

Universidade Federal de Minas Gerais - UFMG
Instituto de Ciências Biológicas
Programa de Pós-Graduação em Ecologia, Conservação e Manejo de Vida Silvestre

Cássio Cardoso Pereira

**ESTRUTURAS SUTIS COM FUNÇÕES NÃO TÃO SUTIS:
efeitos indiretos dos construtores de abrigos na organização das
comunidades de artrópodes de plantas terrestres em diferentes
escalas espaciais, temporais e nas interações tróficas**

**Belo Horizonte,
novembro de 2023**

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Orientadora: Profa. Dra. Tatiana Cornelissen

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Ata da Defesa de Tese

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Cássio Cardoso Pereira

No dia 23 de novembro de 2023, 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) Cássio Cardoso Pereira, orientando(a) do Professor Tatiana Garabini Cornelissen, intitulada: "**Estruturas sutis com funções não tão sutis: efeitos indiretos dos construtores de abrigos na organização das comunidades de artrópodes de plantas terrestres em diferentes escalas espaciais, temporais e nas interações tróficas**". Abrindo a sessão, o(a) Presidente(a) da Comissão, Doutor(a) Tatiana Garabini Cornelissen, 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: Camila Souza (UNIMONTES), Walter Araújo (UNIMONTES), Walisson Kenedy Siqueira (UFMG), Flávio Camarota (UFMG) 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:

Aprovação da tese, com eventuais correções mínimas e entrega de versão final pelo orientador diretamente à Secretária do Programa, no prazo máximo de 30 dias;
 Reprovação da tese - (marcar se é a primeira ou segunda reprovação): () *primeira reprovação () segunda reprovação

*Conforme o disposto no Artigo 80 da Resolução Complementar do CEPE/UFMG Nº 02/2017, de 04 de julho de 2017, caso seja a primeira reprovação, poderá ser concedido, a critério do Colegiado de Curso, um prazo para a realização de nova defesa de tese.

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, 23 de novembro de 2023.

Assinaturas dos Membros da Banca Examinadora



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Resumo

Esta tese é o resultado de um estudo que investigou a importância de diferentes estruturas criadas por artrópodes em plantas para a organização de comunidades de artrópodes terrestres, assim como os efeitos da engenharia de ecossistemas em diferentes escalas espaciais, temporais e suas consequências indiretas em interações tróficas. Ela se divide em três capítulos. Inicialmente, no capítulo 1, disponibilizamos uma extensa base de dados que incluiu mais de 1000 registros únicos de engenharia de ecossistemas por artrópodes, na forma de estruturas construídas em plantas, como galhas, folhas enroladas e folhas unidas com seda. Todos os registros foram publicados na literatura e abrangem tanto estruturas naturais (91% dos registros) quanto estruturas criadas artificialmente por pesquisadores (9% dos registros). Os dados foram coletados entre 1932 e 2021, em mais de 50 países e vários ecossistemas, desde zonas polares a zona tropical. Além de dados sobre plantas hospedeiras e engenheiros, agregamos dados sobre os tipos de construções e a identidade dos inquilinos que utilizam essas estruturas. Este conjunto de dados destacou a importância dessas estruturas sutis para a organização de comunidades de artrópodes terrestres, permitindo testes de hipóteses em estudos ecológicos que abordam a engenharia de ecossistemas e a facilitação mediada pelos abrigos. No segundo capítulo, avaliamos se as estruturas foliares criadas pelo galhador *Ditylenchus gallaeformans* Oliveira, Santin, Seni, Dietrich, Salazar, Subbotin, Mundo-Ocampo, Goldenberg & Barreto, 2013 (Anguinidae) na espécie *Miconia ligustroides* (DC.) Naudin (Melastomataceae), contribuem para mudanças na diversidade e composição da comunidade de artrópodes em diferentes escalas espaciais (nível da planta e foliar). Avaliamos também os efeitos da ocupação dos abrigos na escolha de colonizadores secundários e a influência dessas estruturas nos níveis de herbivoria nas plantas hospedeiras. A abundância, riqueza e biomassa de artrópodes foram maiores em abrigos foliares em comparação com folhas intactas. Esses efeitos foram observados em magnitudes semelhantes nas escalas das plantas e das folhas. A composição dos artrópodes diferiu entre plantas com abrigos e plantas sem abrigos e entre abrigos desocupados e folhas intactas. Os abrigos aumentaram a abundância, a riqueza e a biomassa dos artrópodes em quase 100% em comparação com as folhas intactas. No entanto, os abrigos ocupados tiveram uma diminuição na abundância, riqueza e biomassa de artrópodes em quase 60% em comparação com os abrigos desocupados. Finalmente, as plantas sem abrigos exibiram níveis mais elevados de herbivoria foliar do que as plantas com abrigos. Nossas descobertas demonstraram os fortes e positivos efeitos indiretos da indução de galhas que facilitaram a criação de abrigos e os efeitos indiretos dos abrigos na diversidade, composição de espécies e na herbivoria foliar, e devem ser replicados em outros sistemas envolvendo plantas e seus respectivos galhadores. Por fim, no terceiro capítulo, avaliamos as consequências da facilitação gerada pelos abrigos foliares de *D. gallaeformans* nas comunidades de artrópodes em *M. ligustroides* ao nível foliar e da planta em um ambiente sujeito a forte sazonalidade climática durante dois anos consecutivos. Os abrigos aumentaram a diversidade de artrópodes e modificaram a composição de espécies em *M. ligustroides* ao nível foliar e das plantas, e nas estações seca e chuvosa. Comparando os abrigos nas diferentes estações, os abrigos da estação seca exibiram maior abundância, riqueza e biomassa de artrópodes em comparação aos abrigos da estação chuvosa em ambos os anos avaliados. Finalmente, os efeitos globais dos abrigos na diversidade da comunidade de artrópodes associados a *M. ligustroides* foram positivos e

moderadamente fortes na estação seca, aumentando a abundância, a riqueza e a biomassa dos artrópodes em uma média de 65% em ambos os anos. Nosso estudo contribuiu para uma melhor compreensão dos padrões de variação e magnitude da engenharia de ecossistemas em diferentes escalas espaciais e temporais e forneceu novos insights sobre a importância dos abrigos para espécies sensíveis à aridez.

Palavras-chave: Abrigos foliares, Cerrado, colonizadores secundários, diversidade de artrópodes, engenheiros ecossistêmicos, facilitação indireta, galhas foliares, herbivoria, insetos, refúgios microclimáticos, sazonalidade.

Abstract

This thesis is the result of a study that investigated the importance of different structures created by arthropods on plants for the organization of terrestrial arthropod communities, as well as the effects of ecosystem engineering at different spatial and temporal scales and their indirect consequences on trophic interactions. It is divided into three chapters. Initially, in Chapter 1, we provided an extensive database that included more than 1000 unique records of ecosystem engineering by arthropods, in the form of structures built on plants, such as galls, leaf rolls and leaf ties. All records were published in the literature, and cover both natural structures (91% of records) and structures artificially created by researchers (9% of records). The data was collected between 1932 and 2021, in more than 50 countries and various ecosystems, from polar zones to tropical zone. In addition to data on host plants and engineers, we aggregate data on the types of buildings and the identity of the secondary colonizers using these structures. This dataset highlighted the importance of these subtle structures for the organization of terrestrial arthropod communities, enabling hypothesis testing in ecological studies that address ecosystem engineering and shelter-mediated facilitation. In the second chapter, we evaluated whether the leaf structures created by the galler *Ditylenchus gallaeformans* Oliveira, Santin, Seni, Dietrich, Salazar, Subbotin, Mundo-Ocampo, Goldenberg & Barreto, 2013 (Anguinidae) in the species *Miconia ligustroides* (DC.) Naudin (Melastomataceae), contribute to changes in the diversity and composition of the arthropod community at different spatial scales (plant and leaf level). We also evaluated the effects of shelter occupancy on the choice of secondary colonizers, and the influence of these structures on herbivory levels on host plants. Arthropod abundance, richness and biomass were higher in leaf shelters compared to intact leaves. These effects were observed at similar magnitudes at the plant and leaf scales. Arthropod composition differed between plants with shelters and plants without shelters, and between unoccupied shelters and intact leaves. Shelters increased arthropod abundance, richness and biomass by almost 100% compared to intact leaves. However, occupied shelters had a decrease in arthropod abundance, richness and biomass by almost 60% compared to unoccupied shelters. Finally, plants without shelters exhibited higher levels of leaf herbivory than plants with shelters. Our findings demonstrated the strong and positive indirect effects of gall induction that facilitated shelter creation and the indirect effects of shelters on diversity, species composition and leaf herbivory, and should be replicated in other systems involving plants and their respective gallers. Finally, in the third chapter, we evaluate the consequences of the facilitation generated by *D. gallaeformans* leaf shelters on arthropod communities in *M. ligustroides* at the leaf and plant level in an environment subject to strong climatic seasonality during two consecutive years. The shelters increased arthropod diversity and modified the species composition on *M. ligustroides* at the leaf and plant level, and in the dry and rainy seasons. Comparing shelters in different seasons, the shelters of dry season exhibited higher abundance, richness and biomass of arthropods compared to rainy season shelters in both years evaluated. Finally, the overall effects of shelters on the diversity of the arthropod community associated with *M. ligustroides* were positive and moderately strong in the dry season, increasing arthropod abundance, richness, and biomass by an average of 65% in both years. Our study contributed to a better understanding of the patterns of variation and magnitude of ecosystem engineering at different

spatial and temporal scales, and provided new insights into the importance of shelters for aridity-sensitive species.

Keywords: Arthropod diversity, Cerrado, ecosystem engineers, herbivory, indirect facilitation, insects, leaf galls, leaf shelters, microclimatic refuges, seasonality, secondary colonizers.

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Introdução Geral

Os engenheiros de ecossistemas (EE) alteram a distribuição de recursos ao criarem novos habitats para outras espécies por meio de interações não-tróficas, geralmente com efeitos ecológicos positivos em outras espécies (*i.e.*, facilitação) (Jones *et al.* 1994, 2010). Os organismos atuam como EE ao modular a oferta de outros recursos que não eles próprios, sendo chamados de engenheiros autogênicos quando alteram o ambiente através de suas próprias estruturas físicas (*e.g.*, corais e árvores), e de alogênicos quando alteram o ambiente transformando materiais vivos ou não vivos em diferentes conformações (*e.g.*, lagartas que enrolam folhas) (Jones *et al.* 1994, 2010).

A magnitude da facilitação dos EE é influenciada por vários fatores, como o tamanho do habitat construído, estresse ambiental e risco de predação de colonizadores secundários. Esses fatores também variam de acordo com a complexidade arquitetônica e o número de EE encontrados na natureza (Romero *et al.* 2015). Essas mudanças físicas variam ao longo dos gradientes ambientais, alterando processos biogeoquímicos e a disponibilidade de recursos (Jones *et al.* 1994, 2010; Romero *et al.* 2015; Kozlov *et al.* 2016). Assim, efeitos da engenharia de ecossistema são detectáveis não apenas em parâmetros das comunidades biológicas, mas também em processos ecossistêmicos, como a decomposição (Kozlov *et al.* 2016).

Diversos artrópodes constroem abrigos em plantas para reprodução, desenvolvimento e escape de condições climáticas adversas e/ou predadores, sendo considerados manipuladores de microhabitats (Romero *et al.* 2015; Cornelissen *et al.* 2016). Lagartas de Lepidoptera e aranhas são responsáveis por construir uma infinidade de estruturas diferentes, cortando, dobrando, tecendo, unindo e enrolando folhas com seda (Cornelissen *et al.* 2016). Coleópteros perfuram buracos nos troncos para oviposição e suas larvas se desenvolvem construindo extensas galerias (Novais *et al.* 2018). Larvas de insetos minadores utilizam o mesênquima das folhas como recurso alimentar, construindo diversos túneis nas folhas (Connor & Taverner

1997). Os insetos indutores de galhas induzem diferenciação celular (por hiperplasia e/ou hipertrofia) nos tecidos de suas plantas hospedeiras pela sua atividade de alimentação e criam uma enorme variedade de estruturas de diferentes tipos nos caules, nas folhas, nas flores e nos frutos (Sanver & Hawkins 2000; Shorthouse *et al.* 2005; Cornelissen *et al.* 2016). Todas essas estruturas criadas pelos EE são usadas em pelo menos uma parte de suas vidas. Quando abandonadas, elas podem persistir nas plantas e podem mais tarde serem usadas como abrigo por outros artrópodes, denominados colonizadores secundários (Romero *et al.* 2015; Cornelissen *et al.* 2016).

Os abrigos alteram a arquitetura da planta hospedeira e sugere-se que aumentem a abundância e a diversidade de organismos associados, tendo o potencial de alterar a composição da comunidade e a estrutura funcional em diferentes escalas espaciais e temporais (Romero *et al.* 2015). Os efeitos locais dos engenheiros podem se estender em diferentes escalas espaciais (*e.g.*, efeitos ao nível de folhas e plantas), uma vez que as comunidades de artrópodes se modifiquem no interior dos abrigos, acarretando uma composição de espécies diferente de plantas que não possuem abrigos (Vieira & Romero 2013). Além disso, os efeitos na diversidade de artrópodes podem ser mais pronunciados ao longo de uma determinada estação do ano (Vieira & Romero 2013). Por exemplo, em ambientes com sazonalidade bem definida, a estação seca pode ter um efeito negativo pronunciado na abundância de artrópodes (Wolda 1988), e os abrigos foliares poderiam representar importantes microambientes que poderiam proteger os artrópodes da radiação UV e da dessecação (Vieira & Romero 2013).

Os EE podem também influenciar os níveis de herbivoria das plantas, podendo impactar o *fitness* delas de maneira indireta pelo resultado das diferentes cascatas de interações tróficas que podem ocorrer de acordo com a composição das guildas dos colonizadores (Henriques *et al.* 2019). Dessa forma, um aumento no número de herbívoros poderia aumentar os níveis de herbivoria das plantas se eles as utilizarem também como alimento. Em contrapartida, uma

maior colonização de predadores (como as aranhas) possivelmente diminuiria o número de herbívoros nas plantas e conseqüentemente a herbivoria foliar (Henriques *et al.* 2019).

Os efeitos dos artrópodes terrestres EE ainda são pouco explorados, sobretudo na região tropical (Romero *et al.* 2015). Apenas um estudo examinou como as comunidades respondem aos impactos em diferentes escalas espaciais (*i.e.*, no nível de folha e de planta) e temporais (*i.e.*, estações seca e chuvosa) (Vieira & Romero 2013). Outro estudo avaliou como a presença e o número de abrigos influenciam os níveis naturais de herbivoria em plantas (Henriques *et al.* 2019), e outro avaliou como a engenharia de ecossistemas por besouros influencia a organização das comunidades de formigas (Novais *et al.* 2017). Assim, cenários experimentais ainda estão para serem explorados em sistemas tropicais, que, assim como os sistemas temperados, podem fornecer modelos para se avaliar as hipóteses e mecanismos que governam a engenharia de ecossistemas e seus efeitos em comunidades e ecossistemas.

Nesta tese, investigamos a importância de diferentes estruturas criadas por artrópodes em plantas para a organização de comunidades de artrópodes terrestres, assim como os efeitos da engenharia de ecossistemas em diferentes escalas espaciais, temporais e suas conseqüências indiretas em interações tróficas em sistemas tropicais. Inicialmente, no capítulo 1, disponibilizamos uma extensa base de dados que incluiu mais de 1000 registros únicos de engenharia de ecossistemas por artrópodes, na forma de estruturas construídas em plantas, como galhas, folhas enroladas e folhas unidas com seda. Destacamos a importância dessas estruturas, mesmo que sutis, para a organização de comunidades de artrópodes, permitindo assim futuros testes de hipóteses em estudos ecológicos que abordam a engenharia de ecossistemas e a facilitação mediada pelos abrigos. No capítulo 2, realizamos um estudo experimental com o intuito de avaliar os efeitos dos engenheiros de ecossistemas em diferentes escalas espaciais e suas conseqüências indiretas na herbivoria foliar em um sistema tropical envolvendo uma inédita interação entre um arbusto e um nematóide galhador. Por fim, no

capítulo 3, investigamos os padrões de variação e magnitude dos efeitos dos abrigos criados por esse galhador em comunidades de artrópodes nesse arbusto em diferentes escalas espaciais e temporais em um ambiente sujeito a forte sazonalidade climática durante dois anos consecutivos.

Chapter I. Subtle structures with not-so-subtle functions: a dataset of arthropod constructs and their host plants*

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Abstract

The construction of shelters on plants by arthropods might influence other organisms via changes in colonization, community richness, species composition and functionality. Arthropods, including beetles, caterpillars, sawflies, spiders, and wasps often interact with host plants via the construction of shelters, building a variety of structures such as leaf ties, tents, rolls, and bags; leaf and stem galls, and hollowed out stems. Such constructs might have both an adaptive value in terms of protection (i.e., serve as shelters) but may also exert a strong influence on terrestrial community diversity in the engineered and neighboring hosts via colonization by secondary occupants. While different traits of the host plant (e.g., physical, chemical and architectural features) may affect the potential for ecosystem engineering by insects, such effects have been, to a certain degree, overlooked. Further analyses of how plant traits affect the occurrence of shelters may thus enrich our understanding of the organizing principles of plant-based communities. This dataset includes more than a thousand unique records of ecosystem engineering by arthropods, in the form of structures built on plants. All records have been published in the literature, and span both natural structures (91% of the records) and structures artificially created by researchers (9% of the records). The data were gathered between 1932 and 2021, across more than 50 countries and several ecosystems, ranging from polar to tropical zones. Besides data on host plants and engineers, we aggregated data on the type of constructs and the identity of inquilines using these structures. This dataset highlights the importance of these subtle structures for the organization of terrestrial arthropod communities, enabling hypotheses testing in ecological studies addressing ecosystem engineering and facilitation mediated by constructs.

Keywords: arthropods, caterpillars, ecosystem engineering, inquilines, insects, leaf galls, leaf rolls, leaf tents, leaf ties, plant constructs, shelters

Resumo

A construção de abrigos em plantas por artrópodes pode influenciar outros organismos através de mudanças na colonização, riqueza da comunidade, composição e funcionalidade de espécies. Os artrópodes, incluindo aranhas, besouros, lagartas, moscas-serra e vespas, muitas vezes interagem com plantas hospedeiras através da construção de abrigos, construindo uma variedade de estruturas, como laços foliares, rolos foliares, tendas, galhas foliares, galhas caulinares e galerias nos troncos. Tais construções podem ter um valor adaptativo em termos de proteção (i.e., servir como abrigos), mas também podem exercer uma forte influência na diversidade da comunidade terrestre nos hospedeiros modificados e vizinhos através da colonização por ocupantes secundários. Embora diferentes características da planta hospedeira (e.g., características físicas, químicas e arquitetônicas) possam afetar o potencial de engenharia de ecossistemas por insetos, tais efeitos têm sido, até certo ponto, negligenciados. Análises adicionais de como as características das plantas afetam a ocorrência de abrigos podem, assim, enriquecer a nossa compreensão dos princípios organizadores das comunidades baseadas em plantas. Este conjunto de dados inclui mais de mil registros únicos de engenharia de ecossistemas por artrópodes, na forma de estruturas construídas em plantas. Todos os registros foram publicados na literatura e abrangem tanto estruturas naturais (91% dos registros) quanto estruturas criadas artificialmente por pesquisadores (9% dos registros). Os dados foram coletados entre 1932 e 2021, em mais de 50 países e vários ecossistemas, desde zonas polares a zona tropical. Além de dados sobre plantas hospedeiras e engenheiros, agregamos dados sobre os tipos de construções e a identidade dos inquilinos que utilizam essas estruturas. Este conjunto de dados destaca a importância dessas estruturas sutis para a organização de comunidades de artrópodes terrestres, permitindo testes de hipóteses em estudos ecológicos que abordam a engenharia de ecossistemas e a facilitação mediada pelos abrigos.

Palavras-chave: abrigos, artrópodes, construções em plantas, engenharia de ecossistemas, galhas foliares, inquilinos, insetos, laços foliares, lagartas, rolos de folhas, tendas foliares.

Introduction

Plants rarely interact with single species or mutualists, more typically hosting a diverse set of interaction partners. Such multiple interactions are dynamic in nature, often overlap in time, and occur along a *continuum* of interaction types, with outcomes ranging from positive (e.g., for mutualisms involving pollinators) to negative (e.g., for antagonistic interactions involving herbivores and florivores). These interactions can shape terrestrial community structure by influencing plant richness, diversity and composition (Schuldt et al. 2019) and ultimately ecosystem processes and services including pollination, decomposition and nutrient cycling (Ameixa et al. 2018, Noriega et al. 2018).

An important but often overlooked interaction between insects and plants is facilitation mediated by ecosystem engineers. While the term ecosystem engineering was described almost 30 years ago (Jones et al. 1994, 1997), much research has focused on large ecosystem engineers whose constructs are persistent and have great longevity (e.g., beavers). Only recently have ecologists begun to acknowledge how more subtle habitat manipulations might influence other organisms via their effects on colonization, that in turn affects community richness and composition (Lill and Maquis 2003, Romero et al. 2015, 2021). One subtle type of ecosystem engineering consists of constructs on plants built by arthropods, including beetles, caterpillars, sawflies, spiders, and wasps. Such constructs span an enormous variety of structures including leaf ties, tents, rolls, and bags (Cornelissen et al. 2016, Calixto et al. 2021); leaf and stem galls (Cintra et al. 2020) and hollowed out stems (Novais et al. 2018). For the arthropod engineers that build or initiate the construct through their feeding, the constructs may have an adaptive value in terms of protection from abiotic and/or biotic factors. Subsequently, they may be colonized by a wide range of secondary users, thus exerting a strong influence on arthropod community diversity on the engineered and potentially neighboring hosts (Lill & Marquis 2003, Vieira and Romero 2013, Pereira et al. 2021). Benefits for the colonizers include protection

against extreme conditions (e.g., Vieira and Romero 2013, Novais et al. 2018) and predators (Manicom et al. 2008, Tvardikova and Novotny 2012, Sendoya and Oliveira 2017), food for herbivores (Henriques et al. 2019), hosts for parasitoids (Shorthouse 1994), resources through frass and/or prey (Baer and Marquis 2020) as well as mating sites (Morse 2021). By accommodating multiple different arthropod guilds, including predators, parasitoids, herbivores and decomposers, constructs also have the potential to influence ecosystem processes through direct and indirect trophic interactions (Zhong et al. 2017, Henriques et al. 2019, Calderón-Cortés 2020, Pereira et al. 2021). Additionally, specific host plant traits can affect the structure of the associated consumer community and mediate species interactions (Tielens and Gruner 2020). However, while different traits of the host plant (including e.g., physical, chemical and architectural features) may affect the potential for ecosystem engineering by insects, such effects have been, to a certain degree, overlooked. Further analyses of how plant traits affect the distribution and abundance of constructs may thus enrich our understanding of the organizing principles of plant-based communities.

