UNIVERSIDADE ESTADUAL DE CAMPINAS
INSTITUTO DE BIOLOGIA

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ANTS IN BROMELIADS: CASCADING EFFECTS ON THE DIVERSITY OF ARTHROPODS, NUTRIENT CYCLING AND ECOPHYSIOLOGY OF THE HOST PLANTS

FORMIGAS EM BROMÉLIAS: EFEITOS EM CASCATA SOBRE A DIVERSIDADE DE ARTRÓPODES, CICLAGEM DE NUTRIENTES E ECOFISIOLOGIA DAS PLANTAS HOSPEDEIRAS

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Thesis presented to *Instituto de Biologia* of *Universidade Estadual de Campinas* as part of the requirements to obtain the degree of Doctor in Ecology

Tese apresentada ao Instituto de Biologia da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Ecologia

ORIENTADOR: Prof. Dr. Gustavo Quevedo Romero
CO-ORIENTADOR: Prof. Dr. Paulo Sérgio Moreira Carvalho de Oliveira

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL DA TESE DEFENDIDA PELA ALUNA ANA ZANGIROLAME GONÇALVES, E ORIENTADA PELO PROF. DR. GUSTAVO QUEVEDO ROMERO.

CAMPINAS

2016

Agência(s) de fomento e nº(s) de processo(s): FAPESP, 2001/10137-8; CNPq; CAPES, BEX 8377/12-0

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca do Instituto de Biologia
Mara Janaina de Oliveira - CRB 8/6972

G586a
Gonçalves, Ana Zangirolame, 1984-
Ants in bromeliads : cascading effects on the diversity of arthropods, nutrient cycling and ecophysiology of the host plants / Ana Zangirolame Gonçalves. – Campinas, SP : [s.n.], 2016.

Orientador: Gustavo Quevedo Romero.
Coorientador: Paulo Sérgio Moreira Carvalho de Oliveira.
Tese (doutorado) – Universidade Estadual de Campinas, Instituto de Biologia.

1. Ciclagem de nutrientes. 2. Ecossistema. 3. Relação inseto-planta. 4. Predação (Biologia). 5. Relações tróficas. I. Romero, Gustavo Quevedo,1974-. II. Oliveira, Paulo Sergio Moreira Carvalho de,1957-. III. Universidade Estadual de Campinas. Instituto de Biologia. IV. Título.

Informações para Biblioteca Digital

Título em outro idioma: Formigas em bromélias : efeitos em cascata sobre a diversidade de artrópodes, ciclagem de nutrientes e ecofisiologia das plantas hospedeiras
Palavras-chave em inglês:
Nutrient cycles
Ecosystem
Insect-plant relationships
Predation (Biology)
Trophic relationships
Área de concentração: Ecologia
Titulação: Doutora em Ecologia
Banca examinadora:
Gustavo Quevedo Romero [Orientador]
André Victor Lucci Freitas
Fernando Rodrigues da Silva
Alexander Vicente Christianini
Sebastian Felipe Sendoya Echeverry
Data de defesa: 29-01-2016
Programa de Pós-Graduação: Ecologia

Campinas, 29 de janeiro de 2016.

Banca examinadora:

Prof. Dr. Gustavo Quevedo Romero

Prof. Dr. Andre Victor Lucci Freitas

Prof. Dr. Fernando Rodrigues da Silva

Prof. Dr. Alexander Vicente Christianini

Prof. Dr. Sebastian Felipe Sendoya Echeverry

A Ata da Defesa com as respectivas assinaturas dos membros da banca encontra-se no processo de vida acadêmica do aluno.

À minha tia Fátima Filomena da Gonçalves (in memoriam),

À minha avó Urbana Gonçalves,

Aos meus pais Gilberto Aparecido Gonçalves e Neusa Aparecida Zangirolame,

Aos meus irmãos Ben Hur Gonçalves e Mila Zangirolame Gonçalves.

"O fim é a oportunidade do recomeço"

AGRADECIMENTOS

Primeiramente, eu gostaria de agradecer ao meu orientador Gustavo e co-orientador Paulo por compartilharem comigo, no momento da minha defesa de mestrado, a ideia de que formigas poderiam contribuir para a nutrição de suas plantas hospedeiras, e confiarem a mim a tarefa desta averiguação. Também, gostaria de agradecer a ambos pelo incentivo, ensinamentos e disposição perante nosso trabalho. Em especial ao Gustavo, gostaria de expressar minha mais sincera admiração e agradecimentos pelos 11 anos juntos. Sem dúvida alguma, a construção da minha carreira dependeu da sua orientação.

Aos amigos de laboratório, eu gostaria de dizer que a ciência não é construída de forma solitária. Eles foram essenciais na construção desta tese, sem os quais não haveria alguns dos *insights* aqui apresentados: Paula Omena, Tiago Bernabé, Pablo Antiqueira, Gustavo Migliorini, Thiago Gonçalves-Souza, Alexande Neutzling, Camila Vieira, Fátima Recalde, Sandra Benavides, Bárbara Mamede, Jennifer Tezuka, Tatiane Dias, Adriano Mendonça, Gustavo Cauê e Rafaela Marques.

Gostaria de agradecer aos colaboradores deste trabalho, Dr. Rafael Oliveira por contribuir com minha pesquisa desde o mestrado, sempre disposto, bem-humorado e me deixando "*pra cima*" com o meu trabalho. Agradeço ao apoio dado em cada momento difícil. À Dra. Diane Srivastava que me recebeu na University of British Columbia e em sua casa. Aos professores que aceitaram participar da minha pré-banca e banca, Dra. Helenice Mercier, Dr. Fernando Silva, Dr. Alexander Christianini, Dr. Sebastian Sendoya, Dr. André Freitas, Dra. Simone Vieira e Dr. Rodrigo Cogni. A todos os meus amigos e companheiros de pós-graduação, em especial à Vanessa Villanova. Todos vocês foram fundamentais para meu desenvolvimento como Doutora em Ecologia.

À minha família, aos quais dedico minha tese, e que são responsáveis pela construção da minha personalidade como pessoa e profissional. Meu "papis" Gilberto, minha "mamis" Neusa, minha irmã Mila "Milosinha" e meu irmão Ben Hur "smel". Agradeço minha avó Urbana por cada prece para que eu concluísse meu doutoramento. Dedico minha tese, em especial, à minha tia Fátima Filomena (*in memoriam*), que me deu meu primeiro livro de biologia antes que eu iniciasse a graduação: “Biodiversidade”, EO Wilson (1997). Não sabíamos, mas eu seguiria a carreira na Ecologia e a concluiria trabalhando com formigas, o ponto mais forte das pesquisas de Wilson. Às vezes penso que nem tudo na vida é mera coincidência! Agradeço à minha segunda família, meus amigos fiéis que compartilharam comigo o dia-a-dia em Campinas, todas as minhas alegrias e frustrações, Atila Moretto, Elen Peres, Paula Omena, André Billia e Vinicius Baronni. Sem família, nada somos! Em especial à Paula, a amiga que minha carreira me deu, a amizade começou assim, discreta, porque a Paula é muito discreta, mas ela se mostrou a melhor companheira que alguém pode ter!

Esta tese foi financiada pela Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, Processo 2011/10137-8), pelo CNPq e pela CAPES-DFAIT (Processo BEX 8377/12-0).

ABSTRACT

The most commonly *top-down* effect associated in designing communities and food webs is predation. Predators can consume their prey, but their presence in the environment can alter the morphology, behavior and habitat use by prey. Ecological studies have considered the identity of predators and their body size in determining their roles in ecosystems. Since many animals have complex life cycles in more than one ecosystem, predators that feed on these organisms can cause cross-ecosystem cascade effects. Bromeliaceae are among the most common plants used as a shelter for ants in the Neotropics and also are occupied by numerous terrestrial and aquatic metazoans, which many of them have complex life cycles. Since *Odontomachus hastatus*, *Gnamptogenys moelleri* and *Camponotus crassus* establish their nests in *Vriesea procera* and *Quesnelia arvensis*, they may change the diversity of species in terrestrial and aquatic bromeliad ecosystems through predation. As a result, these ants can cause cross-ecosystem effects and may change ecosystem processes in bromeliads (e.g., nutrient cycling and nutrient availability for plants). In this study, we surveyed in the field and developed greenhouse and field experiments using isotopic and physiological methods to investigate, in the first chapter, how each ant species contributes to the nutrition and development of its host plant through nest debris. In the second chapter, we investigated the effect of *O. hastatus* on the terrestrial and aquatic diversity of metazoans in *V. procera* bromeliads at three different localities of the Atlantic Forest. In the third chapter, we investigated the effect of the three ant species on the aquatic diversity of metazoans, on the detritus processing and nutrient cycling from detritus to bromeliads. Our results demonstrate that ants, especially *O. hastatus*, affected the diversity of aquatic metazoans, and *O. hastatus* contributed more to the nutrition and development its host bromeliads (*Vriesea procera* and *Quesnelia arvensis*) through nest debris. On the other hand, *C. crassus* favored the processing of organic matter and nitrogen flow from detritus to *Q. arvensis* bromeliads through the tank.

RESUMO

O efeito *top-down* mais comumente associado no delineamento de comunidades e de redes tróficas é a predação. Predadores podem consumir suas presas, mas também sua presença no ambiente pode alterar a morfologia, o comportamento e o uso do habitat pelas presas. Estudos ecológicos têm considerado a identidade dos predadores e seu tamanho corporal como relevantes na determinação das suas funções nos ecossistemas. Uma vez que muitos animais tem ciclos de vida em mais de um ecossistema, predadores que se alimentam destes organismos podem causar cascatas tróficas inter-ecossistemas. Plantas da família Bromeliaceae estão entre as mais utilizadas como abrigo para formigas na região Neotropical e, possuindo tanque, essas plantas são ocupadas por inúmeros organismos terrestres e aquáticos, muitos dos quais possuem ciclos de vida complexos e fazem conexões inter-ecossistemas. Uma vez que a formiga *Odontomachus hastatus* estabelece seus ninhos nas raízes das bromélias *Vriesea procera* e *Quesnelia arvensis*, enquanto as formigas *Gnamptogenys moelleri* e *Camponotus crassus* estabelecem seus ninhos nas folhas dessas bromélias-tanque, estas espécies de formigas podem alterar a diversidade de metazoários nos ecossistemas terrestre e aquático das bromélias por meio da predação. Como consequência, essas formigas podem causar cascatas tróficas inter-ecossistemas e interferir em processos ecossistêmicos nas bromélias (e.g., ciclagem de nutrientes e sua disponibilidade para as plantas). No presente estudo, fizemos coletas em campo e desenvolvemos experimentos em casa de vegetação e em campo utilizando métodos isotópicos e fisiológicos para averiguar, no primeiro capítulo, como a identidade de cada uma das espécies de formigas contribuiu para a nutrição e desenvolvimento de suas plantas hospedeiras por meio dos rejeitos dos ninhos. No segundo capítulo, investigamos o efeito da formiga *O. hastatus* sobre a diversidade de metazoários aquáticos e terrestres presentes nas bromélias *V. procera* em três diferentes localidades da Mata Atlântica. No terceiro capítulo, investigamos o efeito das três espécies de formiga sobre a diversidade aquática de metazoários, sobre o processamento dos detritos no tanque das bromélias e a ciclagem de nutrientes dos detritos para as bromélias. Nossos resultados demostram que as formigas, especialmente *O. hastatus*, afetaram a diversidade de metazoários aquáticos, e alteraram apenas a composição de metazoários terrestres. *Odontomachus hastatus* foi a espécie que mais contribuiu para a nutrição e desenvolvimento das bromélias por meio dos detritos dos ninhos presentes nas raízes, enquanto *C. crassus* favoreceu o processamento da matéria orgânica e o fluxo de nitrogênio dos detritos para as bromélias via tanque.

SUMÁRIO

INTRODUÇÃO GERAL

Estudos ecológicos têm se focado cada vez mais em como espécies podem exercer efeitos *top-down* e *bottom-up* sobre outros organismos com os quais interagem, sendo responsáveis pelo delineamento de comunidades ecológicas e alteração do funcionamento dos ecossistemas (Power 1990; Loreau *et al.* 2001; McIntosh *et al.* 2005). O efeito *top-down* mais comumente associado no delineamento de comunidades e de redes tróficas é a predação (Power 1992; Schmitz *et al.* 2000; Preisser *et al.* 2005). Por outro lado, comunidades inteiras podem ser moldadas por efeitos *bottom- up* quando, em sistemas autotróficos ou em cadeias tróficas cuja base são detritos foliares, a densidade de plantas controla os níveis tróficos superiores (Rosemond *et al.* 2001).

Predadores podem exercer efeitos letais sobre suas presas, alterando numericamente a abundância das mesmas, enquanto exercem efeitos não letais por meio de suas características (e.g., comportamento, odor, formato do corpo). Ambos efeitos podem alterar a morfologia, o comportamento e o uso do habitat pelas presas e podem se propagar para níveis tróficos inferiores por meio de cascatas tróficas (Pace *et al.* 1999; Werner & Peacor 2003; Hill & Weissburg 2013). Em sua meta-análise, Preisser *et al.* (2005) demostraram que ambos efeitos letais e não-letais exercem impacto semelhante sobre as presas, mas devido as cascatas tróficas, os efeitos não-letais foram responsáveis por 85% das mudanças na teia alimentar. As cascatas tróficas originadas por efeitos *top-down* podem surtir inúmeros resultados nas populações, comunidades e ecossistemas como, por exemplo, redução de populações de polinizadores, aumento da performance das plantas quando predadores reduzem populações de herbívoros, fornecimento de rejeitos que favorecem a nutrição de plantas, e alteram o funcionamento ecossistêmico como a decomposição e a ciclagem de nutrientes (Treseder *et al.* 1995; Schmitz 2003; Romero *et al.* 2006; Ngai & Srivastava 2006; Schmitz 2008; Hammill *et al.* 2015).

Normalmente encontra-se mais de um predador nas comunidades. A interação entre predadores pode afetar as comunidades e o comportamento tanto dos predadores quanto das presas (Sih *et al.* 1998; Griswold & Lounibos 2006; Huxel 2007), devido a atuação de cada predador na comunidade, sinergismo de suas ações ou devido à predação intra-guilda (Polis *et al.* 1989; Finke & Denno 2004; Schmitz 2007). Tanto os predadores quanto as presas podem modificar seu comportamento na presença de uma espécie adicional, modificando a magnitude e a direção das interações intra e interespecíficas (Griswold & Lounibos 2006). Adicionalmente, a interação entre os predadores é relevante para identificar os efeitos causados nas teias alimentares, em cascatas tróficas, na estrutura de comunidades e em processos ecossistêmicos (Griswold & Lounibos 2006; Schmitz 2006, 2008).

A identidade dos predadores e seu tamanho corporal, muitas vezes associado à sua ontogenia, têm sido considerados os fatores mais importantes na determinação das suas diversidades funcionais dentro dos ecossistemas (Werner and Gilliam 1984, Rudolf et al. 2014). Entretanto, ainda pouco se sabe como a identidade de predadores e seu tamanho corporal podem afetar, por meio de efeitos letais e não-letais, os organismos dos níveis tróficos inferiores. A identidade de predadores pode ditar seu modo de caça, enquanto seu tamanho corporal pode revelar a taxa de consumo de presas, uma vez que é esperado que predadores maiores sejam capazes de consumir mais presas (Werner & Gilliam 1984; Henry *et al.* 2010). Ainda, predadores com maior tamanho corporal podem se alimentar preferivelmente de presas maiores para satisfazer suas elevadas demandas metabolicas quando comparados com predadores menores (Emmerson & Raffaelli 2004). Assim, espera-se que predadores com maior tamanho corporal exerçam efeitos de maior magnitude sobre suas presas, com consequências significativas no funcionamento ecossistêmico (e.g., decomposição e ciclagem de nutrientes).

Efeito de predadores que cruza as fronteiras dos ecossistemas

Outro fator relevante no delineamento da dinâmica e estrutura de comunidades é a ligação entre diferentes ecossistemas por meio do fluxo de organismos entre eles (Polis *et al.* 1997). Estima-se que mais de 80% dos animais têm ciclos de vida em mais de um ecossistema, como, por exemplo, insetos holometábolos, anfíbios e invertebrados marinhos (Werner 1988). Estas espécies conectam ecossistemas, o que pode interferir na dinâmica e estrutura de comunidades (Polis *et al.* 1997), especialmente se predadores se alimentarem destes organismos. Uma vez que predadores podem se alimentar de organismos com ciclos de vida complexos, eles podem promover cascatas tróficas inter-ecossistemas, ampliando a interferência de um ecossistema em outro (Knight *et al.* 2005; Romero & Srivastava 2010). Desta forma, os predadores afetariam a abundância de organismos em um dos ecossistemas e teriam efeito reverberante no outro (Knight *et al.* 2005; Schmitz *et al.* 2010).

Por exemplo, Knight *et al.* 2005 mostraram que peixes reduzem a abundância de larvas aquáticas de libélulas e, consequentemente, a população adulta deste inseto, diminuindo a predação das libélulas sobre polinizadores terrestres (e.g., abelhas), aumentando a polinização e o sucesso reprodutivo das plantas terrestres próximas aos lagos com peixes. Em outro exemplo, Romero & Srivastava (2010) demonstraram que aranhas em bromélias reduzem a abundância de invertebrados terrestres com ciclo de vida complexo e indiretamente aumentam a abundância de invertebrados com ciclo de vida exclusivamente aquático (e.g., ostracoda), uma vez que muitos invertebrados terrestres podem ser predadores durante sua fase larval no ambiente aquático. Portanto, predadores terrestres que habitam as fronteiras dos ecossistemas podem interceptar presas que emergem do ecossistema aquático ou podem se alimentar e/ou inibir fêmeas adultas que visitam o ecossistema aquático para oviposição (Romero & Srivastava 2010). Enquanto a maior parte dos trabalhos ecológicos ressaltam a importância dos predadores em seus próprios ecossistemas, pouco se sabe sobre a magnitude dos efeitos dos predadores dentro e além dos limites dos seus ecossistemas.

Efeito de predadores no funcionamento ecossistêmico

Estudos ecológicos têm se focado cada vez mais na ligação entre o padrão de distribuição e abundância das espécies (i.e., biodiversidade) e a ciclagem biogeoquímica e fornecimento de recursos para os organismos (i.e., funções ecossistêmicas) (Chapin *et al.* 1997; Loreau *et al.* 2001). Os predadores podem alterar a produção de matéria orgânica (i.e., detritos) que nutrem os sistemas se eles exercerem efeitos indiretos na produtividade primária a partir da redução de populações de herbívoros (Paine 2002; Schmitz 2006). Os detritos muitas vezes são a principal fonte de nutrientes e energia em ecossistemas terrestres e aquáticos, mas também podem desempenhar estas funções em reservatórios naturais de água, como bromélias tanque (Cummins 1974; Wallace *et al.* 1999; Ngai & Srivastava 2006; González *et al.* 2014). Zonas de deposição de detritos em ambientes aquáticos tem o papel de alimentar as comunidades aquáticas, mas também fornecem refúgios para macro-invertebrados reduzirem o risco de predação (Woodward & Hildrew 2001; McIntosh *et al.* 2005). Além de interferir indiretamente na produção de detritos, os predadores podem aumentar ou reduzir direta ou indiretamente o processamento desta matéria orgânica e a ciclagem de seus nutrientes se exercerem efeitos letais e/ou não letais sobre detritívoros cuja função é o processamento dos detritos (Malmqvist 1993; Konishi et al. 2001; Ngai & Srivastava 2006). Neste sentido, se predadores podem alterar teias tróficas, ocasionar cascatas tróficas em um ecossistema ou até mesmo inter-ecossistemas, eles também podem alterar processos ecossistêmicos não somente em seus ecossistemas, mas nos ecossistemas adjacentes.

Interações entre predadores e plantas

Inúmeros predadores interagem com plantas e estas interações podem ser benéficas e/ou prejudiciais para pelo menos um dos participantes. A presença de predadores sobre as plantas pode prejudicar sua aptidão quando estes afastam ou se alimentam de polinizadores (Suttle 2003; Dukas 2005; Gonçalves-Souza *et al.* 2008), enquanto podem beneficiá-las quando capturam herbívoros, como mastigadores, sugadores e endófagos (Rico-Gray & Oliveira 2007; Romero *et al.* 2008a). Outra forma de benefício ocorre quando predadores deixam seus rejeitos nas plantas e estes detritos se tornam uma fonte de nutrientes em plantas capazes de absorvê-los. Este evento foi observado entre os hemípteros *Pameridae* (Miridae) e sua planta hospedeira *Roridula* (Roridulaceae) (Ellis & Midgley 1996; Anderson & Midgley 2002, 2003), entre anuros e bromélias (Inselsbacher *et al.* 2007; Romero *et al.* 2010), entre aranhas e bromélias (Romero *et al.* 2006, 2008b; Gonçalves *et al.* 2011), e formigas e diversas espécies de plantas (Treseder *et al.* 1995; Sagers *et al.* 2000; Fischer *et al.* 2003; Solano & Dejean 2004).

Associações simbióticas entre formigas e plantas são muito comuns e envolvem duas categorias, aquela em que o papel das formigas é nutricional e aquela em que é defensivo (Huxley 1980). Estas plantas podem ter órgãos modificados em cavidades (i.e., domáceas) ocupadas pelas formigas, como folhas, hipocótilos e rizomas (Huxley 1980; Dejean *et al.* 1995). A análise de isótopos estáveis (i.e., $\delta^{13}C$, $\delta\ ^{15}N$) tem demonstrado que, enquanto as formigas ocupam as domáceas, seus rejeitos podem ser absorvidos pelas plantas (Treseder *et al.* 1995; Sagers *et al.* 2000; Fischer *et al.* 2003; Solano & Dejean 2004), como no caso de *Dischidia major* (Asclepiadaceae) que obtém 39% do carbono e 29% do nitrogênio necessário para seu desenvolvimento a partir da interação com a formiga *Philidris* (Dolichoderinae) em florestas tropicais da Malásia (Treseder *et al.* 1995). No mutualismo defensivo, as formigas forrageiam nas plantas, que em muitos casos fornecem recursos alimentares (e.g., açúcares, lipídeos ou proteínas), enquanto as formigas atacam herbívoros ou removem plantas competidoras ou fungos que entram em contato com a planta hospedeira (Janzen 1966; Huxley 1980; Sagers *et al.* 2000; Rosumek *et al.* 2009).

Plantas da família Bromeliaceae estão entre as mais utilizadas como abrigo por formigas na região Neotropical (Dejean *et al.* 1995; Blüthgen *et al.* 2000). Poucas espécies de bromélias são mirmecófitas (Huxley 1980; Benzing 1990), i.e., possuem estruturas morfológicas modificadas nas quais as formigas podem formar seus ninhos (Janzen 1966; Huxley 1980), estabelecendo relações espécie-específicas (Blüthgen *et al.* 2000). Bromélias tanque (i.e., com fitotelmata) também são muito utilizadas por colônias de formigas (Dejean *et al.* 1995; DaRocha *et al.* 2015), mas estes animais ocorrem principalmente nas bromélias que apresentam características intermediárias entre fitotelmata e mirmecófitas (Dejean & Olmsted 1997), ocorrendo nas cavidades interfoliares que não acumulam água (Benzing 1990).

Em áreas de restinga da Mata Atlântica, as bromélias *Vriesea procera* (Tillandsioideae) e *Quesnelia arvensis* (Bromelioideae) são colonizadas pelas formigas *Odontomachus hastatus* (Ponerinae), *Gnamptogenys moelleri* (Ectatomminae) e *Camponotus crassus* (Formicinae) (Cogni & Oliveira 2004; Oliveira *et al.* 2011; Camargo & Oliveira 2012; Rodrigues & Oliveira 2014). *Vriesea procera* e *Q. arvensis* são bromélias tanque que obtém nutrientes não somente pelas raízes, mas também pelos tricomas foliares (i.e., estruturas epidérmicas especializadas em absorver água e nutrientes), os quais são capazes de absorver compostos nitrogenados complexos (e.g., aminoácidos) (Benzing & Burt 1970; Benzing 2000). Dentre as três espécies de formigas, *O. hastatus* apresenta maior tamanho e biomassa (≈ 1,3 cm; 7,89 ± 1,57 mg, média ± EP) e seus ninhos encontram-se principalmente entre as raízes das bromélias (Oliveira *et al.* 2011, Rodrigues & Oliveira 2014). *Gnamptogenys moelleri* tem aproximadamente 0,5 cm de comprimento e 1,15 ± 0,32 mg de biomassa e seus ninhos são encontrados especialmente entre as folhas das bromélias que não acumulam água (Lattke 1995, Cogni & Oliveira 2004). Ambas espécies têm ferrão , são consideradas predadoras e caçam artrópodes para compor a maior parte de suas dietas (Cogni & Oliveira 2004; Camargo & Oliveira 2012). *Camponotus crassus* (≈ 0,5 cm de comprimento e 1,04 ± 0,09 mg de biomassa) estabelece seus ninhos nas folhas das bromélias e se alimenta em nectários extraflorais, exsudatos de homópteros e frutos, mas também pode caçar artrópodes para complementar sua necessidade nutricional por proteínas (Oliveira & Freitas 2004, Sendoya *et al.* 2009). Como animais predadores que estabelecem suas colônias próximas às fronteiras dos ecossistemas aquático e terrestre das bromélias, estas espécies de formigas podem afetar as comunidades de metazoários com as quais elas interagem, podendo, por meio de cascatas tróficas, alterar os processos ecossistêmicos tanto do ecossistema terrestre quanto do aquático.

