al., 2019; Boege, 2005)

Variações de temperatura, incidência luminosa, qualidade do solo são alguns fatores abióticos relacionados com a distribuição e sobrevivência das plantas (Figueroa-Macías et al., 2021; Lázaro-Nogal et al., 2015; Lin et al., 2021). Estes múltiplos fatores fizeram com que as plantas desenvolvessem algumas estratégias adaptativas para sobreviver, tais como modificações nas taxas de crescimento das folhas, esclerofilia e produção de frutos, que estão diretamente relacionadas com a disponibilidade de água e nutrientes (Lázaro-Nogal et al., 2015; Poorter et al., 2009). Por exemplo, a taxa de crescimento das folhas está relacionada com uma maior eficiência no uso de água, geralmente as plantas que sofrem de stress hídrico apresentam menor área foliar (Poorter et al., 2009). Assim, estas alterações nas características das plantas podem ter um efeito direto nas comunidades herbívoras associadas (Novotny et al., 2010), plantas com folhas mais escleróﬁlas, por exemplo, sofrem menos danos por algumas guildas de insetos herbívoros (Novotny et al., 2010).

A baixa disponibilidade de nutrientes no solo dificulta a capacidade regenerativa dos tecidos das plantas, devido ao elevado custo energético para a substituição destes tecidos (Janzen, 1970). Estratégias adaptativas, como associações mutualistas com outros grupos, evoluíram para minimizar os efeitos causados por herbívoros. A exemplo, as associações mutualistas com formigas, sendo que a presença de formigas é capaz de reduzir a abundância dos insetos herbívoros em 50% e consequentemente as taxas de herbivoria (Rosumek et al., 2009), além de diminuir a presença de outros agentes patogénicos na planta (Gomes et al., 2021). O comportamento agressivo destas formigas torna-se um serviço de proteção útil para as plantas, em troca, as plantas fornecem um exsudado rico em carboidratos, os chamados néctares extraflorais (NEFs), que servem de alimento para as formigas (Camarota et al., 2015).

Plantas que prosperam em ambientes hostis desenvolveram adaptações para lidar com a privação de água e nutrientes (Silveira et al., 2016). A exemplo os ambientes de Cangas, que possuem solos pobres, pouco profundos e ácidos com elevada concentração de ferro e baixa capacidade de retenção de água (Silveira et al., 2016). As respostas fisiológicas das plantas à água do solo e à disponibilidade de nutrientes estão intrinsecamente relacionadas com a sobrevivência de muitos insetos herbívoros (Awmack & Leather, 2002; Gao et al., 2008; Mattson, 1980). Pois, estas alterações formam microclimas específicos nas plantas hospedeiras que afetam o estabelecimento, sobrevivência e interações das comunidades de insetos (Boege, 2005).

Em geral, a suplementação nutricional de plantas com Nitrogênio, carbono, potássio e a maior disponibilidade de agua, intensificam as taxas fotossintéticas das plantas (Shehata et al., 2011), que também alocam estes recursos para o crescimento e reprodução (Negreiros et al., 2009; Lázaro-Nogal et al., 2015). Plantas com ampla distribuição geográfica sofrem diferentes pressões ambientais e interagem com uma ampla diversidade de insetos, sendo, portanto, bons modelos para explorar o papel das variações abióticas e abióticas nas comunidades de insetos. A espécie vegetal *Copaifera langsdorffii* (Fabaceae) é um bom exemplo. Esta planta ocorre em diversos biomas brasileiros e tem desenvolvido estratégias adaptativas para lidar com as variações nos fatores ambientais nos habitats que ocupa (Souza et al., 2018). A elevada plasticidade fenotípica em diferentes ambientes modifica as interações com as comunidades de insetos (Fagundes et al., 2020; Ramos et al., 2019; Souza et al., 2018). Além disso, esta planta possui EFNs ativos que atuam apenas no início do desenvolvimento foliar, quando se observa intenso forrageamento de formigas (Oliveira & Isaias, 2010; Queiroz et al., 2013). Assim, a utilização desta planta em experiências de campo com suplementação de nutrientes e água e disponibilidade de NEF ao longo de todo o ciclo reprodutivo pode elucidar como os fatores bottom-up e top-down atuam na dinâmica da comunidade e nas interações inseto-planta.

Sabemos que as redes de interações tróficas das comunidades de insetos são sensivelmente modificadas entre ambientes, contudo, ainda não sabemos como as manipulações das condições abióticas (nutrição e hidratação vegetal) e bióticas (adição de NEFs para atrair formigas) alteram a regulação e o funcionamento destas cascatas de interações e como afeta estas comunidades. Compreender como as interações bióticas e abióticas atuam sinergicamente pode fornecer uma direção de como alterações antropogênicas modificam as comunidades vegetais e a estabilidade das suas interações ecológicas, especialmente em cenários de declínio da diversidade de insetos.

Objetivos

Neste estudo, pretendemos compreender como a suplementação de água, disponibilidade de nutrientes e NEFs em *C. langsdorffii* em ambientes naturais afeta as características das plantas e os seus efeitos em cascata nas comunidades de insetos. Especificamente, testamos como as manipulações experimentais modificam as características das plantas e os seus efeitos sobre as redes de interações das comunidades de insetos de vida livre as suas taxas herbívoras. Ao longo desta tese, apresentamos o seguinte manuscrito: "Dinâmica da interação inseto-planta sob manipulação biótica e abiótica: o papel das formigas no controle das taxas herbívoras" (Capítulo 1) e "Fatores bióticos e abióticos modificam as redes de interação inseto-planta" (Capítulo 2).

Capítulo I: Experimental manipulation of biotic and abiotic parameters changes the outcome of insect-plant interactions

Artigo em revisão na revista “Basic and Applied Ecology”

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Abstract

Plants usually respond to environmental shifts with morpho-physiological adjustments, which trickles down to biotic interactions in the insect-plant system. We evaluate how *Copaiffera langsdorffii,* a widespread tree species adapted to multiple ecosystems, responds to shifts in nutrient and water availability through experimental supplementation and how it affects its insect communities. We also evaluate how the presence of extrafloral nectaries (EFN) exudates modifies galling insect diversity and herbivory rates. Such experimental approach is scarce, as we simultaneously evaluate biotic and abiotic factors and cover the entire reproductive cycle of a species in natural environments, bringing important contributions to better understand how bottom-up factors drive community interactions and possibly community assembly. The experiment was set in an ironstone outcrop vegetation, where we deployed supplementation treatments in 80 plant individuals divided into the following field treatments: (T1 = Fertilizer, T2 = EFN simulation (Eppendorf tubes with a 20% sugar solution), T3 = fertilizer + EFN simulation, T4 = water spray, T5 = EFN control (microtube with water), and T6 = Control. We observed lower sclerophylly and greater leaf area in plant individuals that were supplemented with nutrients and water. Herbivory rates were lower and ant abundance was higher in plants with artificial EFNs available. While we did not observe variations in richness and abundance of galling insects, the patterns of co-occurrence of galls varied with the availability of resources (nesting space) in the plant. This study illustrates how variations in nutrient availability to plants modify interactions with insect communities. Ant-plant interactions can have a negative impact on general herbivory rates, however ants seem to have a more harmonious relationship with the galling insects.

Keywords: Ants, Co-occurrence patterns, Extrafloral nectaries, Galls insect, Herbivory, Mutualist interactions, Nutrient supplementation

Introduction

Insect-plant interactions have fostered discussions in ecology and evolution at least since the mid-1960s (Hairston, Smith, Slobodkin, et al., 1960), with pioneering studies seeking to understand how insects selected their plants (Price, 1991), how plants defend themselves (Coley & Barone, 1996; Hanley et al., 2007; Janzen, 1970) and how these interactions coevolved (Cárdenas et al., 2015; Carmona et al., 2011; Ehrlich & Raven, 1964; Hairston, Smith, & Slobodkin, 1960; Herms & Mattson, 1992; Whitham, 1983). Plants, being the chief primary producers in most ecosystems, are some of the basic resources for the terrestrial food webs and interact with a wide range of organisms, and it is estimated that insects are responsible for consuming between 10 and 15% of all plant biomass per year (Cyr & Pace, 1993). The evolution of plant-herbivorous insects interactions' outcomes is driven by a combination of biotic and abiotic factors, challenging researchers that study them (Kos et al., 2015; Lin et al., 2021; Ruiz-Guerra et al., 2021). Therefore, understanding how plant and insect communities establish, assemble, and interact among themselves and with other groups and the environment is key to uncover the role of global changes in modifying interactions and ultimately favor or extinguish some groups (Wilkinson & Sherratt, 2016).

Potentially adverse environmental characteristics, such as low soil fertility, extreme temperatures, water deprivation are the main sources of stress directly affecting plant life and development (Cock & Hierro, 2020; Pennington & Collins, 2007). The adaptive responses of plants to environmental stress include reduced leaf growth (Lázaro-Nogal et al., 2015) and less nutritional quality of their tissues (Awmack & Leather, 2002). Additionally, such abiotic pressures are directly related to plant responses and their ability to interact with other species (Carmona et al., 2011). For instance, plants with high nitrogen and high water content in their leaves are more palatable and prone to chewing herbivory (Hanley et al., 2007; Mattson, 1980). Conversely, the more sclerophyllous leaves are less damaged by chewers (Agrawal, 2007), while favoring specialist herbivore groups, such as galling insects (Fernandes & Price, 1988).

Delving into the biotic spectrum, the pressure exerted by herbivores can affect plant species communities, as shown by herbivore exclusion or suppression experiments (Souza et al., 2016). Also, limited soil resource availability hampers the regenerative capacity of plants due to the high energy investment required in tissue replacement (Janzen, 1970). Thus, mutualistic interactions have been observed in plants as defensive strategies, such as the production of Extrafloral Nectaries (EFNs), which are a valuable resource food for ants (Rosumek et al., 2009). As a result, the aggressive behavior of ants turns into a protective service to the plants since ants are known to remove herbivores through predation (e.g., Rosumek et al., 2009; Camarota et al., 2015).

Plants growing in stressful environments, such as in rupestrian ironstone outcrops, are adapted to nutrient- and water-deprivation (Silveira et al., 2016). Also, the physiological responses of plants to water availability and nitrogen content determine the survival of many herbivorous insect groups (Awmack & Leather, 2002; Gao et al., 2008; Mattson, 1980). In general, supplementation with Nitrogen, Potassium and Carbon reduces the effects of stress on plants and increases their photosynthetic rates (Shehata et al., 2011) while high water availability increases vegetative growth (Lázaro-Nogal *et al.*, 2015). The experimental supplementation with artificial EFNs, on the other hand, attracts more ants that tend to provide a better protective service (Rosumek et al. 2009; Ribeiro et al. 2018). Few studies have yet used a joint experimental manipulation of abiotic and biotic factors at the community level and considered the phenological behavior of plants in natural environments to explain herbivore insect use of plant resources. The plant species *Copaifera langsdorffii* (Fabaceae) is a good model for this experiment, since the plant exhibits high phenotypic plasticity and the interspecific interactions with galling insect communities, which are affected by the environmental conditions in which the host plant is found (Souza et al. 2018; Ramos et al. 2019; Fagundes et al. 2020).

In this study, we aim to understand how experimentally changing water and nutrient contents and the availability of EFNs (using artificial EFNs that mimicked natural nectar composition) of *C. langsdorffii* in natural environments affect plants' quality as a host and its cascading effects on their insect communities (Fig. 1). We sought to answer the following questions: (1.) Does nutrient and water supplementation affect sclerophylly, leaf area, and fruit production in *C. langsdorffii*?; and (2.) Are herbivory rates, galling insect diversity, and gall co-occurrence patterns in communities affected by nutrient and water supplementation and artificial EFNs? Our first hypothesis is that increased nutrient and water availability directly affects plant quality and indirectly affects both, the diversity and co-occurrence of galling insect, and on herbivory. Our second hypothesis is that increased artificial EFNs, by attracting ants, directly affect herbivory rates and indirectly affect plants.

Material and methods

Study area

The experiment was conducted in an area of environmental protection of approximately 1,100 ha, located in the municipality of Brumadinho, Minas Gerais state, Brazil (Fig. 2A). The region's climate is high altitude subtropical according to Koppen's classification (Alvares et al., 2013), with average altitude of 1,200 m, rainfall of 1490.00 mm/year, average temperature of 20.74 °C and high sun incidence. In this mountaintop ecosystem ironstone outcrops are found on poor soils with high iron content that form banded ironstone locally known as *canga* (*campo rupestre* in Portuguese). This ecosystem has low water-holding capacity and nutrients, such as Nitrogen and Phosphorous that are in very low availability (Schaefer et al., 2016; Silveira et al., 2016).

Study system

Copaifera langsdorffii Desf (Fabaceae) is a tropical tree species that varies in height from 2 to 35 m (Almeida et al., 1998). This species has a wide geographic distribution, in Brazil occurring in the biomes Caatinga, Cerrado, Atlantic Forest and Amazon, and shows high phenotypic plasticity (Almeida et al., 1998). The tree has composite leaves with 4 to 12 alternate leaflets. During the expansion phase, the leaflets are reddish and have two active EFNs attractive for various ant species (Queiroz et al., 2013).