We here consider the full range of natural constructs reported in the literature since 1932, as well as artificial (man-made) constructs in the form of leaf shelters (Romero et al. 2021), commonly used to test the hypothesis that these subtle constructs influence community composition and functionality. With this dataset, we aim to advance knowledge regarding these arthropod constructs on: 1) their global distribution; 2) their frequency of occurrence in host plants with different traits; and 3) characteristics of the constructs built by arthropods; as well as the 4) the diversity of secondary occupants occupying constructs; and the 5) major outcomes of shelter construction for other occupants. The strength of the impact of such structures in the community of associated organisms and on plant processes such as herbivory and trophic cascades or decomposition after changes in leaf and subsequent litter quality is still a matter of exciting debate. The dataset will aid future investigations on how the characteristics of the

shelters themselves, as well as host plant phylogeny, foliar traits or habitats might influence the responses of potential colonists to these constructs.

Metadata

Class I. Dataset descriptors

A. Dataset identity:

Title: Subtle structures with not-so-subtle functions: a dataset of arthropod constructs and their host plants

B. Dataset identification code:

Suggested Dataset Identity Code: Engineer_constructs_data.csv

C. Dataset description

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Abstract:

The construction of shelters on plants by arthropods might influence other organisms via changes in colonization, community richness, species composition and functionality. Arthropods, including beetles, caterpillars, sawflies, spiders, and wasps often interact with host plants via the construction of shelters, building a variety of structures such as leaf ties, tents, rolls, and bags; leaf and stem galls, and hollowed out stems. Such constructs might have both an adaptive value in terms of protection (i.e., serve as shelters) but may also exert a strong influence on terrestrial community diversity in the engineered and neighboring hosts via colonization by secondary occupants. While different traits of the host plant (e.g., physical, chemical and architectural features) may affect the potential for ecosystem engineering by insects, such effects have been, to a certain degree, overlooked. Further analyses of how plant traits affect the occurrence of shelters may thus enrich our understanding of the organizing principles of plant-based communities. This dataset includes more than a thousand unique records of ecosystem engineering by arthropods, in the form of structures built on plants. All records have been published in the literature, and span both natural structures (90.6% of the records) and structures artificially created by researchers (9% of the records). The data were gathered between 1932 and 2021, across more than 50 countries and several ecosystems,

ranging from polar to tropical zones. Besides data on host plants and engineers, we aggregated data on the type of constructs and the identity of inquilines using these structures. This dataset highlights the importance of these subtle structures for the organization of terrestrial arthropod communities, enabling hypotheses testing in ecological studies addressing ecosystem engineering and facilitation mediated by constructs.

D. Keywords: arthropods, caterpillars, ecosystem engineering, inquilines, insects, leaf galls, leaf rolls, leaf tents, leaf ties, plant constructs, shelters

E. Description:

The dataset encompasses more than a thousand records (n=1,009) of ecosystem engineering by arthropods in the form of plant constructs published in the literature - both natural (90.6% of the records) and also artificially created by researchers (9% of the records). Data were gathered from 52 countries, ranging from polar to tropical zones and spanning 70° of latitude North and more than 40° of latitude South. Brazil was the country with the most records in the tropics, whereas the USA accounted for the most records in the temperate zone (Figure 1a). Constructs were recorded on 83 plant families and 326 plant species occurring in several vegetation types worldwide, but forests were the biomes more frequently examined, regardless of region (Figure 1b). Among life forms, most constructs (70.5%) were found on trees (Figure 1c), followed by herbs (13.3%) and shrubs (11.2%). Seven main types of constructs have been found on those 326 plant species and are distributed among 36 plant orders. Galls and leaf rolls were the two most frequent construct types (Figure 2) and *Quercus* species hosted the most types of constructs.

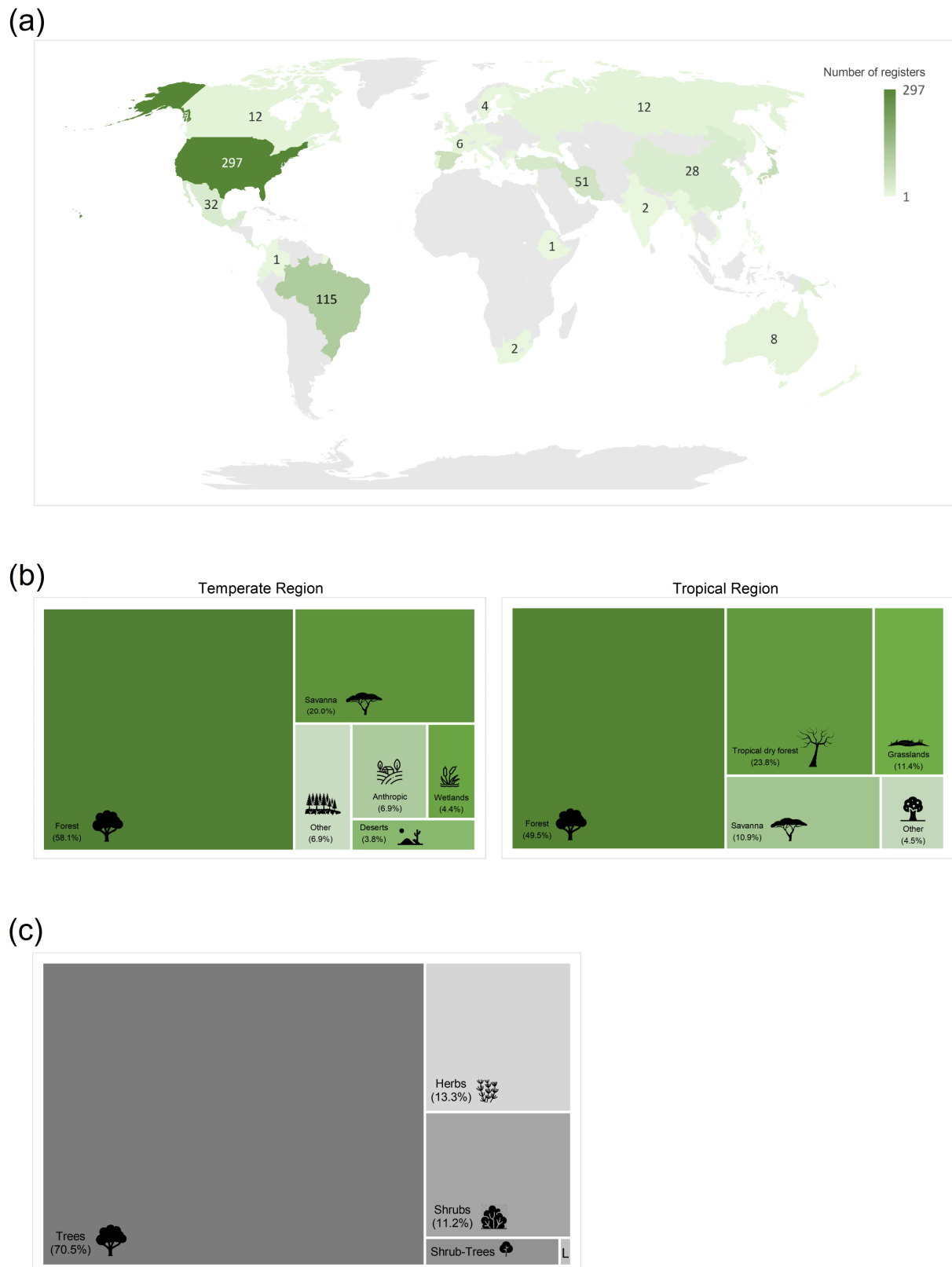


Figure 1. (a) Geographic distribution of records of constructs by country. Green shades indicate the number of records, from 1 (lightest green) to 297 (darkest green). (b) Distribution of constructs in the database by vegetation types in Temperate and Tropical regions. (c) Distribution of constructs according to plant growth forms. Shrub-trees represented 2.3% of the records and lianas (L) represented 0.2%.

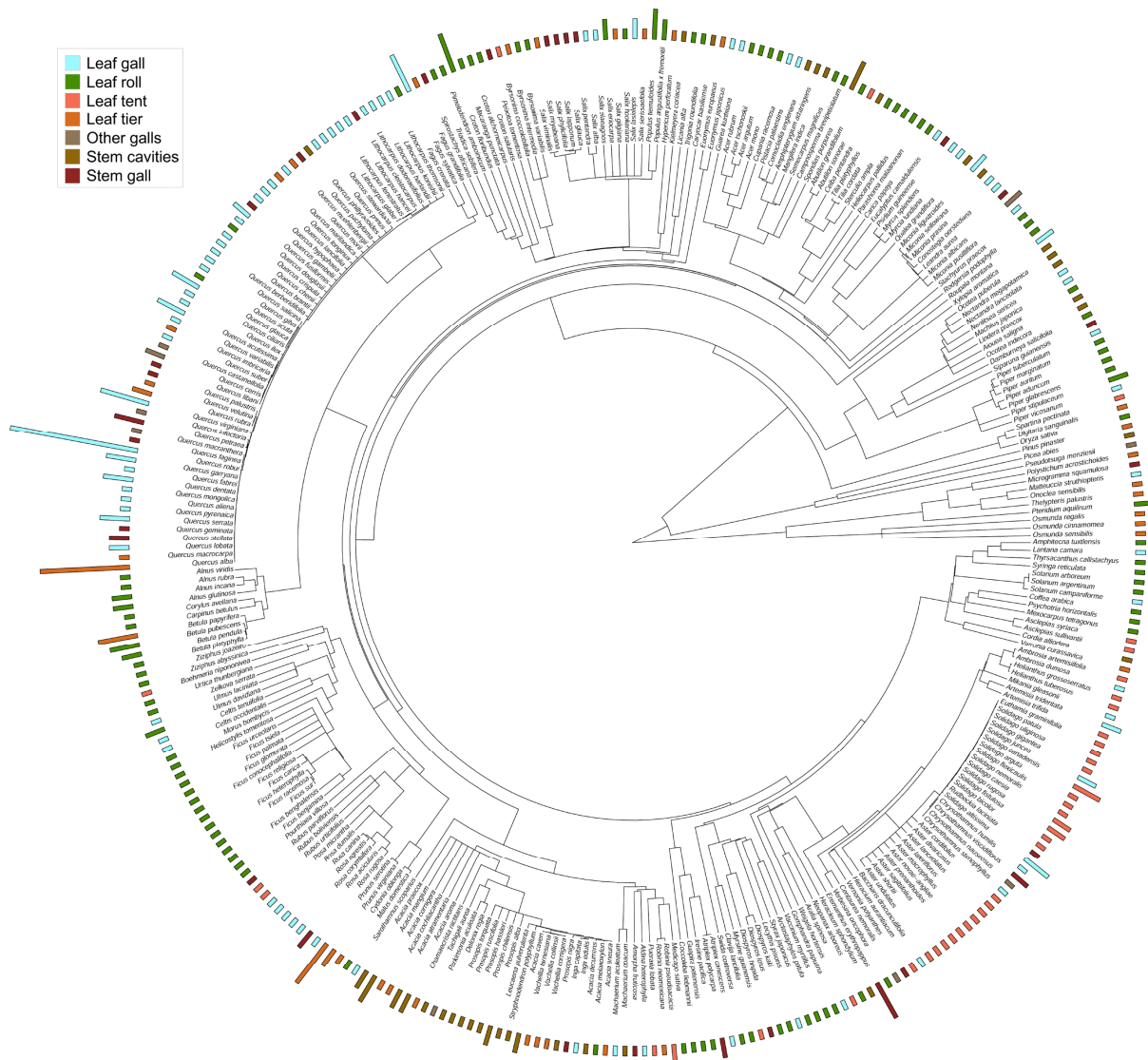
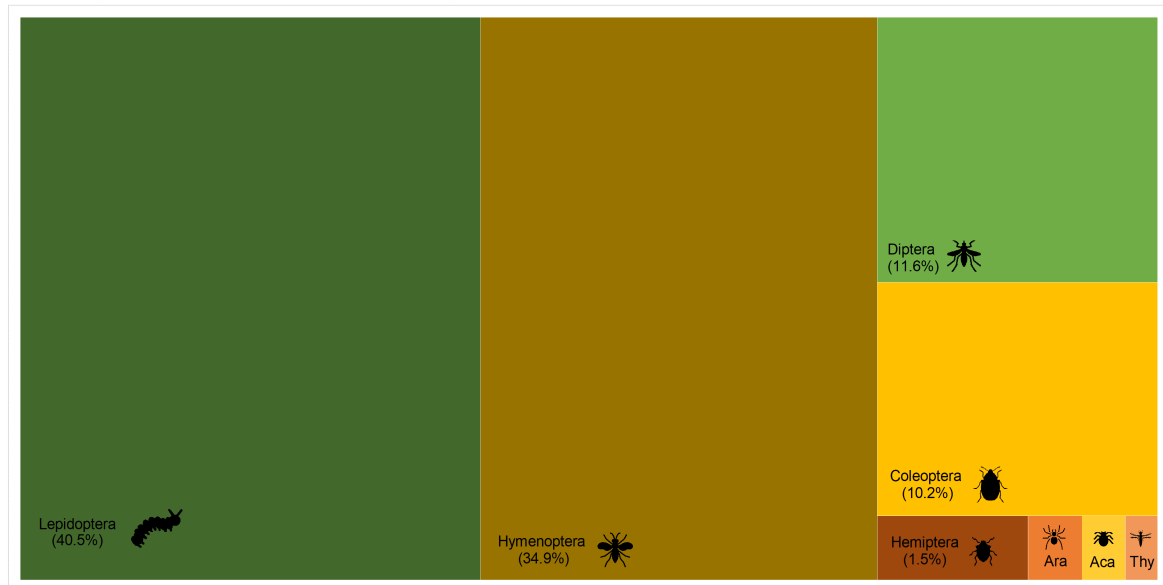


Figure 2. Phylogenetic distribution of constructs in the database mapped onto the phylogeny of host plant species. Phylogeny was reconstructed based upon Qian and Jin (2016). The colored bars indicate the eight main construct types registered in the literature.

Constructs were made by species in 10 arthropod orders, but Lepidoptera and Hymenoptera together represented more than 75% of all records in the database (Figure 3a) and were responsible for the construction of most of the leaf rolls (22.2%) and leaf galls (23.2%). Other constructs created by arthropods on plants included leaf ties, leaf tents and stem cavities (Figure 3b). In one-third of the cases, these constructs were used by the engineers for the development of larval stages and offspring (33%), but also served as shelters (19.8%) and as an

adequate place to ambush and capture prey (15%). Almost 70% of the studies compiled evaluated the role of shelters as a home for other arthropods in the form of primary and secondary secondary occupants, and secondary occupants are recorded in our database as orders. One-third of the studies in our database reported Hymenoptera, especially ants (Formicidae), as the main and only inquiline in these constructs (36.5%), followed by Coleoptera as sole inquilines (7.8% of the cases) or together with other arthropod groups (13.5%) such as spiders (7.7%) (Figure 4). In most cases, however, several arthropod orders were found colonizing these constructs for reasons as diverse as feeding, sheltering and mating sites.

(a)



(b)

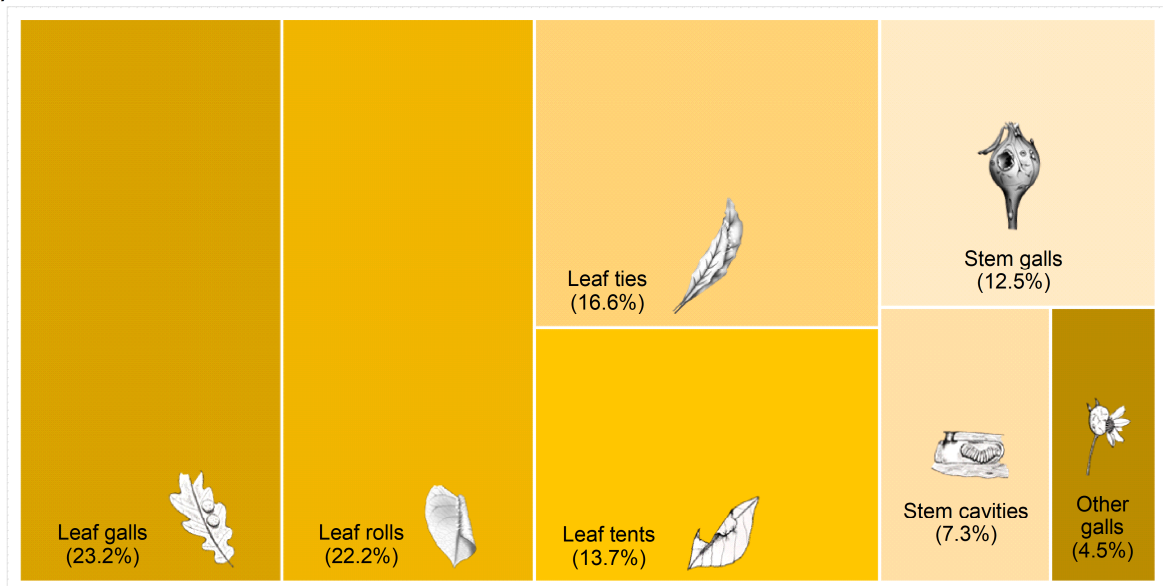


Figure 3. (a) Distribution of constructs according to Arthropod taxonomic groups. Ara=Arachnida (0.5% of the records), Aca=Acari (0.4% of the records) and Thy=Thysanoptera (0.3% of the records). (b) Distribution of the main construct types. Flower and fruit galls were grouped into "Other galls".

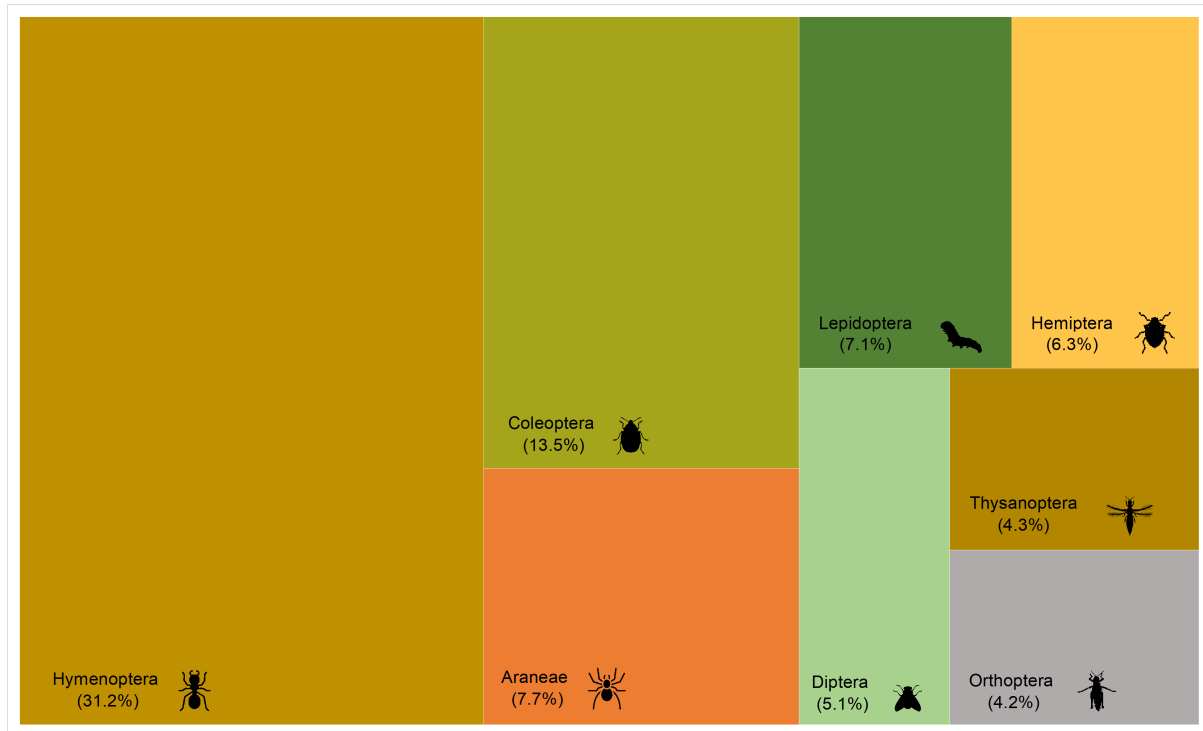


Figure 4. Distribution of secondary occupants found in constructs, according to taxonomic orders.

Our dataset highlights the importance of these subtle structures for the organization of terrestrial communities. Several studies included were designed to directly and experimentally evaluate the role of constructs as shelters and drivers of arthropod community diversity, structure and ecosystem function, thus allowing the testing of general hypotheses regarding the roles of ecosystem engineering mediated by constructs. The compilation of this dataset and the evaluation of the importance of these arthropod constructs for plants appears particularly relevant during the Anthropocene, an era marked by sharp declines in insect richness worldwide (Wagner et al. 2021) and changes in climate, which might reinforce the adaptive value of shelters against extreme conditions (Romero et al. 2021).

Class II. Research origin descriptors

A. Overall project description

1. Identity: Arthropod constructs in host plants worldwide.

2. Originators: The project “Subtle structures with not-so-subtle functions: a dataset of arthropod constructs and their host plants” is part of the doctoral thesis of Cássio Pereira at Universidade Federal de Minas Gerais (UFMG) and part of a global project coordinated by Gustavo Romero (UNICAMP) to evaluate the effects of artificial leaf shelters on colonization and community attributes. The compilation of data on constructs published in the literature was led by Cássio Pereira, Samuel Novais and Milton Barbosa, coordinated by Dr. Tatiana Cornelissen (UFMG) and GW Fernandes (UFMG). Compilation of data on artificial shelters was led by Gustavo Romero (UNICAMP) and the database was assembled with contributions from all other authors.

3. Period of Study: Data collection spans from 1932 to 2021.

4. Objectives: Provide data on the occurrence, type, and secondary occupation of arthropod constructs and related plant data. These data can be further used to test general hypotheses related to facilitation and ecosystem engineering.

5. Abstract: Same as above.

6. Sources of funding: The compilation of this dataset was supported by grants and scholarships from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre - Universidade Federal de Minas Gerais (UFMG) and UNICAMP.

B. Specific subproject description

1. Network establishment: The Ecosystem Engineering (EE) network of researchers was established in 2018, led by Gustavo Romero (UNICAMP).

2. Site description: Data was collected in several sites across the globe, listed in the database with latitude and longitude coordinates (when available), country, region, study location and vegetation type. Site data regarding artificial leaf rolls are individually mapped in Romero *et al.* (2021), Supplementary Material.