As bromélias tanque são ocupadas por inúmeros organismos, como microorganismos, invertebrados e vertebrados (Benzing 2000). Estas plantas agem como um habitat aquático em uma matriz terrestre (Romero & Srivastava 2010) e podem ser ocupadas por organismos que possuem ciclos de vida complexos que se movimentam entre os ecossistemas. As bromélias tanque retêm água das chuvas e detritos que são utilizados como fonte de nutrientes para as cadeias tróficas nelas presentes, assim como para as próprias bromélias, por meio dos seus tricomas foliares (Ngai & Srivastava 2006; Romero & Srivastava 2010; Romero *et al.* 2010). Por exemplo, aranhas predadoras que ocorrem em bromélias do Cerrado deixam seus rejeitos (i.e., carcaças de presas, fezes, restos de teia) nas plantas e este material é uma importante fonte de nutrientes que favorece o desenvolvimento das bromélias (Romero *et al.* 2006; Gonçalves *et al.* 2011). Uma vez que as formigas *O. hastatus, G. moelleri* e *C. crassus* estabelecem seus ninhos nas raízes (i.e., *O. hastatus*) ou nas folhas (i.e., *G. moelleri* e *C. crassus*) das bromélias, os rejeitos dos ninhos podem favorecer a nutrição de suas bromélias hospedeiras, permitindo que as plantas tenham maior desenvolvimento.

Nesta tese apresentamos os efeitos das formigas *O. hastatus*, *G. moelleri* e *C. crassus* sobre as comunidades de metazoários presentes nas bromélias e também o efeito das formigas para a nutrição e desenvolvimento de suas plantas hospedeiras. Especificamente, avaliamos os efeitos e a magnitude dos efeitos das formigas sobre a diversidade de metazoários terrestres e aquáticos, e seus efeitos sobre o fornecimento de nutrientes para suas plantas hospedeiras por meio do tanque (via processamento dos detritos e/ou rejeitos dos ninhos presentes nas folhas), ou por meio dos rejeitos dos ninhos presentes nas raízes.

Hipóteses e objetivos dos capítulos

Capítulo I. Uma vez que *O. hastatus*, *G. moelleri* e *C. crassus* capturam presas, estas formigas podem coletar organismos na floresta e concentrar as carcaças das presas em suas colônias nas raízes (e.g., *O. hastatus*) ou nas folhas (e.g., *G. moelleri* and *C. crassus*) das bromélias, translocando nutrientes de um ambiente da floresta para outro e fornecendo nutrientes para as plantas. Neste capítulo apresentamos resultados experimentais com o objetivo de avaliar os efeitos da identidade de predadores (i.e., intensidade de predação e localização dos ninhos) na nutrição e desenvolvimento de suas bromélias hospedeiras. Assumindo que as três espécies de formigas apresentam ninhos com números semelhantes de indivíduos, nossa hipótese é que as formigas *O. hastatus* e *G. moelleri* contribuirão mais para a nutrição e crescimento de *Q. arvensis*, uma vez que estas formigas são predadoras e espera-se que elas concentrem mais carcaças de presas em seus ninhos, fornecendo mais nutrientes para as bromélias. Por outro lado, esperamos que *C. crassus* contribua menos para a nutrição e crescimento das bromélias, uma vez que sua dieta é composta de poucas presas.

Capítulo II. A formiga predadora *O. hastatus* pode reduzir a abundância de metazoários terrestres com efeitos em cascata no ecossistema aquático, mas também pode afetar as comunidades aquáticas quando se alimenta ou inibe fêmeas adultas que visitam as bromélias para oviposição. Em campo, nós amostramos bromélias *V. procera* com e sem ninhos de *O. hastatus* e identificamos os metazoários dos ecossistemas aquático e terrestre das bromélias, a fim de averiguar se *O. hastatus* altera a composição de organismos nos ecossistemas bromelícolas, alterando a composição de grupos funcionais aquáticos e de guildas terrestres.

Capítulo III. Uma vez que as três espécies de formigas podem se alimentar ou inibir a oviposição de fêmeas no ecossistema aquático bromelícola, e que *O. hastatus* apresenta o maior tamanho corporal e, portanto, espera-se que consuma mais presas, nossa hipótese é que *O. hastatus* exercerá maior impacto nas comunidades bromelícolas, podendo ocorrer efeitos em cascata que alterem as funções ecossistêmicas no tanque das bromélias (e.g., ciclagem de nutrientes). Por outro lado, nós esperamos que *G. moelleri* e *C. crassus*, que necessitam de menor quantidade de presas para sua nutrição, tenham menor efeito na diversidade aquática, não alterando a decomposição da matéria orgânica ou a ciclagem de nutrientes no ecossistema aquático das bromélias.

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CAPÍTULO I

Gonçalves AZ, Oliveira RS, Oliveira PS, Romero GQ

Species-specific effects of ant inhabitants on bromeliad nutrition

Plos ONE (Artigo aceito; doi: 10.1371/journal.pone.0152113)

Research paper

Species-specific effects of ant inhabitants on bromeliad nutrition

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Abstract

Predator activities may lead to the accumulation of nutrients in specific areas of terrestrial habitats where they dispose of prey carcasses. In their feeding sites, predators may increase nutrients availability in the soil and favor plant nutrition and growth. However, the translocation of nutrients from one habitat to another may depend on predator identity and diet, as well as on the amount of prey intake. Here we used isotopic (^{15}N) and physiological methods in greenhouse experiments to evaluate the effects of the identity of predatory ants (i.e., the consumption of prey and nest sites) on the nutrition and growth of the bromeliad *Quesnelia arvensis*. We showed that predatory ants with protein-based nutrition (i.e., *Odontomachus hastatus*, *Gnamptogenys moelleri*) improved the performance of their host bromeliads (i.e., increased foliar N, production of soluble proteins and growth). On the other hand, the contribution of *Camponotus crassus* for the nutritional status of bromeliads did not differ from bromeliads without ants, possibly because this ant does not have arthropod prey as a preferred food source. Our results show, for the first time, that predatory ants can translocate nutrients from one habitat to another within forests, accumulating nutrients in their feeding sites that become available to bromeliads. Additionally, we highlight that ant contribution to plant nutrition may depend on predator identity and its dietary requirements. Nest debris may be especially important for epiphytic and terrestrial bromeliads in nutrient-poor environments.

Key words: *Odontomachus*, *Gnamptogenys*, *Camponotus*, *Quesnelia*, animal-plant interaction, nitrogen flux, mineral nutrition, nutrient-poor environments

Introduction

Ecological research has increasingly focused on the links between species interactions and ecosystem functioning [1, 2]. Predators have a significant influence on ecosystems by affecting prey populations, and controlling the dynamics of nutrients, either by cascading effects through herbivore-plant-decomposers or directly through their excreta and activity of nutrient translocation [2]. As predators capture prey and transport them to their feeding sites, they may redistribute nutrients between habitats [3, 4]. Nutrient translocation by predators has been observed in riparian zones by bears that release marine and freshwater nutrients through deposition of salmon carcasses, raising ammonium and nitrate concentrations in the soil [3]. Similarly, seabirds and sea turtles feeding on marine organisms may excrete nutrients or leave egg remains in their breeding nests and nourish nearby soils [5, 6]. In addition, crows that feed in urban areas transfer nutrients to their roosts in adjacent forests [7]. These translocated nutrients may become available for plants and impact their performance and community diversity and structure [4].

The identity of predators and their hunting and feeding modes play a key functional role within communities and can determine the amount of nutrients that are translocated to soils [2, 8]. However, little is known about how predator identity and hunting mode can affect the nutrition of plants. In the absence of predators, some plants are able to capture or shelter a diversity of species that are not used by them as a direct source of nutrients. For example, the carnivorous plant *Roridula* captures prey but lack digestive enzymes, and depends on the activity of predatory hemipterans for prey digestion and release of nutrients [9]. Similarly, Romero *et al.* [10, 11] showed that while predatory spiders and frogs provide nutrients to their host bromeliads, entire prey carcasses contribute less to plant nutrition. Predatory ants may also contribute to plant nutrition, and many plants have modified hollow structures (i.e., domatia) that provide shelter to ant colonies, establishing symbiotic relationships [12-16]. Because ants have hunting sites often distinct from their feeding sites (i.e., colonies), ants can translocate nutrients from one habitat to another, similarly to bears, seabirds, sea turtles and the crows.

Symbiotic associations between plants and ants have been described for many species. Some plants are myrmecophytes (i.e., specialized ant-plants) that have modified hollow cavities (i.e., domatia) that house ant colonies in an obligate association, while other plants are myrmecophilous in a non-specialized association with ants [13-19]. In both cases, nest debris with food remains and ant feces are stored in contact with plant tissue, and nutrients can be obtained by plants [13-16]; ant-assisted plant nutrition is called myrmecotrophy. In the Neotropics, bromeliads are among the most used plants by ants [17, 18]. Some myrmecophytic bromeliads have bulb-shaped leaves that house ant colonies (e.g., *Brocchinia acuminata*, *Tillandsia bulbosa*, *T. butzii*), while others have ant nests constructed among roots or in inter-foliar cavities that do not accumulate water [18-21]. Tank bromeliads shelter many aquatic and terrestrial organisms, from microorganisms to vertebrates and retain leaves from the forest canopy [22, 23]. Therefore, tank bromeliads can be considered a complete ecosystem with debris, decomposers, detritivores, omnivores and predators [1]. Because they possess foliar trichomes capable of absorbing water and nutrients, bromeliads can obtain nutrients from the trophic network they shelter [10, 11, 22, 24]. Many ants that occupy bromeliads are predators that forage in the vicinity of their host plants and bring arthropod prey into their colonies. Thus, nest debris can be a source of nutrients for bromeliads, as Leroy *et al.* [15, 16] suggested by comparing leaf nitrogen isotopes between bromeliads with and without ant colonies.

In the nutrient-poor soils of coastal Atlantic Forests [25], the bromeliad *Quesnelia arvensis* hosts colonies of *Odontomachus hastatus*, *Gnamptogenys moelleri*, *Camponotus crassus* and two other ant species [26]. Among the ants that interact with this plant, nests of *O. hastatus* are located mainly among bromeliad roots [21, 28]. On the other hand, *G. moelleri* and *C. crassus* have their nests among bromeliad leaves [29, 30, 32, 33, AZG personal observations]. While *O. hastatus* and

G. moelleri have a predatory habit and feed mainly on arthropods, *C. crassus* feeds mostly on extrafloral nectaries, homopteran exudates, and fruits, but may also hunt for arthropods to supplement its diet [30-33]. As *O. hastatus*, *G. moelleri* and *C. crassus* capture and bring prey into their nests among bromeliad roots (*O. hastatus*) or bromeliad leaves (*G. moelleri* and *C. crassus*), they may translocate nutrients from one environment to another and improve plant nutrition, as shown for other predators [3-7]. Here we report the results of an experiment designed to evaluate the effects of the identity of ants and their diet (i.e., the consumption of prey and nest sites) on the nutrition and development of their host bromeliads. Assuming that these three ant species have nests with similar number of ants (see Methods), we hypothesized that *O. hastatus* and *G. moelleri* will contribute more to the nutrition and growth of *Q. arvensis*, since they have a predatory habit and their diet is based mainly on arthropods. On the other hand, we expect that *C. crassus* will contribute less to the nutrition and growth of host bromeliads because its diet is based on fewer prey items.

Methods

Ethics Statement

This study was conducted according to relevant national and international guidelines. Permit number 12.429/2011 for Ana Z. Gonçalves, issued by the *Secretaria do Meio Ambiente* and *Instituto Florestal*, in accordance with the *Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA)* and *Ministério do Meio Ambiente (ICMBio MMA)*.

Organisms and field sampling

Colonies of *Odontomachus hastatus*, *Gnamptogenys moelleri* and *Camponotus crassus* were collected in the restinga forest of the Parque Estadual da Ilha do Cardoso (Cardoso Island), São Paulo State, Brazil (25°04’ S, 47°55’ W). The understory of the restinga is covered mostly by *Quesnelia arvensis*, whereas *Vriesea procera* is the most common epiphytic species [21, 23]. Nests of *O. hastatus* are frequently constructed in *V. procera*, but *Q. arvensis* can also host colonies [21]. *Quesnelia arvensis* is a tank bromeliad that obtains nutrient not only by its roots, but also by foliar trichomes that are capable of absorbing nitrogen compounds (e.g., amino acids) [22, 27]. Among the ants that interact with *Q. arvensis, O. hastatus* has the greatest size (≈ 1.3 cm) and biomass (mean ± SE, 7.89 ± 1.57 mg) and their nests are abundant (ca. 33 colonies. ha^{-1}) among bromeliad roots [21, 28, 31]. This nocturnal ant species is arboreal and occurs from Central to South America [35]; its nests are rarely found on the ground [31, 36]. Rodrigues and Oliveira [28] showed that *O. hastatus* workers can move more than 8 m away from their nests, but nearly half of the foraging activity occurs within 3 m around the nest. *Gnamptogenys moelleri* has ≈ 0.5 cm in length, 1.15 ± 0.32 mg in biomass, occurs in Neotropical plains, its nests can be found on the ground and in terrestrial bromeliads among 1-3 leaves, and workers forage almost exclusively on their host plants [29, 30]. *Camponotus crassus* (≈ 0.5 cm in length, 1.04 ± 0.09 mg in biomass) nests among bromeliad leaves and feeds mostly on extrafloral nectaries, homopteran exudates and fruits, and can suppress herbivores from plants that have extrafloral nectaries [32, 33, 37, AZG personal observations]. On Cardoso Island, nests of this ant are found among 2-3 leaves of *Q. arvensis*, where there is no water accumulation (AZG personal observations).

The number of ants per colony did not differ among treatments of the experiment described below (One-way ANOVA, P = 0.261; mean ± SE, 129.3 ± 7.6 ants *O. hastatus* colonies; 141.7 ± 4.7 ants in *G. moelleri* colonies; and 149.0 ± 11.4 ants in *C. crassus* colonies). However, the nest biomass differed among species (One-way ANOVA, $P < 0.001$; mean ± SE, 1020.1 ± 60.1 mg of *O. hastatus*; 162.9 ± 5.5 mg of *G. moelleri*; and 154.9 ± 11.8 mg of *C. crassus*).

Greenhouse experiment

Previously to the experiment, *Tenebrio* larvae (simulating prey of ants) were enriched with ^{15}N stable isotope to quantify the flux of nitrogen from *Tenebrio* to bromeliads. *Tenebrio* were grown in a substrate in the proportion of 100 g of rat chow Labina-Purina®, 100 g cassava flour and 20 mL of a solution of 5 g enriched ammonium sulfate [$(^{15}NH_4)_2SO_4$, 10% excess atoms, Cambridge Isotope Laboratories, MA] per liter of distilled water. This substrate was dried for 24 h at 60 °C before being offered to *Tenebrio*.

In order to test whether ants alter the availability of nutrients to their host bromeliads due to prey intake and nest sites, a two-month (10-Jan-2012 to 18-Mar-2012) greenhouse experiment was carried out at the Department of Plant Biology at Universidade Estadual de Campinas, Brazil. Since *O. hastatus* can be found in *Q. arvensis* [21], this species was chosen for the experiment and was obtained at Veiga Arquitetura e Paisagismo®, CEASA, Campinas, Brazil. All bromeliads were young and had similar biomass and size (e.g., foliar length varying from 25 to 30 cm). Bromeliads were planted in pots (14.5 cm in diameter, 14.5 cm high) with *Pinus* sp. bark (simulating the poor soil of restinga forest; Martins *et al.* 2015) and were watered with limited amounts of water to avoid desiccation through an automatic irrigation system with a capacity of $8L.h^{-1}$, which worked for 10 min every 2 h. Each pot was kept individually in a white plastic tray (40.7 x 60.8 x 9.8 cm) with Tanglefoot® resin around its border to prevent ants leaving their host bromeliads. All pots had four holes at their bases (approximately 1 x 1 cm) to allow ants to access the bromeliad roots. Colonies of *O. hastatus* collected in the field were placed in the plastic trays and quickly sought refuge and entered the holes of the pots in which bromeliads were planted. In about 30 min, all ants entered the pots and carried eggs and pupae to establish the colony within the roots. Colonies of *G. moelleri* and *C. crassus* were manually placed in the rosette of potted bromeliads, protecting eggs and pupae with *Pinus* sp. barks (the same used inside pots among bromeliad roots).

Experimental bromeliads had the following treatments (*n* = 10 each treatment): (1) control; (2) no ants but with *Tenebrio* larvae placed among roots (to test the amount of nitrogen that bromeliads acquire through roots); (3) no ants but with *Tenebrio* placed on leaves (to test the amount of nitrogen derived from insects that fall into the rosettes); (4) with *C. crassus* colony and *Tenebrio* placed on leaves (to test if ants accelerate the acquisition of nitrogen through leaves); (5) with *G. moelleri* colony and *Tenebrio* placed on leaves (to test if ants accelerate the acquisition of nitrogen through leaves); and (6) with *O. hastatus* colony among roots and *Tenebrio* placed on leaves (to test if ants accelerate the acquisition of nitrogen through roots). Two *Tenebrio* larvae were cut into six pieces and were applied every other day on each bromeliad (except on control plants), in the center of the rosette above the tank in treatments *3, 4, 5 and 6*. *Tenebrio* was the only nutrient source for the colonies, and ants were very fast at collecting *Tenebrio* on leaves. *Tenebrio* was placed among roots (treatment *2*) through a PVC pipe (2 cm in diameter) inserted in the root mass. At the end of the experiment, two leaves of the second inner node of each bromeliad rosette were collected for isotope analyses, and three leaves of the fifth inner node of the rosettes were collected for soluble protein analyses.

Analyses of plant protein

Total soluble protein concentration was determined using the colorimetric Bradford assay [38]. Fresh leaves of *Q. arvensis* were cut into small pieces (1 cm^2), and 1 g of these leaves was frozen in liquid nitrogen and homogenized with 3 mL of ultra-pure water. The homogenate was centrifuged at 12.000 rpm (g) for 10 min, and the supernatant (15 µL) was mixed with Comassie Brilliant Blue G-250 dye solution (185 µL), obtained from 100 mg of the dye dissolved in 95% ethanol and 100 mL of 85% phosphoric acid. The absorbance was measured using a spectrophotometer (Ultrospec 3000; Cambridge, England) at 595 nm, and the concentration of protein was determined by plotting the absorbance of the sample *vs.* a standard curve obtained with bovine serum albumin.

Bromeliad growth

To determine the relative contribution of nitrogen derived from *Tenebrio* only or from ant nests on bromeliad growth, two leaves (i.e., fifth inner node of the rosettes) from each bromeliad were randomly chosen and their lengths were measured at the beginning and the end of the experiment. The bromeliad leaf length was directly related to the leaf biomass (Linear regressions: *Q. arvensis*: $r^2 = 0.73$, $P < 0.001$); leaves showed continuous growth during the experiment and their relative growth rate (RGR, Ln(cm)/day) was calculated using the following equation: $RGR = [\ln(L_{final}) - \ln(L_{initial})]/(t_2 - t_1)$. The $\ln(L_{final})$ and $\ln(L_{initial})$ are, respectively, the natural logarithm of the foliar final length and the natural logarithm of the foliar initial length, with $(t_2 - t_1)$ being the number of days between the initial and final measurements.

Isotopic and statistical analyses

The total N concentration (µg mg^{-1} dry leaf tissue) of bromeliad leaves and the $\delta^{15}N$ of *Tenebrio*, ants and bromeliads were determined with an isotope ratio mass spectrometer (20 – 20 mass spectrometer; PDZ Europa, Sandbach, UK) after sample combustion to N_2 at 1000°C by an on-line elemental analyzer (PDZ Europa ANCA-GSL) in the Stable Isotope Facility at the University of

California, Davis. The nitrogen fraction in bromeliads with and without ants that received *Tenebrio* (f_A) were calculated using mixing model equations with two sources of nitrogen (*i.e.*, soil and *Tenebrio*) and one single isotopic signature (*e.g.*, $\delta^{15}N$; see [39]). Since control bromeliads only had *Pinus* sp. bark substratum as a source of nutrients, leaves of these bromeliads were considered the soil end-member in the equation. The fractionation of ^{15}N during its assimilation and metabolic processing in plants [40] was considered in the following equation: $f_A = (\delta_M - \delta_B - \Delta\delta^{15}N)/(\delta_A - \delta_B)$. The f_A is the proportionate contribution of labelled *Tenebrio* absorbed by bromeliads (%), δ_M is the isotope ratio of bromeliads that received *Tenebrio*, δ_A is the isotope ratio of *Tenebrio* while δ_B is the isotope ratio of control bromeliads, and $\Delta\delta^{15}N$ is the trophic shift for nitrogen between *Tenebrio* or control bromeliads and consumer (*e.g.*, bromeliads). The values of $\Delta\delta^{15}N$ used were + 1.4 ± 0.21‰ (mean ± SE) [41]. All the response variables were compared using ANOVA and Tukey HSD *post-hoc* test were used for pair-wise comparisons.

Results

The $\delta^{15}N$ values of enriched *Tenebrio* larvae, of the ants *Odontomachus hastatus*, *Gnamptogenys moelleri* and *Camponotus crassus* that fed on the enriched *Tenebrio* indicate that these materials were enriched during the experiment (Table 1). As expected, *Odontomachus hastatus* contributed more to the nutrition of its host bromeliad, accounting for 19.3 ± 1.8% (mean ± SE) of the total nitrogen of *Quesnelia arvensis* (Tables 1, 2; Fig. 1A). *Gnamptogenys moelleri* contributed 16.1 ± 1.9% of the nitrogen of its host bromeliad, whereas *C. crassus* contributed with 10.7 ± 2.4% (Tables 1, 2; Fig. 1A). In the absence of ants, *Tenebrio* on leaves contributed with 5.3 ± 2.6% of the nitrogen of bromeliads, and *Tenebrio* on roots contributed with 3.9 ± 0.5% (Tables 1, 2; Fig. 1A). Despite differences among treatments in the contribution to bromeliad nutrition, only *O. hastatus* contributed to the increase of total nitrogen and soluble protein concentrations in bromeliad leaves

(Table 2; Fig. 1B, Fig. 2). Bromeliads with *G. moelleri* and *C. crassus* colonies had total nitrogen and soluble protein concentrations similar to bromeliads with prey carcasses or control bromeliads (Table 2; Fig. 1B, Fig. 2). Additionally, *O. hastatus* and *G. moelleri* improved foliar growth of their host plants compared to control bromeliads while *C. crassus* had no effect on bromeliad growth (Table 2; Fig. 3).

Discussion

In this study, we assessed how the identity and diet of ant inhabitants can affect the nutrition of host plants through the translocation of prey carcasses and feces by predators. We found that the ant species with protein as their main food source (i.e., *Odontomachus hastatus*, *Gnamptogenys moelleri*) had a higher contribution to the performance of their host bromeliads (i.e., plant nutrition, production of soluble proteins, and growth). Regardless of the ant species, nitrogen physically stored in the body of prey becomes available to bromeliads.

We demonstrate experimentally that predatory ants play a role on bromeliad nutrition since they concentrate prey carcasses in their feeding sites (i.e., colonies in bromeliads) and release nutrients from prey bodies through nest debris and feces. We also demonstrate that predators that have arthropod prey as their main source of protein contribute more to the bromeliad performance (i.e., *O. hastatus* and *G. moelleri*). Although *G. moelleri* and *O. hastatus* have similar predatory habit [30, 31], the former species showed an intermediate contribution to its host plant nutrition, possibly due to its small biomass (similar to *C. crassus*). These results reinforce that the dietary requirements of each species must be taken into account to evaluate the potential nutrient- enrichment by predators in a community [8].

Tenebrio larvae (simulating prey that fall into the tank of bromeliads) contributed less to plant nutrition than the presence of predatory ants on bromeliads. This result emphasizes the role of ants in processing the organic matter of prey bodies, releasing compounds through the nest debris and their feces, which can be obtained by bromeliad roots (e.g., when plant interact with *O. hastatus*) or by leaf trichomes (e.g., when interacting with *G. moelleri* or *C. crassus*). Other studies also showed that predator-plant interactions could release organic matter from prey bodies retained by plants [10, 43, 44, 45]. In the absence of predators, *Tenebrio* need to be mineralized by microorganisms present in the bromeliad rosettes [24, 46], a process that should be slower than predator activity, which could explain the lower contribution of *Tenebrio* to bromeliad nutrition during the experiment.