C. langsdorffii shows marked leaf fall in the dry period with new leaves budding soon after, has a supra-annual reproduction in the rainy period (Freitas & Oliveira, 2002). This species also has a diverse fauna of associated insects, mainly gall-inducing insects (Fagundes, 2014), the high geographical distribution of the species probably favored the diversification of the gall-inducing insects on this species (Fagundes et al., 2020).

Experimental Design

A total of 80 *C. langsdorffii* adult trees with an average height of 2 m were selected, with a minimum distance between them of 10m. The trees were haphazardly assigned into six treatments of water fertilization and EFN simulation (T1 = Fertilizer, T2 = EFN simulation, T3 = fertilizer + EFN simulation, T4 = water spray, T5 = EFN control (microtube with water), and T6 = Control. All experiments were initiated in the budding phase of the plants, which corresponds to the period that the EFNs are active in the young leaves of *C.langsdorffii*, lasting on average 1 month (Queiroz et al., 2013), and maintained until the senescence phase of the leaves. We kept our manipulative experiments running for two consecutive years to follow the entire reproductive cycle of this plant species.

To improve plant nutrient quality, fertilization was done with an organo-mineral leaf biostimulant (Aminon-25 Technes ®) that has water-soluble nutrients (11% Nitrogen (N), 1% Potassium (K_2O) and 6% Total Organic Carbon (TOC). The main function is the biostimulation of the plant to increase its photosynthetic capacity and consequently increase its primary production. At the same period of the emission of new leaves in *C. langsdorffii* we began to apply the biostimulant to the 15 experimental plants in the field. Following the manufacturer's recommendation, we used a hand-held sprayer containing a 0.1% solution of Aminon-25; all leaves were sprayed and we avoided letting the product run off to avoid phytotoxicity. The application was always made in the morning (period when the stomata are open and absorb the nutrients more easily) (Berry et al., 2019) and was repeated every 15 days.

To simulate the continuous presence of EFN on the plants, we used microtubes (2 ml) containing a cotton bud soaked with a 20% sucrose solution (Fig. 2BC) (Camarota et al., 2015). We fixed each microtube haphazardly at the end of the branches near the leaves, where the ants had free access. We installed a minimum of 30 tubes per plant and above that, to account for plant size, we calculated a ratio of 10 microtubes per number of branches on the plant, for this treatment we used 15 plants. At every 15 days the microtubes were replaced with new ones with the same solution to avoid contamination by fungi. As the control treatment, on 5 plants we added microtubes containing only potable water (under the same conditions as the EFN supplementation).

Following the same procedures for nutrient supplementation, we manually sprayed only clear water on 15 plants, with the aim of decreasing the water deficit when absorbed by the stomata (Berry et al., 2019). Finally, we settled a treatment combining experimental EFNs + fertilizer applied to another 15 plants and this treatment followed the same application frequency as the others. Other 15 plants remained pristine in normal environmental conditions to be our control treatment.

Biological data collection

The biological data were collected in the months of March/April 2019 and March/April 2020. In each plant, 10 terminal branches (30 cm each) were collected and packed in plastic bags and taken to the laboratory and were immediately sorted. On each branch collected we counted the number of green fruits that were fully developed. To determine the specific leaf mass (SLM) we selected the second pair of leaflets from each branch (300 leaflets for each treatment), a disk (0.38 cm^2) was removed from each leaflet, dried in an oven at 40 °C for 92 h, and individually weighed on an analytical balance, (see Cornelissen et al 2003) for details). Using a scanner we created digital images of each leaflet (300 leaflets per treatment) that were calibrated at 0.01 mm and measured using imageJ 1.6.0 software (Rasband, 1997). Herbivory was determined as a percentage of area removed, given by the formula: Herbivory = [area lost / total leaf area] * 100.

To determine the patterns of galling insects co-occurrence between treatments, we selected the last three leaves of the branches that had at least one gall (Ramos et al., 2019). Thus, it was possible to create a presence and absence matrix for each experiment, where each gall morphospecies represents the columns and each leaflet represents the rows. The galls present in each leaflet were counted and identified according to their morphology, color, texture, and size (see Costa *et al.*, 2011 for details).

We sampled the ants from all trees in each experiment by the tapping method, we arbitrarily selected 1 branch from each plant and tapped 10 times hand-collected the dislodged ants that fell onto an entomological umbrella (Ribeiro *et al.*, 2019). All plants were sampled in the period between 7:00 and 11:00 hours. In this study, we used only the total number of ants that were present on the plants in each treatment, since our goal was to measure the general level of ant attractiveness and activity and to how it would reflect on the herbivory rates.

Statistical Analyses

To test if specific leaf mass (sclerophylly) and leaf area vary between experiments we created linear mixed-effect models (LMM) (Harrison et al., 2018), using the experiments as explanatory variables and SLM and leaf area as response variables. For the galls richness and abundance tests we used generalized linear mixed models (GLMM), with Poisson error distribution. As explanatory variables we used the treatments and as response variables we used richness or abundance of galling insects. To evaluate the herbivory among experiments we used GLMM, with a binomial error distribution. We used the treatments as explanatory variables and the proportion of area removed as a response variable. To show whether there was a difference in ant recruitment between the experiments, we used GLMM, with normal error distribution. As explanatory variables we used the Experiments and as response variables we used the number of ants found. In all these cases we considered the "individual plants" as a random effect. For the test of mean fruit yield per plant, we created generalized linear models (GLM), followed by ANOVA with Experiments as explanatory variable and mean fruits per plant as response variable. All analyses were performed in R software v 3.5.3 (R Core Team, 2017). We used the lme4 v 1.1-12 package (Bolker et al., 2009) to construct the GLMM and LME. The models were tested by comparison with a null model using a chi-square test.

To assess interspecific interactions of the galling insects among treatments we used null model analyses based on co-occurrence patterns (Gotelli, 2000). This approach has often been used to estimate the patterns of competitive interactions among sessile organisms (Gotelli, 2000). We quantified the spatial overlap in the development of each gall morphospecies on each leaflet and performed these analyses separately for each treatment. Galls were scored as either "present" (1) and "absent" (0) on each individual leaflet (see Ramos *et al.*, 2019 for details). We used the C-score index (Stone and Roberts 1990), which quantifies co-occurrence patterns in "checkerboard units", the indices obtained in each matrix were compared with those derived from the 500 randomly assembled null matrices. Model analyses were performed using the co-occurrence platform in EcoSim 7.22 (Gotelli & Entsminger, 2001).

Results

The specific leaf mass varied among the treatments ($\chi2 = 32.199$, N = 5, $p < .001$, Fig. 3). Plant individuals that did not receive nutritional supplementation (Control, and EFN simulation) presented more sclerophyllous leaves compared to plant individuals in the other treatments (EFN simulation +Fertilizer, Fertilizer, Spray Water, and Microtubes with water). Plant individuals belonging to treatments Fertilizer, EFN simulation +Fertilizer, and Spraying water had greater leaf area than plants of the treatments EFN simulation, Control, and microtube with water ($\chi2 = 83{,}525$, N = 5, $p < .001$, Fig. 4). The fruit crop also varied among treatments (Deviance = 138.65, F5 = 2.351, p = 0.04, Fig. 5). The individuals that received only fertilizer addition showed a high fruit production, which differed from all other treatments.

The proportion of leaf area removed by herbivores also varied among treatments ($\chi2 = 39{,}948$, N = 5, $p < .001$, Fig. 6). Plants of the Control, Fertilizer, and Spray water treatment showed higher rates of herbivory compared to the other treatments. The individuals that were supplemented with some kind of nectar (EFN simulation, EFN simulation + fertilizer, and microtubes with water) showed lower rates of damage, although the simulated EFN plus fertilizer treatment showed more damage than treatments with nectar supplementation alone. We also observed a difference in ant numbers between treatments ($\chi 2$ = 18.35 N = 5, $p < 0.001$). The EFN simulation treatment showed 33.84 % (988 ants) out of the total number of ants found (2.919 ants), EFN simulation +fertilizer showed 30.2% (883 ants), while microtubes with only water showed 10.8% (316 ants). On the other hand, the Control, Fertilizer, and Spray water treatments showed 11% (340 ants), 11.64 % (340 ants), and 1.78 % (52 ants), respectively. Therefore, a greater presence of ants on plants seems to decrease the damage caused by herbivorous insects.

Richness (χ^2 = 8.15, N = 5, p = 0.3191, Mean = 3, Total = 10) and abundance (χ^2 = 7.38, N = 5, p = 0.1939, Mean = 19, Total = 2.919) of gall-inducing insects did not change among the treatments. However, the observed c-score values were higher than expected by chance ($p < .001$; Table 1) only in the treatments Control, and EFN simulation. In the other treatments, these values did not vary statistically ($p > 0.05$).

Discussion

In this study, we examined how a plant species adapted to multiple environmental conditions responds locally to supplementation of nutrient and water, and how this response affects the organization of insect communities in an extreme ecosystem, the Ironstone rupestrian grassland. We also asked whether the presence of EFNs throughout the plant's phenological cycle alters herbivory rates and galling insect diversity (Fig. 1). First, we showed that foliar traits (e.g., sclerophylly, leaf area) where modified by water/nutrient supplementation of the plant. We also observed associated variations in the co-occurrence of the galling insect community and herbivory rates. We then show that the higher ant activity, as a result of EFNs supplementation, decreases herbivore rates but shows no effect on galling insect communities.

Our results confirm our first hypothesis, as plants that did not receive water and nutrient supplements showed more sclerophyllous leaves (see Fernandes & Price, 1991), whereas supplemented plants showed greater leaf expansion and higher fruit production. Rocky outcrops are environments with low water availability and poor soils (Silveira et al., 2016), and when the availability of these resources increases, the plant is able to allocate them for growth and reproduction, as seen experimentally in the greenhouse by Negreiros et al. (2009). The positive effect of increased nutrition on leaf growth and fruit production observed in this study had already been shown for cultivated species (Shehata et al., 2011) that relate the increased contents of N, P, K in the decrease of abiotic stress damage to plants (Maestre et al., 2009). High nutrient availability can also increase plant growth capacity and compensate for the possible negative effect of pathogens (Figueroa-Macías et al., 2021). Other studies have also shown that leaf sclerophylly is positively related to water and nutrient stress (Fernandes & Price, 1991; Wright et al., 2017), with the plants' strategy being to increase the number of cells in the leaf epidermis to minimize water loss by the leaves in these environments (e.g., Bussotti et al., 2015).

Possibly, this lower sclerophylly may be related to the momentary hydration by the leaves, commonly called foliar water uptake (FWU). This is a common strategy in several species in different biomes, which means that there is rapid hydration in the leaf, the main organ responsible for the carbon assimilation and biomass accumulation (Berry et al., 2019). The fact of spraying water on the leaves can actually cause it to enter through the leaves or this water can stay on the leaf surface and prevent transpiration (Holanda et al., 2019). In addition to benefiting the water status of the leaf, the FWU can assist in nutrient input (Wójcik, 2004). This makes nutrition even more effective and quick for the leaf, not requiring the roots and, consequently, the xylem to make this transport. According to the proposed treatments, we can be more confident that the leaves used in the study actually experienced greater hydration and nutrition.

Apparently ants played an important defense role in our study, since the treatments that had increased resources for ants (EFN simulation), there was a higher recruitment of ants and herbivory rates were lower. Even plants that had nutrient supplementation and EFN simultaneously showed higher rates of leaf damage, but much less damage compared to plants that had no ant supplement. Previous work has shown that removing ants from plants increases herbivory rates by almost 50% (Rosumek et al., 2009), and in plants with higher abundance of aggressive ants herbivory rates are lower (Camarota et al., 2015). In addition, Gomes et al. (2021) showed that ants play an important role in decreasing herbivory, pathogenic fungi and even in plant quality, such as increased growth and higher leaf nitrogen concentration and decreased trichomes. *C. langsdorffii* maintains active EFNs only in young leaves (Queiroz et al., 2013), although our results show a compensatory effect in maintaining

EFNs until the leaf senescence phase, this mutualistic interaction involving EFNs and ants are not so specific and the results of this interaction are uncertain for plants (Rico-Gray & Oliveira, 2007). Therefore, despite the protective effect of ants shown here, the mutual defense strategies developed by plants could be more compensatory than a single defense investment. Despite this crude measure of ant activity showing significance for plant defense, we argue that future experiments should attempt to focus specifically on ants, with an emphasis on identifying species and functional groups.

Ant-plant interactions did not have any effect for the galling insect communities, with richness and abundance unaffected by the treatments described here. Although the presence of ants on plants tends to reduce general herbivory (Rosumek et al., 2009), the abundance of galls are not affected (Fagundes et al., 2005). Otherwise, studies measuring the impact of ants on sessile herbivore communities are still very scarce. Galling insects may induce deep differentiation at the cellular level in their host plant (Fernandes & Price, 1988) and the plant-galling insect relationships are evolutionarily stable (Fagundes et al., 2020; Fernandes & Price, 1988). Furthermore, when the gall larvae hatch, the abandoned structure may serves as a refuge for various insect groups (Barbosa et al., 2019; Pereira et al., 2020), including ants that use these shelters for protection and nesting. We already expected that the diversity of galls would not vary within the population in a xeric environment; however, the presence of ants was a questionable factor.

