

Effects of predatory ants within and across ecosystems in bromeliad food webs

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Summary

1. Predation is one of the most fundamental ecological processes affecting biotic communities. Terrestrial predators that live at ecosystem boundaries may alter the diversity of terrestrial organisms, but they may also have 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 colonies among roots of *Vriesea procera*, an epiphytic bromeliad species with water-filled tanks that shelters many terrestrial and aquatic organisms. Ants may impact terrestrial communities and deter adult insects from ovipositing in the water of bromeliads via consumptive and non-consumptive effects. Ants do not forage within the aquatic environment; thus, they may be more efficient predators on terrestrial organisms. Therefore, we predict that ants will have stronger effects on terrestrial than aquatic food webs. However, such effects may also be site contingent and depend on the local composition of food webs.

3. To test our hypothesis, we surveyed bromeliads with and without *O. hastatus* colonies from three different coastal field sites in the Atlantic Forest of southeast Brazil, and quantified the effect of this predatory ant on the composition, density and richness of aquatic and terrestrial metazoans found in these bromeliads.

4. We found that ants changed the composition and reduced the overall density of aquatic and terrestrial metazoans in bromeliad ecosystems. However, effects of ants on species diversity were contingent on site. In general terms, the effects of the ant on aquatic and terrestrial metazoan communities were similar in strength and magnitude. Ants reduced the density of virtually all aquatic functional groups, especially detritivore insects as well as metazoans that reach bromeliads through phoresy on the skin of terrestrial animals (i.e. Ostracoda and *Helobdella* sp.).

5. Our results suggest that the cross-ecosystem effect of this terrestrial predator on the aquatic metazoans was at least as strong as its within-ecosystem effect on the terrestrial ecosystem, and demonstrates that the same predator can simultaneously initiate cascades in multiple ecosystems.

Key-words: Bromeliaceae, composition, cross-ecosystem, diversity, *Odontomachus hastatus*, richness, *Vriesea procera*, within-ecosystem

Introduction

Ecological research has increasingly focused on how top-down and bottom-up effects structure ecological

communities and change ecosystem function (Power 1990; McIntosh *et al.* 2005). Top-down effects occur when higher trophic levels limit the abundance of lower trophic levels through consumption or the threat of consumption (Power 1992; Preisser, Bolnick & Bernard 2005). Bottom-up effects occur in autotrophic systems in which plant densities control the higher trophic levels, or in detritus-based

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food webs where the inputs of allochthonous 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 in tank bromeliads (Farjalla *et al.* 2016). While previous research has addressed the role of aquatic predators on detrital food webs, considerably less attention has been given to the direct and indirect effects of terrestrial predators on detritus-based food webs in aquatic systems.

Predators can have lethal effects on their prey, reducing their numerical abundance, and such consumptive effects of predators can propagate downwards in the form of trophic cascades [density-mediated interactions (DMI) Hill & Weissburg 2013]. Non-consumptive effects of predators alter prey behaviour, morphology and habitat use [trait-mediated interactions (TMI) Werner & Peacor 2003], and can also induce trophic cascades. In a meta-analysis, Preisser, Bolnick & Bernard (2005) showed that DMI and TMI exert similar direct impact on prey, but when considering the cascading effects of predators, TMI were responsible for 85% of changes in the food chain. Trophic cascades not only affect the biomass of lower trophic levels, but can also alter diversity (Schmitz 2003) and ecosystem functions (Ngai & Srivastava 2006; Schmitz 2008; Atwood *et al.* 2013; Hammill, Atwood & Srivastava 2015).

The effects of predators may also cross ecosystem boundaries (Knight *et al.* 2005). Many animals have life cycles in more than one ecosystem (e.g. holometabolous insects, amphibians and marine invertebrates; Werner 1988), and the predators that feed on such 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 larval abundance of aquatic dragonflies, and consequently, the adult abundance of this insect, reducing dragonfly predation on terrestrial pollinators and increasing pollination and plant fitness. Fish are also known to induce strong trophic cascades within the aquatic ecosystem (Devlin *et al.* 2015), but to the best of our knowledge, there is no literature comparing the effects of the same predator on aquatic and terrestrial ecosystems. Here, we contrast the top-down effects of a terrestrial predator on terrestrial and aquatic prey communities.

