UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Instituto de Biociências – Câmpus de Botucatu/Rio Claro
Seção Técnica de Pós-Graduação

PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA VEGETAL (INTERUNIDADES)

Recursos florais como mediadores das interações planta-polinizador: organização e distribuição espaço-temporal em múltiplas escalas

CAIO SIMÕES BALLARIN

Tese apresentada ao Instituto de Biociências, Câmpus de Botucatu, UNESP, para obtenção do título de Doutor no Programa de Pós-Graduação em Biologia Vegetal, Interunidades entre o Instituto de Biociências do câmpus de Botucatu e Instituto de Biociências do câmpus de Rio Claro.

BOTUCATU – SP
2024

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ATA DA DEFESA PÚBLICA DA TESE DE DOUTORADO DE CAIO SIMÕES BALLARIN, DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA VEGETAL, DO INSTITUTO DE BIOCIÊNCIAS - CÂMPUS DE BOTUCATU.

Aos 15 dias do mês de agosto do ano de 2024, às 14:00 horas, no(a) Anfiteatro - Herbário - IBB, realizou-se a defesa de TESE DE DOUTORADO de CAIO SIMÕES BALLARIN, intitulada **Recursos florais como mediadores das interações planta-polinizador: organização e distribuição espaço-temporal em múltiplas escalas**. A Comissão Examinadora foi constituida pelos seguintes membros: Prof. Dr. FELIPE WANDERLEY DE AMORIM (Orientador(a) - Participação Presencial) do(a) Departamento de Biodiversidade e Bioestatística / Instituto de Biociências de Botucatu - UNESP, Profa. Dra. LEONOR PATRICIA CERDEIRA MORELLATO (Participação Presencial) do(a) Departamento de Biodiversidade / Instituto de Biociências - Unesp Rio Claro, Prof. Dr. PEDRO JOAQUIM BERGAMO (Participação Presencial) do(a) Departamento de Biodiversidade / Instituto de Biociências - UNESP Rio Claro, Dr. LEANDRO FREITAS (Participação Virtual) do(a) Instituto de Pesquisas Jardim Botânico do Rio de Janeiro., Profa. Dra. ISABELA GALARDA VARASSIN (Participação Virtual) do(a) Departamento de Botânica / Universidade Federal do Paraná. Após a exposição pelo doutorando e arguição pelos membros da Comissão Examinadora que participaram do ato, de forma presencial e/ou virtual, o discente recebeu o conceito final: APROVADO. Nada mais havendo, foi lavrada a presente ata, que após lida e aprovada, foi assinada pelo(a) Presidente(a) da Comissão Examinadora.

Prof. Dr. FELIPE WANDERLEY DE AMORIM

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Resumo

BALLARIN, C, S. **Recursos florais como mediadores das interações planta-polinizador: organização e distribuição espaço-temporal em múltiplas escalas**. 2024. Defesa de Tese de Doutorado – Instituto de Biociências de Botucatu, Universidade Estadual Paulista "Júlio de Mesquita Filho", Botucatu, 2024.

Recursos florais são considerados atributos cruciais para a interação entre plantas e polinizadores. Contudo, independentemente do nível de organização biológica, há diversas lacunas do conhecimento relacionadas à influência dos recursos florais na interação planta-polinizador. Neste trabalho, utilizamos três abordagens em distintos níveis de organização biológica para determinar: 1) ao nível da biosfera, padrões biogeográficos de distribuição do néctar e da diversidade de recursos florais ao redor do globo. Ao nível da comunidade, avaliamos a fauna apícola e a flora visitada por abelhas de um fragmento de Cerrado em Botucatu, São Paulo, Brasil, a fim de investigar 2) a influência da variação espaço-temporal da diversidade de recursos florais na estrutura da rede de interação planta-abelha; 3) a influência de diferentes tipos de recurso floral na estrutura da rede de interação; 4) a influência de atributos funcionais apresentados por abelhas na conectividade de redes de interação montadas a partir de interações planta-abelha mediadas por diferentes recursos florais (*e.g.*, néctar, pólen e óleo); e 4) a influência do fogo, um importante distúrbio em ecossistemas inflamáveis, tal como o Cerrado, na distribuição espaço-temporal dos recursos florais oferecido pelas plantas aos polinizadores e seu potencial efeito na estrutura de interação planta-abelha. Ao nível da população, avaliamos 5) como a oferta de néctar ao longo do dia afeta a interação planta-polinizador no ecótipo anão do pequi, *Caryocar brasiliense* subps. *intermedium*. Esperamos, ao desvendar as questões supra elencadas, contribuir com a compreensão da importância dos recursos florais para a definição de padrões ecológicos na interação planta-polinizador em diferentes níveis e escalas de organização ecológica.

Palavras-chave: diversidade funcional, ecologia do fogo, interações planta-abelha, néctar, óleo, pólen, rede de interação, recursos florais, síndrome de polinização, troca de polinizador.

Abstract

BALLARIN, C. S. **Floral resources as mediators of plant-pollinator interactions: organization and spatiotemporal distribution across multiple ecological scales**. 2024. PhD thesis defense– Institute of Biosciences of Botucatu, São Paulo State University "Júlio de Mesquita Filho," Botucatu, 2024.

Floral resources are considered crucial attributes for plant-pollinator interactions. However, regardless of the level of biological organization, there are numerous knowledge gaps related to the influence of floral resources on plant-pollinator interactions. In this work, we employed three approaches at distinct levels of biological organization to assess: 1) at the biosphere level, the biogeographic patterns of nectar distribution and the diversity of floral resources globally. At the community level, we assessed the bee fauna and bee-visited plants of a Cerrado fragment in Botucatu, São Paulo, Brazil, to investigate: 2) the influence of the spatiotemporal variation in floral resource diversity on the structure of the plant-bee interaction network; 3) the influence of different types of floral resources on the structure of plant-bee interaction network; 4) the influence of functional attributes presented by bees on the connectivity of plant-bee interaction networks mediated by different floral resources (e.g., nectar, pollen, and oil); and 5) the influence of fire, a significant disturbance in flammable ecosystems such as the Cerrado, on the spatiotemporal distribution of floral resources provided by plants to pollinators and its potential effect on the plant-bee interaction network structure. At the population level, we evaluated 6) how the daily nectar supply affects plant-pollinator interactions in the dwarf ecotype of *Caryocar brasiliense* subsp. *intermedium*. By addressing these questions, we aim to contribute to the understanding of the importance of floral resources in defining ecological patterns in plant-pollinator interactions across different levels and scales of ecological organization.

Keywords: fire ecology, floral resources, functional diversity, interaction network, nectar, oil, plant-bee interactions, pollen, pollination syndrome, pollinator shift.

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1 INTRODUÇÃO GERAL

1.1 INTRODUÇÃO

1.1.1 Interação planta-polinizador mediada por recursos florais como modelo de estudos ecológicos

Um dos objetivos centrais da ecologia é entender como as interações entre as espécies e o meio no qual habitam moldam seus nichos ecológicos, influenciando a estrutura e o funcionamento das comunidades às quais pertencem (Vandermeer, 1972; Wiens, 2011). Uma questão chave para aprimorar tal entendimento é investigar os motivos pelos quais espécies desempenham papéis ecológicos distintos dentro de uma comunidade ecológica (*e.g.*, Shmida & Wilson, 1985; Tilman et al. 1997; Tilman, 2001; Rundle & Nosil, 2005). A polinização biótica apresenta uma particularidade interessante para a investigação dos distintos papéis ecológicos das espécies dentro da comunidade onde elas encontram-se inseridas, haja vista que ela compreende uma relação na qual organismos móveis (*i.e.*, animais) são responsáveis pela dispersão do pólen, componente crucial para reprodução de organismos sésseis (*i.e.*, as plantas; Ghazoul 2005). Esse fenômeno oferece pressões seletivas responsáveis por moldar os caracteres das flores, órgãos reprodutivos das plantas, de modo que elas apresentem atributos funcionais responsáveis pela atração e manutenção dos polinizadores (*i.e.*, atributos funcionais mediadores da interação planta-polinizador; Schaefer et al. 2004; Harder & Johnson 2009). Sendo assim, o papel ecológico das espécies de plantas pode ser investigado através de como, e com que frequência no espaço e no tempo, determinada espécie vegetal interage com polinizadores mediante a presença de determinados atributos florais.

Dentre tais atributos, os atrativos e recursos florais se destacam uma vez que o primeiro é responsável por sinalizar o potencial de interação ao visitante floral e, o segundo, responsável por promover a manutenção da interação no espaço e no tempo, dado que este representa uma recompensa ao visitante floral. Enquanto os atrativos florais estabelecem a comunicação entre plantas e polinizadores por meio de sinais químicos e visuais emitidos pelas flores e interpretados pelos polinizadores, recursos florais contribuem para a sobrevivência e reprodução das espécies de animais interagentes, uma vez que, invariavelmente, os alimentam, os protegem, ou os permitem se reproduzir. Deste modo,

recursos florais, o foco desta tese de Doutorado, tem fundamental importância para as interações planta-polinizador, pois permite que essas interações sejam consideradas interações ecológicas mediadas por recurso.

1.1.2 Recursos florais como mediadores de interações consumidor-recurso

Indivíduos conseguem colonizar determinada comunidade biológica somente quando suas tolerâncias e seus requerimentos são atendidos (Hutchinson, 1957). Isso implica que tais organismos precisam não apenas ser capazes de superar filtros ambientais, mas também de adquirir recursos para sobreviver e se reproduzir em tal habitat (Laliberté et al. 2014). Assim sendo, indivíduos que não encontrem recursos disponíveis para sobreviver e se reproduzir não estarão presentes nestas comunidades, mesmo que as condições ambientais virtualmente os permitissem existir neste meio. Deste modo, a base de recursos que uma comunidade pode oferecer tem papel significativo na definição da identidade e distribuição das espécies que estarão presentes neste meio (Ollerton, 2006). Para além de definir a identidade de espécies presentes na comunidade, a base de recursos que uma comunidade oferece também determina a configuração e frequência das interações entre espécies, uma vez que grande parte dos recursos consumidos por uma determinada espécie é adquirido através da exploração de uma outra espécie (*e.g.*, interações consumidor-recurso, veja Worm et al. 2002).

Uma vez mediadas por recursos florais que são finitos no espaço e tempo, e que atendem à demandas impreteríveis para os animais interagentes sobreviverem e se reproduzirem, interações planta-polinizadores podem ser entendidas também como clássicas interações consumidor-recurso (Holland et al. 2005; Valdovinos & Marsland III, 2021). A abordagem consumidor-recurso apresenta uma base teórica e mecanicista sólida, fundamentada no amplo estudo das relações ecológicas antagonistas, como predação e competição (Lotka, 1926, Volterra, 1926; Gause, 1934; Elton, 1946; MacArthur & Levins, 1967; Abrams, 1987; Murdoch et al. 2003; Turchim et al. 2003). Desbruçar-se nesta base teórica se mostra relevante para melhor compreensão de interações mutualísticas e, em especial, interações planta-poliniziador, pois esta leva em consideração que tanto a disponibilidade de espécies consumidoras (*e.g.*, predadores) quanto a de recursos (*e.g.*, presas) são dinâmicas, ou seja, flutuam no espaço e no tempo (Abrams, 1987; Ballarin et al.

2022a; Sponsler et al. 2023). Igualmente, interações planta-polinizadores mediadas por recurso também possuem dois componentes cujas disponibilidades flutuam no espaço e no tempo: espécies interagente e recursos florais mediadores (Sponsler et al. 2023). A oscilação destes dois componentes implica que a organização das interações entre as espécies envolvidas, por consequência, também oscilará espaço-temporalmente (Ballarin et al. 2022b; Hervías-Parejo et al. 2023; Sponsler et al. 2023).

O desafio de antecipar padrões de interações planta-polinizador se torna mais complexo quando estas são entendidas como dinâmicas e não estáticas. Em contrapartida, por debruçar-se em uma base mecanicista sólida, exaustivamente explorada no estudo de interações antagonisticas, investigar os padrões de interação planta-polinizador através da abordagem consumidor-recurso pode oferecer justificativas amplamente conhecidas, porém pouco empregadas no estudo deste modelo ecológico (i.e., polinização). Por exemplo, como explicar o fato de que, em dias consecutivos, um observador enfocado em coletar a frequência de interações entre um determinado indivíduo arbóreo em floração e uma determinada espécie de abelha pode obter frequências de interação drasticamente diferentes? A consideração deste tipo de interação mutualística como uma interação consumidor-recurso pode oferecer potenciais explanações para este fato. Em dias diferentes, este mesmo indivíduo arbóreo pode exibir quantias dramaticamente diferentes de flores, e isso influenciará a quantidade de abelhas capazes de visitar essas flores antes que o recurso seja deplecionado por completo (veja Ballarin et al. 2022b como exemplo). De modo similar, mesmo se o indivíduo arbóreo exibisse quantidade de flores semelhantes em dias consecutivos, o conteúdo de néctar que cada flor abriga pode variar naturalmente (Lanza et al. 1995; Guimarães et al. 2018; Maldonado et al. 2023), ou de acordo com a taxa de visitação de abelhas – quanto mais abelhas consumindo esse recurso, menos néctar cada uma destas flores abrigará (Ballarin et al. 2022b; Figura 1.1).

Figura 1.1 Abelha *Bombus morio* visitando a flor da espécie vegetal *Borreria poaya* (Rubiaceae). Na foto, é possível observar os dois cenários hipotéticos que poderiam servir como justificativas ao fato do observador ter obtido frequências de visitação tão distintas de uma abelha a um mesmo indivíduo vegetal em dias consecutivos. No primeiro cenário, pode se observar que este indivíduo de *B. poaya* apresenta cicatrizes de botões florais (em roxo) com nectários sem néctar (centros esbranquiçados). Isso indica que em diferentes dias, este indivíduo de *B. poaya* pode exibir distintas quantidades de flores com nectários ativos. Já no segundo cenário, vê-se que a abelha *B. morio* está consumindo o néctar de uma flor de *B. poaya* de maneira tal que outros indivíduos de *B. morio* tenham menos néctar a sua disposição ao visitar esse mesmo indivíduo de *B. poaya*. Foto: Diego S. Podadera.

Embora a aplicação de teorias fundamentadas a partir de modelos de interações ecológicas antagonistas seja profícua para o melhor entendimento sobre interações mutualísticas, somente em períodos recentes o estudo de relações consumidor-recurso foi aplicado à mutulismos (Chase & Leibold, 2003; Holland et al. 2005; Holland & DeAngelis, 2010), e, em particular, às interações planta-polinizador (Valdovinos et al. 2013; Valdovinos, 2019; Sponsler et al. 2023). Ainda assim, neste espaço de duas décadas, observa-se que diversos são os ecólogos que adotam uma perspectiva dinâmica ao estudar os mutualismos entre plantas e animais (Bascompte & Jordano, 2014), levando em conta variações nessas relações ao longo do espaço e do tempo (Olesen et al. 2007, 2010; CaraDonna & Waser, 2020; Hervías-Parejo et al. 2023). Entretanto, embora este avanço seja perceptível em

número de estudos, diversas questões relacionadas ao dinamismo das interações planta-polinizador persistem. Deste modo, ainda não é possível antecipar de maneira completamente acurada como essas interações se configuram e como se modulam no espaço e no tempo.

Um dos fatores que contribui para dificultar o acréscimo de acurácia de modelos preditivos empenhados em antecipar padrões configuracionais de interação planta-polinizador é o fato de que, para além das flutuações das espécies interagentes taxonomicamente distintas e das flutuações quantitativas na disponibilidade de recursos, há que se levar em conta as flutuações qualitativas na disponibilidade de recursos. Em outras palavras, um observador não somente pode notar diferenças na frequência de interação entre abelhas e um indivíduo arbóreo devido as variações na disponibilidade de flores ou néctar. Além disso, diferenças marcantes na taxa de visitação de uma abelha a um indivíduo arbóreo podem se dar pelo comportamento da abelha ao coletar diferentes tipos de recursos. Por exemplo, esta determinada espécie de abelha pode estar empenhada em coletar recursos diferentes que esta planta oferece, tal como pólen ou néctar, nos diferentes dias em que a frequência de interação foi aferida pelo observador (veja Hagbery & Nieh, 2012). Finalmente, mesmo coletando o mesmo recurso, esta abelha pode exibir frequências de visitação diferentes uma vez que, em uma mesma árvore, o conteúdo químico deste recurso, tal como néctar, pode variar entre diferentes flores (veja Herrera et al. 2006) e entre diferentes períodos do dia (Amorim et al. 2013; Ballarin et al. 2022b).

Ampliando a escala de interações pareadas (*i.e.*, interação entre uma espécie de planta e uma espécie abelha) para redes de interação entre comunidade de plantas e abelhas ou mesmo para o conjunto de interações planta-abelha em diferentes ecossistemas no globo, vê-se que se torna gradativamente mais complexo compreender todos os fatores subjacentes à configuração e reconfiguração de interações mutualísticas, tal como a polinização, no espaço e tempo. Deste modo, faz-se necessário compreender como variações na: i) identidade de espécies de plantas e polinizadores (*i.e.*, flutuação taxonômica); ii) na quantidade de recursos florais mediadores, e, sobretudo; iii) na qualidade dos recursos florais, atuam em conjunto para organizar a composição e diversidade de interação entre plantas e polinizadores.

1.1.3 Variações qualitativas de recursos florais

Néctar, pólen, óleo, tecidos vegetais, fragrâncias e resinas são típicos recursos florais oferecidos pelas plantas aos polinizadores (Figura 1.2). Entretanto, pelo fato de atenderem de maneira distinta as demandas de diferentes visitantes florais, a produção e a apresentação destes recursos florais pode variar amplamente entre as angiospermas (i.e., plantas com flores), uma vez que estas estão sujeitas às pressões seletivas mediadas pelos principais polinizadores que os acessam (Bernardello et al. 2007; Armbruster, 2012). Deste modo, a composição e a apresentação no espaço e no tempo de um recurso floral por uma determinada espécie de planta estão relacionadas com a disponibilidade de polinizadores na paisagem onde ela está inserida (Caruso et al. 2019; Sletvold 2019). Por outro lado, a presença de determinados grupos de polinizadores também é influenciada pelo conjunto de recursos florais oferecido pela comunidade de plantas (Ollerton, 2006; Armbruster, 2012). Assim, da mesma maneira que o tipo, a quantidade, e a qualidade de recursos florais que uma espécie vegetal oferece é influenciado pelo conjunto de polinizadores que a visita, a assembleia local de polinizadores é também fortemente influenciada pela abundância, composição química e acessibilidade de recursos florais que o conjunto de espécies vegetais apresenta (Woodard & Jha, 2017; Kelly & Elle, 2021). Em outras palavras, recursos florais intermediam a interação entre plantas e polinizadores, sendo, portanto, fundamentais para configurar a composição e diversidade de: i) plantas; ii) polinizadores e, iii) interações planta-polinizador. Portanto, indivíduos de diferentes populações de uma mesma espécie vegetal, populações de diferentes espécies vegetais em uma mesma comunidade, e comunidades vegetais de diferentes ecossistemas na biosfera apresentam padrões de produção e apresentação de recursos florais que refletem o contexto ecológico presente nestes diferentes níveis de organização biológica.

Figura 1.2 Exemplo de flores oferecendo diferentes tipos de recursos florais aos polinizadores. A: *Lippia lupulina* (Verbenaceae); B: *Borreria verticillata* (Rubiaceae); C: *Cuphea micrantha* (Lythraceae), D: *Oxypetalum aequaliflorum* (Apocynaceae), E: *Galactia decumbens* (Fabaceae), F: *Mandevilla longiflora* (Apocynaceae); e G: *Cayaponia espelina* (Cucurbitaceae) oferecendo néctar aos visitantes florais. H: *Sida ciliaris* (Malvaceae); I: *Mimosa gracilis* (Fabaceae); J: *Krapovickasia macrodon* (Malvaceae); e K: *Lessingianthus brevifolius* (Asteraceae) oferecendo néctar e pólen aos visitantes florais. L: *Chamaecrista ramosa* (Fabaceae); M: *Solanum palinacanthum* (Solanaceae); N: *Tibouchina gracilis* (Melastomataceae); O: *Kielmeyera coriacea* (Calophyllaceae); P: *Psidium australe* (Myrtaceae); e Q: *Commelina erecta* (Commelinaceae) oferecendo pólen aos visitantes florais. R: *Annona nutans* (Annonaceae) oferecendo pólen e tecidos florais aos visitantes florais. S: *Sisyrinchium weirii* (Iridaceae); e T: *Banisteriopsis campestris* (Malpighiaceae) oferecendo pólen e óleo aos visitantes florais. Fotos: A, B, C, F, K, N e O: Diego S. Podadera; D, E, G, H, I, J, L, M, P, Q, R, S, T: Caio S. Ballarin.

1.1.4 Influência dos recursos florais na organização de interações planta-polinizador em múltiplas escalas ecológicas: questões em aberto

Embora recursos florais representem o elo entre plantas e polinizadores, diversas questões subjacentes aos padrões de produção e apresentação de recursos florais nos distintos

níveis de organização biológica e como recursos florais moldam a interação entre plantas (*i.e.*, recurso) e polinizadores (*i.e.*, consumidores) ainda permanecem abertas. Por exemplo, ao nível da biosfera, ainda não se sabe qual é o padrão de diversidade de recursos florais e de produção de néctar nas comunidades distribuídas ao redor do globo e quais os fatores macroecológicos e climáticos que definem estes padrões biogeográficos. Ao nível da comunidade, não está claro se a diversidade de recursos florais (i.e., a distribuição das abundâncias relativas de diferentes recompensas oferecidas pelas plantas ao polinizadores tal como néctar, pólen, e óleo) se altera no espaço e no tempo e se esta suposta variação espaço-temporal afeta a estrutura da rede de interação entre plantas e polinizadores (Figura 1.3). Também permanece não elucidado se grupos de espécies de plantas e abelhas que, respectivamente, oferecem e coletam diferentes recursos florais constituem recortes de espécies interagentes com estrutura e diversidade diferentes daqueles observados ao considerar a comunidade de plantas e polinizadores em unidade. Similarmente, permanece uma questão em aberto se organismos coletando recursos florais específicos (e.g., óleo) são mais eficientes em conectar a rede de interação entre plantas e polinizadores que compõem diferentes recortes de espécies interagentes. Além disso, não é evidente se distúrbios ambientais, tal como o fogo, afetam a oferta de recursos florais no espaço e no tempo, impactando subsequentemente a estrutura e a estabilidade das interações planta-polinizadores. Já ao nível da população, são poucos os estudos que investigaram os processos pelos quais indivíduos de uma mesma espécie exibem diferenças marcantes na produção de recursos florais e na apresentação destes no espaço e no tempo (Castaneda-Zarate et al. 2021).

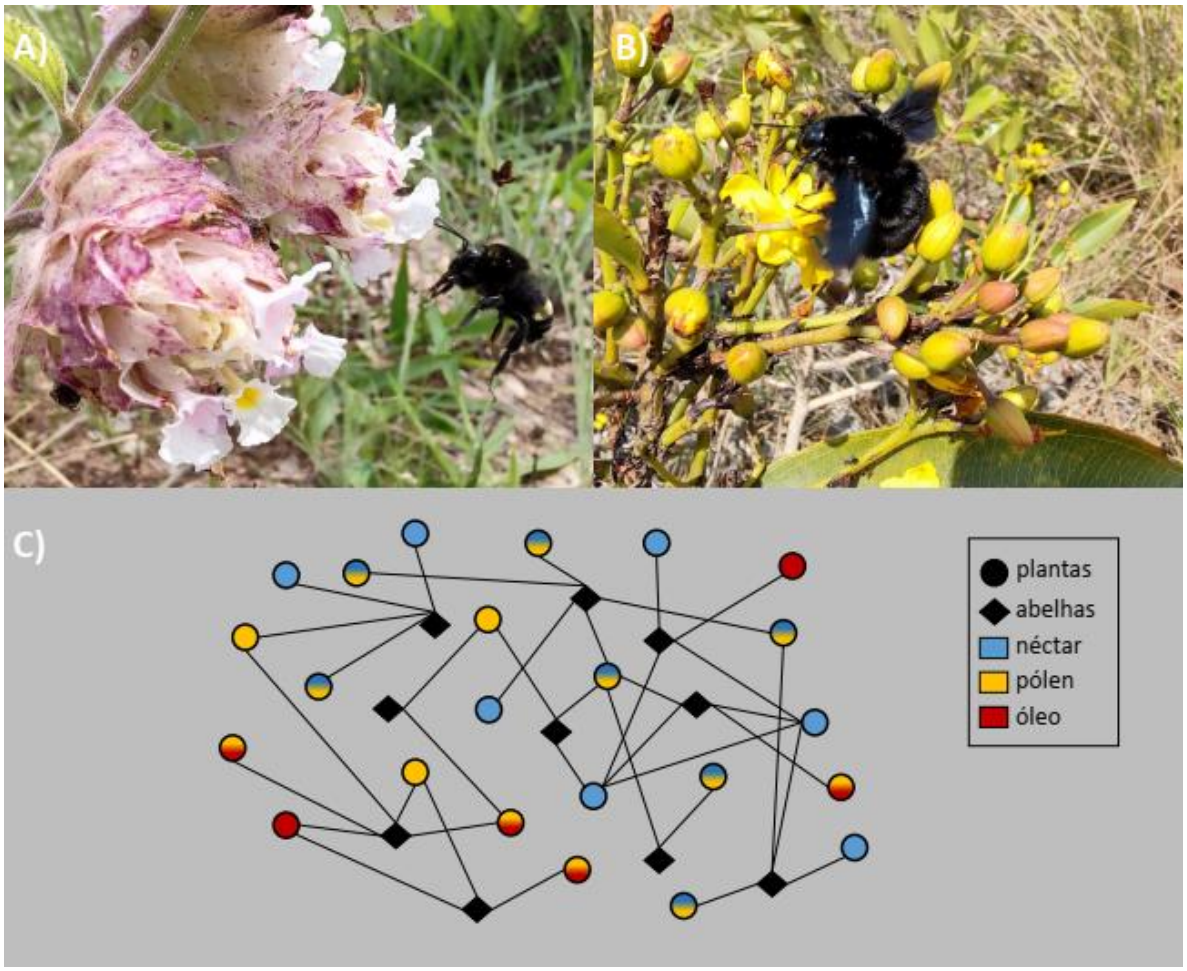

Figura 1.3 Interações planta-abelha mediada por diferentes recursos florais. A abelha *Bombus pauloensis* visita as flores da espécie vegetal *Lippia lupulina* (Verbenaceae) em busca de néctar (A). Já a abelha *Xylocopa* sp., visita as flores da espécie vegetal *Ouratea spectabilis* (Ochnaceae) com intuito de obter o pólen alojado em suas anteras poricidas por meio de vibração (B). Apesar de representarem eventos similares (i.e., visita de uma abelha de porte grande a uma flor), estes eventos de interação planta-abelha refletem contextos ecológicos diferentes. No primeiro caso, embora esteja sendo visitada por *B. pauloensis*, a espécie vegetal *L. lupulina* também recebe a visita de diversas outras abelhas que buscam em suas flores um néctar nutritivo e de fácil acesso. Já no segundo caso, a espécie vegetal *O. spectabilis* oferece pólen para uma gama mais restrita de abelhas que são capazes de sonicar e remover o pólen de suas anteras poricidas. Torna-se uma questão relevante compreender a abundância relativa e a distribuição dessas plantas no espaço e no tempo, uma vez que a diversidade de recursos florais pode reconfigurar a identidade e a diversidade de interações entre plantas e abelhas, reconfigurando a rede de interação entre esses organismos (C). Na rede de interação esquemática (C), as diferentes cores representam o tipo de recurso oferecido pelas plantas (círculos) às abelhas (losangos): azul – néctar, amarelo – pólen, e vermelho – óleo. As interações entre cada tipo de planta e abelha são representadas pelas linhas pretas que conectam os círculos aos losangos. Fotos: Caio S. Ballarin

Todas essas lacunas relacionadas à atuação dos recursos florais como mediadores da interação planta-polinizador fornecem enredo para esta tese de Doutorado. Contudo, considerando que algumas dessas lacunas requerem modelos empíricos para serem preenchidas, é essencial selecionar modelos de estudo (e.g., ecossistema, espécies de plantas e de polinizadores) capazes de fornecer dados e informações relevantes para o desenvolvimento e esclarecimento das perguntas subjacentes a essas lacunas de conhecimento.

1.1.5 Abelhas, sua ampla gama de utilização dos recursos florais, e importância para o funcionamento do ecossistema

Considerando toda a guilda de polinizadores, as abelhas compõem o grupo que explora a maior amplitude de recursos florais oferecidos pelas plantas. Essa diversidade no uso de recursos se deve à sua dependência de distintas recompensas florais ao longo de todo seu ciclo de vida – larvas consumindo pólen e adultas consumindo néctar, por exemplo (Figura 1.4). Por serem amplamente distribuídas em diversos domínios fitogeográficos globais e apresentarem uma alta quantidade de espécies (Michener 2000), abelhas são responsáveis por assegurar a polinização de uma parcela significativa de plantas ao redor do mundo (Hung et al. 2018; Klein et al. 2018), incluindo diversas culturas de relevância econômica (Brown & Paxton, 2009; Khalifa et al. 2021).

A dependência das principais culturas globais em relação aos polinizadores, em particular as abelhas, representa uma questão de extrema relevância para a sociedade, diretamente impactando o bem-estar humano (Klein et al. 2018). Cerca de 75% das culturas fundamentais para alimentação da humanidade contam com o serviço ecossistêmico de polinizadores, sendo que mais de 90% delas, em alguma medida, dependem das abelhas para a polinização (Klein et al. 2007; Potts et al. 2016). Em um cenário carente de polinizadores, especialmente as abelhas, as perdas econômicas chegam a aproximadamente U$ 160 bilhões anualmente (Gallai et al. 2009). O aumento dessas perdas sobe para U$ 334 bilhões por ano, ao considerar outros serviços ecossistêmicos prestado pelas abelhas que são relevantes para as metas de desenvolvimento sustentável global (Bauer & Wings, 2010; Patel et al. 2021).

A maior biodiversidade global de abelhas é encontrada na região Neotropical. Pelo menos 5.000 das 20.000 espécies descritas em todo o mundo estão presentes nessa região (Moure et al. 2007; Almeida et al. 2023), tornando-a um centro prioritário para conservação de polinizadores e, em particular, de abelhas. No Brasil, por exemplo, se a crise dos polinizadores se agravar, as estimativas indicam uma possível perda econômica anual que varia de U$ 4,86 a 14,56 bilhões (Novais et al. 2016) e um déficit nutricional previsto de até 30% ao povo brasileiro (Porto et al. 2021).

Dentro do contexto ecológico-evolutivo, sendo as abelhas imprescindíveis para garantir a reprodução de uma grande quantidade de plantas globalmente, muito da diversidade de formas, cores, odores e recursos florais das angiospermas se deve em parte às pressões seletivas diretas em indiretas mediadas por elas (Pellmyr 1982). Desta maneira, as abelhas representam um grupo modelo chave para entender como a diversidade e distribuição de recursos florais no espaço e no tempo influencia a interação entre plantas e polinizadores, especialmente onde tal assembleia tem papel crucial para a reprodução de porção considerável da comunidade de angiospermas, como no Cerrado brasileiro (Gottsberger & Silberbauer-Gottsberger 2006).

Figura 1.4 Espécie vegetal *Byrsonima coccolobifolia* (Malpighiaceae) sendo visitada por abelhas em busca de recursos florais distintos. A espécie de abelha *Paratrigona* sp. se acerca da flor de *B. coccolobifolia* em busca de pólen (A). A abelha da tribo Tapinotaspidini raspando os elaifóros de *B. coccolobifolia* e alojando o óleo floral oferecido pela espécie vegetal em suas escopas corbiculares (B). Nota-se, neste conjunto de figuras, que em uma mesma flor, diferentes grupos de abelhas podem coletar distintos recursos florais, fazendo deste grupo de polinizadores aquele que usa o mais amplo leque de recursos florias oferecidos pelas plantas aos seus visitantes. Nota-se, ainda, que uma mesma planta se apresenta como um recurso comum (i.e., pólen) ou raro (i.e., óleo) a depender da identidade da abelha que a visita. Faz-se portanto necessáro não apenas identificar a identidade das espécies interagentes mas também o tipo de recursos mediador desta interação, uma vez que cada tipo de recurso floral, tal como pólen e óleo, podem mediar interações mais generalistas ou especializadas, respectivamente. Fotos: Caio S. Ballarin

1.1.6 O Cerrado como ecossistema modelo para investigar interação entre plantas e abelhas em diferentes níveis de organização biológica

O Cerrado é um mosaico fitofisionômico (Oliveira & Marquis 2002) que apresenta uma alta diversidade de plantas, que por sua vez, possuem sistemas variados de polinização (Gottsberger & Silberbauer-Gottsberger 2006). Por distribuir-se ao longo de dois milhões de km² do território brasileiro, ocupando uma ampla extensão latitudinal, longitudinal e altitudinal, o Cerrado apresenta grandes contrastes climáticos, cuja influência na composição de atributos florais de uma determinada espécie vegetal faz-se presente. Neste sentido, o Cerrado se apresenta como um modelo interessante para avaliar como a distribuição e diversidade de recursos florais influencia a assembleia de polinizadores. Isto é válido especialmente quando considerando espécies vegetais visitadas por abelhas, que forrageiam

em uma diversidade significativa de flores, e também considerando espécies vegetais com distribuição ampla dentro do ecossistema e que, portanto, estão sujeitas a condições bióticas e abióticas diversas.

Considerando as espécies vegetais amplamente distribuídas no Cerrado, o pequi, *Caryocar brasiliense* (Caryocaraceae), se destaca como uma espécie-chave por interagir com várias espécies de animais que utilizam suas folhas, flores e frutos como recurso alimentar. Por ser abundante e apresentar uma floração massiva, o pequi atrai um grande número de visitantes florais, incluindo espécies de grupos funcionais distintos, tais como, beija-flores, abelhas, vespas, esfingídeos, morcegos, e até mesmo marsupiais, os quais utilizam o néctar e o pólen produzido em abundância como recursos alimentares. No Cerrado, o pequi apresenta dois ecótipos: *Caryocar brasiliense* subsp. *brasiliense*, que tem porte arbóreo (*i.e.*, fanerófito) e possui uma ampla distribuição ao longo de todo o ecossistema; e o *Caryocar brasiliense* subsp. *intermedium* (pequi-anão) que possui porte arbustivo com tronco principal sob o solo (*i.e.*, hemicriptófito), e uma distribuição mais restrita, limitando-se a áreas de altitudes mais elevadas na região meridional do Cerrado (Prance & Da Silva 1973; Figura 1.5). Espécies com distribuição geográfica ampla e sujeitas a condições climáticas, topográficas e edáficas distintas podem apresentar diferenças fenotípicas marcantes entre as distintas populações ao longo de sua distribuição (Etterson & Mazer, 2016). Tais diferenças podem se refletir nos processos ecológicos nos quais a espécie está envolvida. Por exemplo, observações prévias apontam que não apenas a forma de vida, mas também os órgãos reprodutivos do ecótipo anão do pequi apresentam alterações morfológicas significativas (*observações pessoais* Amorim, F. W.). Essa particularidade torna o pequi um modelo de estudo interessante para também preencher lacunas relacionadas às populações de espécies que potencialmente apresentam variações na apresentação de recursos florais aos polinizadores ao longo de sua distribuição geográfica.

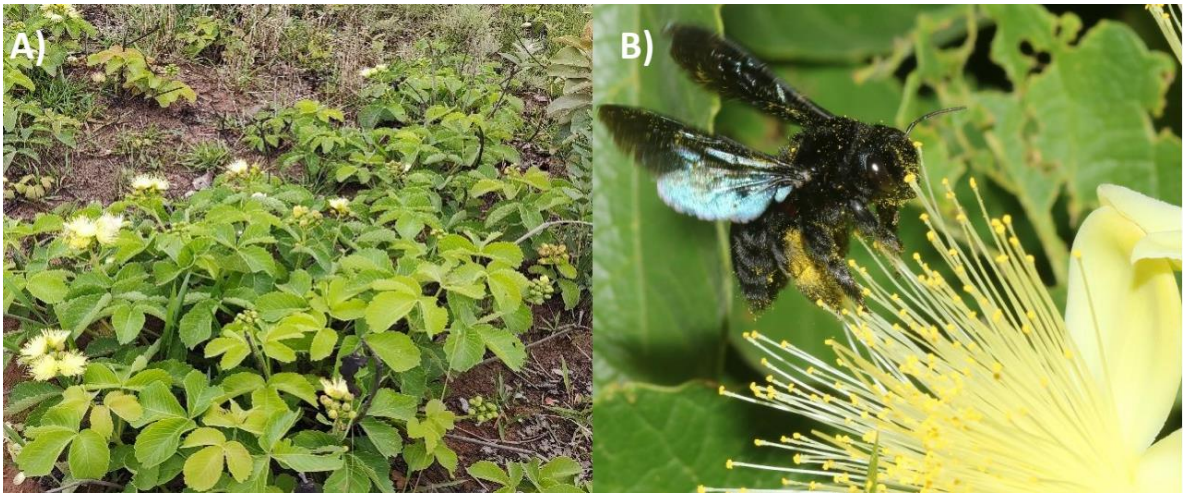

Figura 1.5 Ecótipo anão do pequi (*Caryocar brasiliense* subsp. *intermedium*, Caryocaraceae) e seu principal visitante floral. O ecótipo anão do pequi tem todo seu tronco escondido sob o solo (A). Após um evento de queimada, somente sua folhas e flores estão expostas sobre o solo. Sua parte aérea pode alcançar cerca de no máximo 1,5 metros de altura. Os principais visitantes do pequi-anão são abelhas de grande porte, tal como a espécie de *Xylocopa* sp. (B). Fotos: CSB (A); Felipe Amorim (B).

1.1.7 A preciosidade da Floresta Estadual de Botucatu (FEB): um mosaico fitofisionômico de Cerrado preservado no município

Embora demonstre ser um modelo promissor para a pesquisa das interações entre plantas e polinizadores, o Cerrado, um *hotspot* de biodiversidade para conservação da biodiversidade (Myers et al. 2000), tem enfrentado múltiplas pressões de origem antrópica que comprometem a dinâmica de suas comunidades ecológicas (Strassburg et al. 2016). Em contrapartida, a implementação de medidas eficazes para conter a degradação ambiental nesse ecossistema tem sido escassa em comparação a outros ecossistemas ameaçados (Klink & Machado, 2005). Por exemplo, atualmente, a cobertura do Cerrado representa apenas 1% do território do estado de São Paulo, contrastando com os 14% que originalmente abrangia (Bitencourt, 2001; Kronka et al. 2005). Do reduzido percentual remanescente, apenas 18% encontra-se resguardado em Unidades de Conservação e Reservas Legais (Bitencourt, 2001). Portanto, o Cerrado, especialmente no contexto do estado de São Paulo, emerge como um

ecossistema prioritário para investigações relacionadas à conservação da biodiversidade, tal como o estudo da interações planta-polinizador mediadas por recursos florais.

O município de Botucatu está localizado na região centro-sul do estado de São Paulo, Brasil (22°53'09"S, 48°26'42"O; 920 m.a.n.m). Botucatu ocupa uma zona ecotonal de Cerrado e Mata Atlântica (Ishara et al. 2008), abrigando, portanto um mosaico fitofisionomomico de ecossistemas campestres e florestais. Entretanto, seguindo a tendência global que se reflete nacionalmente (Walter et al. 2015; Silveira et al. 2022), a maioria dos estudos relacionados à levantamentos fitossociológicos conduzidos em Botucatu não utilizaram fitofisionomias campestres como sistema de estudo (Silberbauer-Gottsberger et al. 1977; Ishara et al. 2008, porém veja Pilon et al. 2017). Embora abrigue substancial parcela da biodiversidade global (Bond & Parr, 2010; Murphy et al. 2016), no território brasileiro, estes ecossistemas campestres integram somente 10% dos remanescentes de Cerrado (Durigan & Ratter, 2016). Estando ameaçados nacional e globalmente (Parr et al. 2014; Strömberg & Staver, 2022), faz-se necessário, pois, desenvolver novas investigações sobre estes tipos de ecossistemas no cenário brasileiro.

A Floresta Estadual de Botucatu (FEB) é um área remanescente de Cerrado sob a administração da prefeitura do Município de Botucatu com cerca de 33,4 hectares, que apresenta três tipos de fitofisionomias: campo sujo, campo limpo úmido e mata de galeria inundável (Pilon et al. 2017). Enquanto a mata de galeria congrega majoritariamente espécies vegetais de hábito arbóreo, as duas fitofisionomias remanescentes (i.e., campo sujo e campo limpo úmido) são majoritariamente representadas por espécies vegetais de hábito herbáceo-arbustivo (Pilon et al. 2017; Figura 1.6). As vegetações campestres da FEB abrigam mais de 250 espécies vegetais (Pilon et al. 2017 e *observações pessoais* Ballarin, C. S.), que são expostas a eventos recorrentes de fogo e eventos esporádicos de geada (Figura 1.7).

Figura 1.6 Florestal Estadual de Botucatu (FEB). Abrigando três fitofisionomias – campo sujo, campo limpo úmido e mata de galeria inundável – a FEB é predominantemente ocupada por vegetação de hábito herbáceo-arbustivo. Foto: Diego S. Podadera

Por estar recorrentemente submetida à eventos de fogo, muitas das espécies vegetais presentes na FEB possuem adaptações evolutivas em resposta às pressões seletivas desse distúrbio que: 1) lhes confere tolerância ao fogo, tais como casca espessa, gemas protegida sobre ou sob o solo, e resistência de sementes (Keeley et al. 2011; Fichino et al. 2016; Chiminazzo et al. 2021, 2023) ou 2) as induz adentrar em estágios ecofisiológicos distintos tais como, rebrota, germinação de sementes, floração e surgimento de nectários extraflorais (Alves-Silva & Del-Claro, 2014; Fidelis & Blanco, 2014; Fidelis et al. 2016; Zirondi et al. 2019; Fidelis & Zirondi, 2021; Pilon et al. 2021). Em especial, a rebrota, a germinação de sementes, a floração, e o surgimento de nectários extraflorais permitem com que novas interações entre plantas e animais sejam estabelecidas, uma vez que novas plântulas, folhas, flores e nectários ativos estão disponíveis para herbívoros, polinizadores e defensores (i.e., formigas patrulheiras) interagirem (Ballarin et al. 2024). Todavia, estudos relacionando

efeitos do fogo na reconfiguração das interações entre plantas e animais, tal como interações planta-polinizador, são escassos no Brasil (Ballarin et al. 2024).

Para além de ser uma área de estudo que se mostra profícua para preencher lacunas do conhecimento sobre interações planta-animal, tal como plantas e polinizadores mediadas por recursos florais, e efeitos do fogo na configuração espaço-temporal de espécies vegetais, a FEB possua uma notável diversidade de plantas e abelhas. Observações decorrentes desta Tese de Doutorado e de revisões da literatura sugerem que, apesar de sua pequena extensão, a FEB abriga ao menos oito espécies vegetais raras e/ou ameaçadas de extinção no estado de São Paulo: *Evolvulus fuscus* (Convolvulaceae), *Curtia tenuifolia* (Gentianaceae), *Camarea hirsuta* (Malpighiaceae), *Andropogon hypogynus* (Poaceae), *Borreria argentea* e *Richardia stellaris* (Rubiaceae), *Xyris brevifolia* (Xyridaceae), e *Oxypetalum capitatum* (Apocynaceae; Pilon et al. 2017; *observações pessoais* Ballarin C. S.); e ao menos 52 espécies de abelhas, com elevada diversidade funcional e filogenética (e.g., abelhas generalistas e eussociais, abelhas coletoras de óleo, abelhas coletoras de fragrância florais, abelhas cortadeiras e abelhas capazes de sonicar; *observações pessoais* Ballarin C. S.).

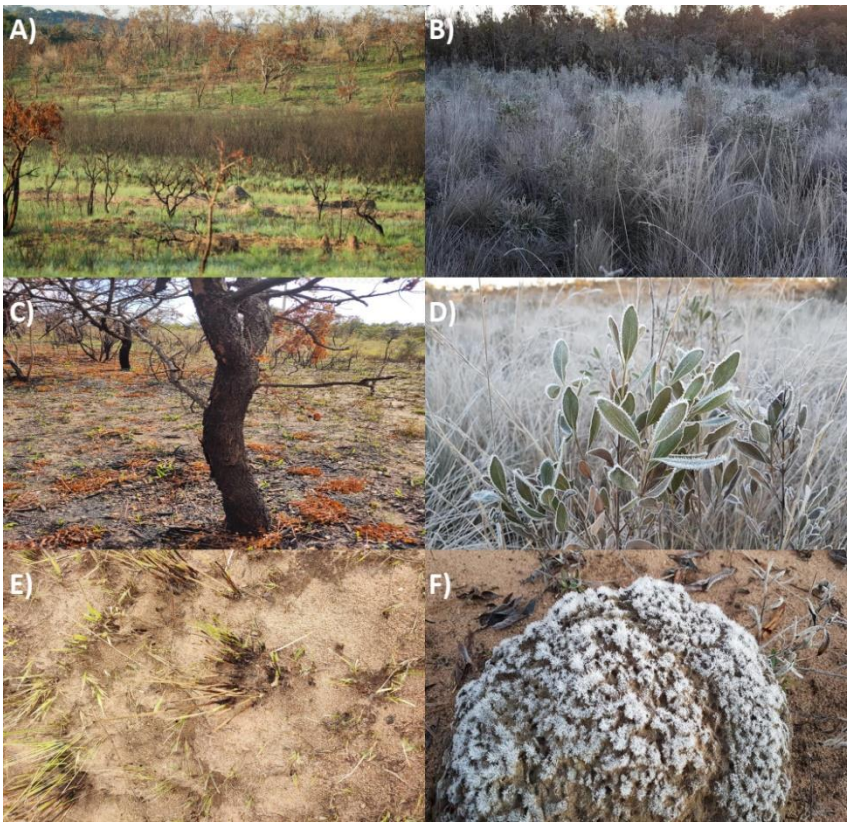

Figura 1.7 Eventos de fogo e geada na FEB. Ambos os distúrbios ambientais (i.e., fogo e geada) afetam a vegetação. A recorrência destes definirá se eles representam uma pressão seletiva às plantas sujeitas a esses impactos. De maneira mais recorrente, o fogo influencia a comunidade vegetal (A). Algumas espécies de plantas apresentam cascas grossas que as permitem resistir ao fogo (C). Já outras, podem não possuir tecido de crescimento secundário e perdem sua parte aérea. Entretanto, são resilientes ao fogo pois logo rebrotam a partir da indução de crescimento de tecidos jovens (E). A geada por sua vez é um evento menos recorrente na FEB, ocorrendo em intervalos maiores de tempo. Ainda assim, ela também afeta a parte área da comunidade vegetal (B). Essa espécies de planta podem ter suas folhas cobertas por camadas de gelo (D). Ao depositar-se sobre o solo (F), camadas de gelo também podem atingir plantas herbáceas e rasteiras. Fotos: Felipe Amorim (B, D, e F); Diego S. Podadera (A); Caio S. Ballarin (C; E).

1.1.8 Implicações do estudo sobre recursos florais para o avanço epistemológico e aplicado da Ecologia em múltiplos níveis de organização biológica.

O preenchimento das lacunas supracitadas através da utilização do Cerrado como ecossistema, e interações planta-abelha mediadas por recursos florais como modelo de estudo, se mostra cabível uma vez que, embora essenciais à sobrevivência e reprodução do visitantes florais e, particularmente, à reprodução das angiospermas, recursos florais apresentam elevada plasticidade e, portanto, estão sujeitos às pressões antrópicas que ameaçam a biodiversidade globalmente. Por exemplo, entender o padrão de distribuição de recursos florais ao redor do globo pode servir como um dado base para previsões da distribuição de espécies de plantas e animas em cenários futuros de mudanças climáticas, uma vez que diferentes recursos florais atendem às demandas ecológicas de diferentes visitantes florais, e recursos florais variam quanto a sua susceptibilidade à alterações no ambiente ocasionadas por mudanças climáticas. Por exemplo, considerando o néctar uma solução aquosa com presença marcante de compostos orgânicos bastante simples, tais como mono e dissacarídeos (*i.e.*, açúcares; Heil 2011), será que o néctar tem maior susceptibilidade ao aquecimento global que o grão de pólen, composto por células, proteínas, aminoácidos e outras estruturas mais complexas e menos voláteis que os açúcares e a água?

Da mesma forma, compreender como a distribuição de recursos florais no espaço e no tempo afeta a interação entre plantas e polinizadores em uma dada comunidade biológica pode ser fundamental para antever efeitos em cascata gerados por perturbações que alteram a abundância relativa ou mesmo extinguem localmente espécie interagentes. Por exemplo, o preenchimento desta lacuna do conhecimento pode auxiliar na tomada de decisão sobre a conservação de comunidade biológicas que estão em maior risco de colapsarem ecologicamente (Carpenter, 2002). Adicionalmente, entender como se dá a relação de cada espécie de visitante floral e, em especial, as abelhas, com cada tipo de recurso floral presente em uma dada comunidade pode ser importante para assistir na conservação de grupos de espécies de polinizadores com demandas mais específicas ou para apontar espécies vegetais que integram mais eficientemente a rede de interação planta-polinizador devido aos recursos que oferecem (veja Armbruster, 2017). Por exemplo, muitas iniciativas de restauração ecológica se baseiam na estratégia espécie-foco para recuperar um ambiente degradado (Lambeck 1997; Simberloff, 1998). A estratégia de restauração espécie-foco é especialmente atraente por ter justamente o foco em um subconjunto representativo da comunidade

ecológica, minimizando custos e esforços (Lindenmayer et al. 2002). Geralmente, a seleção de espécies focais nesta abordagem prioriza espécies guarda-chuva, cuja sensibilidade garante que ao recuperá-la, espécies menos sensíveis também serão; e espécies-chave, cujo efeito na estrutura da comunidade é muito maior daquele esperado apenas pela sua abundância, devido às múltiplas funções que ela desempenha na comunidade (veja Power et al. 1996). Portanto, entender quais espécies de plantas e de polinizadores possuem demandas mais específicas (*i.e.*, espécies guarda-chuva) e quais espécie integram mais eficiente a rede de interação planta-polinizador ao nível da comunidade (*i.e.*, espécies-chave) representa uma avanço importante também dentro da área da Ecologia da Restauração (Ballarin et al. 2023). Ainda, entender como os recursos florais são impactados por distúrbios recorrentes, tal como o fogo no Cerrado brasileiro (Coutinho, 1990), pode ser importante para diagnosticar a resistência e resiliência de um ecossistema ecológico mediante a alterações no ambiente.

Por fim, comparar a produção e apresentação de recursos florais entre diferentes populações de uma mesma espécie pode servir como modelo para prever caminhos evolutivos que outras populações submetidas à condições abióticas semelhantes podem seguir mediada pela interação com polinizadores. Considerando, por exemplo, que a distribuição das espécies vegetais poderá alterar-se em resposta às mudanças climáticas, a comparação do sistema de polinização de populações distintas de uma mesma espécie pode ser importante também para antever qual será o impacto que a alteração na sua distribuição geográfica vai gerar no serviço de polinização biótica.

1.2 OBJETIVOS E HIPÓTESES

Considerando a pequena atenção dada a diversidade, distribuição espaço-temporal, e influência dos recursos florais nas interações planta-polinizador em distintos níveis de organização biológica, esta Tese de Doutorado tem como objetivo central analisar os potenciais padrões biogeográficos relacionados à distribuição global dos recursos florais, bem como caracterizar a sua importância nas relações entre plantas e polinizadores nos níveis da comunidade e da população. Em particular, os objetivos específicos são:

***(A) Biosfera**:*

Capítulo I – Neste capítulo foi realizada uma revisão sistematizada com intuito de descrever padrões biogeográficos relacionados à distribuição do néctar floral nas comunidades de plantas em um contexto global. Especificamente, foram direcionadas as seguintes perguntas: i) quantas angiospermas polinizadas por animais produzem néctar floral? ii) Também foi investigado se a distribuição de angiospermas com néctar e a diversidade de recursos florais ao redor do globo estão relacionadas a fatores biogeográficos, tal como latitude, altitude, bioma, e a fatores climáticos, tal como temperatura e precipitação. A hipótese examinada é a de que a latitude está relacionada com a distribuição de angiospermas que produzem néctar, dado que ela reflete gradientes climáticos de temperatura e precipitação que exercem influência sobre a distribuição de espécies no globo. Tendo em vista que zonas tropicais apresentam maior riqueza de espécies (Brown 2014), associada a uma dissimilaridade funcional ampliada entre as espécies coocorrentes (Reu et al. 2018), comunidades vegetais tropicias podem apresentar estratégias mais dissimilares para atrair polinizadores mediante a oferta de recursos florais. Assim, comunidades tropicais apresentarão maior riqueza e diversidade de recursos mediadores da interação planta-polinizador tal como, pólen, óleo, perfumes e resinas, contribuindo para diminuir a proporção de plantas que oferecem néctar aos seus polinizadores. Portanto, em termos gerais, comunidades localizadas em menores latitudes terão uma menor proporção de plantas produtoras de néctar quando comparadas às comunidades localizadas em latitudes mais elevadas.