We showed that the patterns of co-occurrence of galls varied among the treatments. It is likely that the addition of nutrients could probably affect the competitive interactions among the galling species, and conditions with high resource availability allow for greater coexistence of the species. The interactions that shape the communities of sessile organisms are dependent on the availability of resources; for these groups, physical space is the main cause of competition. For example, plants compete for space with greater availability of nutrients (Sanders et al., 2003), lichens compete for environments with more water (Maestre et al., 2009), and gall-inducing insects compete for oviposition space (Cornelissen et al., 2013; Ramos et al., 2019). Evaluating patterns of co-occurrence among environments with varying stresses, Ramos et al. (2019) showed that in Ironstone outcrops competition is prevalent. The patterns found in this work are similar, as there was variation in co-occurrence of the galls on plants that were supplemented and had their traits modified, such as may leaf area, which affected the random distribution of galls in same type of meristems.

Conclusion

Overall, this study illustrates how variations in nutrient availability to host plants and mutualistic interactions with ants contribute to variation in co-occurrence patterns of gall communities and herbivory levels, respectively. Plants in natural environments are positively modified by nutrient and water addition and this increases the rate of herbivory and decreases competition in the gall communities. Ants play an important role in diminishing herbivory rates, but they do not have an effect on insect gall communities. The study of biotic and abiotic interactions and their implications for ecosystem functioning can provide direction for predicting how the impacts of global change, such as changes in rainfall and water scarcity, modify plant communities and the stability of their ecological interactions, mainly, under scenarios of declining insect diversity.

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Author contributions

LR, RS, GWF and MF conceived the ideas and designed the experiment; LR, RS and DB developed the methodology; LR and RS collected and analysed the data; LR and RS led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data accessibility

Upon acceptance, the full set of raw data used to generate this manuscript will be made publicly available in one of the recommended repositories (Dryad, Zenodo or Figshare).

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Figures

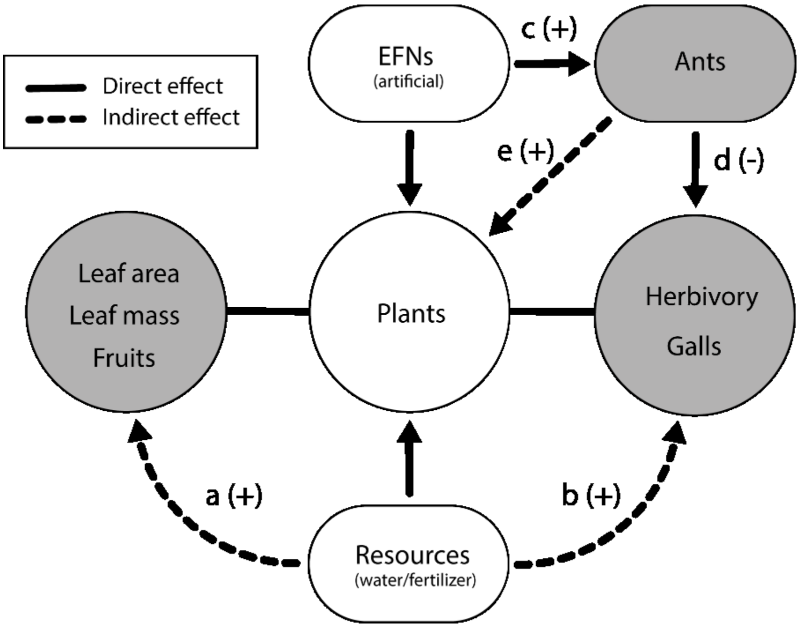

Fig. 1: Path diagram summarizing hypothesized biotic and abiotic effects on trophic interactions. Symbols with white fill (EFNs and Resources) correspond to supplementation used on plants . The gray-filled symbols correspond to the variables measured in this study. Arrows indicate a direct (solid lines) and indirect (dashed lines) effect of one level on another. The (+) and (-) signs indicate a positive or negative effect respectively. The letters next to the arrows represent the effect we expect to find in this study. For example, in A we expect nutrient and water supplementation to decrease plant sclerophylly, increase leaf area and fruit production, and in B increase herbivory rates and the co-occurrence of galling insects. In C, the addition of EFNs (using artificial EFNs that mimic the composition of natural nectar) has a positive effect on ants and (D) ants have a negative effect on herbivory rates and galling diversity. In (E) a positive indirect effect on plants, with higher fruit production and less tissue loss (low herbivory), as ants protect the tissues.

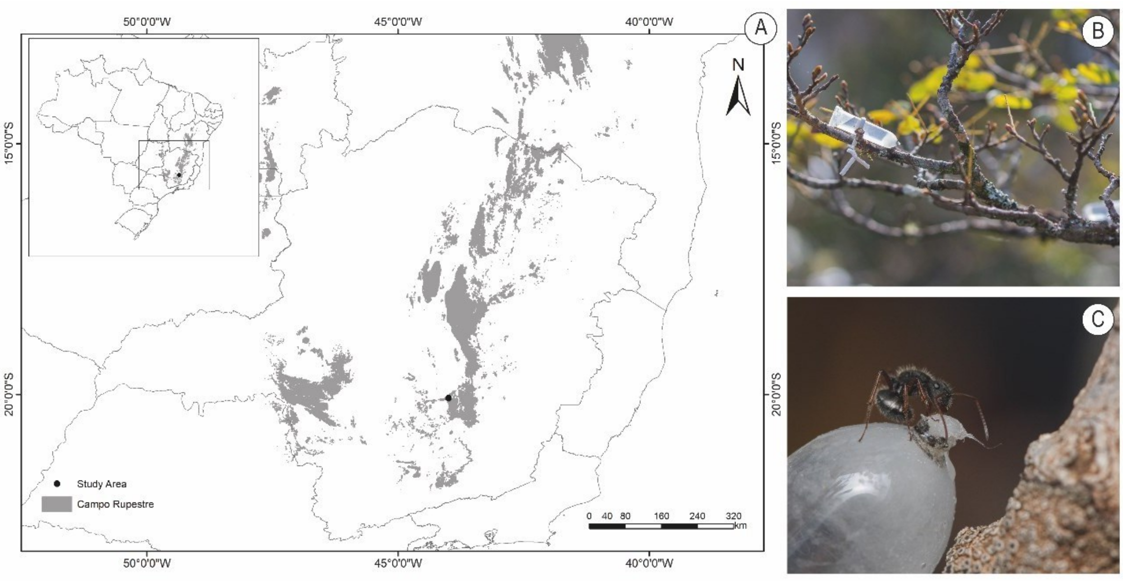

Fig. 2: (A) - In gray is the estimated area of Campo rupestre in Brazil with the sampling area of this study. (B) - Microtube with sugar and water for the Extrafloral Nectary simulation experiment. (C) - *Camponotus rufipes* ant feeding in the EFN experiment. Photo credit: Tomás Oliveira

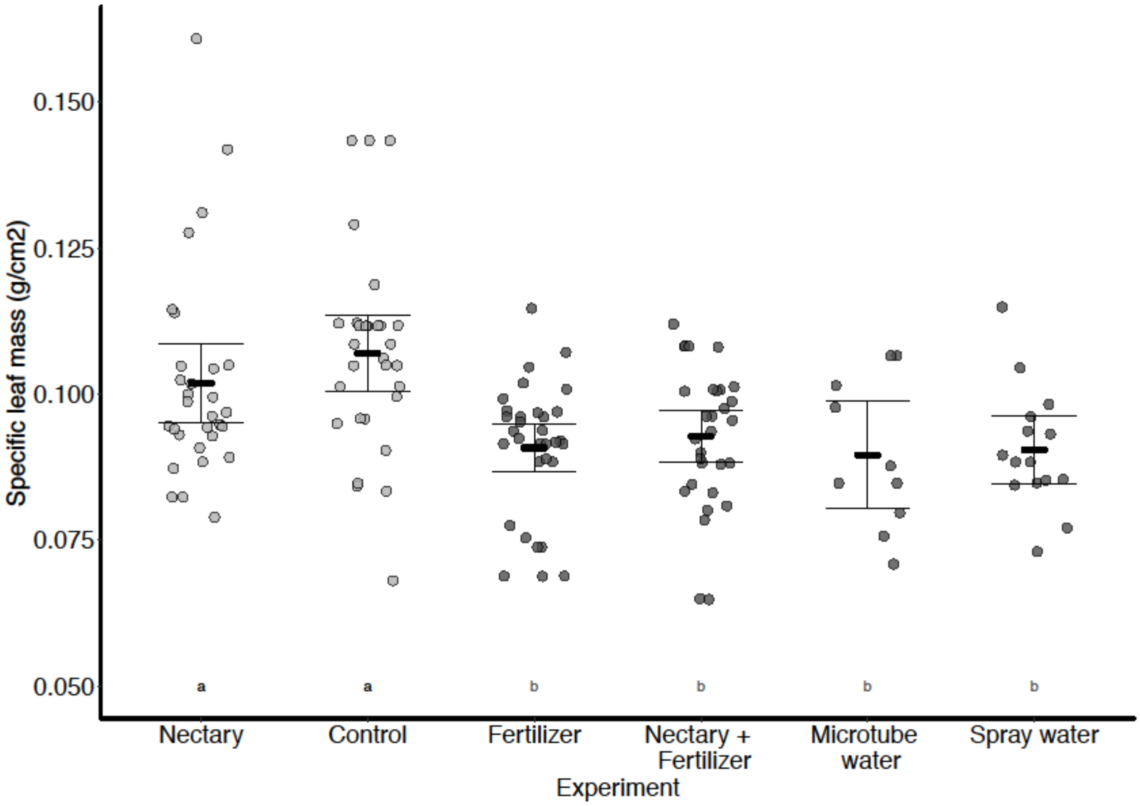

Fig. 3: Variation in specific leaf mass of plants of *Copaifera langsdorffii* among the experiments in ironstone outcrops. Same letters in bars represent grouping by contrast analysis (p.value < 0.05).

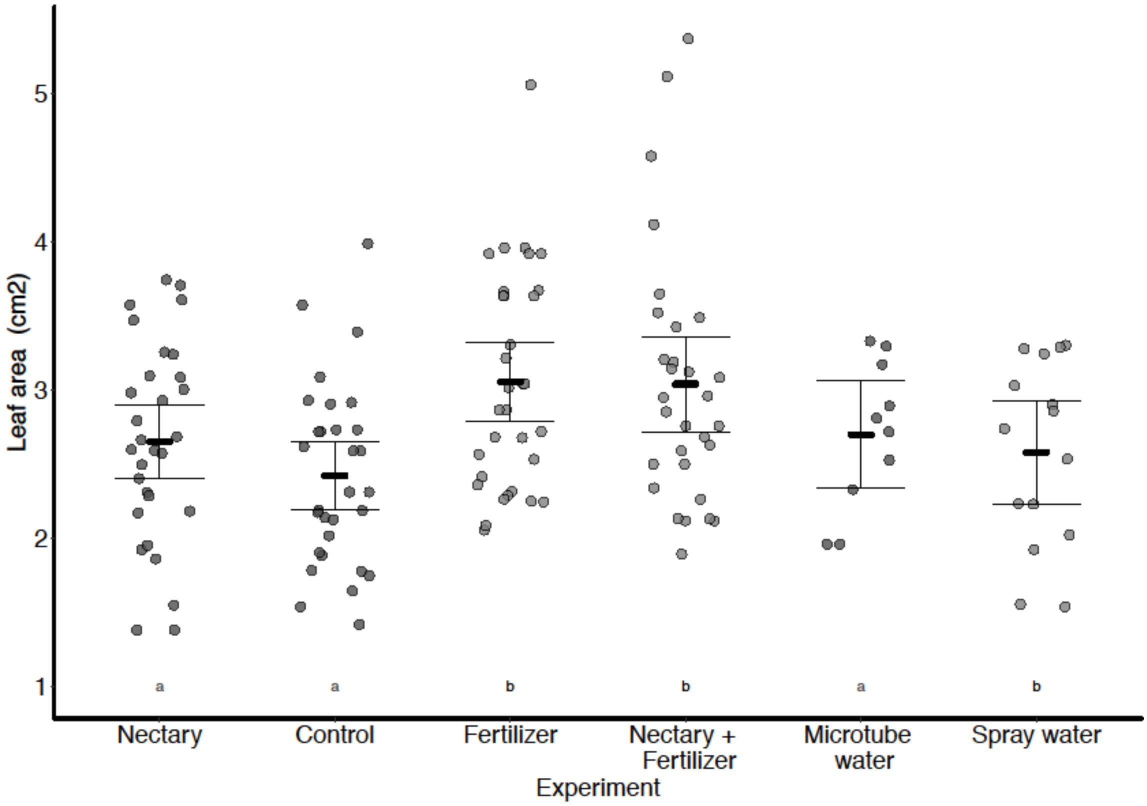

Fig. 4: Variation leaf area of plants of *Copaifera langsdorffii* among the experiments in ironstone outcrops. Same letters on bars represent grouping by contrast analysis (p.value < 0.05).