Tank bromeliads are suitable systems to study how terrestrial and aquatic metazoans cross ecosystem boundaries (Romero & Srivastava 2010). The arrangement of their leaves in a rosette creates a complex detrital-based aquatic system that shelters bacteria, algae, protists, terrestrial and aquatic invertebrates and vertebrates (Benzing 2000). Many ant species in the Neotropics use bromeliads as shelters and nest sites, and the interaction between ants and bromeliads can even be species-specific (Huxley 1980; Dejean, Olmsted & Snelling 1995; Blüthgen *et al.* 2000). In the Atlantic Forest, the predatory ant *Odontomachus hastatus* establishes its colonies mainly in the roots of

epiphytic tank bromeliads, and most workers forage near their host bromeliads (Oliveira, Camargo & Fourcassie 2011; Rodrigues & Oliveira 2014). Since the diet of *O. hastatus* is predominantly composed of arthropods (e.g. Diptera, Lepidoptera, Hemiptera, Coleoptera, Arachnida and other ants; Camargo & Oliveira 2012), and some of these organisms may have life cycles that span both the terrestrial and aquatic bromeliad ecosystems, *O. hastatus* may alter the structure of both terrestrial and aquatic communities.

In this study, we sampled bromeliads with and without *O. hastatus* colonies in three distinct field sites in southeastern Brazil, and we identified all metazoans that were in the aquatic and terrestrial parts of the bromeliad ecosystem. The two motivating research questions are: (i) do ants change the composition, density and richness of bromeliad inhabitants? (ii) If so, are the effects of predatory ants as strong on the aquatic as terrestrial ecosystem? We predict that the effect of *O. hastatus* ants will be stronger on terrestrial than aquatic food webs because, being a terrestrial predator, ants will be in contact with terrestrial prey for longer, thus increasing the encounter frequency with these prey. Consequently, ant presence can trigger both stronger consumptive and non-consumptive responses on terrestrial prey communities. We predict a weaker magnitude effect of ants on aquatic food webs because ants do not forage within aquatic environments, thus they have no direct access to aquatic prey. In addition, ants may deter adult insects from ovipositing in the water of bromeliads via non-consumptive effects, but since the ant activity is mostly nocturnal (Camargo & Oliveira 2012), these deleterious effects can be minimized by temporal displacements of prey and predator activities. However, the predicted effects may be site contingent and depend on the local food web composition.

Materials and methods

STUDY SITES AND ORGANISMS

We collected the epiphytic bromeliad *Vriesea procera* (Tillandsioideae, Bromeliaceae) with and without colonies of *O. hastatus* (Ponerinae, Formicidae) in restinga (sand-based) forest, a domain of the Atlantic Forest, Brazil, at three distinct sites covering the extent of the coast of São Paulo State: Parque Estadual da Serra do Mar, Núcleo Picinguaba (Picinguaba, 23°36'S, 44°836'W); Estação Ecológica Juréia-Itatins (Juréia, 24°25'S, 47°06'W); and Parque Estadual da Ilha do Cardoso (Cardoso, 25°04'S, 47°55'W). Picinguaba and Juréia are separated in distance by 440 km while Juréia and Cardoso are separated by 200 km.

The three field sites differ in their climate and the dominant bromeliad species, although all sites contain epiphytic *V. procera* bromeliads. The climate of Picinguaba is considered rainy-tropical, and it is humid throughout the year. Temperatures from September to March are 24 °C on average, dipping to an average of 20 °C in the period from April to August. Rainfall from September to March is 4000 mm, decreasing to 1500 mm on average from April to August (INPE/CPTEC 2015). The sand-based

forest of Picinguaba has flooded soil in the rainy season, and the forest canopy is less than 20 m high. The understory has terrestrial and epiphytic bromeliads with no dominant species. The climate of Juréia is classified as tropical hot and humid all the year, with temperatures exceeding 18 °C (September to March: 4700 mm rainfall on average, and from April to August, 1300 mm rainfall on average (INPE/CPTEC 2015). The sand-based forest at 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*. Unlike the previous two sites, Cardoso is marked by two seasons, a cold and less humid season (April–August, average temperature: 13 °C, average rainfall: 500 mm), and a hot and humid season (September–March, average temperature: 32 °C, average rainfall: 1800 mm). The sand-based forest on Cardoso 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, Camargo & Fourcassé 2011). *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 detrital collectors, detrital shredders, detrital scrapers, filter feeders and predators (see Gonçalves *et al.* 2017). In addition, bromeliads shelter numerous terrestrial organisms of different guilds, as detritivores, phytophages, omnivores and predators (see Gonçalves *et al.* 2017). Among vertebrates, anurans can use bromeliads temporarily or may depend on these plants for their survival and reproduction (Silva, Britto-Pereira & Caramaschi 1989).