The terrestrial bromeliad *Quesnelia arvensis* is very abundant in the sandy nutrient-poor soils of Cardoso Island [23, 25]. Through the interaction with predators, this plant can obtain nutrients from the soil via its roots or leaf trichomes that are in contact with debris and feces from ant nests. In fact, we showed that the interaction between *Q. arvensis* and ants favored plant nutrition and enabled a higher production of soluble protein in its leaves. High soluble protein concentration is associated with the N availability in plants indicating their favorable nutritional status [48], which may have allowed a higher growth of *Q. arvensis* when interacting with ants. In addition, the increase in N concentration in leaves of bromeliads can favor the production of amino acids usually associated with N storage [49], and can be especially important for epiphytes whose roots are not in contact with soil and have intermittent access to water and nutrients. At Cardoso Island, nearly 70% of the *O. hastatus* nests were recorded in the epiphytic *Vriesea procera* [31]. Therefore, it is expected that *V. procera* can benefit even more from interactions with ants than the terrestrial *Q. arvensis*.

The contribution of *O. hastatus* and *G. moelleri* to bromeliads were similar to the N values that the carnivorous plants *Cephalotus*, *Drosera*, *Philcoxia* and *Sarracenia* derived from insect digestion [50-52]. Despite the existence of carnivorous bromeliads (i.e., *Catopsis*, *Brocchinia*), *Q. arvensis* is considered a saprophytic species because it lacks an active way to attract, immobilize and digest prey [53]. The interactions described here represent a by-product digestive mutualism between ants and bromeliads where ants can find a suitable habitat in bromeliads and, in turn, contribute nutritionally to their hosts [10, 11, 43]. Finally, some researchers have argued that ants preferably associate with bromeliads with longer leaves and larger rosettes and root mass [21, 30]. We suggest that the larger size of ant-inhabited bromeliads could be a result of this by-product mutualism, and not necessarily the cause mediating plant colonization by ants.

In conclusion, our results emphasize the effects of predator identity and diet in the translocation and processing of prey nutrients that contribute to plant nutrition. Predatory ants with that feed on prey as their main source of protein contributed more to the nutrition, protein production and growth of their host bromeliads. These results emphasize that ants can play a role in redistributing nutrients between habitats, from different areas of the forest to their feeding sites (i.e., colonies). We highlight the importance of predator activities in concentrating wastes near their feeding sites and their potential to provide nutrients to plants, and we also reinforce that the by- product digestive mutualism can be relevant to plants that occur in oligotrophic environments.

Acknowledgements

We thank Dr. Paula M. de Omena, Renan F. Awata, Wendell Correia, Rodolfo F. Silva and Dr. Gustavo C. Piccoli for help with sampling of ants in the field.

Author Contributions

Conceived and designed the experiment: AZG PSO GQR. Performed the experiment: AZG. Contributed reagents/materials/analysis tools: RSO PSO GQR. Analyzed the data: AZG RSO PSO GQR. Wrote the paper: AZG RSO PSO GQR.

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Table 1. Average δ^{15}N values of natural abundance and enriched *Tenebrio* larvae, *Camponotus crassus*, *Gnamptogenys moelleri* and *Odontomachus hastatus* ants, and *Quesnelia arvensis* leaves receiving the following treatments: control; *Tenebrio* among roots; *Tenebrio* on leaves; *Camponotus crassus* ants and *Tenebrio*; *Gnamptogenys moelleri* and *Tenebrio*; and *Odontomachus hastatus* and *Tenebrio*

	δ^{15}N values (SE)	*n*
Tenebrio		
Natural abundance	3.13 (0.12)	5
Enriched	76156.0 (2940.62)	5
Camponotus crassus		
Natural abundance	0.8 (1.06)	5
Enriched	36713.5 (392.19)	5
Gnamptogenys moelleri		
Natural abundance	2.6 (0.37)	5
Enriched	47087.9 (714.54)	5
Odontomachus hastatus		
Natural abundance	2.4 (0.41)	5
Enriched	55959.6 (1208.23)	5
Control		
Natural abundance	6.33 (0.60)	10
Larvae on roots		
Enriched	3025.0 (371.28)	10
Larvae on leaves		
Enriched	4079.6 (1902.84)	10
Bromeliad with *C. crassus*		
Enriched	8167.8 (1748.73)	10
Bromeliad with *G. moelleri*		
Enriched	12272.8 (1292.63)	10
Bromeliad with *O. hastatus*		
Enriched	14715.3 (1194.74)	10

The standard errors of means are in parenthesis.

n, Number of replicates.

Table 2. Analyses of variance (ANOVA) summarizing the effects of different treatments (control; *Tenebrio* larvae among roots; *Tenebrio* on leaves; *Camponotus crassus* ants and *Tenebrio*; *Gnamptogenys moelleri* and *Tenebrio*; and *Odontomachus hastatus* and *Tenebrio*) on the % of N derived from *Tenebrio*, the total N and soluble protein concentrations, and relative growth rate of *Quesnelia arvensis* leaves. Significance of $P < 0.05$ is highlighted in bold

Source of variation	d.f.	MS	*F*	*P*
% N derived from *Tenebrio*				
Treatments	4	441.80	10.84	**<0.001**
Error	45	40.77		
Total N concentration				
Treatments	5	65.38	8.91	**<0.001**
Error	54	7.33		
Soluble protein concentration				
Treatments	5	8007.70	48.04	**<0.001**
Error	54	167.70		
Relative growth rate				
Treatments	5	0.01	5.46	**<0.001**
Error	54	0.01		

Figure legends

Fig. 1. (a) Percentage of nitrogen derived from *Tenebrio* larvae and (b) total nitrogen concentration of *Quesnelia arvensis* leaves of different treatments (control; *Tenebrio* among roots; *Tenebrio* on leaves; *Camponotus crassus* ants and *Tenebrio*; *Gnamptogenys moelleri* and *Tenebrio*; and *Odontomachus hastatus* and *Tenebrio*). Bars indicate the standard error and letters indicate Tukey *post-hoc* test ($\alpha < 0.05$).

Fig. 2. Soluble protein concentration for *Quesnelia arvensis* leaves of different treatments (control; *Tenebrio* larvae among roots; *Tenebrio* on leaves; *Camponotus crassus* ants and *Tenebrio*; *Gnamptogenys moelleri* and *Tenebrio*; and *Odontomachus hastatus* and *Tenebrio*). Bars indicate the standard error and letters indicate Tukey *post-hoc* test ($\alpha < 0.05$).

Fig. 3. Relative growth rate of the *Quesnelia arvensis* leaves from different treatments (control; *Tenebrio* larvae among roots; *Tenebrio* on leaves; *Camponotus crassus* ants and *Tenebrio*; *Gnamptogenys moelleri* and *Tenebrio*; and *Odontomachus hastatus* and *Tenebrio*). Bars indicate the standard error and letters indicate Tukey *post-hoc* test ($\alpha < 0.05$).

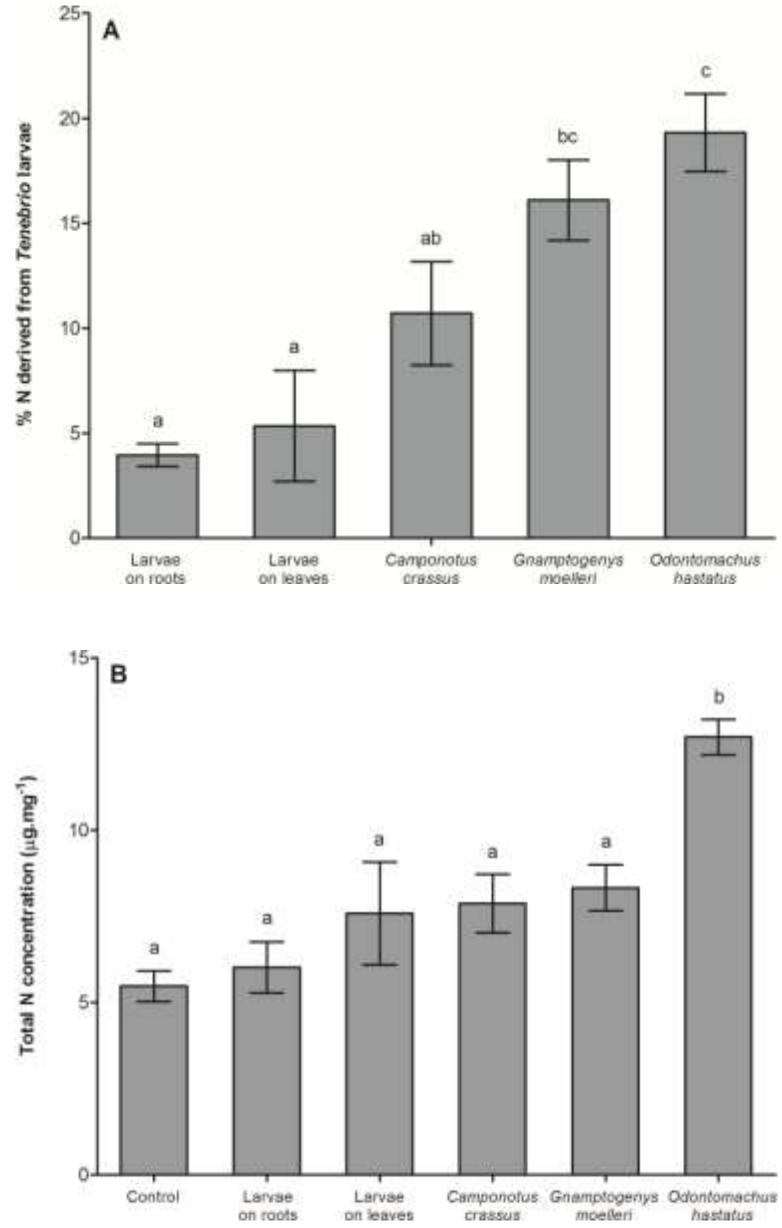
A
% N derived from *Tenebrio* larvae
0
5
10
15
20
25
a
a
ab
bc
c
Larvae on roots
Larvae on leaves
Camponotus crassus
Gnamptogenys moelleri
Odontomachus hastatus
B
Total N concentration (μg.mg⁻¹)
0
5
10
15
a
a
a
a
a
b
Control
Larvae on roots
Larvae on leaves
Camponotus crassus
Gnamptogenys moelleri
Odontomachus hastatus

Figure 1.

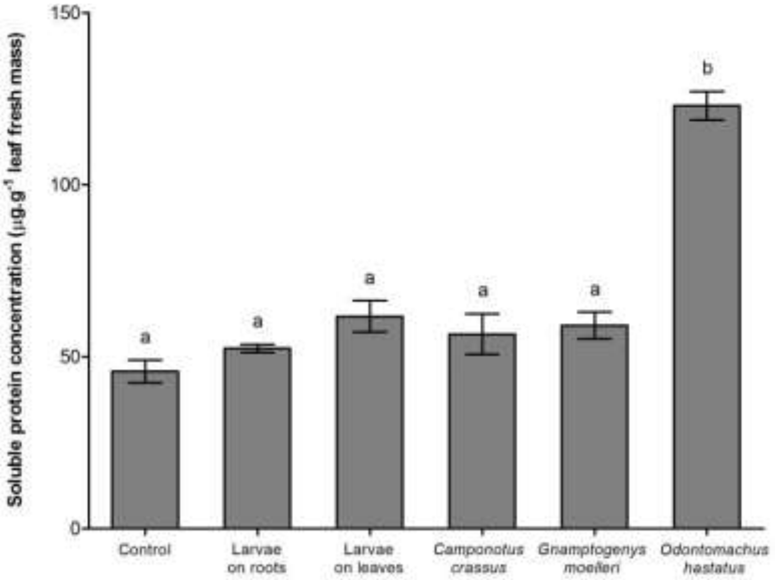
Soluble protein concentration (μg.g⁻¹ leaf fresh mass)
150
100
50
0
a
a
a
a
a
b
Control
Larvae on roots
Larvae on leaves
Camponotus crassus
Gnamptogenys moelleri
Odontomachus hastatus

Figure 2.

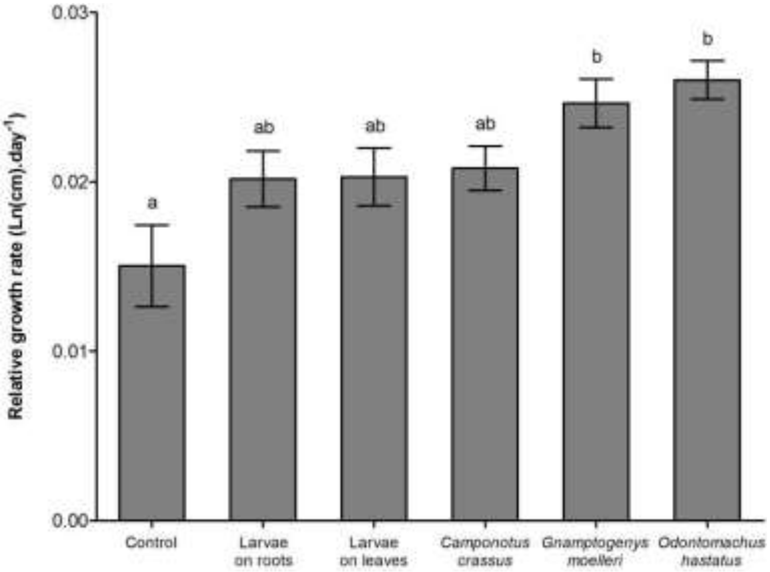
0.03
0.02
0.01
0.00
Relative growth rate (Ln(cm).day-1)
a
ab
ab
ab
b
b
Control
Larvae on roots
Larvae on leaves
Camponotus crassus
Gnamptogenys moelleri
Odontomachus hastatus

Figure 3.

CAPÍTULO II

Gonçalves AZ, Srivastava DS, Oliveira PS, Romero GQ

Predatory ants decrease aquatic and terrestrial diversity in a detritus-based food web

Predatory ants decrease aquatic and terrestrial diversity in a detritus-based food web

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Summary

1. Predation is one of the most fundamental ecological processes in designing biotic communities. Terrestrial predators that live in ecosystem boundaries may alter the diversity of terrestrial organisms through consumptive or non-consumptive effects, but they may also have a cross-ecosystem cascading effects when they feed on organisms with complex life cycles (i.e., organisms that shift from aquatic juvenile stages to terrestrial adult stages) or inhibit female oviposition in the aquatic environment.

2. The predatory ant *Odontomachus hastatus* establishes its nests among roots of the epiphytic bromeliad *Vriesea procera* that possesses a tank and shelters many terrestrial and aquatic organisms. Since this predatory ant occurs in bromeliad ecosystem boundaries, we compared its effects and effect sizes on terrestrial and aquatic diversity.

3. We surveyed bromeliads with and without *O. hastatus* colonies from three different locations in the Atlantic Forest (Picinguaba, Juréia and Ilha do Cardoso, southeast Brazil) and compared the density, richness and composition of terrestrial and aquatic metazoans found in these bromeliads.

4. *Odontomachus hastatus* reduced the density, richness and altered composition of aquatic organisms, affecting especially collectors of detritus and predators that reach bromeliads through phoresy in the skin of terrestrial animals. Moreover, our study reveal that the effect size of this predatory ant was greater on the aquatic ecosystem and *O. hastatus* reduced the density of other ant species and terrestrial detritivores in bromeliads.

5. Our results suggest that the cross-ecosystem effect of this predator was stronger than its within-ecosystem effect. As *O. hastatus* reduced the occurrence of terrestrial detritivores and aquatic collectors of detritus, we suggest that this ant can decrease the processing of

organic matter on this detritus-based food web. Future studies may reveal whether predatory ants can reduce nutrient cycling because of its effect on detritivores.

Key-words: *Odontomachus hastatus*, effect size, within-ecosystem, cross-ecosystem, phoresy, decomposition, ecosystem functioning

Introduction

Ecological research has focused on how top-down and bottom-up effects structure ecological communities (Power 1990; McIntosh *et al.* 2005). The more common top-down effect described in the literature is associated with higher trophic levels consuming lower levels (Power 1992; Preisser, Bolnick & Bernard 2005). On the other hand, bottom-up effects occur in autotrophic systems in which plant densities control the higher trophic levels, or in detritus-based food webs wherein input of exogenous debris control the entire food chain (Rosemond *et al.* 2001). This allochthonous detritus is the main source of energy in stream food webs (Cummins 1974; Wallace *et al.* 1999) and water reservoirs as tank-bromeliads (Ngai & Srivastava 2006; González, Romero & Srivastava 2014). Detritus depositional zones in aquatic environments have the role of feeding the community of aquatic macroinvertebrates, but also provide refuges in which macroinvertebrates can reduce predation risk (Woodward & Hildrew 2001; McIntosh *et al.* 2005). However, the role of predators and their top-down effects are still poorly known in detritus-based food webs, especially whether terrestrial predators exert direct effects on the aquatic food chains.

Predators can have lethal and non-lethal effects on their prey. Lethal effects alter the numerical abundance of prey, but also can propagate downward through trophic cascades (density- mediated interactions, DMI, Hill & Weissburg 2013). Moreover, non-consumptive effects of predators alter prey behavior, morphology and habitat use as an antipredator response that also can affect the base of chains (trait-mediated interactions, TMI, Werner & Peacor 2003). In a meta- analysis, Preisser *et al.* (2005) showed that DMI and TMI exert similar direct impact on prey, but when considered the cascading effects of predators, TMI were responsible for 85% of changes in the food chain. The trophic cascade originated from predators may enhance plant diversity as predators suppress herbivores and dominant plant species used by prey as a refuge (Schmitz 2003), but subsequently trophic cascade may enhance or reduce ecosystem functions (Ngai & Srivastava 2006; Schmitz 2008; Atwood *et al.* 2013; Hammill, Atwood & Srivastava 2015).

The effects of predators may cross ecosystem boundaries. Whereas many animals have life cycles in more than one ecosystem (e.g., holometabolous insects, amphibians and marine invertebrates; Werner 1988), predators that feed on organisms with complex life cycles can promote trophic cascades across ecosystems (Knight *et al.* 2005). For example, Knight *et al.* 2005 showed that fish reduced the abundance of aquatic dragonfly larvae and consequently the adults of this insect, reducing dragonfly predation on terrestrial pollinators (e.g., bees) and increasing pollination and plant fitness near fishponds. One may expect that predatory fish, in the mentioned example, have a larger effect size on the aquatic trophic chains instead of on terrestrial ecosystem. However, to the best of our knowledge, there is no literature comparing the effect sizes of predators between aquatic and terrestrial ecosystems where terrestrial predators structure terrestrial communities with trophic cascades to the aquatic ecosystem.

Tank-bromeliads are suitable systems to study how communities are structured and how terrestrial and aquatic metazoans cross ecosystem boundaries and interact (Srivastava *et al.* 2004). The arrangement of their leaves in a rosette create a complex aquatic detrital-based aquatic ecosystem biologically realistic (Ngai & Srivastava 2006). Tank-bromeliads shelter bacteria, algae, protists, terrestrial and aquatic invertebrates and vertebrates (Benzing 2000; Hammill *et al.* 2015). Many ant species in the Neotropics use bromeliads as shelters and nests, and the interaction between these two groups can be even species-specific (Huxley 1980; Dejean, Olmsted & Snelling 1995; Blüthgen *et al.* 2000). While bromeliads can provide shelter for ant colonies, ants can change the bromeliad morphology and can improve their nutrition and development through nest debris (Céréghino *et al.* 2010, 2011; Leroy *et al.* 2013; see Chapter 1). In the Atlantic Forest, the predatory ant *Odontomachus hastatus* establishes its colonies mainly in the roots of epiphytic tank-bromeliads, and workers can forage up to eight meters from the nests, but most workers forage near their host bromeliads (Oliveira, Camargo & Fourcassié 2011; Rodrigues & Oliveira 2014). Whereas the diet of *O. hastatus* is basically composed by arthropods (e.g., Diptera, Lepidoptera, other ants, spiders; Camargo & Oliveira 2012), and some of these organisms may connect the terrestrial and aquatic bromeliad ecosystems, *O. hastatus* may structure both communities.

In the field, we sampled bromeliads with and without *O. hastatus* colonies and we identified all metazoans that were in bromeliad ecosystems. *Odontomachus hastatus* can reduce the abundance of terrestrial metazoans in bromeliads (i.e., DMI) with cascading effects on the aquatic ecosystem, but can also affect the aquatic communities feeding on or inhibiting the oviposition of adult winged insects in bromeliad tanks (i.e., TMI). Here, we hypothesize that ants will change the composition of organisms in bromeliad ecosystems, altering the composition of functional groups and terrestrial guilds. Specifically, we are interested to know whether (1) ants will reduce the diversity in bromeliad communities, (2) change the composition of bromeliad inhabitants, and (3) the effect size of ants will be higher in terrestrial than in aquatic environment.

Materials and methods

Study sites and organisms

We collected the epiphytic bromeliad *Vriesea procera* (Tillandsioideae, Bromeliaceae) with and without colonies of *Odontomachus hastatus* (Ponerinae, Formicidae) in the restinga sandy- forest, a domain of the Atlantic Forest, Brazil, at three distinct areas from north to south of the coast of São Paulo State: Parque Estadual da Serra do Mar, núcleo Picinguaba (Picinguaba, 23° 20’ S, 44° 46’ W), Estação Ecológica Juréia-Itatins (Juréia, 24° 25’ S, 47° 06’ W), and Parque Estadual da Ilha do Cardoso (Ilha do Cardoso, 25° 04’ S, 47° 55’ W). Ilha do Cardoso is marked by two seasons, a cold and less humid season (April to August, average temperature: 13 °C, average rainfall: 500 mm), and a hot and humid season (September to March, average temperature: 32 °C, average rainfall: 1800 mm). Ilha do Cardoso sandy forest has an open canopy formed by trees of 5-15 m tall, whose understory is covered mainly by *Quesnelia arvensis* (Bromeliaceae), while the most commonly epiphytic species is *V. procera* (Romero & Srivastava 2010; Oliveira *et al.* 2011). Picinguaba climate is considered rainy-tropical, humid throughout the year. Temperatures from September to March remain on average 24 °C and rainfall is 4000 mm on average, and the period from April to August has an average of 20 °C and rainfall of 1500 mm on average (INPE/CPTEC 2015). Picinguaba sandy forest has flooded soil in the rainy season, and the forest canopy does not reach 20 m. The understory has terrestrial and epiphytic bromeliads without a predominant species (AZG personal observations). Juréia climate is classified as tropical hot and humid all the year, with temperatures exceeding 18 °C, and does not have a cold and dry season (September to March: 4700 mm on average of rainfall, and from April to August: 1300 mm on average of rainfall, INPE/CPTEC 2015). Juréia has a 5-18 m tall canopy and has several species of bromeliads in the understory, with few epiphytic bromeliads, of which most are *V. procera* (AZG personal observations).

Vriesea procera is a tank bromeliad (i.e., with phytotelmata) that can shelter numerous aquatic and terrestrial invertebrates and vertebrates. Many aquatic invertebrates of distinct functional groups are associated with tank bromeliads, including collectors, detrital shredders, detrital scrapers, filter feeders and predators (see Table S1). In addition, bromeliads shelter numerous terrestrial organisms of different guilds, as detritivores, phytophages, omnivores and predators (see Table S2). Among vertebrates, anurans can use bromeliads temporarily or may depend on these plants for their survival and reproduction (Silva *et al.* 1989).

The *Odontomachus hastatus* is arboreal, occurs from Central to South America, has twilight and nocturnal habits, and build their nests especially in the roots of epiphytic bromeliads (Kempf 1972, Gibernau *et al.* 2007, Oliveira *et al.* 2011, Rodrigues and Oliveira 2014). It is a large ant species (≈ 1.3 cm in size and 7.89 ± 1.57 mg of biomass, mean ± SE), has sting, predatory and scavenger habits (Camargo & Oliveira 2012).

Field sampling

In order to investigate the effect of *O. hastatus* on the density (number of individuals/mL of tank volume), richness (number of metazoans/mL of tank volume) and composition of aquatic and terrestrial metazoans in epiphytic bromeliads, ten bromeliads with and ten bromeliads without *O. hastatus* colonies were collected in December 2011 at Picinguaba and Ilha do Cardoso, and in December 2013 at Juréia. Bromeliads and their roots were carefully inspected for the presence or absence of *O. hastatus* nests before sampling. Once a bromeliad with ant nest was found, another bromeliad without nest was chosen within one meter in order to avoid environmental effects on the diversity of metazoans. All bromeliads were collected from 1-1.5 m height from the ground.