(B) Comunidade:

Capítulo II – Neste capítulo foi avaliado se a alteração no espaço e no tempo da diversidade de recursos florais afeta a topologia da rede de interação planta-abelha em um Cerrado brasileiro. A hipótese considerada é a de que, em se tratando de um ecossistema inflamável, sazonal e que possui agregados florísticos, há

alteração marcada na diversidade de recursos florais no espaço e no tempo. Devido ao fato de diferentes espécies de abelhas buscarem distintos recursos florais, a alteração na diversidade de recursos florais tem influência na estrutura e configuração da rede de interação planta-abelha. Desta maneira, presume-se que quanto maior a diversidade de recursos em uma unidade espaço-temporal, mais compartimentalizada será a rede de interação emergente da relação entre as plantas e abelhas. Em contrapartida, quanto menor a diversidade de recursos, mais aninhada será a rede de interação planta-abelha (*i.e.*, espécies interagentes generalistas compõe um núcleo de espécies que interagem entre si e com espécies especialistas, que por sua vez, somente interagem com as espécies generalistas).

Capítulo III – Neste capítulo, redes de interação envolvendo plantas e abelhas que coletam diferentes recursos florais foram descritas e comparadas. Tendo em vista que diferentes grupos taxonômicos de abelhas podem forragear em busca de distintos recursos florais, neste capítulo, a topologia e a composição da rede de interação formada por intra-guildas de abelhas que visitam flores oferecendo distintos tipos de recursos florais (*e.g.*, néctar, pólen e óleo) foram estudadas. Ainda, foi aplicado uma abordagem de rede de interação multicamadas (i.e., redes de interação com duas ou mais camadas, assim representadas por apresentarem diferentes categorias de, por exemplo, tipos de interação ou unidades espaço-temporais; Pilosof et al. 2017) com o intuito de avaliar se grupos de abelhas distintos são responsáveis por conectar e manter estável as diferentes redes de interação mediadas por distintos recursos florais (i.e., camadas de rede). A hipótese examinada é a de que redes de interação mediadas por néctar ou pólen apresentam assembleias de abelhas mais similares entre si do que quando comparadas com a assembleia de abelhas que interage com flores mediante a oferta de óleo. As redes de interação planta-abelha mediadas por óleo serão mais aninhadas e terão menor grau de especialização, uma vez que este tipo de interação é composto por grupos funcional e filogeneticamente concisos de plantas e abelhas (Bezerra et al. 2009; Davis et al. 2014). Por sua vez, redes de interação planta-abelha mediadas por néctar e pólen apresentarão maior nível de

especialização por contemplar interações entre grupos de flores e abelhas com mais elevado grau de dissimilaridade funcional. Finalmente, devido ao fato de visitarem uma ampla gama de espécies vegetais (Giannini et al. 2016), ainda foi hipotetizado que abelhas super-generalistas são reponsáveis por conectar as camadas da rede interação mediada por diferentes recursos florais e mantê-las estáveis.

Capítulo IV – Neste capítulo foi avaliado se o fogo, distúrbio endógeno do Cerrado brasileiro, afeta a diversidade de recursos florais no espaço e no tempo bem como a estrutura e estabilidade da rede de interação planta-abelha. Testou-se a hipótese de que o fogo aumenta a dissimilaridade funcional de recursos florais no espaço e no tempo. A alteração na disponibilidade de recursos florais no espaço e tempo modificará a estrutura da rede de interação planta-abelha por meio da reconfiguração espaço-temporal das interações entre esses organismos, alterando também sua estabilidade.

(C) População

Capítulo V – Neste capítulo, nós caracterizamos as variações intraespecíficas na biologia reprodutiva, bem como na dinâmica de produção do néctar entre os ecótipos anão e arbóreo de *Caryocar brasiliense*, considerando populações distribuídas em regiões distintas do Cerrado brasileiro. Foram dirigidos os seguintes questionamentos: i) Considerando o "princípio do polinizador mais efetivo" de Stebbins (1970), será que o ecótipo anão do pequi está submetido à pressões seletivas mediada por polinizadores de modo a promover a troca de polinizador em relação ao ecótipo arbóreo? ii) Existem mudanças na dinâmica de produção do néctar entre os distintos ecótipos de *C. brasiliense*? iii) Caso haja diferença, isso afeta a frequência e a qualidade do serviço de polinização realizado por visitantes diurnos (*i.e.*, abelhas) e noturnos (*i.e.*, morcegos)? A hipótese a ser examinada aqui é que o pequi-anão está sofrendo uma pressão seletiva em direção aos visitantes florais diurnos. Assim sendo, os atributos florais do pequi-anão

estão sujeitos à seleção natural mediada por abelhas, que, dessa forma, diferem-se daqueles observadas no ecótipo arbóreo do pequi, majoritariamente polinizado por morcegos (Gribel & Hay 1993).

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2 CAPÍTULO I

How many animal-pollinated angiosperms are nectar-producing? *

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Research

How many animal-pollinated angiosperms are nectar-producing?

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Summary

• The diversity of plant–pollinator interactions is grounded in floral resources, with nectar considered one of the main floral rewards plants produce for pollinators. However, a global evaluation of the number of animal-pollinated nectar-producing angiosperms and their distribution world-wide remains elusive.

• We compiled a thorough database encompassing 7621 plant species from 322 families to estimate the number and proportion of nectar-producing angiosperms reliant on animal pollination. Through extensive sampling of plant communities, we also explored the interplay between nectar production, floral resource diversity, latitudinal and elevational gradients, contemporary climate, and environmental characteristics.

• Roughly 223 308 animal-pollinated angiosperms are nectar-producing, accounting for 74.4% of biotic-pollinated species. Global distribution patterns of nectar-producing plants reveal a distinct trend along latitudinal and altitudinal gradients, with increased proportions of plants producing nectar in high latitudes and altitudes. Conversely, tropical communities in warm and moist climates exhibit greater floral resource diversity and a lower proportion of nectar-producing plants.

• These findings suggest that ecological trends driven by climate have fostered the diversification of floral resources in warmer and less seasonal climates, reducing the proportion of solely nectar-producing plants. Our study provides a baseline for understanding plant–pollinator relationships, plant diversification, and the distribution of plant traits.

2.1 INTRODUCTION

The resource base for interacting species forms the foundation of plant-pollinator mutualisms, shaping species traits and interaction composition (Ollerton, 2006). For instance, a community's diversity of floral visitors depends on various floral resources like nectar, pollen, oil, resins, fragrances, brood sites, tissues, and heated trysting places (Vogel, 1963, 1971; Fægri & van der Pijl, 1979; Armbruster, 1984, 2012; Gottsberger, 1989). Notably, floral nectar, an aqueous sugar solution, is consumed by a broad spectrum of floral visitors worldwide, including different groups of mammals, birds, lizards, and various insect clades (Fægri & Pijl, 1979, but see also Amorim *et al*., 2023, Cozien *et al*., 2019, Figure 2.1). Nectar and nectarlike secretions in flowering and non-flowering plants serve as a crucial link to understanding the evolution of animal pollination (Lloyd & Wells, 1992; Kato & Inoue, 1994; Ren *et al*. 2009; Ollerton & Coulthard, 2009; Nepi *et al*., 2009, 2018; Nepi, 2017; Parachnowitsch *et al*., 2018).

Despite the overwhelming importance of nectar in the ecology and evolution of plant-pollinator mutualisms, there are still elementary unanswered questions regarding nectar spatiotemporal distribution and its role in shaping ecological communities (Pacini *et al*., 2003; Mitchell, 2004; Pyke, 2016; Roy *et al*., 2017; Pyke & Ren, 2023). For instance, over decades, numerous authors have recurrently asserted that "most flowering plants produce nectar", often concluding that most animals visiting flowers rely on nectar as one of the main floral resources angiosperms produce for pollinators (e.g. De la Barrera & Nobel, 2004; Brandenburg *et al*., 2009; Heil, 2011, Parachnowitsch *et al*., 2018). Nevertheless, there is still a lack of estimates for the proportion of animal-pollinated plants that produce nectar as a floral reward and the distribution of nectar-producing plants across plant communities worldwide. Such quantitative estimates can serve as a heuristic basis to discuss macroecological questions related to the organisation of plant-pollinator interactions, the energy flow in ecological communities, and the ecosystem services these interactions can provide.

Recently, an extraordinary effort has been made to gather data on plant distribution and their functional traits across the globe (Kier *et al*., 2005, 2009; Kreft & Jetz, 2007;

Weigelt *et al*., 2019; Kattge *et al*., 2020; Cai *et al*., 2021; Tietje *et al*., 2023). Despite these efforts, it is widely acknowledged that many important gaps remain to be filled (Kattge *et al*., 2020; Conti *et al*., 2023). Specifically, several plant functional traits related to pollination are underrepresented in datasets (Kier *et al*., 2005; Ollerton *et al*., 2015; Kattge *et al*., 2020; E-Votjkó *et al.*, 2020). Therefore, macroecological studies regarding the floral traits related to angiosperm pollination are still needed (Kreft & Jetz, 2007; Rech *et al*., 2016; Kuppler *et al*., 2020; Song *et al*., 2022). For instance, if most flowering plants produce nectar as a primary floral resource, variations in the proportion of plants producing nectar across the globe can significantly affect the identity and diversity of anthophilous animals supported within a given community. Exploring such data can help identify macroecological patterns in communities worldwide and support decision-making to conserve plant-pollinator interactions, which are crucial for preserving undisturbed ecosystems and restoring degraded ones (Cusser & Goodell, 2013; Devoto *et al.*, 2012; Ollerton, 2017).

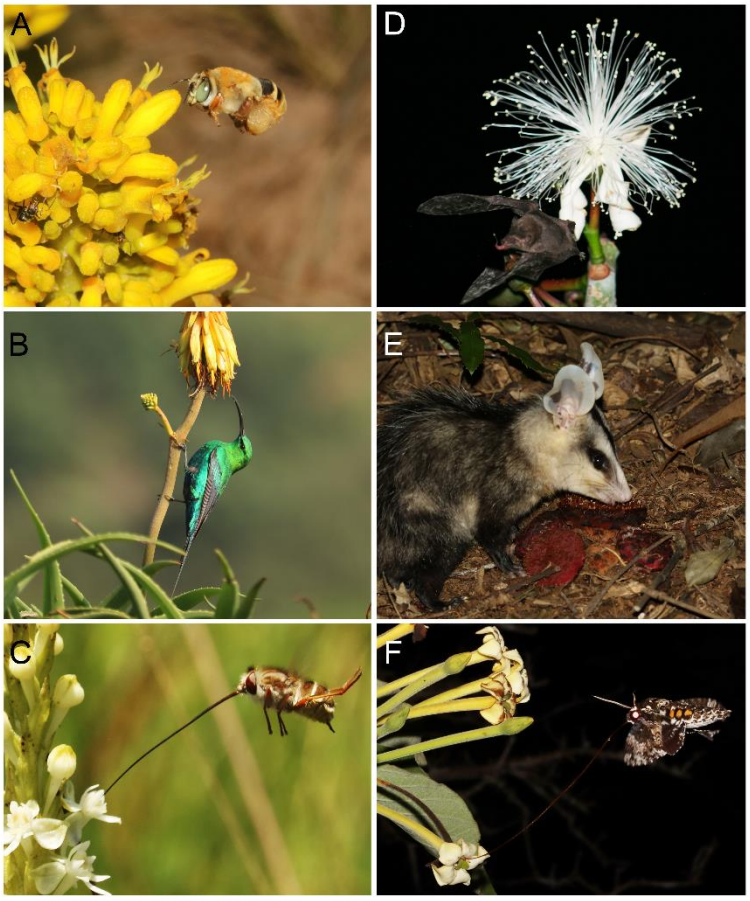

Figure 2.1 Diversity of diurnal (left panel) and nocturnal (right panel) pollinators that feed on floral nectar of different groups of plants. A) The oil-collecting bee, *Centris nitens*, approaching the nectar-producing flowers of *Palicourea rigida* Kunth (Rubiaceae). B) The Malachite sunbird, *Nectarinia famosa*, visiting the flowers of *Aloe striatula* Haw (Asphodelaceae). C) A long-tongued Nemestrinidae fly (possibly an undescribed *Prosoeca* species) visiting the flower of *Habenaria dives* Rchb.f. (Orchidaceae). D) The great fruit-eating bat, *Artibeus lituratus*, visiting the flower of *Pseudobombax longiflorum* (Mart.) A.Robyns (Malvaceae). E) The White-eared opossum, *Didelphis albiventris*, licking nectar on the inflorescence of the holoparasitic *Scybalium fungiforme* Schott & Endl. (Balanophoraceae). F) The long-tongued hawkmoth, *Manduca rustica* (proboscis 13 cm long), visiting the flower of *Tocoyena formosa* (Cham. & Schltdl.) K.Schum. (Rubiaceae). Photos: Felipe Amorim.

Understanding how latitudinal variation and environmental factors influence plant-pollinator interactions is a topic of ongoing debate (Olesen & Jordano, 2002; Ollerton & Cranmer, 2002; Schleuning *et al*., 2012). Over a decade ago, a similar study by Ollerton and associates extended this debate by estimating how many flowering plants are pollinated by animals and how these plants are distributed across the globe (see Ollerton *et al.*, 2011). This study raised multiple biological questions on plant-pollinator interactions and contributed more accurate statistics to promote better decision-making to conserve these relationships. In the present study, to address the question of "how many animal-pollinated angiosperms are nectar-producing", we conducted a comprehensive compilation of the nectar-producing plants across the globe, encompassing plant species and plant communities in all continents and biomes except Antarctica. We used the available information on the number of nectar-producing angiosperms to (1) estimate the number of animal-pollinated plants that offer nectar as a floral reward, (2) evaluate whether there is a relationship between latitude, altitude, and the distribution of nectar-producing animal-pollinated plants in communities worldwide and (3) assess what climate and environmental variables influence the distribution of nectar-producing angiosperms globally.

2.2 MATERIALS AND METHODS

2.2.1 Floral nectar production in flowering plants

We thoroughly examined existing research on floral biology and rewards to construct a comprehensive dataset on animal-pollinated nectar-producing angiosperms. We compiled both focal-species studies that focused on the reproductive biology of specific plant species, irrespective of the local or regional plant community, and community-level studies, which reported surveys that sampled a significant portion of plant species locally (Figure 2.2a). As we concentrate on nectar as a reward for pollination services, we restricted our analyses to plant families with at least one animal-pollinated species (following Dodd *et al*., 1999). Additionally, we considered nectar produced by floral and extrafloral nectaries with nuptial function, *i.e.*, those involved in the pollination process (Bernardello, 2007). We used the World Flora Online platform (hereafter WFO Plant List 2022) as a taxonomic reference.

2.2.2 Focal-species and community-level datasets

To compile data about plant species belonging to different families (i.e., focal-species dataset), we employed a standard search method using key phrases that included the name of a specific plant family and terms related to reproductive biology: [*The name of plant family* (e.g. Cactaceae)] AND (*pollinat* OR *floral resources* OR *floral biology*; Supplementary Figure S2.1), examining publications spanning from 1913 to November 2022 in the Google Scholar and Scopus databases. We included only studies that explicitly indicated animal pollination on the studied plant species. Since we aimed to include at least 1% of each sampled plant family (Figure 2.2b), the WFO Plant List platform was used to record the total number of species within each family. Although we only considered families that contained animal-pollinated species, some families have a few wind-pollinated species, e.g., *Thalictrum* L. in Ranunculaceae (Bernardello, 2007). Conversely, other families, such as Moraceae (Datwyler & Weiblen, 2004), Cyperaceae (Wragg & Johnson, 2011), and Juncaceae (S.Q. Huang *et al*., 2013), primarily consist of wind-pollinated species but also include a few animal-pollinated species. We accounted for both contrasting cases in our analysis.

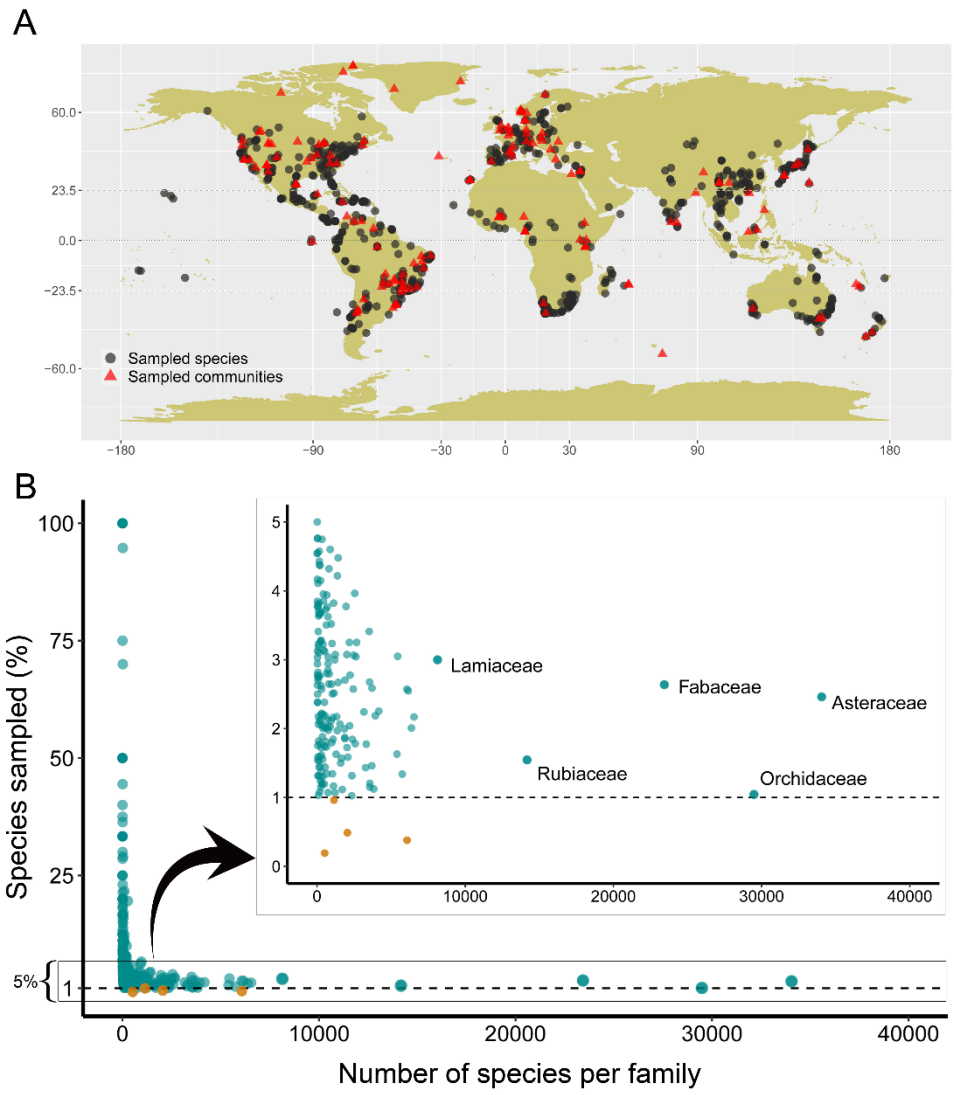

Figure 2.2 Overview of the spatial distribution of sampled plant species and communities globally and the representation of the sampling effort among angiosperm families in the dataset. A) Global map indicating the location of all plant species (dark grey circles) and plant communities (red triangles) sampled for this study, using both the taxonomic-based approach and the community-level (macroecological) approach. B) Sampling effort in each angiosperm plant family, represented by the percentage of species sampled per family. Plant families with more than 1% of total species samples are shown in blue, while those with less are shown in orange. Notably, sampling effort reached more than 1% in most plant families, including the five plant families that rely on animal pollination and have the largest number of species, such as Asteraceae, Orchidaceae, Fabaceae, Rubiaceae and Lamiaceae. There were only four families with a sampling below 1%, including Cyperaceae (0.4%), Fagaceae (0.9%), Juncaceae (0.2%), and Urticaceae (0.5%).

We verified the presence of nectar production in each compiled plant species. Most studies we used already contained information on nectar as a floral reward in the plant species included in our analyses. However, such information was missed in some studies. Consequently, we conducted further investigations for the species with missing information by reviewing related articles that dealt with the same plant species. Despite the additional effort, a small percentage of species (approximately 6% of the surveyed species) still lacked information on nectar production. In such exceptional cases, we relied on additional data available at the genus or family levels. This perusal search was standardised using the family or the genus name and pollination biology-related terms as key phrases (i.e., *pollinat* OR *floral resources* OR *floral biology*). The species was included in the dataset if we found reliable and precise information. Otherwise, it was excluded.

To compile data on plant communities worldwide, we focused on studies that surveyed the local plant community (i.e., community-level dataset). Our primary selection criteria included the surveys involving animal-pollinated species. Therefore, to avoid potential sampling biases from wind-pollinated species, we prioritised including community-level studies that addressed plant-pollinator interactions. Nevertheless, we also included extensive local floristic surveys in which information about plant-pollinator relationships could be assessed. We included plant-pollinator studies that evaluated all pollinator groups, irrespective of their taxonomic relatedness or focused explicitly on bee species. Studies with pollinator assemblages other than bees were excluded due to their strict reliance on nectar as a reward, such as hawkmoths and hummingbirds. Moreover, we also conducted an additional thorough review and excluded wind-pollinated species when necessary. Nevertheless, some studies recorded insect visits on wind-pollinated plants, and these plants were included because ambophily (i.e., pollination by both biotic and abiotic vectors) is known to be more common than previously thought (Culley *et al*., 2002).

2.2.3 Data analyses

We adopted a taxonomic approach to provide a global proportion of nectar-producing angiosperms worldwide by including our samples from focal species and community-level studies in the final dataset. Then, all recorded plant species were organised at the family level.

For each family, we calculate the proportion of plants producing nectar (P_{family}) by dividing the number of nectar-producing species ($n_{familiy}$) by the total number of recorded plant species (t_{family}). To estimate the number of nectar-producing plant species in each family (X_{family}), we extrapolated this fraction to the total number of species in each family (N_{family}; according to WFO Plant List). Finally, we pooled the estimated number of nectar-producing species for all recorded families (T_{global}) and derived the global ratio of nectar-producing species (P_{global}) by dividing the resulting value by the sum of species found in all registered families, as follows:

$$P_{family} = \frac{n_{family}}{t_{family}};$$

$$X_{family} = P_{family} \times N_{family};$$

$$T_{global} = \Sigma X_{family};$$

$$P_{global} = \frac{T_{global}}{\Sigma N_{family}}.$$

To obtain a global estimate of how many animal-pollinated angiosperms are nectar-producing, we used the estimation of animal-pollinated plant species provided by Ollerton *et al*. (2011). Given our goal to estimate floral nectar production globally, Ollerton *et al*. (2011) estimation was the most appropriate approach to avoid biases from including wind-pollinated species. Therefore, we calculated the number of nectar-producing plants among animal-pollinated species by multiplying Ollerton *et al*. (2011) estimate of 308,006 animal-pollinated plants by the proportion of nectar-producing plants obtained through our taxonomic approach (Supplementary Figure S2.1 Flow diagram of the search routine to estimate how many animal-pollinated plants are nectar-producing. **The red box shows an example of the keywords used in this study.**). Nonetheless, global angiosperm richness estimates vary based on data sources. Hence, the estimation of nectar-producing plants may also depend on the species count considered by each data source. To address this variation, we created a confidence interval (CI) for nectar-producing plants among animal-pollinated species. To obtain a 95% CI for our estimation, we used a bootstrap method with 1000

replications based on WFO, GBIF, APG IV, Paton *et al*. (2008), Christenhusz & Byng (2016) angiosperm richness estimation, and the recent update on animal-pollinated plants (Tong *et al*., 2023).

We also followed Ollerton *et al*. (2011) to estimate the proportion of nectar-producing angiosperms in different latitudinal zones. For this, we focused solely on community-level studies. Then, we categorised the plant communities following Ollerton *et al*. (2006) classification: Tropical communities (0º to 29º), Subtropical communities (30° to 39°), Temperate communities (40° to 64°), and Arctic communities (65° to 90°). For this analysis, we excluded species-level studies, as they did not provide a comprehensive survey of plant communities, making it challenging to obtain an accurate proportion of nectar-producing plants by latitude.

To assess the presence of latitudinal and altitudinal gradients in the proportion of nectar-producing angiosperms worldwide, we exclusively used data from native plant species from community-level studies (n = 162). We extracted the proportion of nectar-producing plant species ($P_{community}$) for each survey as our response variable. Then, for each community-level study, we recorded the latitude, longitude, altitude, biome (following Whittaker, 1962), insularity, and plant richness for each sampled plant community. Moreover, to avoid potential biases related to the sampling method of each study, we classified each community according to their sampling effort. For the sampling effort, we considered each study "full" or "limited" based on whether it sampled the entire plant community (e.g., surveys not encompassing the whole flowering season or sampling a subset of the plant community).

We developed 105 candidate models by incorporating the biogeographical fixed factors, i.e., latitude, altitude, insularity, and biome, along with various combinations of random effects. For random effects, we considered (1) plant richness to account for potential biases arising from significant differences in the sample size between non-tropical and tropical communities, as tropical communities tend to have higher plant richness (Kier *et al*., 2005); (2) sampling effort to address potential biases resulting from the adopted sampling method of the plant community (see Rahbek, 1995); and (3) biome, as ecological communities within the same latitude may exhibit distinct plant formation profiles, for

instance, the Brazilian Cerrado (i.e., savannah vegetation) and deserts in Namibia at parallel 20º S. To answer our research question from another perspective, we also used a subsample of the plant communities (n = 87) and computed the floral resource diversity for each of them using the Shannon diversity index (*H'*). To assess *H'*s diversity, we considered each floral resource offered by a given plant species (e.g., nectar, pollen, oil, resin, fragrance) as a different "species" following the regular use of the Shannon diversity index. We employed a subsample for this analysis since it was not feasible to obtain floral resource diversity data for all sampled communities. Finally, considering that latitudinal and altitudinal gradients may reflect Earth's climatic patterns, we repeated the same procedure described above to evaluate whether the proportion of nectar-producing animal-pollinated angiosperms and the floral resource diversity (*H'*) in communities worldwide are influenced by environmental variables, namely, annual mean temperature (MAT), annual precipitation (AP), evapotranspiration (ET) and vegetation coverage (vegetation index; VI). We used the ERA5 dataset for global historical climate data (i.e., MAT, AP and ET; Hersbach *et al*., 2020) and the MODIS dataset for the VI (Didan, 2015).

Further, for the four approaches, we performed Generalized Additive Mixed-effects Models (GAMM) and selected the best model based on the lowest Akaike information criterion corrected for small samples (AICc), comparing the full model (including the main effect, fixed factors, and random effects) with reduced models (all possible combinations among the main effect, fixed factors, and random effects). Model averaging across all models was also assessed by estimating each predictor variable's relative importance ($\Sigma\omega_i$) following Burnham & Anderson (2004). According to Symonds and Moussalli (2011), ωi values represent the likelihood of a given model having the best fit, whereas Σωi values represent the probability of a given predictor variable belonging to the best-fit model. We fitted GAMM models in R using the 'gamm4' package (Wood & Scheipl, 2014) in R 4.3.1 (R Core Team, 2023).

Spatial autocorrelation in the best model residuals was assessed through univariate spline correlograms and partial Mantel correlograms using the 'spdep' (Bivand, 2014) and the 'mpmcorrelogram' (Matesanz *et al*., 2011) R packages for these analyses. Additionally,

we used univariate spline correlograms to evaluate each model's efficiency with spatial autocorrelation and partial Mantel correlograms to assess the significance of spatial autocorrelation at each distance class (13 to 15 distance classes with equal distance intervals). To ensure that the best-fit models were not affected by multicollinearity, we examined model concurvity using the 'mgcv' package (Wood & Wood, 2015). Model concurvity is a suitable method for assessing multicollinearity in GAMM models as it considers the smooth terms, which are not accounted for in the usual approach using the variance inflation factor. Concurvity values range from 0 to 1, where values close to 1 indicate a lack of identifiability and potential issues with multicollinearity.

2.3 RESULTS

We selected 977 studies that met our inclusion criteria until we reached 1% of sampling coverage in 98.8% of our sampled angiosperm families. Building upon these studies, we assessed nectar production on 7,621 plant species distributed along 322 angiosperm families, which accounted for 2.2% of the global angiosperms' richness (352,000; Paton *et al*., 2008; Figure 2.2). Based on Ollerton *et al*. (2011) estimation, these species represented 2.5% of flowering plants pollinated by animals and covered 77.4% of angiosperm families (Christenhusz & Byng, 2016). Based on our database of nectar-producing plant species and the estimates of the number of animal-pollinated species proposed by Ollerton *et al*. (2011) and Tong *et al*. (2023), we estimate that 74.4% of animal-pollinated angiosperms produce nectar, which represents a mean of 223,308 (95% CI: 212,045 to 232,770) nectar-producing animal-pollinated plants, representing 65.1% to 66.9% of all angiosperms following the estimates of Ollerton *et al*., (2011) and Tong *et al*., (2023), respectively. We also observed that the proportion of flowering plants producing nectar as floral reward decreased towards the tropics (Figure 2.3a-b). Approximately 95.5% of animal-pollinated plants produced nectar in communities from the Arctic zone. In contrast, communities from temperate zones had 91.6% of nectar-producing species, subtropical zones had 88%, and tropical zones had 77.6%, showing a progressive decline in the proportion of nectar-producing species as we move closer to the equator (Figure 2.4a).

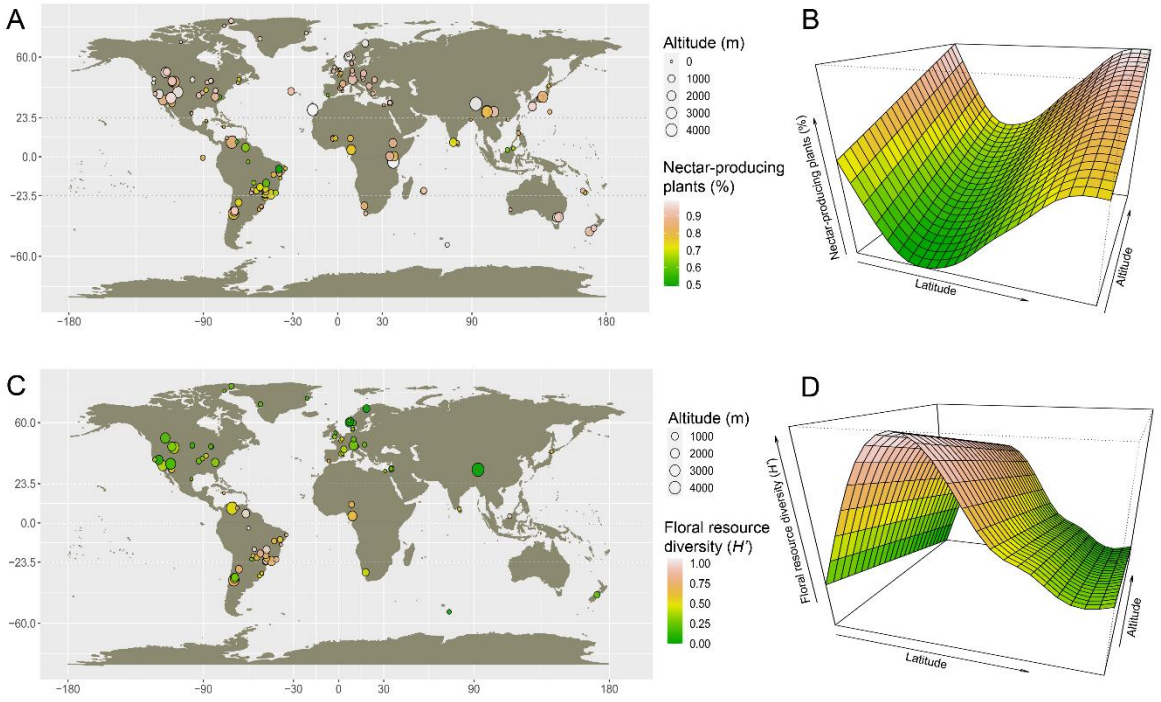

Figure 2.3 Global distribution of nectar-producing animal-pollinated plants and Shannon's diversity (*H'*) of floral resources. A) Distribution of nectar-producing plants (n = 162 plant communities). B) Proportion of nectar-producing plants in response to variations in latitude and altitude. C) Distribution of floral resource diversity (n = 87 plant communities). D) Floral resource *H'*s diversity in response to variations in latitude and altitude. In both maps, point size represents the variation in altitude, and the colour gradient represents the proportion of nectar-producing plants (A) or the *H'*s diversity of floral resources (C).

Among all candidate models, our comparisons revealed that only models including latitude and altitude as fixed factors had ΔAICc < 2, indicating that these two factors were the most influential in determining the proportion of animal-pollinated nectar-producing plants across the globe (latitude: $F_{1,162} = 9.77$, $p<0.001$, altitude: $F_{1,162} = 29.83$, $p<0.001$, $R^2 = 0.379$, $p<0.001$; Table 2.1 and Figure 2.3a-b). The best-fitting model included sampling effort, plant species richness, and biome as random effects. However, for floral resource *H'*s diversity, models including only latitude or latitude and insularity as fixed effects had ΔAICc < 2 (0 and 1.90, respectively; Table 2.1 and Figure 2.3c-d). The best-fitting model for this analysis included latitude as a fixed effect and only sampling effort as a random effect ($R^2 = 0.646$, $F_{1,87} = 30.16$, $p<0.001$). These findings mean that the ratio of nectar-producing plants

increased towards higher latitudes and altitudes, whereas the diversity of floral resources decreased significantly towards these regions, which indicates a negative correlation between these variables (rho = -0.98, p <0.001, Figure 2.4b).

Table 2.1 Model selection of the multi-predictor additive mixed-models explaining the variation of the proportion of nectar-producing plants and Shannon's diversity of floral resources (*H'*) within sampled communities following variations in biogeographical predictors. Evaluation of the multiple predictor variables is indicated through $\Sigma\omega_i$. Variables with $\Sigma\omega_i > 0.8$ are in bold and represent the most probable predictors in explaining variations in nectar production and floral resource diversity across plant communities worldwide. ωi depicts the probability of the model with the lowest Akaike information criterion corrected for small samples (AICc) being the best fit.

	Proportion of nectar-producing plants	Shannon's diversity of floral resources (*H'*)
	$\Sigma\omega_i$	$\Sigma\omega_i$
Latitude	**0.999**	**1**
Altitude	**1**	0.148
Insularity	0.185	0.254
Biome	0.009	0.000
Observations	162	87
ω_i	0.806	0.614
R^2_{adj}	0.379	0.646
AICc	444.913	-36.916

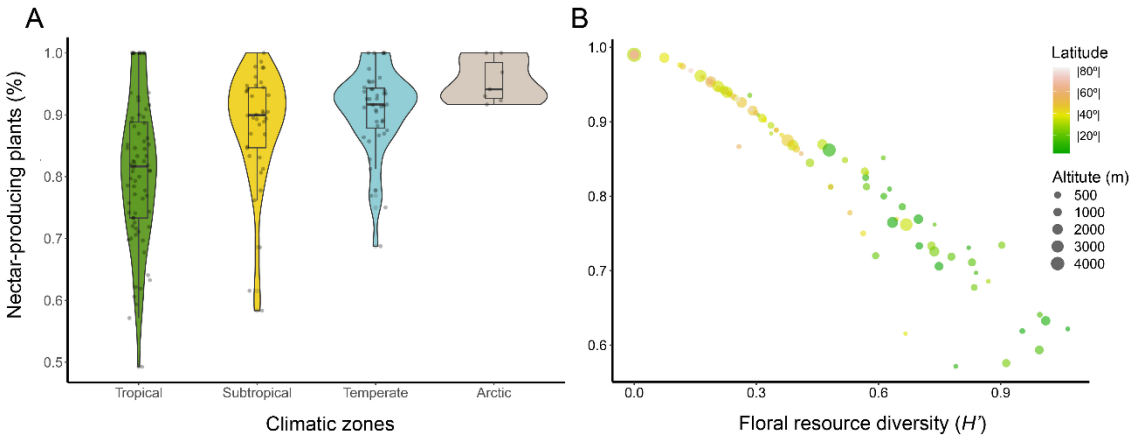

Figure 2.4 Global trends in nectar production across world climatic zones and its correlation with the Shannon's diversity (*H'*) of floral resources. A) Proportion of animal-pollinated nectar-producing plants in plant communities across the climatic zones worldwide. The mean proportion of biotic-pollinated plants that produce nectar progressively increases from the Tropical zone to the Arctic. In boxplots, the upper, central, and lower horizontal lines represent the upper quartile, the median, and the lower quartile, respectively. The whiskers extend to the maximum and minimum observed values. The violin plots overlaying the boxplots display the data density reflecting the distribution shape. B) Correlation between the proportion of nectar-producing plants and floral resource *H'*s diversity (rho = -0.98; n = 87). This analysis examines the co-variation between the proportion of nectar-producing in a sampled plant community and its floral resource *H'*s diversity. As shown in Figure 1, the correlation plot also reveals clear patterns of variations in floral resources plants offer to pollinators across different latitudes and altitudes. Communities located at lower latitudes or elevations (presented by smaller and greenish points) generally exhibit a lower proportion of nectar-producing plants and higher floral resource diversity compared to communities located at higher latitudes and/or higher elevations (represented by bigger and reddish points).

Likewise, our evaluation concerning the environmental variables responsible for driving the distribution of animal-pollinated nectar-producing angiosperms worldwide showed that only annual mean temperature is responsible for influencing the variation in the proportion of nectar-producing plants in communities globally (R^2 = 0.369, $F_{1,160}$ = 8.48, p<0.001; ΔAICc < 2; Table 2.2 and Figure 2.5a). Besides annual mean temperature explaining most of the variation ($F_{1,87}$ = 33.92, p<0.001), annual precipitation ($F_{1,87}$ = 13.96, p<0.001) was also influential in determining the *H'*s diversity of floral resources worldwide (R^2 = 0.630, p <0.001; Table 2.2 and Figure 2.5b). The best-fitting model for this analysis

included only sampling effort (for floral resource *H'*s diversity) or sampling effort along with biome (for the proportion of nectar-producing plants) as random effects.

Finally, multicollinearity and spatial autocorrelation did not affect the results. Partial Mantel correlograms indicated low levels of autocorrelation, with only two out of 15 distance classes showing some autocorrelation for the proportion of nectar-producing angiosperms (Supplementary Figure S2.2a-b) and no autocorrelation for *H'*s diversity of floral resources (Supplementary Figure S2.2c-d) within the biogeographical models (e.g., models including latitude and altitude). Furthermore, the smooth terms showed low levels of concurvity (Supplementary Table S2.1). Similarly, spatial autocorrelation and multicollinearity did not strongly affect the environmental models (e.g., models including MAT and AP). Four out of 15 distance classes showed low levels of autocorrelation in the proportion of nectar-producing plants in partial Mantel correlograms. No spatial autocorrelation was observed in the floral resource diversity (Supplementary Figure S2.3a-d). GAMM models showed low levels of concurvity within their smooth terms (Supplementary Table S2.2).

Table 2.2 Model selection of the multi-predictor additive mixed-models explaining the variation of the proportion of nectar-producing plants and Shannon's diversity of floral resources (*H'*) within sampled communities following variations in environmental predictors. Evaluation of the multiple predictor variables is indicated through $\Sigma\omega_i$. Variables with $\Sigma\omega_i > 0.8$ are in bold and represent the most probable predictors in explaining variations in nectar production and floral resource diversity across plant communities worldwide. ωi depicts the probability of the model with the lowest Akaike information criterion corrected for small samples (AICc) being the best fit. MAT is the mean annual precipitation; AP is the annual precipitation; ET is the evapotranspiration; VI is the vegetation coverage.

	Proportion of nectar-producing plants	Shannon's diversity of floral resources (*H'*)
	$\Sigma\omega_i$	$\Sigma\omega_i$
MAT	**1**	**1**
AP	0.089	**0.986**
ET	0	0
VI	0	0
Observations	160	87
ω_i	0.911	0.986
R^2_{adj}	0.369	0.630
AICc	437.042	-28.657

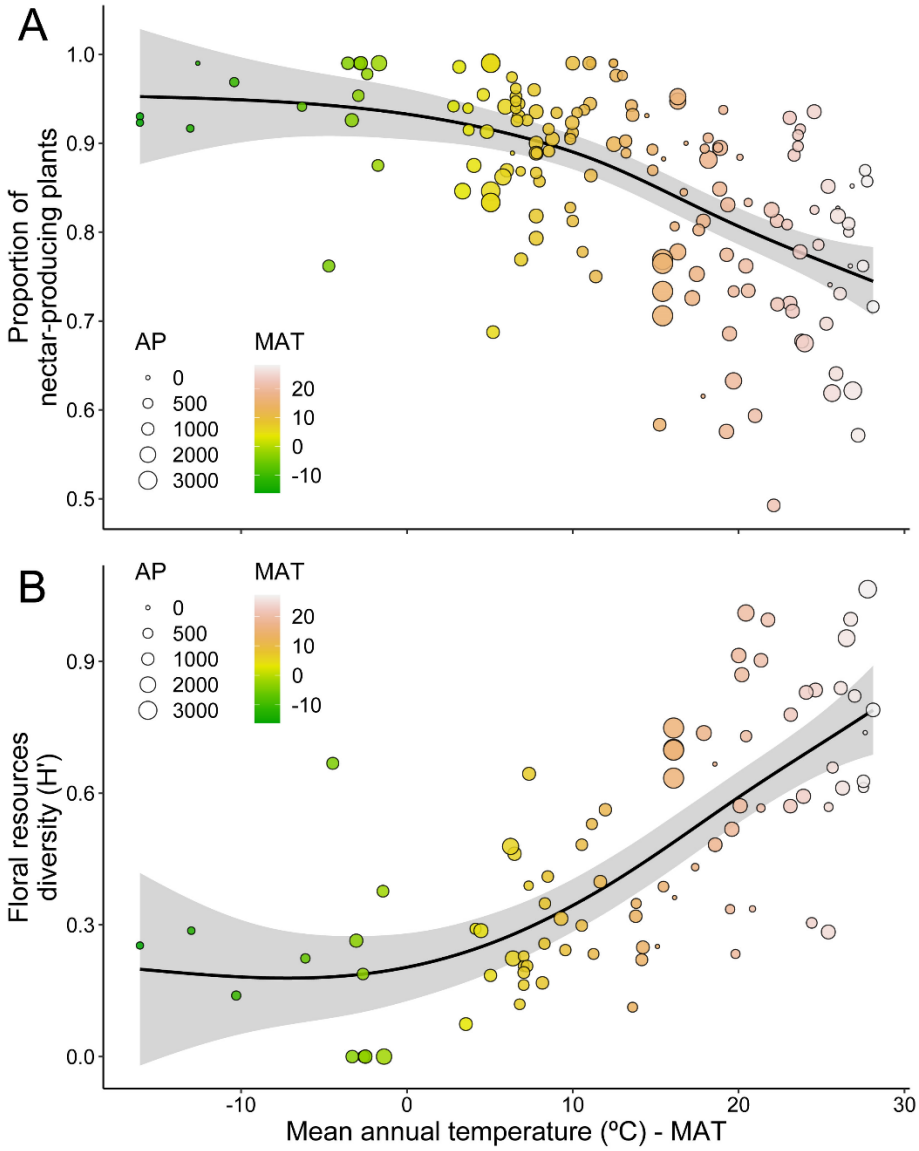

Figure 2.5 Influence of climatic variables on floral resource distribution in plant communities worldwide. A) Proportion of nectar-producing plants in response to variations in annual precipitation (AP; mm/year) and mean annual temperature (MAT; °C). B) Floral resource *H'*s diversity in response to variations in annual precipitation (AP; mm/year) and mean annual temperature (MAT; °C). In both plots, point size represents the variation in AP, and the colour gradient represents the variation in MAT.

2.4 DISCUSSION

Floral nectar serves as a crucial resource for various pollinator groups. However, an estimate of flowering plants producing nectar was previously unknown. Here, we have shown that most angiosperms are indeed nectar-producing. Although most flowering plants (except for Asclepiadoideae and most Orchidaceae) have accessible pollen as a potential resource to different kinds of floral visitors, e.g., pollinators, pollen robbers and florivores, our study shows that, alongside pollen, nectar is the most common floral reward offered to pollinators by plants. Furthermore, our findings indicate a remarkable global trend of increasing proportions of nectar-producing angiosperms in high latitudes and altitudes. Conversely, warmer and wet communities closer to the equator and at lower elevations exhibited a lower prevalence of nectar-producing plants but a higher diversity of floral resources. These findings reveal that nectar production and the diversity of floral resources follow latitudinal and altitudinal gradients worldwide.

Although the prevalence of nectar-producing plants among animal-pollinated angiosperms is not unexpected, it is important to highlight that the global proportion of nectar-producing plants may vary slightly due to the taxonomic focus on angiosperm families of our data collection method. While this approach enhances coverage, it may compromise the accuracy of identifying nectar production within specific plant families. Nonetheless, owing to the extensive sampling coverage of our study, our estimation represents the first comprehensive assessment of nectar production in animal-pollinated plants (see Bernardello, 2007 for a systematic approach). This assessment also offers a simple yet consistent framework for future studies to evaluate the distribution of reproductive attributes in flowering plants, which may provide insights into plant and pollinator evolution and diversification.

For instance, while pollen and other floral parts are likely to have played crucial roles in early plant-pollinator interactions (Pellmyr & Thien, 1986; Peris *et al*., 2017; Tihelka *et al*., 2021), the presence of pollen as the male gametophyte of the spermatophytes (*i.e.*, seed plants) presents challenges in understanding the evolutionary pathways that led to the emergence of floral rewards facilitating pollination. Notwithstanding the importance of

pollen and other floral parts, the evolution of animal pollination, even before the dawn of the flowering plants, can also be related to the production of nectarlike pollination drops by some groups of gymnosperms and the close adaptation of insects to feed on this fluid, which dates from the mid-Mesozoic (Nepi *et al.*, 2009, 2021; Ren *et al*., 2009, Nepi, 2017; but see also Ollerton & Coulthard 2009). The secretion of nectarlike rewards related to unspecialised entomophily also seems to have a crucial role in the evolution of biotic pollination, even in non-angiosperm seed plants (Lloyd & Wells, 1992; Kato & Inoue, 1994). Hence, alongside pollen and other floral parts, nectar and nectarlike secretions can also be regarded as important traits in the evolution of animal pollination before the high diversification of angiosperms in the Cretaceous. In this sense, recognising the widespread production of nectar among angiosperms allows for a more comprehensive exploration of factors influencing the evolution of floral resources and the pollinator-mediated selection of flowering plants in different communities. For example, the assumption that three-quarters of animal-pollinated angiosperms produce nectar suggests that a significant number of plants employ alternative strategies to attract floral visitors (Simpson & Neff, 1981; Armbruster, 2012, 2017). Furthermore, the increasing proportion of floral resource diversity towards the tropics implies that floral visitors who also rely on other floral resources besides nectar, such as specific bee and beetle clades that depend on oil, resins, fragrances, tissues, brood and mating sites, are more taxonomically representative in these regions. Despite these groups of specialised pollinators feeding on diversified resources to satisfy different aspects of their reproductive biology, they also rely on nectar as a key resource to support their daily energetic demands, such as long-distance flight (see Armbruster, 2017).

It is well established that species richness and diversity in terrestrial ecosystems generally increase towards lower latitudes and altitudes (Stevens, 1992; Willig *et al*., 2003; Hillebrand, 2004; Cai *et al*., 2021; Liang *et al*., 2022; Nishizawa *et al*., 2022). However, our study reveals a unique and somehow counterintuitive trend regarding the proportion of nectar-producing angiosperms globally. This is attributed to plants employing an unspecialised strategy to attract pollinators, resulting in a lower ratio of nectar-producing plants in communities with higher richness and diversity of other floral rewards. Considering that we observed a significant influence of mean annual temperature and annual precipitation

on the distribution and dominance of nectar-producing plants globally, we believe this finding can be explained by the fact that both latitude and altitude are related to climate, which in turn is the primary driver of the overall species richness and functional diversity in ecological communities (Jansson & Davies, 2008; Reu *et al*., 2011; Brown, 2014; Gillman & Wright, 2014; Cai *et al*., 2021; E. Huang *et al*., 2021).

Nevertheless, being the most common floral resource in plant-pollinator relationships, nectar fulfils the energetic demands of most pollinator guilds regardless of their location on the planet. Thus, nectar could support both tropical-rich and arctic-depauperate pollinator communities. In this sense, we propose that not only climate variability but also four climate-based ecological trends facilitated floral resource diversification in the tropics and contributed to nectar dominance in non-tropical communities, which include (1) higher productivity in tropical communities compared to non-tropical ones (Gillman & Wright, 2006); (2) prominent negative density-dependent effects in the tropics (LaManna *et al*., 2017); (3) greater importance of biotic interactions in the tropics (Schemske *et al*., 2009); and (4) higher rates of diversification (e.g., evolutionary pace) in the tropics (Mittelbach *et al*., 2007).

The first premise implies that in more productive areas with more stable climates and more diverse pollinator fauna, plant species can invest in different functional trait dimensions related to the interaction with a diverse pollinator fauna, producing more complex floral rewards, such as pollen, oils, resins, and perfumes (Bauer *et al*., 2021). Nectar production, in turn, tends to be less predominant in habitats where pollinators are abundant (McPeek *et al*., 2021; Ratnieks & Balfour, 2021). Conversely, in seasonal, cold and dry regions, such as temperate and elevated habitats, where pollinator assemblages are less predictable, plants produce nectar as a more energy-efficient floral reward (Rech *et al*., 2016; Cunha *et al*., 2022), ensuring their reproduction in depauperate pollinator environments. Still, nectar production is also related to plants' photosynthetic assimilation (e.g., Southwick, 1984), meaning plants growing in open habitats have higher photosynthetic productivity that can be allocated to floral nectar production.

The second premise addresses population dynamics, where larger and denser populations lead to competition for resources (LaManna *et al*., 2017), driving species to colonise areas with different or complementary floral resources, promoting coexistence opportunities (Olesen & Jordano, 2002; Chesson *et al*., 2000; Bergamo *et al*., 2020; Zobel *et al*., 2022; Conti *et al*., 2023). The third premise implies that, at the community level, different plant species that share pollinators indirectly compete for their services (Rathcke, 1983; Armbruster *et al*., 1994). This may generate divergent selection exerted by pollinators to reduce competition among plants sharing pollinators (Sargent & Ackerly, 2008; Fantinato *et al*., 2018; Moreira-Hernández & Muchhala, 2019; Ramos, 2020; Arceo-Gómez, 2021). Hence, tropical communities with richer pollinator faunas experience stronger selection pressures, leading to greater floral diversification and specialisation (Waser *et al*., 1996; Armbruster & Muchhala, 2009; Van Der Kooi *et al*., 2021; Helmstetter *et al*., 2023; Munoz *et al*., 2023). The fourth premise implies that, as tropical plant populations present higher levels of genetic differentiation (Gamba & Muchhala, 2020), they experience higher diversification rates and faster evolutionary responses (Mittelbach *et al.*, 2007). This allows for ecological colonisation and the evolutionary diversification of plant species, increasing community richness and diversity. As tropical communities present higher diversification rates, this may also enhance floral resource diversity. Yet, in addition to climate-based trends, the age of a biome might also influence patterns of floral resource diversity. Older biomes in warm tropical habitats serve as plant diversification sources, as they offer a larger evolutionary window, accumulating species, thereby increasing the evolution of species' niches (Donoghue & Edwards, 2014).

There is a heated debate concerning biogeographical trends in plant-pollinator community specialisation. Until today, it has not been entirely established whether the degree of specialisation of this mutualism has a latitudinal gradient. While some studies point to a negative relationship between latitude and specialisation degree (Olesen & Jordano, 2002; Armbruster, 2006; Dalsgaard *et al*., 2011), others found no or even a positive relationship (Ollerton & Cranmer, 2002; Schleuning *et al*., 2012). Our results may offer further insights into this debate as we tracked the geographical distribution of one of the most common floral resources provided for angiosperms to pollinators and evaluated floral resource diversity

globally. We observed that the niche dimensions involving floral resources are more diverse towards decreasing latitudes and warmer temperatures (Armbruster, 2006). This indicates that tropical plant communities also host a broader range of pollination systems (Ollerton *et al*., 2006).

On the other hand, despite tropical communities being substantially composed of plant species offering a broader range of resources to pollinators, pollinators that are specialised in collecting more complex floral resources usually also visit nectar-producing plants to fulfil their daily energetic demands (referred to as "network asymmetry" in plant-pollinator networks; Bascompte *et al*., 2003; Vázquez & Aizen, 2004). For example, buzz-pollinating and oil-collecting bees commonly visit flowers of other plant species seeking for nectar (see Figure 2.1a). These events may show that, in contrast, floral visitors may contribute to softening the specialised relationships between plants and pollinators in communities with higher floral resource diversity by establishing more generalist interactions with plants offering nectar (Armbruster, 2017).

We acknowledge that identifying global patterns in plant reproductive traits by evaluating plant-animal interactions is quite challenging. These relationships are sometimes ephemeral or facultative and may be overlooked within standardised sampling protocols to assess global patterns in plant-pollinator interactions. For example, some plant species may be absent from the sampling survey because no interactions with pollinators could be tracked (Dorado *et al*., 2011). This could have led to patterns that our results did not show. For instance, fire-prone and open ecosystems in temperate or subtropical latitudes, such as Mediterranean-climate regions, present a diverse flora comparable to tropical plant communities (Cowling *et al*., 1996; Rundel *et al*., 2016, 2018). Thus, they may present a proportion of nectar-producing plants that deviate from the latitudinal and elevational gradient shown in this study. For example, the sand scrubland from the Mediterranean coast in Spain presents 61.5% of nectar-producing plants (Herrera, 1988). Finally, our results point to an important random effect of sampling effort, which indicates that sampling completeness affects the proportion of nectar-producing plants and floral resource diversity. Nevertheless, our results are consistent with several studies that found an increasing trend of ecological

interactions in areas closer to the equator (Rech *et al*., 2016; LaManna *et al*., 2017; Roslin *et al*., 2017), which might collaborate to increase floral resources diversity at lower latitudes (Armbruster, 2006).