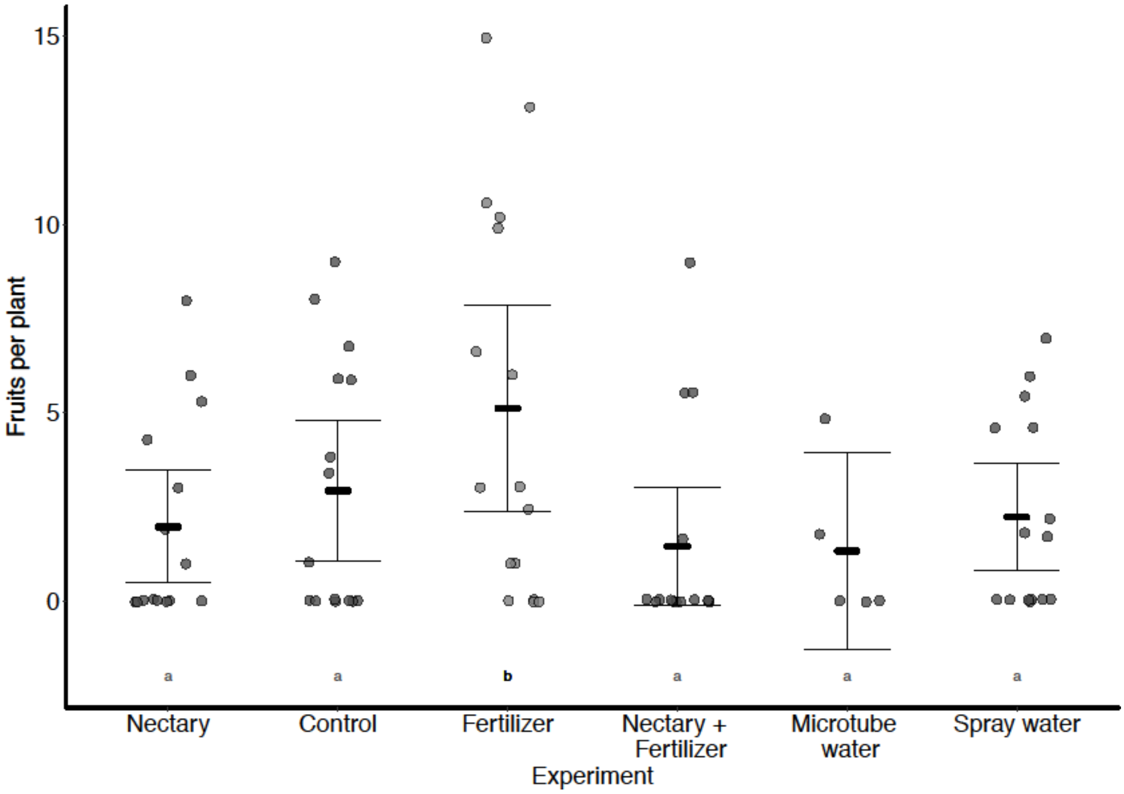

Fig. 5: Variation in media fruits per plants of *Copaifera langsdorffii* among the experiments in ironstone outcrops. Same letter on bar represent grouping by contrast analysis (p-value< 0.05).

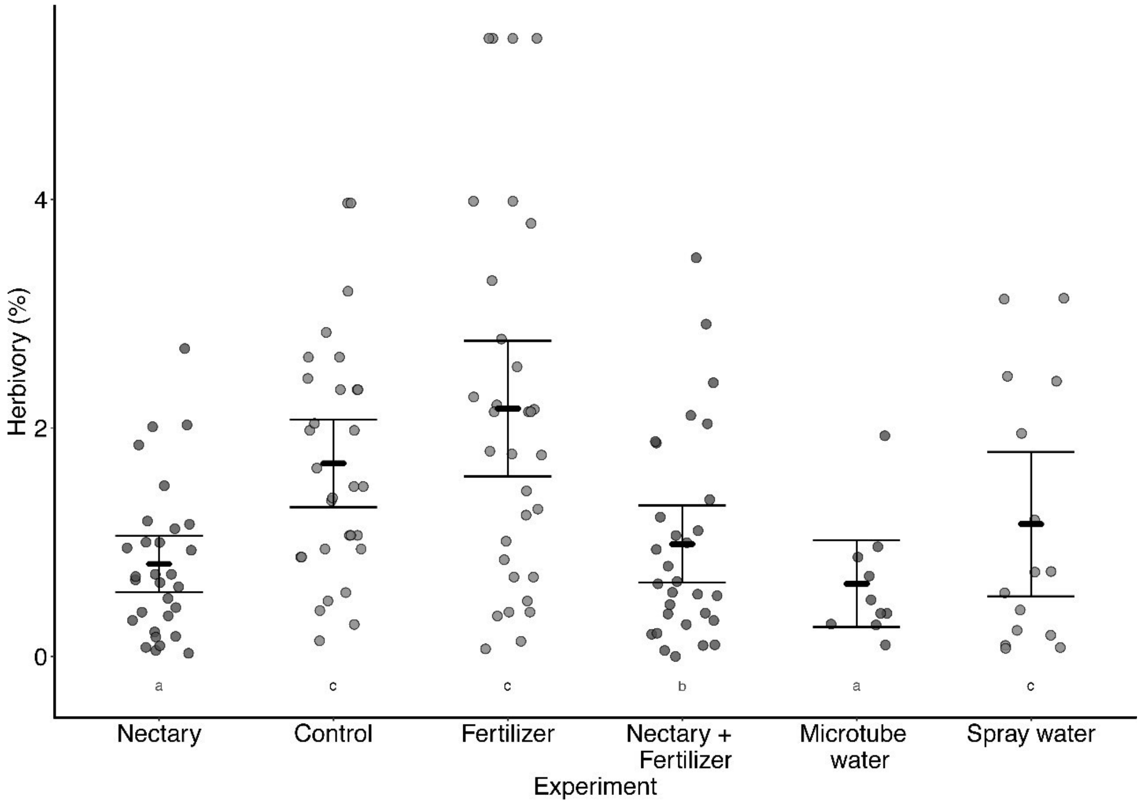

Fig. 6: Variation in percentage of herbivory of plants of *Copaifera langsdorffii* among the experiments in ironstone outcrops. Same letters on bars represent grouping by contrast analysis (p-value < 0.05).

Tables

Table 1: *C-score* indices of the species occurrence of galls in *Copaifera langsdorffii* in six experiments evaluated in ironstone outcrops. Maximum and minimum indices were calculated from 5,000 randomizations of the original matrix. O *p*-values were obtained by bi-flow test and represent the probability that the observed index is greater than, less than or equal to that expected at random matrices. Observed (obs), expected (exp)

	Indices for randomized matrices			p. values	
Treatment	Minimum	Maximum	Observer index	Obs. > exp	Obs. < exp
Control	0.8891	0.9017	0.8955	**0.01**	0.975
Nectary	0.8566	0.8647	0.8652	**0.004**	0.995
Fertilizer	0.9359	0.9743	0.9473	0.766	0.236
Nectary + Fertilizer	1.1051	1.157	1.139	0.193	0.822
Microtube Water	0.990	1.077	1.009	0.917	0.158
Spray water	0.955	1.003	0.965	0.9152	0.094

Capitulo 2: Title: Biotic and abiotic factors modify insect-plant interaction networks

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Abstract

Interactions involving insects and plants are of great relevance to understand how biotic and abiotic changes interfere with trophic interaction networks and community dynamics. We evaluated how *Copaiffera langsdorffii* (Fabaceae) responds to changes in nutrient and water availability through experimental supplementation, and how the presence of extrafloral nectaries (EFN) throughout the phenological cycle of the plant modifies the interaction networks of free-living insect and ant communities. Experiments were set up in an ironstone outcrops vegetation , where we applied supplementation treatments to 80 plant individuals divided into the following field treatments: (T1 = Fertilizer, T2 = EFN simulation (Eppendorf tubes with a 20% sugar solution), T3 = fertilizer + EFN simulation, T4 = water spray, T5 = EFN control (microtube with water), and T6 = control (plants without manipulation)). We classified free-living insects and ants at the level of feeding guilds according to foraging habit. We evaluated the properties of all groups at the level of overall networks between treatments, at the level of species within treatments, and at the level of food guilds between treatments. All networks showed high modularity and specialization, but the network of the treatment with nutritional supplementation showed the greatest number of interactions and was the least specialized. Ants showed higher specialization and free-living insects were more generalists. The treatments with EFNs supplementation showed a greater affinity with opportunistic ants and negative relationships with free-living insect guilds. Moreover, sucking and chewing herbivorous insect guilds were positively related to plants with nutrient supplementation. We show that nutrient supplementation and EFNs in the plant can alter insect interaction networks. However, when we disentangle these networks at the guild level, we to show that the quality of the plant tissues can alter the preference of herbivorous insects. Hence, both abiotic and biotic factors have a role in shaping the interaction networks between plants and free-living insect.

key words: Antagonistic networks, Mutualistic networks, Abiotic, Biotic, Ants, Free-living insects, Insect-plant interaction

Introduction

Plants are embedded in a complex network of interactions and, throughout their life cycle, they maintain mutualistic and antagonistic associations with various organisms (Ehrlich & Raven, 1964; Herms & Mattson, 1992; Hutchinson et al., 2017). It is estimated that 40% of the earth's biodiversity is interrelated with the interactions between herbivores and plants (Price, 2002), and these interactions maintain the upward flow of energy to higher trophic levels (Futuyma & Agrawal, 2009). Changes in plant species composition and individual quality can modify herbivore communities and scale-up to interactions with predators, parasitoids, omnivores, and even mutualisms such as pollinators (Biesmeijer et al., 2006; Novotny & Basset, 2005). In this context, interaction networks are a useful tool for studying the patterns, structure and dynamic of multi-species assemblages connected by their interactions (Dunne et al., 2002), as well as understanding how abiotic and biotic factors modify such interactions.

The different feeding habits and life forms of insects allows for classification into trophic guilds (Lewinsohn et al., 2005; Novotny et al., 2010), and these guilds show different degrees of specialization, from generalists that feed on many plants to others highly specific to their host plant (Novotny et al., 2010). Such specialization is linked to host plant quality, which among other factors, is influenced by changes in water and nutrient availability, consequently affecting herbivore communities that are sensitive to intraspecific variations in plant characteristics (Barbour et al., 2019; Boege, 2005). Just as bottom-up factors have consequences by changing resource availability for herbivores, top-down factors also trigger changes in these communities. Mutualistic associations between ants and plants are a good example to understand such effects, as plants provide a food resource to ants (extrafloral nectaries, EFN), while ants provide anti-herbivore protection (Rosumek et al., 2009). Because these complex effects act at the same time, it is essential to simultaneously assess the effects of abiotic and biotic factors to understand the dynamics of insect-plant interactions.

In general, ecological research with interaction networks tends to focus on specific groups of species with a single type interaction in isolation, such as mutualistic or antagonistic networks. Network structural properties (e.g. Nestedness, Modularity, Specialization) tend to differ with the type of interaction observed. For example, nestedness quantifies the tendency of specialist species, which tend to be more numerous, to interact with subsets of generalist species that are rare (Bascompte et al., 2003; Lewinsohn et al., 2005) and this pattern generates stability in mutualistic networks (Thébault & Fontaine, 2010). In contrast, modularity characterizes the prevalence of interactions in subgroups of species that interact more with each other than with other species, and herbivore-plant antagonistic networks tend to be more stable when they exhibit a more modular pattern (Stouffer & Bascompte, 2011; Thébault & Fontaine, 2010). On the other hand, specialization is characterized by whether or not the frequency of species interactions are restricted from those expected randomly based on partner availability, being at the network level (H'2) and at the level of species in the network (d') (Blüthgen et al., 2006).

Plants generally have physiological adaptations that allow them to withstand water and nutrient scarcity (Fernandes, 2016; Silveira et al., 2016) such as modified leaf traits that decrease evapotranspiration (Boanares et al., 2019), which in turn impact the communities of insects that use these plants as resources (Ramos et al., 2019). Nutritional supplementation with Nitrogen, Potassium, Carbon and increased water availability, increase its plant photosynthetic rates and vegetative growth, thus abridging the effects of environmental stress to these plants (Lázaro-Nogal et al., 2015; Shehata et al., 2011). On the other hand, the experimental addition of EFN-like structures attract more ants that tend to increase patrolling activity and improve protection service against herbivores (Pacelhe et al., 2019; Ribeiro et al., 2018; Rosumek et al., 2009). As an example, *Copaifera langsdorffii* (Fabaceae) is a widespread tropical plant that presents different strategies to cope with environmental variations (Souza et al., 2018), and a high diversity of insects use it as a resource (Fagundes et al., 2020). In addition, this plant has extrafloral nectaries (EFN) found only on young leaves, that attract several ant species (Oliveira & Isaias, 2010; Queiroz et al., 2013). This interplay of biotic and abiotic factors makes this plant an excellent experimental model for testing how these variations influence insect-plant-ant interaction networks.

In this study, we aimed to understand how abiotic and biotic factors affect the interaction networks of a model plant, *C. langsdorffii,* with its associated insects. To do so, we experimentally manipulated in the field the availability of water, nutrients and EFN in the plant. We asked the following questions: (1.) is the stability of insect/plant interactions affected by biotic and abiotic factors?; (2.) does the constant availability of EFN sugary exudate reduce the frequency of insect-plant interactions? We hypothesize that resource availability for plants (nutrients and water) will increase interactions with free-living insects and networks will be less specialized (H'_2). Our second hypothesis is that greater availability of EFN will increase the frequency of interactions with ants, networks will be more nested, and ants will be more specialized (d') than free-living insects.