3. Data sampling: Published data was sampled in the literature, using the search engines Web of Science, Scopus and Google Scholar, and the keywords TS= ("Leaf shelter*" OR "Leaf roll*" OR "Leaf tie*" OR "Leaf tent*" OR inquiline* OR inquilinism OR "Ecosystem Engineer*" OR "Gall inquilines" OR "Insect engineer" OR "Arthropod engineer" OR "Shelter building insect*s"). Stars (*) substituted any single character in the search engines used and searches were conducted in English only. Studies found were individually examined following the PRISMA guidelines (Page *et al.* 2021) for database construction and transparency and added to the database accordingly. Other sources of studies included the bibliographic lists of reviews of arthropod ecosystem engineering (e.g., Romero *et al.* 2015, Cornelissen *et al.* 2016). From each study we recorded data on i) characteristics of the site where the study was conducted, ii) taxonomic data and life forms of the host plants and iii) taxonomic data and guild of the ecosystem engineers and iv) characteristics of the constructs and their effects on secondary occupation. When studies evaluated more than one host plant and/or more than one construct type, each one was included as a separate record in the database. Missing information is indicated as NA (not available).

Data on artificial leaf rolls were sampled following Romero *et al.* (2021) protocol. In brief, 15 plant pairs of broadleaf trees or shrubs, native to each area, were marked in the field and leaves were rolled to form a cylinder, transversally to the leaf axis. Leaves were kept rolled using stainless steel hairclips and all occupants were recorded after 10 days.

4. Taxonomic data: All species records had their taxonomic classification revised and updated.

For taxonomic information, we used <https://www.gbif.org> and APG IV.

Class III. Dataset status and accessibility

A. Status

Latest update: May 2021

Latest archive date: To be defined

Metadata status: Updated, corrected

Data verification: We checked all the information such as species records and localization.

Taxonomic information was homogenized. Transcription errors were corrected.

B. Accessibility

Contact person: Tatiana Cornelissen (taticornelissen@gmail.com), Centro de Síntese Ecológica e Conservação, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (UFMG), Avenida Presidente Antônio Carlos 6627, 31270-910, bloco I3. Belo Horizonte, Minas Gerais, Brazil.

Copyright restrictions: None.

Proprietary restrictions: Please cite this paper when using the current data in publications and let us know how the data is used in the publications.

Costs: None

Class IV. Data structural descriptors

A. Dataset File

Identity:

Size: 22 columns, 1009 records, 329kB

Format and storage mode: Engineer constructs data (CSV file) in a compressed folder (.RAR) named "Data S1".

Header information: See column descriptions in Metadata worksheet

Alphanumeric attributes: Mixed.

Data anomalies: Missing information was classified as “NA”.

B. Variable information

1) Data Source and Study Site Information

Variables	Description	Number of Levels	Example
Study author	Author Last Name	224	Barbosa et al. 2019
Bibliographic source	Electronic and printed reference sources	224	https://doi.org/10.1111/1365-2656.13025
Construct origin	Origin of the data regarding constructs	2	Artificial
Geographic Coordinates	Geographic location of the area where the studies were carried out	145	19°15'S and 43°31'W
Country	Name of the country where the study was conducted	52	Brazil
Region	Geographic areas according to latitude	3	Tropical
Study location	Local name of the study site	256	Serra do Cipó, Minas Gerais
Vegetation type	Type of vegetation of the study site	15	Grasslands

2) Plant Species Information

Variables	Description	Number of Levels	Example
Plant order	Taxonomic order	36	Asterales
Plant family	Taxonomic family	83	Asteraceae
Plant genus	Taxonomic genus	184	<i>Baccharis</i>
Plant species	Scientific species name (specific epithet)	326	<i>Baccharis dracunculifolia</i>
Authority (Plant)	Authority of plant species name	104	(Willd.) Sweet
Life form	Plant growth form	5	Shrub

3) Construct Information

Variables	Description	Number of Levels	Example
Engineer order	Taxonomic order	10	Hemiptera
Engineer family	Taxonomic family	47	Psyllidae
Engineer genus	Taxonomic genus	193	<i>Baccharopelma</i>
Engineer species	Specific epithet	349	<i>Baccharopelma dracunculifoliae</i>
Authority (Engineer)	Authority of engineer species name	254	Burckhardt, Espirito-Santo, Fernandes & Malenovský
Guild	Functional guild of ecosystem engineers responsible for construct	4	Galler
Construct type	Types of constructs based upon type and plant organ	8	Leaf gall
Construct persistence	Duration of the construct	2	< 1 year
Inquiline identity	Taxonomic group(s) of inquilines found on constructs	38	Acari, Araneae, Diptera, Hemiptera, Neuroptera and Thysanoptera
Construct use	Reason for construct use	3	Occupation for development and Occupation for feeding
Construct effect	Indicates the outcome of construct presence	3	Arthropod composition

Class V. Supplemental descriptors

A. Data acquisition

1. **Data request history:** None.

2. **Data set updates history:** None.

3. **Data entry/verification procedures:** done by first author.

B. History of data set usage: None.

Acknowledgments

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Chapter II. Gallers as leaf rollers: ecosystem engineering in a tropical system and its effects on arthropod biodiversity*

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Abstract

1. Gall-inducers can be considered ecosystem engineers when they modify leaf morphology and create shelters for secondary users, altering properties of ecological communities.

2. In this study we evaluated whether shelter structures created by leaf-galling contribute to changes in the diversity and composition of the community at plant and leaf scales, the effects of leaf roll occupation on the choice of secondary colonizers and the influence of leaf shelters on herbivory levels on host plants.

3. The arthropod abundance, richness and biomass were higher in leaf shelters compared to intact leaves. These effects were observed at similar magnitudes at both plant and leaf scales. The composition of arthropods differed between plants with leaf rolled galls and plants with galls removed and between unoccupied leaf rolls and intact leaves. Artificial leaf rolls increased arthropod abundance, richness and biomass by almost 100% compared to intact leaves. However, occupied artificial leaf rolls had a decrease in abundance, richness and biomass of arthropods by almost 60% compared to unoccupied leaf rolls. Finally, plants with galls removed exhibited higher levels of leaf herbivory than plants with leaf rolled galls.

4. Our findings demonstrated the strong and positive indirect effects of gall induction facilitating shelter creation and the indirect effects of shelters on the diversity, species composition and on leaf herbivory, and should be replicated in other systems involving plants and their respective galls.

Keywords: arthropods, Cerrado, ecosystem engineers, facilitation, guilds, herbivory

Resumo

1. Os indutores de galhas podem ser considerados engenheiros de ecossistemas quando modificam a morfologia foliar e criam abrigos para colonizadores secundários, alterando propriedades de comunidades ecológicas.

2. Neste estudo avaliamos se as estruturas de abrigo criadas por galhas contribuem para mudanças na diversidade e composição da comunidade de artrópodes em diferentes escalas espaciais (nível da planta e foliar), os efeitos da ocupação do enrolamento de folhas na escolha de colonizadores secundários, e a influência dos abrigos foliares nos níveis de herbivoria nas plantas hospedeiras.

3. A abundância, riqueza e biomassa de artrópodes foram maiores em abrigos foliares em comparação com folhas intactas. Esses efeitos foram observados em magnitudes semelhantes nas escalas das plantas e das folhas. A composição dos artrópodes diferiu entre plantas com rolos foliares criados pelas galhas e plantas com galhas removidas, e entre rolos foliares desocupados e folhas intactas. Os rolos foliares artificiais aumentaram a abundância, a riqueza e a biomassa dos artrópodes em quase 100% em comparação com as folhas intactas. No entanto, os rolos foliares artificiais ocupados tiveram uma diminuição na abundância, riqueza e biomassa de artrópodes em quase 60% em comparação com os rolos foliares desocupados. Finalmente, as plantas com galhas removidas exibiram níveis mais elevados de herbivoria foliar do que as plantas com rolos foliares criados pelas galhas.

4. Nossas descobertas demonstraram os fortes e positivos efeitos indiretos da indução de galhas que facilitaram a criação de abrigos e os efeitos indiretos dos abrigos na diversidade, na composição de espécies e na herbivoria foliar, e devem ser replicados em outros sistemas envolvendo plantas e seus respectivos galhadores.

Palavras-chave: artrópodes, Cerrado, engenheiros de ecossistema, facilitação, guildas, herbivoria

Introduction

Ecosystem engineering influences the diversity of biological communities (Romero *et al.*, 2014) through changes in community composition, species abundance and richness. By creating new habitats for other species, ecosystem engineers (EE) alter the distribution of resources through non-trophic interactions that might show positive ecological effects (e.g., facilitation) on other species (Jones *et al.*, 1994; 2010). Organisms act as EE when modulating the supply of resources other than themselves, being called autogenic engineers when they change the environment through their own physical structures and allogenic engineers, when they change the environment by transforming living or non-living materials from one physical conformation to another (Jones *et al.*, 1994; 2010).

Many arthropods manipulate plant leaves to build shelters (Cornelissen *et al.*, 2016) acting therefore as EE. These structures can be secondarily occupied by other arthropods, that sometimes co-occur with the EE (Cornelissen *et al.*, 2016). Gallers are seldom suggested as EE, representing only 4.3% of EE records for invertebrates in a global meta-analytic review (Romero *et al.*, 2015), but they are important allogenic engineers because by inducing cell differentiation - by hyperplasia and/or hypertrophy - in their host plants (Price *et al.*, 1986; Fernandes *et al.*, 2014), they create a variety of structures that can provide shelter for several arthropods, such as ants who use galls as nests (e.g., Fernandes *et al.*, 1988; Fukui, 2001; Shorthouse *et al.*, 2005; Maruyama *et al.*, 2012; Barbosa *et al.*, 2019; Novais *et al.*, 2020).

Galls are primarily induced by insects (Gagné, 1994; Espírito-Santo & Fernandes, 2007) but also by nematodes (Giblin-Davis *et al.*, 2004; Maruyama *et al.*, 2012) and they alter the architecture of host plants by providing additional shelter and resources for several organisms, including beetles (Sugiura & Yamazaki, 2009), caterpillars (Cooper & Riskie, 2009), hemipterans (Fernandes *et al.*, 1987), spiders (Wetzel *et al.*, 2016), springtails (Novais *et al.*, 2020) and thrips (Lindner *et al.*, 2018). These structures can increase the diversity of associated

organisms, changing for example arthropod community richness and/or composition (Crawford *et al.*, 2007; Maruyama *et al.*, 2012; Vieira & Romero, 2013; Novais *et al.*, 2020). These changes might, in turn, change the functional structure of these communities when functional groups such as herbivores and predators become more or less frequent in plants, changing ecosystem processes such as herbivory and decomposition at different spatial - leaves, plants or patches - and temporal scales (*i.e.*, over seasons) (see Vieira & Romero, 2013; Barbosa *et al.*, 2019).

Although the effects of EE are now well established, patterns and sources of variation in the magnitude of effects of EE on communities and ecosystems remain largely unknown (Romero *et al.*, 2015). Shelters created by arthropods, especially those built using leaves or leaf parts, can be artificially created and also manipulated to investigate the effects of EE on communities over time (Lill *et al.*, 2007) and space (Vieira & Romero, 2013) and to assess changes in trophic interactions (Henriques *et al.*, 2019). These experimental scenarios, however, have not been fully examined and ecosystem engineering by gall-inducers needs to be further explored. Gall-inducers have been considered ecosystem engineers themselves (*e.g.*, abandoned large galls might serve as ant nests, Fernandes *et al.*, 1988, Santos *et al.*, 2017), with an important role in community organization through non-trophic interactions, as well as due to their enormous abundance in many ecosystems (*e.g.*, see Espirito-Santo & Fernandes, 2007 and references therein). However, no study has shown that galls can facilitate ecosystem engineering when the leaves are rolled and serve as a shelter for secondary colonization.

In this study, we evaluated the consequences of facilitation of leaf-sheltering by gall-inducers on arthropod communities at different spatial scales and on trophic interactions. The host plant *Miconia ligustroides* (DC.) Naudin (Melastomataceae) is galled by the nematode *Ditylenchus gallaeformans* Oliveira, Santin, Seni, Dietrich, Salazar, Subbotin, Mundo-Ocampo, Goldenberg & Barreto, 2013 (Anguinidae) which induces leaf rolled galls, modifying

leaf morphology to convey leaf blades closer together forming conspicuous leaf shelters. We investigated whether these sheltered structures contribute indirectly to changes in community diversity at the plant level and leaf level and the composition and effects of leaf roll occupation in the choice of secondary colonizers. We also explored whether shelters can affect herbivory levels via changes in the occurrence of functional guilds on these plants.

We aimed to answer the following questions: 1) Do shelters created by galls increase the diversity and modify the composition of arthropods on *M. ligustroides* at the plant level and leaf level?; 2) What is the effect of shelter occupancy on arthropod colonization of leaf rolled galls? and 3) How does the presence and the number of shelters influence herbivory levels on *M. ligustroides*? We used three separate experiments to answer these questions by comparing (i) the abundance, richness and biomass of arthropods on plants with leaf rolled galls created by *D. gallaeformans* (control) and plants with galls removed (treatment), (ii) the role of shelter itself on the abundance, richness and biomass of arthropods by comparing intact leaves (control) and artificially rolled leaves, as well as the preference of secondary arthropods when choosing shelters, comparing occupied and unoccupied leaf shelters, and (iii) the level of herbivory on control plants (with leaf rolled galls) and treatment plants (with galls removed).

Material and Methods

Study site

The study was carried out in the Área de Proteção Ambiental (APA) São José (21° 03'S and 44° 06'W), Minas Gerais, Brazil (Fig. 1), which has 4,758 ha of original vegetation. The altitudes range from 800 to 1,400 m (Henriques & Cornelissen, 2019) and the climate is subtropical in altitude (Cwb, average annual rainfall: 1,435 mm, average annual temperature: 19°C). Data were collected in an area of 10 ha in the northern portion (21° 02' 52.5"S, 44° 07'

01.8"W), in the municipality of Prados, where the cerrado *sensu stricto* (Brazilian savanna) is dominant (Pereira *et al.*, 2020).

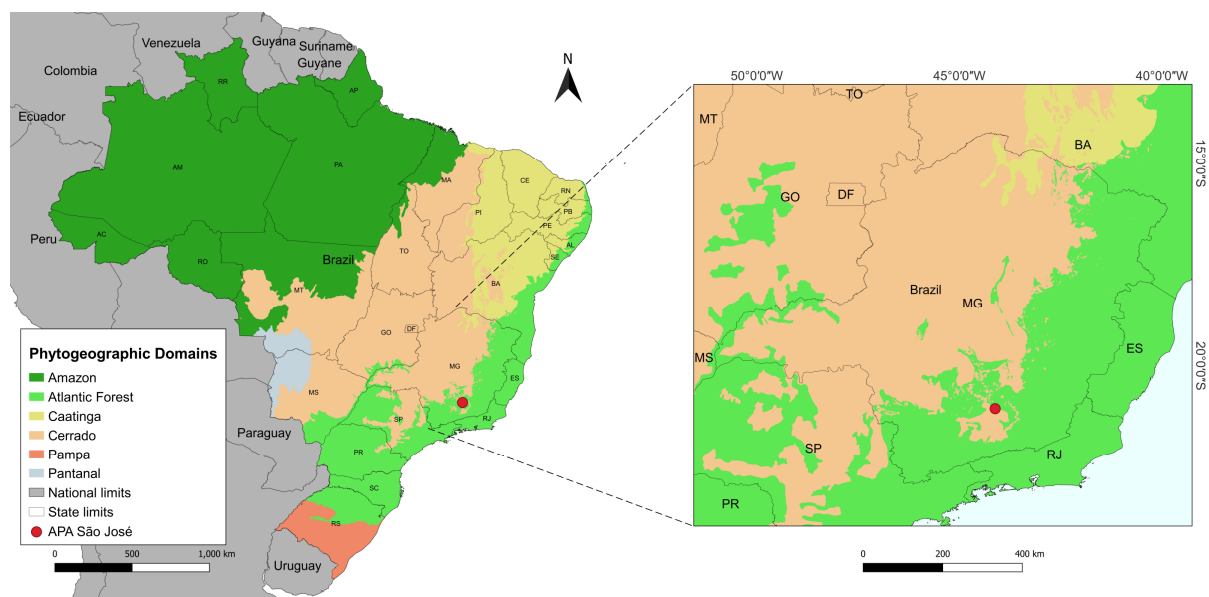


Figure 1. Map of the geographic location of the Área de Proteção Ambiental (APA) São José, Minas Gerais, Brazil. The boundaries of Brazilian Phytogeographic Domains were adapted from shapefiles available from the Instituto Brasileiro de Geografia e Estatística (2022), from the global ecoregions of Dinerstein *et al.* (2017), and from the map of vegetations on rocky outcrops of the Cerrado domain by Newton Barbosa. Map design: Cássio Cardoso Pereira.

Study system

M. ligustroides is a shrub-tree species (Supporting Information S1, Fig. S1a), evergreen, apomictic (Maia *et al.*, 2016), with membranous leaves without trichomes, occurring in several savanna and forest environments in the tropics (Martins *et al.*, 1996). At the study site, this species has leaf rolled galls induced by *D. gallaeformans* which curl their leaves, forming a roll of approximately 20 mm in diameter (Fig. 2a-f and Supporting Information S1, Fig. S1b and Fig. S1c). Leaves are rolled from the abaxial to the adaxial surface, from the edges to the midvein, and leaf rolled galls are often colonized by several arthropods and remain on the plants for approximately 8 months until they fall to the ground (Cássio Pereira, *personal observation*). It is important to note that, unlike leaf rolls produced by other arthropods such as spiders or

caterpillars, these rolls formed on galls are not occupied by the gall-former, which is microscopic ($\sim 600 \mu\text{m}$), and occupies the leaf blade. In the studied system, the secondary colonizers find unoccupied shelters initially, regardless of the state of development of the galls.

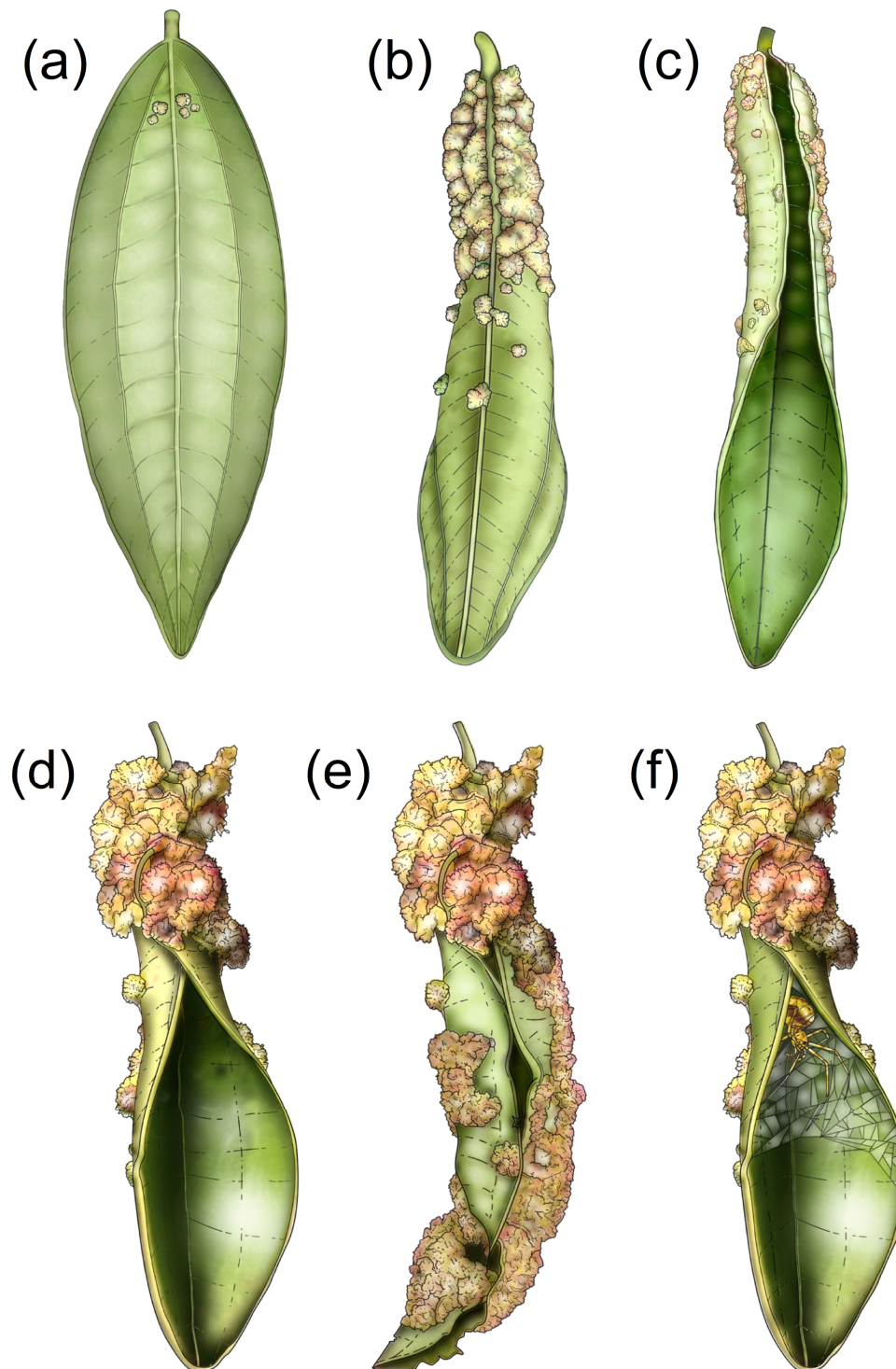


Figure 2. Schematic representation showing the formation of leaf shelters on *Miconia ligustroides* after galling. The leaves of these species are modified by *Ditylenchus gallaeformans*, which induces gall formation on the abaxial leaf face (a). As the infestation increases, the leaf curls from the abaxial to the adaxial face, forming a leaf rolled gall (b - e), which then serves as shelter for different arthropods, such as spiders (f).

Data sampling

Effects of ecosystem engineers on M. ligustroides - Plant scale

To assess whether shelters increase the diversity of arthropods on *M. ligustroides*, 60 plants at least 10.0 m apart were marked in the field and two groups were selected, i) control plants (n = 30, with leaf rolled galls) and ii) treatment plants (n = 30, with galls removed). All plants were between 1.0 and 1.5 m tall. Plants in the control group had about 10 leaf rolled galls of similar age and for plants in the treatment group, we removed all the leaves that had galls. This represents approximately only 1% of biomass removal, as plants on the treatment group had an average of 1021 (± 106.99 SE) leaves. A month after marking the groups of plants and removing galls from the treatment group, the plants were visually inspected for 25 minutes and the arthropods were collected using entomological forceps and aspirators. The shelters were collected in plastic bags with a zipper closure and frozen for occupancy evaluation and arthropod sorting.

Effects of ecosystem engineers on M. ligustroides - Leaf scale

To evaluate the effects of shelters on arthropod colonization and the effects of shelter occupancy on arthropod occurrence, thirty plants, spaced approximately 10.0 m from each other were marked in the field in 2018. In each plant, 3 pairs of paired leaves were marked with colored tags and 2 pairs were selected as treatments and one pair as the control (intact leaves) (see Supporting Information S1, Fig. S2). Treatments were applied into different branches in each plant. The treatment pairs consisted of a rolled leaf with an empty artificial shelter and a rolled leaf with an occupied shelter. To create the artificial shelters, young, intact but fully-developed leaves were used. We opted to use intact leaves to create the shelters because the gall leaves are extremely fragile and break easily, being unviable to be manipulated. In addition, we

previously inspected the galls with a stereomicroscope and found no arthropods associated with them, with the exception of the nematode EE itself.