The ant *O. hastatus* is arboreal, occurs from Central to South America, has twilight and nocturnal activity, and builds its colonies especially in the roots of epiphytic bromeliads (Kempf 1972; Gibernau *et al.* 2007; Oliveira, Camargo & Fourcassé 2011; Rodrigues & Oliveira 2014). It is a large, stinging ant species (\approx 1.3 cm in size and 7.89 ± 1.57 mg of biomass, mean \pm SE), with predatory and scavenger habits (Camargo & Oliveira 2012).

FIELD SAMPLING

In order to investigate the effect of *O. hastatus* on the composition, density (number of individuals per mL of ecosystem volume) and richness 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, nine bromeliads with and nine without ants were collected in December 2011 at Cardoso, and ten bromeliads with and ten without ants were collected in December 2013 at Juréia. Bromeliads and their roots were carefully inspected for the presence or absence of *O. hastatus* colonies before sampling. Once a bromeliad with an ant colony was found, another bromeliad without a colony was chosen within 8–10 m of the first, sufficient distance to avoid ant effects on the diversity of metazoans, but not sufficient to change environmental effects on metazoans. All bromeliads were collected from 1 to 1.5 m height from the ground.

All bromeliads in each location were collected on the same day, and we ensured that both collection days the preceding day were without rain. Only bromeliads with similar aquatic tank sizes were collected (aquatic ecosystem maximum volume, V_{TA} : Picinguaba: 481.4 ± 1.8 mL; Juréia: 490.1 ± 20.4 mL; Cardoso: 497.1 ± 10.9 mL; mean \pm SE) in order to avoid the influence of

the tank volume on the number of metazoans interacting with bromeliads. The total (i.e. aquatic and terrestrial) ecosystem volume (V_{TO}) was calculated assuming that bromeliads approximated a cone in volume ($V_{TO} = (\pi r^2 h)/3$), where r is half of the diameter of bromeliad rosettes and h is the height of the plant. We measured r directly in the field, whereas h was obtained using the formula for a triangle ($h^2 = T^2 - r^2$) in conjunction with field measurements of T , the length of the largest leaf of each bromeliad. From this calculation of V_{TO} (aquatic and terrestrial ecosystem volume) and our field measurements of V_{TA} (aquatic ecosystem maximum volume), we could calculate V_{TE} , the volume of the terrestrial ecosystem of bromeliads, as $V_{TE} = V_{TO} - V_{TA}$. The terrestrial ecosystem maximum volume (V_{TE}) of bromeliads from Picinguaba was: 1142.7 ± 9.5 mL; Juréia: 1099.9 ± 20.4 mL; and Cardoso: 1092.8 ± 10.9 mL.

After bromeliad measurements, all bromeliads were dissected and washed with distilled water in the field, and the debris and the tank water were collected in white trays and separated by two sieves (mesh diameter, 125 and 800 μ m). Each metazoan visible to the naked eye (>0.5 mm) was collected and preserved in 70% ethanol and we recorded abundances of each morphospecies. In laboratory, morphospecies were identified to the lowest taxonomic level, and assigned to functional group or guild based on the literature and direct observations (Merritt & Cummins 1996; Triplehorn & Johnson 2005). Aquatic metazoans were separated into functional groups (collectors, shredders, scrapers, filter feeders and predators), while the terrestrial metazoans were separated in guilds (detritivores, phytophages, omnivores and predators) (see Gonçalves *et al.* 2017).