Material and Methods

Study area

Our sampling site is located in an environmental protection area of approximately 1,100 ha, locally known as Serra da Calçada (approximately 19°16′S, 43°35′W), municipality of Brumadinho, state of Minas Gerais, Brazil. The predominant climate is high altitude subtropical according to Koppen's classification (Alvares et al., 2013), with an average altitude of 1,200 m, rainfall of 1490.00 mm/year, average temperature of 20.74 °C and high solar incidence. The predominant vegetation belongs to the rupestrian grassland ecosystem (Campo rupestre in Portuguese) see Fernandes et al., 2016). In this mountain ecosystem, ironstone outcrops are found on poor soils with high iron content that form banded ironstone locally known as Canga. This environment has low water holding capacity and nutrients such as Nitrogen and Phosphorus are in very low availability (Schaefer et al., 2016; Silveira et al., 2016). Plant species are mostly sclerophillous and adapted to abrupt variations in temperature, insolation, strong winds, and deprivation of water and nutrients in the soil (Fernandes, 2016; Giulietti, 1987; Oliveira-Filho & Ratter, 2002; Schaefer et al., 2016; Silveira et al., 2016).

Study system

Copaifera langsdorffii Desf (Fabaceae) is a tropical tree species that varies in height from 2 to 35m, depending on environmental conditions (Carvalho, 2003). This species has high phenotypic plasticity across its wide geographic distribution, occurring in the Caatinga, Cerrado, Atlantic Forest, and Amazon biomes in Brazil (Almeida et al., 1998; Souza et al., 2018). This species has compound leaves with 4 to 12 alternating leaflets. At the beginning of the leaf expansion phase, the leaflets have a reddish coloration and two EFN at the base that remain active until the mature phase of the leaves (dark green coloration), attracting several ant species (Queiroz et al., 2013).

C. langsdorffii has a striking characteristic of total leaf abscission in the dry period with the emission of new leaves soon after (Costa, et al., 2016; Pedroni et al., 2002). This plant has a supra-annual reproduction during the rainy season, and every two years it produces flowers with a sweet scent that is attractive to many insects contributing to high fruit set (Freitas & Oliveira, 2002). Seeds are dispersed by ants and birds and plants also harbor a diverse fauna of insects, which use its leaves as food and shelter (Costa et al., 2010; Fagundes, 2014).

Experimental Design

We marked a total of 80 *C. langsdorffii* adult trees with an average height of 2m in the study area that were divided into six treatments of water fertilization and EFN simulation (T1 = Fertilizer, T2 = EFN simulation, T3 = Fertilizer + EFN simulation, T4 = Water spray, T5 = EFN control - microtube with water, and T6 = Control). We kept our manipulative experiments running for two consecutive years to follow the entire reproductive cycle of this plant species.

To supplement plant nutrients, we use organo-mineral leaf biostimulant (Aminon-25 Technes ®) which contains water-soluble nutrients (11% Nitrogen (N), 1% Potassium ($K2O$) and 6% Total Organic Carbon (TOC). The main function of this fertilizer is the biostimulation of the plant to increase its photosynthetic capacity and consequently increase its primary production. At the beginning of the leaf sprouting phase in *C. langsdorffii,* we started to apply the biostimulant in the experimental plants in the field until the stage of total leaf senescence. Following the manufacturer's recommendation, we used a manual sprayer containing a 0.1% solution of Aminon-25; and all leaves were sprayed uniformly. The application was always made in the morning of sunny days, which is the period when the stomata are open and absorb nutrients more easily (Roelfsema & Hedrich, 2005) and was repeated every 15 days.

To maintain EFN production until the leaf senescence phase, we simulated EFN production using microtubes (2 ml), perforated at the base, containing a cotton bud soaked in a 20% sucrose solution (Camarota et al., 2015). We attached each microtube randomly at the end of the branches near the leaves, where ants had free access. We installed a minimum of 30 tubes per plant, to account for plant size, we calculated a ratio of 10 microtubes per number of branches on the plant. Every 15 days, the microtubes were replaced with new ones with the same solution to avoid fungal contamination. As a control, on 5 plants we added microtubules containing drinking water (under the same conditions as the EFN supplement).

We also manually sprayed clear water on 15 plants, with the goal of decreasing water deficit when absorbed through the stomata (Schreel & Steppe, 2020). We used a hand sprayer containing clear water, ensuring that all leaves had good application coverage. This application was also performed in the morning (when the stomata are open) and was repeated every 15 days. Finally, we established a treatment combining experimental EFNs + fertilizer on another group of 15 plants, which was also repeated every 15 days strictly in the morning. Another 15 plants remained under normal environmental conditions, to be our control treatment.

Sampling of arthropods and classification into trophic guilds

Sampling of arthropods on the plants occurred at the end of the rainy season in March and April 2019/2020. In each experiment we collected ants and free-living insects from all trees by the beating method (Neves et al., 2013), all sampling occurred in the morning between 7:00 and 11:00 am. We arbitrarily selected one branch of approximate size on all plants, where we tapped 10 times and dislodged insects dropped into an entomological umbrella (Ribeiro et al., 2018). The sampled insects were packed into individualized plastic jars and taken to the lab for identification. Ants were classified by genera following the auxiliary keys of Baccaro et al. (2015), and classified into morphospecies, or when possible, identified to the species level. All sampled ants were classified into functional groups following the classification of Paolucci et al. (2016). All collected insects were identified to the family level with a taxonomic key (Rafael et al., 2012) and classified into morphospecies. The collected insects were also classified into guilds according to their life/feeding habits as: chewers, suckers, galling parasitoids, predators, xylophagous and saprophytes; with the help of specialized literature (Rafael et al. 2012, Novotny et al., 2010).

Network Analyses

We built a total of six individual plant – insects interaction networks, one for each treatment (spray-water, nectary+fertilizer, nectary, fertilizer, ep-water and control), and one network aggregating all individual plants irrespectively of treatments. We evaluated the sampling completeness of all networks considering each combination of a plant and insect species as 'species' and the frequency of each pairwise interaction as their 'abundances' (Vizentin-Bugoni et al., 2016). We have estimated the diversity of interactions using the Chao 1 estimator of species richness (Chao, 1984; Colwell & Coddington, 1994). Then, we calculated sampling completeness as the ratio of the observed and estimated richness of interactions (Chacoff et al., 2012). The Chao 1 estimator was computed using the iNEXT package (Hsieh et al., 2016) in R (R Core Team, 2017).

We evaluated how species partition their interactions in the different networks constructed using three quantitative metrics. Network-wide specialization was estimated by the H_2' index that describes whether or not species restrict their interactions from those randomly expected based on partner availability (Blüthgen et al., 2006). Modularity indices quantify the prevalence of interactions within subsets of species in the community and was calculated using the DIRTLPAwb+ algorithm (Beckett, 2016) using the computeModules function in Bipartite package (Dormann et al., 2008). Nestedness quantifies the degree to which interactions of specialized species are subsets of interactions of the more generalized species in the network, and was quantified by the wNODF index (Almeida-Neto & Ulrich, 2011). Additionally, we also calculate the robustness of each network, as first proposed by Memmott et al., (2004), with an implementation of the quantitative version proposed by Burgos et al., (2007), where extinction simulations were carried out randomly and with a single parameter R, defined as the area under the extinction curve. This index varies from 0 to 1, where R = 1 corresponds to a curve that decreases very mildly until the point at which almost all individual plant species in each treatment are eliminated. We ran the simulation by removing only plants randomly. Network indices may be affected by the number of interacting species and sampling effort (Fründ et al., 2016). Therefore, in addition to using the 'raw' modularity, specialization and nestedness values, we used Δ-transformed with 1000 randomizations for each network. We used the Patefield null model, which fixes the network size and the marginal totals, which is species richness and species total number of interactions, while shuffling interactions randomly (Patefield, 1981), and compared Δ-transformed metrics between networks. Δ transformation is done by subtracting the mean value of a metric obtained by multiple randomizations from the observed value and has been shown to minimize differences owed to sampling in network metrics used here (Dalsgaard et al., 2017).

In addition to these three network-level indices, we also calculated four node-level indices that captured distinct properties of a species/plant individuals in the network: (1) degree, expressing the number of interaction partners' that each node is linked to in the network; and (2) strength, which is the sum of the proportions of interactions performed by a given node across all its interaction partners (Bascompte et al., 2006); (3) partner diversity calculated as the exponential Shannon diversity and interpreted as a measure of generality of interactions, with the advantage of down weighing rare interactions (Dormann, 2011); and (4) specialization d', which quantifies how frequencies of interactions of a given node deviate in relation to the availability of partners in the network, with higher values indicating higher specialization (Blüthgen et al., 2006). We calculated all network-related indices with the Bipartite package v.2.15 (Dormann et al., 2008) in R.

Statistical analysis

We evaluated whether the treatments correspond to the occurrence of insects and ants guild using a Chi-square test to contrast the proportion of distinct combinations of insects guilds occurring in the different treatments. We then illustrated the proportion/contribution of each insect's and ants guild occurrence on treatments graphically using Pearson residuals from the Chi-square test with the "carplot" package in R (Wei and Simko, 2017). Second, we compared the role of ants, insects and *C. langsdorffii* individuals between different treatments using a Tukey test with the function glht in the "multcomp" package (Hothorn et al., 2008). All statistical analyses were conducted in R (R Development Core Team, 2019).

Results

We recorded 2.148 free-living insects representing 43 families and 2934 ants representing nine genera among all treatments (Table:1, Table: 2 in the supplementary material). The treatment where the plants received only nutritional supplementation showed the highest abundance of free-living insects: Fertilizer 679 insects, followed by Control with 520, Nectary+Fertilizer 394, Nectary 310, Ep-water 155 and Spray-water 90. On the other hand, the treatments with EFNs supplementation attracted more ants, with 994 ants in the Nectary treatment, followed by Nectary+Fertilizer with 890 ants, 345 in Control, 332 in Fertilizer, 322 in Ep-water and 51 in Spray-water. We sampled eight free-living insect guilds and six ant guilds. The proportion of occurrence of the guilds of free-living insects and ants were significantly related among the treatments ($X^2 = 1156$, df = 65, $p < 0.0001$) (Fig.1).

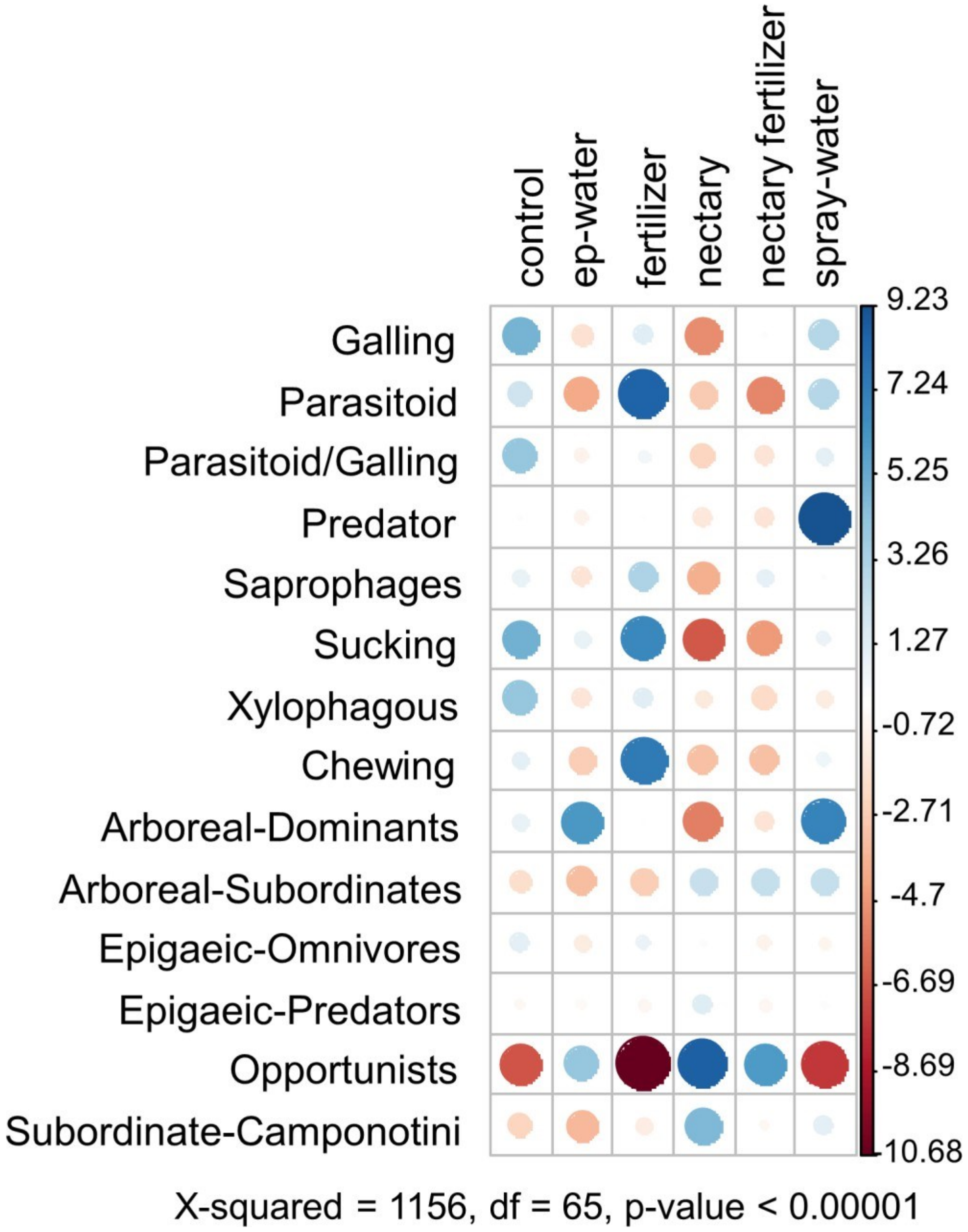

Figure 1: Proportion of occurrence of free-living insect guilds and ants among the treatments evaluated. The sizes of the circles and the colours indicate intensity of occurrence, with blue scale indicating positive associations (the darker the blue colour, the higher is the correlation), and red negative associations (darker the red colour, lower is the correlation).