The effect size (%) of ants on the density and richness of aquatic and terrestrial metazoans was obtained by the mean reduction of individuals in bromeliads with ants compared to bromeliads without ants. All bromeliads in each location were collected on the same day and it did not rain on the day or the day before of field samplings. Bromeliads with similar aquatic tank sizes were collected (aquatic maximum volume, V_{TA}, Picinguaba: 481.4 ± 1.8 ml; Juréia: 490.1 ± 20.4 ml; Ilha do Cardoso: 497.1 ± 10.9 ml; mean ± SE) in order to avoid the influence of the tank volume on the number of metazoans interacting with bromeliads. The volume of the terrestrial bromeliad ecosystem (V_{TE}) was calculated from measurements of the largest leaf of each bromeliad (T) and the diameter of the rosettes (half of this measurement was considered the radius, r) and from triangle formula (to obtain the height of bromeliads, h) and cone volume; triangle $T^2 = h^2 + r^2$, and then the total volume (V_{TO}) of bromeliads (considering the terrestrial and aquatic ecosystems using the cone volume formula): $V_{TO} = (\pi r^2 h)/3$. Later, with the V_{TO}, it was obtained the volume of the terrestrial ecosystem of bromeliads (V_{TE}): $V_{TE} = V_{TO} - V_{TA}$. The terrestrial maximum volume (V_{TE}) were Picinguaba: 1142.7 ± 9.5 ml; Juréia: 1099.9 ± 20.4 ml; Ilha do Cardoso: 1092.8 ± 10.9 ml.

All bromeliads were dissected and washed with distilled water in the field and debris and the tank water were collected in white trays and separated by two sieves (mesh diameter, 125 and 800 µm.) Each metazoan visible to the naked eye (> 0.5 mm) was preserved in 70% ethanol and had it abundance and morphospecies recorded. In laboratory, they were identified to the lowest taxonomic level through literature and direct observations (Merritt & Cummins 1996, Triplehorn & Johnson 2005). Aquatic metazoans were separated into the following functional groups: (1) collectors, (2) shredders, (3) scrapers (4) filter feeders, and (5) predators, while the terrestrial metazoans were separated in guilds as follow: (1) detritivores (2) phytophages, (3) omnivores and (4) predators (see Tables S1, S2).

Statistical analyses

The response variables of density, richness and abundance of functional groups or guilds of aquatic and terrestrial metazoans were compared using Analyses of Variance (ANOVA) and Tukey HSD *post-hoc* test for pair-wise comparisons between bromeliads with and without ants. The composition of aquatic and terrestrial organisms in bromeliads was obtained by Permutation Multivariate Analyses of Variance (PERMANOVA), with the “adonis” function of the vegan package of R (Oksanen et al. 2013), with dissimilarity calculated as Bray-Curtis distances and 9999 permutations. Nonmetric Multidimensional Scaling Plot (NMDS) was performed to visualize similarities or dissimilarities. All statistical analyses were conducted with the statistical platform R (R Core Team, 2014).

Results

The presence of *Odontomachus hastatus* reduced the density of aquatic metazoans in *Vriesea procera* tanks but did not change the density of terrestrial organisms (Table 1, Fig. 1). The ants reduced the richness of the aquatic organisms at Picinguaba and Juréia, but did not alter the richness of aquatic metazoans in the bromeliad tank at Ilha do Cardoso or the richness of terrestrial metazoans (Table 1, Fig. 2). Additionally, the mean effect size of *O. hastatus* on the density of aquatic metazoans were more than 50% at the three localities, while ant effect sizes on the richness of aquatic metazoans were less than 50% at Picinguaba and Juréia (Fig. 1, 2).

Odontomachus hastatus altered the composition of aquatic and terrestrial metazoans in bromeliads at Picinguaba and Juréia, but only affected the composition of aquatic metazoans at Ilha do Cardoso (Table 2, Fig. 3). Specifically, the ants reduced the density of aquatic collectors at the three localities and the density of aquatic predators at Ilha do Cardoso (Table S3, Fig. S1). Additionally, ants reduced the density of terrestrial omnivores at Picinguaba and Juréia, and terrestrial detritivores at Juréia (Table S3, Fig. S1). The organisms most affected by ants were Ostracoda, *Polypedilum* sp., Hirudinea, Muscidae, Isopoda and other species of ants (Table S1, S2).

Discussion

Our results indicate that the predatory ant *Odontomachus hastatus* exert a top-down effect on the detritus-based food webs of the bromeliad *Vriesea procera*, as it reduces the diversity of species in bromeliad ecosystems. We showed that part of the aquatic metazoans affected (i.e., Ostracoda, Hirudinea) were those described to move among bromeliads by phoresy in the skin of terrestrial animals. These results highlight that predatory ants may consume prey and inhibit visitation o terrestrial animals in bromeliads, but the cascading effect through ecosystems boundaries is stronger than the ant effect on the terrestrial ecosystem.

Odontomachus hastatus reduced the density, richness and altered the composition of terrestrial and aquatic species in its host bromeliads. In fact, no species had an increase in its density in bromeliads with ants. This ant is known as a trap-jaw predator that uses its fast jaw to subdue especially adult dipterans, lepidopterans, spiders and other ants (Spagna *et al.* 2008; Camargo & Oliveira 2012). Foragers of *O. hastatus* depart from nests at sunset and capture especially alive canopy-dwelling prey usually in the tree that host its nest bromeliad (Camargo & Oliveira 2012; Rodrigues & Oliveira 2014). However, most foragers remain near their colonies, where they can find their preferred prey since these organisms are the most frequent inhabitants of bromeliads (Richardson 1999; Gonçalves-Souza *et al.* 2010). Whereas ants of this genus have a good vision (Oliveira & Hölldobler 1989), *O. hastatus* would be able to capture even flying prey or inhibit terrestrial winged female to oviposit in bromeliads, what may explain the lower occurrence of bromeliad inhabitants in plants with ants.

Many studies highlight connections of the aquatic and terrestrial ecosystems by flows of organisms, energy and nutrients and most of them reveal how allochthonous subsidies connect terrestrial and aquatic environments (Roth *et al.* 2007; Bartels *et al.* 2012). Nonetheless, few studies have reported the link between ecosystems by predator's effect even with top-down effects being transferred to more trophic levels (Borer *et al.* 2008). For example, Knight *et al.* (2005) showed the effect of aquatic predators on aquatic larvae affecting terrestrial plants while Romero & Srivastava (2010) suggested that predatory spiders reduce the abundance and richness of aquatic insect larvae in bromeliads through consumptive and non-consumptive effects over flying insects. Here, our results suggest that the consumptive and non-consumptive effects of *O. hastatus* in the terrestrial metazoans are less strong than its cross-ecosystem direct and indirect effects on the aquatic organisms. For instance, some of the most affected organisms by ants were Ostracoda and Hirudinea, which have an exclusively aquatic life cycle. These two groups of organisms are described by dispersing among freshwater habitats through phoretic hosts like anurans and reptiles (Lopez, Rodrigues & Rios 1999; Tiberti & Gentilli 2010; Sabagh & Rocha 2014). As the foraging period of *O. hastatus* is predominantly nocturnal like anurans are, and this ant species is not able to reach the bromeliad tanks to catch aquatic prey, we suggest that ants are inhibiting the visitation of frogs in bromeliads, possible hosts of Ostracoda and Hirudinea.

The most affected aquatic functional groups by *O. hastatus* were collectors (i.e., Ostracoda and *Polypedilum* sp.) and predators (i.e., Hirudinea and Muscidae), while the terrestrial guilds were omnivores (i.e., other species of ants) and detritivores (i.e., Isopoda). Despite the *Polypedilum* larvae occur in the water tank, some leaf axils accumulate a greater amount of debris and moisture that both larvae and ants are able to access. In fact, we observed during field samplings *O. hastatus* carrying on its jaw *Polypedilum* larvae from these full-detritus leaf axils, and we believe that the same event might occur with Muscidae larvae. Collectors sweep tiny debris with their mouthparts that was previously processed by other species of aquatic larvae and terrestrial detritivores (Paradise & Dunson 1998; Paradise & Kuhn 1999). With fewer collectors, bromeliads with *O. hastatus* may have an increment of debris deposition in their tanks. Furthermore, *O. hastatus* possibly reduced the abundance of other ants and isopods through consumptive effects as these animals are in the terrestrial ecosystem of bromeliads and ants are among the preferred prey of *O. hastatus* (Camargo & Oliveira 2012). Regarding isopods, the consequences of their consumption by predatory ants may be conspicuous in bromeliad ecosystem. Terrestrial isopods are described to increase the decomposition of litter and the activity of bacteria and fungi in the processing of organic matter thereby increasing the rate at which nutrients can be incorporated into the soil (Hassall, Turner & Rands 1987; Hättenschwiler & Bretscher 2001; Zimmer 2002; Bastow 2011). Therefore, the reduction of isopod density might reduce the canopy litter decomposition in bromeliad tank, reducing the activity of microorganisms and decreasing the available nutrients to the whole food web and to bromeliads, since these plants can be favored by microorganism activities on their leaf surfaces (Inselsbacher *et al.* 2007; Gonçalves *et al.* 2014). In a long timeframe, we expect that the net effect of *O. hastatus* over nutrient cycling in bromeliad tank may be negative; however, this ant species seems to contribute to bromeliad nutrition through the roots (see Chapter 1).

In conclusion, our results emphasize that *O. hastatus* decrease the diversity of terrestrial and aquatic organisms in food webs of *Vriesea procera*, and its effect size seems to be greater in the aquatic environment. We suggest that the effect of a predator near ecosystem boundaries may be strong across its ecosystem instead of within-ecosystem. However, future studies are needed to compare these largely unexplored effects. We also showed that *O. hastatus* might have a direct consumptive effect on other ant species and Isopoda, a consumptive and/or a trait-indirect effect on flying adults inhibiting oviposition in bromeliads and a trait-indirect effect on frogs inhibiting phoresy of Hirudinea and Ostracoda. Consequently, we suggest that this predatory ant can reduce the decomposition of debris in *V. procera* tanks, reducing the most important source of nutrient and energy to bromeliad food webs. However, future studies are needed to show the effects of predatory ants on the ecosystem functioning of detritus-based food webs.

Acknowledgments

We thank Dr. Paula Omena, Bárbara Mamede, Jennifer Tezuka, Adriano Mendonça, Renan Awata, Wendell Correia, Rodolfo F. Silva and Gustavo C. Piccoli for help with field samplings. This study was supported by the FAPESP scholarship to A.Z. Gonçalves [2011/10137-8], and by FAPESP and CNPq research grants to G.Q. Romero and P.S. Oliveira.

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Table 1. Analyses of variance (ANOVA) summarizing the effects of the presence of *Odontomachus hastatus* ants in the density and richness of aquatic and terrestrial metazoans of *Vriesea procera* bromeliads at Parque Estadual da Serra do Mar (Picinguaba), Estação Ecológica Juréia-Itatins and Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil. The (*) symbol means statistical interaction between ants and aquatic/terrestrial metazoans

Source of variation	Ants			Aquatic/Terrestrial metazoans			Ants* Aquatic/Terrestrial		
	df	F	P	df	F	P	df	F	P
Picinguaba									
Density	1	24.10	**<0.001**	1	23.75	**<0.001**	1	5.86	**0.020**
Richness	1	6.44	**0.015**	1	20.07	**<0.001**	1	1.95	0.170
Juréia									
Density	1	37.33	**<0.001**	1	81.69	**<0.001**	1	22.64	**<0.001**
Richness	1	17.25	**<0.001**	1	72.78	**<0.001**	1	3.63	0.064
Ilha do Cardoso									
Density	1	6.96	**0.013**	1	60.25	**<0.001**	1	8.70	**0.006**
Richness	1	0.05	0.823	1	117.25	**<0.001**	1	0.57	0.456

Table 2. Permutation Multivariate Analyses of Variance (PERMANOVA) summarizing the effects of the presence of *Odontomachus hastatus* ants in the composition of aquatic and terrestrial metazoans of *Vriesea procera* bromeliads at Parque Estadual da Serra do Mar (Picinguaba), Estação Ecológica Juréia-Itatins and Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil

Source of variation	df	MS	*F*	*R^2*	*P*
Picinguaba					
Aquatic metazoans					
Ants	1	1.02	3.02	0.14	**<0.001**
Residuals	18	0.34		0.85	
Total	19			1.00	
Terrestrial metazoans					
Ants	1	0.66	2.42	0.12	**0.005**
Residuals	17	0.27		0.87	
Total	18			1.00	
Juréia					
Aquatic metazoans					
Ants	1	0.83	4.32	0.19	**<0.001**
Residuals	18	0.19		0.80	
Total	19			1.00	
Terrestrial metazoans					
Ants	1	1.33	7.04	0.28	**<0.001**
Residuals	18	0.18		0.71	
Total	19			1.00	
Ilha do Cardoso					
Aquatic metazoans					
Ants	1	0.48	1.79	0.10	**0.035**
Residuals	16	0.26		0.89	
Total	17			1.00	
Terrestrial metazoans					
Ants	1	0.54	2.13	0.15	0.067
Residuals	12	0.25		0.84	
Total	13			1.00	

Figure legends

Fig 1. Density of aquatic and terrestrial metazoans (n°/mL) in the tank of *Vriesea procera* with and without *Odontomachus hastatus* nests at (A) Parque Estadual da Serra do Mar (Picinguaba), (B) Estação Ecológica Juréia-Itatins and (C) Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil. Bars indicate SE and asterisks (*) indicate statistical differences between treatments (ANOVA/Tukey HSD *post-hoc* test, α = 0.05). Percentage values (%) indicate differences between the mean densities of metazoans in bromeliads without ants compared to bromeliads with ants.

Fig 2. Richness of aquatic and terrestrial metazoans per volume (mL) of the tank of *Vriesea procera* with and without *Odontomachus hastatus* nests at (A) Parque Estadual da Serra do Mar (Picinguaba), (B) Estação Ecológica Juréia-Itatins and (C) Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil. Bars indicate SE and asterisks (*) indicate statistical differences between treatments (ANOVA/Tukey HSD *post-hoc* test, α = 0.05). Percentage values (%) indicate differences between the mean richness of metazoans in bromeliads without ants compared to bromeliads with ants.

Fig 3. Nonmetric multidimensional scaling plot (NMDS) of the composition of metazoans at Parque Estadual da Serra do Mar (Picinguaba) (A) aquatic and (B) terrestrial, at Estação Ecológica Juréia-Itatins (C) aquatic and (D) terrestrial, and Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil (E) aquatic and (F) terrestrial in *Vriesea procera* with and without *Odontomachus hastatus* nests.

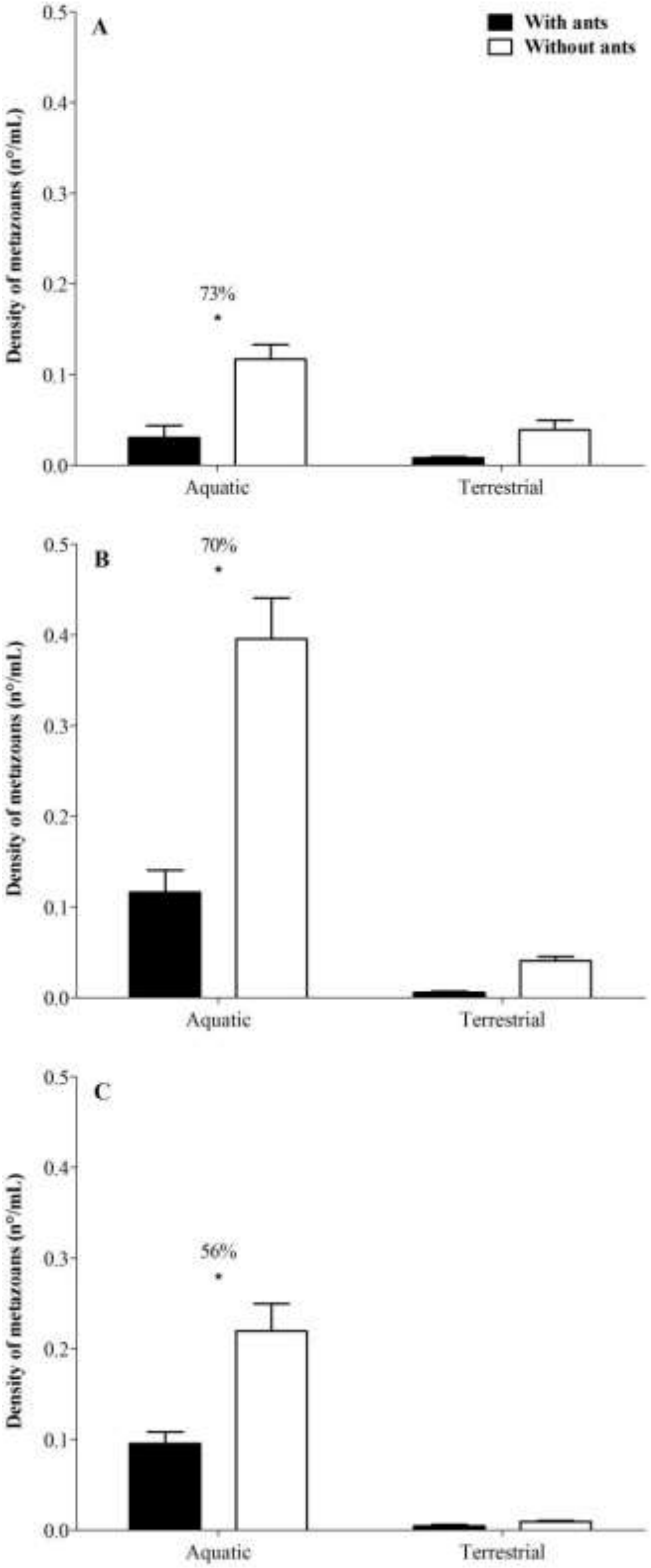
A
■ With ants
□ Without ants
Density of metazoans (n°/mL)
0.5
0.4
0.3
0.2
0.1
0.0
73%
*
Aquatic
Terrestrial
B
70%
*
Density of metazoans (n°/mL)
0.5
0.4
0.3
0.2
0.1
0.0
Aquatic
Terrestrial
C
Density of metazoans (n°/mL)
0.5
0.4
0.3
0.2
0.1
0.0
56%
*
Aquatic
Terrestrial

Fig. 1.

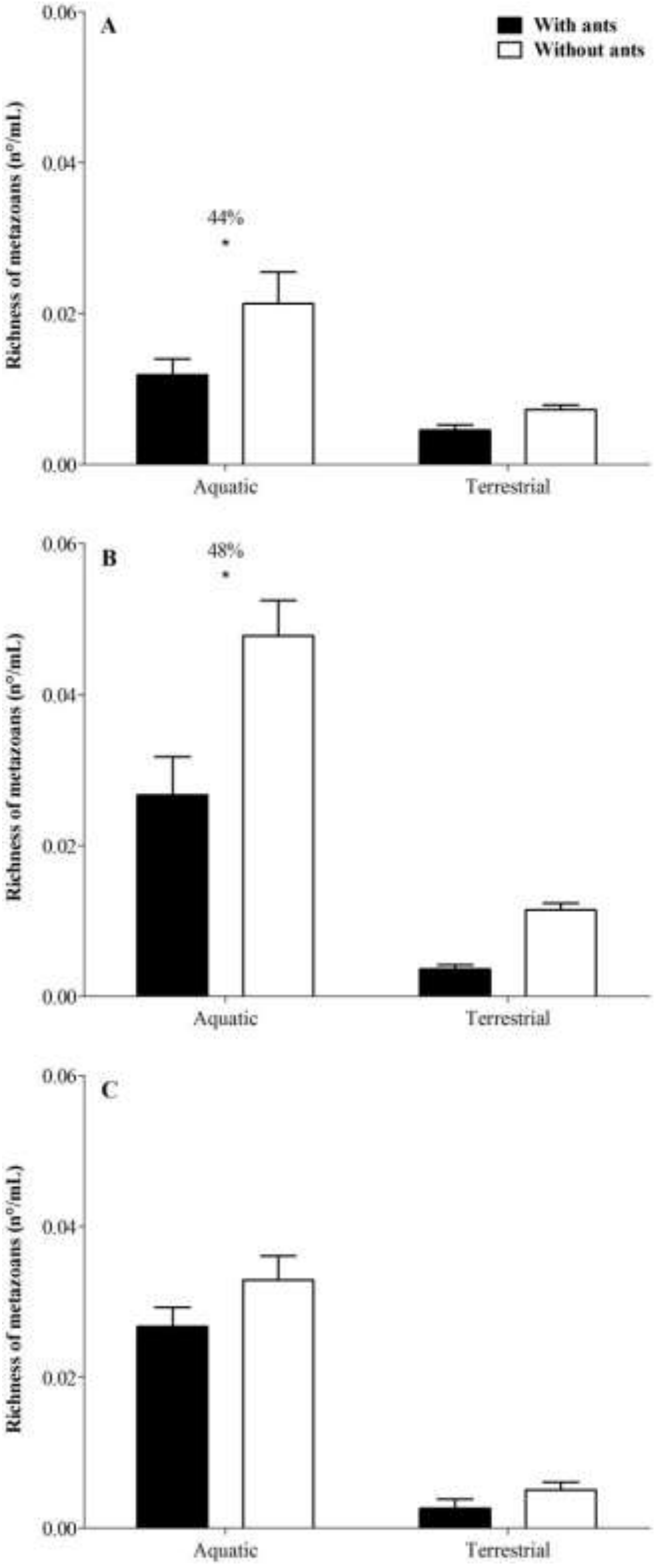
A
B
C
■ With ants
☐ Without ants
Richness of metazoans (n°/mL)
0.06
0.04
0.02
0.00
44%
*
48%
*
Aquatic
Terrestrial

Fig. 2.

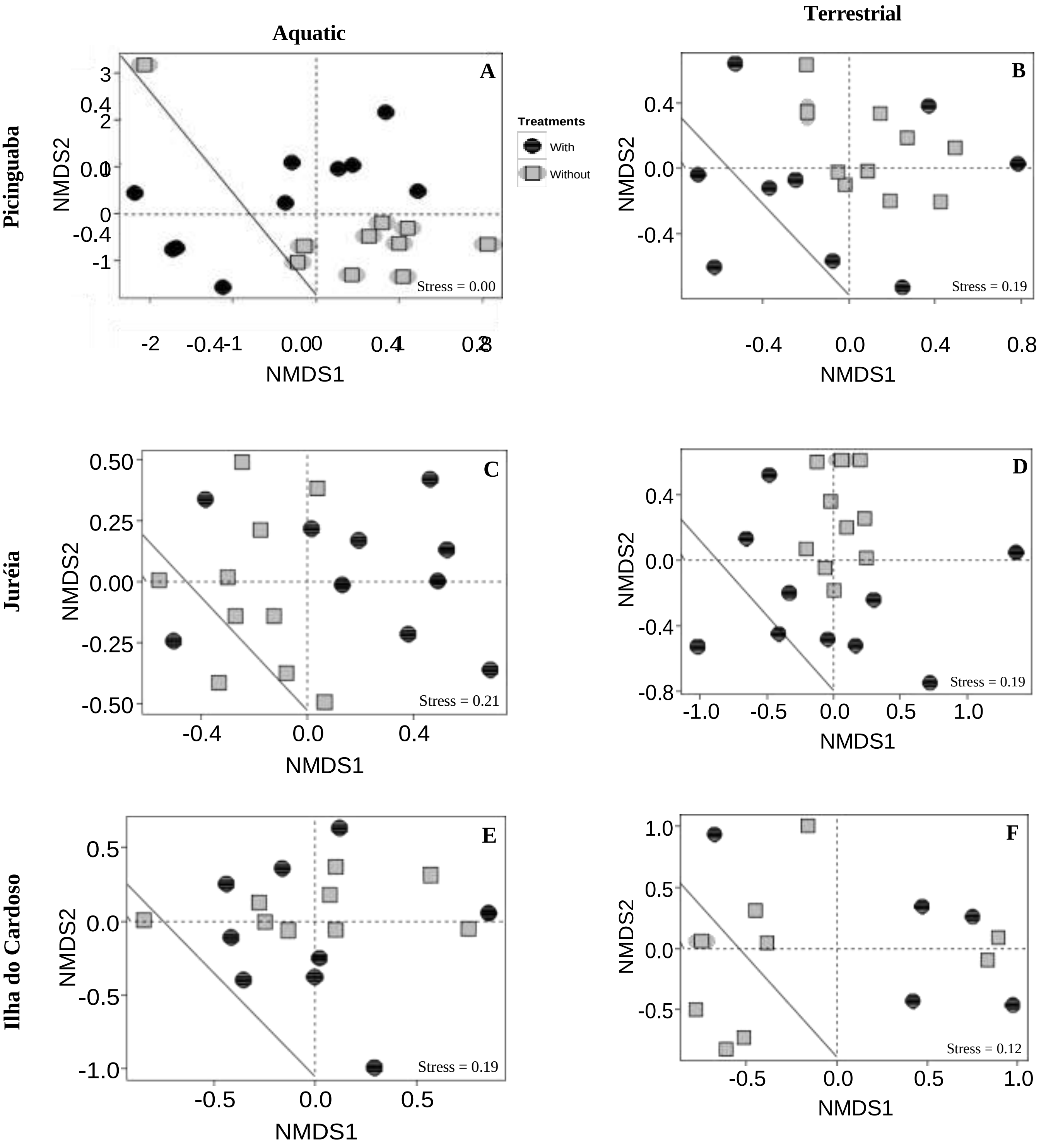
Aquatic
Terrestrial
Picinguaba
Juréia
Ilha do Cardoso
A
B
C
D
E
F
Treatments
With
Without
NMDS1
NMDS2
Stress = 0.00
Stress = 0.19
Stress = 0.21
Stress = 0.19
Stress = 0.19
Stress = 0.12
3
2
1
0
-1
0.4
0.0
-0.4
-2
-1
0
1
2
3
-0.4
0.0
0.4
0.8
0.50
0.25
0.00
-0.25
-0.50
-0.8
-1.0
-0.5
0.5
1.0

Fig. 3.