STATISTICAL ANALYSES

The global effect of *O. hastatus* (considering the three field sites) on the composition of aquatic and terrestrial metazoans in bromeliads, and its effect within each site, were compared by 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. We visualized differences in composition between bromeliad communities using nonmetric multidimensional scaling (NMDS). As analyses of community composition require communities, we removed bromeliads that contained either no aquatic or no terrestrial metazoans from the respective multivariate analysis (Picinguaba: one bromeliad without terrestrial metazoans; Cardoso: one bromeliad without aquatic metazoans and five bromeliads without terrestrial metazoans). The global effect of *O. hastatus* in the density of aquatic and terrestrial metazoans at the three field sites was assessed with linear mixed effect models (*O. hastatus* presence was a fixed effect and field sites were the random effect). Following a significant effect of ants at the global level, we further explored this effect. The density of metazoans within each site and within each aquatic functional group or terrestrial guild were compared using ANOVA and Tukey HSD *post-hoc* test for pair-wise comparisons between bromeliads with and without ants. The species richness at each field site was estimated with a combined individual-based interpolation and extrapolation approach (Colwell *et al.* 2012). Extrapolating the species-accumulation curves (SAC) of the less complete samples (i.e. with a lower richness) instead of rarefying the more complete (i.e. more rich) allow us to make robust inferences comparing samples of equal completeness (Chao & Jost 2012; Colwell *et al.* 2012). The SAC analyses were configured at 999 bootstraps, and calculated using the iNEXT (Hsieh, Ma &

Chao 2013). All statistical analyses were conducted with the statistical platform R (R Core Team, 2014).

Results

The overall community composition of aquatic and terrestrial metazoans differed between *V. procera* bromeliads with and without *O. hastatus* ant colonies (Table 1). *Odontomachus hastatus* altered the community composition of both aquatic and terrestrial metazoans at Picinguaba and Juréia (Table 1, Fig. 1a, b, c and d). At Cardoso, ants also altered the aquatic community but had marginally insignificant effects on the terrestrial community composition (Table 1, Fig. 1e and f). In addition to

Table 1. Summary of the effects of *Odontomachus hastatus* ants on the composition of aquatic and terrestrial metazoans of *Vriesea procera* bromeliads at Parque Estadual da Serra do Mar (Picinguaba), Estação Ecológica Juréia-Itatins (Juréia) and Parque Estadual da Ilha do Cardoso (Cardoso), São Paulo State, Brazil. Ant effects were tested using PERMANOVA, first at the global scale (i.e., three sites, with site as a random effect), and then, following a significant global effect, at each site independently

Source of variation	d.f.	MS	F	R ²	P
Global analyses					
Aquatic metazoans					
Ants	1	1.07	3.15	0.05	<0.001
Residuals	56	0.33		0.94	
Total	57			1.00	
Terrestrial metazoans					
Ants	1	1.24	4.44	0.08	<0.001
Residuals	51	0.28		0.91	
Total	52			1.00	
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	
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	

Bold values indicate statistical significance ($P < 0.05$).

effects on composition, the presence of this ant species also reduced the overall density of aquatic and terrestrial metazoans in bromeliad tanks (Table 2). Aquatic metazoans were reduced by 74% at Picinguaba, by 70% at Juréia and by 56% at Cardoso (Table 2, Fig. 2a, c, and e). *Odontomachus hastatus* also reduced the density of terrestrial metazoans by 79% at Picinguaba and 85% at Juréia, but the reduction of 51% at Cardoso was marginally insignificant (Table 2, Fig. 2b, d, and f).

The effects of *O. hastatus* differed between functional groups and between field sites (Table S1). Specifically, at Picinguaba *O. hastatus* reduced the density of aquatic collectors (77%; e.g. Ostracoda, *Polypedilum* sp.) and shredders (86%; e.g. *Trentepohlia* sp.) (Fig. 2a, Gonçalves *et al.* 2017). Similarly, at Juréia, the ant not only reduced collectors (75%; e.g. Ostracoda, *Polypedilum* sp.), shredders (74%; e.g. Sciaridae), but also reduced scrapers (51%; e.g. *Scirtes* sp.) and aquatic predators (56%; e.g. *Helobdella* sp., *Monopelopia caraguata*) (Fig. 2c, Gonçalves *et al.* 2017). At Cardoso, *O. hastatus* reduced density of shredders (77%; e.g. *Phylloicus bromelium*, *Trentepohlia* sp.) and aquatic predators (74%; e.g. *Helobdella* sp., Muscidae) (Fig. 2e, Gonçalves *et al.* 2017). This predatory ant also reduced the density of terrestrial detritivores (77%; e.g. Collembola) and omnivores (91%; e.g. Formicidae) at Picinguaba, and the density of detritivores (83%; e.g. Isopoda, Collembola), phytophages (93%; e.g. Lepidoptera), omnivores (88%; e.g. Formicidae) and terrestrial predators (77%; e.g. Araneae) at Juréia (Fig. 2b and d, Gonçalves *et al.* 2017). On the other hand, *O. hastatus* did not significantly reduce any terrestrial guild at Cardoso, although all terrestrial guilds tended to be lower in bromeliads with ants (Fig. 2f, Gonçalves *et al.* 2017).