Networks from all treatments had similar sampling completeness (average 53% of sampling) from all interactions. The fertilizer network showed the highest number of ants and insect species, followed by the control network and nectary+fertilizer network (Table: 3 Fig.2). In addition, all networks were more specialized and modular than expected by null models (see Table 3). However, the nestedness differed from that expected by the null model only for the spray-water network. Moreover, the spray-water network was the most modular and specialized, and the ep-water and fertilizer networks were the ones with the most generalized structure (Table:3 Fig.2).

Table 3. Comparison of metrics between the networks considering all treatments performed (fertilizer, nectary, nectary+fertilizer, spray-water, ep-water and control). We report the uncorrected as well as network metrics corrected by the null model used (Patefield).

Treatment	**spray-water**	**nectary+fertilizer**	**nectary**	**fertilizer**	**ep-water**	**control**
Ants number	10	22	23	24	13	19
Insects number	39	73	69	96	35	84
Network size	64	112	109	136	53	119
Number of links	80	239	247	364	89	241
Robustness	0.59	0.64	0.66	0.70	0.60	0.64
Modularity Q	0.63*	0.42*	0.48*	0.40*	0.32*	0.53*
ΔPatefield Q	0.59*	0.26*	0.33*	0.29*	0.21*	0.29*
Modules number	8	9	14	8	4	8
Specialization H2′	0.61*	0.50*	0.58*	0.39*	0.36*	054*
ΔPatefield H2′	0.41*	0.42*	0.49*	0.28	0.30*	0.41*
Nestedness WNODF	4.57*	10.8	9.92	11.77	17.19	9.12
ΔPatefield wNODF	4.38*	9.45	7.45	5.51	10.42	5.42
Sampling completeness	43%	57%	56%	56%	57%	47%

* p value < 0.05.

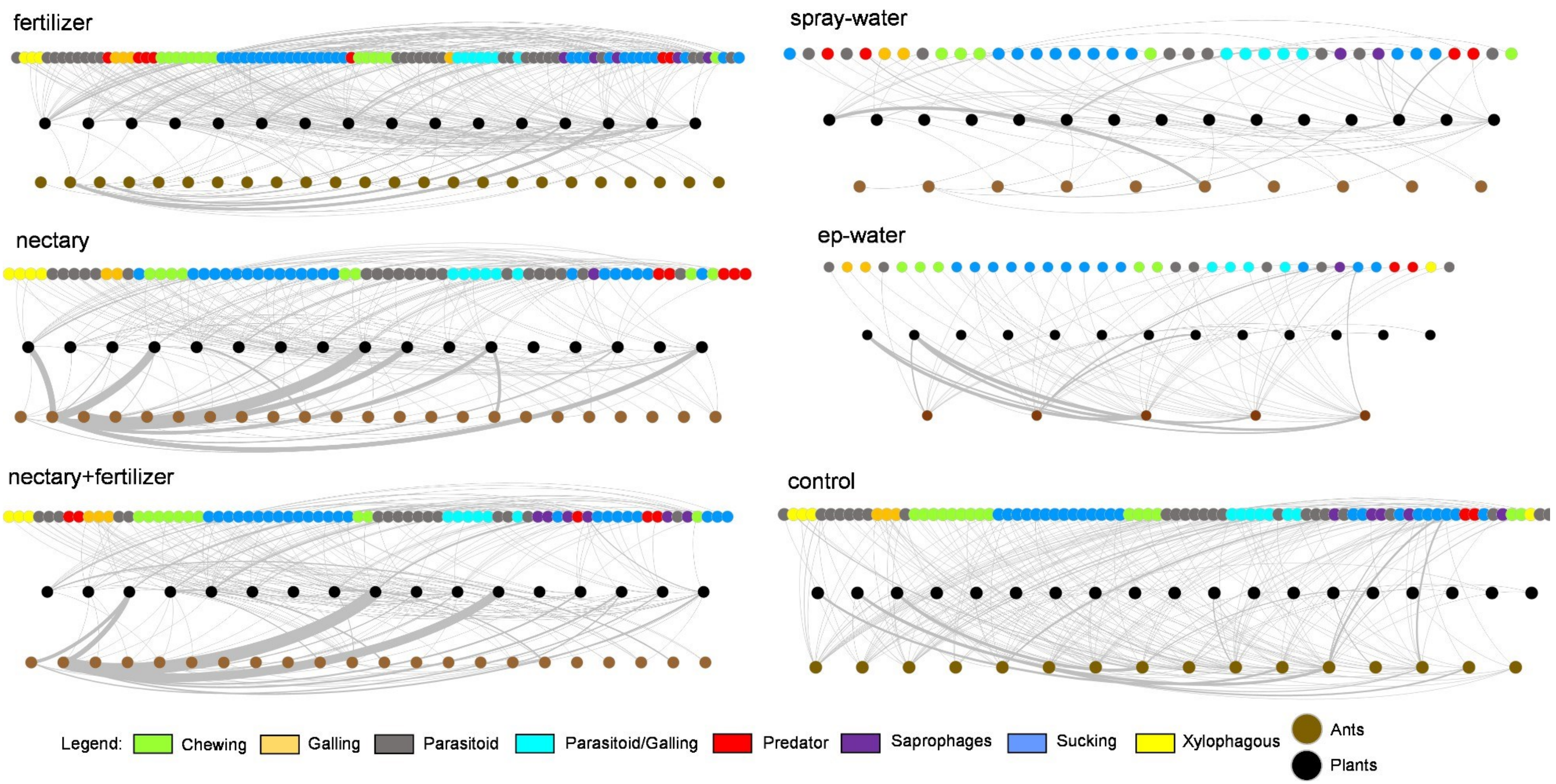

Figure 2: Graphical representation of the networks of interactions of free-living insects and ants on plants in each experiment evaluated. In the networks, the black dots represent the individuals of *Copaifera langdorffii*, the brown dots represent all the ants found in the treatment, and the coloured circles represent the guilds of free-living insects in each treatment. The lines represent the interactions

The role of ants, free-living insects, and plants varied within each network in the treatments. Plants differed in degree (number of partners in the network) among treatments (F= 9.815, $p < 0.0001$, Fig 3). In the treatments with fertilizer, nectary, and nectary+fertilizer had the highest degree, while plants in the treatments with water spray had the lowest degree. Species strength also varied among treatments (F = 6.459, $P < 0.0001$) forming two groups: control, ep-water and fertilizer; and nectary, nectary+fertilizer and spray water. Partner diversity also varied (F = 5.696, $p< 0.0005$) the Fertilizer treatment showed higher values, but did not differ from the control. Specialization (d') also varied across the group of plants (F= 3.326, $p < 0.0001$), with the plants in the spray-water treatment tending to be more specialized. When we evaluated these metrics for free-living insects and ants, we observed that; insects differed in degree between treatments (Fig. 4) (F = 6.623, $p < 0.005$), but ants did not (F = 0.835, p = 0.5257, insects in the fertilizer treatment showed higher degree (Fig. 4). The species strength varied for the insects (F= 7.604, $p < 0.0001$), where the spray-water treatment differed from the others, for the ants it did not differ (F = 0.5907, p = 0.707). The partner diversity of the insects varied among the treatments (F = 8.502, $p < 0.001$), the treatments with Nectary and only with Fertilizer differed from the others, the ants also did not differ (F= 0.5076, p = 0.77). Finally, the specialization (d') of the insects differed (F = 13.95, $p < 0.001$), the treatments with Ep-water and Fertilizer showed lower values, and for the ants also varied (F = 3.771 p = 0.0035), being the treatment with ep-water lower specialization. We also evaluated these indices at the level of insect and ant guilds between the different treatments, however, the only index that had significant variation at the insect level was the partner diversity (F = 2.306, p = 0.0380) (Fig. 5). Ant guilds varied in partner diversity (F = 3.432, p = 0.0304) and specialization (d') (F = 4.102, p = 0.015) with arboreal-dominates showing greater specialization than subordinate-Camponotine (Fig. 6).

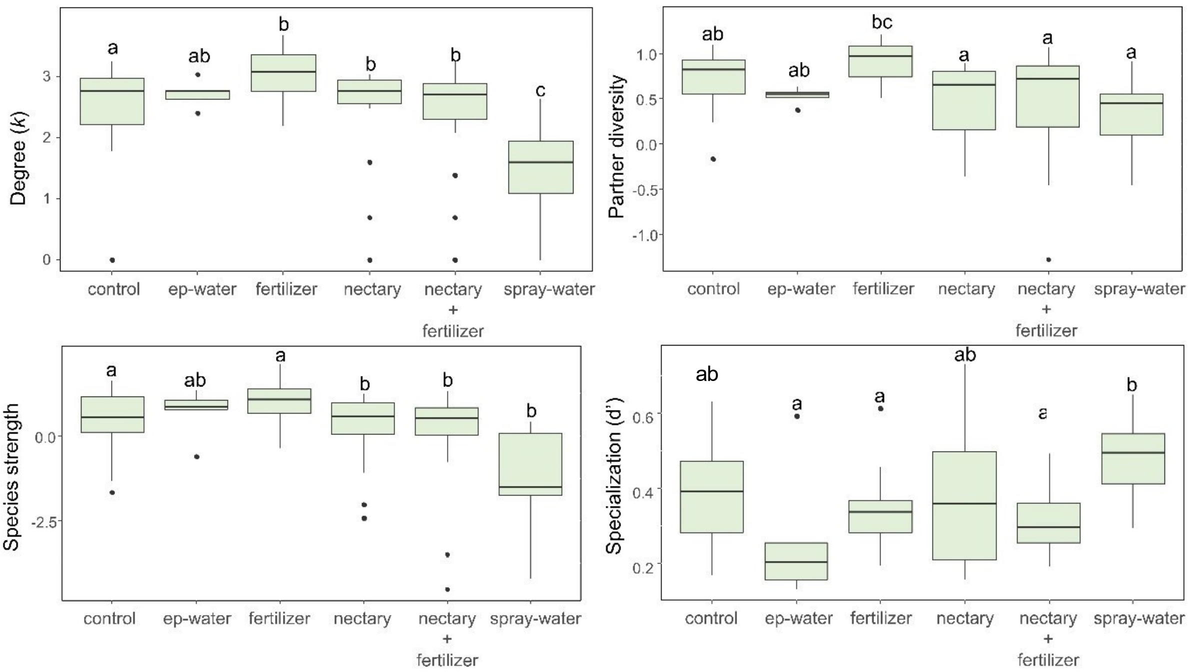

Figure 3: Species-level parameters evaluated among plant individuals in each treatment. The letters on the bars represent differences between treatments for each index evaluated.