Full paper

Predatory ants decrease terrestrial and aquatic diversity in a detritus-based food web

Ana Z. Gonçalves, Diane S. Srivastava, Paulo S. Oliveira and Gustavo Q. Romero

Supporting information Table S1. Abundance of aquatic metazoans collected in *Vriesea procera* bromeliads with and without *Odontomachus hastatus* ants at Parque Estadual da Serra do Mar (Picinguaba), Estação Ecológica Juréia-Itatins (Juréia) and Parque Estadual da Ilha do Cardoso (Ilha do Cardoso), São Paulo State, Brazil. Metazoans were identified to the lowest possible taxonomic level, to their classes, orders or families, and were separated in functional groups

Identification	Class, Order, Family	Functional group	Picinguaba	Juréia	Ilha do Cardoso
Anura	Amphibia	Collector	0	7	0
Atrichopogon sp. 1	Ceratopogonidae	Collector	0	0	3
Atrichopogon sp. 2	Ceratopogonidae	Collector	0	0	2
Atrichopogon sp. 3	Ceratopogonidae	Collector	0	0	2
Atrichopogon sp. 4	Ceratopogonidae	Collector	3	0	2
Atrichopogon sp. 5	Ceratopogonidae	Collector	0	5	0
Bezzia sp. 1	Ceratopogonidae	Predator	16	6	8
Bezzia sp.2	Ceratopogonidae	Predator	0	0	3
Cecidomyiidae sp. 1	Cecidomyiidae	Predator	2	8	0
Cecidomyiidae sp. 2	Cecidomyiidae	Predator	0	10	0
Cecidomyiidae sp. 3	Cecidomyiidae	Predator	0	11	0
cf. Helobdella sp.	Glossiphonidae	Predator	0	58	157
Chironomidae sp. 18	Chironomidae	Collector	0	2	0
Chironomus detriticula	Chironomidae	Collector	1	0	4
Coenagrionidae sp. 5	Coenagrionidae	Predator	0	2	0
Copelatus bimaculatus	Dytiscidae	Predator	13	11	21

Copelatus sp. 2	Dytiscidae	Predator	0	0	13
Copestilum sp. 1	Syrphidae	Scraper	1	0	3
Copestilum sp. 2	Syrphidae	Scraper	0	1	0
Corethrella fulva	Corethrellidae	Predator	0	4	0
Corethrella infuscata	Corethrellidae	Predator	1	19	10
Corethrella sp. 1	Corethrellidae	Predator	0	4	0
Corethrella sp.2	Corethrellidae	Predator	0	4	0
Corynoneura sp.	Chironomidae	Collector	0	1	2
Culicidae	Culicidae	Filter feeder	112	164	50
Culicoides sp.	Ceratopogonidae	Predator	0	8	0
Diptera sp. 30	Diptera	Collector	0	12	0
Diptera sp. 31	Diptera	Collector	0	11	0
Diptera sp. 32	Diptera	Collector	0	1	0
Diptera sp. 33	Diptera	Collector	0	1	0
Diptera sp. 34	Diptera	Collector	0	1	0
Diptera sp. 35	Diptera	Collector	0	5	0
Diptera sp. 36	Diptera	Collector	0	1	0
Diptera sp. 37	Diptera	Collector	0	1	0
Dolychopodidae sp. 1	Dolichopodidae	Predator	3	0	10
Dolychopodidae sp. 2	Dolichopodidae	Predator	0	0	3
Dolychopodidae sp. 3	Dolichopodidae	Predator	0	1	0
Elpidium bromeliarum	Cyheroidae	Collector	88	378	236
Empididae sp. 1	Empididae	Predator	3	1	1
Empididae sp. 2	Empididae	Predator	0	1	0
Ephydridae sp. 1	Ephydridae	Collector	0	6	0
Ephydridae sp. 2	Ephydridae	Collector	0	56	0
Ephydridae sp. 3	Ephydridae	Collector	0	1	0
Fidena rufipilosa	Tabanidae	Predator	0	1	1
Forcipomyia sp.	Ceratopogonidae	Collector	0	4	0
Lachnodacnum luederwaldti	Hydrophilidae	Collector	0	3	0

Lampyridade sp.	Lampyridade	Predator	0	0	2
Leptagrion andromache	Coenagrionidae	Predator	0	3	0
Leptagrion elongatun	Coenagrionidae	Predator	1	10	5
Limoniidae sp. 3	Limoniidae	Shredder	7	0	7
Lymnophies sp.	Chironomidae	Collector	0	0	2
Monopelopia caraguata	Chironomidae	Predator	17	62	20
Muscidae sp.1	Muscidae	Predator	10	0	130
Olbiogaster sp.	Anisopodidae	Collector	24	7	0
Oligochaeta sp. 1	Tubificidae	Collector	37	0	0
Oligochaeta sp. 2	Tubificidae	Collector	0	40	0
Oligochaeta sp. 3	Tubificidae	Collector	0	34	0
Orthocladinae sp. 1	Chironomidae	Collector	1	55	8
Phylloicus bromeliarum	Calamoceratidae	Shredder	5	60	63
Polypedilum kaingang	Chironomidae	Collector	6	51	59
Polypedilum marcondesi	Chironomidae	Collector	66	389	149
Polypedilum sp. 3	Chironomidae	Collector	0	0	1
Psychodidae sp. 1	Psychodidae	Collector	15	4	83
Psychodidae sp. 2	Psychodidae	Collector	2	0	0
Psychodidae sp. 3	Psychodidae	Collector	4	0	0
Psychodidae sp. 4	Psychodidae	Collector	0	4	0
Psychodidae sp. 5	Psychodidae	Collector	0	37	0
Sciaridae sp. 1	Sciaridae	Shredder	0	138	0
Sciaridae sp. 2	Sciaridae	Shredder	0	34	0
Sciaridae sp. 7	Sciaridae	Shredder	0	1	0
Scirtes sp. 1	Scirtidae	Scraper	105	0	10
Scirtes sp. 2	Scirtidae	Scraper	0	431	2
Scirtes sp. 3	Scirtidae	Scraper	0	0	19
Scirtes sp. 4	Scirtidae	Scraper	0	0	5
Scirtes sp. 5	Scirtidae	Scraper	0	0	3
Scirtidae sp. 1	Scirtidae	Scraper	82	0	16

Scirtidae sp. 2	Scirtidae	Scraper	0	0	2
Stenochironomus atlanticus	Chironomidae	Collector	0	5	1
Stibasoma bicolor	Tabanidae	Predator	4	9	2
Syrphidae sp. 2	Syrphidae	Scraper	0	1	1
Syrphidae sp. 3	Syrphidae	Scraper	0	1	17
Tabanidae sp. 2	Tabanidae	Predator	1	0	1
Tabanidae sp. 4	Tabanidae	Predator	0	0	1
Tanytarsus sp.	Chironomidae	Collector	0	78	109
Tipulidae sp. 2	Tipulidae	Shredder	0	2	0
Trentepohlia sp. 1	Limoniidae	Shredder	27	67	39
Trentepohlia sp. 2	Limoniidae	Shredder	11	0	3
Trichoptera sp. 2	Calamoceratidae	Shredder	0	3	2
Tubificidae	Tubificidae	Collector	0	0	70
Planarian	Turbellaria	Predator	0	1	0

Table S2. Abundance of terrestrial metazoans collected in *Vriesea procera* bromeliads with and without *Odontomachus hastatus* ants at Parque Estadual da Serra do Mar (Picinguaba), Estação Ecológica Juréia-Itatins (Juréia) and Parque Estadual da Ilha do Cardoso (Ilha do Cardoso), São Paulo State, Brazil. Metazoans were identified to the lowest possible taxonomic level and were separated in guilds

Identification	**Guilds**	**Picinguaba**	**Juréia**	**Ilha do Cardoso**
Acari	Omnivore	19	21	18
Anura	Predator	0	1	1
Araneae	Predator	80	74	38
Blattodea	Omnivore	17	19	15
Cercopidae	Phytophagous	1	0	0
Chilopoda	Predator	0	9	1
Coleoptera	Omnivore	10	19	9
Collembola	Detritivore	75	22	0
Culicidae (adult)	Omnivore	0	14	0
Diplopoda	Detritivore	10	5	0
Diptera (adult)	Omnivore	4	4	0
Dytiscidae (adult)	Predator	0	2	0
Formicidae	Omnivore	227	103	7
Gastropoda	Phytophagous	0	0	1
Gryllidae	Omnivore	10	3	2
Hemiptera	Phytophagous	13	4	0
Hymenoptera	Omnivore	2	1	0
Hydrophilidae (adult)	Detritivore	0	4	0
Isopoda	Detritivore	15	99	2
Lampyridae (adult)	Predator	0	2	0
Lepidoptera	Phytophagous	2	36	11
Leptagrion (adult)	Predator	0	6	0

Myriapoda	Predator	1	0	0
Neotroponiscus	Omnivore	0	0	5
Oligochaeta	Detritivore	0	32	0
Opiliones	Omnivore	6	6	0
Orthoptera	Phytophagous	0	2	2
Pergidae	Phytophagous	2	0	0
Pholcidae	Predator	1	0	0
Pseudoscorpiones	Predator	19	11	2
Psephenidae	Detritivore	0	1	0
Psocoptera	Omnivore	1	0	0
Salticidae	Predator	1	0	0
Staphylinidae sp. 1	Omnivore	1	0	0
Staphylinidae sp. 2	Omnivore	1	5	0
Staphylinidae sp. 3	Omnivore	7	0	0
Stylommatophora	Phytophagous	0	3	0
Therididae	Predator	1	0	3
Thysanoptera	Phytophagous	1	0	0
Tingidae	Phytophagous	4	0	0
Trichoptera (adult)	Phytophagous	2	0	0
Vespidae	Predator	3	0	0
Zigoptera (adult)	Predator	0	0	4

Table S3. Analyses of variance (ANOVA) summarizing the effects of the presence of *Odontomachus hastatus* ants in the density within each aquatic functional group (collectors, shredders, scrapers, filter feeders and predators) and within each terrestrial guild (detritivores, phytophagous, omnivores and predators) of *Vriesea procera* bromeliads at Parque Estadual da Serra do Mar (Picinguaba), Estação Ecológica Juréia-Itatins and Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil

Source of variation	df	MS	*F*	*P*
Picinguaba				
Aquatic				
Ants	1	0.01	19.24	**<0.001**
Functional groups	4	0.01	7.42	**<0.001**
Ants*functional groups	4	0.01	3.24	**0.015**
Error	90	0.01		
Terrestrial				
Ants	1	0.01	8.19	**0.005**
Guilds	3	0.01	3.80	**0.013**
Ants*guilds	3	0.01	3.55	**0.018**
Error	72	0.01		
Juréia				
Aquatic				
Ants	1	0.07	32.50	**<0.001**
Functional groups	4	0.04	19.67	**<0.001**
Ants*functional groups	4	0.02	8.65	**<0.001**
Error	90	0.01		
Terrestrial				
Ants	1	0.01	74.83	**<0.001**
Guilds	3	0.01	9.22	**<0.001**
Ants*guilds	3	0.01	5.44	**0.001**
Error	72	0.01		
Ilha do Cardoso				
Aquatic				
Ants	1	0.01	15.83	**<0.001**
Functional groups	4	0.01	22.38	**<0.001**
Ants* functional groups	4	0.01	3.17	**0.017**
Error	80	0.01		

Terrestrial				
Ants	1	0.01	4.30	0.051
Guilds	3	0.01	4.30	**0.008**
Ants*guilds	3	0.01	0.41	0.739
Error	52	0.01		

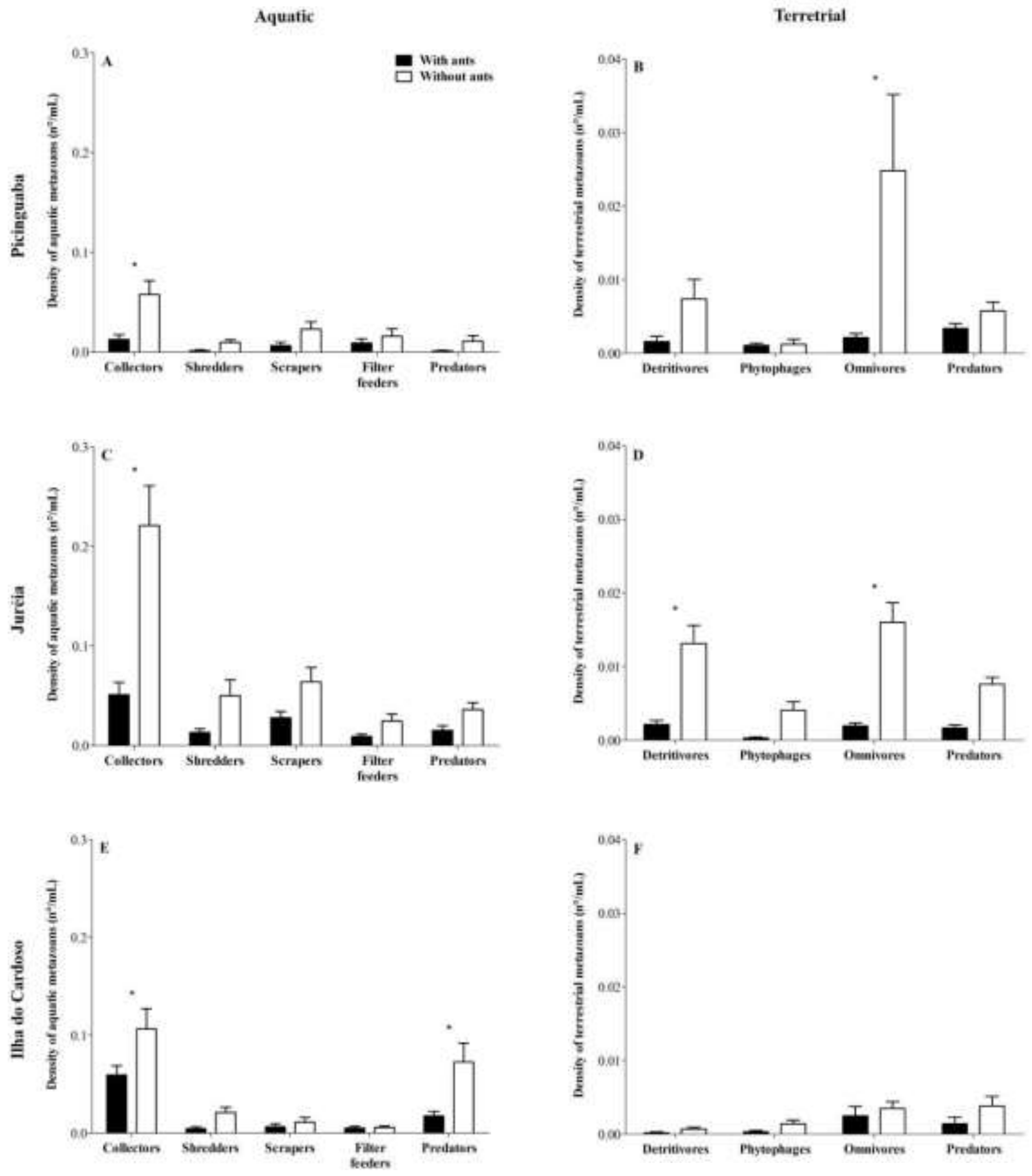

Fig. S1. Density of metazoans (number/mL of the bromeliad tank) within each aquatic functional group and terrestrial guilds at Parque Estadual da Serra do Mar (Picinguaba) (A) aquatic and (B) terrestrial, at Estação Ecológica Juréia-Itatins (C) aquatic and (D) terrestrial, and Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil (E) aquatic and (F) terrestrial in *Vriesea procera* with and without *Odontomachus hastatus* nests. Bars indicate SE and asterisks (*) indicate statistical differences between treatments (ANOVA/Tukey HSD *post-hoc* test, α = 0.05).

CAPÍTULO III

Gonçalves AZ, Oliveira PS, Romero GQ

Contrasting effects of ant identity on diversity and functioning of aquatic bromeliad ecosystems

Contrasting effects of ant identity on diversity and functioning of aquatic bromeliad ecosystems

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Summary

1. Predator identity can structure communities altering prey diversity and behavior, with cascading effects on the lower trophic levels and on ecosystem functions. Predators affect their ecosystem, but if they are on the ecosystem boundaries, they can exert cascading effects across ecosystems.

2. Predatory ants that establish their colonies near aquatic ecosystem boundaries can exert top-down effects within the terrestrial ecosystem with reverberating effects on the aquatic ecosystem whether they prey on or inhibit adult female oviposition on the aquatic habitat. We examined the effects of three ant species (*Odontomachus hastatus*, *Gnamptogenys moelleri* and *Camponotus crassus*) on the aquatic communities and ecosystem functions (i.e., decomposition, nutrient cycling) in the bromeliad *Quesnelia arvensis*.

3. We performed a field experiment with epiphytic and terrestrial *Q. arvensis* bromeliads with and without the three ant species to evaluate the density, richness and composition aquatic metazoans, the total N and chlorophyll concentrations of bromeliad leaves, bromeliad growth, the detritus mass loss on bromeliad tank, the flux of N from detritus to bromeliads, and the quality of the water of the tank of each bromeliad (temperature, pH, concentrations of O_2, chlorophyll and ammonium, turbidity and colored dissolved organic matter).

4. Ants affected the diversity of aquatic organisms in bromeliads and affected the ecosystem functioning of the aquatic environment. While *O. hastatus* reduced aquatic diversity of metazoans, detritus mass loss and the N flux from detritus to *Q. arvensis*, but increased bromeliad N, chlorophyll concentrations and growth, *C. crassus* did not alter bromeliad diversity but increased CDOM, turbidity, chlorophyll and ammonium concentrations in the tank. *Gnamptogenys moelleri* reduced aquatic diversity of metazoans, increased bromeliad

chlorophyll concentration, but did not differ from other treatments concerning the concentration of nitrogen and growth of bromeliads.

5. Our results suggest, for the first time, that ants can affect directly the aquatic ecosystem. Depending on the predator identity that interacts with *Q. arvensis*, it can reduce aquatic diversity and decomposition of organic matter (*O. hastatus*), or may have no effect on metazoans promoting mineralization and decomposition by microorganisms (*C. crassus*).

Key-words: predator identity, trophic cascade, cross ecosystem effects, functional diversity, *Odontomachus*, *Gnamptogenys*, *Camponotus*, *Quesnelia*

Introduction

The most relevant traits of predators in designing communities and ecosystem processes are their size and identity (Rudolf *et al.* 2014). Predator identity can dictate its hunting mode (Henry *et al.* 2010), while its body size can be associated with the rate of prey consumption as it is expected that larger predators have larger consumption of prey (Werner & Gilliam 1984), and may alter prey behavior, morphology and habitat use (Werner & Peacor 2003). Additionally, if prey possesses complex life cycle (Werner 1988), predator's effect may cross ecosystem boundaries affecting the diversity of organisms in both ecosystems (Knight *et al.* 2005; Romero & Srivastava 2010).

Considering the dominant abundance and biomass of ants over other animals in most habitats (Hölldobler & Wilson 1990), ants may play a key role in structuring communities in which they interact, with possible consequences for the ecosystem. Many studies reveal interactions between ants and other species of animals and plants, especially the role of ants in seed dispersal (Youngsteadt *et al.* 2009; Céréghino *et al.* 2010; Leroy *et al.* 2012), plant nutrition and protection (Treseder *et al.* 1995; Fischer *et al.* 2003; Hein & McKey 2003; Rico-Gray & Oliveira 2007), and inhibition of pollinator activity (Ohm & Miller 2014). Other studies bring up that ants tend to increase the concentration of nutrients in the soil (Friese & Allen 1993; Folgarait 1998), but less known is how ants can affect the decomposition process of organic matter, as suggested by Boulton & Amberman (2006) that experimentally showed a higher microbial diversity in ant-nest soils. The prospect of ant effects on communities and ecosystem processes is still poorly known, despite ants are considered ecosystem engineers, and have a great effect on soil processes and function (Folgarait 1998; Romero *et al.* 2015).

Despite ant effects on terrestrial habitats, less intuitive are the effects on aquatic environments near places where ant nests are established. For example, Céréghino *et al.* (2010)

showed that two different ant species indirectly alter the physical structure of the tank of bromeliads through their nesting site preferences as dispersal agents of plants (e.g., sun-exposed or shaded areas). Therefore, these ants indirectly alter the diversity of protozoa, algae and invertebrates in bromeliads due to differences in the water volume of the tanks of these sun-exposed or shaded plants (Céréghino *et al.* 2010; Carrias *et al.* 2012). In another example, Talaga *et al.* (2015) showed that *Odontomachus haematodus* ants favor the accumulation of detritus in bromeliad tanks through nest debris since this species establishes its nests in bromeliad leaves. Thus, *O. haematodus* indirectly reduces the occurrence of detritivores and predators in the tank, since it modifies the structure of bromeliad rosettes, reducing the space available for the occurrence of metazoans (Talaga et al. 2015). Other indirect effects of ants on aquatic communities are poorly studied, but direct effects of ants on aquatic communities and their consequences for ecosystem processes are still unknown.

Ants can play distinct functional roles within communities, being detritivores, granivores, omnivores and predators (Boulton & Amberman 2006). Some predatory ants may feed on invertebrates and vertebrates (Facure & Giaretta 2009; Cogni & Oliveira 2004; Camargo & Oliveira 2012) while others may feed on extrafloral nectaries inhibiting the visitation of invertebrates in their host plants (Oliveira et al. 1995; Oliveira & Freitas 2004). As predators exhibit aggressive behavior, ants can exert top-down effects within their ecosystem, or their effects can cross ecosystems when, for example, their colonies are near aquatic ecosystem boundaries. Most ecological literature abounds with predator effects within their ecosystems; however, little is known about predator’s cross-ecosystem effects (Knight *et al.* 2005; Romero & Srivastava 2010). When ant nests are near aquatic ecosystem boundaries, and considering that many animals have life cycles in more than one ecosystem (e.g., holometabolous insects, amphibians, invertebrates; Werner 1988), we can highlight the effects of predatory and/or aggressive ants on aquatic communities through direct consumption or non-consumptive effects of predators as inhibition of prey behavior. Furthermore, ant effects may cascade and alter the functioning of aquatic ecosystems.

Tank bromeliads occupied by ant colonies are suitable systems to study how predatory ants can structure aquatic communities and their cascading effects on ecosystem functions. Tank bromeliads shelter microorganisms, invertebrates and vertebrates, and many of them use the water of the tank to complete their complex life cycles (Benzing 2000; Hammill *et al.* 2015). In the Brazilian Atlantic Forest, the ants *Odontomachus hastatus*, *Gnamptogenys moelleri* and *Camponotus crassus* establish their nests in tank bromeliads (Cogni & Oliveira 2004; Camargo & Oliveira 2012; AZG personal observations). *Odontomachus hastatus* has the greatest size and biomass while *G. moelleri* and *C. crassus* have similar size and biomass (see Materials and Methods). Furthermore, *O. hastatus* and *G. moelleri* have sting, predatory habit and their diet consists mainly of arthropods (Cogni & Oliveira 2004; Camargo & Oliveira 2012) while *C. crassus* feeds on extrafloral nectaries, homopteran exudates and fruits, but hunts few arthropods to supplement its needs for protein (Oliveira *et al.* 1995; Oliveira & Freitas 2004; personal observations). While *O. hastatus*, *G. moelleri* and *C. crassus* capture prey, these ant species can feed on metazoans or inhibit females to oviposit on the bromeliad tank. We hypothesized that *O. hastatus* will consume more prey as it has the greatest size, having a greater impact on changing the diversity of bromeliad communities, with cascading effects that may reduce some ecosystem functions in the tank (e.g. nutrient cycling). On the other hand, we expect that *G. moelleri* and *C. crassus* that requires few prey items to its nutrition would have less effect on terrestrial and aquatic diversity, and would not change the decomposition of organic matter or the nutrient cycling in aquatic bromeliad ecosystem.