The leaves were cleaned with a brush to exclude all arthropods and then manually rolled from the abaxial to the adaxial face, from the edges to the midrib and secured with hair pins (see Supporting Information S1, Fig. S3a). These shelters were cylindrical rolls, 20 mm in diameter, simulating the leaf rolled galls naturally created on these plants when galled leaves are curled. The leaves were kept rolled with hair clips painted the same color of the leaves with odorless green spray paint. Green modeling clay was used to simulate the occupancy of these artificial shelters, simulating a Lepidoptera caterpillar with approximately 5 mm in diameter, that did not occupy the entire space of the leaf rolls, allowing secondary colonization by arthropods (see Supporting Information S1, Fig. S3b). We conducted a pilot experiment to evaluate how many days were necessary for the colonization of the shelters and concluded that 10 days were sufficient. Thus, two samplings were conducted, the first at 10 days after the experiment was set and the second sampling 10 days after the first. The treatments were reapplied to the same trees and branches and between sampling occasions we used different pairs of leaves. In each sampling occasion, sampled rolls and leaves were collected, stored in plastic bags with a zipper closure, recording the treatment and frozen for later sorting.

The sampled leaves were inspected under a stereomicroscope and all arthropods (except mites) were collected and stored in 70% alcohol. All arthropods were identified to the lowest possible taxonomic level and classified into morphospecies (Oliver & Beattie, 1996) to evaluate abundance, richness and biomass (mg of dry mass). In addition, arthropods were classified into feeding guilds as detritivores, herbivores, omnivores, parasites, parasitoids, predators, based on data from the literature. To estimate biomass, all arthropods were oven-dried at 60°C for 24 hours and weighed on a precision digital scale. The experiments were carried out at the end of

the 2018 rainy season (February and March of 2019) and the plants had no flowers and fruits at the time of the study.

Effects of EE on herbivory levels in M. ligustroides

To evaluate herbivory levels on *M. ligustroides*, 60 plants were marked and divided into three categories: 1) low shelter plants (plants with up to 7 leaf rolled galls, $n = 20$), 2) high shelter plants (plants with 8 to 15 leaf rolled galls, $n = 20$) and treatment plants (plants with galls removed, $n = 20$). All plants were between 1 and 1.5 m tall. The control plants had leaf rolled galls with similar age and for the treatment group, we removed all the leaves that had galls. For each plant in each treatment, 40 fully-intact leaves of similar size were numbered with a permanent felt-tip pen at the beginning of the rainy season and kept on the plants until the end of the season, in order to accumulate herbivory. At the end of the season, after 150 days, we randomly collected 20 out of the 40 leaves initially marked per plant ($n = 1,200$ leaves sampled), packed them into plastic bags, then they were refrigerated and digitized for herbivory measurements.

To determine the total area of the leaf blade and area removed by herbivores, digital images were calibrated to 0.01 mm and measured on the software ImageJ 1.6.0 (Rasband, 1997). Herbivory levels were expressed as the percentage of leaf area lost, estimated according to the formula: $\text{Herbivory} = [\text{area lost}/\text{total leaf area}] * 100$.

All plants marked in this study, including the three experiments, were submitted to similar environmental conditions in an area of 3 ha composed exclusively of cerrado *sensu stricto* under the same type of soil (yellow latosol) and similar altitude (approximately 1020 m).

Data analysis

To evaluate the effects of shelters on arthropod diversity at the plant level, we built generalized linear mixed models (GLMMs) using abundance, richness and biomass as dependent variables, each treatment as the fixed factor and individual plants as random factors. Error distribution was checked through restricted maximum likelihood (REML).

To assess the effects of shelters on arthropod diversity at the leaf level, GLMMs were built using the mean values of richness, abundance and biomass as the response variables, each treatment as the fixed factor and individual plants as a random factor. Error distribution was checked through restricted maximum likelihood (REML). Treatment means were compared using a *post hoc* Tukey test ($\alpha = 0.05$).

The composition of arthropods and guilds at the plant level and leaf level was examined among different treatments using unidirectional ANOSIM, based on Euclidean distances. The one-tailed significance was calculated by permuting the groups with 9,999 permutations. ANOSIMs paired between all pairs of groups were used as a post-hoc test. Nonmetric multidimensional scaling analyses (NMDS) were performed to envision similarities or dissimilarities among treatments, using the species abundances for each individual plant sampled. We also used a unidirectional ANOSIM to check the arthropods composition between the natural shelters collected in the experiment at the plant level and artificial shelters. All analyses mentioned above were conducted using Vegan package (Oksanen *et al.*, 2013) on R software (R Core Team, 2017).

To estimate the magnitude of the effects of the presence and occupation of leaf rolls on the abundance, richness and biomass of arthropods we calculated effect sizes using Hedges' d as a metric to standardize and calculate the cumulative effect size (Hedges & Vevea, 1998). For replicates among treatments, the mean and standard deviation from the 4 leaves per plant, per treatment, were used. Overall effect sizes were calculated on the response variables of

arthropod abundance, richness and biomass. Treatment groups were assigned to intact leaves (control) and leaf rolls (treatment) and between unoccupied leaf rolls (control) and occupied leaf rolls (treatment). The effect size was calculated as:

$$d_i = \frac{\bar{X}_E - \bar{X}_C}{SD} J$$

in which d_i = individual effect for each comparison, X_E and X_C are the sample means of the two groups (E = experimental, C = control) and SD is the pooled standard deviation, expressed as:

$$SD = \sqrt{\frac{S_1^2(n_1 - 1) + S_2^2(n_2 - 1)}{n_1 n_2 - 2}}$$

where n_1 and n_2 are the sample sizes and S_1 and S_2 are the standard deviations of the two groups corrected for sample size with the correction factor J (Rosenberg *et al.*, 2000).

To estimate the cumulative effect ($E++$) of treatments, the individual d_i effects were combined using weighted averages and a random model analysis. A positive effect size indicates that abundance, richness, and biomass of arthropods were lower on control plants compared to experimental plants, whereas a negative effect size implies a lower abundance, richness and biomass of arthropods for the experimental plants compared to controls. As a convention, $E++$ values around 0.2 are considered weak effects, values around 0.5 are considered of moderate magnitude, values around 0.8 are considered strong, and $E++$ larger than 1.0 are considered very strong (Rosenberg *et al.*, 2000). The cumulative effects were considered significant if the confidence intervals (95%) did not overlap with zero. All analyses were conducted in Open MEE (Wallace *et al.*, 2017).






Herbivory levels were calculated for each plant using the number of leaves sampled per plant ($n = 20$) as replicates for each individual and plants were used as replicates on each treatment. To assess variation in herbivory between treatments, we built generalized linear mixed model (GLMM) using herbivory levels as a dependent variable, each treatment as the






fixed factor and individual plants as a random factor. Error distribution was checked through restricted maximum likelihood (REML). A Tukey *post hoc* test was used to assess differences between treatments ($\alpha = 0.05$). These analyses were conducted using Vegan package (Oksanen et al., 2013) on R software (R Core Team, 2017).

Results

A total of 205 arthropods were sampled on *M. ligustroides*, distributed into 43 morphospecies. Observations at the plant level revealed 124 arthropods distributed into 36 morphospecies, being 22 morphospecies found on plants with leaf rolled galls (14 of them found in natural shelters, which housed 67.1% of all arthropods found in this treatment) and 17 on plants with galls removed. Observations at the leaf level showed 81 arthropods distributed into 24 morphospecies, 7 on intact leaves, 5 on unoccupied leaf rolls and 18 on occupied leaf rolls. The artificial shelters exhibited 79% of similarity of species found on natural shelters (leaf rolled galls) (see Table 1).

Table 1. Richness (S) and abundance of arthropods morphospecies sampled at plant level and leaf level on *Miconia ligustroides* (Melastomataceae). Plant level: PRG = Plants with leaf rolled galls, PGR = Plants with galls removed. Leaf level: CL = Control leaves, OLR = Occupied leaf rolls, ULR = Unoccupied leaf rolls. Guilds: D = Detritivorous, H = Herbivorous, On = Omnivorous, P = Predator, Pa = Parasitoid. The morphotypes with an asterisk (*) were found inside the natural shelters (leaf rolled galls).

Order/Morphospecies	Arthropod Abundance					Guild
	Plant level		Leaf level			
	PRG	PGR	CL	ULR	OLR	
Araneae (S = 13) 						
Anyphaenidae sp. 1*	2	-	-	1	-	P
Anyphaenidae sp. 2*	11	-	-	-	-	P
Corinnidae sp.*	2	-	-	1	-	P
Salticidae sp. 1	-	-	1	1	-	P
Salticidae sp. 2	-	1	-	-	-	P
Tetragnathidae sp. 1*	2	-	-	1	-	P
Tetragnathidae sp. 2*	2	-	-	-	3	P
Tetragnathidae sp. 3*	2	-	-	1	1	P
Theridiidae sp. 1*	3	-	-	1	-	P
Theridiidae sp. 2*	1	-	-	2	-	P
Theridiidae sp. 3*	2	-	-	-	-	P
Theridiidae sp. 4	-	2	-	-	-	P
Thomisidae sp.	-	1	-	-	-	P
Blattaria (S = 1) 						
Blattaria sp.*	7	-	-	5	-	D
Coleoptera (S = 5) 						
Chrysomelidae sp.	-	-	3	1	-	H
Curculionidae sp.	1	-	-	2	-	H
<i>Diabrotica</i> sp.	-	1	-	-	-	H
<i>Eumolpus</i> sp.	-	1	-	-	-	H
Torridincolidae sp.*	1	-	-	1	-	H
Hemiptera (S = 4) 						
Aphidae sp.	-	4	9	-	-	H
Cicadellini sp.	1	7	-	-	-	H
Derbidae sp.	4	1	-	-	-	H
Pentatomidae sp.	-	1	-	-	-	H
Hymenoptera (S = 10) 						
<i>Alabagrus</i> sp.	2	-	-	-	-	Pa
<i>Camponotus blandus</i>	6	1	-	4	-	On
<i>Camponotus rufipes</i>	-	-	-	1	2	On
<i>Cephalotes pusillus</i>	-	10	-	24	3	On
Cimbicidae sp.	5	-	-	-	-	H
<i>Creumatogaster</i> sp.*	1	-	1	4	-	On
Myrmaridae sp.	-	-	1	-	-	Pa

<i>Pseudomyrmex pallidus</i>	-	4	-	-	-	On
<i>Pseudomyrmex</i> sp.	-	1	-	-	-	On
Vespidae sp.	-	1	-	-	-	On
Lepidoptera (S = 1) 						
Lepidoptera sp.	-	1	-	1	-	H
Neuroptera (S = 1) 						
<i>Chrysoperla</i> sp.	1	-	-	-	-	H
Orthoptera (S = 2) 						
Orthoptera sp. 1	-	1	-	1	-	H
Orthoptera sp. 2	-	1	-	-	1	H
Psocoptera (S = 1) 						
Asiopsocidae sp.	1	-	-	-	-	On
Thysanoptera (S = 3) 						
<i>Elaphrothrips</i> sp. 1*	27	-	-	2	-	H
<i>Elaphrothrips</i> sp. 2*	1	-	1	-	-	H
<i>Haplothrips gowdeyi</i>	-	-	1	-	-	H

Arthropod diversity at the plant scale

Araneae was the most common order of arthropods found in our studied system, representing a quarter of all specimens sampled (25.0%), followed by Hymenoptera (24.2%), Thysanoptera (22.6%), Hemiptera (15.3%), Blattodea (5.6%), Coleoptera (3.2%), and Blattodea (2.5%). Other orders such as Lepidoptera, Neuroptera, Orthoptera and Psocoptera together represented the other 4.0% of all arthropods sampled.

Plants with leaf rolled galls exhibited on average 2.2 times more arthropods (2.83 ± 0.25 SE) than plants with galls removed (1.30 ± 0.14 SE) ($F_{1,57} = 29.41$, $P = 0.0001$). Similarly, plants with leaf rolled galls had 1.6 times higher species richness (2.03 ± 0.20 SE) than plants with galls removed (1.27 ± 0.13 SE) ($F_{1,57} = 11.13$, $P = 0.002$) and 2.1 times higher arthropod biomass (2.37 ± 0.31 SE) compared to plants with galls removed (1.13 ± 0.25 SE) ($F_{1,57} = 7.72$, $P = 0.003$, see Fig. 3a, b, c).

In plants with leaf rolled galls, predators showed greater abundance ($F_{1,57} = 23.08$, $P = 0.0001$), richness ($F_{1,57} = 23.77$, $P = 0.0001$) and biomass ($F_{1,57} = 22.70$, $P = 0.0001$) than on

plants with galls removed. Similarly, detritivores had greater abundance ($F_{1,57} = 4.48$, $P = 0.039$) richness ($F_{1,57} = 6.28$, $P = 0.015$) and biomass ($F_{1,57} = 4.91$, $P = 0.031$) on plants with leaf rolled galls. Free-living herbivores showed greater abundance ($F_{1,57} = 4.91$, $P = 0.031$) on plants with leaf rolled galls and omnivores showed greater richness ($F_{1,57} = 7.10$, $P = 0.010$) on plants with galls removed. Herbivorous richness and biomass did not differ between treatments, while omnivore abundance and biomass and parasites abundance, richness and biomass did not differ between treatments (all $P > 0.05$). Detritivores and parasitoids were found only on plants with leaf rolled galls (Fig. 3a, b, c and Table 1).

Arthropod diversity at the leaf scale

Hymenoptera were the most common arthropods, representing almost half of all specimens sampled (49.4%), followed by Araneae (16.0%), Hemiptera (11.1%), Coleoptera (8.6%), Blattodea (6.2%), and Thysanoptera (4.9%). Orthoptera and Lepidoptera together represented less than 4.0% of all arthropods sampled.

Unoccupied leaf rolls exhibited on average 3.2 times more arthropods (1.80 ± 0.26 SE) than control leaves (0.57 ± 0.19 SE) and 5.4 times more arthropods than occupied leaf rolls (0.33 ± 0.10 SE) ($F_{2,86} = 16.35$, $P = 0.0001$, Fig. 3). Likewise, unoccupied leaf rolls showed 3.5 times higher richness (1.30 ± 0.17 SE) compared to control leaves (0.37 ± 0.09 SE) and 3.9 times more arthropod species compared to occupied leaf rolls (0.33 ± 0.10 SE) ($F_{2,86} = 18.758$, $P = 0.0001$). Unoccupied leaf rolls had arthropod biomass 2.8 times greater (3.48 ± 0.83 SE) compared to control leaves (1.23 ± 0.61 SE) and 6.3 times higher than occupied leaf rolls (0.56 ± 0.18 SE) ($F_{2,86} = 6.45$, $P = 0.002$). Control leaves and occupied leaf rolls, however, did not differ in any of the variables evaluated (Fig. 3d, e, f).

Detritivores and omnivores showed significantly higher abundance (detritivores: $F_{2,86} = 3.92$, $P = 0.023$; omnivores: $F_{2,86} = 17.01$, $P = 0.0001$), richness (detritivores: $F_{2,86} = 4.46$, $P =$

0.014; omnivores: $F_{2,86} = 18.37$, $P = 0.0001$), and dry biomass (detritivores: $F_{2,86} = 3.41$, $P = 0.037$; omnivores: $F_{2,86} = 6.62$, $P = 0.002$, respectively) in unoccupied leaf rolls than on intact leaves or occupied leaf rolls. Likewise, predators had higher abundance and richness in unoccupied leaf rolls ($F_{2,86} = 3.472$, $P = 0.035$ and $F_{2,86} = 3.472$, $P = 0.035$, respectively), but did not differ in biomass between these treatments ($P = 0.164$). Free-living herbivores, on the other hand, exhibited abundance and richness significantly higher on intact leaves ($F_{2,86} = 3.25$, $P = 0.044$ and $F_{2,86} = 3.41$, $P = 0.038$, respectively). Detritivores were found only in unoccupied leaf rolls and parasitoids were present only on intact leaves, whereas parasitoids did not differ in any of the parameters evaluated between treatments ($P > 0.05$, Fig. 3d, e, f and Table S1, Supporting Information S2).

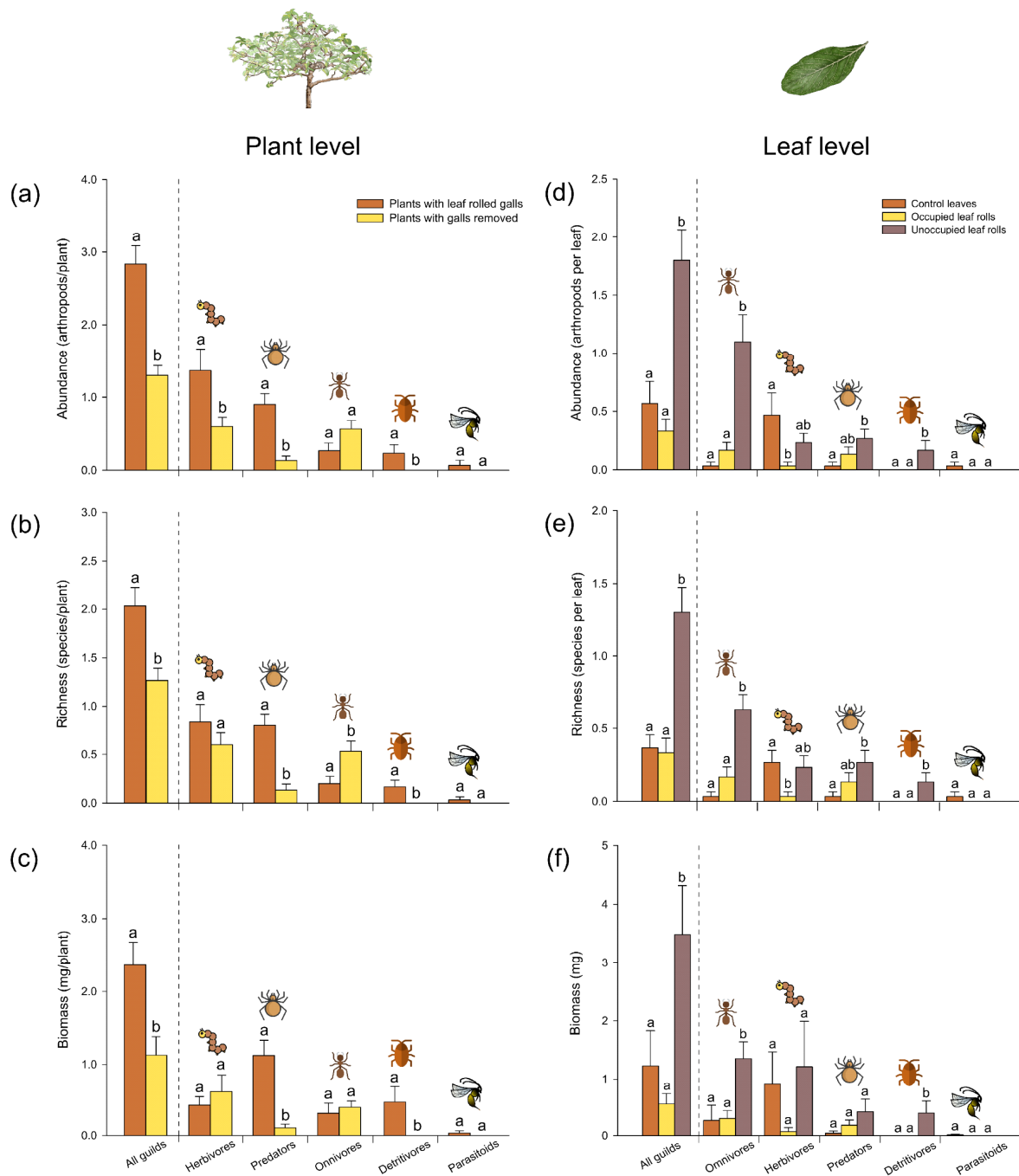


Figure 3. Abundance of arthropods (a, d), richness (b, e) and biomass (c, f) (mean \pm SE) for total arthropods and for different guilds at the plant level and leaf level on *Miconia ligustroides*. Error bars represent \pm SE. Means followed by the same letters do not differ statistically from each other ($P < 0.05$; GLMM/Tuckey's post hoc test, $\alpha = 0.05$).

Arthropod composition among treatments

The composition of arthropods between plants with and without shelters differed significantly (global $R = 0.212$, $P = 0.0001$) (Fig. 4a). In leaf level experiments, unoccupied leaf rolls differed from intact leaves, exhibiting a greater abundance of species for most individuals of *M. ligustroides* sampled (global $R = 0.106$, $P = 0.0001$), but occupied leaf rolls did not differ from intact leaves (Fig. 4b and Table S2, Supporting Information S2). Finally, natural shelters did not differ from artificial shelters on arthropod composition (global $R = 0.0076$, $P = 0.074$) (Fig. 4c).

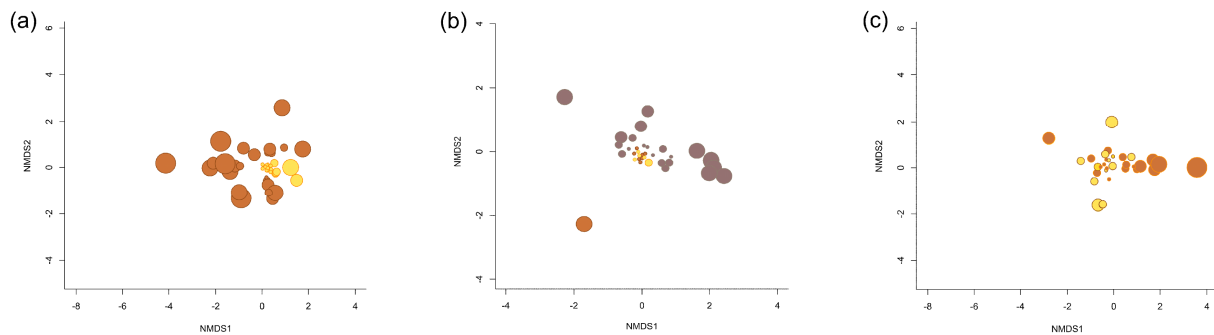


Figure 4. Non-metric multidimensional scale (NMDS) showing variation in species composition of *Miconia ligustroides* between treatments on plant level (a), leaf level (b) and between natural shelters (leaf rolled galls) and artificial shelters (c). The points are scaled to the abundance of arthropods and color-coded by the treatment. (a) Stress value = 0.062, brown circles = plants with leaf rolled galls; yellow circles = plants with galls removed; (b) Stress value = 0.050, brown circle = intact leaves, yellow circles = occupied leaf rolls, dark purple circles = unoccupied leaf rolls; (c) Stress value = 0.108, brown circles = natural shelters (leaf rolled galls); yellow circles = artificial shelters.