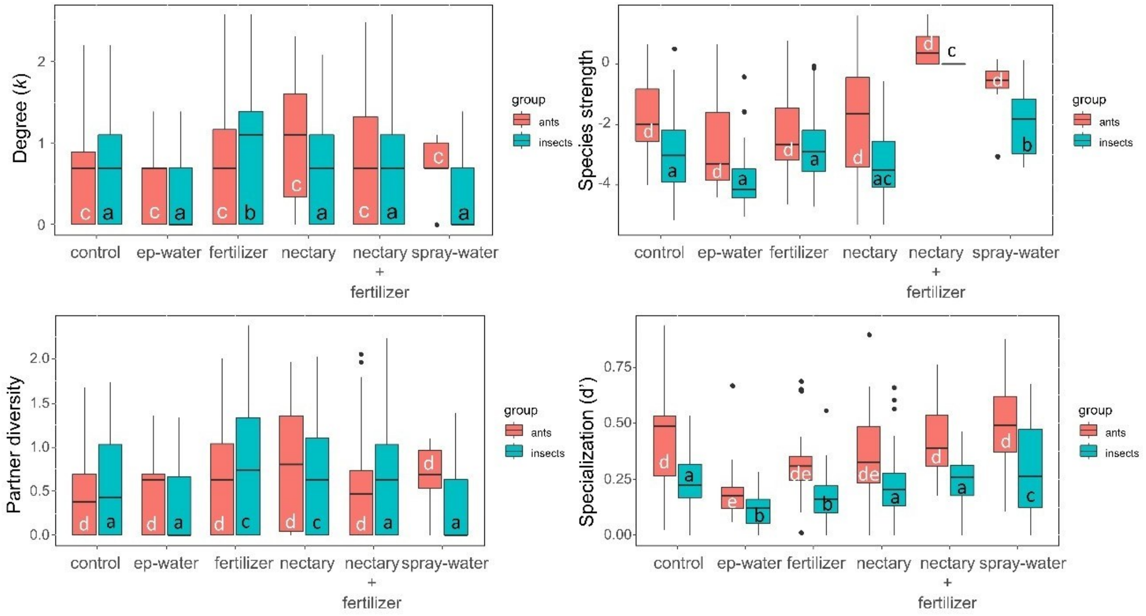

Figure 4: Species-level parameters evaluated among the ant and free-living insect groups among treatments. Pink bars represent the free-living insect group, and blue bars represent ants. The white letters in the pink bars represent differences between treatments for each parameter evaluated in the ant group. The black letters on the blue

bars represent differences between treatments for each parameter evaluated in the insect group

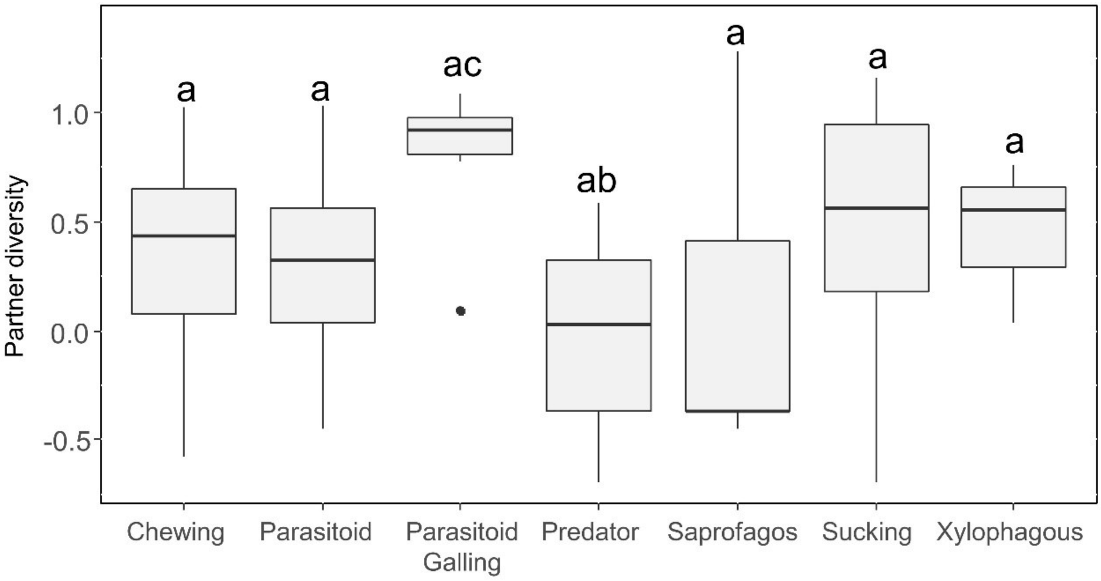

Figure 5: Partner diversity parameters for the free-living insect guilds. The letters above the bars represent the differences between each guild

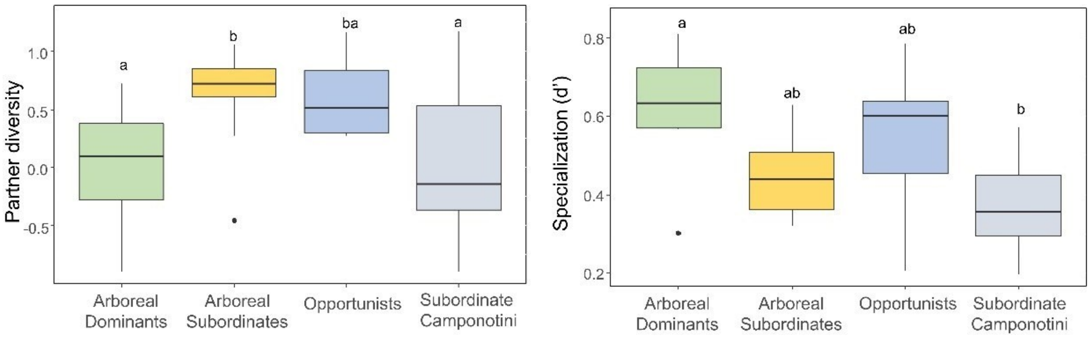

Figure 6: Partner diversity and Specialization (d') parameters for ant guilds. The letters above the bars represent the differences between the groups for each index evaluated

Discussion

Our results showed that abiotic and biotic factors subtly modify insect-plant-ant interaction networks in *C. langsdorffii*. Overall, when we look at the general patterns of Modularity and Specialization, the networks of all treatments show similarities. Our first hypothesis was that increased nutrient and water availability for plants would increase interactions with free-living insects. Indeed, in the treatment with fertilizer supplementation only, the network was larger, more robust, and had a greater number of free-living insects. However, this network showed lower specialization values (ΔPatefield H_2'), than other treatments. Interactions involving herbivorous insects and plants tend to be more specialized than other interactions, due to the coevolutionary processes that guide them (Cárdenas et al., 2015; Carmona et al., 2011; Ehrlich & Raven, 1964; Herms & Mattson, 1992; Whitham, 1983). However, in this work we found no differences among the free-living insect guilds for the specialization index (d'). We expected that only the guilds of chewers and suckers would be less specialized, as observed by Novotny et al., (2010). The chewing insects have high mobility and a wide diet (Novotnony et al. 2010) that allows them to forage on different plants, which may have guided the feeding preference for plants supplemented with fertilizers, which also had the highest number of insects.

In all experiments, networks involving herbivore-plant-ant network showed high modularity values. The occurrence of rare species may have contributed to the modular structure shown (Oliveira et al., 2020), rare species are commonly found in tropical herbivorous insect communities (Lewinsohn et al., 2005). In general, antagonistic networks tend to have modular structure (Cagnolo & Tavella, 2015; Thébault & Fontaine, 2010), which may have interfered with the pattern of networks in all treatments. Contrarily, we observed that nestedness values were not significant in most treatments, which is in agreement with works showing low nestedness in trophic networks (Lewinsohn et al., 2006; Oliveira et al., 2020; Pires & Guimarães, 2013). Networks that feature more specialist species tend not to show patterns of nestedness, while networks with generalist species show nestedness (Memmott et al., 2000). Only our water spray treatment showed nestedness, this does not tell us much about the role that ant species and free-living insects play in each experiment. In fact, we expected that the networks from the nectar simulation treatments would show a higher frequency of interactions and nestedness, as found by Dáttilo et al., (2015) in ant and plant networks with EFNs. According to our results, it is reasonable to evaluate the parameters of the species (ants and free-living insects) separately within each treatment network to understand the patterns of these communities.

When we evaluated the contribution of each species to the network parameters in each treatment, we observed that plants in the Fertilizer, Nectaries and Nectaries + Fertilizer treatments show higher degree (k) and free-living insects also showed higher degree in the fertilizer treatment, while ants showed no differences. So, the experimental manipulation ensures higher number of interaction partners for plants and insects. When we evaluated the proportion of interactions performed by a species with all its interaction partners in the network, plants in the Fertilizer treatment and in the Control showed higher values (species-strength), for insects only in the treatments with nectary+fertilizer and spray-water present showed higher proportion, and ants did not differ. Thus, we assume that these treatments ensured a higher proportion of interactions for both plants and insects. Furthermore, the partner diversity (generality of interactions) of the plants were higher in the fertilizer treatment, and so were the insects in the fertilizer and nectary treatments; overall the ants did not differ. However, when we evaluated the guilds of the ants, we observed that the arboreal subordinate guild had a greater diversity of partners than the others. In addition, the parasitoid/scavenger insect guilds showed higher partner diversity than the predator guild.

Finally, we observed that plants in the Control, Nectary and Spray-water treatments showed higher specialization indices (d'), the insects in the Spray-water treatment also showed higher specialization unlike the insects in the fertilizer and ep-water treatments that showed lower indices. The ants from the ep-water treatment were less specialized than the other treatments, but the groups with higher specialization did not differ from the control (Fig. 4). However, the ants of the arboreal-dominat genus are more specialized than the subordinate-camponotini. Since we sampled a single plant species in the same environment, we expected that ant interactions with plants without EFN supplementation would be more specialized, since there is already the predictability of resource, however, the treatments with EFN supplementation did not differ from the control. In general, interactions involving ants-EFN in multiple plant species that show low specialization (Cagnolo & Tavella, 2015). On the other hand, previous work shows that herbivorous insects are more specialized on plant families (Blüthgen et al., 2006). However, only the insects in the spray-water treatment showed this higher specialization, we expected the herbivorous chewing and sucking insect guilds to be less specialized, however, there were no differences in specialization with the other guilds.

When we evaluated the proportion of feeding guilds of herbivores and ants we noticed very interesting differences. In the treatments with EFN supplementation (EFNs, EFNs + Fertilizer, and ep-water) we observed a negative relationship with all free-living insect guilds and a highly positive relationship with opportunistic ants (genus *Brachymyrmex* and *Pseudomyrmex*). Ants consume food rewards (e.g EFN) opportunistically (Costa, et al., 2016), and EFN availability over time (supplementation experiment) seems to determine their feeding strategies, increasing their frequency on these resources.

Opportunistic ants (genus *Brachymyrmex* and *Pseudormymex*) and *Camponotus* (subordinate-camponotine) were strongly associated with EFN supplementation treatment and negatively related to fertilizer treatments (Fig. 1). *Crematogaster* ants (arboro-dominates) showed a highly positive relationship in the ep-water treatment. The proportion of occurrence of the herbivorous insect guilds were lower in these treatments with EFN supplementation, being a good indicator that ants play an important role in patrolling these plants. Ants of the genus *Camponotus* are highly aggressive, and are considered true mutualists of Cerrado (Del-Claro & Oliveira, 1999) and Campo Rupestre (Fagundes et al., 2012) plants, so it is possible that these ants have the ability to defend their host plants against potential herbivores. In fact, there are several studies in the literature that highlight the potential of *Camponotus* species to defend plant nectar (Rico-Gray & Oliveira, 2007).

On the opposite way, we observed that the treatments only with Fertilizer supplementation had a significant proportion of herbivores feeding on leaves, the plant nutrition was highly positive for the guilds of chewers and suckers and parasitoids. The greater availability of resources and better tissue nutrition favored these guilds that use tissue as food, on the other hand, the increase in parasitoids is also related to the greater availability of resources, in this case insects. We can assume that the plants in this treatment were more attractive to insects, since the availability of nutrients alters the tissue quality of host plants favoring leaf-feeding insects (Boege, 2005) significantly increasing herbivory.

The sprayed water treatment showed a positive relationship for ants, parasitoids and predators, however, insects that feed on plant tissues (leaves) were weakly related in this experiment. On the other hand, ants of the genus *Crematogaster* (Arboreal-dominants) were also positively related and opportunistic ants were negatively related, which makes sense since there was no resource supply for this group. Plants that did not receive any supplementation (control) also had a positive relationship with herbivores and the galling insects were also more evident in this treatment. Since galling insects have a high specificity with the host plant (Fernandes & Price, 1988; Höglund, 2014), this galling-plant interaction is evolutionarily so well established that it is not affected by improving plant tissue quality. Furthermore, opportunistic ants (genus *Brachymyrmex* and *Pseudomyrmex*) were negatively related to this treatment without food rewards.

This is one of the first studies to evaluate a multi-layered network integrating ants that use the plant's EFNs (mutualistic network) with free living insects that also use the plant as resources (antagonistic network). In addition, we create scenarios with EFN supplements to attract more ants (and improve plant defense) but also supplement the plants with nutrients and water, which would turn them more palatable and to insects. Overall, the structure of the networks between treatments showed similar patterns with the nutrient supplementation network being less specialized. When we separately evaluated the contribution of species and guilds in the networks, we showed that plants, insects, and ants showed different contributions at all levels in the networks. With the insects having low specialization in the fertilizer treatments, and the ants having low specialization overall. Furthermore, the insect and ant guilds showed distinct occurrences among treatments, with free-living insects being highly related to the treatments with fertilizer addition, and ants related to the treatments with EFN supplementation. Thus, this work illustrates how biotic and abiotic variations modify species interactions and occurrence. Furthermore, we emphasize the protective role of ants, which decreased the occurrence of free-living insects in the treatments with EFN.

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Considerações finais

Neste estudo avaliámos como variações abióticas altera os traços funcionais da planta *C. langsdorffii* e os efeitos em cascata para os níveis tróficos superiores. Além disso, também avaliamos como variações bióticas, aqui tratados pelo recrutamento de formigas mediados pela suplementação de nectários extraflorais, alteram as interações insetos planta. Compreender os fatores determinantes das interações plantas inseto, bem como os fatores que perturbam estas interações, é importante para avançar o conhecimento da biodiversidade e dos mecanismos que podem causar perturbações e mesmo extinções destes grupos.

Mostramos que as variações bióticas e abióticas são importantes na organização das comunidades de insetos presentes nesta planta. Somos os primeiros a mostrar experimentalmente como as forças abióticas e bióticas alteram as interações inseto planta e estruturam redes mutualistas e antagónicas simultaneamente no campo. Observamos que as características das plantas (esclerofila e área foliar) e a produção de frutos são modificadas com a maior disponibilidade de recursos. Assim, encontramos menor esclerofilia e maior área foliar nos indivíduos de plantas suplementados com nutrientes e água. As taxas de herbivoria foram menores e a abundância de formigas foi maior nas plantas com nectários extraflorais artificiais disponíveis. Não observamos variações na riqueza e abundância de insetos galhadores, no entanto, os padrões de co-ocorrência de galhas variaram com a disponibilidade de recursos (espaço de nidificação) na planta, mas não houve relação com as formigas.