2.5 CONCLUSION

Our study sheds light on the intricate interplay between nectar production, community functional composition and ecological gradients, providing valuable insights into the dynamics of plant-pollinator relationships and floral resource diversity across diverse ecosystems worldwide. In addition, we provide compelling evidence that, indeed, most flowering plants produce nectar as a floral reward offered to pollinators. However, its dominance varies along latitude and altitude. Although nectar is a crucial energy source for floral visitors in all ecosystems globally, the diverse pollinator fauna in tropical communities is related to a greater diversity of floral resources offered by plants in these regions. This observation suggests that climate-driven ecological trends have facilitated the diversification of floral resources and floral visitors in older, warmer and less seasonal climates, thereby decreasing the proportion of nectar as a sole floral reward but increasing the diversity of other floral resources alongside nectar. These findings highlight the importance of considering climate-driven ecological trends in understanding floral resource diversification and plant evolution.

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Author contributions

FWA and CSB originally formulated the idea. CSB and FWA conceived and developed the methodology. CSB, FEF, and FWA performed the statistical analyses. FEF and FWA validated statistical analysis and study design. CSB, FWA, FEF, ARR and PEO wrote the manuscript. All authors revised/edited the manuscript. FWA supervised the research.

Competing interests

None declared.

Data and code availability

The files containing the sampled plant species, communities, raw data used in the analysis, source codes and the trained models are available via GitHub: https://github.com/CaioSBallarin/Ms.nectarworldwide.git

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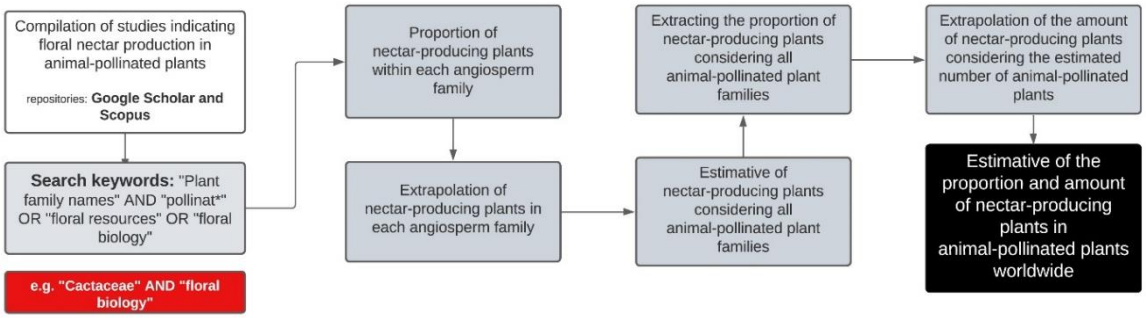
2.8 SUPPLEMENTARY MATERIAL

Supplementary Table S2.1 Concurvity between latitude and altitude. Summary values of concurvity among the numerical predictors. Concurvity measures the multicollinearity between variables in additive models. That is, to what extent smooth terms can be approximated or equaled to other smooth terms in the model. Values lower than 0.8 indicates low levels of concurvity.

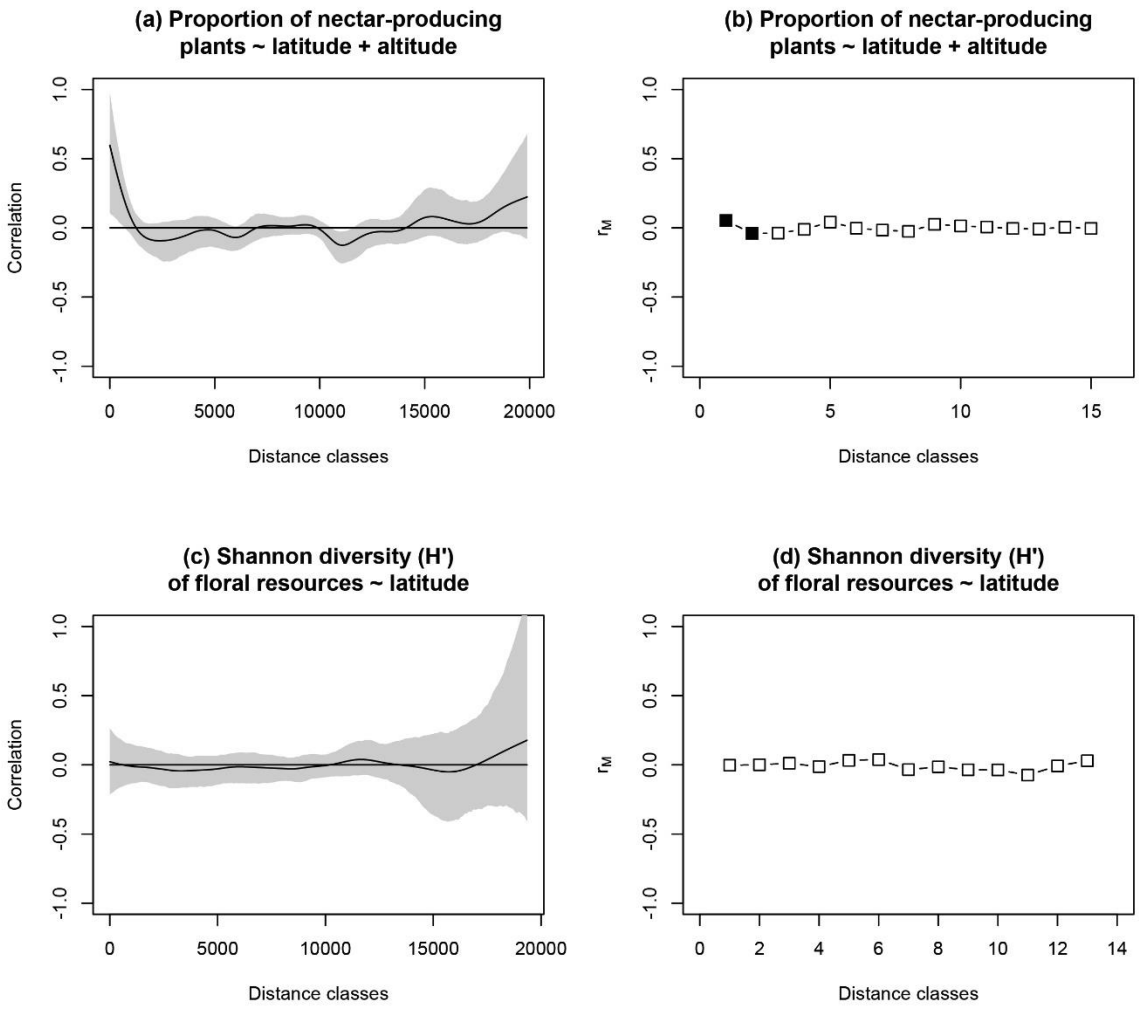
	Proportion of nectar-producing plants		Shannon's diversity of floral resources (*H'*)	
	Latitude	Altitude	Latitude	Altitude
worst	0.29806	0.29806	0.25169	0.25169
observed	0.09579	0.20832	0.15198	0.09199
estimate	0.09973	0.19463	0.12761	0.09878

Supplementary Table S2.2 Concurvity between mean annual temperature (MAT) and annual precipitation (AP). Summary values of concurvity among the numerical predictors. Concurvity measures the multicollinearity between variables in additive models. That is, to what extent smooth terms can be approximated or equaled to other smooth terms in the model. Values lower than 0.8 indicates low levels of concurvity**.**

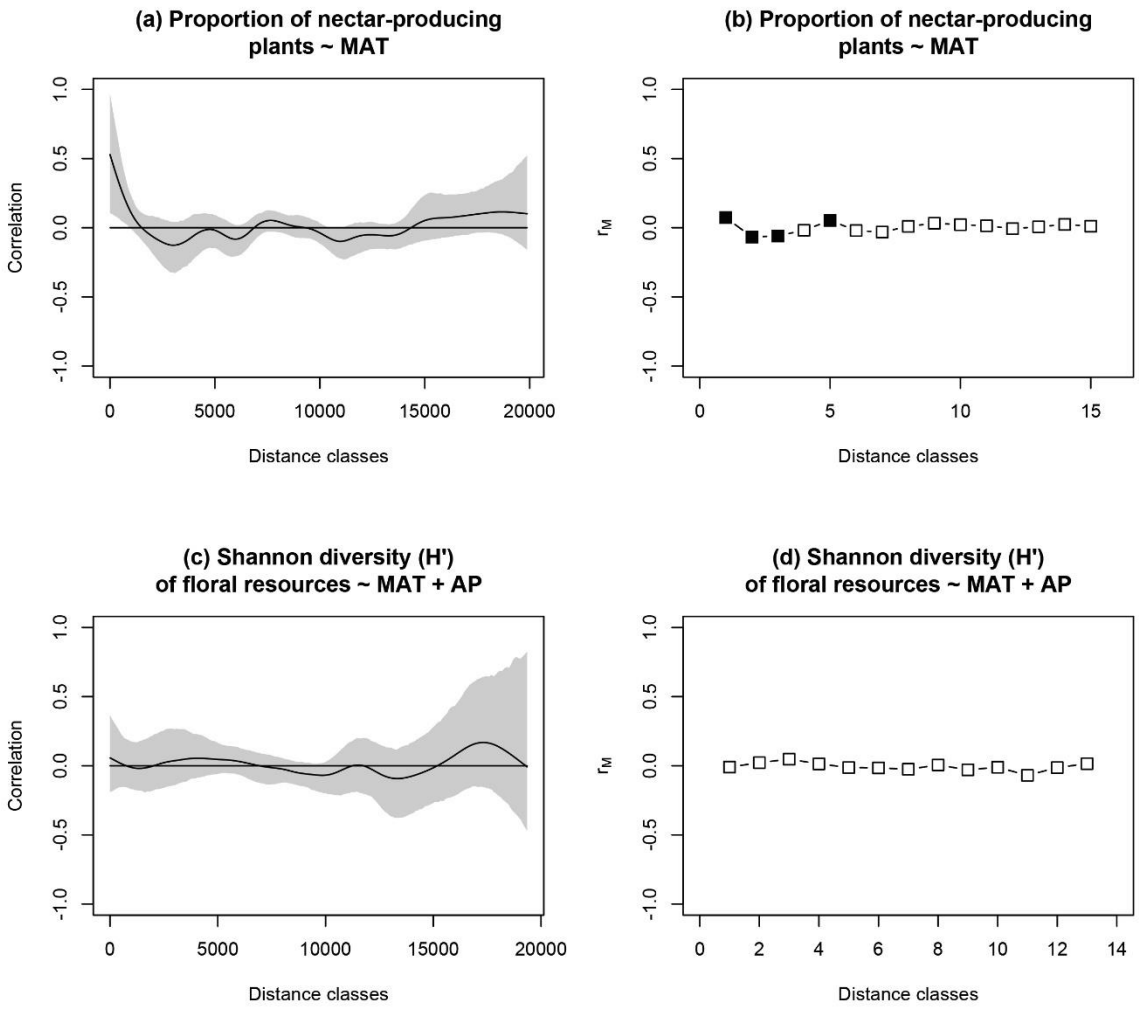
	Proportion of nectar-producing plants		Shannon's diversity of floral resources (*H'*)	
	MAT	AP	MAT	AP
worst	0.36213	0.36213	0.54885	0.54885
observed	0.16962	0.16640	0.29822	0.13985
estimate	0.11512	0.18349	0.21395	0.23712



Supplementary Figure S2.1 Flow diagram of the search routine to estimate how many animal-pollinated plants are nectar-producing. The red box shows an example of the keywords used in this study.



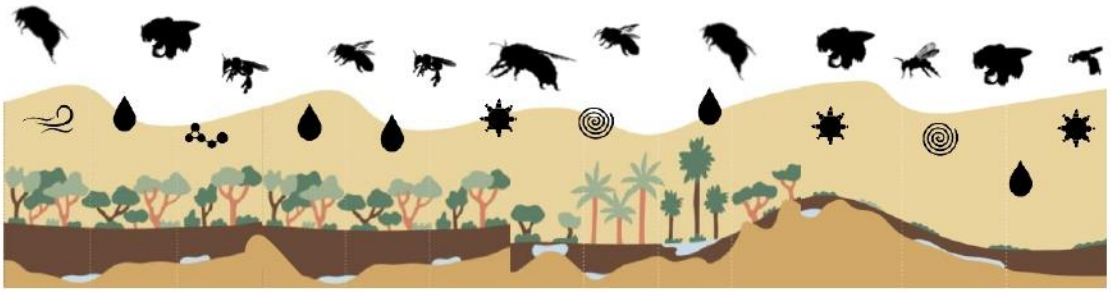
Supplementary Figure S2.2 Spatial autocorrelation in generalised additive mixed models related to the biogeographical predictor variables. In (a) spline correlograms and in (b) Partial Mantel correlogram showing autocorrelation in the first 2 of 15 distance classes for the proportion of nectar-producing angiosperms. In (c) spline correlograms and in (d) Partial Mantel correlogram showing no autocorrelation in any of the 14 distance classes for the Shannon diversity of floral resources.



Supplementary Figure S2.3 Spatial autocorrelation in generalised additive mixed models related to the environmental predictor variables. In (a) spline correlograms and in (b) Partial Mantel correlogram showing autocorrelation in the 4 of 15 distance classes for the proportion of nectar-producing angiosperms. In (c) spline correlograms and in (d) Partial Mantel correlogram showing no autocorrelation in any of the 14 distance classes for the Shannon diversity of floral resources. Acronyms "MAT" refers to mean annual temperature, and "AP" refers to annual precipitation.

3 CAPÍTULO II

Floral resource diversity shapes spatiotemporal plant–pollinator networks across levels of structural organization *



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Floral resource diversity shapes spatiotemporal plant–pollinator networks across levels of structural organization

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Abstract

1. Mechanisms underlying community assembly, including species interactions, vary across space and time. Plant–pollinator networks exemplify these dynamics, where variable link rewiring and turnover mediate adaptations to environmental changes within the community. Phenological and morphological matching, alongside species abundance, are proposed as drivers of plant-pollinator assembly, yet their roles are context-dependent.

2. Bees rely on diverse floral resources (e.g., nectar, oil, pollen, resins and fragrances) for distinct life-history aspects. However, how spatial and temporal variations in the distribution of these floral resources influence bee preferences, and the structure and assembly of plant–bee networks is still understudied.

3. Using finely resolved plant–bee interaction networks in a Cerrado ecosystem, we investigated whether variations in the diversity of floral resources influence plant–bee network structure (e.g., modularity and nestedness), and plant assembling roles (i.e., participation in motifs of different classes, and contribution to nestedness and modularity).

4. We demonstrated that temporal and spatial variations in floral resource diversity modulate network structure. Specifically, while the overrepresentation of a given floral resource increased nestedness—indicative of a more cohesive interaction network, a balanced occurrence of different floral resources increased network compartmentalisation (i.e., modularity). In scenarios featuring higher floral resource diversity, plant species with specialised resources (e.g., oil) had more interactions than in those with lower diversity. This reduced their contribution to nestedness but increased their contribution to modularity, as they were more frequently associated with the motif (i.e., a distinct type of interaction mode) class promoting modularity (i.e., fully–connected motif class) than those increasing nestedness (i.e., core–periphery motif class). Therefore, we found that the relationship between floral resource diversity and network configuration can be tracked in different levels of network organization, as plant species roles, participation in motifs, and network structure were often correlated.

5. Synthesis: Our findings emphasise the importance of accounting for spatiotemporal changes in floral resources when studying plant-pollinator networks. Beyond generalist interactions, plant-bee relationships mediated by specialised floral resources play a pivotal role in diversifying interactions, network structure and assembly. This illustrates how including information at the floral resource level adds to our understanding of plant–pollinator network configuration.

Keywords: Floral rewards, Functional traits, Modularity, Nestedness, Network motifs, Plant–bee interactions, Reproductive ecology, Specialised resources

3.1 INTRODUCTION

Ecological interactions among species are quintessential to community assembly, yet understanding the drivers of their spatiotemporal changes remains a central question in ecological studies (Vázquez et al. 2009a, b). Countless structural patterns can be generated due to interaction rewiring or species turnover, influencing interaction diversity, and pervasively modulating community spatiotemporal dynamics (Poisot et al. 2015; Trøjelsgaard et al. 2015; Klomberg et al. 2022; Estay et al. 2023). For instance, plant–pollinator networks exhibit high spatiotemporal flexibility (Olesen et al. 2008, 2010; Carstensen et al. 2014; Chacoff et al. 2018; CaraDonna & Waser 2020; Bramon Mora et al. 2020; Hervías-Parejo et al. 2023) due to properties like interaction asymmetry (Bascompte & Jordano, 2007; Vázquez et al. 2007), or prevalence of weak links (Jordano, 1987) allowing these webs to dissolve, form and rewire links, and track environmental changes (Petanidou et al. 2008; Poisot et al. 2015).

Phenological and morphological matching between species, alongside the distribution of species abundance, are hypothesised to underlie interaction probability and assembly of plant–pollinator interactions (Vizentin-Bugoni et al. 2014; Olito & Fox, 2015; Sazatornil et al. 2016; CaraDonna et al. 2017). Nonetheless, the evaluation of processes entangling the assembly of plant–pollinator interactions may be more complex to understand than morphological matches or forbidden links, as the importance of species' traits on structuring interactions also vary spatiotemporally due to e.g., partner flexibility (Tinoco et al. 2017; Klomberg et al. 2022; Morán-López et al. 2022). The adjustment of pollinator visits according to their preferences for a given plant species translates into attachments and detachments within the network, altering its structure (Gómez-Martínez et al. 2022; Yahaya et al. 2024). All the above generates fine-scale variation in interactions that may escape appropriate analysis and documentation in snapshot samplings of plant-pollinator assemblages.

There are two most commonly reported structural patterns in plant–pollinator networks: nestedness and modularity. A given network is nested when a specialist species interacts with a properly defined subset of generalist species interacting with other generalists

(Bascompte et al. 2003). A given network is modular when species interactions form compartments (i.e., modules), with species subsets interacting more among themselves than with species composing other compartments (Olesen et al. 2007). Both nestedness and modularity are two sides of the structure of complex interaction networks, and their correlation varies according to the amount of interactions established between species (Fortuna et al. 2010). In plant–pollinator relationships, interaction establishment varies mainly according to plant phenology and the pollinators' preferences for a given floral resource. Floral phenology determines when a plant species remains available for pollinators to interact with (Armstrong et al. 2016; Labonté et al. 2023). Meanwhile, pollinators' preferences for a given floral resource determine what plant will be visited in detriment of other co-flowering plants (Blüthgen & Klein, 2011; Junker et al. 2013; Suárez-Mariño et al. 2022). Thus, subtle changes in the distribution of interactions at fine spatiotemporal scales may lead to shifts between these two structural extremes of network configuration (i.e., nestedness and modularity), depending on changes in the floral resource-base.

The resource base supporting pollination, or any other type of plant resource-based mutualism with animals, is one of the main drivers of interaction diversity (Ollerton, 2006). Plants can offer different types of floral resources for pollinators, such as nectar, pollen, oil, fragrances, resins, and floral tissues. Each resource attracts different pollinators, especially bees that can use these floral resources for distinct ecological demands (Armbruster, 2017). For example, nectar and pollen are floral resources commonly offered by plants as rewards for many bee species (Ballarin et al. 2024a). Yet oil, floral fragrances, resins, and pollen offered in poricidal anthers are used by a shorter spectrum of bees that are behaviorally and morphologically adapted to collect them (Armbruster, 2012, 2017). The partitioning of floral resource use among different bee species across space and time according to their ecological preferences may affect the degree of generalisation in plant–pollinator relationships and, thus, the structure of the interaction network (Carstensen et al. 2016; Gómez-Martínez et al. 2022).

Nevertheless, the importance of different floral resources in influencing bees' preferences and, thus, the structure of the plant–bee interaction network is virtually unknown

for spatiotemporal series in which the abundance of plant species offering nectar, pollen, oil, resins and fragrances (i.e., floral resource diversity) varies. Representing interaction networks in spatial or temporal slices is a powerful approach to evaluate the fine-grained seasonal dynamics of plant and pollinator relationships and investigating the drivers of such dynamism (Olesen et al. 2008, 2010; Bramon Mora et al. 2020; CaraDonna et al. 2021). Here, we used empirical, highly resolved plant–bee interaction networks build in spatiotemporal slices to evaluate 1) whether floral resource diversity varies in space and time; 2) whether changes in floral resource diversity through space and time influence network structure, and 3) if plant species offering floral resources used by specific bee groups play distinct roles in assembling plant–bee interactions by contributing differently to nestedness and modularity. 4) Finally, we explored if the influence of floral resource diversity in modulating network structure and assembly can be tracked in different levels of network organization. Considering that nectar and "regular" pollen (i.e., pollen of non-poricidal plants) are the most common floral resources offered by plants to bees, we hypothesised that lower floral resource diversity, caused by the high representativeness of nectar-offering or regular pollen-offering plants, would increase the likelihood of species entering networks to interact with well-connected species, ultimately increasing network nestedness (i.e., preferential attachments; Olesen et al. 2007). In contrast, we expected network modularity to increase at higher levels of floral resource diversity due to the emergence of high-specificity relationships (e.g., oil-mediated plant–bee interactions) in which different bees could select their most valuable floral resource while avoiding over-exploited nectar or pollen sources (i.e., opportunistic attachments; Valdovinos et al. 2016; Ponisio et al. 2017). Finally, we predicted that changes in plant–bee interaction establishment following a changing resource base would leave imprints in different levels of network organization (i.e., scaling up from species roles to the overall network architecture).

3.2 MATERIAL AND METHODS

3.2.1 Study site

We conducted this study during the flowering seasons of 2021-2022 and 2022-2023 at the Botucatu State Forest (BSF), a Cerrado area located in the peri-urban zone of Botucatu

municipality, São Paulo state, Brazil (22°56'20"S, 48°27'29"W; 860 m a.s.l.). BSF covers an area of 34 ha and exhibits a diverse range of vegetation profiles, including floodable gallery forests, moist *campo limpo* with a dominant herbaceous layer, and *campo sujo* comprising varying degrees of scattered trees, shrubs, herbs, and grasses, where this study was conducted. The climate of Botucatu is high-altitude tropical (Aw according to Köppen classification; Franco et al. 2023), with two distinct seasons: a cold and dry winter, from April to September, and a hot and wet summer, from October to March, during which most plant species bloom.

3.2.2 Floral resource diversity and plant–bee interactions in spatiotemporal units

To assess plant community composition and plant–bee interactions, we set twelve 100m² (10m x 10m) plots spaced at least 30 m apart to account for compositional differences among them (55.7 ± 8.3 bee-pollinated plant species per plot; mean ± SD). During two flowering seasons, from mid-July to early March, we quantified weekly the number of flower units per individual plant at the flowering stage (i.e., anthesis) within each plot. Additionally, we performed visual censuses of plant–bee interactions weekly (see Supplementary Figure S3.1 for details about the sampling protocol). Each week, two observers sampled legitimate interactions defined as instances where bees accessed the flowers and contacted anthers and/or stigmas. Sampling in each plot occurred from 06:00h to 12:00h and from 12:00h to 18:00h, spanning two consecutive days, and totaling 1440 hours across all plots and flowering seasons (120 hours per plot). Within each plot, we recorded the number of legitimate plant–bee interactions in all individual plants within plots we were able to see (212.9 ± 339.3 plant individuals per species within each plot). To mitigate bias due to sampling time, we alternated the order of plots in consecutive days. Plant species were identified *in situ* using a floral guide list specific to BSF (Pilon et al. 2017) or taken to the Herbarium of São Paulo State University (Herbarium BOTU) for further identification by specialists. Bee specimens were collected using entomological nets and identified using identification keys and assistance from specialists (Silveira et al. 2002).

The duration of the flowering phenophase markedly differs among species in the Cerrado vegetation (Morellato et al. 2013). Some species offer few flowers per day over

multiple months (i.e., steady state; *sensu* Gentry, 1974*)*. Others, last a single month when up to hundreds of flowers are produced (*i.e.*, cornucopia; Gentry, 1974), or show just one or more short blooming peaks lasting only two weeks (i.e., big or multiple bangs; Gentry, 1974). Therefore, to capture variations in floral resource diversity with sufficient precision to account for the duration of flowering plants with reduced intervals in the reproductive stage, we built plant–pollinator networks fortnightly for each plot (each fortnight/plot corresponds hereafter to one spatiotemporal sampling census; Supplementary Figure S3.1). To this end, we sampled plant–bee interactions during 15 fortnights per flowering season, totalling 30 sampling censuses for each of the twelve plots (4 h of sampling effort per spatiotemporal sampling censuses). We then built 360 plant–bee interaction networks by creating adjacency matrices *M*, where elements m_{ij} represent the frequency at which a given bee species *i* interacts with a given plant species *j*. We excluded 149 (41.4%) matrices *M* due to the resulting network size being lower than four plant or bee species. Network size of the remaining networks was on average 18.2 ± 7.2 (mean ± SD). We further evaluated the sampling completeness of the overall network (i.e., pooling data from fortnights and plots in each flowering season) and for the two sampling scales used: spatial and temporal (i.e., spatiotemporal sampling censuses) with the iNEXT package in R (Hsieh et al. 2016). For each network, sampling completeness was measured as the proportion of the estimated richness of plant–bee interactions (Chao 1 estimator; Chao, 1984) which was actually observed. Sampling completeness (%) of the overall network was 80.64 ± 0.02 (Supplementary Figure S3.2a, b). Networks built in each spatiotemporal sampling census (i.e., the highest resolution in this study) presented a modal completeness value of 66% (Supplementary Figure S3.2c). We found no significant differences in completeness in spatiotemporal sampling censuses among plots and differences in just a minor fraction of the comparisons across fortnight periods (Supplementary Figure S3.3).

3.2.3 Spatiotemporal changes in floral resource diversity

To evaluate intraseasonal spatiotemporal changes in floral resource diversity, we first calculated the Shannon (*H'*) diversity of floral resources for each spatiotemporal sampling census. *H'*s diversity of floral resources was calculated by considering the abundance of

flowers possessing each type of floral resource. Nectar, pollen, oil, fragrance, floral tissues, and nectar alongside pollen were considered as different floral resource types (n = 6). Then, to explore how different floral resources provided by plants varied over space and time, we created an adjacency matrix *Y* compiling data from each spatiotemporal sampling census. Cells y_{ij} in *Y* represent the quantity of different floral resources available in a given plot and fortnight (i.e., spatiotemporal sampling census). We then created a distance matrix by estimating the Bray-Curtis distance, which captures changes in floral resource diversity among spatiotemporal sampling censuses. A permutational multivariate analysis of variance (PERMANOVA) was conducted to evaluate the significance of differences in the average distances among plots (i.e., space) and fortnights (i.e., time) according to the floral resources offered by plant species and the accompanying *H'* diversity of floral resources within each spatiotemporal sampling census. We ran PERMANOVA with 1000 permutations in the package vegan in R (Oksanen et al. 2022). Finally, we also tested whether the coefficient of variation in *H'* diversity among plots and fortnights differs by extracting the ratio between the logarithm of the variance of *H'* diversity for plots and fortnights (Lewontin, 1966). This would indicate which spatiotemporal component (i.e., space or time) are more relevant to influence variation in floral resource diversity among spatiotemporal sampling units. To visualize changes in floral resource diversity across sampling units, we applied a non-metric multidimensional scaling (NMDS) in the vegan package.

3.2.4 Floral resource diversity and network structure

To evaluate whether spatiotemporal changes in floral resource diversity influence network structure, we first estimated nestedness and modularity in each matrix *M*. Nestedness was examined by calculating the network weighted nestedness (wNODF, Almeida-Neto & Ulrich, 2011). wNODF values indicate that species within the network core (*sensu* Bascompte et al. 2003) also engage in interactions more frequently (Almeida-Neto & Ulrich, 2011). We calculated a weighted measure of modularity (Q'_w; DIRTLPAwb+ algorithm; Beckett, 2016), which ranges from 0 to 1, with higher values indicating greater compartmentalisation of interactions. Both metrics were estimated using the bipartite R package (Dormann & Strauss, 2014). As wNODF and Q'_w values show sensibility to

sampling biases, network size, and connectance, i.e., the proportion of potential links between plants and bees that are realised (Dalsgaard et al. 2017), we employed a Δ-transformation, which accounts for how much empirical values depart from random ones, generated by a given null model (Schleuning et al. 2012). In Δ-transformations, the mean value obtained by random networks is subtracted from the empirical value, allowing proper comparisons between networks with different sizes and connectance (Fründ et al. 2016). To generate the null models, we used the Patefield algorithm (1981) that fixes network size and the marginal totals for rows and columns, meaning that species richness and interaction count are conserved when generating the null matrices (Dormann et al. 2008). Then, we performed two linear mixed models (LMM), considering ΔwNODF and ΔQ'w as variables responding to variations in Shannon (*H'*) diversity of floral resources, floral abundance and plant species richness. Floral abundance and plant species richness were included as predictors to account for the influence of the probability of encounters among plant and bee species as well as the situations in which plant species pool outweighs the effect of floral resource diversity. Models' fortnights and plots were included as random effects in a crossed structure. The performance of the LMMs built with floral resource diversity, floral abundance, and plant species richness as predictor variables was compared by building candidate models considering all combinations among the predictor variables and a null model (built with the intercept and the random effects only). The best model was further selected according to the Akaike Information Criterion corrected for small samples (AICc). Collinearity among predictors were checked using the car package in R (Fox & Weisberg, 2019).

3.2.5 Floral resource diversity and interaction configuration

To assess whether plant species providing different floral resources to bees assume distinct roles in interaction configuration and, consequently, contribute differently to network structure, we first categorised plant species into two groups based on floral resource specialisation. Plant species that offered nectar, pollen (accessible to either non- and buzz-pollinating bees), or both nectar and pollen were assigned to the generalised floral resource group (hereafter referred to as the generalised group, n = 80 taxa; Supplementary Table S3.1). Plants providing oil, floral fragrances, floral tissue, or pollen (exclusive to buzz-pollinating

bees) were categorised under the specialised floral resource group (specialised group; n = 25 taxa; Supplementary Table S3.1). We differentiated between non-buzz-pollinated and buzz-pollinated plant species because, even though they offer the same type of resource, pollen from buzz-pollinated plants is exclusively collected by vibration-producing bees (Buchmann 1983; Vallejo-Marín, 2019). Further, we assessed whether floral resource richness and diversity influenced the number of interactions established by each floral resource specialisation group in networks of each spatiotemporal sampling census. We assessed floral resource richness by counting the number of floral resources available to bees in the plant community. For example, if a spatiotemporal sampling census contains two plant species offering nectar and pollen, floral resource richness sums four. We used floral resource richness or diversity as predictors alongside floral resource specialisation (generalised *versus* specialised plant groups) in two separate LMMs in which the log-transformed number of interactions was the response variable. Plot and fortnights were treated as random effects. Best fit models were selected according to the lowest AICc.

3.2.6 Plant assembling roles

Additionally, as the assembly of pairwise interactions exert an escalating influence on the architecture of interaction networks (Bascompte & Stouffer, 2009), we set up a framework to study the influence of floral resource diversity on plant assembling roles and on network structure from the micro- to the macro-scale of network organization (similar to Ballarin et al. 2024b). We first studied the meso-scale of network organization by inspecting the influence of floral resource specialisation on the assembly of network motifs. Network motifs are interconnected sub-networks that, when combined, form the entire network (Milo et al. 2004). Ecologically, they unveil cues about network assembling through paths of interactions that stem from each pairwise relationship (Rodríguez-Rodríguez et al. 2017; Simmons, 2019a, 2021). Recently, motifs were categorised based on their interaction structure and conformation similarity (Simmons et al. 2021). The "core–peripheral" motifs involve interactions among a core of generalist species interacting with specialist ones, potentially contributing to network nestedness. On the other hand, "fully–connected" motifs (defined as “complete” in Simmons et al. 2021) represent patterns where all possible links

are realised, potentially promoting modularity, as species tightly connected tend to create modules with high level of interaction exclusivity. Therefore, we separated the "core–peripheral" and "fully-connected" motifs in two different assembling classes that reflect their distinct contribution to network nestedness and modularity (nested class and modular class, respectively). To inspect whether floral resource specialisation influences sub-network assembling, we assessed the relative frequency in which the generalised and specialised plant groups were involved in each class of motifs for each spatiotemporal sampling census using the package bmotif in R (Simmons et al. 2019b). This approach allowed us to inspect whether specialised or generalised plant groups differ in their frequency of inclusion in nested and modular motif classes following variations in floral resource diversity. Differential participation in the two classes of motifs might indicate different signatures on the dynamics of network assembly, as well as different topological roles. We tested whether floral resource diversity and floral resource specialization influence plants' participation in different motif classes by building two generalised additive mixed models (GAMM) using the package gamm4 in R (Wood et al. 2017). GAMMs enable the exploration of both linear and non-linear responses (e.g., relative frequency; Zuur et al. 2009). In GAMMs, the relative frequency of plants' participation in motifs of each class (i.e., nested and modular classes) were considered as response variables, with floral abundance, floral resource diversity, and floral resource specialisation as predictors. Fortnights and plots were treated as random effects. To avoid zero-inflation effects and the use of small datasets, we excluded from this analysis those networks from spatiotemporal sampling censuses where neither motifs in the nested class nor the modular class were present. The best-fit model was that with the lowest AICc value.

We then studied the micro-scale of network organization by assessing the influence of floral resource diversity, and floral resource specialisation (generalised *versus* specialised groups) on the role of plant species in structuring the plant–bee interaction network. For this purpose, we computed the mean contribution of each floral resource group to network nestedness and modularity using the bipartite R package. Nestedness contribution gauges how a particular species (i.e., node) contributes to network nestedness, taking into account

the interactions it is involved in (Saavedra et al. 2011). Nestedness contributions are expressed as z-scores through the comparison between the empirical value obtained for each species and the values obtained through randomisation (Bascompte et al. 2003). Plant species positively contributing to community nestedness will have values greater than 0, whereas those negatively contributing will have values lower than 0. Contribution to modularity were assessed by calculating the within module degree, which reflects the level of connections a species maintains within its module (Olesen et al. 2007). Species tightly connected with its own module exhibit higher values of within module degree. We conducted two LMMs to evaluate whether variations in floral resource diversity influence the contribution of plant species to nestedness and modularity. We used floral abundance, floral resource diversity, and floral resource specialisation as predictors and nestedness or modularity contribution as response variables. Random effects included fortnights and plots, and the best-fit model was identified through the AICc procedure.

3.2.7 Integrating network assembly and structure across levels of network organization

Finally, we developed four structural equation models (SEM) using the lavaan package (Rosseel, 2012) in R to examine the joint influence of floral resource diversity and floral resource specialisation on the assembly and structure of plant–bee interaction networks. SEM models enable assessing cascading effects in intricate systems by examining multiple relationships among predictors and response variables. Given that we evaluated topological properties across multiple levels of network organization (macro-scale – network structure; meso-scale – motif assembly; and micro-scale – plant species' contribution to nestedness and modularity), the employment of SEM can elucidate how floral resource diversity influences network assembly and structure through processes operating at different levels of network organization (Ballarin et al. 2024b). For each floral specialisation group (generalised and specialised), we fitted two SEMs examining its impact on network nestedness or modularity. SEMs for nestedness included the influence of floral resource diversity on the micro- and macro-scales of network organization (i.e., plants' contribution to nestedness and network nestedness). Additionally, we considered the upscaling effect from micro-scale to macro-scale by incorporating the influence of plants' contribution to

nestedness on their participation in the nested motif class and on network nestedness, as well as the impact of plants' participation in the nested motif class on network nestedness. SEMs exploring the influence of floral resource diversity on network modularity followed a similar logical structure but included plants' contribution to modularity, plants' participation in the modular motif class, and network modularity as operational variables. We evaluated SEM performance by examining $\chi 2$, the standard root mean squared residual (SRMR), and the comparative fit index (CFI). Low values of $\chi 2$, $p > 0.05$, SRMR < 0.08, and CFI > 0.9 suggest suitable model fit (Shipley, 2002).

3.3 RESULTS

3.3.1 Spatiotemporal changes in floral resource diversity

We recorded 44 plant species offering nectar and pollen, 35 nectar, 17 pollen (16 of them as buzz-pollinated species), seven oil, one fragrances, and one floral tissue (n = 105 plant species; Supplementary Table S3.1). Floral abundance and plant species richness greatly varied across spatiotemporal sampling censuses (550.8 ± 695.6 floral units; and 8.4 ± 4.1 plant species; mean ± SD). There was low to moderate evidence that the composition and diversity of floral resources varied between plots (PERMANOVA, $F = 3.73$; $R^2 = 0.08$; $p < 0.001$; Figure 3.1a) and fortnights (PERMANOVA, $F = 12.78$; $R^2 = 0.03$; $p < 0.001$; Figure 3.1b). Finally, the coefficients of variation in Shannon (*H'*) diversity of floral resources in plots or fortnights were very different (CV= 5.83 % and 33.03 %, respectively; $F = 48.14$; $p < 0.001$). Thus, variations in Shannon (*H'*) diversity of floral resources were less detectable among plots than among fortnights.

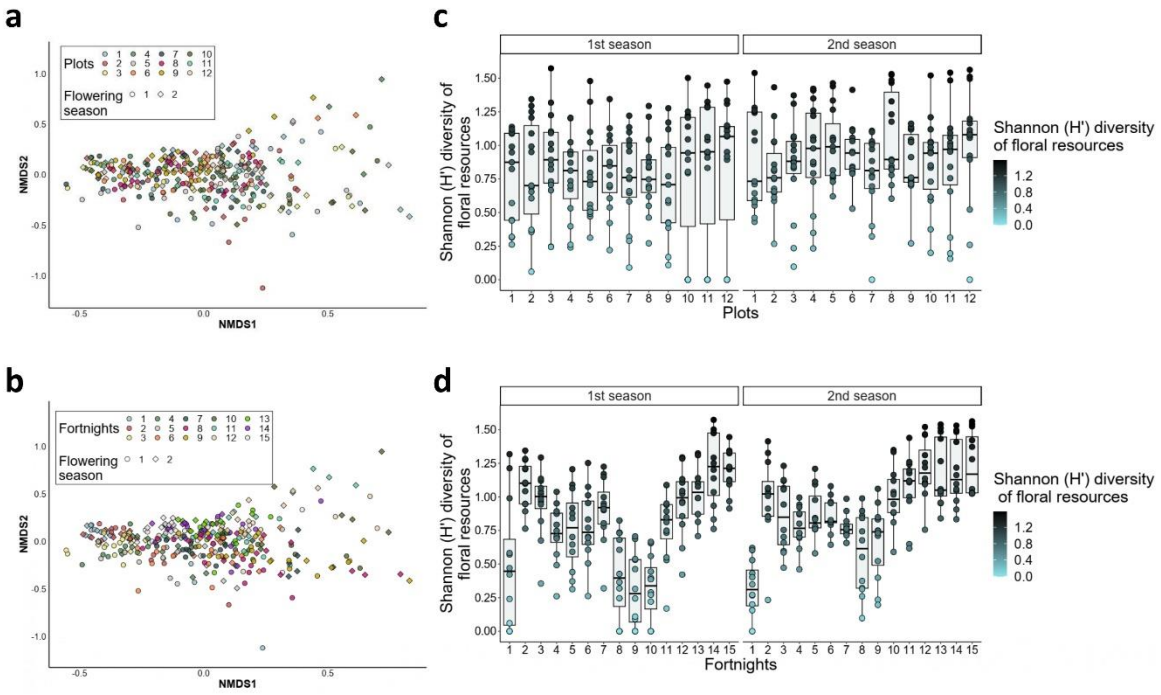

Figure 3.1 Variations in floral resource diversity through space and time in two consecutive flowering seasons. NMDS visualization of the floral resource diversity variations in space (A, among plots) and time (B, among fortnight censuses). Point dispersion depicts variations in the composition and Shannon (*H'*) diversity of floral resources in different plots (A) and fortnights (B), discriminated by colour. Point shape depicts different flowering seasons. Variation in space (C, among plots) of *H'* diversity of floral resources, measured in each plot in a given fortnight. Variation in time (D, among fortnight censuses) of *H'* diversity of floral resources measured in each fortnight in a given plot. The colour gradient from blue to black represents *H'*s diversity of floral resources (low levels in blue; high levels in black).

3.3.2 Floral resource diversity and network structure

Considering both flowering seasons, we recorded 24,546 legitimate interactions between 105 plant species and 52 bee species (1,023 ± 610 plant–bee interactions per plot and flowering season). We found that variation in floral resource diversity across spatiotemporal sampling censuses influenced network structure. Models including only *H'*s floral resource diversity exhibited the lowest AICc in the models considering network nestedness and modularity as responses. Network nestedness decreased with increases in floral resource diversity ($F_{1, 209} = 13.84$, $R^2_{adjusted} = 0.26$, $p < 0.001$; Figure 3.2a). In contrast,

network modularity increased with increases in floral resource diversity ($F_{1, 209}$ = 110.19, $R^2_{adjusted}$ = 0.62, p < 0.001; Figure 3.2b). Collinearity between predictor variables, expressed by the variation inflation factor, were always lower than 2.

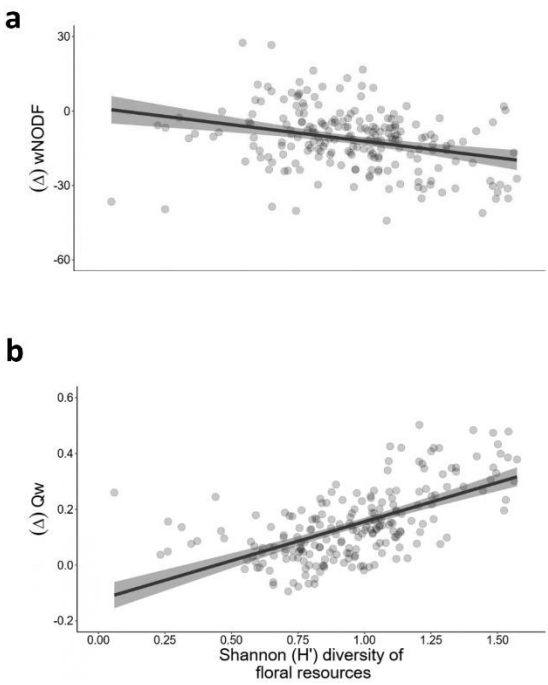

Figure 3.2 Variation in nestedness (A) and modularity (B) in plant–bee interaction networks as a function of changes in floral resource diversity. Floral resource diversity was expressed as the Shannon (*H'*) diversity of floral resources. Network nestedness (A) was expressed as the transformed weighted nestedness (ΔwNODF), while modularity (B) was expressed as the transformed weighted modularity ($\Delta Q'_w$). Points correspond to raw data in each spatiotemporal sampling census (i.e., a plot in a specific fortnight period).

3.3.3 Floral resource diversity and interaction configuration

Variations in the composition and diversity of floral resources influenced interaction patterns (Figure 3.3a,b). Floral resource richness affected the number of interactions performed by plants belonging to the generalised or specialised floral resource group ($R^2_{adjusted}$ = 0.59; p < 0.001; Figure 3.3c). Increasing the richness of floral resources increased the total number of interactions within the community ($F_{1,617}$ = 115.23; p < 0.001), although the number of interactions established by the plant species in the generalised or specialised floral resource

group differed ($F_{1,617}$ = 456.44; $p < 0.001$). Nonetheless, no interaction between floral resource richness and floral resource specialisation was detected ($F_{1,617}$ = 1.17; $p > 0.05$), meaning that increasing floral resource richness did not imply that the specialised plant group contributed more to the total number of interactions. In contrast, floral resource diversity differently influenced the number of interactions performed by each plant group ($F_{1,617}$ = 70.29; $p < 0.05$). Higher floral resource diversity resulted in more interactions established by plant species with specialised floral resources in relation to the number of interactions performed by plants with generalised floral resources (Figure 3.3d).

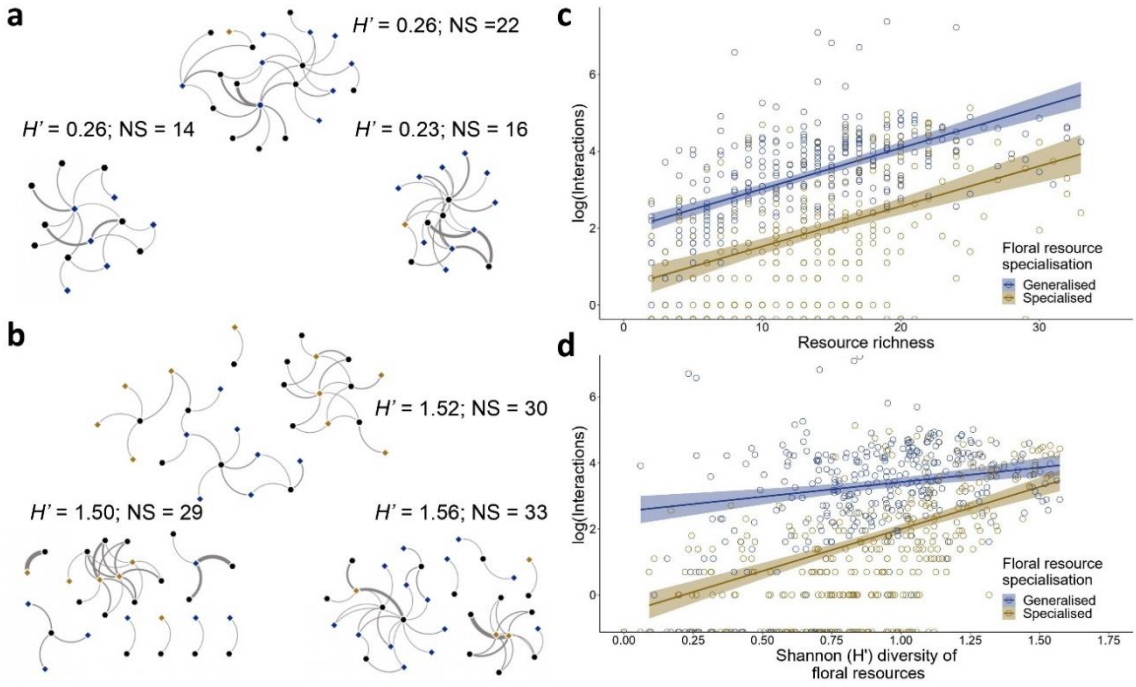

Figure 3.3 Spatiotemporal influences of the floral resource diversity on interaction patterns within plant–bee interaction networks. Three networks in (A) and (B) depict examples of plant–bee interactions sampled in three spatiotemporal sampling censuses with very low (A) or high (B) Shannon (*H'*) diversity of floral resources. Black nodes represent bees, and blue and beige nodes indicate plants offering generalised or specialised floral resources, respectively. *H'*s diversity of floral resources and network size (NS) are described in each network. Networks in (A) and (B) highlight the higher participation of plant species with specialised floral resources in scenarios where the diversity of floral resources is higher. Networks in (A) and (B) also highlight that network modules (i.e., interaction compartmentalisation) are more common in scenarios with higher floral resource diversity (in B). Figure (C) and (D) represents the relationship between floral resource richness (C) or floral resource diversity (D) and the number of interactions performed by plants offering generalised (blue) or specialised (beige) floral resources.

3.3.4 Plant assembling roles

Floral resource diversity, and floral resource specialisation influenced the frequency of plant species participating in motifs from the nested ($R^2_{adjusted} = 0.09$; $p < 0.001$; Figure 3.4a), and modular classes ($R^2_{adjusted} = 0.12$; $p < 0.001$; Figure 3.4b). Increasing floral resource diversity reduced the frequency of plant species with specialised floral resources participating in motifs of the nested class but maintained stable the participation of plant species with generalised floral resources in those motifs ($F_{1, 450} = 10.84$; $p < 0.001$). Contrarily, increasing floral resource diversity increased the frequency of plant species with specialised floral resources participating in motifs of the modular class while reducing the participation of plants with generalised floral resources in those motifs ($F_{1, 306} = 6.55$; $p < 0.001$). This indicates that flower resource specialisation influences the likelihood of plant species in participating in motifs of different classes ($F_{1, 450} = 9.90$; $p < 0.01$; Figure 3.4a - nested motifs; $F_{1, 306} = 18.38$; $p < 0.001$; Figure 3.4b - modular motifs).

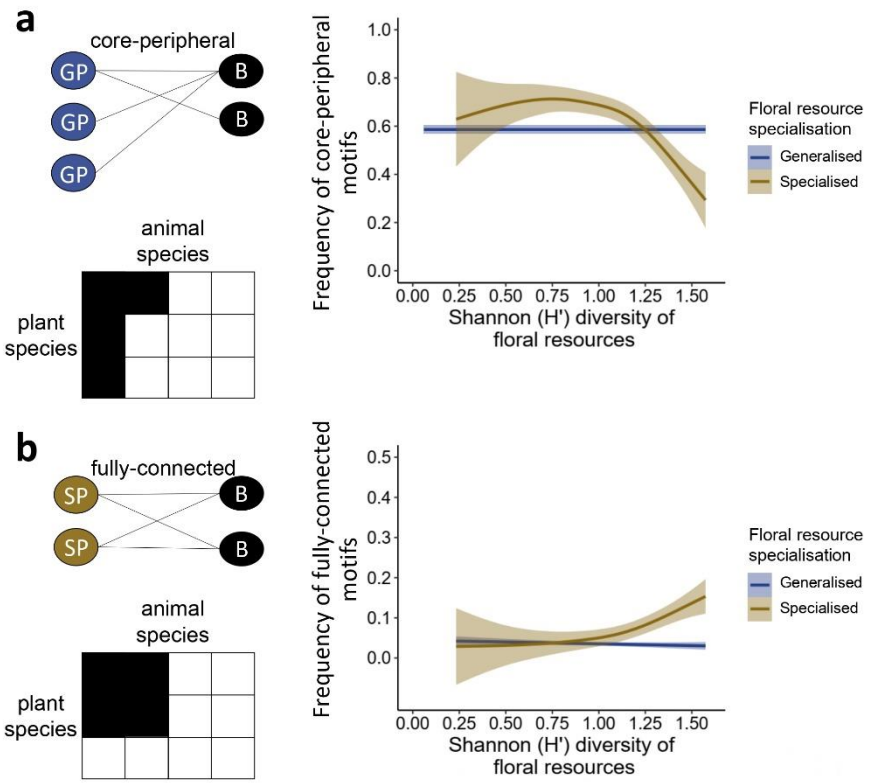

Figure 3.4 Relative frequency of plant species offering distinct types of floral resources in different motif classes. Positions in core–peripheral motifs (i.e., nested motif class; A) are more occupied by plant species offering generalised floral resources (in blue) in high floral resource diversity scenarios, where species offering specialised floral resources show a lower frequency of these interaction motifs. Fully–connected motifs (i.e., modular motif class; B) are more represented by plant species offering specialised floral resources (in beige) in high floral resource diversity scenarios. Both species groups respond differently to increases in Shannon (*H'*) of floral resources by diverging in the type of motifs where they more frequently participate. Black dots in the right panels depict bee species. Blue dots depict plants offering generalised floral resources (GP), whereas beige dots depict plants offering specialised floral resources (SP). Adjacency matrices in (A) and (B) help to illustrate how the core–peripheral motif favours network nestedness, whereas the fully–connected motif favours network compartmentalisation (i.e., modularity).

Nested contribution by each plant species was influenced by variations in floral resource diversity and floral resource group ($R^2_{adjusted}$ = 0.21; $p < 0.001$). Increasing the diversity of floral resources reduced the contribution of plant species to nestedness ($F_{1, 539}$ = 8.65; $p < 0.001$; Figure 3.5a), but plant species presenting specialised floral resources often

exhibited a higher negative contribution to network nestedness ($F_{1, 539} = 13.76$; $p < 0.001$). Contribution to modularity (i.e., within module degree) by plant species was influenced by floral resource diversity but differed between generalised and specialised plant groups. Increasing the diversity of floral resources increased the contribution of plants presenting specialised floral resources to modularity while maintained stable the contribution of plants with generalised resources ($F_{1, 340} = 4.40$; $R^2_{adjusted} = 0.04$; $p < 0.05$; Figure 3.5b).

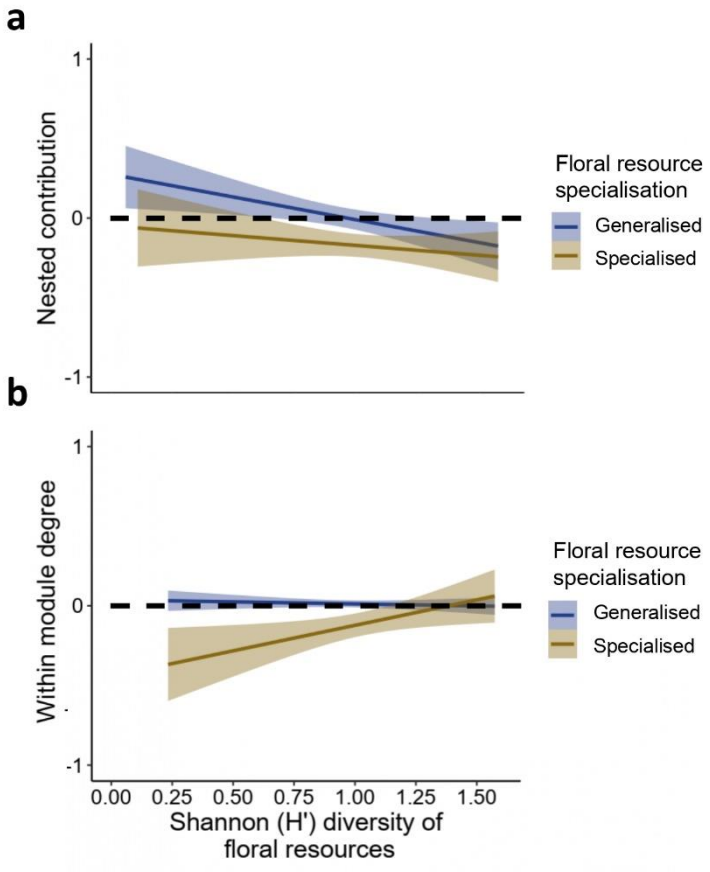

Figure 3.5 Variation in nestedness and modularity contribution by plant species in relation to changes in Shannon (*H'*) resource diversity. Blue lines represent changes in the nested (A) or modularity (B) contribution of plant species offering generalised types of floral resources (i.e., nectar- or non-buzz-pollinated-pollen plants). Beige lines represent the contribution of plant species offering specialised types to nestedness (A) and modularity (B). The dashed line in both graphs depicts that the contribution to nestedness or modularity equals zero. Values above this threshold represent a positive contribution to nestedness or modularity, whereas values below this threshold represent a negative contribution to both topological patterns. Within module degree was used as a proxy for modularity contribution.