Magnitude of the effects of artificial leaf rolls on arthropod community

Artificial leaf rolls simulating galled leaves increased arthropod abundance by almost 95.0% ($E_{++} = 0.935$, CI 0.562 to 1.309, $P < 0.05$), arthropod richness by 99.0% ($E_{++} = 0.992$, CI 0.575 to 1.409, $P < 0.05$), and arthropod biomass in 84.0% ($E_{++} = 0.845$, 0.442 to 1.247, $P < 0.05$) compared to control intact leaves (Fig. 5).

Artificially occupied leaf rolls had a negative effect on arthropod colonization. There was a decrease in arthropod abundance of 59.0% ($E_{++} = -0.589$, CI -1.027 to -0.150, $P < 0.05$), decreased richness ($E_{++} = -0.601$, CI -1.043 to -0.158, $P < 0.05$), and lower biomass ($E_{++} = -0.554$, IC -0.759 to -0.352, $P < 0.05$) when compared to unoccupied leaf rolls (Fig. 5).

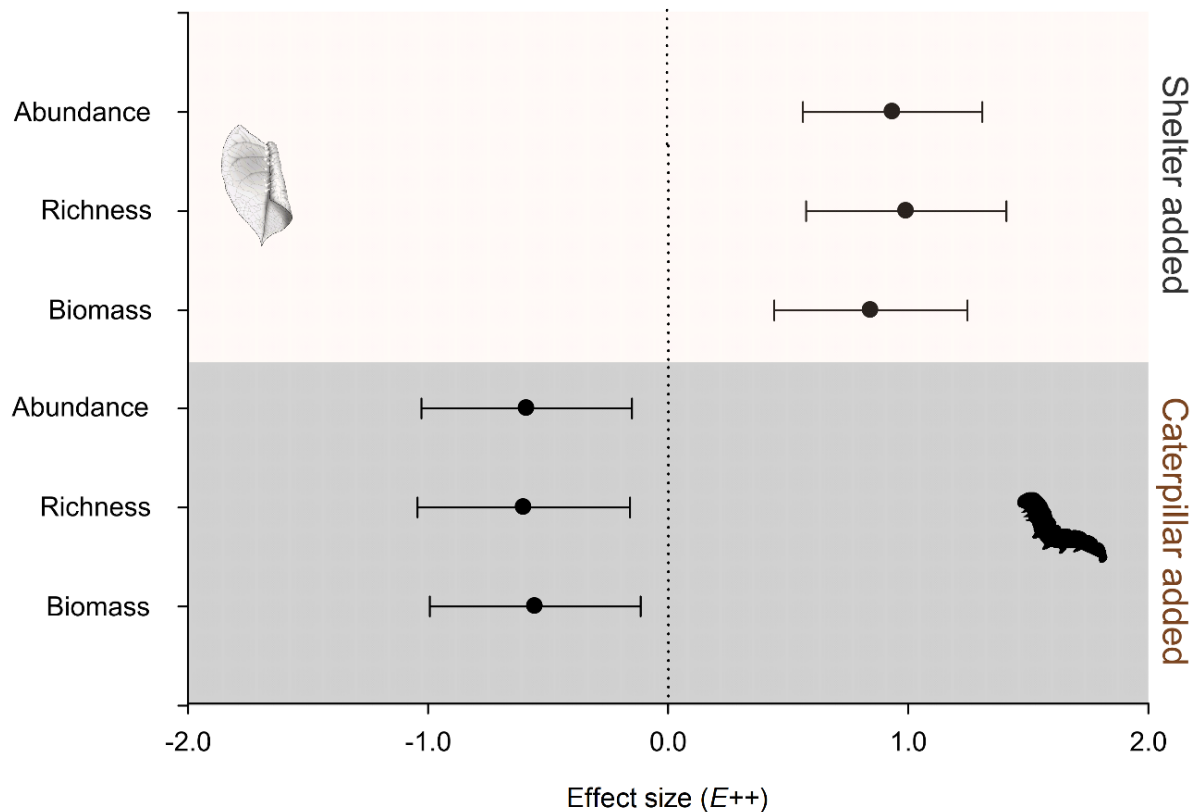


Figure 5. Effects of the addition of shelters (expanded leaves as controls) and the occupation of shelters (unoccupied leaf rolls as controls) on the abundance, richness and biomass of arthropods in *Miconia ligustroides*. The cumulative effect size is reported with its 95% confidence interval and the effects are significant if the confidence intervals do not overlap to zero.

Effects of EE on herbivory levels in M. ligustroides

Herbivory levels on *M. ligustroides* varied between zero (intact leaves) and 65.0% of leaf area lost by chewing herbivores. Plants with galls removed exhibited higher levels of

herbivory on its leaves compared to galled plants with leaf rolled galls ($F_{2,56} = 17.71$, $P = 0.0001$) (Fig. 6 and Table S3, Supporting Information S2).

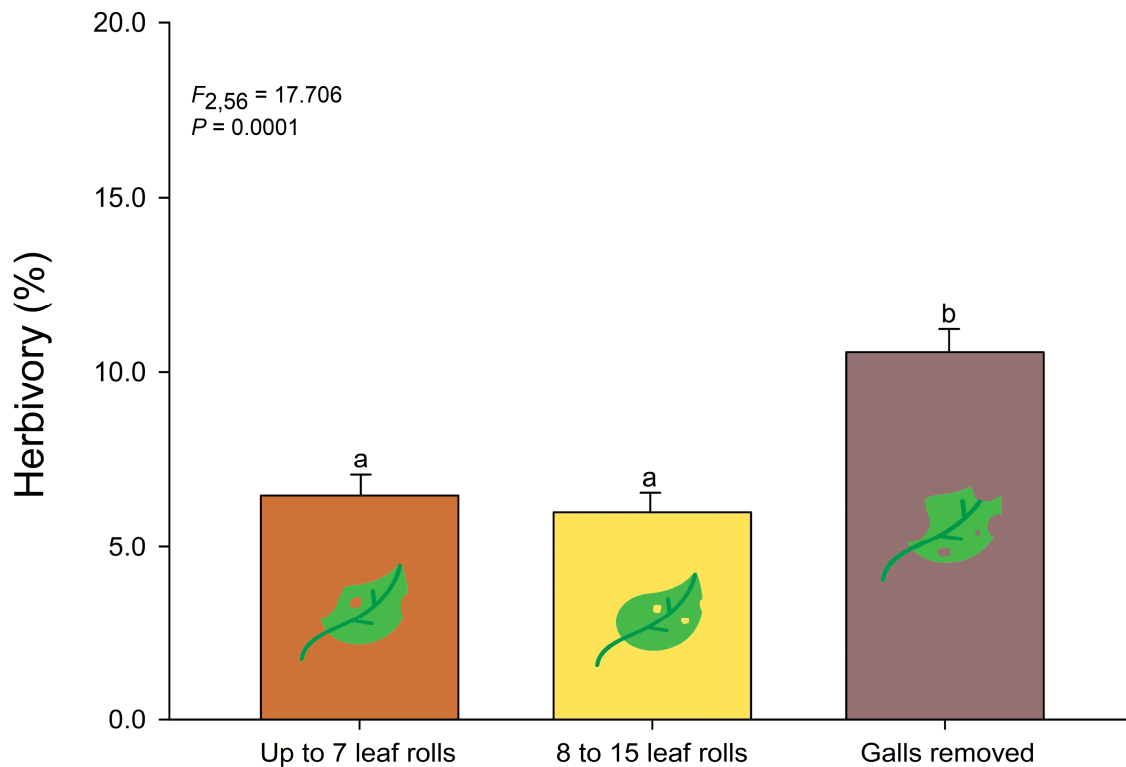


Figure 6. Herbivory (percentage of leaf area removed) in galled plants with up to seven leaf rolled galls, galled plants with eight to fifteen leaf rolled galls (control), and in plants with galls removed of *Miconia ligustroides* (treatment). Error bars represent \pm SE. Means followed by the same letters do not differ statistically from each other ($P < 0.05$; GLMM/Tuckey's post hoc test, $\alpha = 0.05$).

Discussion

We showed that gallers can also act as leaf rollers, facilitating ecosystem engineering when curled and galled leaves act as shelters, with consequences for the components of arthropod community diversity. The leaf shelters created by the *D. gallaeformans* are subtle and ephemeral structures, but with strong effects in community organization, as previously shown to other ecosystem engineered structures created by arthropods (e.g., Lill & Marquis, 2003; Nakamura & Ohgushi, 2003; Crawford *et al.*, 2007; Wang *et al.*, 2012; Vieira & Romero, 2013; Baer & Marquis, 2014; Wetzel *et al.*, 2016; Henriques *et al.*, 2019; Novais *et al.*, 2020).

Our results demonstrated the strong and positive indirect effect of gall-induction and leaf sheltering on diversity and composition of arthropod species at different spatial scales, revealing the positive effect of shelters induced by galls on arthropod diversity and its indirect effects on leaf herbivory. Although most herbivorous species forage by chewing or piercing fully-intact, unaltered leaves (Lill & Marquis 2003; Lill *et al.*, 2007), we suggest the importance of these altered, galled leaves forming shelters to arthropods in this tropical savanna and discuss the relevance of galls as ecosystem engineers.

Plants with leaf rolled galls serving as shelters exhibited greater abundance, richness and biomass of arthropods than plants with galls removed, a pattern that was also observed for detritivores and predators. Arthropods sheltered inside the leaf rolled galls may represent important and readily available groups of prey that attract predators to these engineered structures, which is supported by the large number of spiders found in these plants, which also exhibited species composition different from plants with galls removed, indicating that arthropod communities can be influenced by these structures. These results show that the effects of galls as EE can go beyond merely providing habitat for specialists and influencing the structure of communities that do not directly interact with habitat modifications (Wetzel *et al.*, 2016; Novais *et al.*, 2020). In addition, we suggest that leaf shelters can be an adequate place to ambush and capture prey, in addition to having an adaptive value, as predators resting and nesting inside them can save time and energy by occupying already existing structures (Fukui, 2001; Vieira & Romero, 2013; Slinn *et al.*, 2017). In addition, shelters can provide small predators with protection from larger predators, such as insectivorous birds that forage around the canopies sometimes looking for damaged and modified leaves (Romero & Vasconcellos-Neto, 2005; Rogy *et al.*, 2019).

The presence of leaf shelters decreased the levels of herbivory experienced by these plants. Although the abundance of herbivores did not differ between treatments, the vast

majority of herbivores found on plants with leaf rolled galls were not chewing herbivores, but mainly phloem feeders such as true bugs and thrips. Lower levels of herbivory on plants with leaf rolled galls also reinforce that the use of shelters by predators can negatively influence the damage to the leaves of *M. ligustroides*, inhibiting herbivores that chew upon the leaves of this plant species, similar to what was found for wasp galls on oak trees that age and are then occupied by jumping spiders, which suppressed the density of herbivores (Wetzel *et al.*, 2016). These results differ from previous studies in which caterpillars that built leaf rolls on *Trigonia rotundifolia* leaves also cause extensive damage on the same host plants by feeding on neighbouring leaves and returning to shelters for rest and protection (Henriques *et al.*, 2019).

Our findings on herbivory levels in these plants show that the gall inducer *D. gallaeformans* indirectly influences the loss of leaf tissue in *M. ligustroides*. Studies evaluating the impact of *D. gallaeformans* on fitness of plants within the genus *Miconia* have shown variable and wide effects on plant performance. In *M. albicans*, for instance, galls negatively affected inflorescence length and fruit production in galled compared to healthy shoots. However, galled seedlings did not show significant reduction in germination or germination time (Viana *et al.*, 2013). In addition, *D. gallaeformans* induces an increase in the number of carpels in *M. albicans*, offsetting a potential impact on the fruits of this plant species (Ferreira *et al.*, 2017), whereas galls induced by *D. gallaeformans* did not cause changes in fluctuating asymmetry in relation to the ungalled leaves of *M. fallax* (Alves-Silva 2012). These findings indicate a high homeostasis capacity of these gallers with their host plants, favoring, at least partially, the aptitude of the host plant under different scenarios. Thus, an important future step to be investigated in our system is whether host plants can also allow colonization of leaf rolling galls at a threshold density where the plants outweigh the potential negative effects of galls by attracting other arthropods (*i.e.*, predators) within leaf gall shelters that might contribute to the reduction in herbivory via predation on leaf herbivores.

With our experimental design, we have shown that leaf rolls exhibited different species composition than fully-intact leaves, indicating that arthropod communities are different within shelters. Artificial shelters functioned as ecosystem engineered structures that positively changed abundance, richness and biomass of arthropods when compared to intact leaves. This pattern held true for detritivores, herbivores, omnivores, and predators. Previous studies carried out with leaf shelters have shown that shelters provide their occupants with a more suitable environment than intact leaves, mainly due to their effects against UV radiation, desiccation, rain and wind (Lill & Marquis 2003; Wang *et al.*, 2012; Vieira & Romero, 2013; Henriques *et al.*, 2019; Novais *et al.*, 2020). Temperature in this tropical savannah reaches 35°C in the summer and seasonal importance of the shelters to other arthropods is also expected. In our studied system, the average temperature inside the leaf rolls was about 2.86°C lower (29.50°C \pm 0.82 SE) than temperature in the leaf lamina (32.36°C \pm 0.98 SE) (GLM, $F_{1,14} = 4.98$, $P = 0.042$, see Table S4, Supporting Information S2), indicating that the leaf rolls can act as shelters against high temperatures, wind and precipitation, besides being adequate hiding places for arthropods to rest. Artificial leaf shelters hosted not only inquilines or secondary colonizers, but also other possible leaf-roller builders (Cornelissen *et al.*, 2016). The leaf shelters in our study were also used by one caterpillar (Lepidoptera) and six species of spiders, whose nests were found inside shelters, some with oothecas full of eggs (Cássio Pereira, *personal observation*).

Overall effects of leaf shelter on the diversity of the arthropod community associated to *M. ligustroides* were strong and positive. Our experimental design showed that the artificial shelters increased the abundance, richness and biomass of arthropods by almost 100%, and emphasize the facilitation process of leaf shelters created by galling of *D. gallaeformans*. Our findings demonstrate the important role these galls play in the diversity and structure of arthropod community and reveal they might be as important as other shelters such as those

created by leaf-rollers, leaf-tiers and leaf-tenters (Cornelissen *et al.*, 2016). Positive effects of vertebrate and invertebrate EE have been globally reviewed (but included only two studies with galls; Crawford *et al.*, 2007 and Maruyama *et al.*, 2012) with increases of about 25% in species richness in shelters and stronger effects on the tropics, where increases in species richness in ecosystem engineered plants reached almost 85% (Romero *et al.*, 2014). In the tropical liana *Trigonia*, leaf shelters built by *Pandemis* caterpillars increased the richness of arthropods by almost 50.0% and caused a 63.0 % increase in abundance, indicating that the presence of these subtle and sometimes ephemeral structures in the form of leaf rolls may be enough to increase plant colonization and also influence herbivory levels experienced by plants (Henriques *et al.*, 2019). Changes in arthropod community and differences in the frequency of predators and herbivores might change bottom-up and top-down forces on host plants, influencing herbivory levels and interactions with predators and pollinators, ultimately influencing ecosystem processes. Although the role of shelters and the role of EE have been globally evaluated, galls have been virtually ignored as important EE, although their impact on plant morphology and fitness has been long acknowledged (Price *et al.*, 1986).

Empty and occupied shelters also differed in their effects on arthropod community structure. Overall effects of already occupied shelters were negative, decreasing abundance (-59.0%), richness (-60.0%) and biomass (-87.0%) of arthropods when compared to unoccupied shelters. Secondary colonizers prefer unoccupied shelters, therefore avoiding competition for resources such as food or space. The costs of sharing oak shelters in caterpillars have been demonstrated, resulting in negative behavioral interactions and increased competition (Lill *et al.*, 2007), suggesting that the co-occurrence of caterpillars in leaf shelters might happen only when other shelters are not available. Occurrence in already occupied shelters might be adaptive only when risk of predation is high enough to compensate for the negative impacts of competition, such as in the case of spiders. Many spiders were found building nests inside the

shelters, and the presence of these predators seems to limit the space for late colonizers, forcing them to colonize other parts of the plant or other unoccupied shelters. We suggest that arthropods in this community avoided occupied shelters (experimentally mimicked using model caterpillars) due to the danger of predation by spiders. In experiments with occupied shelters by caterpillars in *Quercus*, Wang *et al.* (2012) observed that the inquiline species responded mainly to the habitat structure instead of the presence of caterpillars themselves. However, in *Quercus* plants, spiders also avoided colonizing leaf shelters, preferring intact leaves for web building and foraging sites, in a way that leaf shelters could serve as enemy free space (*sensu* Jeffries & Lawton, 1984) for some arthropods (Berdegue *et al.*, 1996). Arthropods found in our study were generally quite small and many of them, mainly ants, co-occupied the same shelter. The greater negative effect of occupied shelters in arthropod biomass suggests that the occupied shelters may be limiting the space for larger arthropods, and may also be a barrier for those shelter-building arthropods who use them not only to hide, but also to nest.

The presence of galls and their effects on host plant morphology, architecture and performance have been previously investigated in the literature (Fernandes & Santos, 2014), but no study has shown that galls might facilitate ecosystem engineering when galled leaves are rolled and act as shelters for secondary colonization. After experimental manipulation of leaf galls in the shrub *Baccharis dracunculifolia*, Barbosa *et al.* (2017) have shown increased parasitism in the gall *Baccharopelma dracunculifoliae* and reduced connectance, evenness and robustness of arthropod food webs, indicating the central role gallers have in interaction engineering (Barbosa *et al.*, 2019). Our study reinforces and highlights this important role, as gall presence facilitated ecosystem engineering, changing community composition, richness and biomass. Results were even stronger when shelters created by galls were unoccupied. Arthropod communities associated to galls are not discrete ecological communities and interactions with other members might occur via habitat availability (Barbosa *et al.*, 2017;

2019). Leaf rolling by gallers and changes in leaf morphology and plant architecture are frequent, but facilitation of shelter creation by gallers has been rarely studied, although many galls curl leaves during development (Harris & Pitzschke, 2019). We here suggest that reductions in gall abundance in the studied system might be enough to change plant architecture due to effects of galls on shelter creation, causing secondary and cascading extinctions via indirect non-trophic interactions and this might hold true for other plant-gall systems.

Our initial observation of galls facilitating shelter creation by rolling plant leaves and our experimental design led to insights we believe are relevant for future studies with leaf galls, which tend to be more focused on the harmful role galls have on plants as hosts (Harris & Pitzschke, 2019). Leaf galls are very common (Redfern, 2011; Fernandes & Santos, 2014) and in a recent research agenda for plant galls, researchers have highlighted many open questions such as “are gallers better than other types of plant associates at controlling future access to the plants?” (Harris & Pitzschke, 2019). We suggest that shelters created by gallers can be manipulated experimentally and investing in experimental studies within this guild of herbivores represents a viable and fundamental alternative that can lead to a better understanding of the effects of indirect facilitation and ecological engineering created by gallers. We also suggest with this study that gallers should be treated as ecosystem engineers and that these shelters that are ubiquitous in several biological communities should receive deeper attention in order to better understand patterns of species distribution and interactions.

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Contribution of authors

CCP, TC conceived the ideas; CCP, NRH, VFS, AANS and TC designed the methodology; CCP, NRH, VFS, AANS performed the experiments; CCP and TC analysed the data; and CCP, GWF and TC led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication. Conflict of interest: The authors declare that they have no conflict of interest.

Data Availability Statement

The data that supports the findings of this study are available in the supplementary material of this article.

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Chapter III. From leaves to whole plants: effects of shelter-builders on arthropod communities are stronger in dry seasons*

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Abstract

Leaf shelters act as microclimatic refuges, reducing arthropod exposure to the climatic fluctuations of surrounding habitats. Although facilitation is expected to increase under stressful conditions, empirical studies are still needed to investigate the patterns of variation and magnitude of effects of ecosystem engineering at different spatial and temporal scales. In this study we evaluated the facilitation consequences of leaf shelter created by gall-inducers on arthropod communities of *Miconia ligustroides* (DC.) Naudin (Melastomataceae). We evaluated how such effects change at the leaf and plant level in an environment subject to strong climatic seasonality over two consecutive years. A total of 525 arthropods were sampled on *M. ligustroides*, distributed into 54 morphospecies. The presence of leaf shelters increased arthropod diversity and modified the species composition on *M. ligustroides* at the leaf and plant level, and in wet and dry seasons. However, artificial leaf shelter added during the dry season exhibited higher abundance, richness, and biomass of arthropods compared to shelters during the wet season. Finally, the global effects of artificial leaf shelters on the diversity of arthropods associated to *M. ligustroides* were positive and moderately strong in the dry season, increasing the abundance, richness, and biomass of arthropods by an average of 65% for both years. Our study contributes to a better understanding of the patterns of variation and magnitude of ecosystem engineering at different spatial and temporal scales and provides new insights into the importance of shelters for aridity-sensitive species.

KEYWORDS

Arthropod diversity, Cerrado, ecosystem engineers, galls, indirect facilitation, leaf shelter, microclimatic refuges, seasonality

Resumo

Os abrigos foliares poderiam atuar como refúgios microclimáticos, reduzindo a exposição dos artrópodes às flutuações climáticas dos habitats circundantes. Contudo, embora se espere que a facilitação aumente sob condições estressantes, estudos empíricos precisam ser realizados para investigar os padrões de variação e magnitude desses efeitos em diferentes escalas espaciais e temporais. Aqui, avaliamos as consequências da facilitação gerada por abrigos foliares de indutores de galhas em comunidades de artrópodes em *Miconia ligustroides* (DC.) Naudin (Melastomataceae) ao nível foliar e da planta em um ambiente sujeito a forte sazonalidade climática durante dois anos consecutivos. Um total de 525 artrópodes foram amostrados em *M. ligustroides*, distribuídos em 54 morfoespécies. Os abrigos foliares aumentaram a diversidade de artrópodes e modificaram a composição de espécies em *M. ligustroides* ao nível foliar e das plantas, e nas estações chuvosa e seca. Comparando os abrigos nas diferentes estações, os abrigos foliares artificiais da estação seca exibiram maior abundância, riqueza e biomassa de artrópodes em comparação aos abrigos foliares artificiais da estação chuvosa em ambos os anos avaliados. Finalmente, os efeitos globais dos abrigos foliares artificiais na diversidade da comunidade de artrópodes associados a *M. ligustroides* foram positivos e moderadamente fortes na estação seca, aumentando a abundância, a riqueza e a biomassa dos artrópodes em uma média de 65% em ambos os anos. Nosso estudo contribui para uma melhor compreensão dos padrões de variação e magnitude da engenharia de ecossistemas em diferentes escalas espaciais e temporais e fornece novos insights sobre a importância dos abrigos para espécies sensíveis à aridez.