The presence of *O. hastatus* reduced the overall richness of aquatic metazoans but did not alter the overall richness of terrestrial metazoans in bromeliad tanks (Fig. 3a and b). At the site level, however, *O. hastatus* had very different effects. This ant species did not alter the richness of aquatic metazoans at Picinguaba but increased the richness of terrestrial metazoans in this locality (Fig. 3c and d). Conversely, ants reduced the richness of aquatic metazoans but did not alter the richness of terrestrial metazoans at Juréia (Fig. 3e and f). Finally, ants did not change the richness of either aquatic or terrestrial metazoans at Cardoso (Fig. 3g and h).

Discussion

Our results indicate that the predatory ant *O. hastatus* exerts a strong top-down effect on the detritus-based food webs of the bromeliad *V. procera*. In general, this ant species reduced the density and altered the composition of both aquatic and terrestrial metazoans. The effect of the ant on species diversity was more variable between ecosystems and sites. In all sites, ants had similarly strong and negative effects on aquatic as on terrestrial metazoan density (although the reduction in terrestrial density was

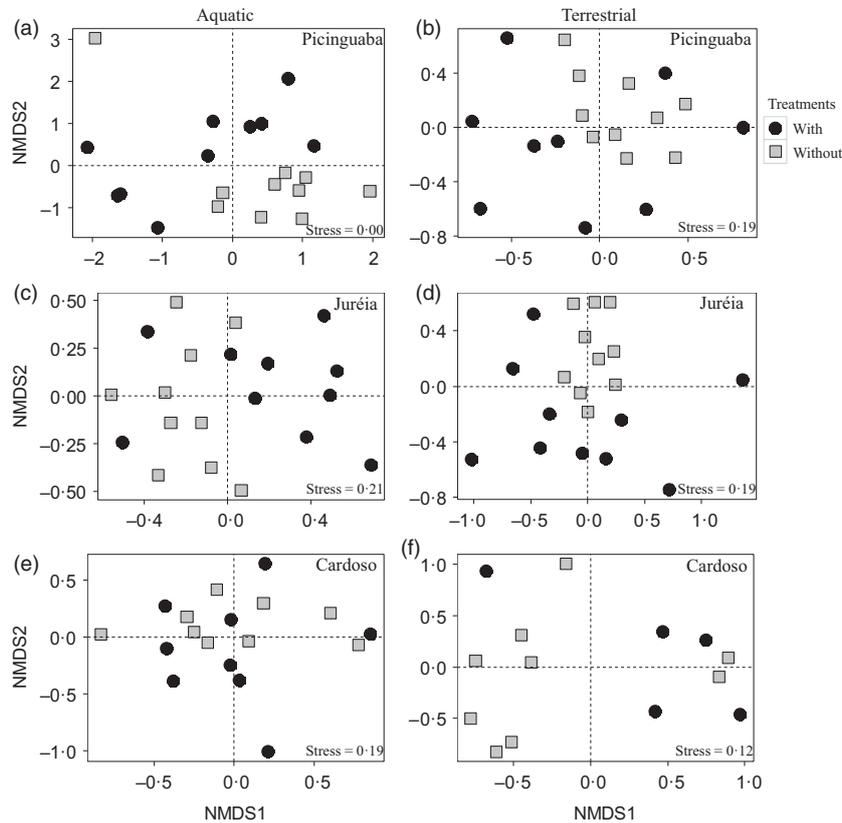


Fig. 1. The composition of aquatic and terrestrial metazoans in *Vriesea procera* bromeliads differs between those with and without *Odontomachus hastatus* colonies. (a) Aquatic and (b) terrestrial metazoan communities at Parque Estadual da Serra do Mar (Pinguaba), (c) aquatic and (d) terrestrial metazoan communities at Estação Ecológica Juréia-Itatins (Juréia), and (e) aquatic and (f) terrestrial metazoan communities at Parque Estadual da Ilha do Cardoso (Cardoso), São Paulo State, Brazil. The nonmetric multidimensional scaling (NMDS) plots attempt to display communities such that distances are proportional to their dissimilarity, with the stress value (lower right of each plot) indicating the degree of distortion in this process. The most abundant organisms found in Pinguaba were Culicidae (bromeliads with *Odontomachus* ants) and *Scirtes* sp.1 (Scirtidae, bromeliads without ants) in the aquatic ecosystem, and Araneae (bromeliads with ants) and non-*Odontomachus* Formicidae (bromeliads without ants) in the terrestrial ecosystem; in Juréia were *Scirtes* sp.2 (with ants) and *Polypedium marcondesi* (Chironomidae, without ants) in the aquatic ecosystem, and Isopoda (with ants) and Formicidae (without ants) in the terrestrial ecosystem; and in Cardoso were *Polypedium marcondesi* (with ants) and *Elpidium bromeliarum* (Cyberoidae, without ants) in the aquatic ecosystem, and Acari (with ants) and Araneae (without ants) in the terrestrial ecosystem.