3.3.5 Integrating network assembly and structure across levels of network organization

Floral resource diversity influenced the topological aspects of plant–bee networks across various levels of network organization (Figure 3.6). As evidenced across micro-, meso-, and macro-scales of network organization, floral resource diversity exhibited a negative correlation with network nestedness but a positive correlation with network modularity. Furthermore, SEMs revealed a clear cascade effect from micro- to macro-scale, wherein plant species' contribution to nestedness (or modularity), their participation in motifs within nested (or modular) classes, and overall network nestedness (or modularity) were frequently correlated. However, the impact of floral resource diversity on the arrangement of plant species with generalised or specialised floral resources within the network architecture differed. While the negative effects of floral resource diversity on nestedness across micro-, meso-, and macro-scales were more pronounced in plants with generalised floral resources (Figure 3.6a), the positive effects of floral resource diversity on modularity at distinct levels of network organization were more prominent in plants with specialised floral resources (Figure 3.6b). Contrary to expectations, nested and modular motif classes exhibited opposing effects on network nestedness and modularity, respectively. This negative association was notably stronger for plants with generalised floral resources. SEMs consistently presented a suitable model fit (Figure 3.6).

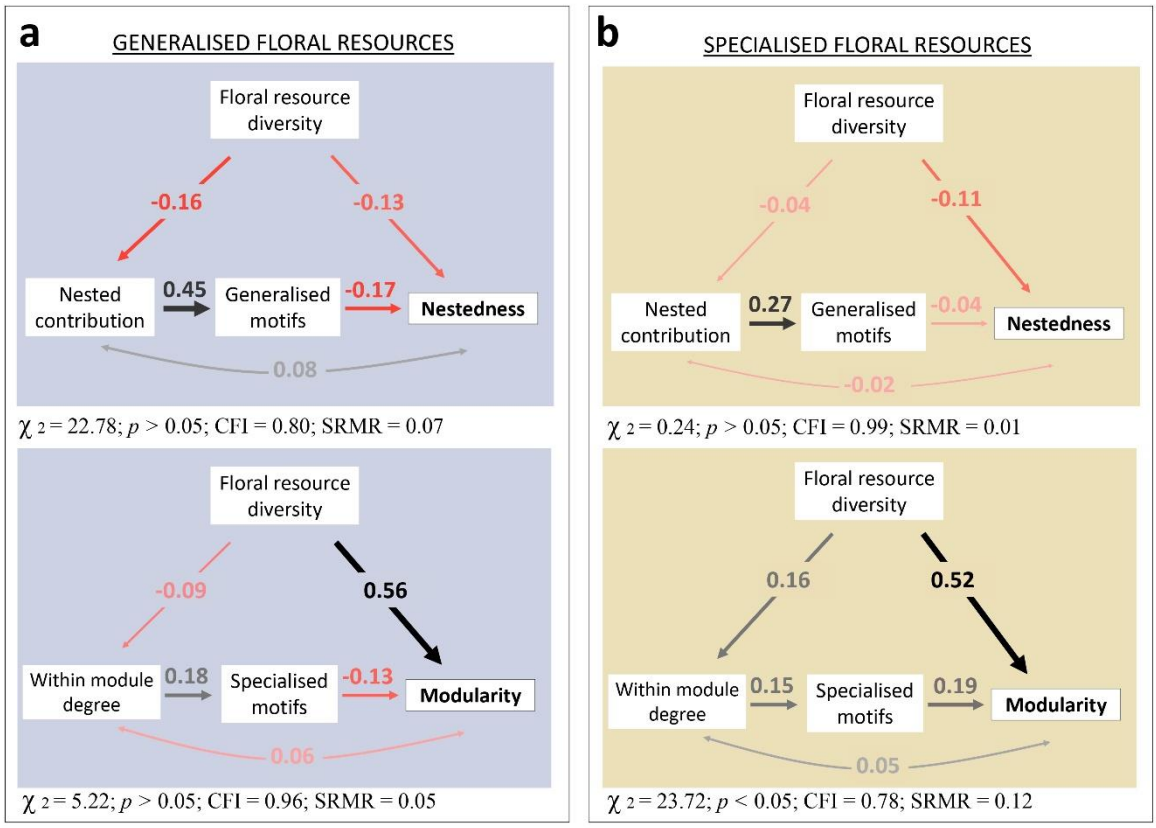

Figure 3.6 Structural equation models (SEMs) depicting the influence of floral resource diversity on network structure and assembly at multiple levels of network organization. SEMs were built for each plant specialisation group. Blue (A) and beige (B) panels show the influence of floral resource diversity on plants providing generalised (e.g., nectar) or specialised floral resources (e.g., oil), respectively. The thickness and color intensity of each arrow illustrate the standardized path coefficients. Arrows in black and red indicate positive and negative correlations between variables, respectively.

3.4 DISCUSSION

Ecologists are increasingly adopting a dynamic perspective when studying plant-animal interactions (Bascompte & Jordano, 2014), recognising that these relationships vary across both space and time (Olesen et al. 2010; CaraDonna & Waser, 2020; Hervías-Parejo et al. 2023). Nonetheless, questions persist regarding how variations in organism traits, from within populations to between populations, influence observed changes in species interactions (Hachuy-Filho et al. 2020; Ballarin et al. 2022; Arroyo-Correa et al. 2023). Here, we illustrate how fine-grained spatiotemporal changes in floral resource diversity leave

imprints in multiple levels of network organization, shaping the structure and assembly of plant–bee interaction networks.

Flowering plant diversity varies over space and time in plant communities worldwide. Nevertheless, few studies have examined the spatiotemporal changes in the floral resource base at the local scale, where it represents a key element sustaining pollinator visits (Ollerton, 2006). Thus, beyond the turnover of plant and pollinator species in seasonal habitats, the composition and diversity of floral resource types also change in time and space and may represent major shifts affecting pollinator preferences. However, existing research aiming to track spatiotemporal changes in floral resources has been mainly conducted in non-tropical regions, focusing on nectar or pollen, i.e., two floral resources typically associated with generalist strategies employed by plants to reward pollinators (Armbruster, 2017). We monitored the fine-scale spatiotemporal variations of floral resources in a tropical ecosystem harbouring plant species providing a diversified set of floral resources beyond nectar/pollen, which, in turn, are linked to specialised plant-pollinator interactions (e.g., oil- and fragrance-mediated plant–bee relationships; Armbruster, 2017). This allowed us to track greater spatiotemporal variability in pollinator rewards offered by the plant community, which is especially important for bees, which reportedly forage on a broad range of floral resources to meet their diverse biological demands.

While closely linked to flowering plants, evolutionary pathways shaping plant–bee relationships vary among bee lineages (Almeida et al. 2023), and they can predict a bee's foraging preferences (Raiol et al. 2021). Overrepresentation of one floral resource, such as nectar, regarded as the most common floral resource offered by plants to pollinators (Ballarin et al. 2024a), may influence community-wide plant–bee relationships differently than when nectar is evenly distributed alongside pollen, oil, and fragrances. Pollen is equally and commonly used by bees but can also promote specialised plant–bee relationships (Minckley & Roulston, 2006). Oil and fragrances, in turn, generally attract specific groups of bees specialised in collecting them (Armbruster, 2012). We demonstrate that more evenly distributed and diversified floral resources (e.g., oil, pollen, nectar) result in higher compartmentalisation of plant–bee interactions. In contrast, overrepresenting a single

resource increases nestedness in plant–bee networks. Importantly, our results show a much larger temporal variance in resource-based diversity than its spatial variance, reflecting a strong seasonal component in the participation of plant species with different floral resources in this assemblage.

Variations in bee life-history traits may also shed light on how they adapt to spatiotemporal changes in floral resources. Polylectic and super-generalist bee species are more resilient to temporal shifts in the composition of plant species blooming in a given community (Ogilvie & Forrest, 2017). Hence, bee species with broader diets tend to tolerate variations in floral resource diversity driven by community-wide floral phenology dynamics (Morán-López et al. 2022). In fact, preferential attachments between generalist plant species and bee species persist consistently across space and time (Resasco et al. 2021), shaping plant–bee network structures (Olesen et al. 2008). However, we assert that interactions involving specialised plant and bee species and opportunistic attachments also influence plant–bee networks (Ponisio et al. 2017). Higher resource diversity leads to less competitively skewed scenarios among plant species offering nectar, pollen, oil, or fragrances, allowing bees to either form more specialised relationships or engage in opportunistic associations (Ponisio et al. 2017). Indeed, we observed that plant species offering specialised floral resources generally contributed negatively to network nestedness. In scenarios with increased levels of floral resource diversity, in turn, they positively contributed to modularity by stretching their relationships with counterparts from their own module (i.e., increased within module degree), and participating more frequently in network motifs associated with the formation of modules. This suggests that floral resource diversity and bee dietary specialisation also contribute to shaping the dynamic topology of plant–bee interaction networks by increasing niche partitioning (Glaum et al. 2021; Gómez-Martínez et al. 2022). In fact, our results demonstrate that increases in floral resource diversity results in a much higher increase of the number of interactions involving more specialised partners relative to the concurrent change observed in the generalised species. This might explain why plants offering specialised floral resources exhibit a much more consistent signal on module formation across levels of network organization than plants offering generalised floral

resources. Indeed, the meso-scale signatures of interaction assembling only translated into the macro-scale structure when considering specialised plants and their contribution to modularity. Thus, interactions between pairs of species mediated by specialised floral resources might be much more relevant to modulate network topology than previously thought (Pinheiro et al. 2019).

Interestingly, our study shows that in the Brazilian Cerrado, a megadiverse ecosystem, neither floral abundance nor plant species richness were the best predictor for explaining variations in plant–bee network structure and assembly. The early evolution of major bee lineages occurred in landmasses in the southern hemisphere (Almeida et al. 2023), implying that certain plant and bee lineages developed highly specialised relationships in the Neotropical region (e.g., plants from Malpighiaceae and Centridini bees). Therefore, even plant species with very high floral abundances do not necessarily interact with most bee species. Instead, specific plant species with reduced floral abundances but offering rare food resources, such as the buzz-pollinated *Tibouchina gracilis* and *Kielmeyera coriacea*, or the oil-offering *Sisyrinchium weirii* and *Camarea hirsuta*, interact with a particular subset of the community adapted to collect these resources, which may contribute to softening the relevance of floral abundance and plant species richness in explaining network structure and assembly. This finding underscores the significance of functional diversity within a plant–pollinator system and suggests that the higher the functional diversity in a given plant–pollinator system, the more likely niche-based processes govern its structure (Vizentin-Bugoni et al. 2018). For example, by using a single functional trait (i.e., type of floral resource) within the plant multidimensional functional trait space related to plant–pollinator interaction, we could identify plant functional groups with different topological roles on network assembly and structure, which we believe may be governed by niche-based processes (i.e., pollinator preferences for different set of floral resources and competition avoidance in over-exploited resources).

It is important to note, however, that although plant species offering specialised resources generally contributed to reducing nestedness and increasing modularity, a significant part of these plant species played an opposite role at different scenarios of floral

resource diversity. Nestedness and modularity are non-exclusive architectural patterns within a network (Lewinsohn et al. 2006; Fortuna et al. 2010; Pinheiro et al. 2022), meaning that they might co-occur in compound topologies within plant–animal networks (Lewinsohn et al. 2006; Felix et al. 2022; Pinheiro et al. 2022). This phenomenon could elucidate the somewhat unexpected finding that plants with generalised floral resources influence network topology differently at distinct levels of network organization. This stands in contrast to plants providing specialised floral resources, which consistently played predictable roles at different levels of network organization.

Moreover, both architectures bring stability to the system (Thébault & Fontaine, 2010). On the one hand, nestedness reduces the effects of competition while providing ecological redundancy by creating novel linkage opportunities for interacting species after species loss (Memmott et al. 2004; Okuyama & Holland, 2008; Bastolla et al. 2009). On the other hand, modularity prevents pervasive effects of species loss in one compartment from affecting another (Stouffer & Bascompte, 2011). In this sense, we argue that plant–bee relationships mediated by specialised floral resources might dilute the effect of disturbance events on interaction networks, as they contribute to increasing network compartmentalisation while only slightly reducing network nestedness (as shown by Pinheiro et al. 2019 with theoretical simulations). Further, we show that plant–bee relationships mediated by specialised floral resources consistently influence network topology across various levels of network organization, which points to their potential for future assessments of network structure and stability. In this regard, we argue that their removal could lead to more predictable effects compared to the removal of plants offering generalised floral resources, which are abundant, functionally diverse, and possess network roles on different levels of network organization less readily anticipated.

Finally, we showed that at increasing levels of floral resource diversity, plant species offering different types of floral resources (generalised *versus* specialised resources) acted more frequently in different motif classes and emitted different signals for network conformation. This result underscores that, first, both plant functional groups hold relevance to preserve network dynamical structure. While generalised resources may reinforce the

network core, more specialised resources appear to diversify the secondary structure of the network in terms of adding new modular components. Interestingly, these changes manifest from micro- to macro-scale (Ballarin et al. 2024b), suggesting that significant alterations in the frequency of species-specific interactions might discharge effects relevant to defining nestedness and modularity in plant–bee networks (Pinheiro et al. 2019). Secondly, communities presenting a diverse set of floral resources might bring structural flexibility into plant–pollinator networks, boosting the overall robustness of the system at different levels of organization.

3.5 CONCLUSION

Our study demonstrates that temporal and spatial changes in floral resource composition within plant communities significantly impact the structure of plant–bee interaction networks beyond the simple turnover of species partners. The overrepresentation of specific floral resources within the plant community, such as nectar, fosters network nestedness, while the even distribution of diverse floral resources promotes network compartmentalisation. These findings highlight the complexity of plant–bee interactions, indicating that, beyond the importance of generalist relationships involving plant species offering nectar or pollen, specialised relationships between plants offering less common or challenging-to-obtain resources and oligolectic bees are critical drivers of network structure. In essence, our work demonstrates how incorporating information at the floral resource level enhances our comprehension of the configuration of pollination networks at different scales of structural organization.

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Author contributions

CSB conceived the ideas, designed the methodology, and collected the data. CSB, PJ, and BA-C analyzed the data and validated the results; CSB led the writing of the manuscript. FWA and PJ supervised, and FWA administered the study. All authors contributed critically to the drafts and gave final approval for submission.

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3.8 SUPPLEMENTARY MATERIAL

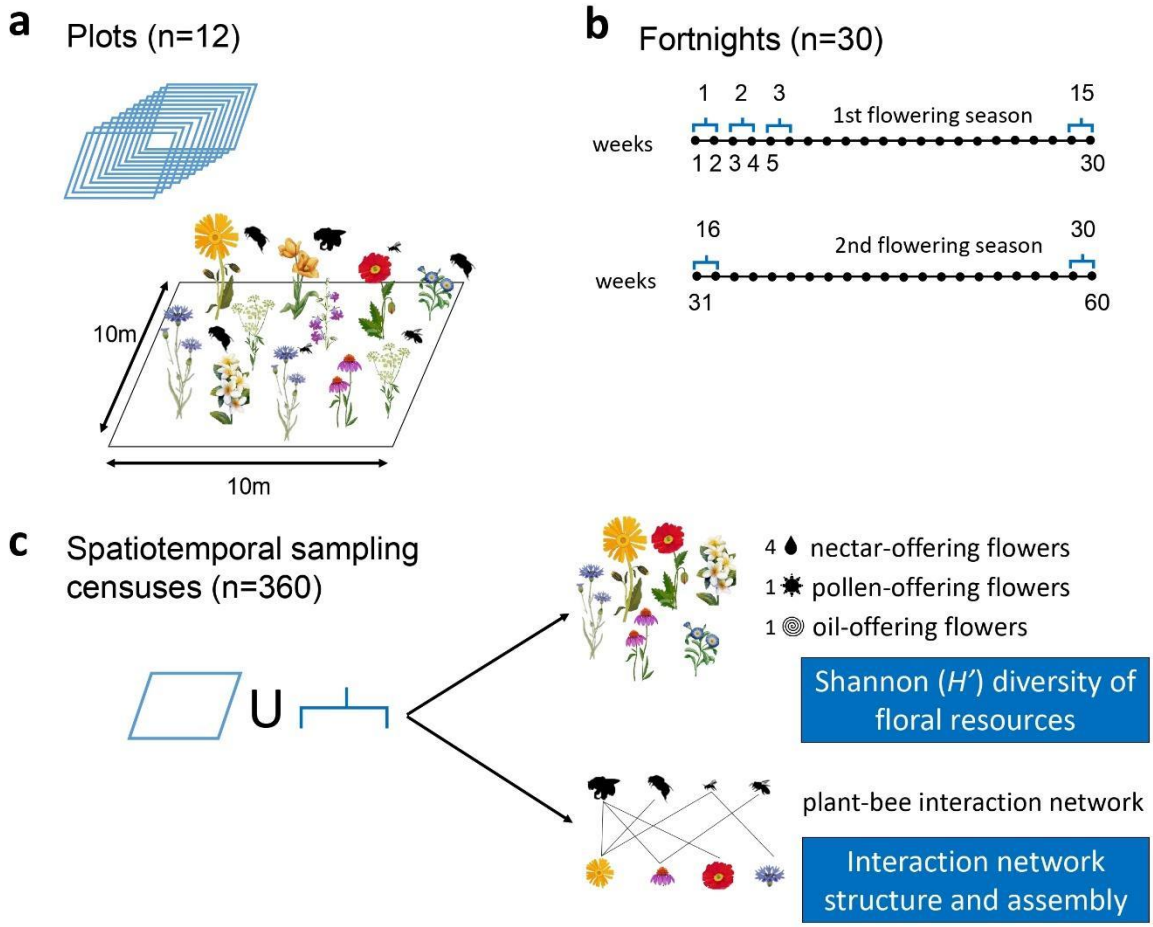
Supplementary Table S3.1 Plant species sampled in the study. Taxonomic identity, and floral resource offered are provided. Moreover, plant species were classified according to its floral resource specialisation. Plants species offering nectar, "regular" pollen (i.e., pollen of non-buzz-pollinated plant species), both were grouped in the generalised floral resource group. Plant species providing pollen (exclusively removable by vibration-producing bee species), oil, fragrances or floral tissues were grouped in the specialised floral resource group.

Family	Plant species	Floral Resource	Floral resource specialisation
Melastomataceae	*Acisanthera alsinaefolia*	Pollen (buzz)	Specialised
Lamiaceae	*Aegiphila verticillata*	Nectar	Generalised
Malpighiaceae	*Aeschynomene falcata*	Nectar	Generalised
Polygalaceae	*Asemeia hirsuta*	Nectar and pollen	Generalised
Asteraceae	*Aspilia foliacea*	Nectar and pollen	Generalised
Malpighiaceae	*Banisteriopsis campestris*	Oil and pollen	Specialised
Malpighiaceae	*Banisteriopsis stellaris*	Oil and pollen	Specialised
Asteraceae	*Barrosoa betonicaeformis*	Nectar and pollen	Generalised
Rubiaceae	*Borreria argentea*	Nectar	Generalised
Rubiaceae	*Borreria brachystemonoides*	Nectar	Generalised
Rubiaceae	*Borreria poaya*	Nectar	Generalised
Rubiaceae	*Borreria verticillata*	Nectar	Generalised
Orobanchaceae	*Buchnera lavandulacea*	Nectar	Generalised
Malpighiaceae	*Byrsonima coccolobifolia*	Oil and pollen	Specialised
Malpighiaceae	*Byrsonima intermedia*	Oil and pollen	Specialised
Asteraceae	*Calea triantha*	Nectar and pollen	Generalised
Malpighiaceae	*Camarea hirsuta*	Oil and pollen	Specialised
Myrtaceae	*Campomanesia adamantium*	Pollen (buzz)	Specialised
Caryocaraceae	*Caryocar brasiliense*	Nectar and pollen	Generalised
Cucurbitaceae	*Cayaponia espelina*	Nectar	Generalised
Fabaceae	*Chamaecrista calycioides*	Pollen (buzz)	Specialised
Fabaceae	*Chamaecrista desvauxii* var. *mollissima*	Pollen (buzz)	Specialised

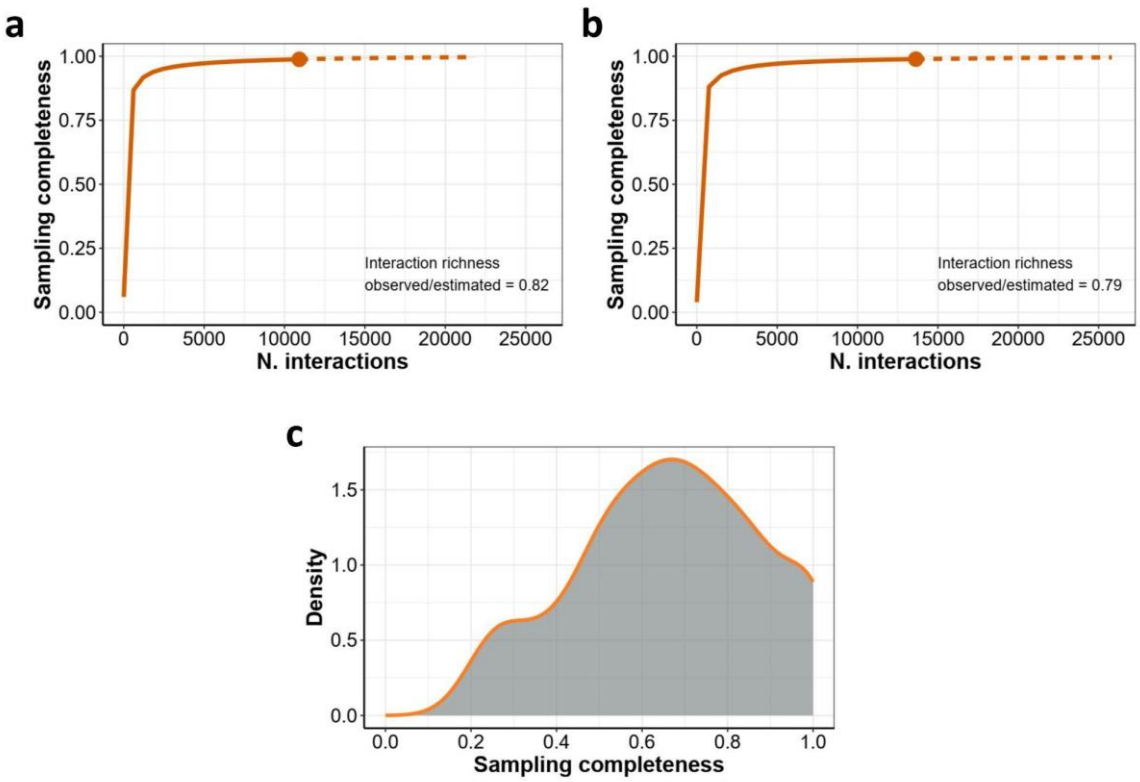
Fabaceae	*Chamaecrista ramosa*	Pollen (buzz)	Specialised
Asteraceae	*Chaptalia integerrima*	Nectar and pollen	Generalised
Asteraceae	*Chresta sphaerocephala*	Nectar and pollen	Generalised
Asteraceae	*Chromolaena campestris*	Nectar and pollen	Generalised
Asteraceae	*Chromolaena congesta*	Nectar and pollen	Generalised
Asteraceae	*Chromolaena squalida*	Nectar and pollen	Generalised
Asteraceae	*Chrysolaena obovata*	Nectar and pollen	Generalised
Asteraceae	*Chrysolaena platensis*	Nectar and pollen	Generalised
Asteraceae	*Chrysolaena simplex*	Nectar and pollen	Generalised
Commelinaceae	*Commelina erecta*	Pollen	Generalised
Asteraceae	*Conyza primulifolia*	Nectar and pollen	Generalised
Rubiaceae	*Cordiera obtusa*	Nectar	Generalised
Fabaceae	*Crotalaria balansae*	Nectar	Generalised
Fabaceae	*Crotalaria pallida* var. *obovata*	Nectar	Generalised
Euphorbiaceae	*Croton campestris*	Nectar and pollen	Generalised
Lythraceae	*Cuphea micrantha*	Nectar	Generalised
Fabaceae	*Dalbergia miscolobium*	Nectar	Generalised
Rubiaceae	*Declieuxia fruticosa*	Nectar	Generalised
Annonaceae	*Duguetia furfuracea*	Floral tissue	Specialised
Asteraceae	*Emilia fosbergii*	Nectar and pollen	Generalised
Lamiacea	*Eriope crassipes*	Nectar	Generalised
Fabaceae	*Eriosema campestre* var. *macrophyllum*	Nectar	Generalised
Fabaceae	*Eriosema crinitum*	Nectar	Generalised
Fabaceae	*Eriosema longifolium*	Nectar	Generalised
Apiaceae	*Eryngium juncifolium*	Nectar	Generalised
Erythroxylaceae	*Erythroxylum campestre*	Nectar and pollen	Generalised
Erythroxylaceae	*Erythroxylum deciduum*	Nectar and pollen	Generalised
Erythroxylaceae	*Erythroxylum microphyllum*	Nectar and pollen	Generalised
Erythroxylaceae	*Erythroxylum suberosum*	Nectar and pollen	Generalised
Myrtaceae	*Eugenia bimarginata*	Pollen (buzz)	Specialised

Myrtaceae	*Eugenia dysenterica*	Pollen (buzz)	Specialised
Myrtaceae	*Eugenia punicifolia*	Pollen (buzz)	Specialised
Convolvulaceae	*Evolvulus fuscus*	Nectar and pollen	Generalised
Convolvulaceae	*Evolvulus sericeus* var. *sericeus*	Nectar and pollen	Generalised
Fabaceae	*Galactia decumbens*	Nectar	Generalised
Apocynaceae	*Hemipogon acerosus*	Nectar	Generalised
Asteraceae	*Inulopsis scaposa*	Nectar and pollen	Generalised
Convolvulaceae	*Ipomoea procurrens*	Nectar and pollen	Generalised
Bignoniaceae	*Jacaranda oxyphylla*	Nectar	Generalised
Calophyllaceae	*Kielmeyera coriacea*	Pollen (buzz)	Specialised
Malvaceae	*Krapovickasia macrodon*	Nectar and pollen	Generalised
Asteraceae	*Lessingianthus bardanoides*	Nectar and pollen	Generalised
Asteraceae	*Lessingianthus brevifolius*	Nectar and pollen	Generalised
Asteraceae	*Lessingianthus grandiflorus*	Nectar and pollen	Generalised
Verbenaceae	*Lippia lupulina*	Nectar	Generalised
Convolvulaceae	*Merremia digitata*	Nectar and pollen	Generalised
Asteraceae	*Mikania oblongifolia*	Nectar and pollen	Generalised
Fabaceae	*Mimosa dolens* subsp. *eryophylla*	Nectar and pollen	Generalised
Fabaceae	*Mimosa dolens* var. *rigida*	Nectar and pollen	Generalised
Fabaceae	*Mimosa gracilis*	Nectar and pollen	Generalised
Fabaceae	*Mimosa* sp. 1	Nectar and pollen	Generalised
Fabaceae	*Mimosa* sp. 2	Nectar and pollen	Generalised
Myrtaceae	*Myrcia bella*	Pollen (buzz)	Specialised
Ochnaceae	*Ouratea spectabilis*	Pollen (buzz)	Specialised
Oxalidaceae	*Oxalis conorrhiza*	Nectar and pollen	Generalised
Apocynaceae	*Oxypetalum aequaliflorum*	Nectar	Generalised
Apocynaceae	*Oxypetalum capitatum*	Nectar	Generalised
Rubiaceae	*Palicourea rigida*	Nectar	Generalised
Malvaceae	*Peltaea polymorpha*	Nectar and pollen	Generalised
Celastraceae	*Peritassa campestris*	Nectar and pollen	Generalised
Amaranthaceae	*Pfaffia gnaphaloides*	Nectar and pollen	Generalised

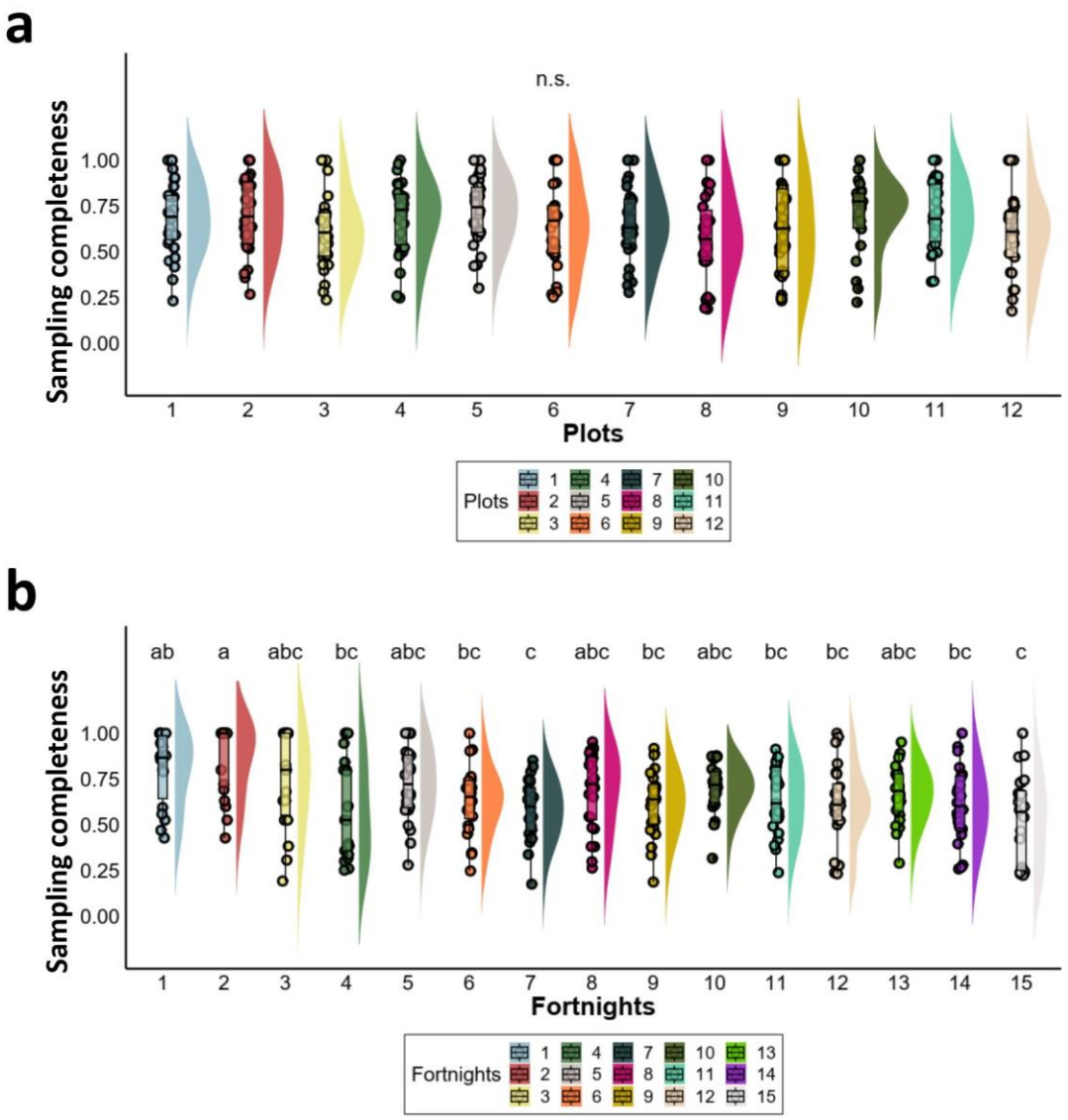
Asteraceae	*Piptocarpha rotundifolia*	Nectar and pollen	Generalised
Melastomataceae	*Pleroma oleifolia*	Pollen (buzz)	Specialised
Polygalaceae	*Polygala tenuis*	Nectar	Generalised
Myrtaceae	*Psidium australe*	Pollen (buzz)	Specialised
Rubiaceae	*Richardia stellaris*	Nectar	Generalised
Acanthaceae	*Ruellia bulbifera*	Nectar	Generalised
Euphorbiaceae	*Sapium obovatum*	Fragrance	Specialised
Sapindaceae	*Serjania erecta*	Nectar and pollen	Generalised
Malvaceae	*Sida ciliaris*	Nectar and pollen	Generalised
Iridaceae	*Sisyrinchium weirii*	Oil and pollen	Specialised
Solanaceae	*Solanum lycocarpum*	Pollen (buzz)	Specialised
Solanaceae	*Solanum palinacanthum*	Pollen (buzz)	Specialised
Fabaceae	*Stryphnodendron adstringens*	Nectar and pollen	Generalised
Fabaceae	*Stylosanthes acuminata*	Nectar	Generalised
Fabaceae	*Stylosanthes bracteata*	Nectar	Generalised
Fabaceae	*Stylosanthes gracilis*	Nectar	Generalised
Melastomataceae	*Tibouchina gracilis*	Pollen (buzz)	Specialised
Celastraceae	*Tontelea micrantha*	Nectar	Generalised
Iridaceae	*Trimezia juncifolia*	Oil and pollen	Specialised
Malvaceae	*Waltheria communis*	Nectar and pollen	Generalised
Fabaceae	*Zornia burkartii*	Nectar	Generalised
Fabaceae	*Zornia reticulata*	Nectar	Generalised



Supplementary Figure S3.1 Framework for data collection and plant-bee network construction. Plots (A) as spatial series of sampling units (n = 12 plots) in which data regarding floral resource diversity and plant-bee interactions was collected. Fortnights (B) defining a temporal series of sampling units (n = 30 fortnights) in which data regarding floral resource diversity and plant-bee interactions were obtained. Plot and fortnight units were joined as spatiotemporal sampling censuses (C) to evaluate floral resource diversity and plant–bee interaction networks.



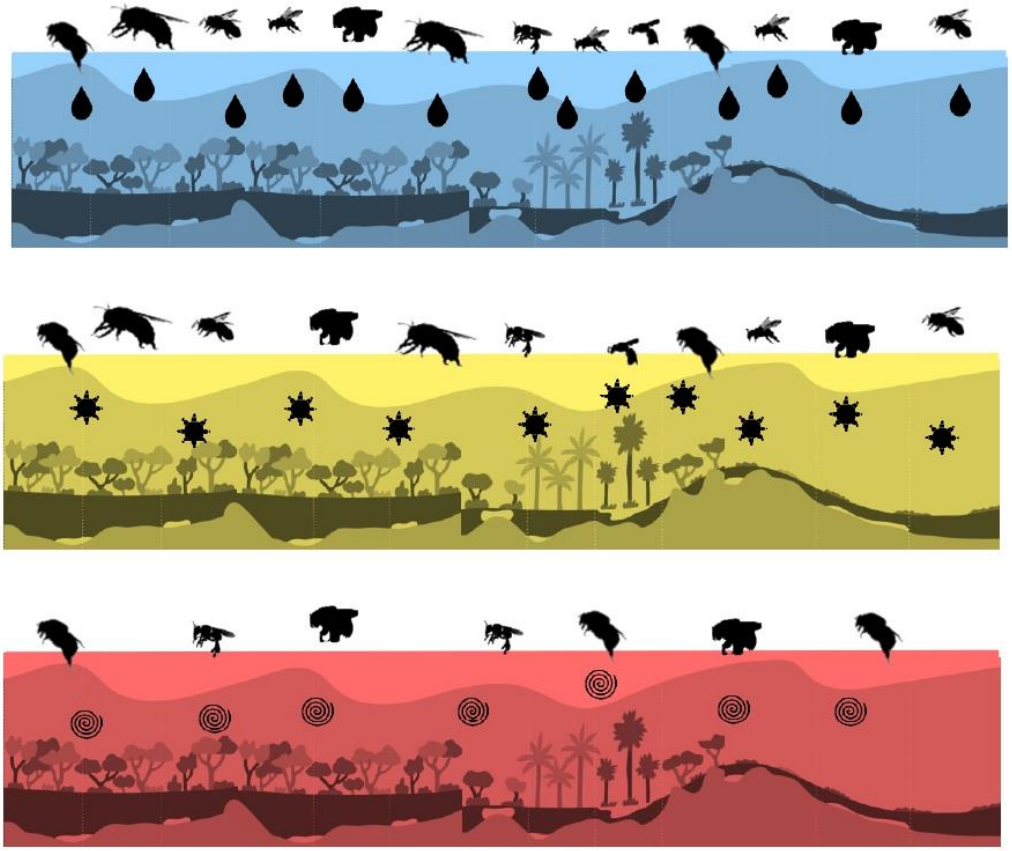
Supplementary Figure S3.2 Sampling completeness of plant-bee interaction networks. The overall plant-bee interaction network, i.e., merging all temporal interaction matrices (n= 180 matrices per year) built for each spatiotemporal census (n= 12 plots in space) in each flowering season, presented high values of sampling completeness both in the first (A) and second (B) flowering seasons. The frequency distribution of sampling completeness values for plant-bee networks constructed in each spatiotemporal sampling census (the finest-scale sampling resolution used in this study, C) varied but generally presented values above 65% completeness (a modal completeness of 66.1 ± 22.4).



Supplementary Figure S3.3 Sampling completeness comparisons among different plots and fortnights. Sampling completeness of plant-bee interaction networks in spatiotemporal sampling censuses was compared to evaluate if they differ between plots (A) and fortnights (B) by performing two generalized linear models (GLM) with quasibimonial error distribution. Sampling completeness did not differ among plots (i.e., with all the temporal censuses pooled for the same plot; $\chi^2_{11,\ 327} = 14.05$; $R^2 = 0.04$; $p = 0.23$). Still, sampling completeness differed in a minor fraction (4 out of 15) of the fortnights (i.e., with all the plot-census data pooled for a given fortnight period; $\chi^2_{14,\ 324} = 54.37$; $R^2 = 0.15$; $p < 0.01$).

4 CAPÍTULO III

Floral resource-mediated multilayer networks: trophic structure and pollinators' roles *



* Manuscrito em fase final de prepação para submissão na revista Functional Ecology.

Floral resource-mediated multilayer networks: trophic structure and pollinators' roles

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Abstract

1. Resource availability shapes species interactions, particularly in plant-pollinator mutualisms where floral resource diversity influences pollinator foraging. Bees, known for utilizing a broad array of floral resources, play a crucial role in this dynamic. Yet, it remains largely unexplored how similar are interaction networks involving bees and plants offering different floral resources, and whether bee abundance and functional traits contribute to explaining bee roles in connecting different floral resource-mediated networks.

2. We examined plant-bee interactions in the Brazilian Cerrado, focusing on nectar, pollen, and oil as floral resources. We compared the structure of plant-bee interaction networks mediated by these floral resources and evaluated bee roles in connecting and bringing stability to the resource-mediated multilayer network resulting from embedding the full diversity of floral rewards.

3. Unique bee assemblages were linked to specific floral resources, resulting in divergent network structures and interaction specificity. At one extreme, oil-mediated interactions exhibited nested, overlapping, and generalized relationships. Nectar- and pollen-mediated networks exhibited modular and specialized relationships at the other extreme. Interestingly, in addition to abundant and generalist bee species, those less abundant that visit plants offering oil or pollen in poricidal anthers played pivotal roles in connecting the resource-mediated layers and maintaining the stability of the multilayer network.

4. Our findings suggest that not only abundant and super-generalist bees but also rare and specialized bees contribute significantly to the structure of the plant-bee multilayer network. Despite variations in network structures mediated by different floral resources, the role of less common bee species emerged as critical in upholding the configuration and stability of plant-pollinator interactions.

5. Our study unveils complex relationships between floral resources, bee abundance, and bee traits in defining the topological signatures of plant-pollinator networks. It highlights the critical role of less common bee species in maintaining the configuration and stability of plant-pollinator interactions. These insights contribute to a better understanding of the intricate dynamics in plant-bee interaction networks in the context of varying floral resource availability and diversity.

Keywords: Bee traits**,** Buzzing, Floral resource-mediated layers, Plant-bee interaction network, Oil-collecting bees

4.1 INTRODUCTION

Abiotic factors represent relevant niche dimensions because they rule community assembly by filtering species that tolerate specific ranges of environmental conditions (Hutchinson, 1957; Chase et al. 2003; Kneitel & Chase, 2004; Lebrija-Trejos et al. 2010; Kraft et al. 2015). Nonetheless, trophic resources are also influential on community assembly because they represent niche dimensions related to species' requirements to survive and reproduce (Hutchinson, 1957; Tilman et al. 2004; Kremen et al. 2018; Ponisio et al. 2019; Gralka et al. 2020; Bauer et al. 2020). For example, a given species cannot thrive in habitats in which environmental conditions fit its tolerances if there are no resources to attend to its dietary demands, offer shelter and protection against antagonists, and favor its reproduction.

In plant-pollinator mutualisms, many plants offer floral resources essential for pollinator survival and reproduction. Given that, several animals' lineages present morphological, physiological, and behavioral traits related to the collection of different floral resources (Ollerton et al. 2017). For example, hummingbirds, phyllostomid bats, and adult hawkmoths heavily depend on floral nectar for energy acquisition. Bees, in turn, require a broad array of floral resources responsible for meeting their ecological demands across multiple niche dimensions (Almeida et al. 2023; Filipiak et al. 2023). They collect nectar to obtain readily spendable energy, accumulate pollen to store food for their offspring, gather oil to build their hives and brood cells, use resins for storing honey and protecting the nest, and manipulate fragrances to attract mates, representing thus the most eclectic group of floral resource users (Simpson & Neff 1981; Armbruster, 1984, 2017; Ollerton, 2006).

However, there are notable intraguild differences among bees that can influence plant-pollinator relationships (Raiol et al. 2021). Disparities in size, behavior, sociality, and resource-use breadth among bees hold significance in the community context, as they determine the number of plant species a particular bee species forage on, the frequency of such foraging, and the proportion of plant species it effectively pollinates (Greenleaf et al. 2007; Martins et al. 2015; Aguiar et al. 2023; Lanuza et al. 2023). Among the functional attributes of bee species that may greatly affect plant-pollinator relationships is the ability to collect different floral resources (Vaudo et al. 2015; Armbruster, 2017; Ogilvie & Forrest

2017). Despite relying on nectar to fulfill their instantly dietary requirements (Nicolson et al. 2009), many bee taxonomic groups have evolved specialized relationships with flowering plants that offer other resources than nectar, such as pollen (removable only by sonication, i.e., buzzing), oil, fragrances, and resins (Cameron, 2004; Minckley & Roulston, 2006; Ramirez et al. 2010; Armbruster, 1984, 2012, 2017; Martins et al. 2015; Pacheco-Filho et al. 2015; Cardinal et al. 2018). For example, in contrast to bee-pollinated nectar-producing plants, plant species offering oil might exhibit some functional constraints that allow only certain specialized bee groups to legitimately visit their flowers (Armbruster 2012). All this suggests that the diversity of floral resources offered by a given plant assemblage, and not just the plant species diversity per se, may have a lasting effect in structuring interaction diversity in bee assemblages (Armbruster, 2017; Ballarin et al. ***submitted to Journal of Ecology – Capítulo II***).

However, questions related to how the division of labor of floral resource collection among bee species affects the structure and functioning of plant-pollinator relationships in the community context remain understudied (Armbruster, 2017). For example, the structure of plant-bee interaction networks mediated by different floral resources has already been examined (oil-mediated - Bezerra et al. 2009; fragrance-mediated – Rocha-Filho et al. 2012; and pollen-mediated – Mesquita-Neto et al. 2018 and Valadão-Mendes et al. 2022). Nevertheless, comparisons among the structural patterns of these networks remain challenging because they were sampled in different natural communities. Therefore, the topological differences among them may not necessarily reflect the direct effects of different floral resources on plant-bee network structure. Instead, they could be attributed to environmental or compositional differences that arise inherently when comparing different plant and bee assemblages. Sampling plant-bee networks mediated by different floral resources in the same plant community — where environmental and compositional effects can be controlled — is thus necessary for a more comprehensive evaluation of the effects of floral resource diversity per se on the structure of plant-bee interaction networks (Armbruster, 2017). For example, it might be expected that a given change in floral resource diversity (e.g., along the season) may differentially contribute to adding more interactions by specialized

than by generalized bee species (Armbruster, 2017; Ballarin et al. ***submitted – Capítulo II***). Thus, the turnover in interaction diversity may respond more to shifts in the diversity of floral resources (i.e., the resource base) than to just plant species turnover.

Each distinct floral reward offered by plants to bees represents a unique niche dimension for these animals, as they consist of different chemical attributes and are associated with distinct life-history events for individual bees within their respective populations (e.g., bees collecting pollen to feed larvae and bees collecting oil to build brood cells). In this sense, the whole complexity of plant-bee interaction networks can be better represented by a multilayer framework where different floral resources have associated distinct forms of interaction, represented as different layers. The evaluation of a multilayer network enables the study of how species occupying different layers within a web due to temporal, spatial, functional, or trophic partitioning (Pilosof et al. 2017; Hutchinson et al. 2018; Souza et al. 2022; Vitali et al. 2023), are indirectly connected, and which functional traits are responsible for linking these different layers (Hervías-Parejo et al. 2020). In this context, a multilayer framework helps representing different floral resources potentially supporting bees in a specific intrapopulation task, which may be valuable for identifying, e.g.,: 1) bee species crucial for maintaining the structure and stability of plant-bee interaction networks mediated by different floral resources (i.e., resource-mediated multilayer network), and 2) bee species connecting with more frequency these trophic, resource-mediated layers.

In this study, we sampled plant-bee interactions in the Brazilian Cerrado, a conservation priority hotspot (Myers et al. 2000), which hosts numerous plants reliant on bee pollination and offering various types of floral resources (Gottsberger & Silberbauer-Gottsberger 2006). We used three sets of plant-bee interaction networks mediated by nectar, pollen, and oil, and also created a plant-bee multilayer network with three layers, each representing the type of resource collected by bees during their foraging bouts on flowers (i.e., nectar, pollen, and oil). To this end, we aimed to answer three key questions: 1) Do bee assemblages collecting different floral resources present compositional differences? 2) Do the structural patterns of plant-bee interaction networks mediated by distinct floral resources differ? 3) Which are the bee species and their associated functional traits responsible for

maintaining cohesive layers composed of pairwise plant-bee interactions mediated by different floral resources? We anticipated that the β-diversity of bees using different floral resources would be highest when comparing oil- to nectar- or pollen-mediated interactions due to constraints oil-flowers impose on floral visitors, and the particular interest of certain taxonomic bee groups adapted to collecting and using oil (Vogel, 1969; Roubik, 1992). Likewise, we hypothesized that oil-mediated plant-bee interaction networks would show higher niche overlap among bees compared to nectar- and pollen-mediated networks (Bezeera et al. 2009). This is attributed to the greater functional differences between nectar- and pollen-offering flowers in contrast to oil-offering flowers, which have undergone pollinator-mediated selection by specialized bee groups (Davis et al. 2014). Consequently, the oil-mediated plant-bee interaction network was expected to have lower specialization and higher network nestedness, indicating more generalist interactions (see Bezerra et al. 2009). In contrast, nectar- and pollen-mediated networks would display lower levels of nestedness but higher specialization and modularity, reflecting networks with more specialized relationships. Finally, considering that super-generalist bees (i.e., those that interact with the highest amount of plant species) are typically represented by the most abundant species (Giannini et al. 2016), we hypothesized that bees with higher abundances would play a role in connecting layers within the multilayer network. In this sense, bee abundance would be responsible for bringing stability to the resource-mediated multilayer network.

4.2 MATERIAL AND METHODS

4.2.1 Study site

We conducted this study in the flowering season between 2022 and 2023 at the Botucatu State Forest (BSF), a Cerrado area located in the peri-urban zone of Botucatu municipality, São Paulo state, Brazil (22°56'20"S, 48°27'29"W; 860 a.s.l.). BSF possesses 34 ha. with a mixture of vegetation profiles encompassing floodable gallery forests, humid *campo* limpo generally presenting a dominant herbaceous layer, and a *campo sujo* represented by varying degrees of scattered trees, shrubs, herbs, and grasses. The climate in BSF is high-altitude tropical (Aw according to Köppen classification; Franco et al. 2023) with two marked seasons: a cold and dry winter subject to sporadic frosts from April to

September, and a hot and wet summer from October to March, when the greater portion of angiosperms flourish.

4.2.2 Plant community composition and interaction sampling

To determine the plant community composition as well as plant-bee interactions, we established twelve 100m² (10m x 10m) plots, which were at least 30 m apart, to cover a broad range of compositional differences among plant communities as they differed in the number and plant species identities. From mid-July 2022 to early March 2023, we sampled flowering plants weekly by counting the number of flower units in each plant individual at the flowering stage and recording the floral resources they offered to bees within each plot. We considered three major types of floral resources: 1) pollen; 2) nectar; and 3) oil as they were offered by more than one plant species. Similarly, we sampled plant-bee interaction every week through visual censuses recorded within each plot from 06:00h to 12:00h and 12:00h to 18:00h on two consecutive days. In each plot, we sampled different flowering units for three to five minutes, counting each bee legitimately visiting the flowers and recording each floral resource it was collected (n = 720 h). In our study, three main resources were collected by bees in more than one plant species: nectar, pollen, and oil. We considered legitimate visits only those events in which bee individuals touched the reproductive organs of the flower. To avoid inherent biases arising from periods of sampling within the day, we alternated plot order on different days of sampling. We identified plant species *in situ* with the aid of a floral guide list for BSF (Pilon et al. 2017). Plant species that we could not identify in the field were collected and taken to the Herbarium of São Paulo State University (Herbarium BOTU) for further identification with the aid of specialists. We collected each different bee specimen with entomological nets and identified each bee species using identification keys (Silveira et al. 2002) and the aid of specialists.

Interaction data at the species level were summarized in the form of an adjacency matrix M in which elements m_{ij} depict the frequency at which a given bee species i interacts with a given plant species j. As we intended to evaluate topological and functional differences in interaction networks built with plant-bee relationships mediated by different floral resources (e.g., nectar, pollen, and oil), we built 39 interaction networks. 36 networks were

built by pooling data of plant-bee interaction frequencies within each plot considering different floral resources offered by plants to bees (i.e., three different floral resource-mediated networks for each plot). This accounts for topological variations in network structure among plots with different network sizes (i.e., varying numbers of plants and bees), thereby enabling the evaluation of the network structure and the dissimilarity among bee species. The remaining three networks were constructed by pooling data of resource-mediated plant-bee interaction frequencies from all plots together, which represented the layers in the multilayer approach and depict a snapshot of each type of trophic interaction.

4.2.3 β-diversity of bee assemblage and network structure

Due to inherent differential patterns of resource use among bees, we first calculated the turnover of bee species participating in networks built with different floral resources. For this purpose, we assessed the uncorrected probability version of the abundance-based Chao-Sørensen indices, namely $\beta_{Chao–Sørensen}$ (Chao et al. 2006; Barwell et al. 2015). $\beta_{Chao–Sørensen}$ ranges from 0 to 1, with values close to 1 indicating substantial species turnover between bee assemblages. These calculations were performed using the *CommEcol* and *vegan* packages in R (Dixon 2003; Melo & Melo 2019).

We also evaluated whether patterns of resource use in plant-bee interaction networks mediated by different floral resources differ. For this end, we extracted niche overlap (R_0), and network-wide specialization (H_2'). R_0 quantifies the resemblance of interaction patterns of bee species in an interaction network and is assessed using Horn's index (Horn, 1966). Values of R_0 vary from 0 to 1. Low values of R_0 depict that bee species are feeding on different plant species to assess a given floral resource within the community. H_2', in turn, quantifies the level of specialization within an interaction network by considering the availability of potential partners and reflects the degree of interaction exclusiveness within a network. H_2' varies from 0 to 1, and values closer to 1 indicate a higher degree of specialization within the interaction network (Blüthgen et al. 2006). Finally, we investigated whether potential differences in resource use among bees collecting different floral resources affect the structure of the plant-bee interactions network built according to different floral resources collected by bees. For this end, we calculated two network metrics related to

network topology: weighted nestedness (wNODF) and weighted modularity (Q'_w). wNODF assesses the extent to which specialized species interactions compose subsets encompassed within generalist interactions (i.e., nestedness), with higher values indicating higher nestedness. Q'_w depicts whether the network presents interaction compartments within which species interact more with each other than with species from other compartments. Q'_w ranges from 0 to 1, where 0 signifies no compartmentalization, whereas 1 indicates complete compartmentalization (Olesen, 2007). We used the DIRTLPAwb+ algorithm to calculate modularity (Beckett, 2016). It has been shown that the comparison of wNODF and Q'_w values between different networks may suffer biases arising from network connectance and size (Dalsgaard et al. 2017; Song et al. 2017). To address this, we applied a Δ-transformation by comparing the observed values of wNODF and Q'w with random structural patterns (i.e., wNODF and Q'w generated by a null model). This allows us to correct the deviations arising from factors other than the structural differences between networks. We assessed Δ-wNODF and Δ-Q'w values by subtracting the mean value of random networks from empirical values. We used the Patefield algorithm to generate the null models, ensuring that species richness (i.e., network size) and the total number of interactions between species are preserved. Sampling completeness, assessed by calculating the ratio between the observed and the estimated plant-bee interaction richness using Chao 1 estimator (Chao, 1984) and the *iNEXT* package (Hsieh et al. 2016), exceeded 70% in each resource-mediated network (i.e., nectar-, oil-, and pollen-mediated; Figure 4.1d). Sampling completeness in oil-mediated plant-bee interaction network built for each plot were higher than nectar- and pollen-mediated plant-bee interaction networks (88.4% ± 11.7; Supplementary Figure S4.1). Still, both nectar-mediated and pollen-mediated networks exhibited consistently high levels of sampling completeness in each plot (nectar - 70.9% ± 12.7; pollen - 59.9% ± 15.7; Supplementary Figure S4.1).