PALAVRAS-CHAVE

Abrigo foliar, Cerrado, Diversidade de artrópodes, engenheiros ecossistêmicos, facilitação indireta, galhas, refúgios microclimáticos, sazonalidade

1. INTRODUCTION

Shelter-building arthropods influence plant communities by creating new habitats for other species (Romero et al., 2015; Pereira et al., 2022a) and by driving changes in community composition and species diversity on host plants (Romero et al., 2015; Cornelissen et al., 2016). This phenomenon fits within the concept of allogenic ecosystem engineering (EE) as these shelter builders alter the distribution of resources through non-trophic interactions that can have positive ecological effects (e.g., facilitation) on other species (Jones et al., 1994; 2010).

Several organisms build a vast array of different types of structures on plants that may serve as shelters for other species (e.g., Askew, 1961; Sanver & Hawkins, 2000; Pereira et al., 2022a). Caterpillars (Reinhardt & Marquis, 2023), beetles (Novais et al., 2018), flies (Almeida et al., 2014), leafhoppers (Barbosa et al., 2023), nematodes (Pereira et al. 2021); spiders (Yoneya et al., 2014), wasps (Wetzel et al., 2016), and other arthropods construct different structures such as leaf galls, leaf rolls, leaf tents, leaf stem cavities, and stem galls (Pereira et al., 2022a). These structures have adaptive value in terms of protection (i.e., serving as shelters, see Price et al., 1987; Fernandes & Price, 1992; Romero et al., 2015; Cornelissen et al., 2016), but they can also exert a strong influence on terrestrial community diversity in modified and neighboring hosts through colonization by secondary occupants (Pereira et al., 2022a), at different spatial scales (i.e., leaf and plant level, see Pereira et al., 2021) and over time (i.e., over the seasons, see Vieira & Romero, 2013). These changes can, in turn, alter the functional structure of these communities when functional groups such as herbivores and predators become more or less frequent in plants (Pereira et al., 2021), altering ecosystem processes such as herbivory (Henriques et al., 2019) and decomposition (Kozlov et al., 2016).

Most studies on insect-plant interactions have focused on antagonistic and mutualistic interactions (e.g., Stam et al. 2014; Ramos & Schiestl, 2019; Zu et al., 2020). However, efforts have been made to try to reveal patterns and sources of variation in the magnitude of the effects

of facilitative interactions, such as those that occur between arthropods and their host plants, in communities and ecosystems around the world (e.g., Romero et al., 2015; Romero et al., 2022; Barbosa et al., 2023). Experimental studies have revealed that shelters created on plants increase arthropod diversity, providing new microhabitats favorable to secondary colonizers due to the provision of resources that are not available in expanded leaves (Vieira & Romero, 2013; Wetzal et al., 2016; Pereira et al., 2021; Romero et al., 2022). However, little is known about these patterns throughout the seasons, especially in environments associated with strong climatic seasonality.

Changes in precipitation patterns is one of the main drivers of changes in the state of ecosystems, as well as decreased productivity (Fischer et al., 2022). Because the dry season has a pronounced negative effect on arthropod abundance in the tropics (Wolda, 1988; Fischer et al., 2022, Queiroz et al., 2023), plant shelters could act as microclimatic refuges, reducing the exposure of arthropods to unfavorable conditions such as strong winds, low temperatures, and UV radiation from surrounding habitats. Thus, climate may be a common driver of such beneficial interactions (Suggitt et al., 2018; Pinsky et al., 2019; Romero et al., 2022). Thus, the role of shelter-building arthropods as amplifiers of biodiversity may be even stronger in dry seasons and might extend beyond the shelter level (Vieira & Romero, 2013). As a result, the EE can influence arthropod assemblages at different temporal and microspatial scales (Vieira & Romero, 2013). Although facilitation is expected to increase under stressful conditions (Romero et al., 2022), more empirical studies are necessary to investigate the patterns of variation and magnitude of these effects at different spatial and temporal scales, especially in current scenarios of constant climate changes (Montgomery et al., 2020; Mathes et al., 2021).

In this study we evaluated the facilitation consequences generated by leaf shelters of gall-inducers on arthropod communities at different spatial scales and over time. The nematode *Ditylenchus gallaeformans* Oliveira, Santin, Seni, Dietrich, Salazar, Subbotin, Mundo-

Ocampo, Goldenberg & Barreto, 2013 (Anguinidae) is the only galler known to create leaf shelters similar to the leaf rolls of lepidopterans and spiders (Pereira et al., 2021; 2022b; 2023), modifying leaf morphology by joining the leaf blades. We investigated whether these unusual leaf-rolling galls indirectly contribute to changes in arthropod diversity and composition on *Miconia ligustroides* (DC.) Naudin (Melastomataceae) at the leaf and plant level in an environment subject to strong climatic seasonality over two consecutive years.

We tested the following hypotheses: (i) The leaf shelters created by gall-inducers increase arthropod diversity and modify the species composition on *M. ligustroides* at the leaf and plant level, both in wet and dry seasons; and (ii) The effect of leaf shelters on arthropod diversity is stronger in dry seasons. In light of these hypotheses, we made the following predictions: (i) Leaf shelters created by the nematode gall inducers provide new microhabitats favorable to secondary colonizers due to the provision of resources that are not available on expanded leaves. Consequently, these effects extend to the entire arthropod community on the whole plant, regardless of seasonality; and (ii) Leaf shelters represent important microclimatic refuges in the dry season, when arthropods are more prone to desiccation.

To test the above-mentioned hypotheses, we used two separate experiments to compare: (i) The role of shelter itself on the abundance, richness and biomass of arthropods by comparing expanded leaves (control) and artificial leaf rolls (treatment); and (ii) The abundance, richness, and biomass of arthropods on plants with leaf roll galls created by *D. gallaeformans* (control) and plants with leaf roll galls removed (treatment). The experiments were carried out in the wet and dry seasons of 2020 and 2021, respectively.

2. METHODS

2.1. Study site

The study was conducted in the Área de Proteção Ambiental (APA) São José (21° 03'S and 44° 06'W), Minas Gerais, Brazil, which has 4,758 há (for more details, see Figure 1 of Chapter II). This environmental reserve comprises a mountain of about 12 km in length within the municipalities of Coronel Xavier Chaves, Prados, Santa Cruz de Minas, São João del-Rei, and Tiradentes. The mountain has a very rugged relief, with outcrops of quartzitic sandstone in the landscape, with altitudes ranging from 800 to 1,400 m (Henriques et al., 2019). The climate is subtropical in altitude (Cwb) according to the Köppen classification (Alvares et al., 2013), with dry winters and mild summers, with an average annual rainfall of 1,435 mm and an average annual temperature of around 19 °C (Pereira et al., 2020). The vegetation is diverse, presenting several Cerrado domain phytophysionomies that occur in mosaics, ranging from forests to grasslands in a radius of less than 1 km along the entire length of the mountain. Furthermore, at the foot of the south face of the mountain range, there is a large extension of Floresta Estacional Semidecidual, a phytophysionomy of the Mata Atlântica domain (Pereira et al., 2020). Data were collected in an area of 10 ha in the northern portion (21° 02' 52.5"S, 44° 07' 01.8"W), in the municipality of Prados, where the Cerrado Rupestre is dominant, presenting a shallow soil and stony (litholic neosol), with tree cover varying between 5 and 20% (Pereira & Fernandes, 2022). During the dry season (May to September) there is a sharp decrease in temperature and a drastic reduction in rainfall in this savanna, which presents marked leaf deciduousness in the plant community (Pereira & Fernandes, 2022).

2.2. Study system

M. ligustroides is a shrub-tree, perennial, and apomictic plant, with membranous leaves without trichomes, which occurs from forest to grassland environments, varying their size according to soil properties (Martins et al., 1996; Maia et al., 2016). At the Cerrado Rupestre in the study area, this plant is shrubby (Figure 1a), and has leaf galls induced by *D.*

gallaeformans (Figure 1b), which curl its leaves forming a leaf roll gall of approximately 20 mm in diameter (see Pereira et al., 2021; 2022b; 2023) (Figure 1c). The leaves are rolled from the abaxial to the adaxial face, from the edges to the midrib and are frequently colonized by spiders and a myriad of other insect groups. Leaves remain on the plants for about eight months when they fall to the ground (Pereira et al., 2021; 2023). Unlike the leaf rolls produced by other arthropods, these structures are not occupied by the engineer, which is microscopic (~600 μm) and occupies the leaf blade. Thus, secondary colonizers always initially find unoccupied shelters, regardless of the stage of gall development (Pereira et al. 2021).

2.3. Data sampling

Leaf scale

To evaluate the effects of leaf shelters on arthropod colonization, 30 plants, spaced approximately 10.0 m from each other were marked in the field in February 2020. All plants were between 1.0 and 1.5 m tall and had no flowers and fruits at the time of the study. In each plant, two pairs of leaves were selected as treatments ($n = 4$, artificial leaf rolls), and two pairs of leaves as the control ($n = 4$, expanded leaves). We used different branches for each pair and marked the branches with colored tags (see Supporting Information S1, Figure S1).

We use different branches for each pair of marked leaves. The treatment pairs consisted of a rolled leaf simulating a shelter created by *D. gallaeformans* (Figure 1d). We use young, intact, and fully developed leaves to create artificial shelters. We opted to use intact leaves to create the shelters because the galled leaves are extremely fragile and break easily. Furthermore, a previous study showed that the use of these artificial shelters resembles natural shelters, as they exhibited 79% of species similarity with natural shelters (Pereira et al., 2021).

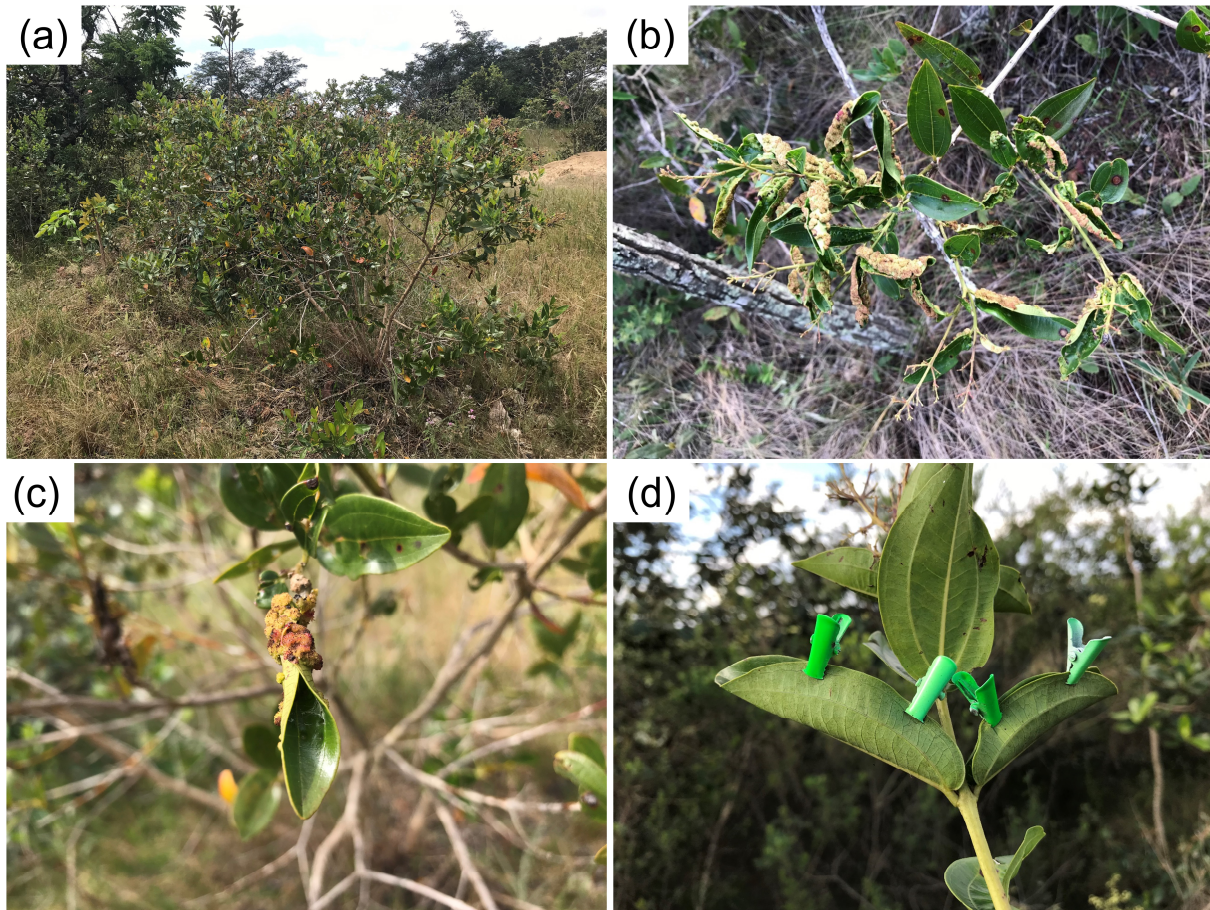


Figure 1. Photos of the study system. (a) Individual of *Miconia ligustroides*. (b) Branch with a high infestation of *Ditylenchus gallaeformans* galls in the leaf blade, showing several leaf roll galls. (c) Leaf roll gall induced by *D. gallaeformans*. As these galls develop, the leaf curls from the abaxial to the adaxial surface, from the edges to the midvein, forming a roll of ~20 mm in diameter. (d) Photo of the experimental design. The artificial rolls were kept rolled with clips, in the same format as the natural rolls. Photo credit: Cássio Cardoso Pereira.

Leaves were manually rolled from the abaxial to the adaxial face, from the edges to the midrib, and secured with hair pins (Figure 1d). These artificial shelters simulate cylindrical rolls, 20 mm in diameter, similar to leaf roll galls naturally created on these plants by the nematode. Hair clips were painted the same color as the leaves with odorless green spray paint. In previous experiments (Pereira et al., 2021), we showed that 10 days were sufficient for the colonization of these shelters. Thus, we carried out two samplings, the first 15 days after the set

of the experiment and the second sampling was done 15 days after the first. On each sampling occasion, artificial rolls and expanded leaves were collected, stored in plastic bags with zipper closure, and frozen for later sorting. The treatments were reapplied to the same trees and branches and between sampling occasions using different pairs of leaves.

To evaluate the effects of shelters over seasons, this experiment was repeated on the same individuals in August 2020 (dry season), as well as in the wet (February) and dry (August) seasons of 2021.

Plant scale

To assess whether leaf roll galls increase arthropod diversity in *M. ligustroides*, 60 plants at least 10.0 m apart were tagged in January 2020 (wet season) and two groups were selected: i) plants with leaf roll galls ($n = 30$, control) and ii) plants with galls removed ($n = 30$, treatment). All plants were between 1.0 and 1.5 m tall and had no flowers and fruits at the time of the study. Individuals of *M. ligustroides* had an average of ten leaves curled from galls of similar age, and for the plants in the treatment group, we removed all leaves that had galls (as there were no plants naturally without galls). This represents approximately only 1% of biomass removal, as plants on the treatment group had an average of 1,021 (± 106.99 SE) leaves (see Pereira et al. 2021). One month after marking the groups of plants (February 2020) and removing the galls from the treatment group, the entire plants were visually inspected for 25 minutes and the arthropods were collected using entomological forceps and aspirators. Collections were carried out between 10 am and 2 pm, the period when we observed the highest arthropod activity in the studied community. We used zippered plastic bags to collect the shelters and then froze them for arthropod screening. To assess the effects of shelters over seasons, this experiment was repeated on the same individuals in July 2020 (dry season), as well as in the wet and dry seasons of the following year (January and July 2021, respectively).

Arthropod screening and identification

We inspected the collected leaves and shelters using a stereomicroscope. All arthropods found were stored in 70% alcohol and identified to the lowest possible taxonomic level or classified into morphospecies (Oliver & Beattie, 1996) to allow the assessment of abundance, richness and dry biomass (mg). Furthermore, we classified arthropods into feeding guilds as detritivores, herbivores, omnivores, parasites, parasitoids, and predators. All arthropods were dried in an oven at 60°C for 24 hours and weighed on a precision digital scale to estimate biomass.

2.4. Data analysis

We used generalized linear mixed models (GLMMs) to evaluate the effects of shelters on arthropod diversity at the leaf level. For this, we used the average values of abundance, richness, and biomass as response variables, each treatment as a fixed factor and individual plants as a random factor.

At the plant level, the effects of shelters on arthropod diversity were also assessed by GLMMs. We used the average values of abundance, richness, and biomass as dependent variables, each treatment as a fixed factor and individual plants as random factors. The distribution of errors in the aforementioned analyzes was verified using restricted maximum likelihood (REML).







We used analysis of similarities (ANOSIMs) based on Euclidean distances to evaluate arthropod composition at the leaf and plant level between different treatments. One-sided significance was calculated by permuting the groups with 9,999 permutations. ANOSIMs paired between all pairs of groups were used as a post-hoc test. To view similarities or

differences between treatments, we used non-metric multidimensional scaling (NMDS) analyses, using species abundance for each individual plant sampled. All analyzes mentioned above were performed using the Vegan package (Oksanen et al., 2013) in the R software (R Core Team, 2022).

We used Hedges'd metric to estimate the magnitude of the effects of adding leaf shelters on arthropod abundance, richness and biomass in the dry and rainy seasons of 2020 and 2021 (Hedges & Vevea, 1998). For replicates among treatments, the mean and standard deviation from the 4 leaves per plant, per treatment, were used. Overall effect sizes were calculated on the response variables of arthropod abundance, richness and biomass. Groups were assigned to expanded leaves (control) and leaf rolls (treatment). To estimate the cumulative effect (E_{++}) of treatments, the individual d_i effects were combined using weighted averages and a random model analysis. A positive effect size indicates that abundance, richness, and biomass of arthropods were lower on expanded leaves compared to leaf rolls, whereas a negative effect size implies a lower abundance, richness and biomass of arthropods for the leaf rolls compared to expanded leaves. As a convention, E_{++} values around 0.2 are considered weak effects, values around 0.5 are considered of moderate magnitude, values around 0.8 are considered strong, and E_{++} larger than 1.0 are considered very strong (Rosemberg et al., 2000). The cumulative effects were considered significant if the confidence intervals (95%) did not overlap with zero. All analyses were conducted in Metawin 3.0 (Rosemberg, 2023).

3. RESULTS

A total of 525 arthropods were sampled on *M. ligustroides*, distributed into 54 morphospecies. Araneae was the most common order of arthropods found, representing more than a quarter of all specimens sampled (27.78%), followed by Hymenoptera (24.07%), Hemiptera (16.67%), Coleoptera (12.96%), Orthoptera and Thysanoptera (both 5.56%),

Hymenoptera (S = 13)																		
<i>Alabagrus</i> sp.		-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1	Pa
<i>Camponotus blandus</i>		-	-	6	4	-	-	4	2	-	-	6	10	-	3	-	-	On
<i>Camponotus rufipes</i>		-	-	2	-	-	-	-	-	-	1	-	-	-	-	-	-	On
<i>Camponotus sericeiventris</i>		-	-	-	3	-	-	-	-	-	-	-	1	-	-	-	-	On
<i>Cephalotes pusillus</i>		3	2	26	9	1	7	10	7	1	1	25	7	-	5	18	12	On
Cimbicidae sp.		-	-	-	2	-	-	-	-	-	1	-	-	-	-	-	-	H
<i>Creumatogaster</i> sp. 1		-	-	1	-	1	-	-	-	-	-	2	-	-	-	-	-	On
<i>Creumatogaster</i> sp. 2		1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	On
Myrmaridae sp.		-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	Pa
<i>Pseudomyrmex oculatus</i>		-	-	1	-	-	-	-	-	-	-	4	3	-	-	-	-	On
<i>Pseudomyrmex pallidus</i>		-	-	-	-	-	-	2	-	-	-	-	-	-	-	5	5	On
<i>Pseudomyrmex</i> sp.		-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	On
Vespididae sp.		1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	On
Lepidoptera (S = 1)																		
Arctiidae sp.		-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	H
Neuroptera (S = 1)																		
<i>Chrysoperla</i> sp.		-	-	-	2	-	-	1	2	1	-	-	-	1	-	-	-	H
Orthoptera (S = 3)																		
<i>Chromacris</i> sp.		-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	H
<i>Ronderosia</i> sp.		-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	H
<i>Schistocerca flavofasciata</i>		-	-	-	-	-	-	1	-	-	-	-	-	-	-	3	-	H
Psocoptera (S = 1)																		
Asiopsocidae sp.		-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	On
Thysanoptera (S = 3)																		
<i>Elaphrothrips</i> sp. 1*		-	-	-	-	-	-	3	-	-	-	-	-	-	3	6	-	H
<i>Elaphrothrips</i> sp. 2*		-	5	1	-	-	-	-	-	-	3	2	-	-	-	-	-	H
<i>Frankliniella</i> sp.		-	-	12	9	-	6	12	6	1	1	9	5	-	-	-	-	H

Arthropod diversity at the leaf scale

At the leaf level, 122 arthropods distributed into 38 morphospecies were found. Araneae was the most common order of arthropods found, representing 34.21% of all specimens sampled, followed by Hymenoptera (21.07%), Hemiptera (18.42%), Coleoptera and Thysanoptera (both 7.89%). Blattodea, Lepidoptera, Neuroptera, and Orthoptera together represented the other 10.52% of all arthropods sampled. See more details about arthropod abundance and richness at the leaf level in Table 1.

Artificial leaf rolls exhibited higher arthropod abundance, richness, and biomass compared to control leaves in both seasons and years (GLMMs, all $P < 0.05$, Figure 2a-f, and Table S1, Supplementary information S1). Comparing the shelters at different seasons, artificial leaf rolls during the dry season exhibited higher abundance, richness, and biomass of arthropods

compared to artificial leaf rolls during the wet season in both years evaluated (GLMMs, all $P < 0.05$, Figure 2a-f, and Table S1, Supplementary information S1).