Materials and methods

Study site and organisms

The experiment was developed at Parque Estadual da Ilha do Cardoso (Cardoso Island), São Paulo State, Brazil (25°04’ S, 47°55’ W). Cardoso Island is marked by two seasons, a cold and more dry season (April to August, average temperature: 13 °C, average rainfall: 500 mm), and a hot and humid season (September to March, average temperature: 32 °C, average rainfall: 1800 mm). It has an open canopy formed by trees of 5-15 m tall, whose understory is covered mainly by *Quesnelia arvensis* (Bromeliaceae, Bromelioideae), while the most commonly epiphytic species is *Vriesea procera* (Bromeliaceae, Tillandsioideae) (Romero & Srivastava 2010; Oliveira *et al.* 2011).

In Cardoso Island, nests of *Odontomachus hastatus* (Formicidae, Ponerinae) are abundant (ca. 33 colonies. ha^{-1}) among roots of epiphytic bromeliads 1.0 to 4.6 m above ground (Camargo and Oliveira 2012). This species is arboreal, occurs from Central to South America (Kempf 1972), is nocturnal and its nests are rarely found on the ground (Gibernau et al. 2007; Camargo and Oliveira 2012). Rodrigues and Oliveira (2014) showed that *O. hastatus* workers can move more than 8 m away from their nests, but about half of them stay within a 3 m radius around their nests. *Gnamptogenys moelleri* (Formicidae, Ectatomminae) occurs in Neotropical plains, its nests can be found on the ground and epiphytic bromeliads among 1-3 leaves and workers forage almost exclusively on their host plants (Lattke 1995; Cogni and Oliveira 2004). *Camponotus crassus* (Formicidae, Formicinae) consumes exudates of plants and insects (Davidson *et al.* 2003) and can suppress herbivores from plants that have extrafloral nectaries (Sendoya *et al.* 2009). In Cardoso Island, nests of this ant can be found among 2-3 leaves of *Q. arvensis*, where there is no water accumulation (personal observations). Among these ants, *O. hastatus* has the greatest size (≈ 1.3 cm) and biomass (mean ± SE, 7.89 ± 1.57 mg), *G. moelleri* has ≈ 0.5 cm in length and 1.15 ± 0.32

mg in biomass and *C. crassus* is the smallest ant with ≈ 0.5 cm in length and 1.04 ± 0.09 mg in biomass.

Quesnelia arvensis has a terrestrial and epiphytic life forms and can obtain nutrients not only from its roots, but also by foliar trichomes, which are capable of absorbing complex nitrogen compounds (e.g., amino acids) (Benzing & Burt 1970; Benzing 2000). This bromeliad species has a tank that can accumulates water (i.e., with phytotelmata) and shelters numerous aquatic and terrestrial invertebrates and vertebrates. Many aquatic invertebrates of distinct functional groups are associated with tank bromeliads, including collectors, detrital shredders, detrital scrapers, filter feeders and predators (see Table S1).

Field experiment

Colonies of *O. hastatus* were collected from epiphytic *V. procera* while *G. moelleri* and *C. crassus* were collected from terrestrial *Q. arvensis*, and were inserted on experimental bromeliads as soon as collected. The number of ants per colony did not differ among treatments (One-way ANOVA, P = 0.261; mean ± SE, 129.3 ± 7.6 ants *O. hastatus* colonies; 141.7 ± 4.7 ants in *G. moelleri* colonies; and 149.0 ± 11.4 ants in *C. crassus* colonies). Since *O. hastatus* can be found in *Q. arvensis* in the field, this species was chosen to the experiment and was bought at Veiga Arquitetura e Paisagismo®, CEASA, Campinas, Brazil. To remove any metazoans or detritus in bromeliad tanks, we washed carefully with abundant water any bromeliad axils and suspended the bromeliads upside down in a line for 24h before the experiment. All bromeliads had similar biomass and size (e.g., foliar length varying from 25 to 30 cm), and were kept in pots (14.5 cm in diameter, 14.5 cm high) with *Pinus* sp. bark, simulating the poor soil of the restinga forest at the same time that allows the movement of ants in bromeliad roots inside the pots. Each pot was kept individually in a white plastic tray (40.7 x 60.8 x 9.8 cm) with Tanglefoot® resin around its border to prevent ants leaving their host bromeliads. All pots had four holes at their bases (approximately 1 x 1 cm) to allow ant to access the bromeliad roots.

We conducted this experiment during three months (January-April 2014), and experimental bromeliads were randomly organized in ten blocks with five treatments each block: (1) bromeliads with *O. hastatus* kept with epiphytic habit; (2) epiphytic bromeliads without ants (epiphytic control); (3) bromeliads with *G. moelleri* and kept with terrestrial habit; (4) bromeliads with *C. crassus* and kept with terrestrial habit; and (5) terrestrial bromeliads without ants (terrestrial control). All ants collected in the field were placed in the white trays and quickly sought a refuge, entering the holes of the pots. In about half an hour, all ants entered the pots and carried their eggs and pupae. In the treatments with *G. moelleri* and *C. crassus* colonies, ants were manually placed among 2-3 leaves of bromeliads with 2-3 *Pinus* sp. barks protecting the colonies. The white trays of epiphytic treatments were suspended at 1.0 m from the ground, through support built with bamboo, wire and string. For treatments with terrestrial bromeliads, white trays were placed on the soil. Each treatment was distant 0.5-1.5 m from each other and blocks were distant about 8 m.

At the end of the experiment, all bromeliads from each treatment were collected on the same day and it did not rain on the day or the day before of field samplings. All bromeliads were dissected and washed with distilled water in the field and debris and the tank water were collected in white trays and separated by two sieves (mesh diameter, 125 and 800 µm.) Each metazoan visible to the naked eye (> 0.5 mm) was preserved in 70% ethanol and had it abundance and morphospecies recorded. In the laboratory, they were identified to the lowest taxonomic level through literature and direct observations (Merritt & Cummins 1996, Triplehorn & Johnson 2005). The density (number of individuals/mL of tank volume), richness (number of species/mL of tank volume) and composition of aquatic metazoans were recorded. Aquatic metazoans have been separated into the following functional groups: (1) collectors, (2) shredders, (3) scrapers (4) filter feeders, and (5) predators (see Table S1).

Processing of detritus and N flow from detritus to bromeliads

In order to test whether the three ant species alter the decomposition of detritus present in the bromeliad tanks and the nitrogen flow from detritus to bromeliads, all experimental bromeliads received six labeled leaves of *Eugenia uniflora* L. (Myrtaceae) enriched with ^{15}N. Every two leaves of *Eugenia* were placed on opposite sides in the tanks, submerged in the phytotelmata. Before the experiment, *Eugenia uniflora* grew in a greenhouse with an automatic irrigation system with a capacity of $8L.h^{-1}$, which worked for 10 min every 2 h. *Eugenia* was enriched during one month through 300 $mL.day^{-1}$ with a solution of distilled water with enriched ammonium sulfate [$(^{15}NH_4)_2SO_4$, 10% excess atoms, from Cambridge Isotope Laboratories, MA, USA], in the proportion of 5 g ammonium $sulfate.L^{-1}$. After enrichment, *Eugenia* leaves were collected, dried at 70 °C for 24 h and their dry weight was determined. At the end of the experiment, each *Eugenia* leaves were recovered from phytotelmata, were dried at 70 °C for 24 h and the remaining mass of leaves was determined in order to estimate the detritus mass loss (%) according to the following equation: detritus mass loss = [(final detritus mass – initial detritus mass) x 100]/ (final detritus mass). In addition, two new leaves of the innermost node of each bromeliad rosette were collected for isotopic analysis to estimate the nitrogen flow from detritus to bromeliads ($\delta^{15}N$‰).

Bromeliad growth, analyses of chlorophyll concentration of bromeliad leaves, and limnological analyses of tank's water

To determine whether the presence of ant nests affect bromeliad growth, two leaves (i.e., fifth inner node of the rosette) from each bromeliad were randomly chosen and their lengths were measured at the beginning and the end of the experiment. The bromeliad leaf length was directly related to the leaf biomass (Linear regression: *Q. arvensis*: $r^2 = 0.73$, $P < 0.001$); leaves showed continuous growth during the experiment and their relative growth rate (Ln(cm)/day) was calculated using the following equation: $RGR = [\ln(L_{final}) - \ln(L_{initial})]/ (t_2 - t_1)$. The $\ln(L_{final})$ and $\ln(L_{initial})$ are, respectively, the natural logarithm of the foliar final length and the natural logarithm of the foliar initial length, with $(t_2 - t_1)$ being the number of days between the initial and final measurements.

To determine the concentration of chlorophyll in bromeliad leaves, we used the hand-held chlorophyll meter SPAD-502 Plus (Soil Plant Analysis Development, Minolta Camera Co., Osaka, Japan). Measurements were made in the central portion of the adaxial leaf blade of four distinct leaves of the seventh inner node of the rosettes. This measurement is used as an indicator of the nutritional status of plants since there is a correlation between nitrogen and chlorophyll concentration in leaves (Evans 1983). The values obtained in SPAD-502 were transformed into total chlorophyll concentration ($mg.cm^{-2}$) according to the following equation (Richardson *et al.* 2002): total chlorophyll concentration = $[(5.52 \times 10^{-4}) + (4.04 \times 10^{-4} \times V) + (1.25 \times 10^{-5} \times V^2)]$, where V is the value obtained by SPAD-502. To determine the temperature (°C), pH, concentration of O_2 ($mg.L^{-1}$), concentration of chlorophyll ($mg.L^{-1}$), turbidity (ntu), colored dissolved organic matter (CDOM, ppb) and concentration of ammonium (µM) of the water of each phytotelmata, we used the hand-held AquaFluor Fluorometer (Turner Designs) and Oakton PC-600 waterproof portable meter kit.

Natural effects of Gnamptogenys moelleri *and* Camponotus crassus *nests*

To investigate whether the effect of *G. moelleri* and *C. crassus* on bromeliads in the field were similar with experimental bromeliads, ten terrestrial *Q. arvensis* with and ten bromeliads without colonies of each ant species were collected at Cardoso Island. We recorded the density (number of individuals/mL of tank volume), richness (number of species/mL of tank volume) and composition of aquatic and terrestrial metazoans. Aquatic metazoans were separated into functional groups as described in the experiment and terrestrial metazoans was separated into the following guilds: (1) detritivores (2) phytophages, (3) omnivores and (4) predators (see Table S1, S2). The effect size (%) of ants on the density and richness of aquatic and terrestrial metazoans was obtained by the mean reduction of individuals in bromeliads with ants compared to bromeliads without ants. All bromeliads of each ant species were collected on the same day and it did not rain on the day or the day before field samplings. Bromeliads with similar aquatic tank sizes were collected (aquatic maximum volume, V_{TA}, with *G. moelleri*: 443.6 ± 8.8 mL; with *C. crassus*: 438.2 ± 9.04 mL; mean ± SE) in order to avoid the influence of the tank volume on the number of metazoans interacting with bromeliads. The volume of the terrestrial bromeliad ecosystem (V_{TE}) was calculated from measurements of the largest leaf of each bromeliad (T) and the diameter of the rosettes (half of this measurement was considered the radius, r) and from triangle equation (to obtain the height of bromeliads, h) and cone volume; triangle $T^2 = h^2 + r^2$, and then the total volume (V_{TO}) of bromeliads (considering the terrestrial and aquatic ecosystems using the cone volume equation): $V_{TO} = (\pi r^2 h)/3$. Later, with the V_{TO}, it was obtained the volume of the terrestrial ecosystem of bromeliads (V_{TE}): $V_{TE} = V_{TO} - V_{TA}$. The terrestrial maximum volume (V_{TE}) were: bromeliads with *G. moelleri*, 829.8 ± 79 mL; with *C. crassus*, 701.3 ± 55.3 mL.

Isotopic and statistical analyses

The total N concentration (µg/mg dry leaf tissue) and $\delta^{15}N$ of bromeliad leaves were determined with an isotope ratio mass spectrometer (20 – 20 mass spectrometer; PDZ Europa, Sandbach, UK) after sample combustion to N_2 at 1000°C by an on-line elemental analyzer (PDZ Europa ANCA-GSL) in the Stable Isotope Facility at the University of California, Davis.

The response variables of density, richness and abundance of functional groups or guilds of aquatic and terrestrial metazoans, total N concentration, total chlorophyll concentration of bromeliad leaves, relative growth rate, detritus mass loss, $\delta^{15}N$, temperature, pH, concentration of O_2, concentration of chlorophyll in the tank water, turbidity, CDOM and concentration of ammonium were compared using linear mixed-effect model (LME) with treatments as fixed effects and blocks as random effects. Tukey HSD *post-hoc* test was used for pair-wise comparisons between treatments. The composition of aquatic and terrestrial organisms in bromeliads was obtained by Permutation Multivariate Analyses of Variance (PERMANOVA), with the "adonis" function of the vegan package of R (Oksanen *et al.* 2013), with dissimilarity calculated as Bray- Curtis distances and 9999 permutations. Nonmetric Multidimensional Scaling Plot (NMDS) was performed to visualize similarities or dissimilarities. All statistical analyses were conducted with the statistical platform R (R Core Team, 2014).

Results

The presence of ants changed the diversity of aquatic metazoans. *Odontomachus hastatus* reduced the density and richness of aquatic metazoans compared to other treatments, while *Gnamptogenys moelleri* reduced the density and richness of metazoans compared to *Camponotus crassus* but did not differ from control bromeliads (Table 1, Fig. 1A, 1B). On the other hand, the presence of *C. crassus* on bromeliads did not alter the diversity of metazoans compared to control bromeliads (Table 1, Fig. 1A, 1B).

The composition of aquatic metazoans differs among treatments (Table 1, Fig. 1C). *Odontomachus hastatus* reduced the density of most functional groups of aquatic metazoans compared to other treatments, except filter feeders (Table 1, Fig. 1D). In general, the presence of *G. moelleri* in bromeliads kept the aquatic functional groups in an intermediate density compared to *O. hastatus* and *C. crassus*, while bromeliads with *C. crassus* showed similar density within functional groups compared to terrestrial-control bromeliads (Table 1, Fig. 1D). Specifically, *O. hastatus* reduced by 100% of Ostracoda compared to any other treatment, and 93% of *Polypedilum* sp. compared to epiphytic control (i.e., aquatic collectors; Table S1). Furthermore, *O. hastatus* reduced by 100% of Hirudinea compared to any other treatment, reduced by 65% of *Leptagrium andromache*, and 95% of *Monopelopia caraguata* compared to the epiphytic control (i.e., aquatic predators; Table S1).

Bromeliads with *O. hastatus* nests showed the highest concentrations of nitrogen and chlorophyll on their leaves and grew more than bromeliads of other treatments (Table 2, Fig. 2). Additionally, bromeliads with *G. moelleri* nests showed higher concentration of chlorophyll on their leaves compared to control bromeliads, but there was no difference in the concentration of nitrogen or growth of *G. moelleri*-bromeliads and bromeliads of other treatments (Table 2, Fig. 2). However, the detritus on tank had the lowest mass loss in the presence of *O. hastatus* (Table 2, Fig. 3A). Also, the nitrogen flow from detritus to bromeliads was lower in the presence of *O. hastatus*, and was also lower in bromeliads with *G. moelleri* and *C. crassus* compared to the terrestrial control (Table 2, Fig. 3B). In the middle and at the end of the experiment, bromeliads with *O. hastatus* showed a lower chlorophyll concentration in the tank, lower turbidity, lower concentrations of dissolved organic matter (CDOM) and ammonium compared to the water of bromeliads with *C. crassus* nests (Table 3, Fig. 4).

The pattern found in the experiment was similar to that found in bromeliads collected in the field. Bromeliads with *G. moelleri* and *C. crassus* collected in the field showed a lower density and richness of aquatic metazoans, but there was no effect of ants on the density and richness of terrestrial organisms (Table S3, Fig. S1, S2). Bromeliads with *G. moelleri* and *C. crassus* nests showed a different composition of aquatic organisms compared to bromeliads without nests, while these ant species did not show an effect on the composition of terrestrial organisms (Table S4, Fig. S3, S4). Bromeliads with *G. moelleri* had a lower density of aquatic collectors and predators, and bromeliads with *C. crassus* showed a reduction in the density of aquatic collectors; however, the effects of *G. moelleri* on the predatory larvae and the effect of *C. crassus* on collector larvae were not observed in the field (Table S5, Fig. S5, S6).

Discussion

Despite ants are terrestrial organisms, their effects were remarkable on the aquatic ecosystem of bromeliads. *Odontomachus hastatus* had a stronger effect on the aquatic diversity in *Quesnelia arvensis* while *Gnamptogenys moelleri* showed an intermediate effect and *Camponotus crassus* did not alter the aquatic diversity. The presence of *O. hastatus* favored the accumulation of nitrogen and chlorophyll in bromeliad leaves, contributing to their growth. However, bromeliads with *O. hastatus* had the lowest detritus mass loss and the lowest flow of nitrogen from detritus to bromeliads possibly because *O. hastatus* reduced the density of detritivores. Therefore, while smaller (e.g., *G. moelleri* and *C. crassus*) and less aggressive predators (e.g., *C. crassus*) did not affect communities and ecosystem functioning of bromeliads, *O. hastatus* seems to contribute to the nutrition and growth of its host plants through nest debris, but not through nutrient cycling in the tank.

Contrary to our expectations, ants had a greater effect on the aquatic environment. Especially *O. hastatus* and *G. moelleri* changed the density, richness and composition of aquatic metazoans. Therefore, this is the first research suggesting that ant activities may affect the diversity of aquatic organisms, with a reduction on the nutrient cycling in the aquatic environment. Ant activities may have affected direct or indirectly aquatic metazoans: directly through consumptive effects on females that visit bromeliads to opposite, or indirectly by non-consumptive effects inhibiting female oviposition through female perception of ant traits (e.g., ant behavior, smell, body shape). For organisms with exclusive aquatic life cycles that move away from one to another bromeliad by phoresy especially in the skin of frogs (e.g., Ostracoda, Hirudinea; Lopez *et al.* 1999; Tiberti & Gentilli 2010), ants should exert non-consumptive, for example, inhibiting the visitation of frogs in bromeliads and reducing the phoresy of Ostracoda and Hirudinea. Additionally, ants may directly fed on some species of larvae (e.g., *Polypedilum* sp.), as these larvae access some bromeliad axils with a lot of moisture and organic matter, where ants also can access (field observations). Similar results were observed in field samplings from Parque Estadual da Serra do Mar (Picinguaba), Estação Ecológica Juréia-Itatins (Juréia) with *O. hastatus*, and Ilha do Cardoso with *O. hastatus, G. moelleri* and *C. crassus*, suggesting that our results are consistent (see Supporting Information and Chapter 2).

Odontomachus hastatus reduced the aquatic diversity, the decomposition of organic matter and the nutrient cycling in the aquatic ecosystems of *Q. arvensis*. Many studies have shown the effects of cross ecosystem movements of subsidies such as invertebrates and leaf litter from the terrestrial to the base of aquatic food webs (Zhang & Richardson 2011; Bartels *et al.* 2012). Few other studies highlight the effects of predators that cascade across ecosystem boundaries (Knight *et* *al.* 2005; Romero & Srivastava 2010). Romero & Srivastava (2010), for example, showed that two species of spiders indirectly facilitate the occurrence of Hirudinea and Ostracoda in bromeliads through the reduction of other larvae of detritivores, and these spiders did not alter the flux of nitrogen from detritus to bromeliads. Our results reinforce that species identity of predators is relevant in structuring communities and altering ecosystem functions as the predatory ant *O. hastatus* reduced the abundance of Hirudinea and Ostracoda, the detritus decomposition and nitrogen flux from detritus to bromeliads.

Although *O. hastatus* decreased nutrient cycling in the tank of *Q. arvensis*, it contributed to the increment of total nitrogen and chlorophyll concentrations in bromeliad leaves, which possibly reflected in the highest growth of these plants. Many inhabitants of bromeliads are the preferred prey of *O. hastatus* (Richardson 1999; Gonçalves-Souza *et al.* 2010) and we observed here that the presence of this ant is reducing the occurrence of some of these organisms in tank, possibly because *O. hastatus* is capturing prey and converting them into nest debris. This nest debris can be a source of nutrient for *Q. arvensis* roots (Leroy *et al.* 2013; see Chapter 1), contributing to bromeliad nutrition and growth. In a previous experiment (see Chapter 1), enriched prey offered to *O. hastatus*, *G. moelleri* and *C. crassus* showed that bromeliads derived approximately 19% of nitrogen from the first ant species. These results suggest that *O. hastatus* colonies play an important role in bromeliad nutrition through plant roots, but radically alter the community of metazoans occurring in the tank.

Quesnelia arvensis with *C. crassus* colonies showed higher concentrations of chlorophyll and ammonium, and higher turbidity and CDOM in the tank water. These results suggest that *C. crassus* did not affect the occurrence of microorganisms in the phytotelmata and did not alter the aquatic ecosystem functioning, while *O. hastatus* seemed to reduce the activity of microorganisms. In fact, we observed the greatest flow of nitrogen from detritus to bromeliads in the presence of *C.*

crassus colonies. Chlorophyll concentration in the water of bromeliads is directly associated with algae biomass in the tank (Moulton *et al.* 2009; Marino *et al.* 2011), while ammonium, turbidity and CDOM are associated with decomposition and mineralization of the organic matter by microorganisms (Inselsbacher *et al.* 2007; Brezonik *et al.* 2015). Since microorganism activities release nutrients and bromeliads are capable of absorbing nutrients through foliar trichomes (Benzing 2000), *C. crassus* may benefit bromeliads through the tank water over long time frames.

In conclusion, we showed for the first time that ant directly affects aquatic ecosystems (i.e., *O. hastatus*). While *O. hastatus* seems to reduce the diversity of metazoans, the decomposition of organic matter and nitrogen flow from detritus to the bromeliad *Q. arvensis*, it seems to contribute nutritionally to its host plants by nest debris. On the other hand, *C. crassus* did not affect the diversity of metazoans and seemed to allow a higher microbial activity in the tank, allowing the aquatic ecosystem functioning.

Acknowledgments

We thank Dr. Paula Omena, Bárbara Mamede, Jennifer Tezuka, Fátima Ruiz, Adriano Mendonça, Monica Paiano and Auro Filho for help with field experiment and samplings. This study was supported by the FAPESP scholarship to A.Z. Gonçalves [2011/10137-8], and by FAPESP and CNPq research grants from G.Q. Romero.

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Table 1. Linear mixed-effect (LME) and Permutation Multivariate Analyses of Variance (PERMANOVA) summarizing the effects of treatments (bromeliads with *Odontomachus hastatus* nests, epiphytic control without ants, with *Gnamptogenys moelleri* nests, with *Camponotus crassus* nests and terrestrial control without ants) in the density, richness, composition and density within functional groups of aquatic metazoans of *Quesnelia arvensis* at Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil

Source of variation	df	MS	*F*	R^2	*P*
Density	4	-	70.28	-	**<0.001**
Richness	4	-	21.40	-	**<0.001**
Composition					
Treatments	4	0.92	6.02	0.34	**<0.001**
Residuals	45	0.15		0.65	
Total	49			1.00	
Density within functional groups					
Treatments	4		84.56		**<0.001**
Functional groups	4		170.98		**<0.001**
*Treatments*functional groups*	16				**<0.001**

Table 2. Linear mixed-effect (LME) summarizing the effects of treatments (bromeliads with *Odontomachus hastatus* nests, epiphytic control without ants, with *Gnamptogenys moelleri* nests, with *Camponotus crassus* nests and terrestrial control without ants) in the total nitrogen concentration, total chlorophyll concentration, relative growth rate and abundance of ^{15}N of *Quesnelia arvensis* leaves, and detritus mass loss of *Eugenia uniflora* (Myrtaceae) leaves after three months on bromeliad tanks at Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil

Source of variation	Treatments		
	df	F	P
Total N concentration	4	40.28	**<0.001**
Total chlorophyll concentration	4	87.81	**<0.001**
Relative growth rate	4	38.57	**<0.001**
Detritus mass loss	4	24.20	**<0.001**
Abundance of ^{15}N (δ^{15}N)	4	19.24	**<0.001**

Table 3. Linear mixed-effect (LME) summarizing the effects of treatments (bromeliads with *Odontomachus hastatus* nests, epiphytic control without ants, with *Gnamptogenys moelleri* nests, with *Camponotus crassus* nests and terrestrial control without ants) in the temperature, pH, concentration of oxygen, concentration of chlorophyll, turbidity, colored dissolved organic matter (CDOM) and concentration of ammonium in the tank of the experimental bromeliad *Quesnelia arvensis* at Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil

Source of variation	Treatment			Time			Treatment*time		
	df	F	P	df	F	P	df	F	P
Temperature	4	11.15	**<0.001**	2	58.79	**<0.001**	8	1.27	0.261
pH	4	11.44	**<0.001**	2	81.05	**<0.001**	8	8.66	**<0.001**
Concentration of O_2	4	15.20	**<0.001**	2	372.51	**<0.001**	8	1.37	0.213
Concentrations of	4	11.37	**<0.001**	2	93.59	**<0.001**	8	1.03	0.414
Turbidity	4	21.33	**<0.001**	2	79.08	**<0.001**	8	1.99	0.052
CDOM	4	16.57	**<0.001**	2	39.63	**<0.001**	8	0.45	0.885
Concentration of ammonium	4	7.75	**<0.001**	2	11.81	**<0.001**	8	0.70	0.688

Figure legends

Fig 1. (A) Density (n°/mL), (B) richness (n°/mL), (C) nonmetric multidimensional scaling plot (NMDS) of the composition of aquatic metazoans, and (D) density of aquatic metazoans within each functional group in the tank of the experimental bromeliad *Quesnelia arvensis* with the following treatments: with *Odontomachus hastatus* nests, epiphytic control without ants, with *Gnamptogenys moelleri* nests, with *Camponotus crassus* nests and terrestrial control without ants at Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil. The error bar indicate SE and different letters indicate statistical differences between treatments (ANOVA/Tukey HSD *post-hoc* test, $\alpha = 0.05$).