4.2.4 Multilayer network and bee species' role in connecting layers

A multilayer network is a representation of the whole complexity of interactions in systems with heterogeneous types of interactions (Hutchinson et al. 2019, De Domenico, 2022). To construct the multilayer network, we pooled the three networks built with plant-

bee interactions for each floral resource offered by plants and used by bees (Figure 4.1a). This type of multilayer is often represented as an edge-colored graph (De Domenico, 2022), i.e., a representation of a system with heterogeneous links between the nodes (in our case the set of nodes V includes all the plant and bee species, V_P and V_B, respectively). Hence, plant-bee interactions (links) were grouped according to the resource type used so that each link was assigned to a color depending on the resource type used (Figure 4.1b). Thus, links of the same color belong to the same layer (Hammoud and Kramer, 2020). Each layer can contain a subset or the complete set of nodes, depending on whether the plants produce the specific resource associated to the layer (color) or the bee species use that type of resource.

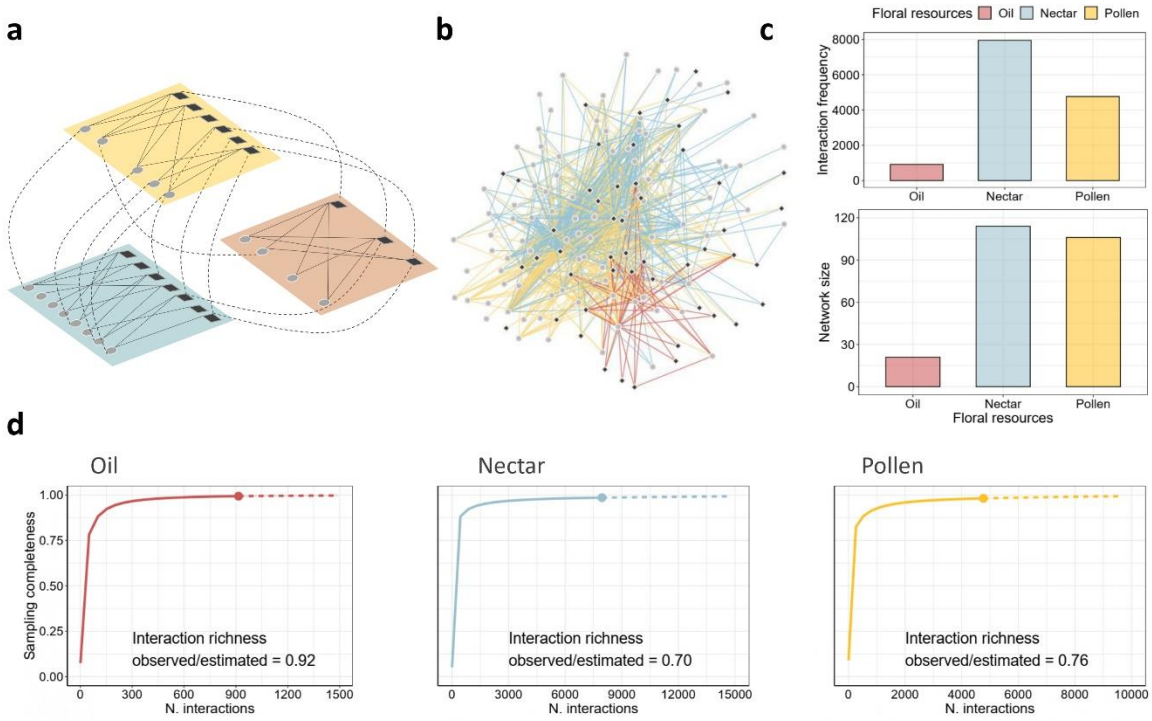


Figure 4.1 Floral resource-mediated plant-bee interaction networks. Layers are color-coded in the schematic design (a) based on the resource type provided by plants to bees (nectar - blue; pollen - yellow; oil - red). Observed multilayer interaction network (b) illustrates plants (gray circles) linked to bees (black diamonds), depending on the resource that mediates the interaction between them. The discrete colors in links follow the same palette as the schematic design. Interaction frequency (i.e., number of links) and network size (i.e., the sum of plants and bees interacting mediated by a given floral resource) of each resource-mediated layer within the multilayer network are depicted in (c). Sampling completeness in all resource-mediated layers exceeded 70% (d).

Plant-bee interactions were arranged as adjacency matrices, where plants were listed as rows and pollinators as columns. In each matrix, each cell represented an individual pairing of a plant and a pollinator. Thus, the resource-mediated plant-bee multilayer network comprised three layers, each one representing one type of resource-mediated plant-bee interaction (i.e., nectar-mediated, pollen-mediated, and oil-mediated). These layers were interlinked through a shared set of plant species offering more than one floral resource (e.g., *Caryocar brasiliense* and *Byrsonima intermedia* offering nectar and pollen, or oil and pollen, respectively) or a set of bee species consuming more than one type of resource (e.g., *Centris* sp. foraging on nectar, pollen, and oil). Consequently, both plants and bees could engage in interlayer links if they participated in interactions mediated by different floral resources. To build the multilayer network, we assumed interlayer strength across all species to be equal, meaning that every species connecting the layers exerts an identical effect in the interlayer process (Pilosof et al. 2017; Timóteo et al. 2018; Souza et al. 2022). Ultimately, we configured our layers to enable interconnection with both adjacent and non-adjacent layers. This decision was based on the fact that certain bee species utilizes all three floral resources, resulting in a lack of clear ordination when connecting them.

We used five multilayer metrics to inspect bee species' roles in connecting layers of plant-bee interactions mediated by different floral resources: node multidegree, node versatility, closeness centrality, k-coreness, and the standard deviation degree (De Domenico, 2022, Frydman et al. 2023). The node multidegree metric expands the conventional concept of degree in bipartite ecological networks (i.e., the number of connections among species within a network) by adopting a multilayer network perspective. This approach considers the possibility that a species may interact with different partners across distinct layers within the network, capturing the number of interactions a given species may have in the multilayer network (Dickison et al. 2016). Node versatility identifies species with high topological importance in shaping the structure of a multilayer network. Node versatility uses Google’s PageRank algorithm, which describes the path length taken by a random walk between adjacent nodes within a layer and among layers (De Domenico et al. 2015a; Timóteo et al. 2018). It assigns an importance score to a given species based on the distance from an

important highly connected node (i.e., root nodes). This procedure illustrates the level of dependence of a given root node on a species being scored (Allesina & Pascual, 2009; Chen et al. 2009). The closeness centrality measures how close a node is to all other nodes, irrespective of whether they represent core and highly connected nodes or not (Freeman 1979, González et al. 2010). Nodes with high closeness centrality values can spread information throughout the entire network faster; in our study case, closeness is related to versatility in the use of floral resources by any bee species. The k-coreness ranks species based on their location within a specific subset of nodes (i.e., k-core). K-cores are subsets of nodes where each node has at least k connections within the same subset (Seidman, 1983). In other words, they are maximal connected sub-networks where every node has at least k connections to other nodes within that sub-network. The k-core is identified by progressively removing nodes and their links if they have less than k connections, stopping when further removal would break the sub-networks connectivity (Dorogovtsev et al. 2006). In ecological networks, k-coreness helps identify densely interconnected regions within a network and which species are important for preventing the structural collapse of the network (García-Algarra et al. 2017; Morone et al. 2019). Species with higher values of k-coreness are more important for maintaining the network structure stable. Finally, the standard deviation degree indicates the evenness of interaction for a given species across different layers. For example, in our study, we were interested in evaluating how bees collecting different resources connect layers. Thus, if a given bee exhibits an identical number of partners in the three layers, it would indicate that it connects layers, exhibiting a standard deviation degree equal to 0. Conversely, if a bee species had numerous plant partners in the layer representing nectar-mediated plant-bee interactions and only a few or no partners in the layers representing oil-mediated and pollen-mediated plant-bee interactions, it would exhibit a high standard deviation degree, indicating an uneven participation across the three layers (Dickison et al., 2016). The exploration of different species level descriptors based on various interaction patterns helps us accurately estimate the importance of bee species in the multilayer network (Sazima et al. 2010). To construct the multilayer network and assess species-level metrics we used the packages *igraph* (multilayer network building; Csárdi et al. 2023), *multinet*

(standard deviation degree assessment; Magnani et al. 2021), and *muxViz* (assessment of the remaining metrics; De Domenico et al. 2015b; De Domenico, 2022) in R.

4.2.5 Data analysis

We performed a generalized linear model (GLM) with quasibinomial error distribution to evaluate whether the β-diversity of bee assemblages formed by bee species collecting distinct floral resources differ. In this model, we considered the pairwise comparison (e.g., nectar-collecting *versus* oil-collecting bee assemblages) as a fixed factor and the Sorensen taxonomic β-diversity as the response variable. Differences among pairwise β-diversity of bee assemblages were inspected through a Tukey *posthoc* test using the package *emmeans* in R (Lenth, 2016).

To evaluate differences among the patterns of resource use and the structure emerging from plant-bee interaction networks built according to different resources collected by bees on flowers, we made four linear mixed models (LMM) considering as response variables the network metrics extracted in each floral resource-mediated network built with plant-bee interaction sampled in each plot (n = 36). We considered for each LMM the following network metrics as response variables: niche overlap (R_0), community-wide specialization degree (H2'), transformed-weighted nestedness (ΔwNODF), and transformed-weighted modularity (ΔQ'w). The floral resources collected by bees (e.g., nectar, pollen, and oil) were considered as a fixed factor, and the plots were treated as a random effect. Differences among network descriptors across networks built according to each floral resource collected by bees were assessed with a Tukey *posthoc* test using the *emmeans* package.

To verify whether bee functional traits and bee abundances reflect their roles in connecting layers, we conducted sixteen linear models for each response variable (i.e., node multidegree, node versatility, closeness centrality, k-coreness, and the standard deviation degree). The models were built considering all possible combinations among four predictive variables: the bee type according to their partner flexibility (i.e., oligoletic or polyletic), the bee size (i.e., small, medium or large), their resource-use breadth, which depicts their capacity of collect different types of resources (i.e., ability to collect pollen through

sonication, ability to collect oil, both or none), and the abundance of bees during the entire flowering season. Bee discrete traits were assessed using a recent dataset on Neotropical bee traits (Borges et al. 2020), literature sources (e.g., Devkota et al. 2024), and input from specialists. Bee abundance was assessed in censuses carried out independently to the visit surveys by counting bees interacting with plants outside the plots or non-interacting bees within the plots on the same days when plant-bee interaction sampling was made. For the linear model using node multidegree and k-coreness as the response variables, we used a Poisson error distribution, for node versatility and closeness centrality, a Gaussian error distribution was applied, and for standard deviation degree we used a Gamma error distribution. We then used the Akaike Information criterion corrected for small samples (AICc) to compare the global models, which included the combination between all four predictive variables with the (i) candidate models, which were represented by the predictive variables used in separate or combined, and (ii) the null model. The model with the lowest AICc represents the best-fit model in all linear model comparisons. We conducted model averaging by determining the relative importance ($\Sigma\omega_i$) of each predictor variable for each linear model. ω_i values represent the likelihood of a model having the best fit, and the cumulative $\Sigma\omega_i$ values indicate the probability of a predictor variable being part of the best-fit model (Burnham & Anderson, 2002; Symonds & Moussalli, 2011). We also assessed multicollinearity in the best-fit models using the *mgcv* package in R (Wood & Wood, 2015). VIF values below 3 suggest acceptable collinearity among predictor variables (Zuur et al. 2010). Further, to evaluate how different information each multilayer network metrics deliver, we applied Spearman's correlation tests among them and bee abundance.

Finally, we explored how bee species' role in the multilayer network varied with their traits by creating an adjacency matrix B where elements b_{ij} represent the multilayer metrics j estimated for each bee species i. We then used Bray-Curtis distance to create a distance matrix that identifies variations in bee multilayer roles with their accompanying functional traits. To evaluate whether bee species' roles in the multilayer network can be anticipated by their functional traits we conducted a permutational multivariate analysis of variance (PERMANOVA) with the 'adonis2' function (1000 permutations) in the *vegan* package in R

(Oksanen et al. 2022). The PERMANOVA was fitted considering the bee type according to their partner flexibility, the bee size, and the resource-use breadth as variables influencing bee species' role in the multilayer network. Furthermore, we investigated whether the variances in bee species' role on the multilayer network homogeneously vary among classes of bee type, size, and resource-use breadth with the function 'betadisper' in package *vegan* (PERMDISP protocol; Anderson et al. 2006). To illustrate changes in bee species' role following differences in bee traits, we employed Non-metric Multidimensional Scaling (NMDS) using the 'metaMDS' function from the *vegan* package.

4.3 RESULTS

Overall, we recorded 13,627 legitimate interactions between 96 plant and 50 bee species (1136 ± 629 plant-bee interactions per plot). Nectar was the most common floral resource collected by bees (7,946 flower visits – 58.3%), followed by pollen (4,766 flower visits – 35.0%) and oil (915 flower visits – 6.7%). At the species level, 43 bee species foraged for nectar on 71 plants, 40 bees foraged for pollen on 66 plants, and 14 bees foraged for oil on 7 plant species. *Eulaema nigrita* and *Euglossa* sp. also visited *Sapium obovatum* for fragrance collection, but were not considered in this study.

As expected, the bee β-diversity was higher when comparing oil-collecting bee assemblages with the nectar- and/or pollen-collecting bee assemblages ($F_{1, 431}$= 3858.1, R^2= 0.90, p< 0.001; Figure 4.2). Niche overlap in resource use was higher in oil-collecting bee assemblages than in bee assemblages collecting other floral resources than oil ($F_{1, 34}$= 128.45, R^2= 0.80, p< 0.001; Figure 4.3b), even though we found no differences of network-wide specialization ($F_{1, 34}$= 3.92, R^2= 0.11, p= 0.140; Figure 4.3c). We found progressively higher compartmentalization of interactions from the oil-mediated interaction networks (i.e., lowest modularity) to the pollen-mediated interaction networks (i.e., highest modularity; $F_{1, 34}$= 150.27, R^2= 0.82, p< 0.001; Figure 4.3d). Also, nestedness differed between oil-, nectar-, and pollen-mediated plant bee interaction networks ($F_{1, 34}$= 6.44, R^2= 0.16, p= 0.039; Figure 4.3e). Nevertheless, no differences in nestedness could be tracked between resource-mediated plant-bee interactions after the Tukey *posthoc* test. Still, a trend emerging from

network structure following differences in the patterns of resource use among bees feeding on different resources could be noticeable even without statistical differences between resource-mediated plant-bee networks. At one extreme, oil-mediated plant-bee networks presented the bee assemblage with the lowest interaction exclusiveness, which resulted in networks that were more nested and less modular. Conversely, nectar- and pollen-mediated plant-bee interaction networks presented increasing levels of specialization in resource use, which resulted in plant-bee networks that were less nested and more modular.

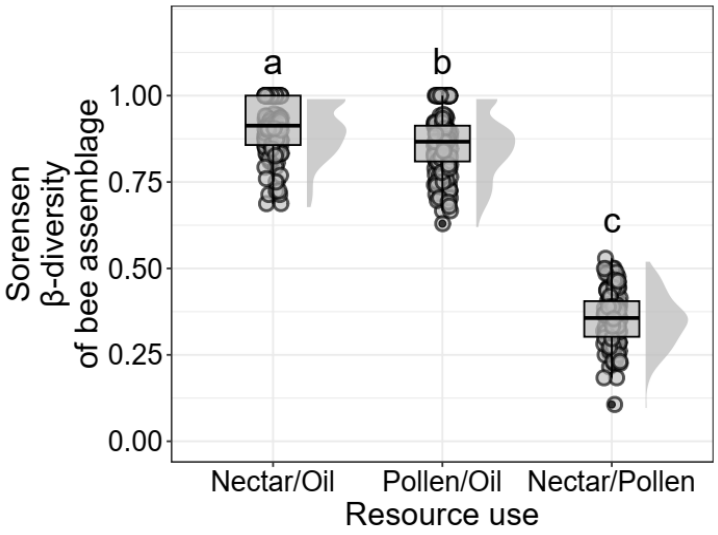


Figure 4.2 β-diversity of bee assemblages that use different floral resources. Dissimilarities among bee assemblages are higher when comparing bee intraguild that collect oil or nectar and oil or pollen. Bee assemblages collecting nectar or pollen show higher taxonomical similarity. The compact letter displays significant differences among the β-diversity of bee assemblages after a Tukey *posthoc* test.

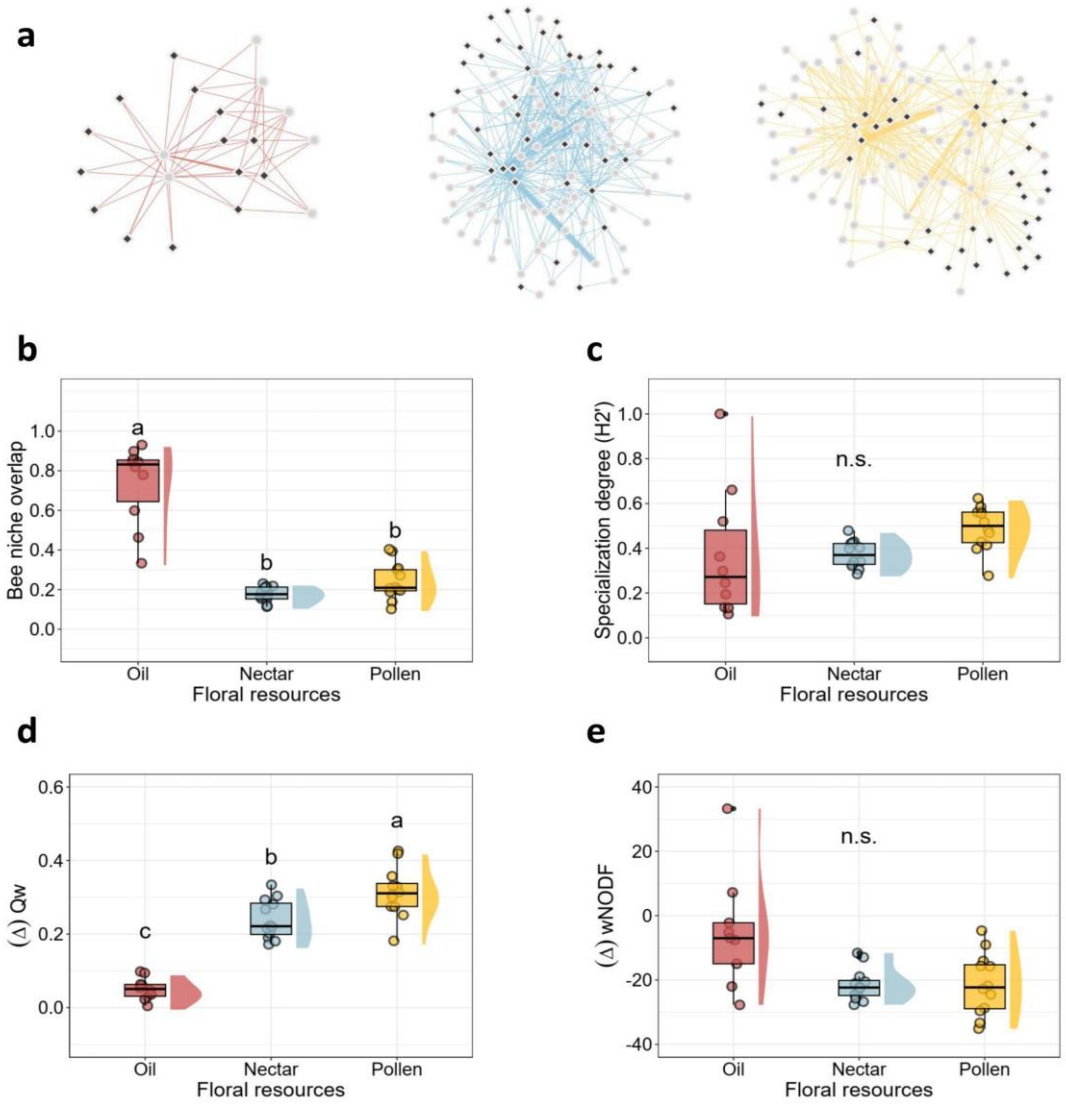


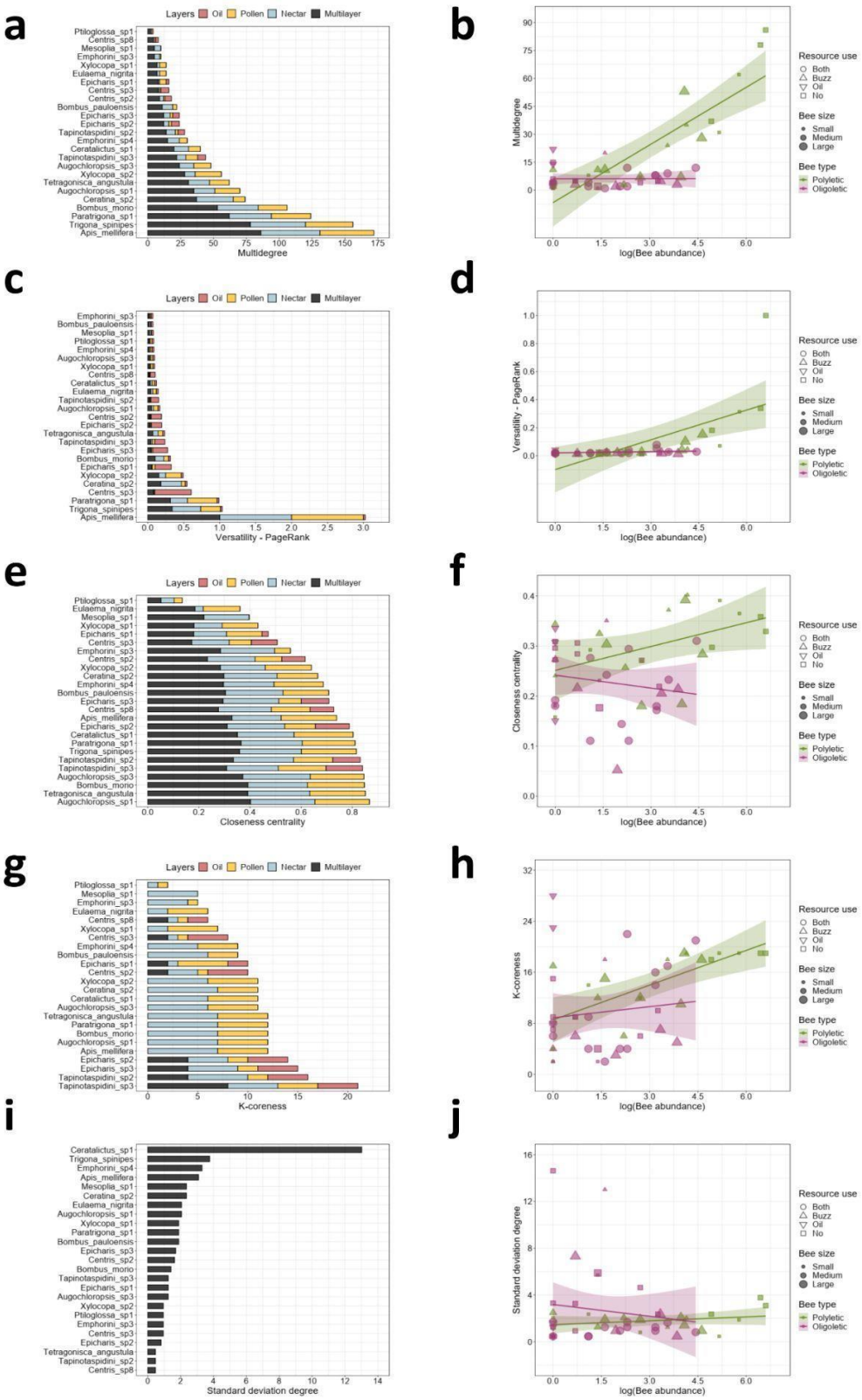
Figure 4.3 Patterns of resource use and network structure in bee assemblages that collect different floral resources within floral resource-mediated plant-bee interaction networks. Resource-mediated plant-bee interaction network presents different topology, size, and interaction frequency (a). Oil-mediated plant-bee interaction networks show higher niche overlap among bees (b). Community-wide specialization degree (c) did not differ among different floral resource-mediated plant-bee interaction networks, whilst there is a noticeable trend pointing to the low level of specialization in the network built with oil-mediated plant-bee interactions. Oil-mediated plant-bee interaction networks show low levels of network compartmentalization (i.e., modularity; $\Delta Q'_{w;}$ d). Despite not significantly differing, oil-mediated plant-bee interaction networks show marginally higher levels of network nestedness (ΔwNODF; e). The compact letter displays significant differences among

network metrics extracted for each network after a Tukey posthoc test. “n.s.” depicts no significant differences among floral resource-mediated plant-bee interaction networks.

As expected, super-generalist bees in high abundance, such as *Apis mellifera* and *Trigona spinipes*, received the highest scores for multidegree (Figure 4.4a) and node versatility (Figure 4.4c). Indeed bee abundance was the best factor explaining variations in both metrics, though bee partner flexibility, bee size, and bee resource-use breadth also influenced bee multidegree (Figure 4.4b, d; and Table 4.1). Nonetheless, the superabundant and super-generalist bees did not receive the highest score for bee closeness centrality (Figure 4.4e), k-coreness (Figure 4.4g), and standard deviation degree (Figure 4.4i). Contrary to what we expected, most of the variation in bee closeness centrality, k-coreness, and standard deviation degree were explained by bee functional traits rather than bee abundance. Still, abundance had a minor contribution to explaining bee k-coreness (Table 4.1). Bee closeness centrality was primarily explained by bee partner flexibility and bee size (Figure 4.4f and Table 4.1). Bee k-coreness and bee standard deviation degree, on the other hand, was predominantly influenced by bee partner flexibility and resource-use breadth (Figure 4.4h,j and Table 4.1). Besides the strong correlation among bee abundance, multidegree, and node versatility (rho > 0.80), the remaining multilayer metrics exhibited lower correlations with each other and abundance. This implies they provide unique insights into bee roles in the multilayer network structure and interaction patterns (Supplementary Figure S4.2).

Table 4.1 Model selection and averaging for linear models explaining the variation bee species role in connecting layers in the floral resource-mediated multilayer network. The likelihood of the predictor variables in explaining multilayer metrics is indicated by Σωi. Superscripted number in each explanatory variable indicate the linear model in which it was found to significant after AICc model selection. Variables with $\Sigma\omega_i > 0.5$ are shown in bold and represent the most probable predictors in explaining variations in bee species role in connecting floral resource-mediated layers. $\chi 2$ goodness of fit and p-value (p) for each variable in linear models are provided. In each model, variables with p-value < 0.05 are shown in bold. ωi depicts the probability of the model with the lowest AICc to be the best fit. AICc and R^2 are also provided.

	Multidegree (1)			PageRank - versatility (2)			Closeness centrality (3)			K-coreness (4)			Standard deviation degree (5)		
	∑ωi	χ2	p	∑ωi	χ2	p	∑ωi	χ2	p	∑ωi	χ2	p	∑ωi	χ2	p
Bee partner flexibility[1,3,4,5]	**1**	56.43	**<0.001**	0.24			**0.832**	7.56	**0.006**	**1**	21.45	**<0.001**	**0.98**	10.64	**<0.01**
Bee size[1,3]	**0.701**	7.26	**0.027**	0.09			**0.934**	11.5	**0.003**	0.084			0.08		
Bee abundance[1,2,4]	**1**	173.20	**<0.001**	**1**	243	**<0.001**	0.432			**0.94**	8.3	**0.004**	0.35		
Bee resource-use breadth[1,4,5]	**1**	29.18	**<0.001**	0.02			0.049			**1**	35.82	**<0.001**	**0.99**	25.69	**<0.001**
Intercept	0			0			0			0			0		
	R^2		0.76			0.83			0.36			0.34			0.12
	ωi		0.70			0.66			0.49			0.87			0.32
	AIC		482.20			-132.03			-121.48			360.41			171.68


a
Layers Oil Pollen Nectar Multilayer
Ptiloglossa_sp1
Centris_sp8
Mesoplia_sp1
Emphorini_sp3
Xylocopa_sp1
Eulaema_nigrita
Epicharis_sp1
Centris_sp3
Centris_sp2
Bombus_pauloensis
Epicharis_sp3
Epicharis_sp2
Tapinotaspidini_sp2
Emphorini_sp4
Ceratalictus_sp1
Tapinotaspidini_sp3
Augochloropsis_sp3
Xylocopa_sp2
Tetragonisca_angustula
Augochloropsis_sp1
Ceratina_sp2
Bombus_morio
Paratrigona_sp1
Trigona_spinipes
Apis_mellifera
0 25 50 75 100 125 150 175
Multidegree
b
Multidegree
0 15 30 45 60 75 90
0.0 1.5 3.0 4.5 6.0
log(Bee abundance)
Resource use
Both
Buzz
Oil
No
Bee size
Small
Medium
Large
Bee type
Polyletic
Oligoletic
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Layers Oil Pollen Nectar Multilayer
Emphorini_sp3
Bombus_pauloensis
Mesoplia_sp1
Ptiloglossa_sp1
Emphorini_sp4
Augochloropsis_sp3
Xylocopa_sp1
Centris_sp8
Ceratalictus_sp1
Eulaema_nigrita
Tapinotaspidini_sp2
Augochloropsis_sp1
Centris_sp2
Epicharis_sp2
Tetragonisca_angustula
Tapinotaspidini_sp3
Epicharis_sp3
Bombus_morio
Epicharis_sp1
Xylocopa_sp2
Ceratina_sp2
Centris_sp3
Paratrigona_sp1
Trigona_spinipes
Apis_mellifera
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Versatility - PageRank
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0.0 1.5 3.0 4.5 6.0
log(Bee abundance)
Resource use
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Buzz
Oil
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Bee size
Small
Medium
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Bee type
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Layers Oil Pollen Nectar Multilayer
Ptiloglossa_sp1
Eulaema_nigrita
Mesoplia_sp1
Xylocopa_sp1
Epicharis_sp1
Centris_sp3
Emphorini_sp3
Centris_sp2
Xylocopa_sp2
Ceratina_sp2
Emphorini_sp4
Bombus_pauloensis
Epicharis_sp3
Centris_sp8
Apis_mellifera
Epicharis_sp2
Ceratalictus_sp1
Paratrigona_sp1
Trigona_spinipes
Tapinotaspidini_sp2
Tapinotaspidini_sp3
Augochloropsis_sp3
Bombus_morio
Tetragonisca_angustula
Augochloropsis_sp1
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Closeness centrality
0.0 0.1 0.2 0.3 0.4
0.0 1.5 3.0 4.5 6.0
log(Bee abundance)
Resource use
Both
Buzz
Oil
No
Bee size
Small
Medium
Large
Bee type
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Layers Oil Pollen Nectar Multilayer
Ptiloglossa_sp1
Mesoplia_sp1
Emphorini_sp3
Eulaema_nigrita
Centris_sp8
Xylocopa_sp1
Centris_sp3
Emphorini_sp4
Bombus_pauloensis
Epicharis_sp1
Centris_sp2
Xylocopa_sp2
Ceratina_sp2
Ceratalictus_sp1
Augochloropsis_sp3
Tetragonisca_angustula
Paratrigona_sp1
Bombus_morio
Augochloropsis_sp1
Apis_mellifera
Epicharis_sp2
Epicharis_sp3
Tapinotaspidini_sp2
Tapinotaspidini_sp3
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K-coreness
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0.0 1.5 3.0 4.5 6.0
log(Bee abundance)
Resource use
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Buzz
Oil
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Bee size
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Medium
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Bee type
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Ceratalictus_sp1
Trigona_spinipes
Emphorini_sp4
Apis_mellifera
Mesoplia_sp1
Ceratina_sp2
Eulaema_nigrita
Augochloropsis_sp1
Xylocopa_sp1
Paratrigona_sp1
Bombus_pauloensis
Epicharis_sp3
Centris_sp2
Bombus_morio
Tapinotaspidini_sp3
Epicharis_sp1
Augochloropsis_sp3
Xylocopa_sp2
Ptiloglossa_sp1
Emphorini_sp3
Centris_sp3
Epicharis_sp2
Tetragonisca_angustula
Tapinotaspidini_sp2
Centris_sp8
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Standard deviation degree
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Standard deviation degree
0 4 8 12 16
0.0 1.5 3.0 4.5 6.0
log(Bee abundance)
Resource use
Both
Buzz
Oil
No
Bee size
Small
Medium
Large
Bee type
Polyletic
Oligoletic

Figure 4.4 Multilayer metrics of the floral resource-mediated multilayer plant-bee network. Ranking of bee species (left panel) and the influence of bee traits on their role in connecting layers and maintaining stable the multilayer resource-mediated plant-bee interaction network (right panel). The multilayer metrics are: multilayer multidegree (a and b), node versatility (PageRank; c and d), closeness centrality (e and f), *k*-coreness (g and h), and standard deviation degree (i and j). Black bars in the left panel represent bee species' roles in the entire multilayer network. The blue, yellow, and red colors represent bee species' role in the nectar-mediated, pollen-mediated, and oil-mediated layers, respectively. Green and purple colors in the right panel represent bee types according to their partner flexibility (i.e., oligoletic and polyletic). The size and the shape of the points in the right panel depict, respectively, the bee size (i.e., small, medium, and large), and the range of their resource use, i.e., whether they are able to sonicate for pollen collection, if they are able to collect oil, both types of resources or do not exhibit any different behavior during floral resource collection

Finally, we observed a low to marginal signal indicating that the role of bee species in the multilayer network varied based on bee partner flexibility (i.e., bee type, PERMANOVA, $F_{1,49} = 6.20$, $p < 0.001$) and resource-use breadth (PERMANOVA, $F_{4,46} = 1.70$, $p = 0.07$), respectively. For instance, while polylectic bees visited in higher frequency and more plants for pollen and nectar (i.e., higher multidegree scores and higher span in NMDS1-axis; Figure 4.5), oligolectic oil-collecting and buzz-pollinating bees generally visited a balanced set of plants to collect oil, pollen and nectar (i.e., lower standard deviation degree scores and higher span in NMDS2-axis; Figure 4.5). Moreover, variance among bee species' roles was not different among bee types ($F_{1, 50} = 2.52$, $p > 0.05$), bee size ($F_{2, 48} = 1.60$, $p > 0.05$), and resource-use breadth ($F_{3,47} = 2.45$, $p > 0.05$). These results indicate that, despite bee traits significantly signaling potential bee roles in the multilayer network, bees with different combinations of functional traits could share similar roles in the multilayer network (Figure 4.5).

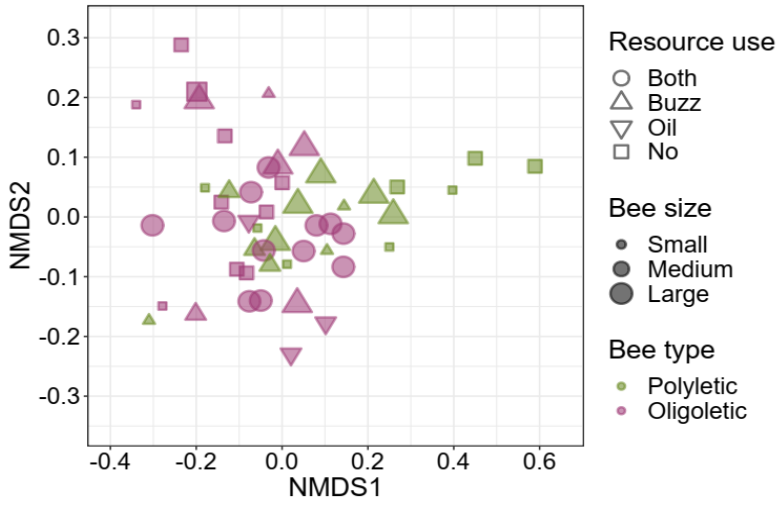

Figure 4.5 Variations in bee species' role in the multilayer network based on bee functional traits. NMDS visualization of the variations of bee species' role on the resource-mediated plant-bee multilayer network. Point colors and shapes differ bees according to their type based on partner flexibility and resource-use breadth, respectively. Point size illustrates bee size.

4.4 DISCUSSION

Within plant-bee mutualisms, floral resource diversity influences bee foraging patterns and thereby, the structure of plant-pollinator networks (Ballarin et al. ***submitted to Journal of Ecology – Capítulo II***). Despite using different types of floral resources, plant-bee interaction networks were never split according to the resources collected by bees when visiting each plant species. This limitation hindered our comprehension of the ecological and evolutionary implications of plants offering various floral resources to bees for community assembly, structure, and functioning. Here, we show that bee assemblages collecting nectar, pollen, or oil exhibit varying levels of dissimilarity and form, together with plants they visit, plant-bee interaction networks that also display varying but consistent and predictable patterns of floral resource use and network structure. Moreover, we show that bee abundance alone, in turn, is not a reliable predictor of bee roles in the floral resource-mediated plant-bee multilayer network. Rather, only in combination with different bee functional traits bee abundance enhances the predictive capacity of bee roles across different layers in a multilayer network. Hence, in addition to the acknowledged and crucial loss of ecological interactions

for ecosystem stability (Valiente-Banuet et al. 2015), our study illuminates the added importance of interaction diversity. Modulated by both resource quality and the traits of interacting species, interaction diversity emerges as a pivotal factor shaping ecosystem structure, function, and stability (see Hiraiwa & Ushimaru, 2024).

The higher species turnover observed in bee assemblages collecting oil, compared to those collecting nectar and pollen, is somewhat expected. Only specific bee taxonomic groups, such as *Centris* spp., *Epicharis* spp., and bees from Tapinotaspidini tribe, possess the capability to scrape oil from oil-producing plant organs (e.g., elaiophores) and store it in specialized body structures (e.g., scopae). As a result, plant-bee interactions mediated by oil generally form well-defined compartments within plant-bee networks, which are commonly built encompassing all types of floral resource-mediated interactions (Olesen et al. 2007; Hachuy-Filho et al. 2020). In contrast, bee assemblages collecting nectar or pollen exhibit high species richness and share lower similarities. These resources are the most common mediators of plant-bee interactions (Ballarin et al. 2024). In this sense, plants with a broader floral morphology offer these resources, which, in turn, are collected by bees with a more diverse range of functional traits.

Differences in the composition and functional diversity of bee assemblages may also explain the structural patterns observed within plant-bee networks. The patterns of resource use (i.e., higher niche overlap and lower specialization degree) and the structure of oil-mediated plant-bee networks (i.e., lower modularity and higher nestedness) suggest more generalized relationships. This implies that different bees able to collect oil share the same plants (Bezerra et al. 2009; Pacheco-Filho et al. 2015). We argue that the high incidence of partner sharing among oil-collecting bees reflects a plant-bee relationship highly stable on the evolutionary scale (Anderson et al. 1979; Davis et al. 2014). In this sense, oil-collecting bees do not face interaction constraints that impair their relationship with oil-offering plants, as both groups are functionally and phylogenetically cohesive (Rasmussen & Olesen, 2000; Davis et al. 2014; Pacheco-Filho et al. 2015; Raiol et al 2021). Therefore, considering that the core region of this network comprised the most abundant oil-offering plants, we argue that the structure of oil-mediated plant-bee interaction networks should be primarily

governed by neutral processes, in which abundant plants and bees tend to concentrate the significant portion of interactions (Jordano et al. 2006; Lewinsohn et al. 2006; Krishna et al. 2008). However, as previously discussed in the preceding paragraph, the oil-mediated plant-bee interactions often compose a cohesive module within plant-bee networks in which different resource-mediated relationships are pooled together (refer to Olesen et al. 2007 and Hachuy-Filho et al. 2020). The modular arrangement of oil-mediated plant-bee interactions in community-wide plant-bee networks (i.e., expanding the scale) and their nested design in resource-mediated plant-bee networks (i.e., reducing the scale) is consistent with prior studies that suggest that nested patterns may manifest within specific compartments in plant-pollinator networks (i.e., nested modules in hierarchical compound topologies; Lewinsohn et al. 2006; Felix et al. 2022; Pinheiro et al. 2022).

In contrast, nectar and pollen-mediated plant-bee interaction networks showed concise lower levels of bee niche overlap and higher network compartmentalization. Nectar and pollen attend to the dietary demands of the largest range of bee species. Nectar is the most common resource offered by plants to pollinators (Ballarin et al. 2024); while pollen is the male reproductive unit of angiosperms, meaning that it is present in virtually all flowering plants a bee can visit (Thorp, 2000). Considering that nectar is valuable to all other pollinator clades, and virtually all flowering plants contain pollen, nectar- and pollen-mediated plant-bee relationships are not limited to a particular and phylogenetically narrow group of plants morphologically adapted to bee pollination. Instead, bees can search for nectar and pollen in flowers with varying morphology whenever they are accessible. This is evident in the frequent pollinator shift towards bee pollination (Fenster et al. 2004; Rosas-Guerrero et al. 2014). In this sense, bee functional traits may play a role in defining the flowers capable of being legitimately visited by bees. Moreover, pollen, in particular, can be stored in poricidal anthers, whose morphological structure only allows bees able to sonicate to collect this resource (Vallejo-Marín, 2019). Thus, constraints in flower morphology and bee behavior may contribute to increased specialization and compartmentalization in nectar-mediated and pollen-mediated plant-bee interaction networks. Indeed, a greater functional diversity of

floral attributes typically leads to increased floral constancy displayed by bee species within a given habitat (Waser, 1986).

Contrary to what we expected, besides abundance, different aspects of bee traits contributed to explaining the multiple facets of the role played by bee species in connecting layers within the floral resource-mediated multilayer network (see Peralta et al. 2024). These results indicate that not only neutral-based rules but also the functional diversity underlying niche-based processes assist in foreseeing how bees connect layers in resource-mediated plant-pollinator networks (Vizentin-Bugoni et al. 2018; Peralta et al. 2024). Multidegree and node versatility were strongly influenced by bee abundance, which is expected since a greater number of network partners (i.e., degree or multidegree) and participation in interactions with generalist and core species are associated with higher encounter rates and organism abundance (Bascompte & Jordano, 2007; Vázquez et al. 2007). Partner flexibility and resource-use breadth played relevant roles in explaining inter-connected bee groups capable of preventing network structural collapse (i.e., k-coreness) and anticipating species with balanced importance across the three layers of floral resource-mediated plant-bee interactions (i.e., standard deviation degree). This implies that those bees visiting different plant species in search of either the same (i.e. polyletic) or different floral resources (i.e., resource-use breadth) play a crucial role in preventing the structural misconfiguration of the layers, as they make a significant contribution to all layers in which they participate. Meanwhile, information flow within the multilayer network (i.e., closeness centrality) was primarily influenced by partner flexibility and bee size. This result aligns with previous studies indicating that polyletic bees have higher behavioral plasticity, enabling them to interact with a diverse range of plant partners and spread information within the network faster (Ogilvie & Forrest 2017). Contrary to studies indicating higher behavioral plasticity in large bees due to their larger brain size or flight capacity (Greenleaf et al. 2007; Lanuza et al. 2023), our study reveals that smaller bees were more closely connected in the network. This suggests that, at the site level, smaller bees can also exhibit significant behavioral plasticity if they are polylectic, such as *Tetragonisca angustula* and *Trigona spinipes* - highly-recruiting eusocial

bees from the Meliponini tribe; or if they have a broader resource-use breadth, as seen in bees from the Tapinotaspidini tribe, which frequently forage for pollen, oil, and nectar.

These results highlight the intricate and multifaceted importance of bee traits in shaping bees' roles in connecting floral resource-mediated layers. While bee abundance contributes to the role of bee species in linking layers, it alone is insufficient to explain many topological properties of the multilayer network (see Simpson et al. 2022). For instance, while super-generalists and abundant bees like *Apis mellifera* and *Trigona spinipes* connect with numerous plant species, particularly with the generalist-core plants, less abundant bees, especially those capable of sonication or oil-collection, such as *Centris* spp., *Epicharis* spp. and bees from Tapinotaspidini tribe, assume central roles in information spread and structural collapse avoidance by engaging in interactions more consistently across the three layers of the multilayer network. These findings underscore the significance of both abundant and less common bee species in shaping network dynamics (Simpson et al. 2022; Peralta et al. 2024).

4.5 CONCLUSION

Our results highlight an innovative approach to assess resource-use patterns among free-living pollinator species. Distinct groups of floral resources were examined as layers of multilayer complex networks describing the multiple dimensions flowering plants use to attract pollinators. Such higher dimensionality of complex ecological networks has been seldom analyzed, yet represents a biologically-meaningful description of the complexity of interactions between resource-based consumers (e.g., bees) and their food plants. We demonstrate the significance of distinct floral resources (i.e., distinct rewards provided by groups of flowering species) as drivers of resource utilization patterns in bees and the resulting structural patterns within plant-bee interaction networks. Further, we show that multiple bee functional traits play a role in connecting layers mediated by floral resources. Hence, it becomes evident that solely predicting bee roles based on their abundance and the number of partners falls short in anticipating the architecture, structure, and stability of plant-bee interaction networks. Instead, less common bee species engaged in relationships with fewer plants, especially those offering oil or pollen collected exclusively through sonication,

also prove pivotal in maintaining the topological signatures of plant-bee interaction networks stable.

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Author contributions

CSB conceived the ideas, designed the methodology, and collected the data; CSB and PJ analyzed the data and validated the results; CSB led the writing of the manuscript. CSB and FWA supervised and administered the study. All authors contributed critically to the drafts and gave final approval for publication.

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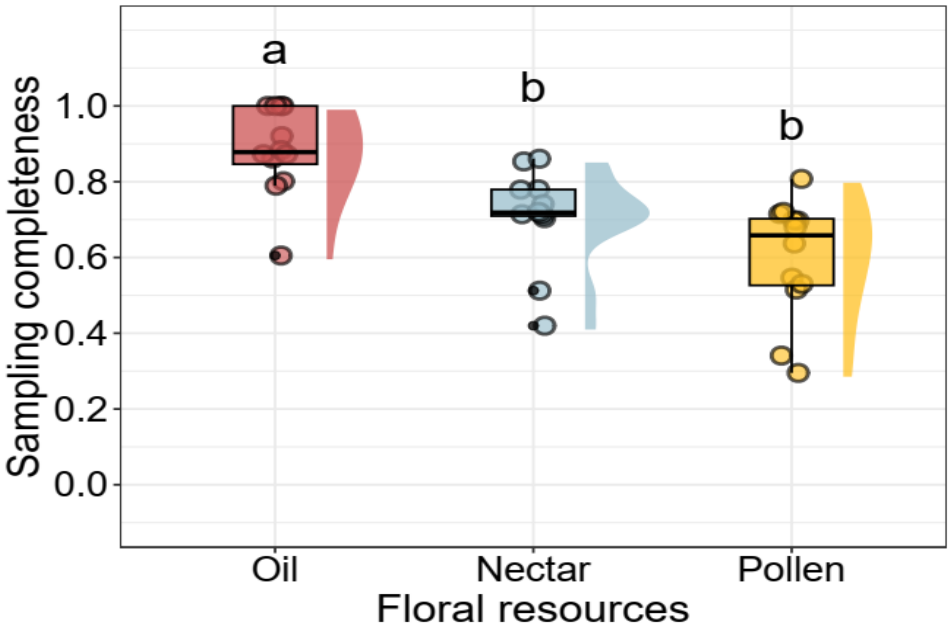
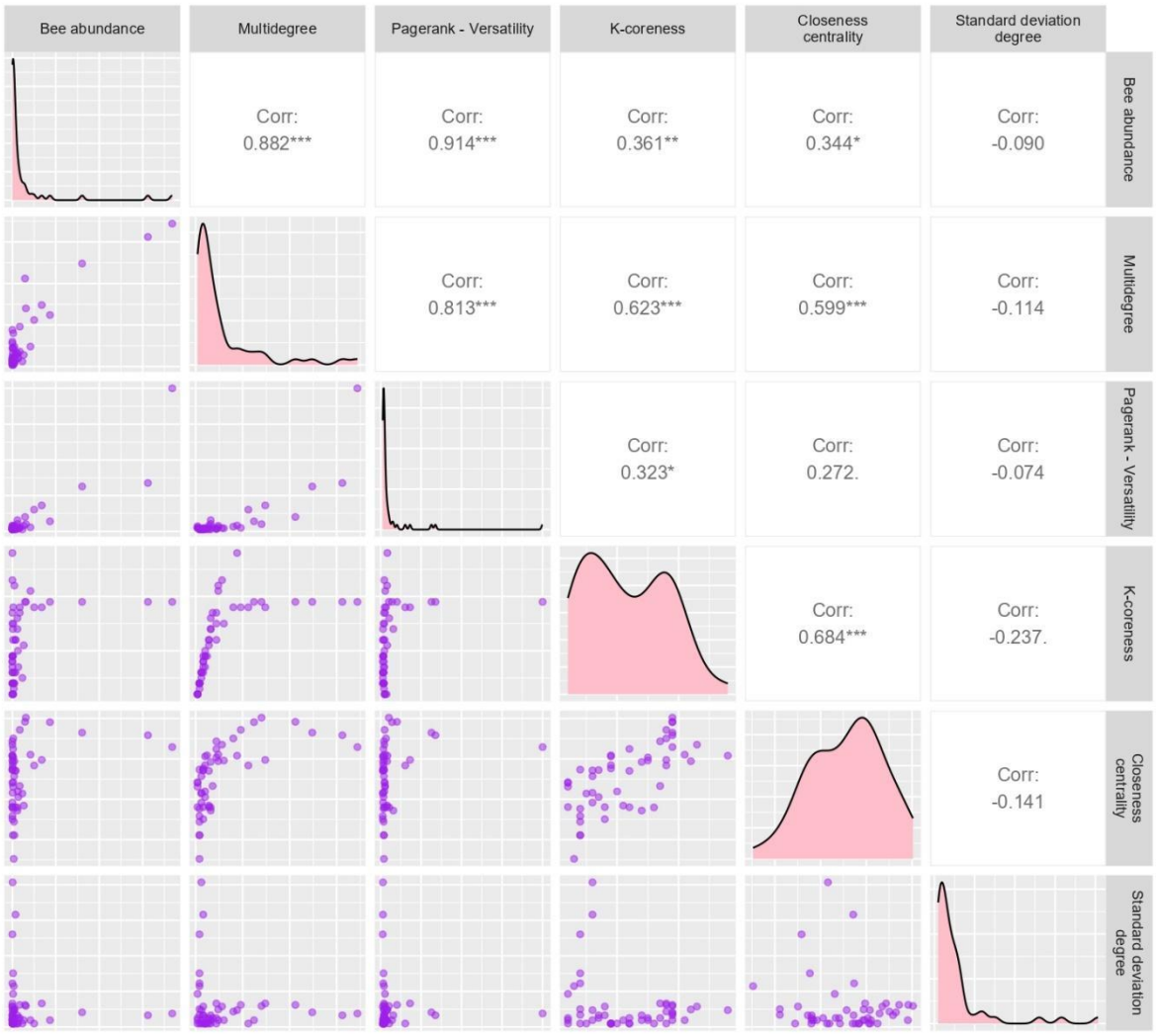
4.8 SUPPLEMENTARY MATERIAL



Supplementary Figure S4.1 Sampling completeness among different resource-mediated network built in each plot. Differences among sampling completeness for each resource-mediated plant-bee interaction networks in plots was compared through a generalized linear model (GLM) with quasibimonial error distribution. Sampling completeness were higher in oil-mediated plant-bee interactions networks; $\chi^2_{1,\ 35} = 25.83$; $R^2 = 0.48$; $p < 0.01$). Nevertheless, nectar-mediated and pollen-mediated networks also exhibited consistently high levels of sampling completeness (70.9% ± 12.7; and 59.9% ± 15.7, respectively).



Supplementary Figure S4.2 Correlations between multilayer network metrics revealing bee roles in the resource-mediated multilayer network. Graphs in purple depict the correlation in scatterplots. Pink density plots depicts the distribution of values obtained by each bee species. Values represent correlation after Spearman's correlation tests and significance (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$).