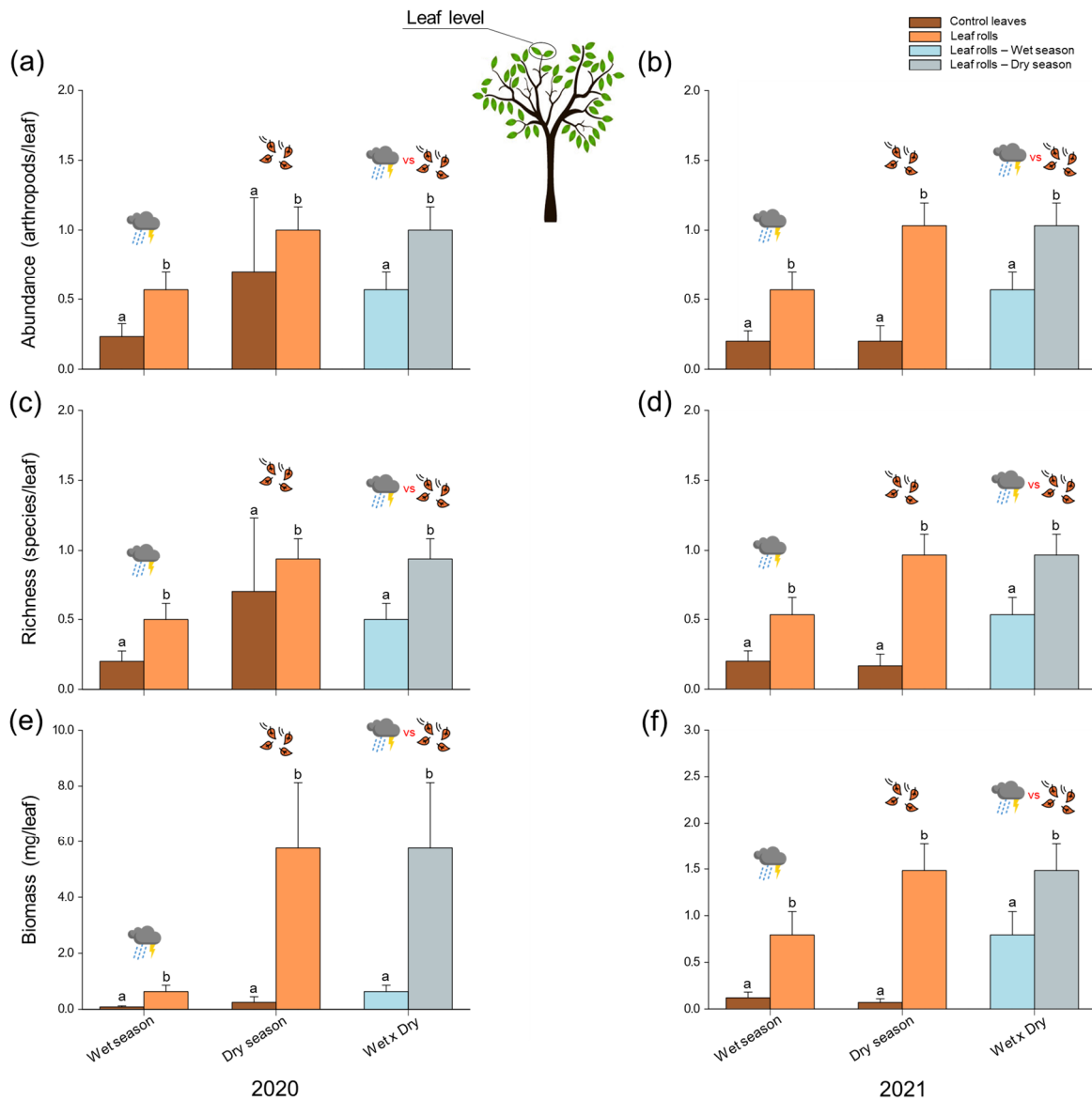


Figure 2. Abundance of arthropods (a, b), richness (c, d), and biomass (e, f) (mean \pm SE) for total arthropods at the leaf level on *Miconia ligustroides* in the wet and dry seasons of 2021 and 2022, respectively. Error bars represent \pm SE. Means followed by the same letters do not differ statistically from each other ($P < 0.05$; GLMM, $\alpha = 0.05$).

Arthropod diversity at the plant scale

Observations at the plant level revealed 403 arthropods distributed into 45 morphospecies. Araneae was the most common order of arthropods found (31.11%), followed by Hymenoptera (24.44%), Coleoptera (15.56%), Hemiptera (8.89%), Orthoptera and Thysanoptera (both 6.67%). Blattodea, Neuroptera, and Psocoptera together represented the other 6.66% of all arthropods sampled. See more details about arthropod abundance and richness at the plant level in Table 1.

Plants with leaf roll galls exhibited higher arthropod abundance, richness, and biomass compared to plants with galls removed in the both seasons throughout the years 2020 and 2021 (GLMMs, all $P < 0.05$, Figure 3a-f, and Table S1, Supplementary information S1). Comparing plants with shelters in different seasons, in the 2020 there were no significant differences between the arthropod abundance and richness between plants with leaf rolls galls ($P > 0.05$). However, arthropod biomass in plants with leaf rolls galls was statistically higher in the wet season (2.52 ± 0.48 SE) than in the dry season (1.22 ± 0.19 SE) ($F_{1,57} = 6.947$, $P = 0.011$). In contrast, in the 2021, plants with leaf rolls galls in the wet season had a higher arthropod abundance ($F_{1,57} = 12.842$, $P = 0.001$) and richness ($F_{1,57} = 6.820$, $P = 0.012$) than the dry season. However, arthropod abundance did not differ between the two seasons ($P > 0.05$) (Figure 3a-f, and Table S1, Supplementary information S1).

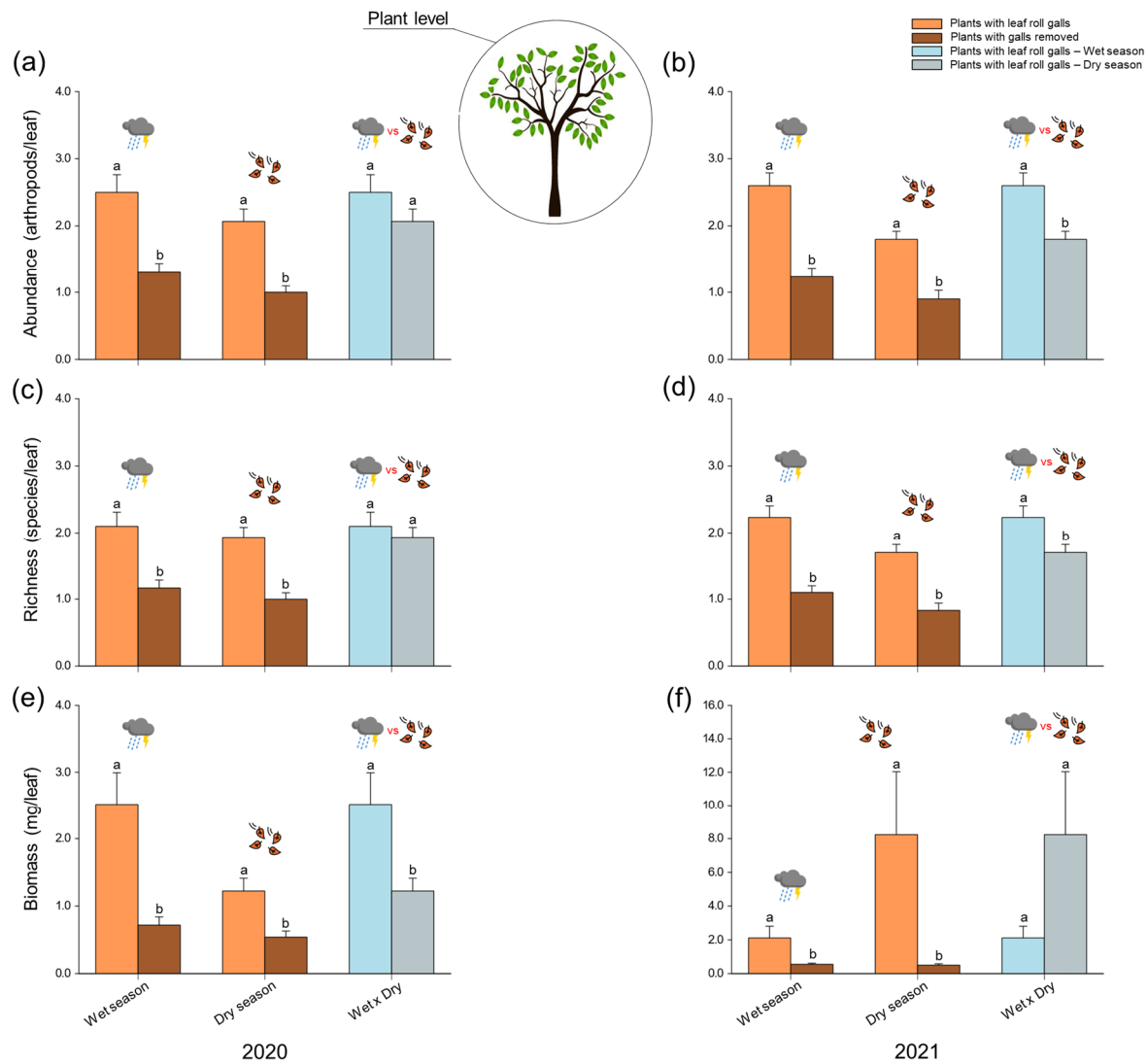


Figure 3. Abundance of arthropods (a, b), richness (c, d), and biomass (e, f) (mean \pm SE) for total arthropods at the plant level on *Miconia ligustroides* in the wet and dry seasons of 2021 and 2022, respectively. Error bars represent \pm SE. Means followed by the same letters do not differ statistically from each other ($P < 0.05$; GLMM, $\alpha = 0.05$).

Arthropod composition among treatments

The composition of arthropods on artificial leaf rolls differed significantly from expanded leaves (control leaves), in both seasons throughout the years 2020 and 2021 (ANOSIM, all $P < 0.05$, Figure 4a-d, and Table S2, Supplementary information S1). Arthropod composition on artificial leaf rolls also differs between wet and dry seasons in both years (ANOSIM, all $P < 0.05$, Figure 4e-f, and Table S2, Supplementary information S1).

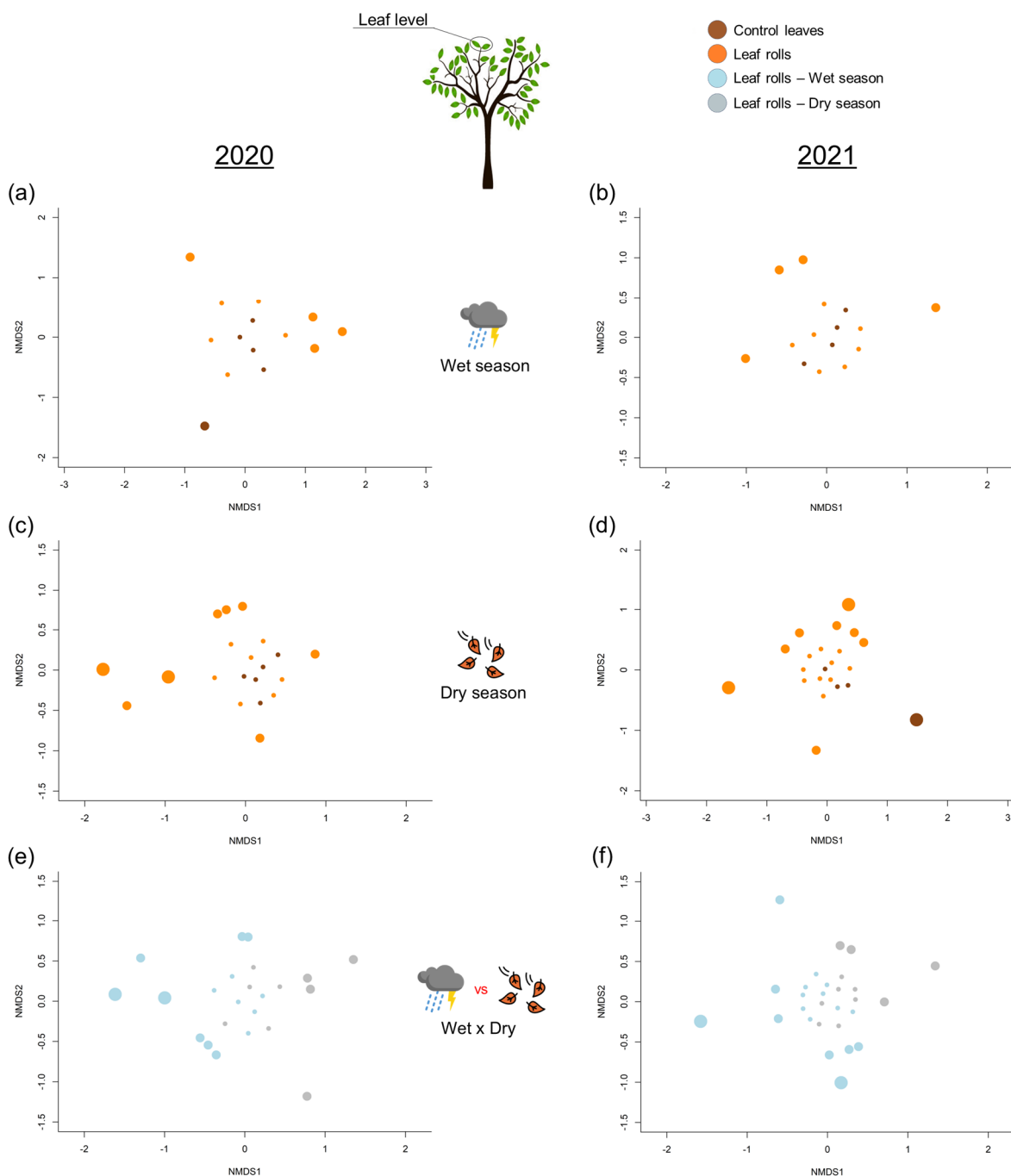


Figure 4. Non-metric multidimensional scale (NMDS) showing variation in arthropod species composition of *Miconia ligustroides* between treatments on leaf level in wet season (a, b), dry season (c, d), and for leaf shelters between the two seasons (e, f) in 2020 and 2021, respectively. The points are scaled to the abundance of arthropods and color-coded by the treatment. (a) Stress value = 0.046; (b) Stress value = 0.060; (c) Stress value = 0.071; (d) Stress value = 0.075. Brown circles = control leaves; orange circles = leaf rolls; (e) Stress value = 0.084; (f) Stress value = 0.106. Light blue circles = leaf rolls in wet season; gray circles = leaf rolls in dry season.

In plant-level experiments, the composition of arthropods between plants with leaf rolls galls and plants with galls removed also differed significantly in both seasons throughout the years 2020 and 2021 (ANOSIM, all $P < 0.05$, Figure 5a-d, and Table S2, Supplementary information S1). Finally, arthropod composition on plants with leaf roll galls also differed between wet and dry seasons in both years (ANOSIM, all $P < 0.05$, Figure 5e-f, and Table S2, Supplementary information S1).

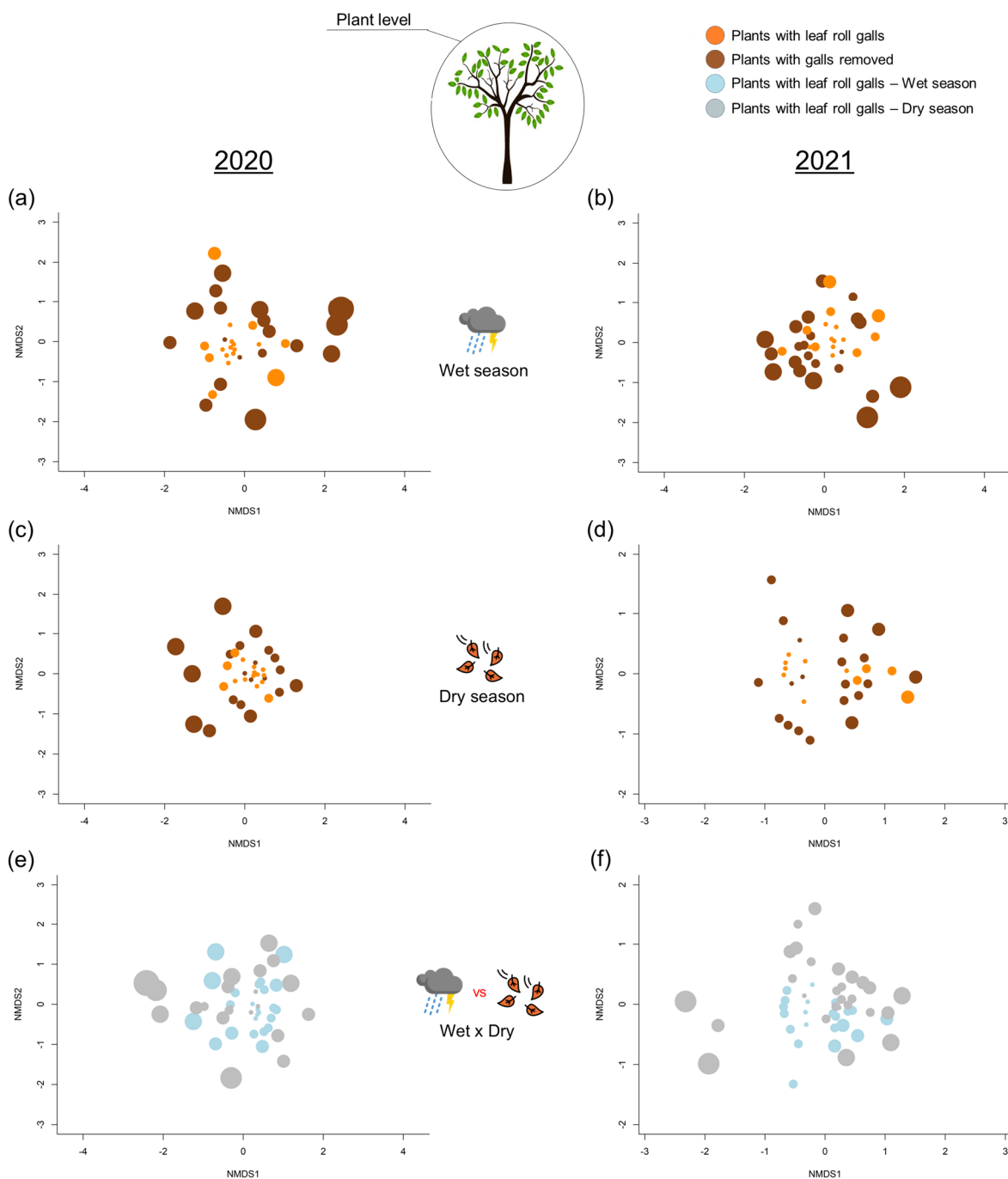


Figure 5. Non-metric multidimensional scale (NMDS) showing variation in arthropod species composition of *Miconia ligustroides* between treatments on plant level in wet season (a, b), dry season (c, d), and for plants with shelters between the two seasons (e, f) in 2020 and 2021, respectively. The points are scaled to the abundance of arthropods and color-coded by the treatment. (a) Stress value = 0.113; (b) Stress value = 0.136; (c) Stress value = 0.195; (d) Stress value = 0.109. Brown circles = plants with galls removed; orange circles = plants with leaf roll galls; (e) Stress value = 0.146; (f) Stress value = 0.136. Light blue circles = plants with leaf roll galls, wet season; gray circles = plants with leaf roll galls, dry season.

Magnitude of the effects of artificial leaf rolls on arthropod community

In the 2020 wet season, artificial leaf rolls simulating leaf roll galls did not increase abundance ($E_{++} = 0.2542$, CI -0.0905 to 0.5989, $P > 0.05$), richness ($E_{++} = 0.2542$, CI -0.0905 to 0.5989, $P > 0.05$), and biomass ($E_{++} = 0.3881$, CI 0.0215 to 0.7546, $P > 0.05$) of arthropods compared to expanded control leaves. In contrast, in the 2020 dry season, artificial leaf rolls increased arthropod abundance and richness by 66.0% (both $E_{++} = 0.6648$, CI 0.3429 to 0.9868, $P < 0.05$), and arthropod biomass by 70.0% ($E_{++} = 0.700$, CI 0.3771 to 1.0213, $P < 0.05$) compared to expanded control leaves (Figure 6).

In a similar way, in the 2021 wet season, artificial leaf rolls did not increase abundance ($E_{++} = 0.3558$, CI -0.0094 to 0.7211, $P > 0.05$), richness ($E_{++} = 0.3558$, CI -0.0094 to 0.7211, $P > 0.05$), and biomass ($E_{++} = 0.3881$, CI 0.0215 to 0.7546, $P > 0.05$) of arthropods compared to control leaves. However, in the 2021 dry season, the patterns were different from the wet season. Artificial leaf rolls increased arthropod abundance by 62.0% ($E_{++} = 0.6233$, CI 0.3093 to 0.9372, $P < 0.05$), arthropod richness by 63.0% ($E_{++} = 0.6305$, CI 0.3167 to 0.9443, $P < 0.05$), and arthropod biomass by 64.0% ($E_{++} = 0.640$, CI 0.3239 to 0.9483, $P < 0.05$) compared to expanded control leaves (Figure 6).

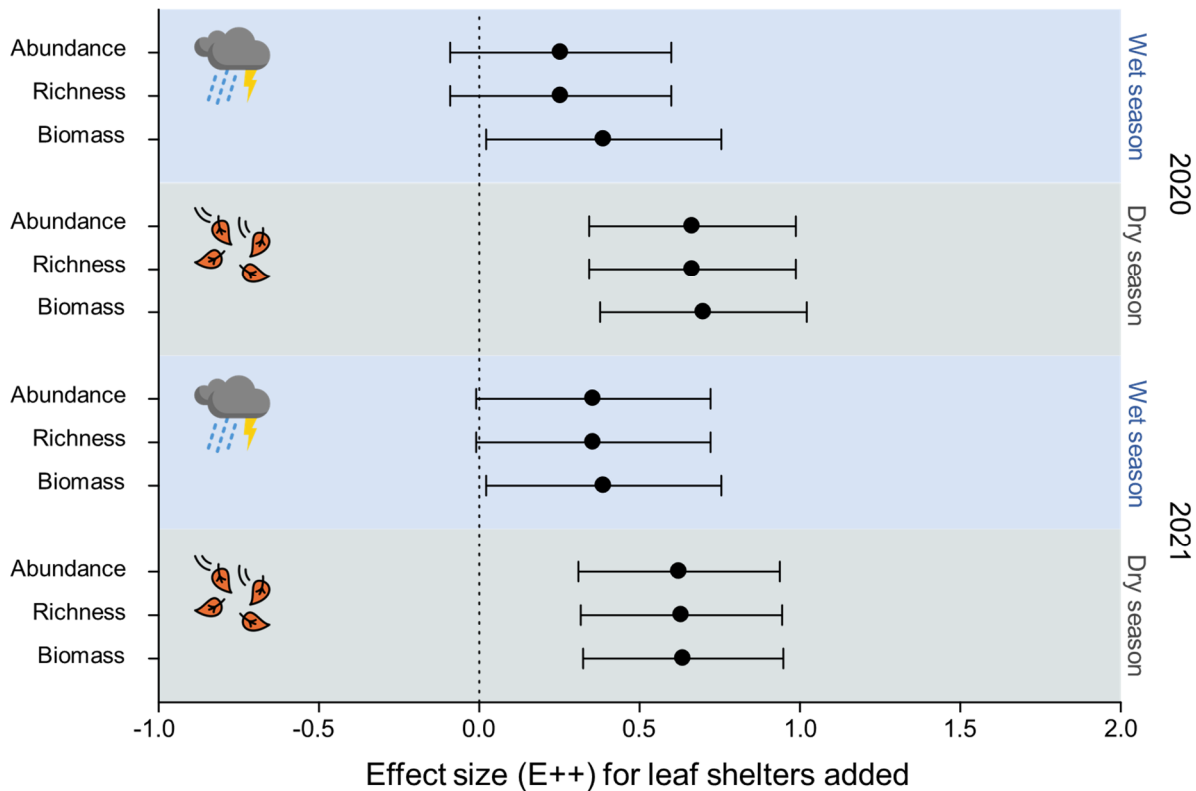


Figure 6. Effects of the addition of shelters (expanded leaves as controls) on the abundance, richness and biomass of arthropods in *Miconia ligustroides* in the wet and dry seasons of 2021 and 2022, respectively. The cumulative effect size is reported with its 95% confidence interval and the effects are significant if the confidence intervals do not overlap to zero.