Fig 2. (A) Total nitrogen concentration, (B) total chlorophyll concentration and (C) relative growth rate of the experimental bromeliad *Quesnelia arvensis* with the following treatments: with *Odontomachus hastatus* nests, epiphytic control without ants, with *Gnamptogenys moelleri* nests, with *Camponotus crassus* nests and terrestrial control without ants at Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil. The error bar indicate SE and different letters indicate statistical differences between treatments (ANOVA/Tukey HSD *post-hoc* test, $\alpha = 0.05$).

Fig 3. (A) Detritus mass loss of *Eugenia uniflora* (Myrtaceae) leaves after three months on bromeliad tanks and (B) abundance of ^{15}N stable isotope ($\delta^{15}N$‰) of the experimental bromeliad *Quesnelia arvensis* with the following treatments: with *Odontomachus hastatus* nests, epiphytic control without ants, with *Gnamptogenys moelleri* nests, with *Camponotus crassus* nests and terrestrial control without ants at Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil. The error bar indicate SE and different letters indicate statistical differences between treatments (ANOVA/Tukey HSD *post-hoc* test, α = 0.05).

Fig 4. (A) Temperature, (B) pH, (C) concentration of oxygen, (D) concentration of chlorophyll, (E) turbidity, (F) colored dissolved organic matter (CDOM) and (G) concentration of ammonium in the tank of the experimental bromeliad *Quesnelia arvensis* with the following treatments: with *Odontomachus hastatus* nests, epiphytic control without ants, with *Gnamptogenys moelleri* nests, with *Camponotus crassus* nests and terrestrial control without ants at Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil. Measurements were taken at the beginning, in the middle (45 days) and at the end of the experiment. The error bar indicate SE and different letters indicate statistical differences among treatments within each experimental time (ANOVA/Tukey HSD *post- hoc* test, α = 0.05).

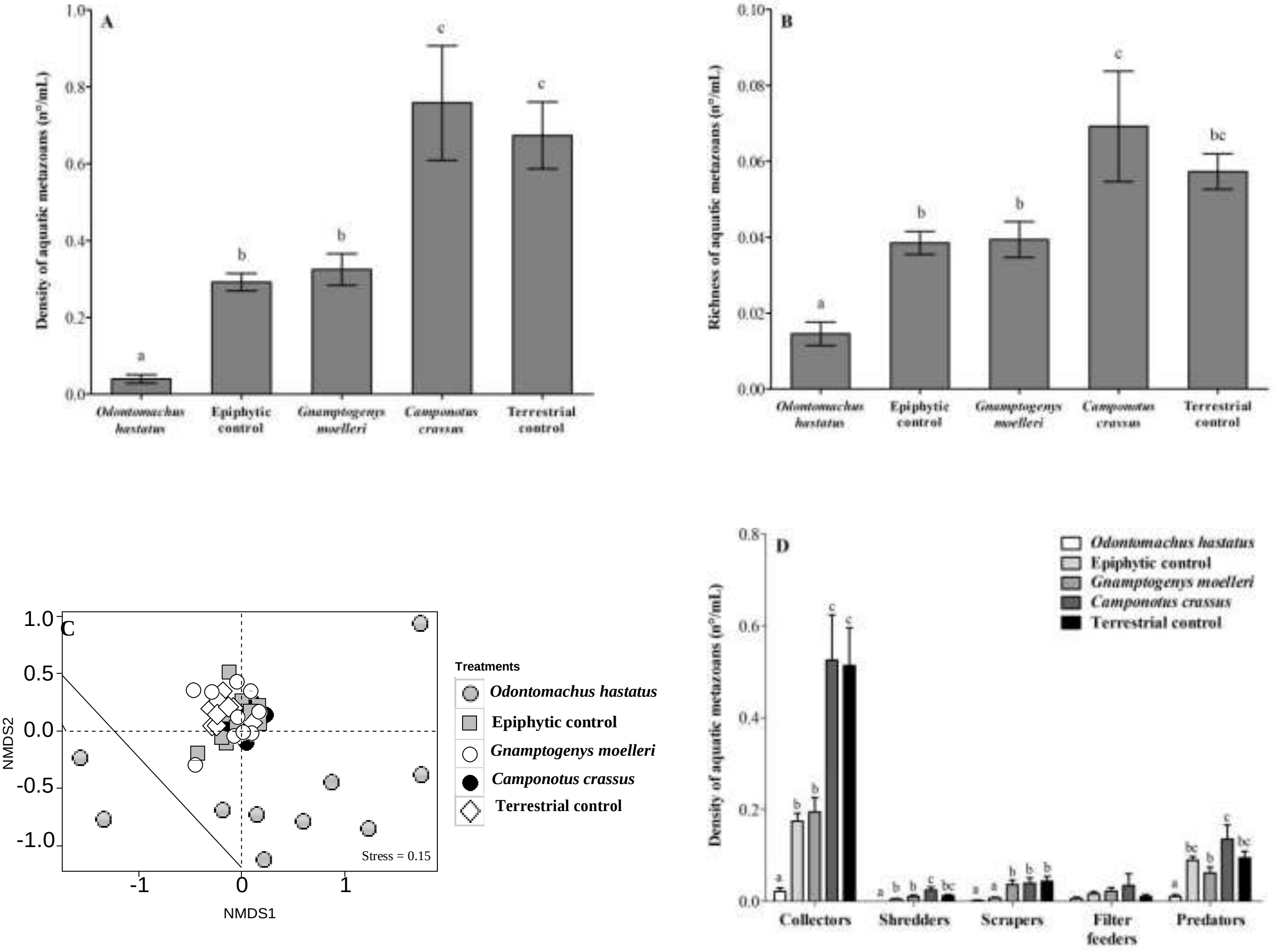
A
Density of aquatic metazoans (n°/mL)
0.0
0.2
0.4
0.6
0.8
1.0
a
b
b
c
c
Odontomachus hastatus
Epiphytic control
Gnamptogenys moelleri
Camponotus crassus
Terrestrial control
B
Richness of aquatic metazoans (n°/mL)
0.00
0.02
0.04
0.06
0.08
0.10
a
b
b
c
bc
Odontomachus hastatus
Epiphytic control
Gnamptogenys moelleri
Camponotus crassus
Terrestrial control
C
NMDS2
NMDS1
1.0
0.5
0.0
-0.5
-1.0
-1
0
1
Stress = 0.15
Treatments
Odontomachus hastatus
Epiphytic control
Gnamptogenys moelleri
Camponotus crassus
Terrestrial control
D
Density of aquatic metazoans (n°/mL)
0.0
0.2
0.4
0.6
0.8
Odontomachus hastatus
Epiphytic control
Gnamptogenys moelleri
Camponotus crassus
Terrestrial control
Collectors
Shredders
Scrapers
Filter feeders
Predators

Fig. 1.

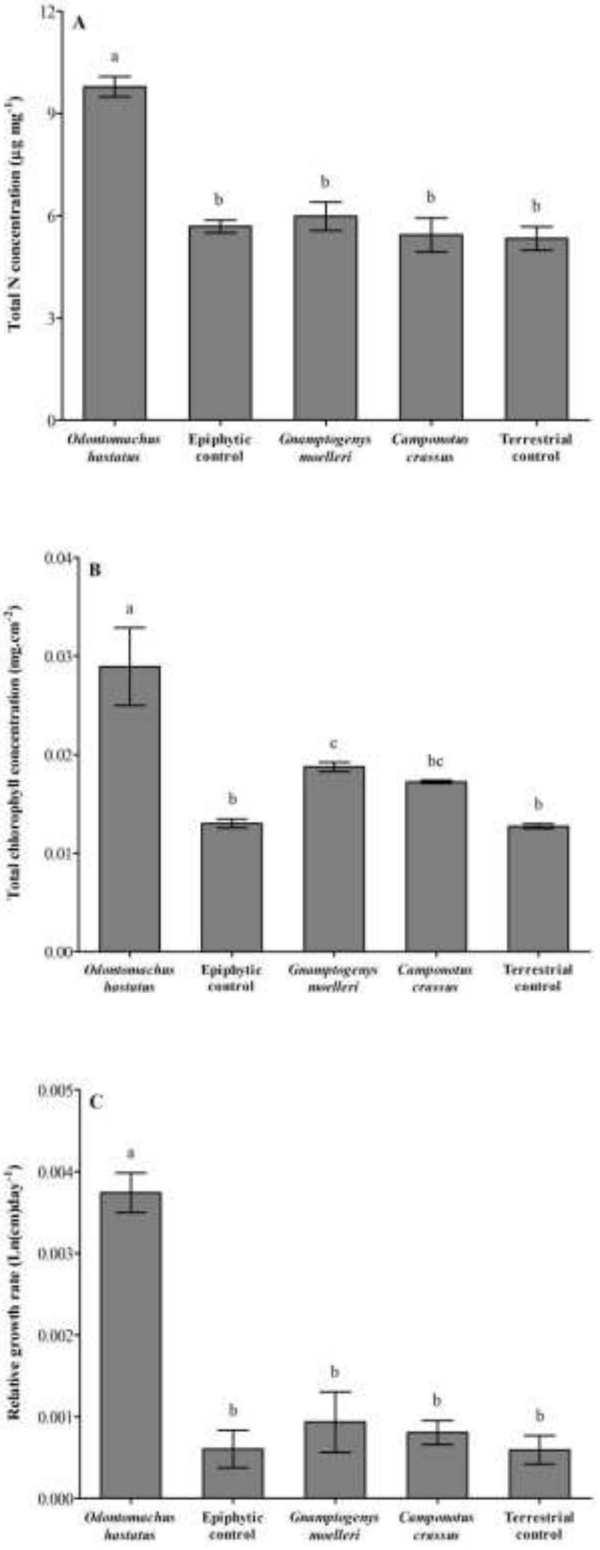
A
Total N concentration (µg mg⁻¹)
12
9
6
3
0
a
b
b
b
b
Odontomachus hastatus
Epiphytic control
Gnamptogenys moelleri
Camponotus crassus
Terrestrial control
B
Total chlorophyll concentration (mg.cm⁻²)
0.04
0.03
0.02
0.01
0.00
a
b
c
bc
b
Odontomachus hastatus
Epiphytic control
Gnamptogenys moelleri
Camponotus crassus
Terrestrial control
C
Relative growth rate (Ln(cm)day⁻¹)
0.005
0.004
0.003
0.002
0.001
0.000
a
b
b
b
b
Odontomachus hastatus
Epiphytic control
Gnamptogenys moelleri
Camponotus crassus
Terrestrial control

Fig. 2.

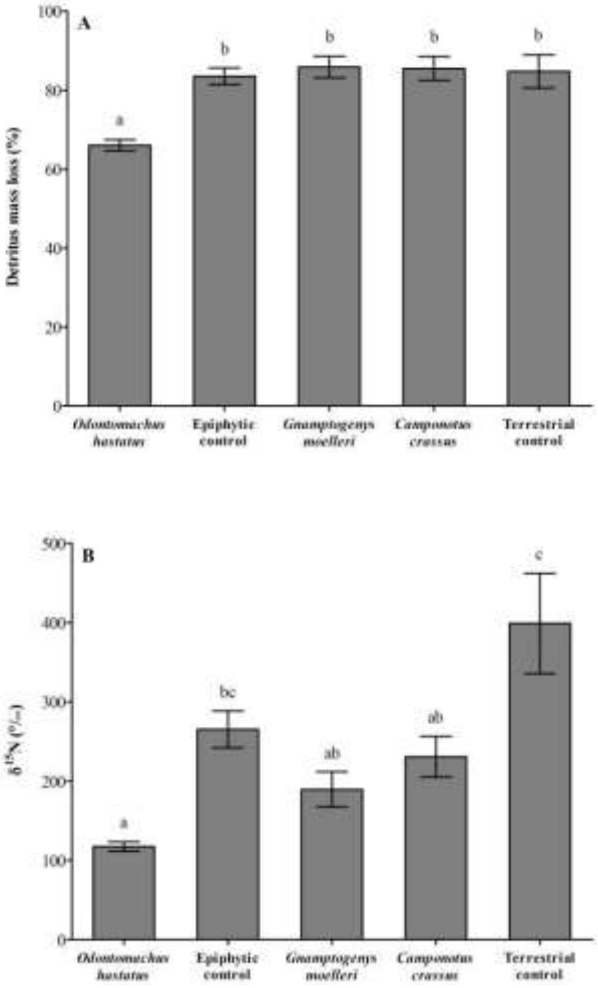
A
Detritus mass loss (%)
0
20
40
60
80
100
a
b
b
b
b
Odontomachus hastatus
Epiphytic control
Gnamptogenys moelleri
Camponotus crassus
Terrestrial control
B
δ15N (‰)
0
100
200
300
400
500
a
bc
ab
ab
c
Odontomachus hastatus
Epiphytic control
Gnamptogenys moelleri
Camponotus crassus
Terrestrial control

Fig. 3.

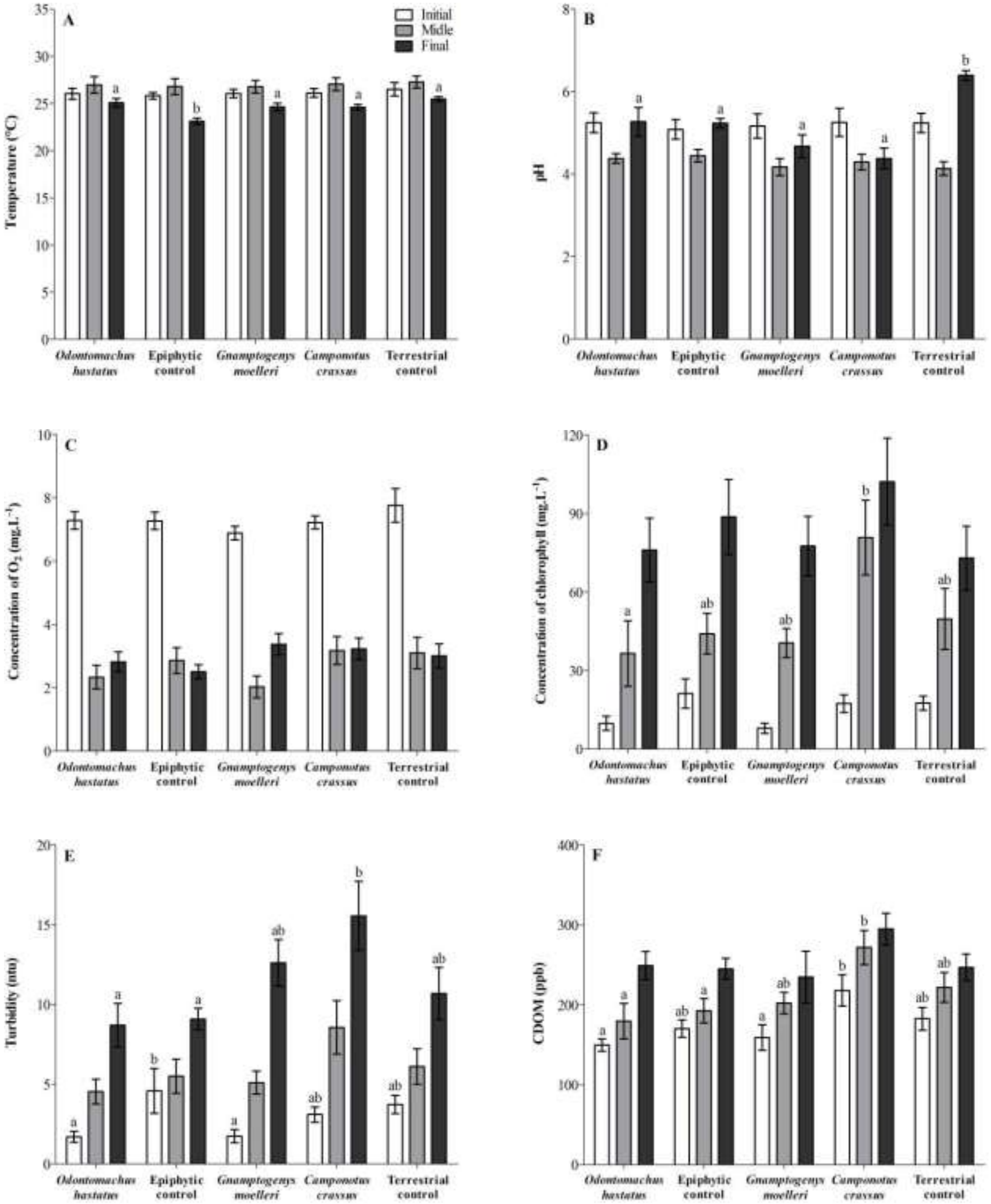
A
Initial
Midle
Final
Temperature (ºC)
0
5
10
15
20
25
30
35
a
b
a
a
a
B
pH
0
2
4
6
8
a
a
a
a
b
C
Concentration of O₂ (mg.L⁻¹)
0
2
4
6
8
10
D
Concentration of chlorophyll (mg.L⁻¹)
0
30
60
90
120
a
ab
ab
b
ab
E
Turbidity (ntu)
0
5
10
15
20
a
a
b
a
a
ab
ab
b
ab
ab
F
CDOM (ppb)
0
100
200
300
400
a
a
ab
a
a
ab
b
b
ab
ab
Odontomachus hastatus
Epiphytic control
Gnamptogenys moelleri
Camponotus crassus
Terrestrial control

Fig. 4.

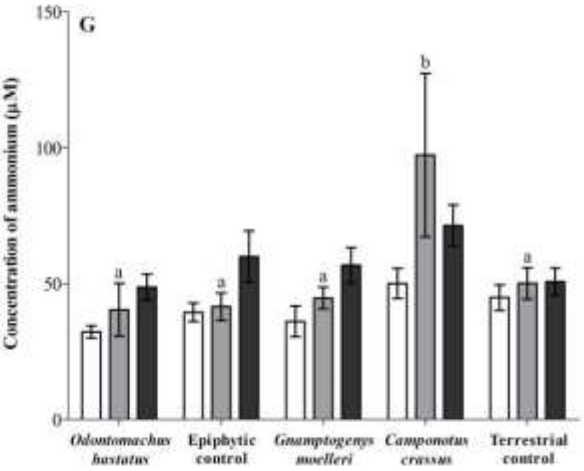
G
Concentration of ammonium (µM)
150
100
50
0
a
a
a
b
a
Odontomachus hastatus
Epiphytic control
Gnamptogenys moelleri
Camponotus crassus
Terrestrial control

Fig. 4.

Full paper

Contrasting effects of ant identity on diversity and functioning of aquatic bromeliad ecosystems

Ana Z. Gonçalves, Paulo S. Oliveira and Gustavo Q. Romero

Supporting information Table S1. Abundance of aquatic metazoans collected in *Quesnelia arvensis* bromeliads from the experiment (treatments: bromeliads with *Odontomachus hastatus* nests, epiphytic control without ants, with *Gnamptogenys moelleri* nests, with *Camponotus crassus* nests and terrestrial control without ants), and field sampling with and without colonies of *G. moelleri* and *C. crassus* at Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil. Metazoans were identified to the lowest possible taxonomic level, to their classes, orders or families, and were separated in functional groups

Identification	Class, Order, Family	Functional group	Experiment	Field sampling
Atrichopogon sp. 1	Ceratopogonidae	Collector	1	32
Atrichopogon sp. 2	Ceratopogonidae	Collector	258	20
Atrichopogon sp. 3	Ceratopogonidae	Collector	1	1
Atrichopogon sp. 4	Ceratopogonidae	Collector	41	52
Cecidomyiidae sp. 1	Cecidomyiidae	Predator	5	0
Cecidomyiidae sp. 2	Cecidomyiidae	Predator	3	1
Cecidomyiidae sp. 3	Cecidomyiidae	Predator	1	1
Cecidomyiidae sp. 6	Cecidomyiidae	Predator	9	6
cf. Helobdella sp.	Glossiphonidae	Predator	275	74
Chironomus detriticula	Chironomidae	Collector	0	378

Corethrella fulva	Corethrellidae	Predator	6	7
Corethrella infuscata	Corethrellidae	Predator	13	23
Corethrella sp.2	Corethrellidae	Predator	1	1
Corethrella sp.4	Corethrellidae	Predator	6	2
Corynoneura sp.	Chironomidae	Collector	10	19
Culicidae	Culicidae	Filter feeder	281	79
Dolychopodidae sp. 1	Dolichopodidae	Predator	9	15
Dytiscidae	Dytiscidae	Predator	2	8
Elpidium bromeliarum	Cyheroidae	Collector	983	84
Empididae sp. 1	Empididae	Predator	3	1
Ephydridae sp. 2	Ephydridae	Collector	25	29
Forcipomyia sp.	Ceratopogonidae	Collector	1	6
Hydrophyllidae	Hydrophyllidae	Collector	5	74
Leptagrion andromache	Coenagrionidae	Predator	206	136
Leptagrion elongatun	Coenagrionidae	Predator	2	0
Lymnophies sp.	Chironomidae	Collector	4	27
Monopelopia caraguata	Chironomidae	Predator	544	28
Olbiogaster sp.	Anisopodidae	Collector	7	6
Oligochaeta sp. 1	Tubificidae	Collector	315	30
Orthocladinae sp. 1	Chironomidae	Collector	341	100
Phylloicus bromeliarum	Calamoceratidae	Shredder	2	21
Polypedilum kaingang	Chironomidae	Collector	70	75
Polypedilum marcondesi	Chironomidae	Collector	1700	44
Polypedilum sp. 3	Chironomidae	Collector	3	0
Psychodidae sp. 1	Psychodidae	Collector	2	1
Psychodidae sp. 2	Psychodidae	Collector	1	0
Psychodidae sp. 3	Psychodidae	Collector	4	20
Psychodidae sp. 4	Psychodidae	Collector	1	2
Psychodidae sp. 5	Psychodidae	Collector	63	7
Sciaridae sp. 1	Sciaridae	Shredder	21	30

Sciaridae sp. 2	Sciaridae	Shredder	7	0
Scirtes sp. 1	Scirtidae	Scraper	0	4
Scirtes sp. 2	Scirtidae	Scraper	376	78
Syrphidae sp. 1	Syrphidae	Scraper	1	45
Syrphidae sp. 3	Syrphidae	Scraper	3	0
Tabanidae sp. 1	Tabanidae	Predator	21	15
Tabanidae sp. 2	Tabanidae	Predator	1	0
Tanytarsus sp.	Chironomidae	Collector	59	16
Tipulidae sp. 2	Tipulidae	Shredder	4	14
Trentepohlia sp. 1	Limoniidae	Shredder	109	52

Table S2. Abundance of terrestrial metazoans collected during field samplings in *Quesnelia arvensis* bromeliads with and without colonies of *G. moelleri* and *C. crassus* at Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil. Metazoans were identified to the lowest possible taxonomic level, to their classes, orders or families, and were separated in guilds

Identification	**Guilds**	**Field sampling**
Acari	Omnivore	14
Anura	Predator	0
Araneae	Predator	58
Auchenorrhyncha	Phytophages	1
Blattodea	Omnivore	71
Chilopoda	Predator	12
Coleoptera	Omnivore	8
Collembola	Detritivore	42
Diplopoda	Detritivore	7
Diptera (adult)	Omnivore	4
Ephemeroptera	-	3
Formicidae	Omnivore	63
Gryllidae	Omnivore	10
Hemiptera	Phytophages	4
Isopoda	Detritivore	14
Lepidoptera	Phytophages	36
Neuroptera	Predator	4
Staphylinidae	Omnivore	12
Thysanoptera	Phytophages	6
Tingidae	Phytophages	5
Trentepohlia (adult)	Phytophages	0
Vespidae	Predator	6

Table S3. Analyses of variance (ANOVA) summarizing the effects of the presence of *Gnamptogenys moelleri* and *Camponotus crassus* ants in the density and richness of aquatic and terrestrial metazoans of *Quesnelia arvensis* bromeliads at Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil

Source of variation	Ants			Aquatic/Terrestrial			Ants* Aquatic/Terrestrial		
	df	F	P	df	F	P	df	F	P
Gnamptogenys moelleri									
Density	1	79.68	**<0.001**	1	263.53	**<0.001**	1	55.11	**<0.001**
Richness	1	36.59	**<0.001**	1	148.72	**<0.001**	1	16.00	**<0.001**
Camponotus crassus									
Density	1	5.49	**0.024**	1	32.24	**<0.001**	1	4.11	**0.047**
Richness	1	33.18	**<0.001**	1	107.16	**<0.001**	1	14.07	**<0.001**