5 CAPÍTULO IV

Spatiotemporal variations in floral resource landscape triggered by fire reorganize but do not affect the structure and stability of plant-bee interaction networks *

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Spatiotemporal variations in floral resource landscape triggered by fire reorganize but do not affect the structure and stability of plant-bee interaction networks

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Abstract

1. Plants can offer different types of floral resources for pollinators, which is primarily important for bees that use different floral resources for distinct ecological demands. Fire can rapidly alter resource availability, affecting plant-pollinator dynamics. However, fire's effects on the availability of different floral resources have never been assessed in the tropics, impairing our comprehension of the responses of highly diverse plant-bee interaction networks to changes in floral resources diversity following fire events.
2. We assessed flower production, plant species richness and diversity, and the Shannon and β-diversity of floral resources following a well-documented fire event in a Brazilian Cerrado to evaluate whether fire impacts floral resource landscape. Further, we investigated whether predicted changes in floral resource landscape affected plant-bee networks by inspecting their structure, robustness to plant loss, interaction β-diversity, and the spatiotemporal organization of pairwise interactions.
3. At broader scale, fire showed no consistent trend in stimulating flower production, plant species richness and diversity, and Shannon diversity of floral resources. Nevertheless, at finer scale, fire influenced the spatiotemporal distribution of floral resources, increasing dissimilarities among floral resource offering in pre-burned and burned situations. Moreover, fire increased the turnover of floral resource in a great degree than expected in unburned situations. Surprisingly, plant-bee interactions maintained their network structure similar even after the fire event. The resilience of the network mirrored its structural stability once burned and unburned plots exhibited similar robustness to plant species loss. Nevertheless, dissimilarities in floral resource landscape triggered by fire promoted higher β-diversity of plant-bee interactions, which exhibited higher interaction rewiring, turnover and drastically reorganized pairwise interactions in space and time.
4. Fire affects the spatiotemporal distribution of floral resources but does not severely disrupt plant-bee interactions. We argue that bees adapt to shifts in floral resources distribution, preserving interaction network robustness in recurrently burned Cerrado ecosystems. Thus, finer-scale changes in floral resource landscape are insufficient to erode plant-bee interaction structure and stability. Nonetheless, the high turnover of floral resources and plant-bee interactions reveals that fire represent a source of patchy variability in Cerrado ecosystems.
5. Opposing to fire suppression policies that promote taxonomical and ecological homogenization, we suggest that fire is beneficial for Cerrado as it brings ecological diversity by promoting finer-scale changes in floral resources that attend different bee species.

Keywords: Brazilian Cerrado, Interaction β-diversity, Rewiring, Robustness, Floral resource turnover

5.1 INTRODUCTION

In ecological communities, resource availability rules the diversity, distribution, and the frequency of interaction that plants engage with animals (Ollerton, 2006; Yang et al. 2010; García et al. 2011; Fontúrbel et al. 2017; Ballarin et al. 2022a). Nevertheless, the availability of resources within the landscape may suddenly change after abiotic and biotic alterations in the habitat (Yang et al. 2010), which may cascade effects on resource-mediated plant-animal relationships (Hoover et al. 2012; Waser & Price, 2016). As disturbances are drivers of environmental changes, they are also remarkable sources of spatiotemporal alterations on resource availability (Hemberger et al. 2023), and therefore, on resource-mediated plant-animal relationships, such as plant-pollinator interactions (Aguilar et al. 2006; Walter, 2020; Moreno-García et al. 2023). Fire is one of the most common disturbances that influence plant-pollinator relationships worldwide through alterations in the resource landscape (Potts et al. 2003; 2005), as it immediately changes the availability, distribution, and quality of resources explored by flowers and floral visitors (Potts et al. 2003; Dantas et al. 2015; Koltz et al. 2018; Goldas et al. 2022a).

For example, in many fire-prone ecosystems (*i.e.*, ecosystems where fire is recurrent enough to be a selective pressure; Keeley et al. 2011), plants can rapidly boost flower production due to bottom-up effects elicited by short-term increases of nutrient input in soil (Pellegrini et al. 2015; Teixeira et al. 2022), smoke stimulation (Lamont & Downes 2011), and resource allocation to reproductive organs (Fidelis et al. 2022). Nevertheless, different plant species offer distinct types of resources to pollinators and, in particular, to bees, such as nectar, pollen, and oil (Simpson & Neff, 1981; Ollerton, 2006). Considering that biochemical pathways to produce each kind of floral resource are different, changes in floral resource production may be expected after fire events (Burkle & Irwin, 2010; Carbone & Aguilar, 2017), which might reorganize plant-pollinator interactions (Sponsler et al. 2023; Ballarin et al. ***submitted – Capítulo II***).

Moreover, it has been hypothesized that plants with generalist or specialist pollination systems may have different responses to fire (García et al. 2018). While plants relying on pollinators belonging to different functional groups may only residually suffer the impact of

fire because the loss of fire-sensitive pollinator species can be buffered by pollinator replacement, the impact of fire on plants with specialized pollination systems might be stronger if the specialist pollinator is lost (García et al. 2018; Lázaro & Gómez-Martínez et al. 2022). Indeed, it has been observed that fire triggers biotic generalization in plant-pollinator networks (Peralta et al. 2017; Barônio et al. 2021). Considering that the type of resources offered by plants to pollinators influences the level of interaction specialization, especially in plant-bee interactions, which are mediated by different floral resources (Ollerton 2006; Armbruster 2012, 2017), it is also expected that interactions involving plants offering and bees consuming different kinds of floral resources may respond differently to fire events (Goldas et al. 2022a), which may also impact network structure.

Bees may rearrange their foraging preferences according to the identity, quality and quantity of floral resources offered by plants (Vaudo et al. 2015; Ballarin et al. 2022b). For example, nectar is the usual resource offered by plants to a myriad of pollinators, including bees (Ballarin et al. 2024), whereas oil attends the foraging demand of a particular bee group behaviorally and morphologically adapted to collect, store and use oil for brood cell construction (Bezerra et al. 2009; Martins et al. 2015; Pacheco-Filho et al. 2015). In a scenario where fire causes changes in the diversity of floral resources within the landscape, interactions involving oil-collecting bees and flowers might respond differently to fire events in comparison with nectar plants and bees. Nevertheless, the effect of fire on the floral resource landscape has never been investigated in the tropical region (but see Potts et al. 2003 for an investigation in the temperate zone), a latitudinal range that harbors the highest floral resource diversity (Ballarin et al. 2024), which impairs our comprehension about fire impacts on plant-bee community-wide relationships.

Bees present an overall positive response to fire events, especially when a fire occurs at long time intervals (Carbone et al. 2019; Ulyshen et al. 2022). However, because bees rely on nesting sites that may be severely altered by architectural changes on vegetation after fire events (Potts et al. 2005) and also present different levels of generalization (oligolectic *vs*. polylectic; Raiol et al. 2021), which might affect foraging routes in altered resource landscapes, the effect of fire on plant-bee interactions ensemble different results. While it has

already been documented that fire increase overall plant-bee interaction richness (Ponísio et al. 2016; Burkle et al. 2022), fire has also already been recognized as an important filter that selects specific functional bee groups that are able to recolonize the habitat (Moretti et al. 2006; Lazarina et al. 2016). Nonetheless, considering that bees are reliant on floral resources for different ecological demands, it remains unclear whether changes in floral resources landscape triggered by fire affect the stability of plant-bee interactions through the rearrangement of interactions among plants offering different kinds of resources and bees (but see Barônio et al. 2021).

We investigated whether the floral resource landscape changes after a fire event within the Cerrado, a well-studied, fire-prone ecosystem in Brazil (Ballarin et al. 2023). We also studied the potential impacts of changes in the floral resource landscape on the plant-bee interaction network. Specifically, we asked if (1) fire trigger changes in floral resource diversity across space and time; if (2) those changes affect the plant-bee interaction network by altering their structure and stability; and (3) whether changes in network properties is related to the spatiotemporal reorganization of the linkage among plants and bees. We hypothesized that fire promotes changes in floral resource diversity by increasing the β-diversity of floral resources across space and time. This would influence the network structure and stability, which would be explained by spatiotemporal rearrangements in resource-mediated plant-bee relationships.

5.2 MATERIAL AND METHODS

5.2.1 Study site

This study was conducted during two flowering periods between 2021 and 2023 at the Botucatu State Forest (BSF), a Cerrado area located in the peri-urban zone of Botucatu municipality, São Paulo state, Brazil (22°56'20"S, 48°27'29"W; 860 a.s.l.). BSF encompasses an area of 34 ha. that harbors a combination of different vegetation types, including floodable gallery forest, moist *campo limpo* mainly occupied by dominant herbaceous plants, and a *campo sujo* composed of scattered trees, shrubs, herbs, and grasses to varying extents. The climate at BSF is classified as high-altitude tropical (Aw according to Köppen classification;

Franco et al. 2023), exhibiting two distinct seasons: a cold and dry winter occurring between April and September, and a hot and wet summer from October to March, during which most angiosperms thrive.

Fire in BSF is mainly occasioned by anthropogenic pressures. While in *campo sujo* and *campo limpo* fire is very common at BSF, the flooded gallery forest within BSF generally does not experience fire events. Despite the overall annual fire return interval in BSF, a fire occurs in limited patches. Hence, fire return interval within patches may vary from annual to triennial depending on the locality within the BSF. For example, in our study site, an anthropogenic fire burned vegetation in the early months of the dry season of 2019. No visual cues of the fire passage could be seen when we set up our study in mid-July 2021. Just before the second year of sampling began, in late May 2022, an anthropogenic fire with high severity burned 20% of the *campo sujo* within BSF. By inspecting 10 tree trunks, flame height reached 2.64 ± 0.14 meters, top-killing some trees and burning the aerial organs of herbs, grasses and shrubs in six of the 12 plots we established to sample plants and bees. The fire passage in half of our plots yielded the opportunity to conduct a before-after-control-impact (BACI) experiment of the fire effects on plants and bees by evaluating the fire influence on the floral resource landscape and on the plant-bee interaction network (Supplementary Figure S5.1).

5.2.2 Floral resource landscape and plant-bee interactions sampling

We assessed plant community composition and plant-bee interaction by establishing twelve 100m² plots at least 30 meters apart. For this end, we conducted weekly surveys during the flowering seasons of 2021-2022 and 2022-2023, from mid-July to early-March. In the beginning of the second flowering season (2022-2023), nearly two months after the fire event, when we started the sampling survey, plants had already resprouted and begun to flower (Supplementary Figure S5.2). To document floral resource availability and plant-bee interactions across space and time, in each plot, we built 100 1m²-grids (subplots of 1m x 1m) and counted flower units per plant as well as plant and bee pairwise interactions. Hence, for each subplot we obtained floral abundance and plant-bee interaction frequency for each encountered plant species on a weekly basis.

Plant-bee interactions were assessed through visual censuses made by two observers from 06:00h to 18:00h, during two consecutive days, totaling 1440 hours of sampling considering both years. We only considered plant-bee interactions when bees contacted the flower reproductive organs. We alternate sampling periods between plots to avoid biases related to the period of sampling. Plant species were identified *in situ* using a floral guidelist (Pilon et al. 2017). Plants not identified *in situ* were taken to the Herbarium (BOTU) for further identification with the aid of specialists. Bee specimens were captured and identified using keys (Silveira et al. 2002) and the aid of specialists.

We built 24 plant-bee interactions networks, each one corresponding to plant-bee interactions sampled in a given plot and in a given flowering season. We created interaction networks by building an adjacency matrix M_{ij} in which m_{ij} corresponded to the frequency of a given bee *i* assessed a given plant species *j*.

5.2.3 Spatiotemporal influence of fire on floral resource landscape

To evaluate whether fire triggers spatiotemporal changes in the floral resource landscape in Cerrado, we first took advantage of our BACI experiment to provide a holistic perspective regarding fire effects on floral resource diversity. For this, we compared the floral abundance, plant species richness, Shannon diversity of plants species as well as Shannon diversity of floral resources among years in burned and unburned plots (Supplementary Figure S5.3a-c). We assessed Shannon diversity of floral resources by considering each floral resource offered by plants to bees as different "species" following the ordinary approach. However, as Cerrado plants exhibit different strategies of resprouting and flourishing after a fire event (Pilon et al. 2021), differences within plots among years may not deeply track changes in floral resource diversity influenced by fire, since changes on the grain scale (*i.e.*, differences among subplots between years), and on temporal fine-scale (*i.e.*, differences among weeks between years) might be blurred by considering the plot and the year as the analytical scales. In this sense, to deeply compare dissimilarities in floral resource diversity triggered by fire, we assessed the β-diversity of floral resources between years across space and time by verifying whether fire influences floral resource diversity in these two different components. Thence, to verify if the fire influenced the diversity of floral resources in space,

we compared changes in floral resource diversity within each grid by evaluating the β-diversity of floral resources in unburned plots among years (before-after-control) and the β-diversity of floral resources in burned plots among years (before-after-impact). We used the mean β-diversity of floral resources within grids in a plot as the spatial β-diversity of floral resources (n = 24; Supplementary Figure S5.4a). To verify if the fire influences the diversity of floral resources over time, we compared changes in floral resource diversity within each plot in a given week of the sampling census performed in both years. To avoid interference of asynchrony caused by sampling surveys made in different weeks in distinct years, we selected 23 pairwise weeks that were evaluated in both years (*e.g.,* the first week of October in the first flowering season *versus* the first week of October in the second flowering season). To this end, we compared changes in floral resource diversity within a pairwise week by evaluating the β-diversity of floral resources in unburned plots among years (before-after-control) and the β-diversity of floral resources in burned plots among years (before-after-impact). We used the mean β-diversity of floral resources within plots in a pairwise week as the temporal β-diversity of floral resources (n = 46; Supplementary Figure S5.4a). To calculate β-diversity we treated floral resources produced by plants (*e.g*., nectar, pollen, oil) as "species identities" and used the Sorenesen index to extract the dissimilarities among floral resources across space and time. Furthermore, β-Sorensen (hereafter, β-total) can be decomposed in two components that reflect the total β-diversity: β-nestedness (hereafter, βnest) and β-turnover (hereafter, βturn). While βnest represent the loss or gain in floral resources richness, *i.e.*, the number of floral resources, βturn depicts the turnover of floral resources, *i.e.*, the changes in the type of floral resources presented by plants. Both components bring relevant information about dynamism in community-wide floral resource presentation. While βnest informs how plant assemblage has lost or gained different floral resources across space and time, βturn informs whether the plant assemblage has changed the floral resources it presents across space and time.

5.2.4 Spatiotemporal influence of fire on plant-bee interactions structure and stability

To evaluate whether fire influenced plant-bee interaction network in Cerrado, we also used our BACI experiment to compare the structure, and the stability of plant and bee

interaction networks (Supplementary Figure S5.3d). For network structure, we employed three distinct but conceptually related measures that assess how bee species distribute their interactions within the plant community. For each network corresponding to a plot in a given sampling year, we first examined the community-wide network interaction specialization (H_2'). H_2' evaluates the extent to which species limit their interactions compared to what would be expected based on the availability of partners (Blüthgen et al. 2006), serving as a measure of interaction exclusiveness. Values of H_2' varies from 0 to 1, values closer to 1 indicate higher network-wide specialization. Second, we inspected network weighted nestedness (wNODF), which assess whether interactions between specialized species form subsets of interactions involving more generalist species (Almeida-Neto & Ulrich, 2011). Higher values of wNODF indicate that species within the network core (*sensu* Bascompte et al. 2003) also exhibit a higher frequency of interactions (Almeida-Neto & Ulrich, 2011). Third, we evaluated network weighted modularity (Q'_w), which indicates whether subset of interacting species compartmentalize interactions in a manner to perform more interactions within their own compartment than with others. Q'_w varies from 0 to 1, with higher values denoting higher compartmentalization of interactions. Q'_w was calculated using the DIRTLPAwb+ algorithm (Beckett 2016). Because wNODF and Q'w values might be biased by differences in network size, connectance and differences in the sampling method, we followed Dalsgaard et al. (2017) and utilized Δ-transformation for both network metrics. This transformation quantifies the deviation of observed values from a random structural pattern generated by a specific null model (Schleuning et al. 2012; Sebastián-González et al. 2015). By subtracting the mean value of random networks from the empirical value, Δ-transformations account for variations in network topology that lack ecological significance. Thence, this approach enables valid comparisons across networks of different sizes and connectance (Fründ et al. 2016). To generate the null models, we used the Patefield algorithm, which ensures that network size and marginal totals for rows and columns remained fixed, ensuring conservation of species richness and total interaction counts between species (i.e., interaction frequencies) in the null matrices. The conservation of species and interaction frequencies are regarded to be one of the best strategies to maintain ecological integrity while generating the null matrices (Dormann et al. 2008).

For network stability, we calculated the robustness (R) of the plant-bee interaction network constructed for each plot and flowering season. R informs how stable a network remains to secondary exclusions after the random simulations of species loss (Memmott et al. 2004). R evaluates the percentage of species in a given trophic level that remain in an interacting network after sequential removal of species from the other trophic level, assuming that a given species becomes extinct when it loses all partners with which it was linked. Values of R range from 0 to 1, with higher values indicating a more stable system (Burgos et al. 2007). As we were investigating the stability of the plant-bee interaction network after changes in the floral resource landscape, R in our study was assumed as the robustness of the plant-bee interaction network after random exclusions of plant species.

5.2.5 Spatiotemporal influence of fire on plant-bee interaction organization

To assess whether fire triggers the reorganization of plant-bee pairwise interactions, we assessed plant-bee interaction dissimilarities between years in burned and unburned plots within Cerrado. For this, we calculated the β-diversity of interactions among plants and bees within the same plot between flowering seasons (n = 12). To compute the β-diversity of interactions, we followed Magrach et al. (2017), which employs the Ruzicka distance coefficient as a quantitative measure. This coefficient considers the shared pairwise interactions between compared webs. We calculated three components of β-diversity: β_{OS}, which represents the portion of β-diversity resulting from changes in the interactions among shared species (*i.e.*, rewiring). $\beta_{ST,}$ which represents the component of β-diversity resulting from dissimilarity in species composition between sampling periods (*i.e.*, turnover; Poisot et al., 2012). β_{WN}, which quantifies the overall dissimilarity between the compared networks (*i.e.*, overall interaction dissimilarity: $\beta_{WN} = \beta_{ST} + \beta_{OS}$). These values range from 0 to 1, with a value of 1 indicating higher temporal β-diversity in plant-pollinator interactions.

Finally, considering our data regarding plant-bee pairwise interactions were spatiotemporally explicit, which means that we had information at both the spatial (i.e., subplot - space) and the fine-temporal scale (i.e., pairwise weeks - time), we also evaluated how pairwise plant-bee interactions rearranged in space and time following the fire event (similar to Li et al. 2023). For this purpose, we constructed two different adjacency matrices

for each plot to represent both the spatial and temporal components of variation in pairwise interactions (S_{ij} matrices for space and T_{ij} for time; n = 12 for each matrix type; Li et al. 2023). In the S_{ij} matrices, s_{ij} corresponded to the frequency of a given plant-bee pairwise interaction *j* sampled in a given subplot *i*. In turn, t_{ij} in the T_{ij} matrices corresponded to the frequency of a given plant-bee pairwise interaction *j* sampled in a given pairwise week *i*. With these types of adjacency matrices, we could compare whether fire triggered the spatial (S_{ij}) and temporal (T_{ij}) reconfiguration of plant-bee interactions. To do this, we followed the same protocol used in the M_{ij} matrices to evaluate the spatiotemporal β-diversity of pairwise plant-bee interactions. In this case, β_{WN} represented the overall dissimilarity of plant-bee pairwise interactions in space or time, β_{OS} indicated that pairwise interactions present in both flowering seasons had occurred in different subplots or pairwise weeks. β_{ST}, in turn, depicted the entrance or exit of new pairwise interactions, subplots, or pairwise weeks that had just appeared in a given flowering season.

5.2.6 Data analysis

To assess whether fire influences floral resource production, plant composition, and the diversity of floral resources (*i.e.*, Shannon diversity of floral resources), we built two generalized linear mixed model (GLMM) and two linear mixed models (LMM) considering the floral resource abundance, plant species richness, plant species diversity, and Shannon diversity of floral resources as the response variables, respectively. In these models, the presence or absence of fire (*i.e.*, fire occurrence) and the flowering season were treated as a fixed factor, while the plot identity was considered as a random factor. The most suitable model for each response variable was assessed by evaluating the Akaike information criterion corrected for small samples (AICc). Specifically, we compared the full model, encompassing the combination of all fixed factors (*i.e.*, fire occurrence and flowering season) and the random effect, with the reduced models, which included the models encompassing the fixed factors alone (*i.e.*, fire occurrence or flowering season) along with the random effect. The GLMMs considering floral abundance or plant richness as the response variables were constructed with the Poisson error distribution, while the LMMs using plant species diversity or Shannon diversity of floral resources as the response variables were constructed using the

standard Gaussian error distribution. Differences among pair of means were assessed by performing a Tukey *posthoc* test using the package *emmeans* in R (Lenth 2016). Further, to evaluate whether the Sorensen β-diversity differs among burned and unburned plots across space and time, we performed six GLMs considering βtotal, βnest, and βturn as variables responding to fire occurrence. All GLMs were constructed using the quasibinomial error distribution. Differences between burned and unburned plots in all components of Sorensen β-diversity were assessed by performing a Tukey *posthoc* test.

We also evaluated whether fire influence patterns of resource use (*i.e.*, community-wide network interaction specialization; H_2') and the network structure emerging from plant-bee interaction networks (i.e., the transformed-network weighted nestedness, ΔwNODF, and the transformed-network weighted modularity, ΔQ'w). To assess if fire influences these abovementioned network metrics, we developed linear mixed models (LMMs) considering the network metrics (i.e., H2', ΔwNODF, ΔQ'w) as the response variables. We considered fire occurrence and the flowering season as fixed factors and the plot identity as a random effect. We assessed the most appropriate model for each response variable by evaluating the AICc. For this purpose, we compared the full model, including fire occurrence and flowering season as fixed factors along with the plot identity as the random effect, with reduced models that only included the fixed factors separately (i.e., fire occurrence or flowering season) along with the random effect. We then performed Tukey *posthoc* tests to assess differences between network metrics arising from plant-bee interaction sampled in burned and unburned plots. We conducted the same procedure mentioned above to evaluate whether fire influence plant-bee interaction network robustness. We built all three LMMs using all possible combinations between fixed factors, namely, fire occurrence and flowering season. Plot identity as used as a random effect. Comparison among models was assessed through AICc. To evaluate whether network robustness differ in burned and unburned plots we performed a Tukey *posthoc* tests.

Finally, to assess potential dissimilarities in plant-bee interaction β-diversity between burned and unburned plots across years, we conducted three GLMs. We used β_{WN}, β_{ST}, and β_{OS} as variables responding to fire occurence. GLMs were conducted using quasibinomial

error distribution. Comparision among burned and unburned plots was perfomed with a Tukey *posthoc* test. Similarly, we conducted six GLMs with quasibinomial distribution considering the three β-diversity components (i.e., β_{WN}, β_{ST}, and β_{OS}) as response variables to assess whether variation of pairwise plant-bee interaction in space (n = three GLMs) and time (n = three GLMs) differed between burned and unburned plots.

5.3 RESULTS

5.3.1 Spatiotemporal influence of fire on floral resource landscape

During this study, flowering plants produced 86,705 and 111,568 flower units in the first and second flowering seasons, respectively. In the first flowering season, unburned plots produced 9,515 ± 2,845 flower units, while plot in pre-burned situations produced 4,936 ± 1,994 flower units. In the second flowering season, when the fire event took place, burning half of our plots, unburned plots increased floral production 1.20-fold (11,461 ± 2,955 flower units), while burned plots increased flower production 1.29-fold (7,133 ± 2,612 flower units). Despite flower production within and among plots severely differed in flowering seasons before and after fire ($R^2_{\text{-adjusted}} = 0.96$; $p < 0.001$; Table 5.1 and Figure 5.1a), no trend regarding floral production stimulation by fire could be tracked, once flowering seasons explained most of the variations in flower production ($\chi 2_{1, 24} = 3{,}082.89$) in comparison with fire occurrence or not ($\chi 2_{1, 24} = 11.61$). Plant species richness did not differed between plots and season ($R^2_{\text{-adjusted}} = 0.03$; $p > 0.05$; Table 5.1 and Figure 5.1b). However, it marginally increased in burned plots and decreased in unburned plots in the second season. Marginal differences among plots were only explained by the fire occurrence ($\chi 2_{1, 24} = 0.60$). Shannon diversity of plant species did not differ between plots ($R^2_{\text{-adjusted}} = 0.61$; $p > 0.05$; Table 5.1 and Figure 5.1c), with marginal differences only explained by differences in the flowering season ($\chi 2_{1, 24} = 2.88$). Still, besides burned and unburned plots differed in the Shannon diversity of floral resources ($\chi 2_{1, 24} = 14.52$; $R^2_{\text{-adjusted}} = 0.40$; $p < 0.001$; Table 5.1 and Figure 5.1d), no pronounced effect of the fire could be detected as plots in pre-burned and burned situations did not differ ($p = 0.17$).

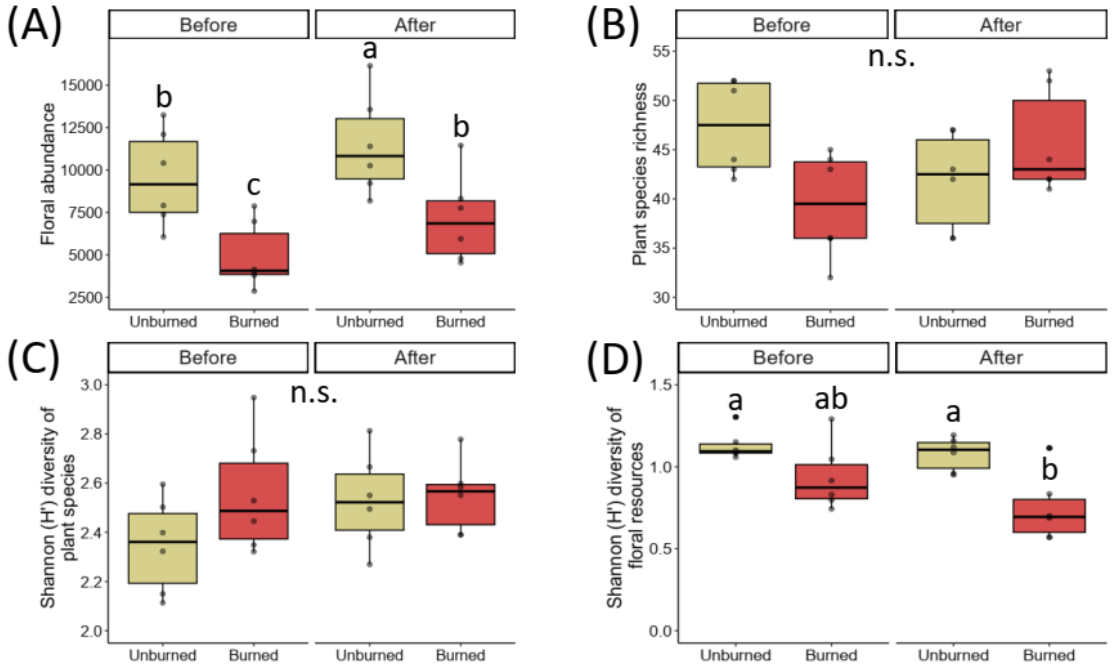

Figure 5.1 Community-wide floral production before and after a fire event in burned and unburned plots. Floral abundance (A), plant species richness (B) and Shannon (*H'*) diversity of flora resources (C) before and after the fire event that burned half of the plots Compact letter displays depicts differences among plots in the before-after-impact-control experiment after a Tukey *posthoc* comparisons of pair of means. Red and beige boxplots refer to burned and unburned plots, respectively. Red boxplots in before panels refer to plots in pre-burned situations."n.s." means no significant differences among treatments.

Table 5.1 Floral resource production before and after the fire event in burned and unburned plots. P-values indicate significant differences through means pairwise comparisons among plots before (*i.e.*, unburned S1and pre-burned plots) and after (*i.e.*, unburned S2 and burned) the fire event.

Floral abundance			
Treatments			*P-value*
Unburned S1	*vs.*	Pre-burned	***< 0.01***
Unburned S1	*vs.*	Unburned S2	***< 0.001***
Unburned S1	*vs.*	Burned	> 0.05
Pre-burned	*vs.*	Unburned S2	***< 0.001***
Pre-burned	*vs.*	Burned	***< 0.001***
Unburned S2	*vs.*	Burned	***< 0.05***
Plant species richness			
Treatments			*P-value*
Unburned S1	*vs.*	Pre-burned	> 0.05
Unburned S1	*vs.*	Unburned S2	> 0.05
Unburned S1	*vs.*	Burned	> 0.05
Pre-burned	*vs.*	Unburned S2	> 0.05
Pre-burned	*vs.*	Burned	> 0.05
Unburned S2	*vs.*	Burned	> 0.05
Shannon (H') diversity of plant species			
Treatments			*P-value*
Unburned S1	*vs.*	Pre-burned	> 0.05
Unburned S1	*vs.*	Unburned S2	> 0.05
Unburned S1	*vs.*	Burned	> 0.05
Pre-burned	*vs.*	Unburned S2	> 0.05
Pre-burned	*vs.*	Burned	> 0.05
Unburned S2	*vs.*	Burned	> 0.05
Shannon (H') diversity of floral resources			
Treatments			*P-value*
Unburned S1	*vs.*	Pre-burned	> 0.05
Unburned S1	*vs.*	Unburned S2	> 0.05
Unburned S1	*vs.*	Burned	***<0.01***
Pre-burned	*vs.*	Unburned S2	> 0.05
Pre-burned	*vs.*	Burned	> 0.05
Unburned S2	*vs.*	Burned	***<0.01***

Nevertheless, fire promoted dissimilarities in floral resource diversity across space ($R^2 = 0.71$; $\chi 2_{1,11} = 23.84$; $p < 0.001$; Figure 5.2a) and time ($R^2 = 0.12$; $\chi 2_{1,45} = 4.84$; $p < 0.05$; Figure 5.2b). Specifically, fire increased the β-diversity of floral resources between grids within plots and between plots among pairwise weeks. Furthermore, fire increased the turnover of floral resources both in space and time. 41.05 ± 12.97% of the β-diversity was explained by the spatial turnover of floral resources in unburned plots, while in the burned plots, the spatial turnover of floral resources explained 60.42 ± 4.99% of the β-diversity (Figure 5.2c). 4.69 ± 15.55% of the β-diversity was explained by the temporal turnover of floral resources in unburned plots, whereas the temporal turnover of floral resources explained 26.73 ± 24.27% of the β-diversity in burned plots (Figure 5.2d).

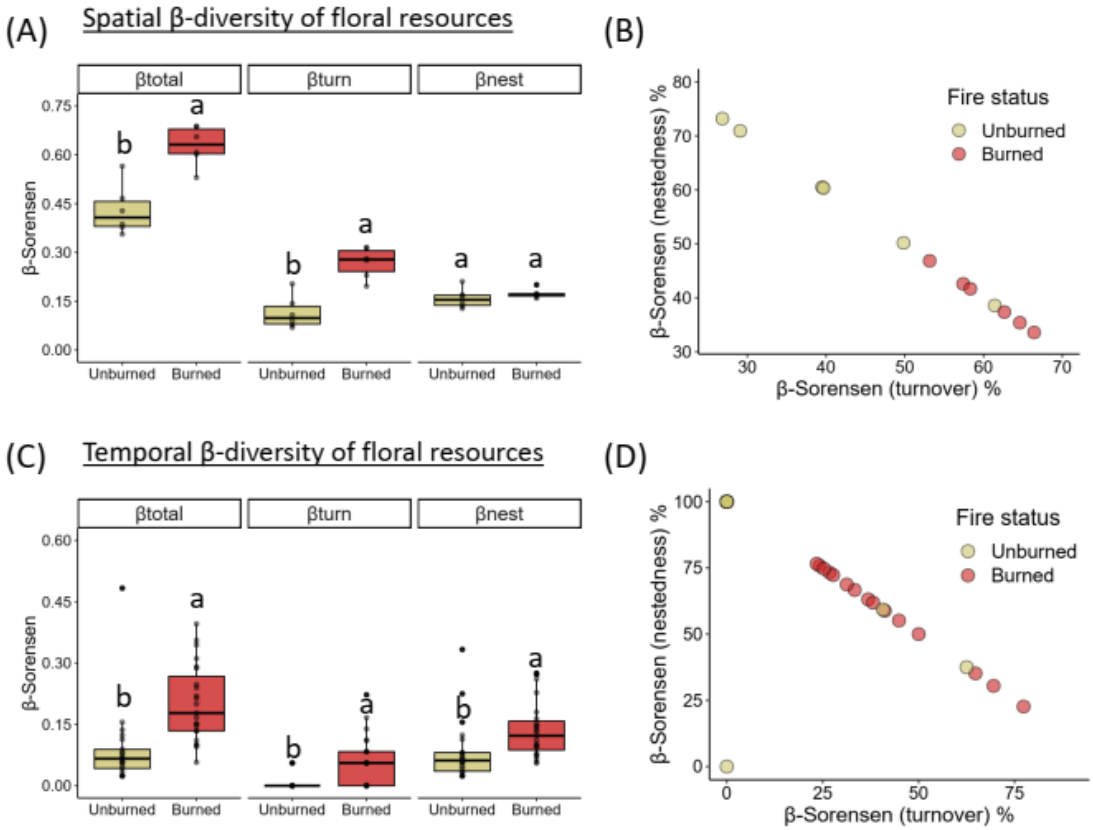

Figure 5.2 *β*-diversity of floral resources in burned and unburned plots across space and time. Sorensen *β*-diversity of floral resources was divided into two components: the component informing whether *β*-diversity was caused due to the turnover of floral resources (*β*turn) or the component informing whether *β*-diversity was caused due to nested assembling of floral resources within communities (*β*nest). The summation of both components gives the entire value of *β*-diversity of floral resources (*β*total). (A) depicts the spatial dissimilarities in floral resources among burned (red) and unburned (beige) plots. (B) depicts the representativeness in percentage of *β*nest and *β*turn in composing the spatial dissimilarities (*i.e.*, *β*total) among floral resources in burned and unburned plots. (C) depicts the temporal dissimilarities in floral resources among burned and unburned plots. (D) depicts the representativeness in percentage of *β*nest and *β*turn in composing the temporal dissimilarities (*i.e.*, *β*total) among floral resources in burned and unburned plots.

5.3.2 Spatiotemporal influence of fire on plant-bee interactions structure and stability

Throughout the two flowering seasons, we documented a total of 24,546 legitimate interactions involving 105 distinct plant species and 52 bee species (equivalent to an average of 1023 ± 610 plant-bee interactions per plot per flowering season). Unexpectedly, the fire did not affect patterns of resource use or the network structure in the plant-bee interactions networks. Community-wide network interaction specialization was only influenced by

season ($\chi 2_{1, 24}$ = 8.43; $R^2_{\text{-adjusted}}$ = 0.47; $p < 0.01$) and no differences in network interaction specialization could be tracked in plant-bee interaction networks collected in burned and unburned plots ($\chi 2_{1, 24}$ = 0.86; $p > 0.05$). Changes in network nestedness (*i.e.*, the transformed-network weighted nestedness; wNODF) were also only influenced by season ($\chi 2_{1, 24}$ = 4.63; $R^2_{\text{-adjusted}}$ = 0.65; $p < 0.05$), whereas neither season ($\chi 2_{1, 24}$ = 0.66) nor fire ($\chi 2_{1, 24}$ = 2.68) explained variations in network modularity when comparing burned and unburned plots ($R^2_{\text{-adjusted}}$ = 0.39; $p < 0.05$). Despite season marginally explaining the variations in patterns of resource use and in the network structure from the webs built with plant-bee interaction, burned and unburned plots did not exhibit significant differences regarding each of these network metrics (Figure 5.3b-d). The robustness of plant-bee interaction networks after the simulation of plant species removal were very similar among years and between burned and unburned plots ($\chi 2_{1, 24}$ = 3.44; $p > 0.05$; $R^2_{\text{-adjusted}}$ = 0.52). In fact, we find no differences in network robustness among burned and unburned plots and years (Figure 5.3e).

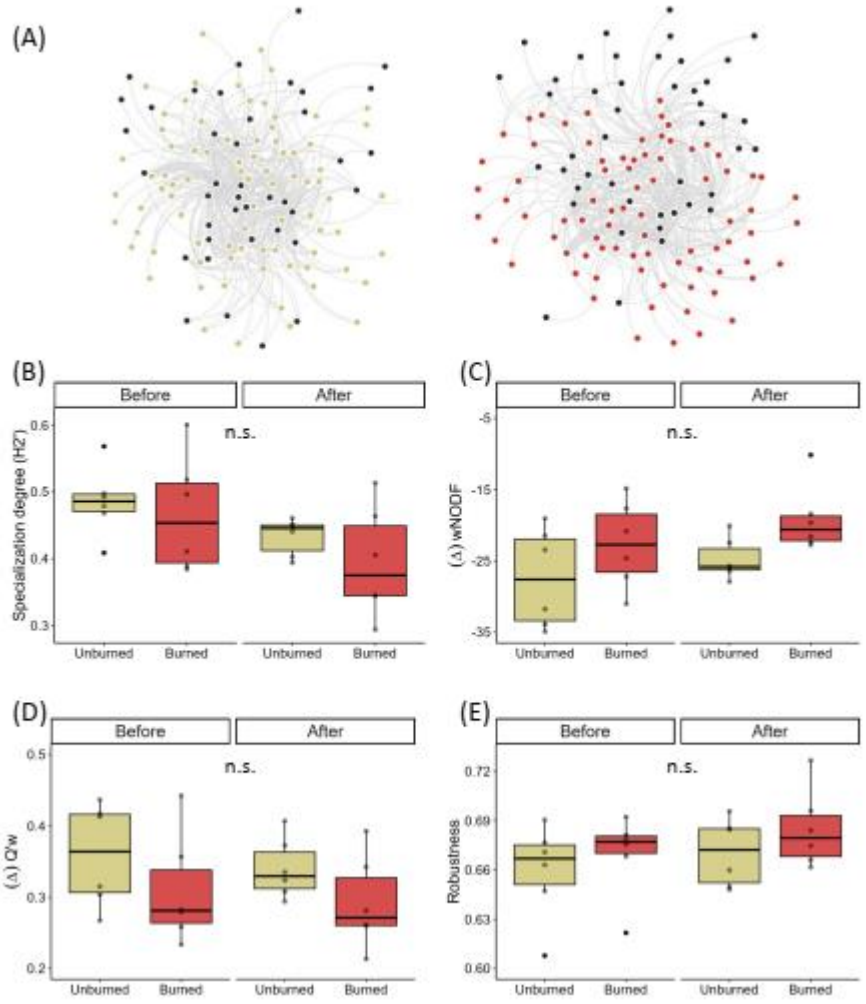

Figure 5.3 Floral resource-mediated plant-bee interactions network structure before and after a fire event in burned and unburned plots. Plant-bee interaction network (A) polling data on unburned and burned plots during the second year of sampling, when the fire took place. Circles in beige (unburned plots) or red (burned plots) represent plant species whereas circles in black represent bee species. The thickness of gray arrows represent pairwise plant-bee interaction frequency. Community-wide specialization degree (B), transformed-weighted nestedness (C), transformed-weighted modularity (D), and network robustness to plant species loss (E) before and after the fire event that burned half of the plots. Compact letter displays the differences among plots in the before-after-impact-control experiment after a Tukey *posthoc* comparisons of pair of means. Red boxplots before panels refer to plots in pre-burned situations. "n.s." means no significant differences among treatments.

5.3.3 Spatiotemporal influence of fire on plant-bee interaction organization

Despite being similarly robust to plant species loss, patterns of interactions before and after the fire event were more dissimilar in burned plots than in unburned plots (R^2 = 0.61; $\chi 2_{1, 12}$ = 13.65; $p < 0.001$; Figure 5.4). Burned plots presented higher values of network rewiring (β_{OS} = 0.65 ± 0.14; R^2 = 0.40; $\chi 2_{1, 12}$ = 6.35; $p < 0.05$) and interaction turnover (β_{ST} = 0.12 ± 0.04; R^2 = 0.74; $\chi 2_{1, 12}$ = 29.31; $p < 0.001$) than unburned plots (β_{OS} = 0.48 ± 0.08 and β_{ST} = 0.03 ± 0.02). Finally, despite being mostly explained by rewires in plant-bee interactions in burned or unburned plots, the turnover of species interaction explained a higher variation of the dissimilarities of plant-bee interactions in burned plots (16.12 ± 6.60%) than in unburned plots (6.40 ± 2.95%). Fire also influenced the overall variations of plant-bee pairwise interactions (i.e., β_{WN}) in space (R^2 = 0.47; $\chi 2_{1, 12}$ = 6.27; $p < 0.05$; Figure 5.5a) and time (R^2 = 0.49; $\chi 2_{1, 12}$ = 7.07; $p < 0.01$; Figure 5.5b). However, with the exception of the higher temporal turnover (i.e., β_{ST}) of pairwise plant-bee interactions in burned plots (R^2 = 0.23; $\chi 2_{1, 12}$ = 4.02; $p < 0.05$), there were no differences among burned and unburned plots in the β_{OS} and β_{ST} components of the spatiotemporal variations of plant-bee pairwise interactions ($p > 0.05$).

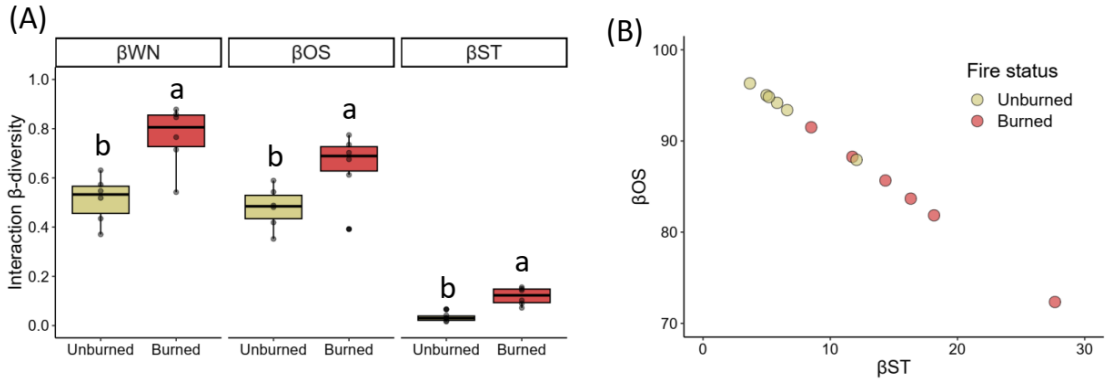

Figure 5.4 *β*-diversity of plant-bee interactions in burned and unburned plots. Interaction *β*-diversity of plant-bee interactions was divided into two components: the component informing whether *β*-diversity was caused due to the rewiring of plant-bee interactions (β_{OS}) or the component informing whether *β*-diversity was caused due to turnover of plant-bee interaction (β_{ST}). The summation of both components gives the entire value of interaction *β*-diversity (β_{WN}). (A) depicts the decomposition of plant-bee *β*-diversity in burned (red) and unburned (beige) plots. (B) depicts the representativeness in percentage of β_{OS} and β_{ST} in composing the plant-bee interaction dissimilarities (*i.e.*, β_{WN}) in burned and unburned plots.

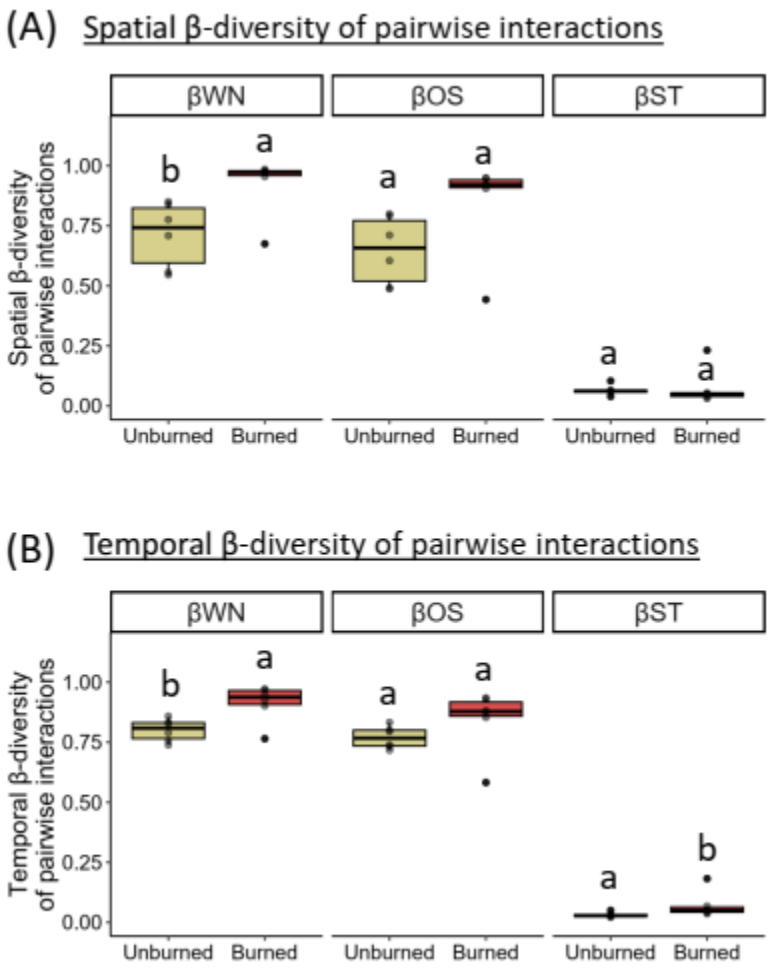

Figure 5.5 Spatiotemporal *β*-diversity of plant-bee pairwise interactions in burned and unburned plots across space and time. Spatial (A) and temporal (B) dissimilarities among plant-bee pairwise interactions was divided into two components: the component informing whether *β*-diversity was caused due to the spatiotemporal movement of pairwise interactions present in both flowering seasons (β_{OS}) or the component informing whether *β*-diversity of pairwise interaction was caused by the entrance or exit the new pairwise interactions, subplots or pairwise weeks (β_{ST}). The summation of both components gives the entire value of pairwise interaction *β*-diversity (β_{WN}). Burned plots are presented in red, and unburned plot, in beige.

5.4 DISCUSSION

Fire is a significant driver of plant compositional changes in ecosystems worldwide (Bond & Keeley, 2005). However, the mechanisms governing when and where flowers resurge in burned habitats and how plant-pollinator interactions respond to these disturbance

remain topics of debate (see Ponísio et al. 2016; LaManna et al. 2021; Burkle et al. 2022; Goldas et al. 2022b; Moreno-García et al. 2023). We demonstrate that, despite not related with variations in floral abundance or plant species composition, fire triggers shifts in the floral resource landscape, which is more apparent when investigated at finer temporal and spatial scales. Interestingly, despite influencing the reorganization of pairwise interactions in space and time, these shifts in floral resources do not seem to impact the structure and stability of the plant-bee interaction network (see Goldas et al. 2022b for a similar result). This resilience can be attributed to the remarkable adaptability of several bee species, enabling them to switch their flower preferences in response to shifts in plant species composition or to search for their specific floral resources in a new scenario with a reorganized floral resource display (see Gaiarsa et al. 2021; Guezen & Forrest, 2021; Burkle et al. 2022; Goldas et al. 2022b). In this context, while fire may influence the availability of various floral resources by delaying, accelerating, or relocating them, it does not appear to disrupt the stability of plant-bee interaction networks in Brazilian Cerrado.

Plants typically rely on ecological dispersal to distribute propagules and, in turn, move within their spatial environment (Jordano, 2017). We present new insights into how fire can promote plant movements within an ecosystem. By altering the floral resource landscape (Fidelis & Zirondi in 2021), fire has the potential to modify the local distribution of flowering species through both individual exclusion and the stimulation of seed banks, propagules (Pilon et al. 2021), and flowering (Zirondi et al. in 2022). This, in turn, creates opportunities for the spatial movement of species into and out of grains within the habitat. These effects are particularly pronounced in fire-adapted ecosystems experiencing species invasions, where fire, in addition to its cleansing role, can reveal plant species that were previously disadvantaged due to local microclimatic conditions resulting from the invasion process (Hachuy-Filho et al. 2019). Furthermore, we demonstrate that fire can also bring temporal mobility to plants, accelerating or delaying the availability of different floral resources. In this context, since we demonstrate that burned plots exhibit high dissimilarities of floral resources, we suggest that patchy fire events can prolong the presentation of different floral resources in the landscape, as burned patches may display floral resources differently

than unburned patches (Richardson & Wagenius, 2022; Richardson et al. 2023). The alteration of temporal flowering synchrony and flowering duration induced by fire becomes particularly relevant in scenarios of climate change, where temporal mismatches among organisms occur (Renner et al. 2018).

Moreover, we demonstrate, for the first time, that fire increases spatiotemporal variations in pairwise plant-bee interactions. In this context, we propose that fire contributes to the spatiotemporal variation in pollination, serving as a crucial initial step in facilitating the redistribution of seeds within the seed bank (de Andrade & Miranda, 2014). This process allows fire to establish a positive feedback in plant mobility over space and time, as it expands the spatial and temporal dimensions where viable seeds can become dispersed and/or part of the seed bank. Expanding spatiotemporally the probabilities of seeds being dispersed or becoming part of the seed bank increases the likelihood of both fire-intolerant seeds escaping during and fire-tolerant seeds germinating after patchily fire events (see Pausas, 2019 for general plant responses to fire). As we establish that plant-bee networks remain stable despite the spatiotemporal reconfiguration of floral resources and plant-bee pairwise interactions (see Ponísio, 2020; Ulyshen et al. 2022), we propose that fire-induced compositional changes in the Cerrado may not only increase spatiotemporal variability in pollination but also in subsequent processes that a plant individual must successfully undergo to reach maturity, such as seed dispersal and seed germination (Miller et al. 2019; Keith et al. 2020; Isla et al. 2024; Quinteiro et al. 2024).

Interestingly, fire enhanced both floral resource and pairwise interaction turnovers (see Burkle et al. 2022 and Moreno-García et al. 2023). While dissimilarities in floral resource presentation and plant-bee interactions in unburned plots were primarily attributed to changes in the combinations of plants providing resources and bees collecting them, burned patches promoted shifts in plant-bee interactions due to the turnover in floral resource-offering. In this context, whereas dissimilarities in floral resource presentation and plant-bee interactions in unburned plots can be linked to inherent differences in floral and bee phenologies between years (CaraDonna et al. 2017) as well as variation in bee foraging preferences though time, we propose that fire may trigger finer adjustments in floral resource

presentation and plant-bee interactions. These finer adjustments contribute to increased variability in both plant composition (Burkle et al. 2015, 2016; Singh et al. 2024), and plant-bee interactions through space and time (Moreno-García et al. 2023), creating ephemeral niche spaces that allow for a higher diversity of plants and pollinators to coexist (Ponísio et al. 2016; Kaiser-Bunbury et al. 2017; Ogilvie & Forrest, 2017; Goldas et al. 2022).

We also demonstrate that a single fire event is insufficient to influence either plant species richness or the structure of the plant-bee network. Despite reconfigurations in the presentation of floral resources over time and space, the spatiotemporal rearrangements among plants and bees do not disrupt their typical structure and do not diminish its stability (Goldas et al. 2022b). Disturbance events can enhance plant-pollinator network generalization, as the changes in floral resource availability might lead to a high overlap of interactions that benefit generalist and dominant pollinators (Hachuy-Filho et al. 2019; Barônio et al. 2021; Rakosy et al. 2022). Although fire represents a disturbance event, we demonstrate that it does not affect the degree of specialization in plant-bee interaction networks in the fire-prone Cerrado (see Goldas et al. 2022b). In our study, through a finer-scale investigation, we show in contrast that fire stimulates the turnover of floral resources, which may be advantageous for different bee lineages adapted to collect various types of floral resources (Vaudo et al. 2015). As a recurrent event in the Cerrado, fire may act as a selective pressure, filtering a diverse group of plants that offer different kinds of resources (Potts et al. 2003; Brown et al. 2016; Burkle et al. 2019). Since floral resource diversity influences network structure (Ballarin et al. ***in progress – Capítulo II***), maintaining relatively stable floral resource diversity at a broader spatiotemporal scale may preserve network structure and cohesion among plant and bee relationships after a fire has occurred.

Nevertheless, several conservation units in the Cerrado still implement a fire suppression policy (Durigan & Ratter 2016), which may facilitate the invasion of dominant herbs or woody encroachment (Buisson et al. 2022), homogenizing resource availability (Durigan 2020) with negative consequences for bees and co-flowering plants (Hachuy-Filho et al. 2019; Ballarin et al. 2022b). Besides not affecting the stability of plant-bee interactions while bringing variability to the floral resource landscape, fire has remarkable effects on

controlling invader seed banks (Assis et al. 2021). By demonstrating no negative effects on plant-pollinator structure and stability, which are relevant for plant fitness (Lázaro et al. 2020), our study contributes to the growing body of research addressing various ecological processes that plants rely on, including seed removal and germination, in which fire might be beneficial (Daibes et al. 2017; Alcolea et al. 2021). This suggests that prescribed fires, following a thorough evaluation of ecosystem responses, could be an interesting management tool for the Cerrado ecosystem to fulfill its potential in terms of structure and functioning.

5.5 CONCLUSION

In the Brazilian Cerrado, fires induce spatiotemporal changes in the floral resource landscape, which become more evident when examined at finer scales. These changes are characterized by a higher incidence of turnover in floral resources compared to unburned patches. However, we demonstrate that these spatiotemporal changes in floral resources do not significantly impact the structure and stability of plant-bee networks. This resilience is attributed to the bees' capacity to reconfigure their interactions by exploring new plant species offering different resources or relocating the specialized relationships they engage with plants in space and time. Consequently, we argue that the spatiotemporal variability in floral resource composition and plant-bee interactions triggered by fire does not pose a threat to these mutualistic relationships. This mechanism also helps prevent taxonomic and ecological homogenization, in contrast to what occurs in Cerrado habitats where fire is suppressed.

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Author contributions

CSB conceived the ideas. CSB and FWA designed the methodology. CSB collected the data; CSB and PJ analyzed the data and validated the results; CSB led the writing of the manuscript. CSB and FWA supervised and administered the study. All authors contributed critically to the drafts and gave final approval for publication.