As diferentes guildas de insetos de vida livre e das formigas mostraram ocorrência distinta entre os tratamentos, contudo, ao contrário do que se esperávamos, não houve especialização destas guildas nas redes. As redes de todos os tratamentos apresentaram alta modularidade e especialização, contudo, a rede do tratamento com adição de fertilizante apresentou o maior número de interações e menos especializada que o esperado pelo acaso. Por outro lado, quando avaliamos o papel dos grupos separadamente observamos que as formigas apresentaram maior especialização e os insetos de vida livre são mais generalistas. As formigas oportunistas foram positivamente relacionadas com os tratamentos com suplementação de EFNs, já os insetos de vida livre apresentaram menor ocorrência nesses tratamentos, porém as guildas dos insetos mastigadores e sugadores foram altamente relacionadas com às plantas que receberam suplementação de nutrientes. Com isso, mostramos que as modificações abióticas e bióticas alteram a estabilidade das interações inseto-planta.

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Supplementary Material

Table S1: List of guild classification of insect species collected from the *Copaifera langsdorffii* plant in each experiment. The numbers indicate the abundance of individuals found in each treatment.

Ordem/Family	Control	Ep-water	Fertilizer	Nectary	Nectary+ fertilizer	Spray-water	Guilda
Collembola							
Entomobryidae							
Entomobryidae-sp1			15		6		Saprófagos
Entomobryidae-sp2	33	8	11	13	28	1	Saprófagos
Coleoptera							
Bostrychidae							
Bostrychidae-sp1	13		3	1	1		Xylophagous
Bostrychidae-sp2	3		5	2	2		Xylophagous
Bostrychidae-sp3	1		4	3	1		Xylophagous
Carabidae							
Carabidae-sp1			1		1		Predator
Carabidae-sp2						1	Predator
Carabidae-sp3					1		Predator
Chrysomelidae							
Chrysomelidae-sp1	2		1	1	1		Chewing
Chrysomelidae-sp2	1		6		2		Chewing
Chrysomelidae-sp3	1		3				Chewing

Continuation Table S1:

Chrysomelidae-sp4	3		8	8	2	3	Chewing
Chrysomelidae-sp5	1	1	5	3			Chewing
Chrysomelidae-sp6	2			1	2		Chewing
Chrysomelidae-sp7	2	1	2		2	1	Chewing
Chrysomelidae-sp8	2		5				Chewing
Chrysomelidae-sp9	1				1	1	Chewing
Chrysomelidae-sp10		1	1		1		Chewing
Coccinellidae							
Coccinellidae-sp1			4				Predator
Curculionidae							
Curculionidae-sp1	2		1		1		Chewing
Curculionidae-sp2	1		4	1		1	Chewing
Curculionidae-sp3	5	5	4	2			Chewing
Curculionidae-sp4	3				1		Chewing
Curculionidae-sp5		1	1				Chewing
Curculionidae-sp6			1				Chewing
Staphylinidae							
Staphylinidae-sp1			1		2		Saprofagos
Staphylinidae-sp2	2						Saprofagos
Tenebrionidae							
Tenebrionidae-sp1		1					Xylophagous
Diptera							
Cecidomyiidae							
Cecidomyiidae-sp1	15	3	14	3	15	6	Galling
Cecidomyiidae-sp2	26	4	17	3	18	4	Galling
Cecidomyiidae-sp3	6		2		1		Galling

Continuation Table S1:

Eurytomidae							
Eurytomidae-sp1		2					Parasitoid/Galling
Muscidae							
Muscidae-sp1	2		4		3	3	Saprofagos
Phoridae							
Phoridae-sp1	3	2	9	16	4	8	Parasitoid
Sciaridae							
Sciaridae-sp1			3		2		Saprofagos
Hemiptera							
Acanthosomatidae							
Acanthosomatidae-sp1						1	Sucking
Cicadellidae							
Cicadellidae-sp	15	11	49	10	16	2	Sucking
Cicadellidae-sp1	18	4	17	17	9	4	Sucking
Cicadellidae-sp2	10	2	12	4	6	2	Sucking
Cicadellidae-sp3	6	3	6	6	5		Sucking
Cicadellidae-sp4	4	1	1		1	1	Sucking
Cicadellidae-sp5	2		1	3			Sucking
Cicadellidae-sp6	8	2	7	19	7		Sucking
Cicadellidae-sp7	1		11		5		Sucking
Cicadellidae-sp8		1		1		2	Sucking
Cicadellidae-sp9			2		1		Sucking
Cicadellidae-sp10	3		11	1	3	1	Sucking
Cicadellidae-sp11	2	2	3	6	2		Sucking
Cicadellidae-sp12	12	2	15	5	11	1	Sucking
Cicadellidae-sp13			1				Sucking

Continuation Table S1:

Cicadellidae-sp14	1		1		2		Sucking
Cicadellidae-sp15				2	3		Sucking
Cicadellidae-sp16	3		2		7		Sucking
Cicadellidae-sp17	9		3	2			Sucking
Cicadellidae-sp18	1	1					Sucking
Cicadellidae-sp19			2	1	1	1	Sucking
Cochonilha							
Cochonilha-sp				1			Sucking
Lygaeidae							
Lygaeidae-sp1					1		Sucking
Membracidae							
Membracidae-sp1		1	2				Sucking
Membracidae-sp2	1						Sucking
Membracidae-sp3	1		2	4			Sucking
Membracidae-sp4			1				Sucking
Piesmatidae							
Piesmatidae-sp1	1		4				Sucking
Psyllidae							
Psyllidae-sp1	6		16	5	11		Sucking
Psyllidae-sp2	4		24	12	7		Sucking
Psyllidae-sp3	99	63	54	23	52	12	Sucking
Psyllidae-sp4	3		3	5	2	1	Sucking
Psyllidae-sp5	3	2	3	3	2	2	Sucking
Reduviidae							
Reduviidae-sp1	1	1	1	1	1	1	Predador
Reduviidae-sp2			1	4			Predador

Continuation Table S1:

Reduviidae-sp3	5					8	Predador
Reduviidae-sp4					1		Predador
Reduviidae-sp5		1					Predador
Scutelleridae							
Scutelleridae-sp1	1		1				Sucking
Tingidae							
Tingidae-sp1			4		1		Sucking
Hymenoptera							
Aphelinidae							
Aphelinidae-sp1	1	1	2				Parasitoid
Braconidae							
Braconidae-sp1			2	1			Parasitoid
Braconidae-sp2	13		4		2		Parasitoid
Braconidae-sp3	7			1			Parasitoid
Braconidae-sp4			4	2	1		Parasitoid
Braconidae-sp5			2		2		Parasitoid
Braconidae-sp6	1		4	5		2	Parasitoid
Braconidae-sp7	1		4	1			Parasitoid
Braconidae-sp8	1		6				Parasitoid
Braconidae-sp9	1		2				Parasitoid
Ceraphronidae							
Ceraphronidae-sp1		1	5		2		Parasitoid
Ceraphronidae-sp2	6		6	5	7	2	Parasitoid
Chalcididae							
Chalcididae-sp1			1	1			Parasitoid
Diapriidae							

Continuation Table S1:

Diapriidae-sp1	1		6	2	2		Parasitoid
Embolemidae							
Embolemidae-sp1	1		4	1			Parasitoid
Embolemidae-sp2				1	1		Parasitoid
Embolemidae-sp3			1	2			Parasitoid
Embolemidae-sp4					1		Parasitoid
Encyrtidae							
Encyrtidae-sp1	2		1	3	2		Parasitoid
Encyrtidae-sp2	1	1		1	1	1	Parasitoid
Encyrtidae-sp3	1		5	1		1	Parasitoid
Encyrtidae-sp4	1		5		1		Parasitoid
Encyrtidae-sp5	4	2	6	3	3	1	Parasitoid
Eulophidae							
Eulophidae-sp1	8	2	8	6	9	1	Parasitoid/Galling
Eulophidae-sp2	13		4	2	2		Parasitoid/Galling
Eulophidae-sp3	7	3	3	3	3	1	Parasitoid/Galling
Eulophidae-sp4	3	3	7	7	5	2	Parasitoid/Galling
Eulophidae-sp5			2	1			Parasitoid/Galling
Eulophidae-sp6	3		4		2	1	Parasitoid/Galling
Eupelmidae							
Eupelmidae-sp1			16		3		Parasitoid
Eupelmidae-sp2	2	1	7	1	2		Parasitoid
Eurytomidae							
Eurytomidae-sp1	6		2	2	3	1	Parasitoid/Galling
Eurytomidae-sp2	1						Parasitoid/Galling
Figitidae							

Continuation Table S1:

Figitidae-sp1			4				Parasitoid
Figitidae-sp2	10		6	5	1		Parasitoid
Figitidae-sp3			3	2			Parasitoid
Figitidae-sp4	1		3	1			Parasitoid
Figitidae-sp5	2		3				Parasitoid
Ichneumonidae							
Ichneumonidae-sp1	1			2			Parasitoid
Ichneumonidae-sp2						1	Parasitoid
Signiphoridae							
Signiphoridae-sp1			5				Parasitoid
Signiphoridae-sp2	8		10	2	2	3	Parasitoid
Torymidae							
Torymidae-sp1			1	1			Parasitoid
Torymidae-sp2		1		1			Parasitoid
Trichogrammatidae							
Trichogrammatidae-sp1	3			1			Parasitoid
Trichogrammatidae-sp2	1			1			Parasitoid
Lepidoptera							
Stenomatinae							
Stenomatinae	7		49	10	10	1	Chewing
Stenomatinae-sp1	11		8	9	10	1	Sucking
Tineidae							
Tineidae-sp1	1						Xylophagous
Orthoptera							
Gryllidae							
Gryllidae-sp1	1		1		2		Saprofagos

Continuation Table S1:

Psocodea							
Psocidae							
Psocidae-sp1	39	14	57	29	56	3	Saprofagos
Thysanoptera							
Tripidae							
Tripidae-sp1			7	1	2		Sucking
Total	520	155	679	310	394	90	

Table S 2 : List of guild classification of ant species collected from the *Copaifera langsdorffii* plant in each experiment. The numbers indicate the abundance of individuals found in each treatment. Guilds adapted from Paolucci et al 2016

Genus / Species	Control	Ep-water	Fertilizer	Nectary	Nectary+fertilizer	Spray-water	Guilda
Brachymyrmex							
Brachymyrmex -sp1	35	89	8	29	82	1	Opportunists
Brachymyrmex -sp2	110	163	141	546	580		Opportunists
Brachymyrmex -sp3	69		48	174	1		Opportunists
Camponotus							
Camponotus-rufipes	15	3	22	33	16	8	Subordinate-Camponotini
Camponotus-sp1	2		1	14	23		Subordinate-Camponotini
Camponotus-sp2			3	19			Subordinate-Camponotini
Camponotus-sp3	1						Subordinate-Camponotini
Camponotus-sp4				6			Subordinate-Camponotini
Camponotus-sp5					1		Subordinate-Camponotini
Camponotus-sp6			1		1		Subordinate-Camponotini
Camponotus-sp7			2				Subordinate-Camponotini
Camponotus-sp8					1		Subordinate-Camponotini
Camponotus-sp9			1		1		Subordinate-Camponotini
Camponotus-sp10				7			Subordinate-Camponotini
Cephalotes							
Cephalotes-sp1	14	7	37	59	55	7	Arboreal-Subordinates
Cephalotes-sp2	17	2	13	45	36	2	Arboreal-Subordinates
Cephalotes-sp3			3				Arboreal-Subordinates
Crematogaster							
Crematogaster-sp1	1		2		25		Arboreal-Dominants
Crematogaster-sp2	7	1	11	6	3	19	Arboreal-Dominants
Continuation Table S2:							
	21	2	8				Arboreal-Dominants

Crematogaster-sp3							
Crematogaster-sp4	2				1		Arboreal-Dominants
Crematogaster-sp5		2					Arboreal-Dominants
Crematogaster-sp6		35					Arboreal-Dominants
Crematogaster-sp7			9				Arboreal-Dominants
Linepthema							
Linepthema-sp1				1	2		Epigaeic-Omnivores
Linepthema-sp2			4	1			Epigaeic-Omnivores
Linepthema-sp3	4			1			Epigaeic-Omnivores
Myrmelachista							
Myrmelachista-sp1	15	14	7	7	28	4	Arboreal-Subordinates
Myrmelachista-sp2	15	1	1	51	29		Arboreal-Subordinates
Pachycondrya							
Pachycondrya-sp1				1			Epigaeic-Predators
Procryptocerus							
Procryptocerus-sp1	1			9	3	3	Arboreal-Subordinates
Pseudomyrmex							
Pseudomyrmex-sp1			5	15	3	7	Opportunists
Pseudomyrmex-sp2	7	2	2	2	12		Opportunists
Pseudomyrmex-sp3	3		3	1	21	2	Opportunists
Pseudomyrmex-sp4	6	1	2	4			Opportunists
Pseudomyrmex-sp5				7	2		Opportunists
Pseudomyrmex-sp6		2					Opportunists
Total Geral	345	322	332	994	890	51	