Table S4. Permutation Multivariate Analyses of Variance (PERMANOVA) summarizing the effects of the presence of *Gnamptogenys moelleri* and *Camponotus crassus* ants in the composition of aquatic and terrestrial metazoans of *Quesnelia arvensis* bromeliads at Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil

Source of variation	df	MS	*F*	R^2	*P*
Gnamptogenys moelleri					
Aquatic metazoans					
Ants	1	0.73	3.84	0.17	**<0.001**
Residuals	18	0,19		0.82	
Total	19			1.00	
Terrestrial metazoans					
Ants	1	0.20	1.09	0.05	0.383
Residuals	18	0.18		0.94	
Total	19			1.00	
Camponotus crassus					
Aquatic metazoans					
Ants	1	0.58	2.33	0.11	**0.008**
Residuals	18	0.25		0.88	
Total	19			1.00	
Terrestrial metazoans					
Ants	1	0.24	0.88	0.04	0.564
Residuals	18	0.27		0.95	
Total	19			1.00	

Table S5. Analyses of variance (ANOVA) summarizing the effects of the presence of *Gnamptogenys moelleri* and *Camponotus crassus* ants in the density within each aquatic functional group (collectors, shredders, scrapers, filter feeders and predators) and within each terrestrial guild (detritivores, phytophages, omnivores and predators) of *Quesnelia arvensis* bromeliads at Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil

Source of variation	df	MS	*F*	*P*
Gnamptogenys moelleri				
Aquatic metazoans				
Ants	1	0.01	68.02	**<0.001**
Functional groups	4	0.00	78.48	**<0.001**
Ants*functional groups	4	0.00	11.10	**<0.001**
Error	90	0.00		
Terrestrial metazoans				
Ants	1	0.01	4.47	**0.037**
Guilds	3	0.00	8.04	**<0.001**
Ants*guilds	3	0.00	1.16	0.327
Error	72	0.00		
Camponotus crassus				
Aquatic metazoans				
Ants	1	0.07	9.01	**0.003**
Functional groups	4	0.01	29.39	**<0.001**
Ants*functional groups	4	0.00	2.47	**0.049**
Error	90	0.00		
Terrestrial metazoans				
Ants	1	0.01	1.67	0.199
Guilds	3	0.00	5.13	**0.002**
Ants*guilds	3	0.00	0.73	0.533
Error	72	0.00		

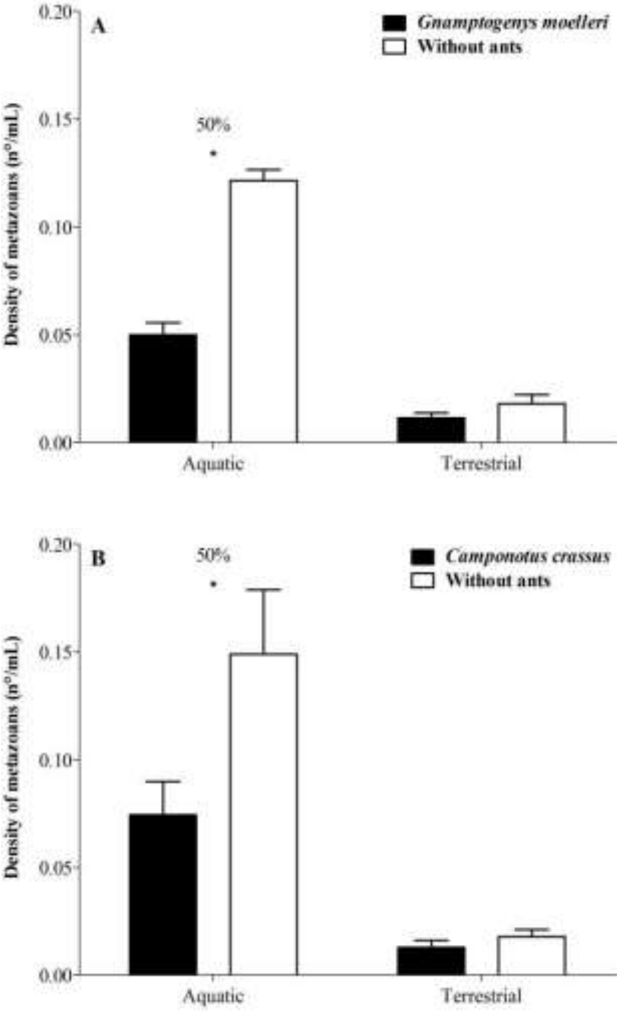

Fig. S1. Density of aquatic and terrestrial metazoans (n°/mL) in the tank of *Quesnelia arvensis* bromeliads collected in the field with (A) *Gnamptogenys moelleri* nests, (B) *Camponotus crassus* nests and bromeliads without ants at Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil. The error bar indicates SE and asterisks (*) indicate statistical differences between treatments (ANOVA/Tukey HSD *post-hoc* test, α = 0.05). Percentage values (%) indicate differences between the mean density of metazoans in bromeliads without ants compared to bromeliads with ants.

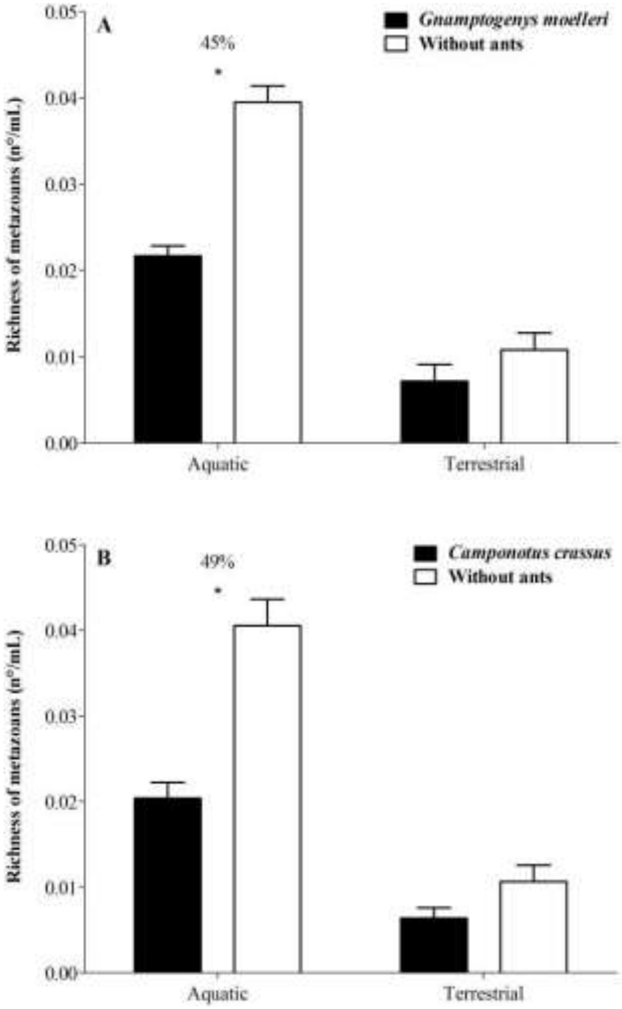

Fig. S2. Richness of aquatic and terrestrial metazoans (n°/mL) in the tank of *Quesnelia arvensis* bromeliads collected in the field with (A) *Gnamptogenys moelleri* nests, (B) *Camponotus crassus* nests and bromeliads without ants at Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil. The error bar indicates SE and asterisks (*) indicate statistical differences between treatments (ANOVA/Tukey HSD *post-hoc* test, α = 0.05). Percentage values (%) indicate differences between the mean richness of metazoans in bromeliads without ants compared to bromeliads with ants.

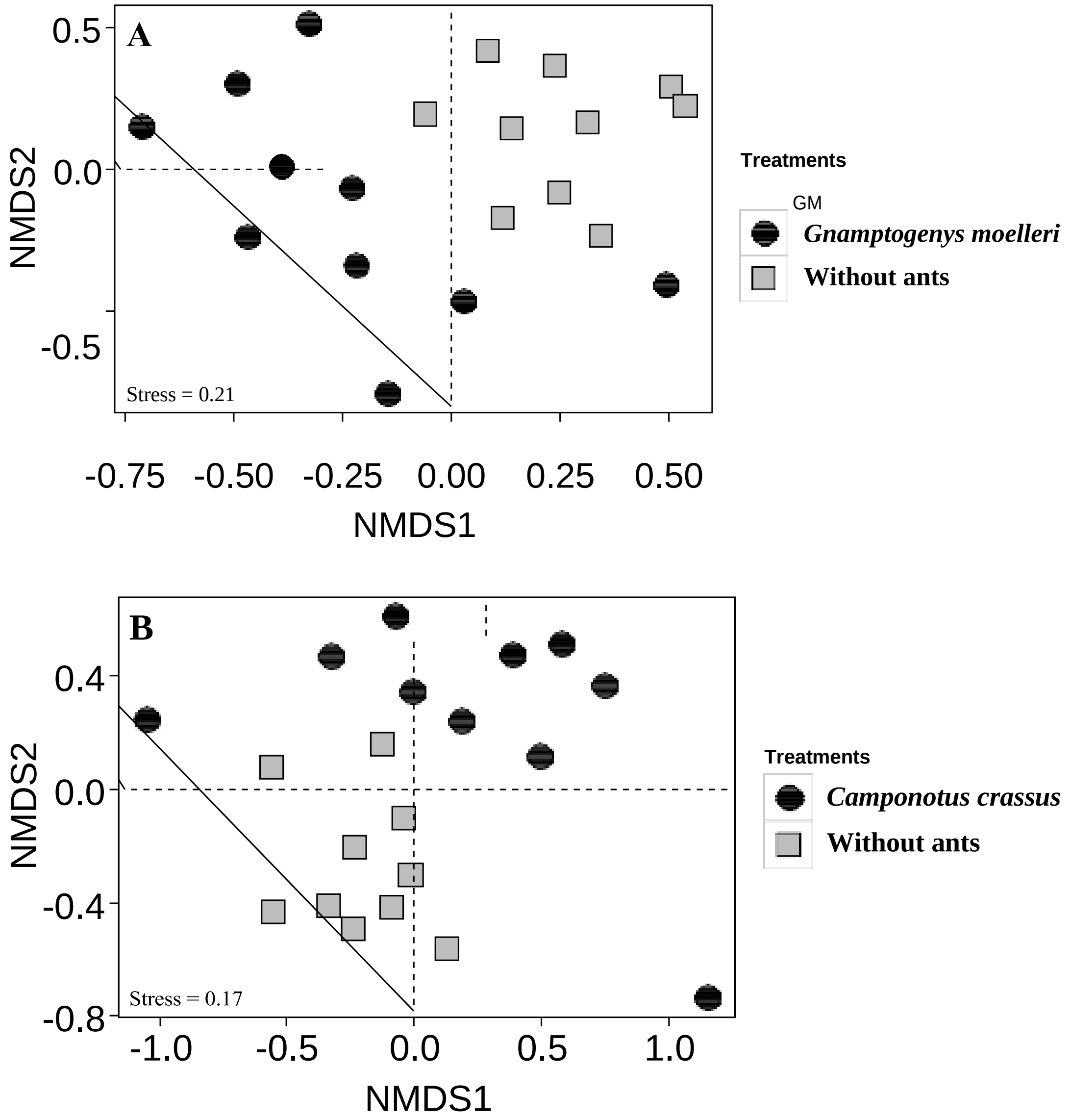

Fig. S3. Nonmetric multidimensional scaling plot (NMDS) of the composition of aquatic metazoans in the tank of *Quesnelia arvensis* bromeliads collected in the field with (A) *Gnamptogenys moelleri* nests, (B) *Camponotus crassus* nests and bromeliads without ants at Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil.

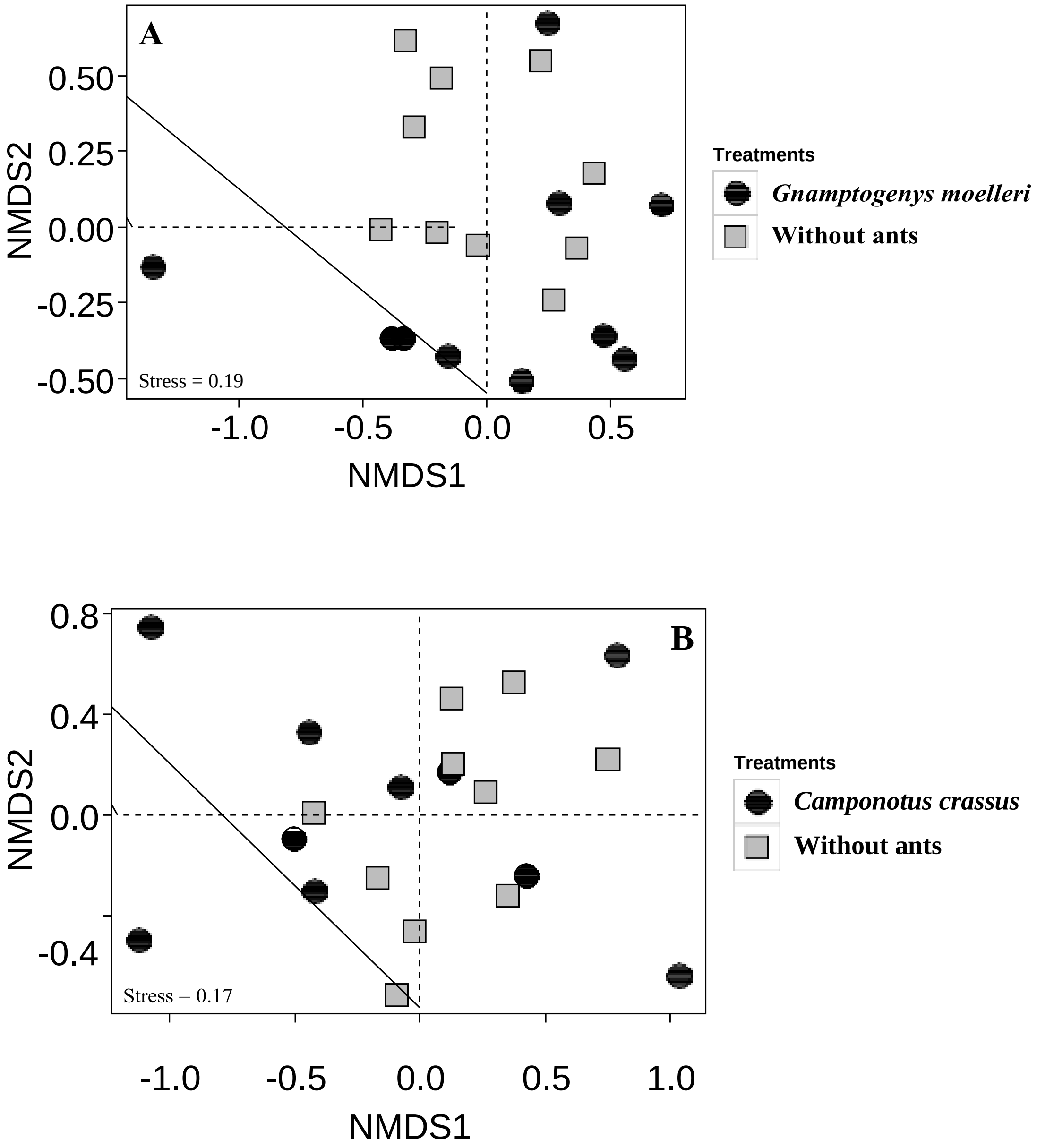

Fig. S4. Nonmetric multidimensional scaling plot (NMDS) of the composition of terrestrial metazoans in the tank of *Quesnelia arvensis* bromeliads collected in the field with (A) *Gnamptogenys moelleri* nests, (B) *Camponotus crassus* nests and bromeliads without ants at Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil.

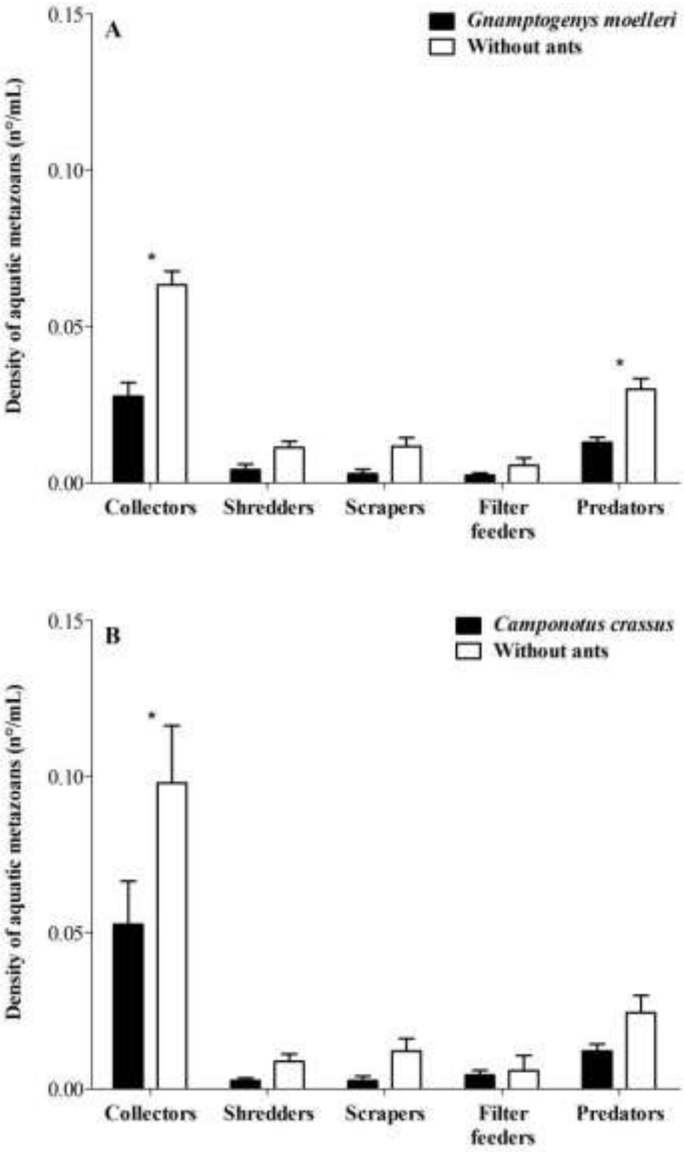

Fig. S5. Density of aquatic metazoans within each functional group in the tank of *Quesnelia arvensis* bromeliads collected in the field with (A) *Gnamptogenys moelleri* nests, (B) *Camponotus crassus* nests and bromeliads without ants at Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil. The error bar indicates SE and asterisks (*) indicate statistical differences between treatments (ANOVA/Tukey HSD *post-hoc* test, $\alpha = 0.05$).

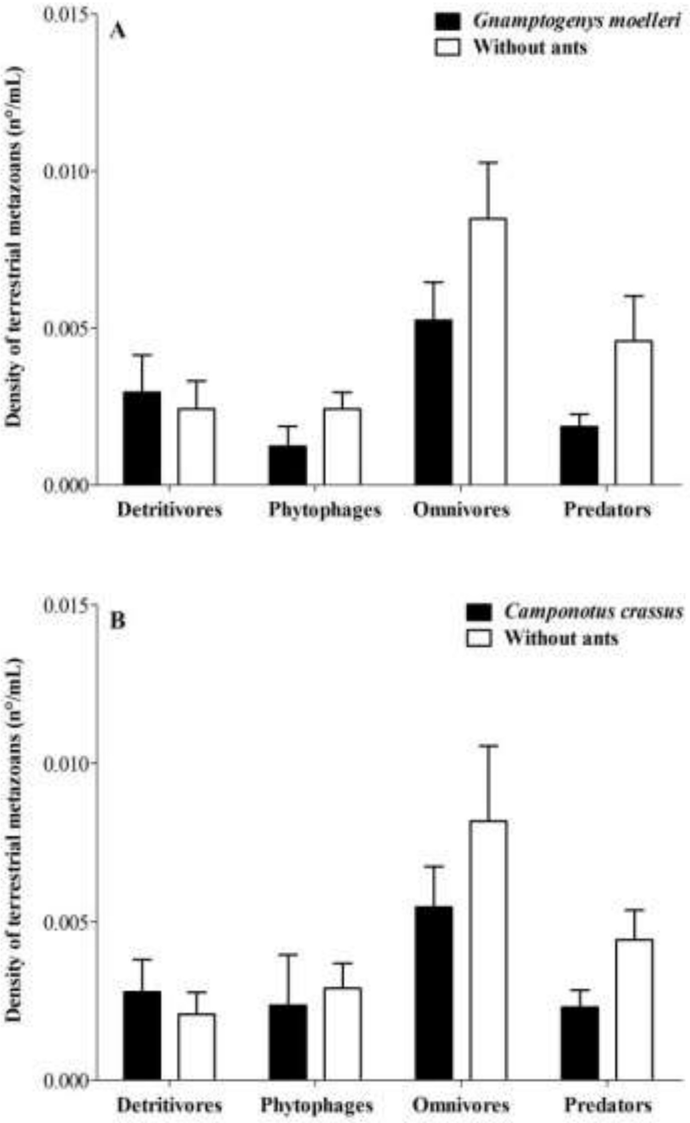

Fig. S6. Density of terrestrial metazoans within each guild in the tank of *Quesnelia arvensis* bromeliads collected in the field with (A) *Gnamptogenys moelleri* nests, (B) *Camponotus crassus* nests and bromeliads without ants at Parque Estadual da Ilha do Cardoso, São Paulo State, Brazil. The error bar indicates SE and asterisks (*) indicate statistical differences between treatments (ANOVA/Tukey HSD *post-hoc* test, α = 0.05).

SÍNTESE

No primeiro capítulo, como esperado, mostramos que as formigas que capturam mais presas devido ao seu maior tamanho corporal (i.e., *O. hastatus*) e que têm uma dieta basicamente proteica (i.e., *O. hastatus* e *G. moelleri*) foram as espécies que mais contribuíram para a nutrição e desenvolvimento de suas bromélias hospedeiras. Este resultado foi reforçado no terceiro capítulo, uma vez que apenas as bromélias experimentais com *O. hastatus* apresentaram maiores crescimento e concentração total de clorofila em suas folhas. Nossos resultados mostram experimentalmente, pela primeira vez, que formigas podem contribuir para a nutrição e atributos fisiológicos de suas bromélias hospedeiras. Esta contribuição é extremamente relevante para estas plantas que podem ocorrer em ambientes pobres em nutrientes, como ambientes epifíticos e o solo das restingas.

No segundo capítulo mostramos que *O. hastatus* reduziu a densidade de metazoários aquáticos de *V. procera* em três localidades distintas de Mata. Ainda, esta espécie de formiga alterou a composição de metazoários terrestres, mas não afetou a densidade ou riqueza destes organismos. Especificamente, *O. hastatus* reduziu as densidades de Ostracoda, *Polypedilum* sp., Hirudinea, Muscidae, Isopoda e outras espécies de formigas. Estes resultados, novamente, foram consistentes com os resultados obtidos no terceiro capítulo. Afetando Ostracoda e Hirudinea, organismos que se movimentam de uma bromélia para outra de forma passiva (i.e., foresia) na pele de anuros, sugere que *O. hastatus* inibe a visitação de anuros nas bromélias com ninhos. Ainda, reduzindo as densidades de detritívoros coletores aquáticos (i.e., Ostracoda e *Polypedilum* sp.) e detritívoros terrestres (Isopoda), *O. hastatus* pode prejudicar o processamento da matéria orgânica no ecossistema bromelícola.

De fato, observamos no terceiro capítulo que *O. hastatus* reduziu o processamento da matéria orgânica no tanque das bromélias, prejudicando o fluxo de nitrogênio dos detritos para as bromélias por meio dos tricomas foliares. Por outro lado, a presença de ninhos de *C. crassus* nas bromélias não alterou a diversidade de metazoários aquáticos e parece aumentar o processamento da matéria orgânica devido às maiores concentrações de turbidez, matéria orgânica dissolvida e amônio nestas bromélias. Ainda, C. crassus contribuiu para uma maior obtenção de nitrogênio pelas bromélias via detritos do tanque, possivelmente por meio dos tricomas foliares.

Apêndice I

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DECLARAÇÃO

Em observância ao **§5º do Artigo 1º da Informação CCPG-UNICAMP/001/15**, referente a Bioética e Biossegurança, declaro que o conteúdo de minha Tese de Doutorado, intitulada "***Formigas em bromélias: efeitos em cascata sobre a diversidade de artrópodes, ciclagem de nutrientes e ecofisiologia das plantas hospedeiras***", desenvolvida no Programa de Pós-Graduação em Ecologia do Instituto de Biologia da Unicamp, não versa sobre pesquisa envolvendo seres humanos, animais ou temas afetos a Biossegurança.

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Data: 25 de novembro de 2015

Apêndice II

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Presidente
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