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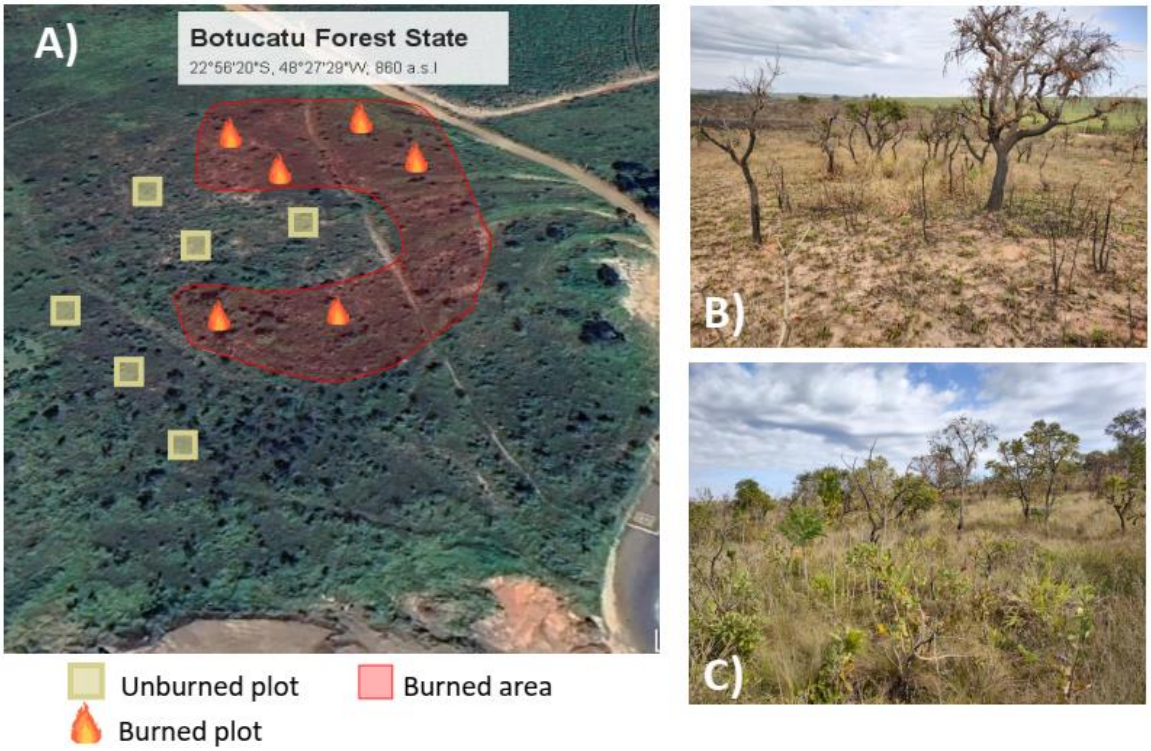
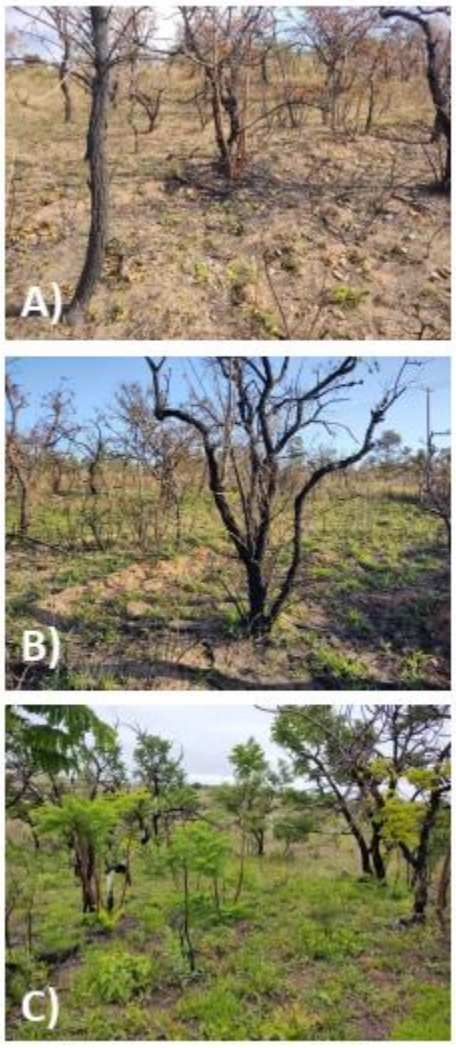
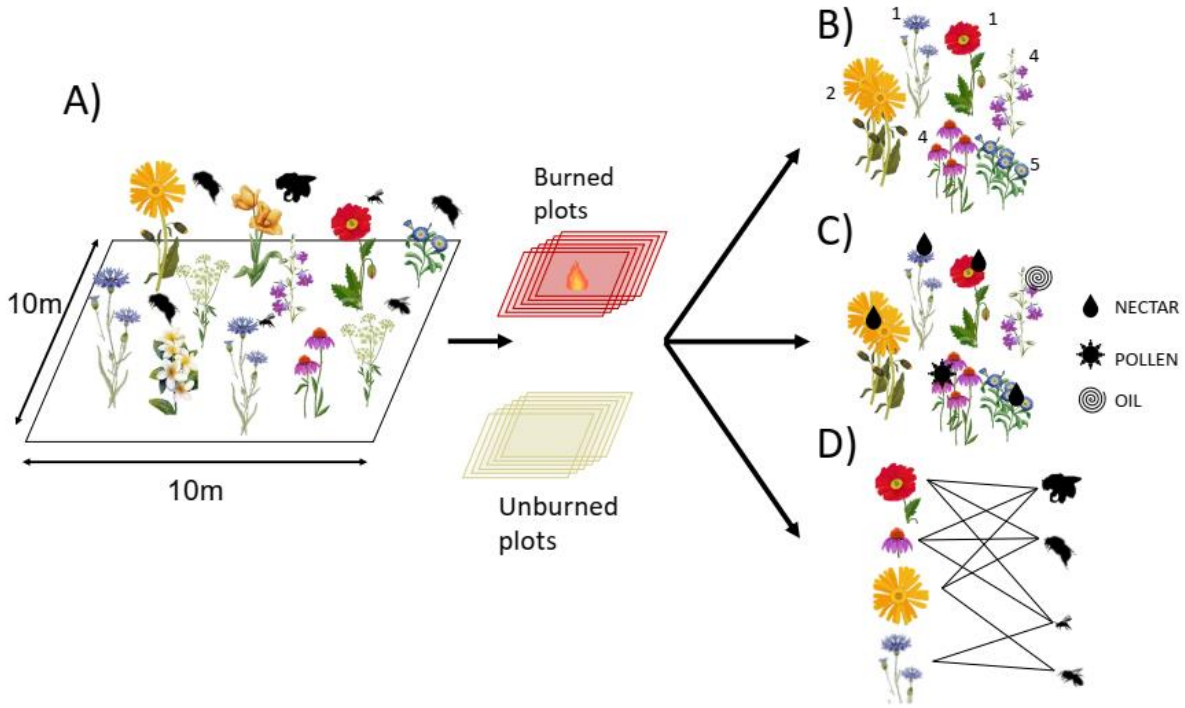
5.8 SUPPLEMENTARY MATERIAL



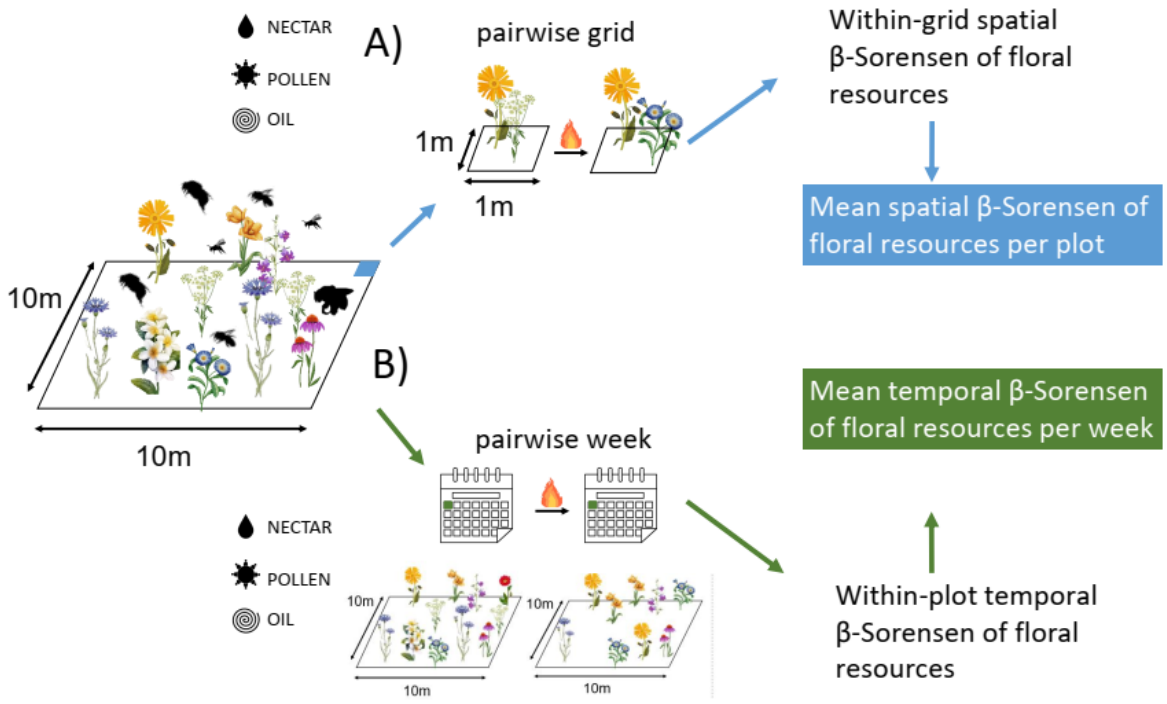
Supplementary Figure S5.1 Study site and fire documentation. Botucatu Forest State (BSF) and the region where the fire took place, burning half of the plots (A). Burned (B) and unburned (C) plot profiles two weeks after the fire event.



Supplementary Figure S5.2 Vegetation responses to the fire event. Vegetation profile two weeks (A), one month (B), and three months (C) after the fire event.



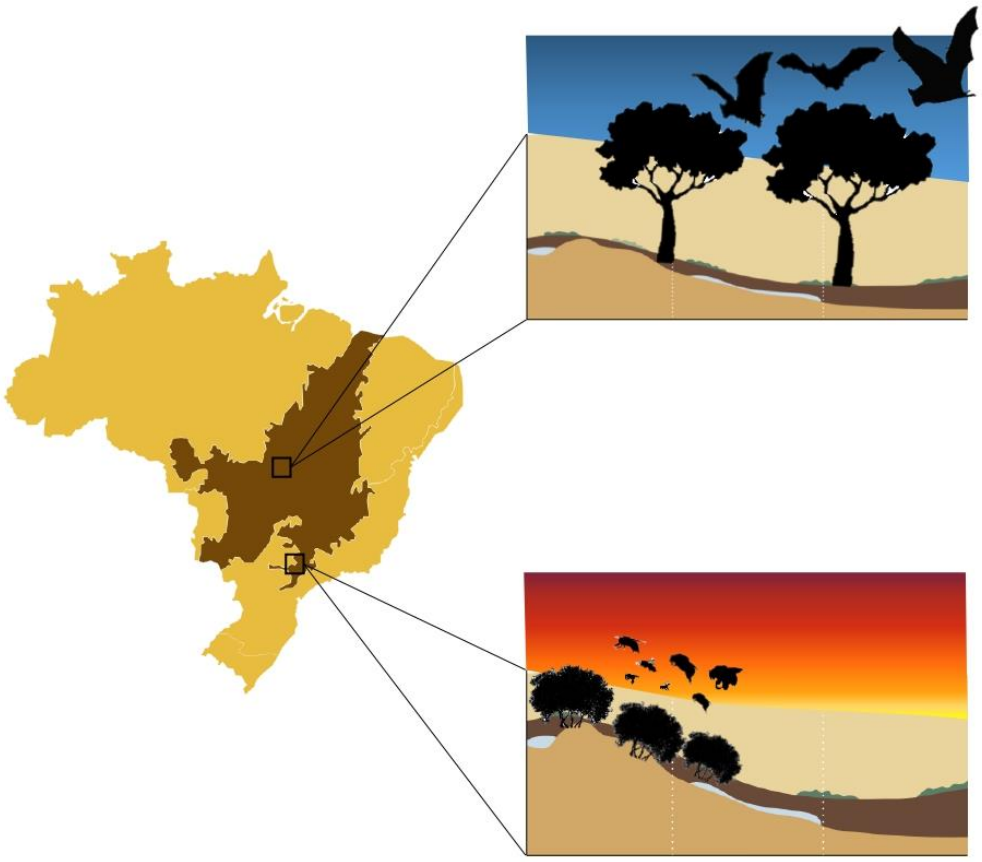
Supplementary Figure S5.3 Framework for evaluating changes in floral resource presentation and plant-bee interactions after the fire event. Figure (A) represents one of the 12 plots we used in this study, of which six plot were burned in the second flowering season while other six plots remained unburned. We evaluated whether fire influenced the floral abundance, and plant species composition (A) and the Shannon diversity of floral resources (B). We also evaluated if fire influenced the patterns of resource use and the structure of the plant-bee interaction network (D).



Supplementary Figure S5.4 Framework for evaluating spatiotemporal changes in floral resource diversity triggered by fire. Figure (A) represents the spatial evaluation of the changes in floral resource *β*-diversity in the grain scale (*i.e.*, among pairwise grids). Spatial *β*-diversity was assessed by the mean *β*-diversity (Sorensen index) of floral resources per plot. Figure (B) represents the temporal evaluation of the changes in floral resource *β*-diversity in the fine-temporal scale (*i.e.*, among pairwise weeks). Temporal *β*-diversity was assessed by the mean *β*-diversity (Sorensen index) of floral resources per week.

6 CAPÍTULO V

From bats to bees: pollinator shift between *Caryocar brasiliense* ecotypes resulting from changes in flower morphology and time of resource provisioning *



* Manuscrito em fase final de prepação para submissão na revista Annals of Botany.

From bats to bees: pollinator shift between *Caryocar brasiliense* ecotypes resulting from changes in flower morphology and time of resource provisioning

Running Title: Pollinator shift in the dwarf *Caryocar brasiliense*

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Abstract

Background and Aims: Plants with extensive ranges face diverse environmental pressures, driving phenotypic adaptations that influence ecological interactions. *Caryocar brasiliense* in the Brazilian Cerrado shows notable phenotypic variation, including a dwarf ecotype (*C. brasiliense* subsp. *intermedium*) in southernmost and high-altitude regions. While bats traditionally pollinate the arboreal ecotype, the main pollinator of the dwarf ecotype is unknown. We investigated whether pollinator-mediated selection has driven a pollinator shift in the dwarf ecotype. We also explored whether environmental, ecological, and genetic factors influenced the generation of pollination ecotypes.

Methods: We studied *C. brasiliense* in three sites within the Brazilian Cerrado. Floral traits related to pollinator rewarding and plant-pollinator matching, such as flower dimensions, and nectar dynamics, were compared between the arboreal and dwarf ecotypes. Pollinators' frequency and exclusion experiments determined pollinators' impact on fruit and seed set. Genome size and chromosome numbers were analyzed to explore genetic influences on phenotypic variation among ecotypes.

Key results: The dwarf ecotype of *C. brasiliense* exhibit smaller flowers. Flower opening occurs later at night, with nectar production peaking in the early morning. Bees are the primary visitors, contrasting with infrequent bat visits. Diurnal visitors, especially large bees, notably enhance fruit and seed set. Consistent genome sizes and diploid chromosome numbers across ecotypes suggest no polyploidy events.

Conclusion: This study highlights a pollinator shift from bats to bees in the dwarf ecotype of *C. brasiliense* in the southernmost Brazilian Cerrado. Past adaptations to environmental pressures likely drove this shift, given the continued presence of bats and the absence of polyploidy events between ecotypes. Changes in flowering anthesis likely explain the adaptation to diurnal visitors (e.g., large bees), enhancing seed set, with the reduced floral organ sizes as possibly phenotypic response. Further research on pollinator shifts in widespread plant populations could enhance our understanding of plant responses to climate-driven habitat changes.

6.1 INTRODUCTION

Plants with a wide geographic distribution are subject to different environmental pressures related to the climatic, edaphic, and topographical conditions where they are inserted (Etterson and Mazer, 2016; Etterson *et al.*, 2016; Hoffman *et al.*, 2020). Such species can play a highly variable ecological role in natural communities located throughout their distribution because local environmental conditions can trigger phenotypic changes in these species that will influence interacting species' responses (Miner *et al.*, 2005; Etterson and Mazer, 2016). For instance, certain plant species may present pollination ecotypes (*sensu* Armbruster, 1985) when their range exceeds the geographical distribution of its main pollinator (Grant and Grant, 1965). In the absence of the most efficient floral visitor, secondary floral visitors can mediate the selection towards their sensory and morphological preferences (Stebbins, 1970). In this phenomenon, ("Grant-Stebbins model" *sensu* Johnson, 2006), the exchange in plant's pollination niches is determined by the distribution of the main pollinator in a variable landscape (Johnson, 2010; Van der Niet *et al.*, 2014).

The absence of the main pollinator is the best-documented driver of pollination ecotypes (see Pérez-Barrales *et al.*, 2007; Bober *et al.*, 2014; Newman *et al.*, 2014). Nevertheless, some situations do not fit the "Grant-Stebbins model" (Van der Niet *et al.*, 2014). For example, past adaptations exerted by abiotic selective forces can induce morphological/physiological changes in reproductive organs affecting pollinator visitation and behaviour (Potts *et al.*, 2003; LoPresti *et al.*, 2018; Pardee *et al.*, 2018; Rodríguez-Sánchez *et al.*, 2024). In these cases, regardless of the occurrence of the main pollinators, past phenotypic changes may prompt the detachment of the interaction between these plants and their main pollinators (Thompson and Wilson, 2008). This occurs because the fitness gain of becoming more specialized for the main pollinator does not exceed the fitness loss of being less adapted to secondary, less effective pollinators (Aigner, 2001). In such scenarios, natural selection might not favor plant phenotypes related to the main pollinator, but rather, plant phenotypes in which the additive contribution of both pollinators increases plant fitness (Aigner, 2001). Thus, if secondary pollinators are more frequent than the main pollinator due

to plant responses to environmental filters that impair main pollinator visitation, plants might exhibit striking adaptations to secondary pollinators.

Additionally, phenotypic variations influencing pollination may derive from genomic changes, such as polyploidy, i.e., multiplication of the number of chromosomes, and genome expansion (Rezende *et al.*, 2020). Polyploidization alters many plant traits that influence pollinator attraction, including plant and flower size, phenology, color, and scent (Porturas *et al.*, 2019; Rezende *et al.*, 2020). Such phenotypic variation can also lead to a pollinator shift when the main pollinators reduce or stop visiting flowers legitimately (Rezende *et al.*, 2020). By doubling the genome, the polyploidy directly affects the genome size, another cytogenetic trait with potential consequences for a set of nucleotypic effects (i.e., phenotypic characteristics influenced by the total amount of DNA; Doyle and Coate, 2019). These effects can be observed from the micro to macro morphological scale and can consequently impact pollination. Still, studies evaluating the evolutionary processes beyond a pollinator shift event are scarce and mainly focused on the traditional case where the main pollinator is absent, which does not raise questions about new or less investigated drivers of pollinator shift.

The Brazilian neotropical savannah (Cerrado) is a great source of biodiversity to evaluate whether pollinator shifts occur following the "Grant-Stebbins model" or due to alternative drivers, such as the past adaptation to an ancient abiotic condition or the polyploidization event. The Cerrado is a fire-prone ecosystem that covers a large territorial extension (almost 2 million km^2; Klink and Machado, 2005), with a high elevational variation (from 100 to 1700 m a.s.l; Silva *et al.*, 2006). Due to Pleistocene climatic fluctuations, frequent fire, and occasional frost events in high sites, Cerrado harbours a high diverse taxa of acaulescent, dwarf or xylopodium-harbouring species (Simon *et al.*, 2009; Simon and Pennington, 2012; Cássia-Silva *et al.*, 2022 but see De Toma *et al.*, 2022 for examples in other stressful ecosystems). Such species successfully prevailed in this seasonal and flammable ecosystem might due to a preadaptation that allowed them to protect their gems by growing belowground (Simon *et al.*, 2009; Simon and Pennington, 2012; Cássia-Silva *et al.*, 2022). Moreover, considering the apparent relationship between the height of trees and

the size of the genome (the larger the genome, the smaller the tree; Knight & Beaulieu, 2008), a high number of polyploidy species in Cerrado is also expected (Forni-Martins and Martins, 2000; Marinho *et al.*, 2014), building an interesting scenario to investigate the processes behind pollinator shifts events.

Caryocar brasiliense (Caryocaraceae) is an endemic species that occurs in a large portion of the Cerrado (Prance and Silva, 1973). Although *C. brasiliense* is a tree in most of its distribution within this ecosystem, in high-altitude southernmost regions of Cerrado, *C. brasiliense* is bushy and dwarf (ecotype *Caryocar brasiliense* subsp. *intermedium*). There is no information regarding the genome size for *C. brasiliense*, but the chromosome number was reported for three populations across the core and southernmost regions Cerrado, all with 2*n*=46 (Ehrendorfer *et al.*, 1984; Maglio *et al.*, 1984). However, whether one of these samples was a dwarf morphotype is unclear, preventing any correlation between ecotype and polyploidisation in *C. brasiliense*. *Caryocar brasiliense* trees in the core region of Cerrado present a yellowish-cream brush-like flower, which houses a large amount of diluted nectar that generally attract a myriad of floral visitors, including bees, hawkmoths, hummingbirds and nectarivorous bats, but especially *Glossophaga soricina* (Chiroptera: Phyllostomidae), its main pollinator (Gribel and Hay, 1993).

Nevertheless, despite the occurrence of *G. soricina* in the meridional region of Cerrado (Diaz *et al.*, 2017) and the floral similarity between the flowers belonging to the arboreal and the dwarf populations of *C. brasiliense,* the flowers of *C. brasiliense* subsp. *intermedium* are exposed close to the ground due its shrubby size. This represents an unwelcome feature for bats, as it hampers their flight maneuvers and exposes them to terrestrial predators, but a meaningless feature for bees. Indeed, our previous observations show that bees frequently visit the dwarf ecotype of *C. brasiliense,* while bats are rarely recorded visiting *C. brasiliense* subsp. *intermedium,* which may show that bees, even representing secondary pollinators, might contribute to plant fitness owing to their high visitation frequency (see Aigner, 2001). Considering that both bats and the dwarf ecotype of *C. brasiliense* occur in the southernmost region of the Cerrado, and thus, the "Grant-Stebbin

model" may not correctly apply to this case, this pollination system is a great opportunity to understand whether pollinator shifts can arise from other eco-evolutionary processes.

In this sense, we asked what alternative forces (past adaptation to climatic constraints *versus* polyploidization) could have generated a pollinator shift in the *C. brasiliense*. For this, we first verified whether the core- and southernmost populations of *C. brasiliense* configure as pollination ecotypes by comparing several floral traits (flower size and dimensions, anthesis, as well as nectar dynamics and composition) among Cerrado populations. Further, we conducted an exclusion experiment to test if a pollinator shift occurred in our study system by evaluating if bees mediated the selection of *C. brasiliense* subsp. *intermedium* floral traits towards diurnal pollination by increasing fruit and seed. Lastly, we examined the genome size of *C. brasiliense* in both ecotypes to determine whether the morphotypes of *C. brasiliense* could be a result of polyploidization, or if the dwarfing observed in the southernmost *C. brasiliense* population is a potential adaptation to frost-prone environments. Both scenarios could lead to subsequent shifts in pollinator dynamics.

6.2 MATERIAL AND METHODS

6.2.1 Study species and sites

Caryocar brasiliense is a typical keystone fire-prone species widely distributed in savannah vegetations within de Brazilian Cerrado. It possesses terminal raceme inflorescences arranged above its canopy. Each inflorescence displays conspicuous actinomorphic white to yellowish-cream scented flowers with a brush-like morphology that bears from 350 to 500 stamens (Gribel and Hay, 1993). As a chiropterophylous species, anthesis in *C. brasiliense* is nocturnal. Flowers open from 17:30 to 19:00h, synchronously with the start of activities by bats, its main pollinators, which fly over the landscape to locate resources and establish their foraging paths for the night (Gribel and Hay, 1993; Bobrowiec and Oliveira, 2012). The sulfuric scent emission, along with the large amounts of nectar and pollen displayed by *C. brasiliense,* also attracts a wide range of floral visitors other than glossophagine bats, such as non-flying mammals, hummingbirds, hawkmoths, bees, wasps, and flies (Paiva *et al.*, 2019; Souza *et al.*, 2022).

Caryocar brasiliense has populations with a disjunct distribution within Cerrado, which are the result of multiple maternal lineages (Collevatti *et al.*, 2003). This genetic differentiation can turn into phenotypic variations among *C. brasiliense* populations occurring in different regions of their range. For example, while the typical autonomous subspecies *C. brasiliense* subsp. *brasiliense* is a tree with up to 15 m and occurs predominantly in the core region of Cerrado, the most recent lineage of this species located in high peripheral areas on the southernmost Cerrado boundaries (*C. brasiliense* subsp. *Intermedium* – dwarf ecotype) is a sub-shrub/shrub with a 1.5 m and trunk hidden belowground or prostrated at the ground level (Prance and Silva, 1973; Collevatti *et al*., 2003).

We study *C. brasiliense* populations in three sites across its range, encompassing both the arboreal and the dwarf ecotypes (Figure 6.1 and Supplementary Table S6.1). We conducted fieldwork in 2015, 2016, and from 2019 to 2022, during *C. brasiliense* flowering peak, which lasts for one or two months and occurs between June and December, depending on the locality.

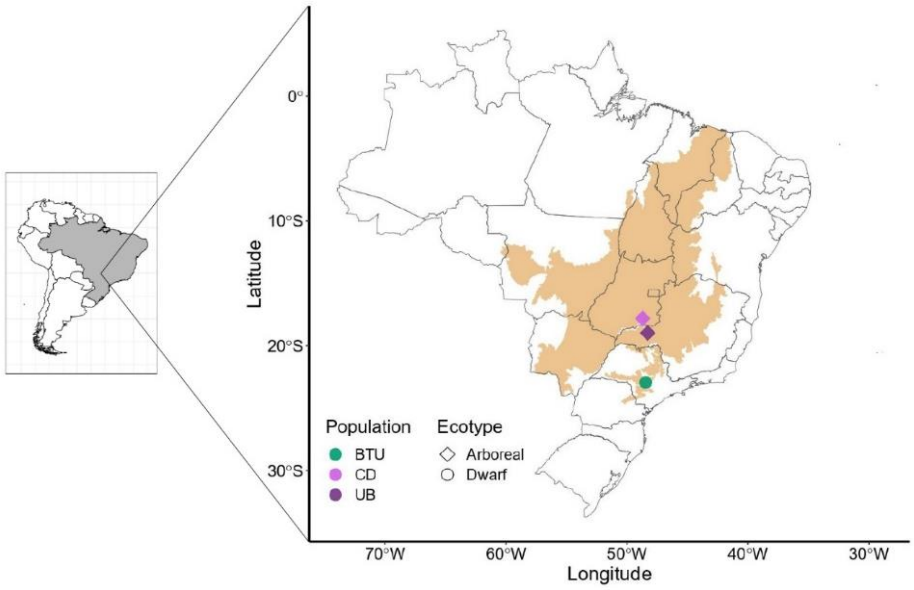

Figure 6.1 Location of the study sites where *Caryocar brasiliense* were studied. Botucatu - BTU: the southernmost region of Cerrado, where *C. brasiliense* present the dwarf ecotype (green circle) and Caldas Novas - CD and Uberlândia - UB, sites in the core region of the Cerrado, where *C. brasiliense* present the arboreal ecotype (purplish diamonds). The polygon in beige represents the Cerrado domain.

6.2.2 Floral traits

To evaluate whether the arboreal and the dwarf populations of *C. brasilience* are different pollination ecotypes, we measured several traits related to plant-pollinator matching (floral size, dimension, and floral anthesis) and pollinator rewarding (nectar production dynamics and nectar composition). Using a digital calipter, we measured i) petals' height and width (mm); ii) stamens' and carpel's length (mm), and iv) petal area. The petal area in *C. brasiliense* was assumed to be an ellipse-like. Thus, we calculated petal area using the following formula: Petal's area = π *[(petal's widht/ 2) * (petal's height/2)]. We randomly choose from three to five mature flowers in each *C. brasiliense* within each population for these measurements. In the dwarf population of *C. brasiliense*, we measured morphological traits in 380 flowers in 121 individuals, while for the arboreal populations, we evaluated 425 flowers in 108 individuals. To test whether morphometric floral traits differed between the dwarf and the arboreal populations, we conducted linear models (LM) considering the arboreal and the dwarf ecotypes as influencing floral morphometric traits, namely, petals' height and width, stamens' and carpel's length, and petal area, which were all considered as response variables. Furthermore, to examine the differences in floral morphology among *C. brasiliense* ecotypes, we conducted a Non-metric Multidimensional Scaling (NMDS) analysis based on the stamen length, anther length and petal area of each *C. brasiliense* belonging to each ecotype presented, using Bray-Curtis distances. To carry out the NMDS, we utilized the 'metaMDS' function from the *vegan* package in R (Dixon, 2003). During this analysis, we categorized *C. brasiliense* individuals according to its ecotype (n = 2) and its population (n = 3). Finally, to assess whether the morphometric floral traits differ between ecotypes and populations in *C. brasiliense*, we conducted a permutational multivariate analysis of variance (PERMANOVA). This test evaluates significant differences in the average distances between *C. brasiliense* individuals based on their morphometric traits, considering the ecotype it represents and the population it belongs to (i.e., using the ecotype and the population and their interaction as fixed factors). We utilized the 'adonis2' function with 10000 permutations from the *vegan* package in R to run the PERMANOVA.

We evaluated nectar production dynamics in the dwarf population of *C. brasiliense* while consulting the literature to assess nectar dynamics in the arboreal ecotype of *C. brasiliense* (Brobowiec and Oliveira, 2002). In the dwarf population, we selected 90 flowers (from two to three flowers per individual) and isolated them with nylon mash bags to avoid the interference of floral visitors. Thence, within an 18-hour period, which corresponds to the entire flower lifespan in *C. brasiliense* subsp. *intermedium*, we sampled nectar in six sets with 15 flowers each in 3-hour time intervals since *C. brasiliense* floral opening to senescence. Volumes of nectar samples were taken using a microliter syringe (Hamilton, NV, USA) and a pocket refractometer (0–90%; Instrutherm - RT-280) was used to quantify nectar sugar concentration (percentage sucrose, mass/mass), following Galetto and Bernardello (2005). We used the one-way analysis of variance (ANOVA) and a Tukey *posthoc* test for multiple comparisons among time intervals to characterize the pattern of nectar production (*i.e*, periods of nectar secretion, cessation or reabsorption; Ballarin *et al*., 2022). For this analysis, we considered nectar volume, concentration and amount of sugar as response variables to test for differences among time intervals throughout the flower lifespan.

6.2.3 Pollinators' frequency and efficiency

We recorded diurnal and nocturnal floral visitors through focal observations or camera traps (Bushnell Natureview model 119740, Bushnell Corporation, Overland Park, Kansas) in the dwarf ecotype of *C. brasiliense*. We discriminate legitimate and illegitimate visits by considering, in the former case, only the events in which visitors contacted *C. brasiliense* reproductive organs during their visits. To assess the visitation rate performed by day and night visitors, we calculated the ratio between the number of visits per unit of time. We considered the time unit as the product between the number of flowers observed and the number of hours sampled (*sensu* Muchhala *et al.*, 2009) since it allows compensating for differences in flowers that can be observed in day and night surveys with camera traps and focal observations. In the dwarf population, in 2015, 2016, 2019, and 2021 floral visitors' recordings totaled 174 h and 166 h in the diurnal and nocturnal periods, respectively. We did not conduct focal observations for the arboreal ecotype floral visitors. However, bat visits to the arboreal ecotype *C. brasiliense* are largely recognized as very frequent and crucial for *C.*

brasiliense reproductive output (Gribel and Hay, 1993; Bobrowiec and Oliveira, 2012; Souza *et al.*, 2022). We also investigated whether bee visits on *C. brasiliense* are linked to the nectar production rhythms of its dwarf ecotype. For this, we followed Ballarin et al. (2022) and conducted a generalized linear model (GLM) to analyze hourly bee visits on *C. brasiliense* in relation to the amount of sugar produced by its flowers throughout the day. The amount of sugar (in milligrams) in the nectar of *C. brasiliense* and bee size (i.e., small, medium, and large bees) were considered predictor variables, while the frequency of bee visits to *C. brasiliense* was the response variable. GLM was fitted using a Poisson distribution for the error terms.

To evaluate the effect of diurnal and nocturnal pollinators on fruit and seed sets in the dwarf ecotype of *C. brasiliense*, we conducted floral visitor exclusion experiments. For this, in 2019, we randomly assigned 31 *C. brasiliense* individuals. We marked from five to 11 flowers for each individual and each treatment, totaling 199 and 200 flowers for the nocturnal and diurnal exclusion, respectively. To exclude diurnal floral visitors, flowers remained exposed to nocturnal visitors at dusk (18:00 h) until dawn (4:30 h), when they were bagged with nylon mesh bags, avoiding diurnal visits. Oppositely, we excluded nocturnal floral visitors by bagging the flowers at dusk (18:00 h) until dawn (4:30 h), when flowers were then unbagged and exposed to diurnal floral visitors. We score fruit and seed sets in each *C. brasiliense* individual by calculating the ratio between the number of fruit or seeds and the number of flowers (as in Van der Niet *et al.*, 2014). To establish whether fruit and seed set were influenced by diurnal and nocturnal floral visitors, we ran two linear mixed models (LMM) considering fruit and seed set as response variables, exclusion treatments (diurnal and nocturnal exclusion) as fixed factors, and *C. brasiliense* individuals as a random effect. Thence, we made multiple comparisons between treatments with a Tukey *posthoc* test with *emmeans* package (Lenth, 2016) in R.

6.2.4 Cytogenetic analysis: genome size estimation and chromosome number

The genome size estimation was based on approximately 50 mg of fresh young leaf tissue macerated with the 25 mg of leaf tissue of the internal reference *Solanum lycopersicum* cv. Stupicke (2C=1.96pg; Doležel *et al.*, 1992). Both leaf materials were macerated in

0.5mL of extraction buffer (Ebihara *et al.*, 2005) supplied with 0.025 µg mL^{-1} RNAse. The nuclei suspensions were filtered and stained with 12.5 µL of a 1 mg mL^{-1} solution of propidium iodide (PI, Sigma). Three individuals from each population were analysed: two populations of dwarf individuals from the southernmost Cerrado (Botucatu, São Paulo State) and one population of arboreal individuals from the core Cerrado (Uberlândia, Minas Gerais State). Each individual was analysed in triplicate. The analysis was performed using the FACSCanto II cytometer (Becton Dickinson, San Jose, CA, USA) made available by the Microbiology and Immunology Department of IBB-UNESP (Botucatu, Brazil). The histograms were obtained with FACSDiva software based on 5,000 events, and the statistical evaluation was performed using FlowJo™ v10.8 Software (BD Life Sciences; https://www.flowjo.com/). The quality control was based on two estimates: the coefficient of variation (CV) of each measurement and the standard deviation (SD) among genome size measurements, which should be below 5%. Such limits ensure that the variations observed inside and among measurements are due to technical factors and should not represent intraspecific variation among individuals.

The chromosome number of dwarf *C. brasiliense* from southernmost Cerrado was analysed on meiotic cells. The floral buds were collected and fixed on 3:1 (absolute alchool: glacial acetic acid, v:v) at room temperature for 24 h and stored at −20 °C, in the same solution. For slide preparation, the anthers were squashed in a drop of 60% acetic acid. The coverslip was removed by freezing in liquid nitrogen and after air-dried, the slide was stained with 2% Giemsa. The slides were analyzed under an Olympus BX51 microscopy, and selected metaphases were captured with a CCD camera and analyzed by the software Image CellSens (Olympus, Inc.). The images were processed for contrast and brightness uniformity using Adobe Photoshop CS5 (Adobe Systems, Inc.).

6.3 RESULTS

6.3.1 Floral traits

Despite presenting similar color and morphology, flowers of the dwarf ecotype of *C. brasiliense* are smaller than those of the arboreal ecotype (Table 6.1; Figure 6.2a).

Specifically, stamen's and carpel's length, petal's height and width and petal area of the dwarf ecotype of *C. brasilense* are smaller than the arboreal ecotype ($P < 0.001$; Table 6.1). The NMDS ordination revealed that dissimilarities among *C. brasiliense* is mainly influence by the ecotype and the population of a given *C. brasiliense* (stress: 0.039; $R^2 = 0.99$; Figure 6.2b). Furthermore, the PERMANOVA analysis indicated that the floral morphometric traits varies in *C. brasiliense* ecotypes and populations. Floral morphometric variations in *C. brasiliense* is mainly explained by the ecotype ($F_{1, 189} = 291.06$; $R^2 = 0.58$; $P < 0.001$) and lastly by the population C. *brasiliense* belongs to ($F_{1, 188} = 19.82$; $R^2 = 0.03$; $P < 0.001$). Unexpectedly, floral anthesis also differs between both ecotypes. While the arboreal flowers open late afternoon, at around 17:00 h, the flower opening of the dwarf ecotype occurred late at night, after 02:00.

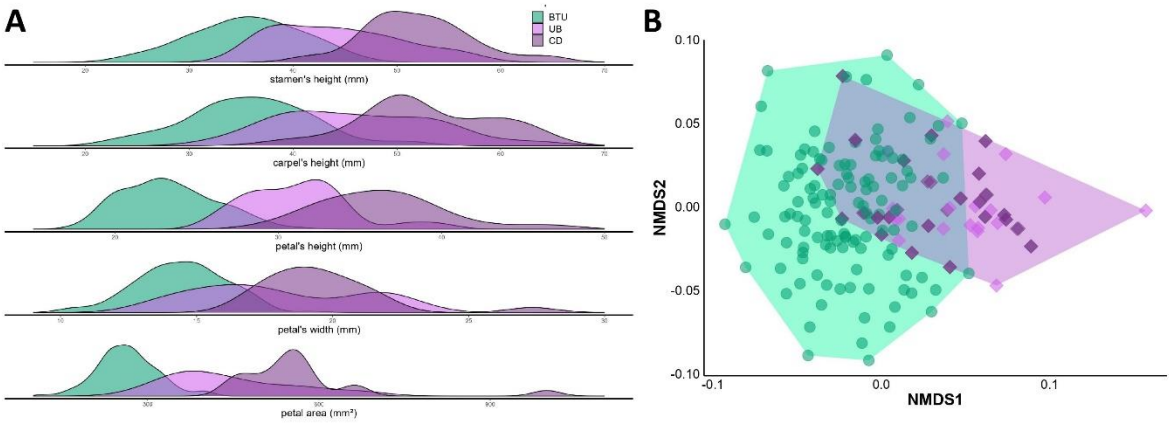

Figure 6.2 Floral attributes of the flowers of *Caryocar brasiliense* ecotypes. Floral dimensions of the dwarf (green) and arboreal (purplish) ecotypes (a). In the non-metric MultiDimensional Scaling (NMDS; b), green circles represent the dwarf ecotype, located in Botucatu (BTU), within, the southernmost region of Cerrado, and purplish diamonds represent the arboreal ecotype, located in Uberlândia (UB) and Caldas Novas (CD),within the core region of the Cerrado.

Nectar secretion in the dwarf ecotype of *C. brasiliense* flowers started concomitantly to its flower opening, at 02:00 h. Newly open flowers possessed 238.90 ± 57.14 µl of nectar, with 11.40 ± 1.71% of sugar concentration, containing, therefore, 29.04 ± 10.27 mg of sugar (Table 6.1, Figure 6.3). The highest amount of sugar in *C. brasiliense* flowers occurred from

05:00 h to 8:00 h, when flowers held 312.10 ± 129.28 µl of nectar, with 13.10 ± 0.99% of sugar concentration and 43.41 ± 20.17 mg of sugar. The dwarf *C. brasiliense* actively produced nectar until 11:00 h, when volume decreased (148.20 ± 46.00 µl; $F_{5,54} = 23.65$, $P < 0.001$) and the amount of sugar started to reduce afterwards (39.76 ± 12.20 mg of sugar; $F_{5,54} = 12.61$, $P < 0.001$). Nectar volume in *C. brasiliense* drastically decreased at the end of the flower lifespan, but sugar concentration increased ($F_{5,54} = 152.30$, $P < 0.001$), which indicates water evaporation instead of nectar reabsorption in these flowers.

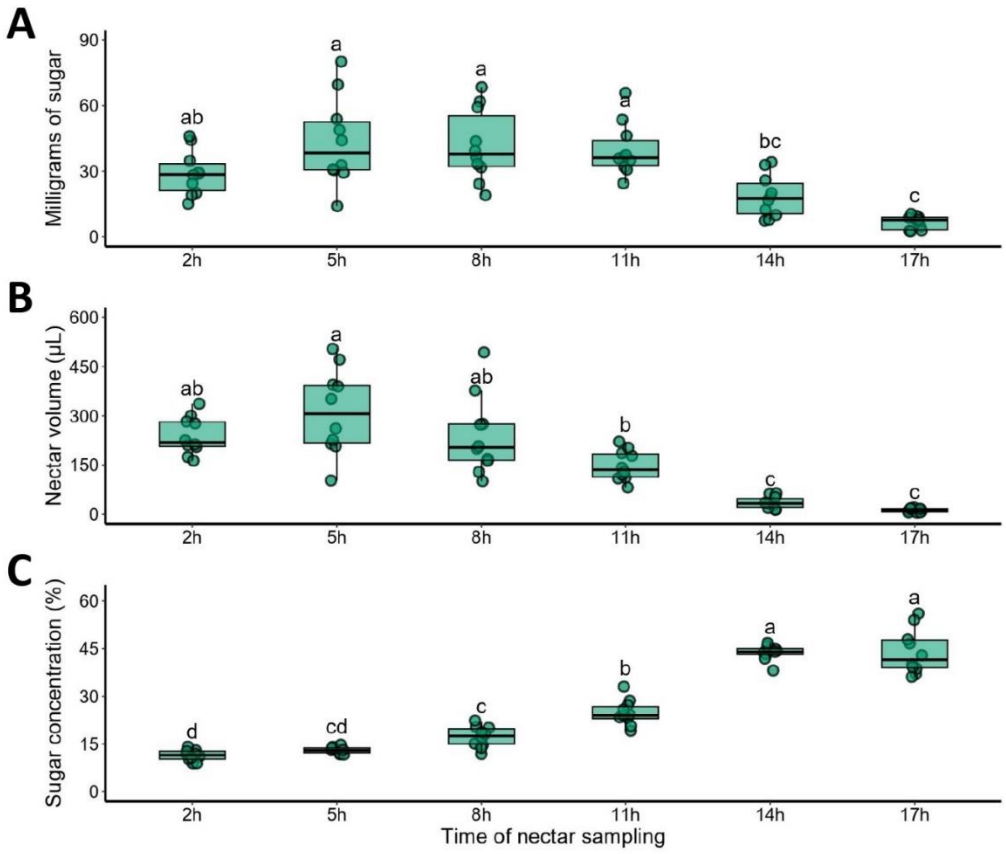

Figure 6.3 Nectar secretion pattern of the dwarf ecotype of *Caryocar brasiliense* over flower lifespan in the southernmost region of the Brazilian Cerrado. (a) amount of sugar (in milligrams), (b) nectar volume (in microliters) and (c) sugar concentration (percentual of mass/mass). Different letters above the boxplot represent significant differences ($P < 0.05$) among each period of nectar sampling after the Tukey *post hoc* test for multiple comparisons among pairs of means.

Table 6.1 Flower morphometric traits of the dwarf and the arboreal ecotypes of *Caryocar brasiliense*. Letters after Mean ± SD values depicts multiple comparisons among ecotypes with a Tukey post-hoc test. Numbers subscript before F values represent degrees of freedom.

	Ecotype (Mean ± SD)		**F value**	**R^2**	***P***
Flower traits	dwarf	arboreal			
stamen' length (mm)	38.71 ± 4.79 **a**	43.25 ± 3.95 **b**	$_{(1, 101)}$ 32.04	0.24	***<0.001***
carpel' length (mm)	34.67 ± 4.68 **a**	45.43 ± 4.61 **b**	$_{(1, 101)}$ 49.61	0.33	***<0.001***
petal' height (mm)	23.67 ± 1.63 **a**	27.50 ± 9.89 **b**	$_{(1, 101)}$ 111.00	0.52	***<0.001***
petal' width (mm)	14.17 ± 1.47 **a**	16.38 ± 0.92 **b**	$_{(1, 101)}$ 42.12	0.29	***<0.001***
petal area (mm)	215.33 ± 24.85 **a**	415.50 ± 44.14 **b**	$_{(1, 101)}$ 75.32	0.43	***<0.001***

6.3.2 Pollinators' frequency and efficiency

Bees massively visited the flowers of the dwarf ecotype of *C. brasiliense*, while bats were much less common (Supplementary Table S6.2 and Supplementary Table S6.3). While we found 926 visits of 28 bee species (0.0107 visits/hour*flower), bats visited the flowers of the dwarf ecotype of *C. brasiliense* only two times (0.0002 visits/hour*flower), which means bees have an almost 32-fold higher frequency of visits per hour per flower than bats. Floral visitation by bees occurred mainly in the morning, from 05:00 h to 11:00 h, with a significant number of large-sized bees visiting *C. brasilense* in the early morning (Figure 6.4a). Nonetheless, we observed a significant number of floral visiting bees also in the evening, with a particular increase of medium-sized bees, mainly due to *T. spinipes* individuals, which landed in flowers to explore the viscous, highly concentrated nectar solution. Milligrams of sugar produced by *C. brasiliense* nectar influenced visitation rates by bees ($\chi 2 = 4.72$; $R^2 = 0.40$; $P < 0.05$; Figure 6.4b). Yet, bees with different sizes did not respond differently to rhythms of nectar production by *C. brasiliense* ($P > 0.05$; Figure 6.4b).

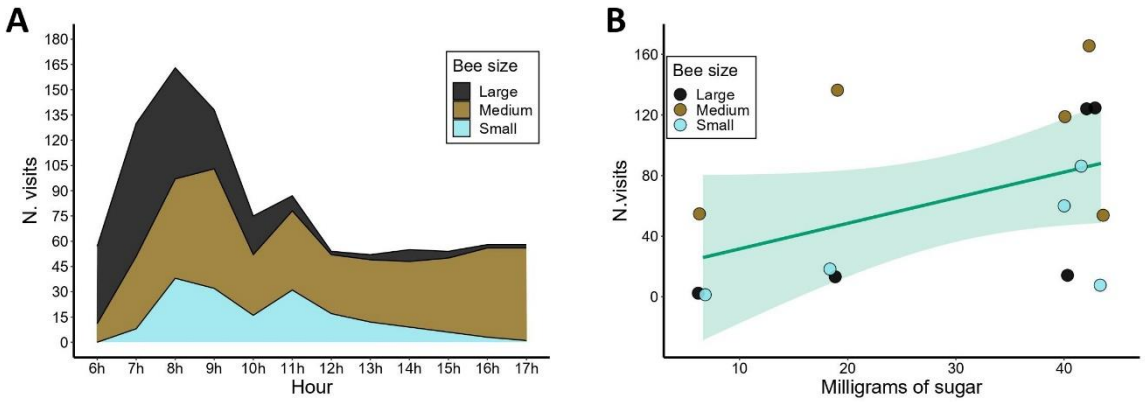

Figure 6.4 Bee visits on *Caryocar brasiliense* throughout the day. Bee visits per hour on the dwarf ecotype of *C. brasiliense* (a). Visits by bees are grouped according to their sizes: large (black), medium (brown) and small (light blue). Relationship between daily rhythm of nectar production (in terms of mass) by the dwarf ecotype of *C. brasiliense* and the number of bee visits on its flowers (b). Black, brown and light blue circles depict the frequency of large, medium and small bees, respectively.

Due to different sizes and behaviour, bees varied in their efficiency in pollinating *C. brasiliense*. Large bees belonging to *Xylocopa*, *Ephicaris*, *Bombus*, *Centris*, *Eulaema*, *Euphrisia* and *Oxaea* genera usually touched the stigmas of *C. brasiliense* while collecting pollen or nectar. In contrast, medium-sized bees (e.g. *Apis mellifera* and *Trigona spinipes*) and small-sized bees (e.g., *Tetragonisca angustula* and *Paratrigona lineata*) touched the stigma of *C. brasiliense* while collecting resources at intermediary and low frequencies, respectively. Fruit and seed set were extremely high in the dwarf ecotype of *C. brasiliense*. 86.15% of daily-pollinated and 32.66% of nocturnal-pollinated flowers develops into fruits. Regarding seed set, 63.50% of daily-pollinated flowers produced one or more seeds, which corresponded to 1.24 seeds per flower. In contrast, 18.09% of nocturnal-pollinated flowers produced one or more seeds, which corresponded to 0.32 seeds per flower. Pollinator exclusion experiments revealed that diurnal flower visitors are the most effective pollinators of the dwarf ecotype of *C. brasiliense*, as nocturnal-excluded flowers produced more fruits ($F_{1, 62}$= 132.27; R^2 = 0.72, P < 0.001; Figure 6.5a), and seeds ($F_{1, 62}$= 151.43, R^2 = 0.77 P < 0.001; Figure 6.5b) than those daily-excluded.

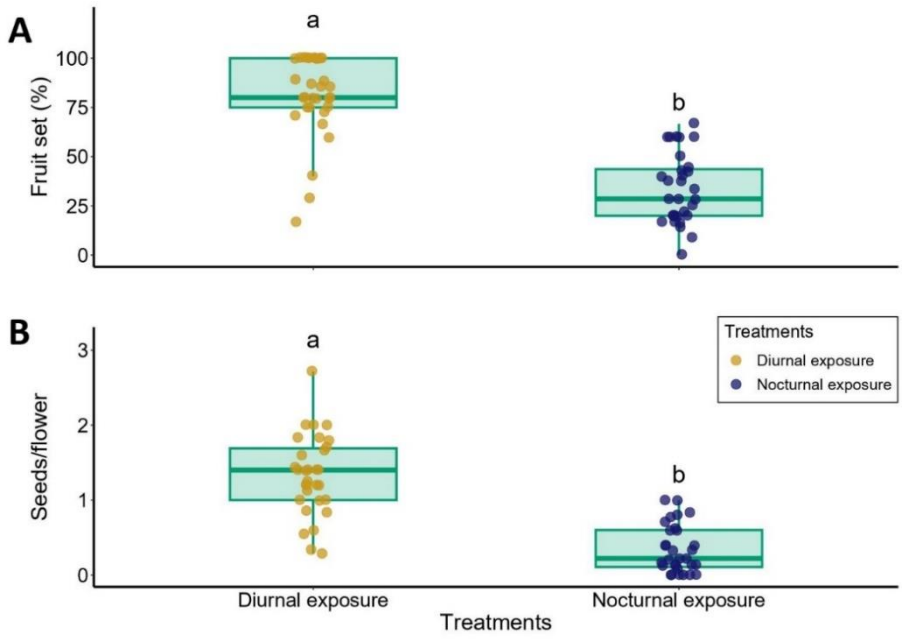

Figure 6.5 Reproductive output of the dwarf ecotype o *Caryocar brasiliense* after exclusion experiment. Fruit set (a) is represented as the percentage of flowers converted into fruits. Seed set (b) is represented as number of seeds in each flower. Blue and yellow circles depict nocturnal and daily excluded treatments, respectively. Different letters above the boxplot represent significant differences ($P < 0.05$) among each period of nectar sampling after the Tukey *post hoc* test for multiple comparisons among pairs of means.

6.3.3 Genome size and chromosome number

The genome size of *C. brasiliense* was estimated to be 1C=0.50 ± 0.025pg (489Mbp), with no discernible variation between dwarf and arboreal ecotypes collected from the southernmost and core Cerrado regions, respectively (F= 0.2625, p= 0.7744; Figure 6.6a-c). This lack of difference in genome size between the two ecotypes suggests the absence of polyploidy event between these morphotypes. Furthermore, the diploid number, *n*= 23, observed in the meiotic cells of dwarf *C. brasiliense* further supports the absence of

polyploidy (Figure 6.6c-d). The small size of chromosomes agrees with the reduced genome size.

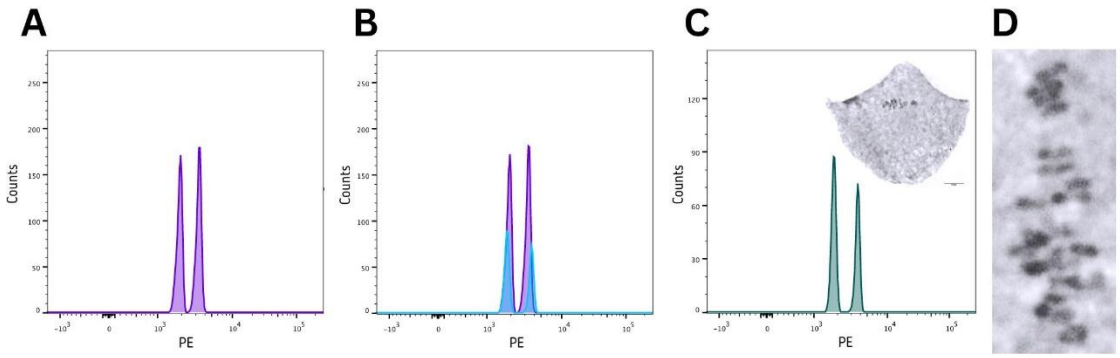

Figure 6.6 Genome size and chromosome number of *Caryocar brasiliense.* (a) Representative histogram of an arboreal individual from core Cerrado. (b) Overlay of histograms (a in purple) and (c in blue). (c) Representative histogram of a dwarf individual from southernmost Cerrado. The insert in (c) showns metaphase I and the below bar represents 10um. (d) Detail of metaphase I showing the 23 chromosome pairs.