4. DISCUSSION

We show that leaf shelters created by *D. gallaeformans* increase the leaf-level arthropod abundance, richness, and biomass, and modify the species composition on *M. ligustroides* shrubs. These effects extend to the entire arthropod community on the whole plant, regardless of seasonality. However, although the wet season exhibits greater arthropod biomass at the plant level in the present study, and this season is often associated with greater arthropod diversity (e.g., Wolda, 1988; Fischer et al., 2022), the dry season showed higher abundance, richness, and biomass of arthropods at the leaf level.

Artificial leaf rolls exhibited more than twice the arthropod abundance, richness, and biomass in relation to expanded leaves in both seasons and years evaluated. These strong and positive indirect effects of leaf sheltering on diversity maybe due to leaf rolls providing new

microhabitats favorable to secondary colonizers due to the provision of resources that are not available in expanded leaves, which probably also influenced differences in species composition found (Lill & Marquis, 2003; Wang et al., 2012; Vieira & Romero, 2013; Henriques et al., 2019; Pereira et al., 2021; Romero et al., 2022). In a previous study, *M. ligustroides* shelters showed milder temperatures compared to the expanded leaves (Pereira et al., 2021). Furthermore, these shelters can protect numerous guilds of arthropods against rain, wind and UV radiation, especially in the dry season, when their bodies are sensitive to desiccation (Vieira & Romero, 2013). In this way, the shelters could provide them with local humidity through the transpiration of the leaves and provide stable microclimatic refuges against long periods of drought (Romero et al., 2022).

These effects extended to the entire arthropod community throughout the whole plants. Leaf roll galls positively influenced population dynamics in the studied community, influencing arthropod assemblages at different microspatial and temporal scales. The natural shelters presented an important resource for the secondary colonizers, increasing the migration of arthropods on these plants, which took refuge, and also found suitable space to seek food, reproduce and develop (Fukui et al., 2001; Pereira et al., 2021). This pattern was clear due to the differences in arthropod diversity and composition with plants without galls removed, therefore showing that the use of shelter did not represent a redistribution of arthropods already present on the plants. Although most of these arthropods forage through the plant's branches, the increase in species diversity can be evidenced by the large number of species found exclusively within the natural shelters created by *D. gallaeformans* ($n = 11$), especially spiders ($n = 8$, see Table 1). As a result, populations of these arthropods tend to increase on these plants, resulting in the patterns found. Furthermore, given the large number of predators found, we also suggest that leaf roll galls may be a suitable place to ambush and capture prey, as predators that rest and nest within them can save time and energy by occupying existing structures (Fukui,

2001; Vieira & Romero, 2013; Pereira et al., 2021). Finally, we suggest here that reductions in gall abundance in the studied system may be sufficient to alter community arthropod assemblages, causing secondary and cascading extinctions through indirect non-trophic interactions (Vieira & Romero, 2013; Pereira et al., 2021).

The most noticeable characteristic found in our study is that these structures sheltered a greater arthropod diversity in the dry season, and different arthropod composition compared to the wet season, in both years. Even though the dry season is generally marked by reduction in the abundance and richness of arthropods (e.g., Wolda, 1988; Fischer et al., 2022, Queiroz et al., 2023), these structures sheltered a great diversity of species, including some that were not found in the shelters during the wet season. These differences in the composition of shelters throughout the seasons can be explained by the ephemeral nature of these species, which end up being replaced within a period of six months (Cássio Pereira, *personal observation*). Furthermore, with increasing aridity, we believe that many species that survive outside these shelters in the wet season have turned to these subtle structures for sheltering, thus reducing their exposure to climate fluctuations in the surrounding habitats, demonstrating the power of adaptation to climate change, especially predators such as spiders (Suggitt et al., 2018; Pincebourde & Casas, 2019; Romero et al., 2022).

The global effects of artificial leaf shelters on the arthropod community diversity associated with *M. ligustroides* were positive and moderately strong in the dry season. Our experimental design showed that artificial shelters increased the abundance, richness and biomass of arthropods by an average of 65% for the 2020 and 2021 dry seasons, in a similar way to other studies carried out with leafrollers in tropical systems, who also evaluated these three diversity parameters (e.g., Pereira et al., 2021; Romero et al., 2022). Our findings reinforce the importance of shelters as microclimatic refuges for many species, especially predators (Fukui et al., 2001; Cornelissen et al., 2016; Morse, 2021; Pereira et al., 2021). The large

presence of predators (e.g., spiders) in artificial leaf shelters also helps to explain the overall effects for body biomass, the parameter that showed the greatest magnitude of shelter effects in the dry season (70% and 64% increase in the 2020 and 2021 dry seasons, respectively). Larger organisms typically require more favorable climatic conditions than their smaller prey, mainly due to greater water loss due to their larger body surface area (Petchey et al., 1999; Brose et al., 2012; Romero et al., 2022). Furthermore, competition between predators could select for larger body sizes in shelter-using species. Therefore, in seasonal environments there may be a tendency to select larger bodies, especially of organisms at higher trophic levels (Romero et al., 2022). Thus, as climate change continues, we predict an increase in the importance of these leaf shelters in maintaining arthropod species, and an increase in the dominance of larger predatory bodies in increasingly arid and climatically variable regions (Romero et al., 2022).

Our study contributes toward a better understanding of the patterns of variation and magnitude of the ecosystem engineers at different spatial and temporal scales (i.e., seasonal differences), and provides new insights about the mechanisms involved. Ecosystem-engineering arthropods like the gallers studied here are ubiquitous in tropical forests and savannas, acting as keystone species in structuring arthropod communities on plants, especially in environments subject to climatic variations. Thus, species sensitive to aridity, high temperatures, and climate variability can find shelter in microclimatic refuges, such as leaf rolls built by *D. gallaeformans*. Given this scenario, more studies that evaluate the role of shelters in population dynamics and intra and interspecific interactions of secondary colonizing arthropods in stressful environments could be a suitable topic for future research.

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Conclusão Geral

Esta tese contribuiu para uma melhor compreensão da organização das comunidades de artrópodes terrestres, para os padrões de variação e magnitude da engenharia de ecossistemas em diferentes escalas espaciais e temporais, e suas consequências indiretas em interações tróficas em sistemas tropicais. Fornecemos uma extensa base de dados que incluiu mais de mil registros únicos de engenharia de ecossistemas por artrópodes, na forma de estruturas construídas em plantas. Além de dados sobre plantas hospedeiras e engenheiros, agregamos dados sobre o tipo de construções e a identidade dos inquilinos que utilizam essas estruturas. Este conjunto de dados destacou a importância dessas estruturas sutis para a organização de comunidades de artrópodes terrestres, permitindo testes de hipóteses em estudos ecológicos que abordam a engenharia de ecossistemas e a facilitação mediada pelos abrigos. Demonstramos ainda com nossas investigações empíricas que os indutores de galhas podem ser considerados engenheiros de ecossistemas quando modificam a morfologia foliar e criam abrigos para colonizadores secundários, alterando propriedades de comunidades ecológicas. Nossos resultados mostraram um padrão claro que corroborou as hipóteses testadas. Mostramos que os abrigos de folhas criados por *Ditylenchus gallaeformans* aumentaram a abundância, a riqueza e a biomassa dos artrópodes no nível foliar e da planta, modificando a composição de espécies e diminuindo indiretamente os níveis de herbivoria nos arbustos de *Miconia ligustroides*. No entanto, embora a estação chuvosa exibisse maior biomassa de artrópodes ao nível da planta, a estação seca apresentou maior abundância, riqueza e biomassa de artrópodes ao nível foliar. Dessa forma, os abrigos foliares poderiam atuar como importantes refúgios microclimáticos, reduzindo a exposição dos artrópodes às flutuações climáticas dos habitats circundantes. Por fim, reforçamos que artrópodes engenheiros de ecossistemas, como os galhadores aqui estudados, são onipresentes em florestas tropicais e savanas, atuando como espécies-chave na estruturação de comunidades de artrópodes em plantas, sobretudo em ambientes submetidos a

variações climáticas. Assim, espécies sensíveis à aridez, às altas temperaturas e à variabilidade climática podem encontrar abrigo em refúgios microclimáticos, como os rolos foliares construídos por *D. gallaeformans*. Diante deste cenário, mais estudos que avaliem o papel dos abrigos na dinâmica populacional e nas interações intra e interespecíficas de artrópodes colonizadores secundários em ambientes estressantes poderiam ser um tema adequado para pesquisas futuras.

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Material Supplementar

Chapter I. Subtle structures with not-so-subtle functions: a dataset of arthropod constructs and their host plants

Our dataset with ecosystem engineering records has more than a thousand records and is too long to be attached at the end of this document as supporting information. Please access our data via this link: <https://doi.org/10.6084/m9.figshare.16879135>

Chapter II. Gallers as leaf rollers: ecosystem engineering in a tropical system and its effects on arthropod biodiversity

Supporting Information S1



Figure S1. Photos of the study system. Individual of *Miconia ligustroides* (a) and natural leaf roll induced by the galler (b). (c) Branch with great infestation of galls in the leaf blade. Photo credit: Cássio Pereira.

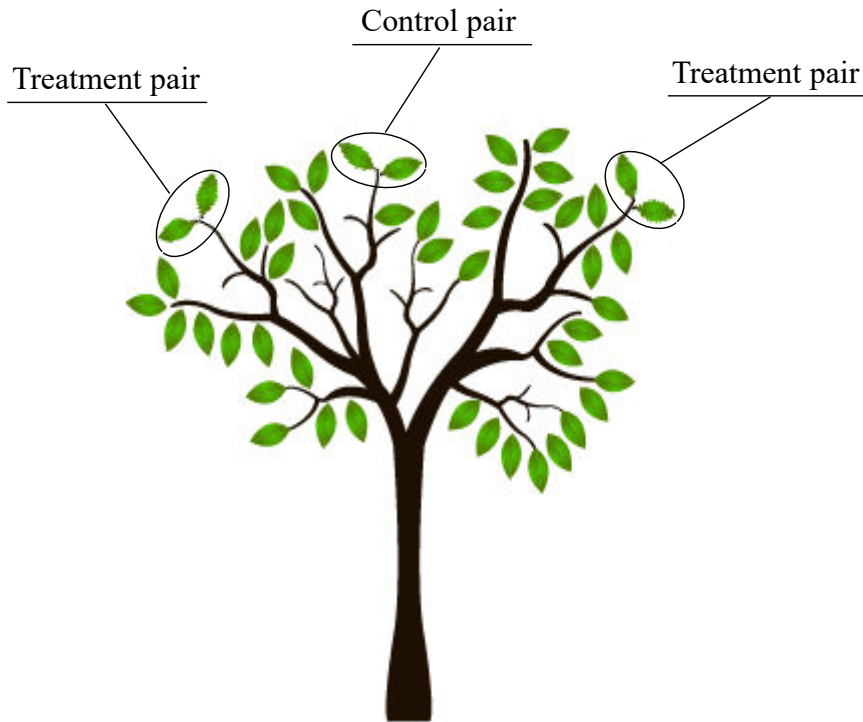


Figure S2. Schematic representation of the treatment arrangement in the branches on *Miconia ligustroides*. In each plant, 3 pairs of paired leaves were marked and 2 pairs were selected as treatments and one pair as the control (expanded leaves). Treatments were applied into different branches in each plant. The treatment pairs consisted of 1) a leaf with an empty artificial shelter and 2) another rolled leaf with an occupied shelter.

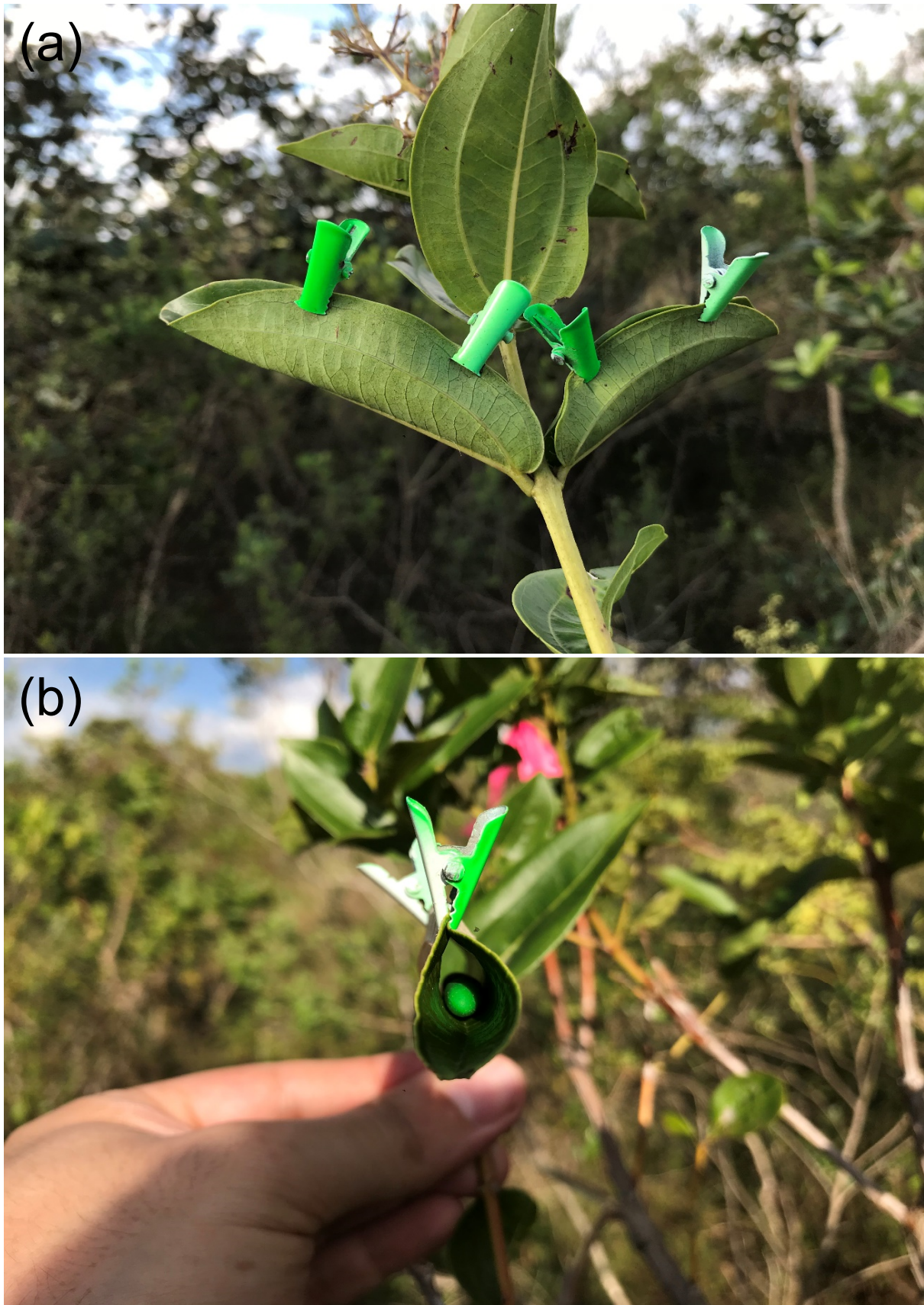


Figure S3. Photos of the experimental design. The artificial rolls were kept rolled with clips, in the same format as the natural rolls, consisting of pairs of shelters (a), one unoccupied and the other occupied with modeling clay, simulating a *Lepidoptera* caterpillar (b). Photo credit: Cássio Pereira.

Supporting Information S2

Table S1. General Mixed Linear Models (GLMMs) and pairwise comparisons (*post hoc* Tukey's test) at leaf level, showing the differences between treatments at control leaves, occupied leaf rolls and unoccupied leaf rolls for all guilds and for each guild separately. CL = Control leaves, OLR = Occupied leaf rolls, ULR = Unoccupied leaf rolls. * = Significant difference between treatments.

GLMs/Tukey's test	Leaf level					
	Abundance		Richness		Biomass	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
All guilds	16.354	0.0001*	18.742	0.000*	6.448	0.002*
CL X OLR	-	0.675	-	0.981	-	0.713
CL x ULR	-	0.0001*	-	0.0001*	-	0.026*
OLR x ULR	-	0.0001*	-	0.0001*	-	0.003*
Detritivores	3.919	0.023*	4.462	0.014*	3.411	0.037*
CL X OLR	-	1.000	-	1.000	-	1.000
CL x ULR	-	0.045*	-	0.030*	-	0.067
OLR x ULR	-	0.045*	-	0.030*	-	0.067
Herbivores	3.248	0.044*	3.407	0.038*	1.131	0.327
CL X OLR	-	0.034*	-	0.047*	-	-
CL x ULR	-	0.361	-	0.937	-	-
OLR x ULR	-	0.471	-	0.102	-	-
Omnivores	17.008	0.0001*	18.374	0.0001*	6.620	0.002*
CL X OLR	-	0.782	-	0.409	-	0.994
CL x ULR	-	0.0001*	-	0.0001*	-	0.005*
OLR x ULR	-	0.0001*	-	0.0001*	-	0.007*
Parasitoids	1.000	0.372	1.000	0.372	1.000	0.372
CL X OLR	-	-	-	-	-	-
CL x ULR	-	-	-	-	-	-
OLR x ULR	-	-	-	-	-	-
Predators	3.472	0.035*	3.472	0.035*	1.849	0.164
CL X OLR	-	0.501	-	0.501	-	-
CL x ULR	-	0.027*	-	0.027*	-	-
OLR x ULR	-	0.296	-	0.296	-	-

Table S2. Pairwise comparisons (*R/P* values) between treatments at leaf level in experiment. * = Significant difference between treatments.

Treatments	Intact leaves	Occupied leaf rolls	Unoccupied leaf rolls
Intact leaves		0.0065/0.1857	0.1655/0.0001*
Occupied leaf rolls	0.0065/0.1857		0.1621/0.0001*
Unoccupied leaf rolls	0.1655/0.0001*	0.1621/0.0001*	

Table S3. Pairwise comparisons (*P* values) between treatments of the leaf herbivory damage experiment. * = Significant difference between treatments. LR = Leaf rolls.

Treatments	Up to 7 LR	8 to 15 LR	Ungalled plants
Up to 7 LR		0.839	0.0001*
8 to 15 LR	0.839		0.0001*
Galls removed	0.0001*	0.0001*	

Table S4. Temperatures related to the interior of the leaf rolls and expanded leaves on *Miconia ligustroides* measured at different times during a summer day in the cerrado *sensu stricto* of the São José Environmental Protection Area, MG, Brazil.

Time (h)	Temperature inside leaf rolls (°C)	Temperature in the leaf lamina (°C)
9 am	25.10	27.20
10 am	26.70	28.80
11 am	29.50	32.90
12 am	30.70	33.30
1 pm	31.00	34.20
2 pm	31.40	34.70
3 pm	31.00	33.80
4 pm	30.60	34.00

Chapter III. From leaves to whole plants: effects of shelter-builders on arthropod communities are stronger in dry seasons

Supplementary information S1

Table S1. General Linear Mixed Models (GLMMs) at leaf and plant level, showing the differences between treatments carried out on *Miconia ligustroides* in the wet season, dry season, and between the two seasons throughout the years 2020 and 2021. Leaf level: CL = Control leaves, LR = Leaf rolls. Plant level: PWLR = Plants with leaf roll galls, PWGR = Plants with galls removed. * = Significant difference between treatments.

Level	Season/Year	Treatments	GLMMS					
			Abundance		Richness		Biomass	
			<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Leaf	Wet/2020	CL X LR	4.253	0.044*	4.804	0.032*	5.994	0.017*
Leaf	Dry/2020	CL X LR	20.468	0.0001*	20.282	0.0001*	5.715	0.020*
Leaf	Wet x Dry/2020	LR X LR	4.152	0.046*	5.211	0.026*	4.839	0.032*
Leaf	Wet/2021	CL X LR	5.800	0.019*	5.292	0.025*	6.810	0.012*
Leaf	Dry/2021	CL X LR	17.894	0.0001*	22.154	0.0001*	27.124	0.0001*
Leaf	Wet x Dry/2021	LR X LR	4.944	0.030*	5.039	0.029*	4.158	0.046*
Plant	Wet/2020	PWLR x PWGR	16.852	0.0001*	15.144	0.0001*	14.101	0.0001*
Plant	Dry/2020	PWLR x PWGR	26.544	0.0001*	27.815	0.0001*	11.872	0.001*
Plant	Wet x Dry/2020	PWLR x PWLR	1.896	0.174	0.429	0.515	6.947	0.011*
Plant	Wet/2021	PWLR x PWGR	34.942	0.0001*	33.302	0.0001*	5.090	0.028*
Plant	Dry/2021	PWLR x PWGR	25.640	0.0001*	29.371	0.0001*	4.128	0.047*
Plant	Wet x Dry/2021	PWLR x PWLR	12.842	0.001*	6.820	0.012*	2.505	0.119

Table S2. Analysis of similarities (ANOSIM) between treatments at leaf and plant level, showing the differences between treatments carried out on *Miconia ligustroides* in the wet season, dry season, and between the two seasons throughout the years 2020 and 2021. Leaf level: CL = Control leaves, LR = Leaf rolls. Plant level: PWLR = Plants with leaf roll galls, PWGR = Plants with galls removed. * = Significant difference between treatments.

Level	Season/Year	Treatments	ANOSIM	
			Global <i>R</i>	<i>P</i>
Leaf	Wet/2020	CL X LR	0.0228	0.0224*
Leaf	Dry/2020	CL X LR	0.0838	0.0001*
Leaf	Wet x Dry/2020	LR X LR	0.0364	0.0064*
Leaf	Wet/2021	CL X LR	0.0219	0.0154*
Leaf	Dry/2021	CL X LR	0.0963	0.0001*
Leaf	Wet x Dry/2021	LR X LR	0.0416	0.0003*
Plant	Wet/2020	PWLR x PWGR	0.0970	0.0003*
Plant	Dry/2020	PWLR x PWGR	0.0853	0.0001*
Plant	Wet x Dry/2020	PWLR x PWLR	0.0712	0.0026*
Plant	Wet/2021	PWLR x PWGR	0.1455	0.0001*
Plant	Dry/2021	PWLR x PWGR	0.0586	0.0101*
Plant	Wet x Dry/2021	PWLR x PWLR	0.1084	0.0003*