6.4 DISCUSSION

In this study, we reported a pollinator shift in the dwarf ecotype of *Caryocar brasiliense*. While *C. brasiliense* trees are commonly and mainly pollinated by bats, the dwarf ecotype of *C. brasiliense* is primarily pollinated by bees, which in turn visit more frequently and efficiently *C. brasiliense* flowers in the southernmost regions of the Brazilian Cerrado. We also verified that a pollinator-mediated selection is on course in the dwarf ecotype of *C. brasiliense* once daily-pollinated flowers exhibited more fruits and seeds, and flowers from this ecotype are smaller and strongly differ in their opening time in comparison with the arboreal ecotype of *C. brasiliense*. Finally, considering that bats, the common pollinator of *C. brasiliense* in the core region of Cerrado, also occurs in the southernmost distribution of this plant, and that there is no polyploidy event between dwarf and arboreal *C. brasiliense* ecotypes, we argue that this pollinator shift report may have arisen from past phenotypic changes. These changes may have promoted the rewiring of plant-pollinator

interactions toward bees, which, although secondary pollinators, have additive effects on plant fitness (Aigner, 2001). Together with bees' high visitation frequency, their additive effect on plant fitness has favored plant phenotypes well adapted to them (Aigner, 2001).

Among the abiotic forces that can exert pressure on a plant's life history, altitude is a very interesting geographic barrier to be studied because altitudinal variations can occur on a very short spatial scale and still promote significant environmental impacts that allow phenotypic changes in population located at an elevational gradient (Körner, 2007). Indeed, dwarf ecotypes of native plants located at high elevations have already been documented (De Toma *et al.*, 2022). In the southernmost regions of the Brazilian Cerrado, habitats located at relatively high elevations are prone to fire and freeze - environmental selective pressures that might favor plant individuals that protect their gems belowground (see Hoffmann *et al.*, 2019 and Pilon *et al*., 2022 for fire effects on Cerrado ecosystem). In fact, several plant lineages located in these regions present dwarfism, which can indicate that, together with the low Cerrado soil fertility due to its dryness, which has already been documented as a factor driving dwarfism in native plants (Oliveira-Filho *et al.*, 1989), fire and freeze represent environmental selective forces that also can reduce plant size and height.

Within the plant-pollinator context, the increase in altitude can affect the abundance of different floral visitors, even when they occur in an extensive spatial context (Hoiss *et al.*, 2012; Rodríguez-Sánchez *et al.*, 2024), which may cause the detachment of certain plant-animal interactions and pollinator shifts when the interacting species do not coexist in the same environment (Pi *et al.*, 2021). Indeed, pollinator shift records worldwide usually occur when the primary pollinators are absent (Van der Niet *et al.*, 2014). However, this is not the case in our study system because glossophagine bats are also common in the southernmost region of the Cerrado. We argue that the flower's position closer to the ground after *C. brasiliense* dwarfization may have discouraged bat visitations as it hampers bats' flight maneuvers and exposes them to terrestrial predators (Diniz *et al.*, 2019). Moreover, considering that nectar-feeding phyllostomid bats use echolocation to identify nectar sources within the landscape (Fleming and Muchhala, 2008), it is possible that the presentation of flowers closer to the ground by the dwarf *C. brasiliense* hinders bats from locating flowers

(Muchhala and Serrano, 2015), synergistically contributing to reducing bat visitation on the dwarf *C. brasiliense*. With increasingly less bats bouts to the dwarf ecotype of *C. brasiliense*, individuals with smaller flowers and offering floral resource primarily during the day may have experienced higher reproductive success due to more frequent bee visits, which in turn, regulates their activity in relation to the amount of nectar resources produced by the dwarf *C. brasiliense*. In this sense, we suggest that the smaller flower dimension and the change in flower anthesis mainly result from pollinator-mediated selection imposed by bees, which act as the primary pollinators of this ecotype of *C. brasiliense*.

Fruit and seed set of the dwarf ecotype of *C. brasiliense* were extremely high in comparison with the arboreal ecotype (Gribel and Hay, 1993; Roque *et al.*, 2023). Moreover, daily-pollinated flowers produced more fruits and seeds than nocturnal-pollinated ones. We argue that the reduction in flower morphometric traits in the dwarf ecotype of *C. brasiliense* may have increased the likelihood of bees, especially the large ones, in exchanging pollen from anthers to stigma when searching for pollen and nectar. For example, bees have been regarded as resource exploiters and floral robbers in the arboreal ecotype of *C. brasiliense*, whose flowers are larger (de Araujo *et al.*, 2020). This is not the case of our study, which shows that bees are the most frequent and efficient pollinators of the dwarf ecotype of *C. brasiliense*. It is possible that the reduction of the flower traits also increased self-pollination in the dwarf ecotype, which may have ensured that nocturnal-pollinated plants produced fruits and seeds even in scenarios where bat visits were very scarce. Nevertheless, nocturnal-pollinated flowers produced fewer seeds than bee-pollinated ones. Despite *C. brasiliense* presenting auto-compatibility, this result is expected once nocturnal-pollinated flowers were possibly auto-pollinated in our study, given the low number of bat visits we recorded. Auto-pollination in *C. brasiliense* has already been suggested as an important cause of seed abortion (Collevatti *et al.*, 2009). Thus, despite ensuring reproduction in scenarios where bats are scarce, we believe that the pollinator shift from bats to bees represented a safer evolutionary path for the dwarf *C. brasiliense* then relying on self-pollination.

Although the shrinkage of floral organs represents and indirect evidence of phenotypic selection mediated by bees, the most frequent floral visitor in the dwarf ecotype

of *C. brasiliense*, more clear evidence that ascertains the pollinator shift we recorded was the change in time of floral opening and in the temporal production and presentation of floral resources. Nectarivorous bats, which live in sympatry with the dwarf ecotype of *C. brasiliense* could easily pollinate flowers in which stamens, carpels and petals were smaller. This means that the reduction in flower traits does not represent a reproductive barrier if the bats could access the nectar inside the flower. In contrast, delaying the floral opening to dawn impairs the visit of nectarivorous bats at higher frequencies once these animals are traplinners and plan their flight routes before the flowers of the dwarf ecotype of *C. brasiliense* are open (Sazima *et al.*, 1999). Without recognizing these flowers as nectar sources, the likelihood to forage on them after 02:00h, rigorously when *C. brasiliense* flowers open, reduce to near zero. It is possible, however, that changes in the floral opening time was a response of the dwarf ecotype of *C. brasiliense* to bees, their main pollinators in the southernmost region of Cerrado. We observed that the period of high nectar production was in the morning, precisely when bees were most active, which in turn may have exerted selective pressures to induce floral specialization toward their foraging preferences (Takimoto *et al.*, 2022). Therefore, we suggest that the temporal decoupling of bats and the flowers of the dwarf ecotype of *C. brasiliense* was a consequence and not the cause of the pollinator shift toward bee pollination.

Bat pollination have been recognized as an evolutionary dead end (Tripp and Manos, 2008). Nonetheless, despite the pollinator shifts involving bats and bees generally involve the opposing direction (from bees to bats – Knox *et al.*, 2008; Tripp and Manos, 2008; Martén-Rodríguez *et al.*, 2010; Rosas-Guerrero *et al.*, 2014), bat-pollination have already been documented to shift to bee-pollination in *Cayaponia* (Curcurbitaceae; Duchen and Renner, 2010). Notwithstanding the rarity of these records in flowering plants, we believe that dwarf ecotype of *C. brasiliense* also holds a particularity that aggrandizes the knowledge about pollinator shifts worldwide: the temporal shift in flower opening and floral resource availability. Pollinator shifts from diurnal to nocturnal pollinators and vice-versa, have already been documented (Luckow and Hopkins, 1995; Perret et al., 2003; Tripp and Manos, 2008; Martén-Rodríguez *et al.*, 2010). However, this shift is generally accompanied by drastic changes in floral attractions and flower morphology. In our study, the decrease in

floral organs was not a determinant to reduce bat and increased bee visits. Instead, the change in flowering opening and in nectar dynamics is what boosted bee visitation to the dwarf ecotype of *C. brasiliense*. Although pollination-mediated changes in flower lifespan have been documented as a mechanism that saves plants' energy to survive in extreme habitats (Arroyo *et al.,* 2022), this is the first study to our knowledge that reports a pollination-mediated change in flower anthesis as a result of pollinator shift.

Curiously, studies prospecting changes in range shifts of *C. brasiliense* and nectarivorous bats suggest that both interacting species will change their current distribution towards the southernmost regions of Cerrado in scenarios under climate change (Collevatti *et al.*, 2011; Aguiar *et al.*, 2016). Therefore, the sympatry among bats and native chiropterophylous dwarf plant populations may be more common than the current expectation. Hence, it is possible that novel pollinator-mediated selection from bats to ubiquitous floral visitors, such as bees, will happen in the future.

6.5 CONCLUSION

In this study, we suggest that past adaptations for singular environmental pressures lead to a pollinator shift from bats to bees in the dwarf ecotype of the cultural keystone species *Caryocar brasiliense* in the southernmost Brazilian Cerrado. We show that flowers from the dwarf ecotype of *C. brasiliense* are smaller and differ in flower opening time, providing floral resources mainly for diurnal floral visitors, such as bees, which are more frequent floral visitors and yield more fruits and seeds than bats. Besides the changes in floral traits we reported here, other floral attributes have already been regarded as important drivers of pollinator shifts, such as changes in floral scent (Peter and Johnson, 2014; Sayers *et al.*, 2021), shifts in floral resources (Castaneda-Zarate *et al.*, 2021), and changes in floral colour (Bradshaw Jr. and Schemske, 2003). Therefore, future studies should explore how changes in chemical and visual cues as well in floral resource quality and quantity could also foster bee attraction and reinforce the shift towards bee pollination in the dwarf ecotype of *C. brasiliense*.

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Author contributions

FWA conceived the idea. FWA and CSB and designed the methodology. CSB, FWA, PALB, and APM collected the data; CSB and FWA analyzed the data and validated the results; CSB led the writing of the manuscript. FWA and CSB supervised and administered the study. All authors contributed critically to the drafts and gave final approval for publication.

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6.8 SUPPLEMENTARY MATERIAL

Supplementary Table S6.1 Study sites. Localities of the study sites for the study with *Caryocar brasiliense* ecotypes.

Study site	Coordinates	Elevation (m)	Vegetation structure	*Caryocar brasiliense* ecotype
Botucatu **(BTU)**	22°56'20"S, 48°27'29"W	860	Cerrado "*campo sujo*"	Dwarf
Uberlândia **(UB)**	18°58'29"S, 48°17'25"W	800	Cerrado "*strictu sensu*"	Arboreal
Caldas Novas **(CD)**	17°48'38"S, 48°41'57"W	970	Cerrado "*lato sensu*"	Arboreal

Supplementary Table S6.2 Sampling effort and visitation frequency. Sampling effort and floral visitation in the dwarf ecotype of *Caryocar brasiliense* during the day and the night.

Period	Sampling effort (h)	Flowers sampled	Floral visitors				Total
			Bees			Bats	
			Large-sized	Medium-sized	Small-sized		
Day	174	492	237	516	173	0	926 (99.78%)
Night	166	78	0	0	0	2	2 (0.21%)

Supplementary Table S6.3 Bee visitors. Species of bees visiting the dwarf ecotype of *Caryocar brasiliense*.

Bee size	Species	N. visits	Relative N. visits
Large	*Xylocopa* sp.1	26	2.81%
	Xylocopa sp.2	130	14.04%
	Bombus morio	8	0.86%
	Bombus pauloensis	2	0.22%
	Ptiloglossa sp.1	16	1.73%
	Oxaea flavescens	14	1.51%
	Epicharis sp.1	8	0.86%
	Centris sp.2	4	0.43%
	Centris sp.3	13	1.40%
	Centris sp.8	3	0.32%
	Eulaema nigrita	6	0.65%
	Eufriesea sp.1	7	0.76%
Medium	*Trigona spinipes*	378	40.82%
	Apis mellifera	74	7.99%
	Ceratina sp.2	17	1.84%
	Augochloropsis sp.1	15	1.62%
	Augochloropsis sp.3	15	1.62%
	Exomalopsis fulvofasciata	8	0.86%
	Agapostemum sp.1	3	0.32%
	Epicharis sp.2	5	0.54%
	Epicharis sp.3	1	0.11%
Small	*Temnosoma* sp.1	14	1.51%
	Tetragonisca angustula	44	4.75%
	Paratrigona sp.1	90	9.72%
	Ceratalictus sp.1	15	1.62%
	Plebeia sp.1	6	0.65%
	Nannotrigona sp.1	1	0.11%
	Tapinodaspidini sp. 3	3	0.32%

7 CONSIDERAÇÕES FINAIS E CONCLUSÃO

7.1 DISCUSSÃO GERAL

A interação entre plantas e polinizadores é amplamente difundida no globo terrestre, de modo que a maioria das plantas com flores depende, em alguma instância, dos animais para se reproduzir (Ollerton et al. 2011; Rech et al. 2016; Rodger et al. 2021; Tong et al. 2023). De maneira equivalente, muitos animais dependem das plantas para desempenhar funções cruciais para manutenção de suas populações (Heinrich, 1975; Ollerton, 2016). Por exemplo, múltiplos grupos de invertebrados e vertebrados, tais como abelhas, besouros, vespas, formigas, borboletas, mariposas, lagartos, aves, morcegos e marsupiais dependem do recurso oferecido pelas flores para preencher parcial ou integralmente seus requerimentos energéticos diários. De fato, a importância dessa relação para plantas e animais é tida como um dos fatores que impulsionaram a diversificação de muitos dos grupos destas espécies interagentes (Eriksson & Bremer, 1982; Pellmyr, 1992, 2002; Kay & Sargent, 2009; Van der Niet & Johnson, 2012). Atualmente, estima-se que ambos, plantas com flores e animais polinizadores, somam cerca de 700.000 espécies (352.000 angiospermas - Paton et al. 2008; e 349.000 polinizadores - Ollerton et al. 2016).

A diversificação pode ser facilmente notada não somente pelo número expressivo de entidades taxonômicas e o rápido e repentino surgimento destas (i.e.,irradiação adaptativa), o que, ao menos para as plantas, Darwin chamou de “abominável mistério” (Darwin, 1871), mas também pela vasta variação dos atributos funcionais que essas espécies interagentes apresentam. Se, por um lado, animais podem exibir aparatos sugadores que variam de microscópico até grandes o suficiente para alcançar o néctar alojado na base de flores com tubo florais enormes (Müller, 1873; Muchhala, 2006), por outro lado, plantas podem exibir flores com grande variedade de formatos, cores, odores, e recursos florais (Faegri & Van Der Pijl, 1979; Crepet & Nicklas, 2009).

A variação imensa nos atributos florais das plantas com flores representa um objeto de investigação central em distintas áreas das ciências naturais que estão voltadas ao estudo destes organismos. Paleontólogos e biogeógrafos estão interessados em entender a origem exata e o padrão de distribuição das angiospermas e seus atributos florais no globo. Botânicos estudam em detalhe a anatomia, morfologia e fisiologia dos órgãos florais reponsáveis por

sinalizar e recompensar os visitantes florais. Ecólogos, por sua vez, se dedicam a entender como a interação entre flores e visitantes florais se organiza no espaço e no tempo e em qual medida ela determina a distribuição das espécies interagentes com diferentes papéis ecológicos. Entretanto, é consenso entre estas múltiplas disciplinas que, à luz da evolução, muitas das perguntas levantadas nessas áreas de investigação somente serão respondidas a partir de uma visão integrada que unifique conceitos desenvolvidos por cada uma destas áreas do conhecimento. Utilizando os recursos florais como eixo central do estudo, esta Tese de Doutorado se dedicou a compreender de maneira integrada aspectos sobre: 1) a distribuição global dos sistemas de interação planta-polinizador; 2) a organização e o funcionamento das interações planta-polinizador no espaço e tempo; e 3) os aspectos funcionais dos órgãos reprodutivos responsáveis pelo acomplamento planta-polinizador.

7.1.1 Sobre a distribuição global dos sistemas de interação planta-polinizador

Apesar do imenso esforço para compilar dados sobre os atributos funcionais das plantas (e.g., hábito, morfologia das folhas, sistemas de reprodução; Kier et al. 2005, 2009; Kreft & Jetz, 2007; Weigelt et al. 2019; Kattge et al. 2020; Cai et al. 2021; Tietje et al. 2023), é consenso que lacunas sobre algumas características fenotípicas, especialmente aquelas relacionadas à polinização, ainda persistem (E-Vojtkó et al. 2020). Flores muitas vezes podem ser diminutas, de modo que se torna difícil aferir muitos de seus atributos funcionais, tais como a quantidade e qualidade dos recursos florais que oferecem. Deste modo, algumas perguntas basais sobre a distribuição global de atributos funcionais nas angiospermas permanecem abertas.

Uma maneira de contornar a dificuldade em aferir atributos funcionais laborosos de se mensurar é trabalhar com dados categóricos. O uso de dados categóricos permite compreender a distribuição de determinado atributo funcional mesmo que suas propriedades não sejam medidas *in locu*. No Capítulo I desta tese, a presença/ausência do néctar em angiospermas de variados contextos filogenéticos e ecológicos foi amostrada para demonstrar que o néctar está amplamenente distribuído nas plantas polinizadas por animais. **Cerca de ¾ das aniospermas com polinização biótica produzem néctar**, o que sugere que esse recurso possivelmente é consumido por uma ampla gama de visitantes florais.

Em diferentes ecossistemas, a produção de néctar representa, portanto, um atributo que confere incremento na capacidade reprodutiva das plantas que o produzem, pois permite que potenciais polinizadores as visitem. Uma vez que a grande maioria dos potenciais visitantes florais têm interesse no néctar, a distribuição desse recurso nas angiospermas parece prevalecer sobre a distribuição de outros recursos que atraem grupos mais específicos de visitantes florais, como o óleo, a resina ou até mesmo o pólen. Embora o pólen seja a unidade masculina de reprodução das plantas com flores e esteja presente nas angiospermas em sua virtual totalidade, ele frequentemente não desempenha a função de um recurso floral produzido com função de recompensa aos polinizadores.

Ao inserir a classificação das plantas entre produtoras e não produtoras de néctar na malha espacial do globo terrestre, o Capítulo I também versa sobre os potenciais fatores climáticos que influenciaram a proporção de plantas produtoras deste recurso mundialmente. Se o néctar é o recurso mais comum apresentado pelas plantas aos polinizadores, ele também representa uma estratégia evolutiva conservadora, que permite que plantas atraiam visitantes mesmo em cenários depauperados de animais (veja Cunha et al. 2022 para discussão similar). A diversidade e riqueza de organismos segue um gradiente latitudinal e altitudinial, onde zonas mais frias e sazonais, tais como as regiões polares, temperadas e topos de montanhas, abrigam menos espécies que zonas mais quentes e climaticamente estáveis, tais como florestas úmidas e savanas (Rahbek, 1995; Brown, 2014). Devido a presença maior e mais constante de animais com que as plantas possam interagir, comunidades vegetais nos trópicos podem investir com mais segurança na produção de diferentes recursos florais (Bauer et al. 2021; Ratnieks & Balfour, 2021), dado que a probabilidade de que ele atenda às demandas de algum visitante floral é superior àquela encontrada em comunidades localizadas em latitudes ou altitudes superiores (veja Nery et al. 2024). Levando isto em conta, o Capitulo I mostra que, **ao nível global, a produção de néctar e a diversidade de recursos florais também segue gradientes latitudinais a elevacionais**. Comunidades tropicais e/ou próximas ao nível do mar apresentam maior diversidade de recursos florais e menor proporção de plantas que produzem néctar quando comparadas a comunidades não tropicais e/ou localizadas em elevadas altitudes.

Os resultados supramencionados podem contribuir para a acalorada discussão sobre a distribuição de sistemas de polinização generalistas e especialistas ao redor do globo. Ainda não há consenso se o grau de especialização nos sistemas de polinização segue um gradiente latitudinal. Enquanto alguns estudos apontam para um relação negativa entre latitude e sistemas especializados de polinização (i.e., quanto menor a latitude, maior o grau de especialização das interações planta-polinizador; Olesen & Jordano, 2002; Armbruster, 2006; Dalsgaard et al. 2011), outros não encontraram qualquer relação, ou até mesmo uma relação positiva (i.e., especialização aumenta com o aumento da latitude; Ollerton & Cranmer, 2002; Schleuning et al. 2012). Neste capítulo mostramos também que **a amplitude do eixo do nicho associado aos recursos florais aumenta em direção às latitudes mais baixas com temperaturas e taxas de precipitação mais elevadas**. Essa observação sugere que as comunidades de plantas tropicais têm a capacidade de hospedar uma diversidade mais abrangente de sistemas de polinização (Armbruster, 2006; Ollerton et al. 2006). Entretanto, sabe-se que mesmo sistemas de polinização bastante especializados podem conter espécies interagentes envolvidas em outra relações pareadas mais generalistas (Bascompte et al. 2003; Bascompte & Jordano, 2007; Vázquez et al. 2007; Krishna et al. 2008). Por exemplo, não é incomum que abelhas envolvidas em interações altamente especializadas com plantas que oferecem fragrâncias também visitem outras espécies vegetais para coletar néctar (Armbruster, 2017). Isso implica que **recursos florais são atributos funcionais fundamentais para reger o quão especializadas serão as interações pareadas entre plantas e polinizadores**. Contudo, a disponibilidade de flores oferendo diferentes recursos florais pode não ser estática espaço-temporalmente. De maneira similar, a escolha de um polinizador por determinada planta em detrimento de outra pode variar de acordo com as demandas de sua população, a fisiologia do seu organismo, o seu comportamento, ou as características do canal comunicativo entre esse e a flor - órgão que aloja seu recurso de interesse (veja Waser, 1986). Se os recursos florais desempenham um papel fundamental na mediação da relação entre plantas e polinizadores, resta compreender como variações quali e quantitativas no espaço e tempo influenciam a identidade, distribuição e composição dessas interações.

7.1.2 Sobre a organização e o funcionamento das interações planta-polinizador no espaço e tempo

Os Capítulos II, III e IV buscam entender, sobre diferente óticas, como a interação entre plantas e polinizadores flutua no espaço e no tempo e qual é o papel dos recursos florais na modulação dessa relação trófica. O ínicio e a duração do período de floração difere amplamente entre as angiospermas (Morellato et al. 2016) e, cada uma delas, pode oferecer recursos florais com características qualitativas únicas. Entretanto, a exploração de como a diversidade de recursos florais flutua espaço-temporalmente ainda é um tema pouco investigado. O Capítulo II destaca que, dentro de uma comunidade vegetal, **a variação na diversidade de recursos florais é ampla, porém, é notavelmente mais expressiva ao longo do tempo do que do espaço**.

A partir desta observação, cabe entender como cada espécie vegetal, quando em floração, contribui para a montagem da interação entre plantas e polinizadores. Como sugerido no Capitulo I, recursos florais são responsáveis por definir o grau de especialização das interações pareadas planta-polinizador. Todavia, ao considerar variações espaço-temporais destes recursos florais, consecutivas novas questões são geradas. Por exemplo, qual é o papel dos recursos florais em um contexto de comunidades de plantas e polinizadores, em que interações pareadas, quando em unidade, formam redes de interações? Os recursos florais são capazes de modular a composição, a estrutura e a arquitetura destas redes de interações?

As repostas para essas perguntas são complexas e contexto-dependentes. Em outras palavras, o papel dos recursos florais na configuração das interações entre plantas e polinizadores varia de acordo com a perspectiva utilizada para interpretar sua contribuição. Ao contabilizar variações espaço-temporais na composição de recursos florais em uma comunidade vegetal, o Capítulo II desta tese mostra que **a diversidade de recursos florais modula a estrutura da rede de interação planta-polinizador no espaço e tempo**. Se, por um lado, recursos florais que intermedeiam interações especializadas contribuem para a compartimentalização das redes de interação planta-polinizador, por outro lado, recursos explorados por uma ampla gama de visitantes florais geralmente promovem a inclusão de

relações mais generalistas na rede como um todo. Dessa forma, cenários nos quais a abundância relativa de flores oferecendo diferentes recursos florais é mais homogênea resultam em redes mais compartimentalizadas. Em contrapartida, a predominância de um recurso mais amplamente distribuído, como o néctar, conduz a redes mais aninhadas e generalistas. Isso implica que o agrupamento de espécies de plantas com base no tipo de recursos florais que oferecem pode aprimorar a compreensão de como a arquitetura da rede de interação planta-polinizador varia no espaço e no tempo.

Contudo, ao buscar entender o papel funcional dos recursos florais nestas redes empregando uma perspectiva trófico-funcional, pode-se alcançar outras reflexões. O Capítulo III mostra que **padrões estruturais drasticamente diferentes podem surgir quando redes de interação são montadas agrupando somente as interações mediadas pelo mesmo recurso** (i.e., redes de interação mediadas por néctar, pólen ou óleo). Dentro desta perspectiva, recursos florais tidos como especializados (e.g., óleo) podem gerar redes de interações generalistas, ao passo que recursos florais tidos como generalizados (e.g., néctar) podem gerar redes mais especializadas e compartimentalizadas. Assim, embora recursos especializados sejam importantes para compartimentalizar as rede de interação planta-polinizador montadas em "fatias" espaço-temporais, eles também acrescem o grau de generalização nas redes de interação planta-polinizador montadas em “fatias” trófico-funcionais. Assim, no nosso modelo de estudo em específico, pode-se notar que recursos especializados podem ser determinantes para geração de “módulos aninhados”. Em contrapartida, recursos generalizados podem ser determinantes para gerar um “aninhamento de módulos” (Figura 7.1).

Mesmo parecendo contraintuitivo, a explicação para esse papel “ambiguo” dos recursos florais é justamente o tipo de abordagem adotada para montar as redes de interação. Ao reunir todos os recursos florais da comunidade vegetal em uma rede de interação planta-polinizador, os recursos especializados atuam como mediadores de um bloco coeso de interações (veja Olesen et al. 2007; Hachuy-Filho et al. 2020). Nesse bloco, as espécies de plantas ou polinizadores compartilharão conjuntos semelhantes de atributos funcionais. Por exemplo, espécies de plantas produtoras de óleo geralmente possuem elaióforos, órgãos

reponsáveis pela produção e armazenamente deste recurso (Vogel, 1969; Cocucci & Vogel, 2001). Abelhas coletoras de óleo, por sua vez, apresentam escopas corbiculares capazes de extrair e armazenar o óleo coletado nos elaióforos (Roubik, 1992). Portanto, a inclusão de plantas com recursos especializados, tal como óleo, contribui para a compartimentalização da rede montada considerando todo o espectro de recursos florais oferecidos pelas plantas e utilizado pelos polinizadores (Olesen et al. 2007). De outro modo, por representar um recurso que intermedeia interações coesas entre grupos funcionalmente similares de plantas ou polinizadores, interações mediadas por óleo representam interações generalistas quando estas são agrupadas em uma rede de interação mediada por este único recurso floral. Isso se dá porque, virtualmente, todas as espécies de abelhas coletoras de óleo são capazes de visitar todas as plantas que oferecem óleo (Bezerra et al. 2009; Pacheco-Filho et al. 2015). Como não há restrições morfológicas para que essas interações se efetivem, já que a seleção mediada por abelhas coletoras de óleo atuou de maneira convergente nas flores que oferecem esse recurso (Davis et al. 2014), a probabilidade de interação planta-abelha mediada por óleo será majoritariamente regida pela abundância das espécies interagentes (Jordano, 1987; Jordano et al. 2006). Neste caso, plantas e abelhas mais abundantes possuirão maior quantidade de parceiros mutualistas e maior frequência de interação (Jordano et al. 2006). Em contraste, apesar de o néctar ser um recurso de baixa especificidade, uma vez que muitos dos potencias polinizadores o visitam (vide Capítuo I), ele foi responsável por gerar redes de interações planta-polinizadores mais compartimentalizadas. Relações plantas-polinizadores mediadas pelo néctar não estão limitadas a um grupo coeso e filogeneticamente restrito de espécies interagentes. Assim, é precisamente por servir como recurso para uma ampla gama de visitantes florais que plantas com diversas morfologias florais produzem néctar. Isso faz com que os animais visitem um subconjunto da comunidade de flores que oferecem o recurso mais acessível e adequado às suas demandas morfológicas e energéticas, respectivamente.

Esses resultados não só demonstram a natureza multifacetada das redes de interação ecológicas, mas também a sua natureza topologicamente hierárquica (Lewinsohn et al. 2006; Felix et al. 2022; Pinheiro et al. 2022). Em outras palavras, o estudo das redes de interação sob a perspectiva trófico-funcional permite revelar padrões de interações que estão ocultos

quando avaliados através da perspectiva espaço-temporal. Por exemplo, os resultados provenientes dos Capítulos II e III, quando compreendidos em conjunto, sugerem que **compartimentos concisos de interações planta-polinizador especializadas podem abrigar, internamente, interações generalistas. Igualmente, compartimentos amplos e relaxados de interações planta-polinizador generalizadas podem abrigar, internamente compartimentos concisos com maior grau de especialização**. Este fenômeno pode ser visto como "redes dentro de redes" que diferem em suas topologias (Newman et al. 2006).

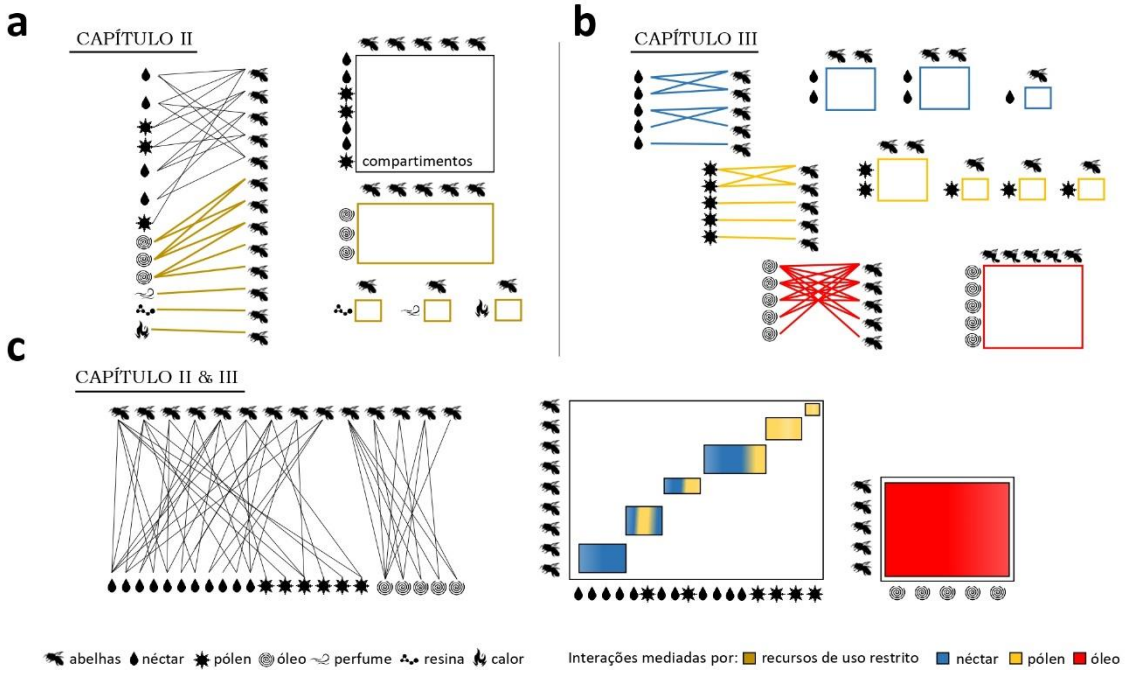

Figura 7.1 Modelo exemplificado de "redes dentro de redes". Diferentes abordagens de avaliação da rede de interação (e.g., espaço-temporal ou trófico funcional) podem revelar diferentes aspectos topológicos da rede. Na perspectiva espaço-temporal (a), observa-se que recursos de uso restrito (i.e., mediando interações especializadas) tal como óleo, perfume e resinas são importantes para criação de compartimentos (i.e., módulos). Já na perspectiva trófico-funcional (b), observa-se que redes construídas pela interação planta-polinizador mediadas por recursos de uso comum (e.g., néctar e pólen) são também capazes de gerar compartimentos quando estudadas separadamente. Em contrapartida, interações mediadas por óleo, recurso floral de uso restrito, quando estudadas isoladamente, geram redes aninhadas, generalistas e sem compartimentalização. Isso exemplifica o potencial da formação de redes dentro de redes (c).

Os recursos florais são fundamentais também para definir os papéis ecológicos dos polinizadores que atuam na rede de interação planta-polinizador. Isso é extremente válido

quando se estuda, em particular, as abelhas, grupo de visitantes que utiliza a mais ampla gama de recursos florais. Por exemplo, para além do néctar, abelhas utilizam outros recursos florais para se alimentar e se reproduzir. Pólen e óleos florais são consumidos como fonte de proteínas, aminoácidos e lipídeos (Vogel, 1969; Simpson & Neff, 1983; Buchmann, 1987; Armbruster, 2012). Óleos florais e resinas também são coletados para construção e proteção das colmeias (Simpson & Neff, 1983; Armbruster, 1984, 2012). Finalmente, fragrâncias são coletadas por grupos específicos de abelhas para atração de parceiros sexuais (Dodson, 1969; Dressler, 1982; Simpson & Neff, 1983; Armbruster, 2012). Embora, em temos gerais, abelhas abundantes sejam consideradas as reponsáveis pela organização e estruturação da rede de interação planta-abelha pois apresentam maior probabilidade de interação com plantas em floração (Giannini et al. 2016), o Capítulo III desta Tese ainda sugere que **abelhas menos abundantes, mas capazes de coletar recursos florais especializados, tal como o óleo ou o pólen alojado em anteras poricidas, são também fundamentais para conectar e trazer estabilidade para a rede de interação**. Isso se dá porque, mesmo sendo menos abundantes, elas utilizam maior ampla gama de recursos florais, criando rotas de interações indiretas entre as plantas que elas visitam em busca de diferentes recursos. A criação de novos caminhos diretos e indiretos conectando espécies interagentes proporciona estabilidade à rede. Isso ocorre pois a probabilidade de extinção em cascata de espécies, após a remoção de um determinado interagente, é reduzida quando as espécies demonstram maior flexibilidade no uso de recursos (Saint-Béat et al. 2015; Spiesman & Gratton, 2016).

Se recursos florais são importantes para definir muitas das propriedades de redes de interação planta-polinizador, tais como, sua estrutura no espaço e tempo, sua estrutura trófica e o papel de abelhas interagentes, o que aconteceria com a rede de interação planta-abelhas mediadas por recurso se um fator ambiental alterasse a disponibilidade de recursos florais? O sistema como um todo também seria alterado? Em qual magnitude? O Capítulo IVexplora justamente como o fogo, distúrbio recorrente em muitos ecossistemas globais, tal como o Cerrado brasileiro, impacta a composição e a distribuição de recursos florais e se tal efeito se transfere a outras propriedades do sistema (e.g., estrutura da rede de interação planta-polinizador). Diversos ecossistemas ao redor do globo, como o Cerrado, são considerados

tolerantes ao fogo (Bond et al. 2005), uma vez que este distúrbio é recorrente o bastante para atuar como uma pressão seletiva sobre muitos dos organismos presentes (Keeley et al. 2011; Keeley & Pausas, 2022). Deste modo, é esperado que o fogo não apresente um efeito severamente destrutivo em comunidades naturais onde ele é recorrente, mas sim, represente um fator ecológico que promova a diversidade (Fidelis, 2020). O Capítulo IV endossa a ideia do fogo como promotor de diversidade em ecossistemas fogo-tolerantes. Com o foco no estudo sobre a potencial alteração da diversidade de organismos, interações, e recursos mediadores no espaço e no tempo após a passagem de um distúrbio, o Capítulo IV mostra que **o fogo influencia a distribuição espaço-temporal de recursos florais e promove a reconfiguração das interações planta-abelha no espaço e tempo**. Entretanto, este capítulo também mostra que **o fogo não é reponsável por alterar a abundância de flores, a riqueza e diversidade de plantas, bem como a estrutura e a estabilidade da rede de interação planta-abelha.**

Uma possível explicação para o fato de a rede de interação planta-abelha se manter semelhante em termos de estrutura e estabilidade é que, embora se reconfigure em finas escalas espaço-temporais, a base de recursos se mantem estável em termos de capacidade de suporte. Como a riqueza e a diversidade de espécies vegetais se mantêm similares, e a abundâcia de flores parece não responder à passagem de fogo, o limite de visitantes florais que a comunidade vegetal suporta parece ser semelhante em condições pré e pós-fogo. Ou seja, ainda que as interações pareadas entre plantas e abelhas se modifiquem no espaço e no tempo devido à reconfiguração espaço-temporal dos recursos florais, redes de interações planta-abelhas parecem ser tolerantes à passagem do fogo em uma área de Cerrado, dado que se mantêm com arquitetura semelhante e apresentam similar robustez a eventos de exclusão de plantas. Assim sendo, **eventos de fogo parecem ser muito mais uma fonte de variabilidade para as interações planta-abelha no Cerrado do que uma ameaça a sua estrutura e estabilidade** (Figura 7.2). Isso se aplica especialmente aos eventos de fogo com características semelhantes às descritas no Capítulo IV, uma vez que diferenças notáveis na intensidade, severidade, duração, altura das chamas e outras propriedades podem refletir

diferenças também marcantes nos efeitos que estes provocam na comunidade de organismos (Fernández-Garcia et al. 2020).

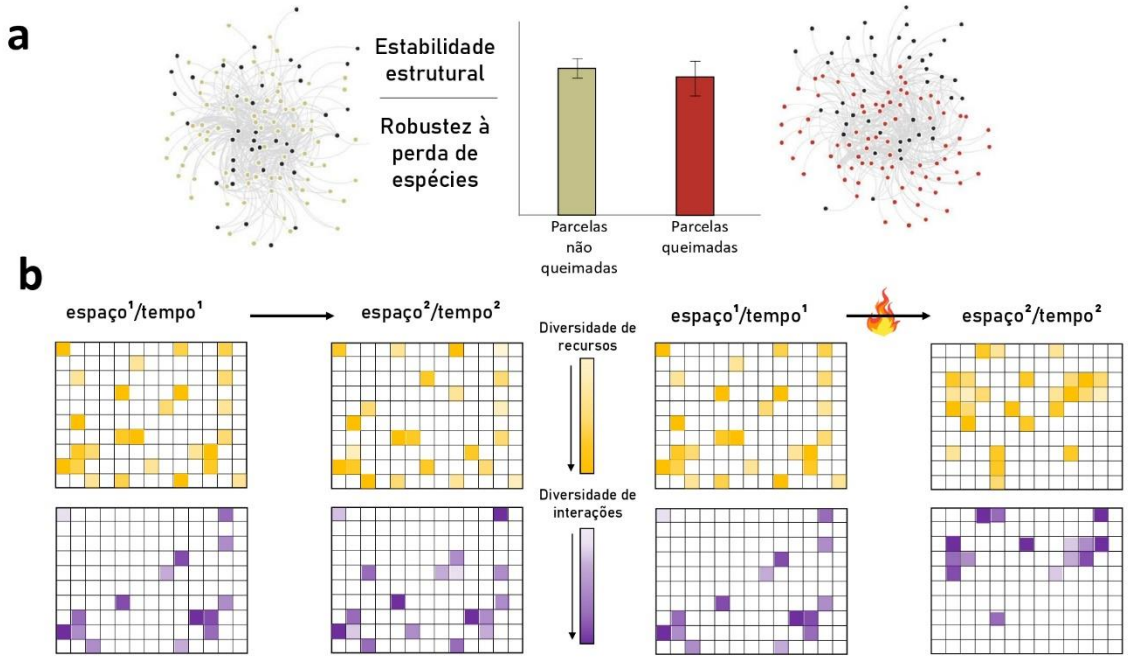

Figura 7.2 O fogo como fonte de variabilidade espacial e temporal na disponibilidade de recursos e interações. Mesmo não apresentando influência na estabilidade estrutural e na robustez da rede de interações planta-abelha à perda de espécies (a), o fogo traaz mobilidade espaço-temporal para interações pareadas planta-abelha (b). A previsibilidade de encontrar um mesmo par de espécies de plantas e abelhas interagindo em um mesmo lugar e em um mesmo período em parcelas não queimadas é maior que em parcelas queimadas, uma vez que há, nestas últimas maior deslocamento espaço-temporal da interação pareada entre plantas e abelhas.

7.1.3 Sobre os aspectos funcionais dos órgãos reprodutivos responsáveis pelo acomplamento planta-polinizador

Embora atributos funcionais de flores, incluindo recursos florais, desempenhem um papel crucial na interação entre uma miríade de plantas com flores e polinizadores em comunidades e na biosfera (vide Capítulos I II, III, e IV), sua influência no acoplamento planta-polinizador pode também ser avaliado ao nível do indivíduo. Essa abordagem é particularmente importante no cenário evolutivo, dado que a seleção natural atua neste nível de organização biológica. Deste modo, o estudo de interações planta-animal com enfoque no indivíduo permite estabelecer vínculos diretos entre padrões de interação ao nível da

comunidade e processos demográficos e evolutivos ocorrendo nas populações de uma mesma espécie (Gómez & Perfectti, 2012, Gómez et al. 2014; Arroyo-Correa et al. 2021, 2023; Isla et al. 2022; Quintero et al. 2023).

Em última instância, interações ecológicas planta-polinizador ocorrem entre indivíduos pertencentes a espécies de diferentes níveis tróficos. Assim, interações planta-polinizador são mediadas pelos fenótipos do indivíduos interagentes, e definem, por sua vez, em que direção e em intensidade as demografias das populações interagentes bem como o conjunto de seus atributos funcionais se modulará (Thompson, 1999; Strauss & Irwin, 2004; Strauss et al. 2005). Por exemplo, indivíduos vegetais apresentando determinado fenótipo que aumente sua frequência de interação com polinizadores frequentes e efetivos apresentarão maior sucesso reprodutivo (Stebbins, 1970). Se tais atributos funcionais deste fenótipo são herdáveis, eles se fixarão na população em maior frequência que atributos funcionais que confiram menor sucesso reprodutivo aos indivíduos que o apresentam. Logo, os atributos que medeiam a interação planta-polinizador influenciam o sucesso reprodutivo dos indivíduos que o apresentam. Contudo, justamente por mediar o sucesso reprodutivo destes indivíduos, atributos mediadores da interação planta-polinizador estão sujeitos a pressões seletivas que moldam a evolução de suas características na população que os carrega.

Em ecossistemas que ocupam extenso território, tal como o Cerrado, algumas espécies de angiospermas com polinização biótica se distribuem de maneira tal que as pressões seletivas bióticas e abióticas atuando em cada uma de suas populações pode diferir significativamente (Hoffman et al. 2020). O mosaico de filtros bióticos e abióticos atuando em cada uma destas populações pode moldar a evolução de atributos de maneira bastante distinta, influenciando a identidade e frequência das interações que os individíduos destas populações realizam (Miner et al. 2005; Etterson & Mazer, 2016). Por exemplo, espécies submetidas à condições ambientais diferentes podem oferecer recursos florais em contrastante quantidade e qualidade, que por sua vez, podem atrair de maneira diferencial distintos grupos de polinizadores (Castañeda-Zárate et al. 2021). Utilizando o pequi (*Caryocar brasiliense*), espécie amplamente distribuída no Cerrado, como modelo de estudo,

o Capítulo V mostra que **diferenças interpopulacionais nos atributos funcionais mediadores da interação planta-polinizador podem estar relacionadas com a troca de sistemas de polinização**. Neste capítulo, o ecótipo-anão do pequi (*C. brasiliense* subsp. *intermedium*) apresentou diferenças marcantes no tamanho de sua flor, no início de abertura floral, e na dinâmica de produção de néctar, quando comparado às populações do ecótipo arbóreo (*C. brasiliense* subsp. *brasiliense*).

Há dois fatores potenciais que podem levar a troca de polinizadores: ausência do principal polinizador e eventos de poliploidia. A ausência do principal polinizador é a justificativa mais recorrentemente reportada como propulsionadora da troca de polinizadores, uma vez que, na ausência do principal polinizador, flores estão submetidas à forças seletivas exercidas por polinizadores secundários (Grant & Grant, 1965; Stebbins, 1970; Van der Niet et al. 2014). Já a poliploidização, pode alterar atributos funcionais relacionados a polinização, alterando subsequentemente a identidade de polinizadores mais interessados no fenótipo resultante (Rezende et al. 2020). Ambos fenômenos não foram verificados neste sistema de estudo, indicando uma alternativa potencial subjacente à troca de polinizadores. O Capítulo V sugere que **adaptações à filtros ambientais também podem promover o desacoplamento da interação de uma espécie vegetal com seu polinizador mais efetivo, tornado-a sujeita a eventos de troca de polinizador**. O ecótipo-anão do pequi esconde seu tronco prostrado abaixo do solo, possivelmente como uma resposta evolutiva às condições adversas de um habitat mais frequentemente sujeito a eventos de fogo e geada. A exposição de suas flores próximas ao solo implicou no mal acomplamento entre essas e morcegos, polinizador mais comum do ecótipo arbóreo, uma vez que estes não costumam visitar flores rentes ao solo (Diniz et al. 2019; mas veja exceção em Amorim et al. 2023). Assim, abelhas de grande porte, polinizadores secundários do ecótipo arbóreo, passaram a representar os polinizadores mais efetivos no ecótipo anão, oferecendo subesquentemente, pressões seletivas que promoveram a redução da morfologia das flores do *C. brasiliense* subsp. *intermedium*.

Diferenças não foram somente encontradas em órgãos visíveis e de fácil aferimento neste Capítulo V. Mudanças no período de apresentação deste recurso aos polinizadores

também foram observadas. Morcegos e abelhas possuem período de forrageio distintos o que pode colaborar pelo mal acoplamento do ecótipo do C. brasiliense anão com os morcegos, umas vez que a apresentam e o consumo deste recurso estão mal-acoplados temporalemntes. Finalmente, morcegos e abelhas também preferências claras por um determinado tipo de néctar. Embora ainda não investigado durante esta tese, pretende-se ainda avaliar se há os ecótipos do C. *brasiliense* diferem quanto a composição química do néctar. Ao passo que morcegos exibem uma preferência por soluções hexose-dominantes durante seus voos noturnos, abelhas dão maior importância a soluções sacarose-dominantes durante suas visitas diurnas (Baker & Baker, 1983), indicando uma possível pressão seletiva capaz de alterar a química de açúcares deste recurso floral. Esta observação (i.e., alteração no período de apresentação do néctar) e esta predição (i.e., suposta diferença na composição de açúcares entre diferenes ecótipos) sugerem que **um único recurso floral pode apresentar inúmeras fontes de variação intraespecíficas relacionadas a sua disponibilidade no tempo, no espaço e a suas características quali e quantitativas**.

7.2 LINHAS DE PESQUISA ABERTAS

Esta Tese de Doutorado traz à tona, e, talvez essa seja sua contribuição mais significativa, que **é possível explorar a importância de recursos florais para interação planta-polinizador em diversas escalas e níveis de organização biológica**, partindo de variações intraindividuais (Herrera et al. 2006; Guimarães et al. 2018) para variações interindividuais, intersepecíficas, e até mesmo entre ecossistemas (como demonstra os Capítulos I a V desta tese). Além disso, esta tese se propõe a interpretar seus resultados obtidos integrando todas estas escalas, uma vez que todas essas regem dinâmicas fundamentais para a biodiversidade (e.g., indivíduos sendo a unidade da seleção natural e ecossistemas definindo padrões globais de distribuição de espécies). Embora pareça se tratar de uma demanda conflitante entre alcance e resolução – quanto mais se abrange a escala, menor o nível de detalhes possíveis de se obter – com os avanços científicos nas múltiplas áreas do conhecimento relacionadas direta ou indiretamente com a biodiversidade, essa tarefa parece estar gradativamente mais acessível, ainda que persista desafiadora. Compilando todo

o debate trazido nesta tese, é possível elencar alguns caminhos que podem render novas linhas de pesquisas com discussões frutíferas:

1- Ao nível da biosfera, a criação de um banco de dados global sobre a produção de recursos florais em angiospermas (em especial o néctar, cujos protocolos de extração são claros; veja Galetto & Bernardello, 2005), a fim de estimar variações intra e interindividuais bem como interespecíficas, poderia ser um conteúdo basilar para futuras questões em múltiplos níveis de organização biológica – desde estudos populacionais até estudos macroecológicos (veja Liu et al. 2024).
2- Ao nível da comunidade, a exploração das interações entre plantas e polinizadores sob uma perspectiva bioenergética, em que a disponibilidade de diferentes tipos de recursos florais pode ser convertida em conteúdo calórico disponível aos visitantes florais, poderia ser um direcionamento proveitoso, capaz de balizar/nivelar a comparação entre plantas oferecendo diferentes recursos florais.
3- Também ao nível da comunidade, compreender como os recursos florais medeiam interações entre plantas e visitantes florais no gradiente mutualismo-antagonismo (e.g., polinizadores e pilhadores de recurso) poderia ser prolífico. Compreender como espécies do mesmo nível trófico, mas com efeitos opostos à espécie interagente de outro nível trófico, demonstram preferência por algum tipo específico de recurso floral é crucial para integrar a interação planta-polinizador em um debate multitrófico que abrange diversos tipos de interações entre consumidores e recursos (veja Camacho et al. 2023 para abordagem similar).
4- Já ao nível da população, estudos que insiram o recurso floral dentro de um espaço multidimensional funcional pode ser fundamental para superar a carência de projetos de pesquisas que os estudem de maneira qualitativa, e não somente quantitativa ou categórica. Isso é de extrema importância ao se considerar que a visita de um determinado visitante floral a uma flor não é mediada somente pela presença e quantidade deste recurso, mas sim pelas suas propriedades químicas, sua apresentação no espaço e tempo, sua acessibilidade e sua completude nutricional.

5- Como uma proposta adicional de revisão de literatura, um debate interessante que se mantém inerte está relacionado a apreciação das flores polinizadas por abelhas como representantes de uma única síndrome de polinização (i.e., melitofilia; Faegri & Van der Pijl, 1979). Nesta tese foi demonstrado o quão diferente é a contribuição de plantas oferecendo distintos recursos florais e sendo visitadas por subconjuntos de abelhas dispares para a estrutura e funcionamento da comunidade de plantas e polinizadores. Explorar esse debate no contexto ecológico-evolutivo, a fim de abordar conceitos seminais raramente revisitados, pode também representar uma rota de investigação proveitosa

7.3 SUMÁRIO DOS PRINCIPAIS ACHADOS DA TESE

1- O néctar é o recurso mais comum oferecido pelas plantas ao polinizadores. No globo terreste, cerca de ¾ das angiospermas polinizadas por animais produzem esse recurso floral. Segundo esta estimativa, isso equivale a mais de 223,000 angiospermas.
2- A proporção de plantas produtoras de néctar e a diversidade de recursos florais em comunidades de angiospermas ao redor do globo seguem um gradiente latitudinal e elevacional. Neste gradiente, comunidades tropicais, mais quentes e úmidas e próximas ao nível do mar, apresentam menor proporção de plantas produtoras de néctar e maior diversidade de recursos florais do que comunidades não-tropicais e/ou localizadas em elevadas altitudes, cujo clima é mais frio e seco.
3- A diversidade de recursos florais influencia a estrutura da rede de interação entre plantas e abelhas no espaço e no tempo. Unidades espaço-temporais em que a abundância relativa de recursos florais generalistas e especialistas é mais equivalente tendem a exibir redes de interação mais compartimentalizadas. Unidades espaço-temporais em que há a predominância de um recurso floral generalista tendem a ser mais aninhadas e menos compartimentalizadas.
4- Redes de interação planta-abelha mediadas por distintos recursos florais diferem e sua topologia e composição. Interações entre plantas e abelhas mediadas por óleo compõe redes ecológicas mais aninhadas e generalistas. Interações entre

plantas e abelhas mediadas por néctar ou pólen compões redes ecológicas mais compartimentalizadas e especialistas.

5- Abelhas menos abundantes e com maior amplitude no uso de diferentes recursos florais também desempenham um papel ecológico fundamental na manutenção da estrutura e estabilidade da rede de interação planta-abelha. Ao criar rotas que conectam direta ou indiretamente plantas e abelhas que, respectivamente, oferecem e utilizam diferentes recursos florais, essas abelhas reduzem a probabilidade do colapso da estrutura da rede de interação após sua reconfiguração.
6- Embora altere a diversidade de recursos florais e reconfigure as interações pareadas no espaço e no tempo, o fogo não afeta a comunidade de flores nem a estrutura e estabilidade da rede de interação planta-abelha.
7- Distintos ecótipos de *Caryocar brasiliense* diferem quanto ao seus atributos florais e seus polinizadores principais. Isto indica que adaptações evolutivas a diferentes condições climáticas podem acerrater no evento de troca de polinizaor em populações não contíguas de uma mesma espécie vegetal.
8- A investigação dos recursos florais em diferentes escalas ecológicas e diferentes níveis de detalhamento pode contribuir para o melhor entendimento sobre a composição e diversidade das interações planta-polinizadores no espaço e no tempo. Por exemplo, ao considerar não somente a disponibilidade de recurso como também sua diversidade (e.g., Shannon, β-Sorensen, e funcional) muitos padrões espaço-temporais e trófico-funcionais provenientes da interação planta-polinizador puderam ser melhor compreendidos dentro das escalas ecológicas estudadas.

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