marginally insignificant in Cardoso). These results are not in agreement with our hypothesis that the effect of terrestrial predators will be stronger on terrestrial than aquatic food webs. Instead, our findings provide evidence that the impact of terrestrial predators can cascade with similar strength across ecological boundaries.

Odontomachus hastatus altered the composition and reduced the overall density of aquatic and terrestrial metazoans in its host bromeliads. In fact, no metazoan species had higher density in bromeliads with ants. We assume that direct consumptive effects are responsible for much of the negative effects of *O. hastatus* ants on the abundance of terrestrial detritivores (e.g. Collembola, Isopoda), other ant species, spiders and lepidopterans. These taxa have previously been identified as among the preferred prey of *O. hastatus* (Spagna *et al.* 2008; Camargo & Oliveira 2012). Furthermore, it is known that most *O. hastatus* individuals hunt for prey on or near the bromeliad hosting their colony (Camargo & Oliveira 2012;

Rodrigues & Oliveira 2014), where there are high densities of invertebrates (Gonçalves-Souza *et al.* 2010).

The negative effect of *O. hastatus* ants on aquatic insects could be due to any combination of two mechanisms: direct consumption of ovipositing terrestrial adults and avoidance of bromeliads with ants by ovipositing adults. Since ants of this genus have fast 'trap-jaw' movements and good vision (Oliveira & Hölldobler 1989), *O. hastatus* has the potential to capture flying insects, reducing oviposition and therefore lowering the density of aquatic insect larvae in plants with ants. For example, most aquatic species in bromeliads are dipterans (Gonçalves *et al.* 2017), and *O. hastatus* is known to prey effectively on dipteran adults (Spagna *et al.* 2008; Camargo & Oliveira 2012). Even the threat of such predation by ants may deter insects from ovipositing in bromeliads.

Some of the aquatic organisms most affected by ants were Ostracoda and *Helobdella* sp. Here, consumption of ovipositing terrestrial adults cannot be relevant as these

Table 2. The effect of *Odontomachus hastatus* ants on the density of aquatic and terrestrial metazoans (number per mL of the bromeliad tank) in *Vriesea procera* bromeliads at Parque Estadual da Serra do Mar (Picinguaba), Estação Ecológica Juréia-Itatins (Juréia) and Parque Estadual da Ilha do Cardoso (Cardoso). Significance of ant effects was determined by linear mixed models at the global scale (i.e., three sites with site as a random effect), and then explored with ANOVA at each location separately

Source of variation	d.f.	MS	F	P
Global analyses				
Aquatic metazoans	1	–	41.43	<0.001
Terrestrial metazoans	1	–	28.82	<0.001
Picinguaba				
Aquatic metazoans	1	0.03	17.2	<0.001
Terrestrial metazoans	1	0.01	8.68	0.008
Juréia				
Aquatic metazoans	1	0.39	29.76	<0.001
Terrestrial metazoans	1	0.01	57.11	<0.001
Cardoso				
Aquatic metazoans	1	0.06	14.45	0.001
Terrestrial metazoans	1	0.01	4.72	0.054

Bold values indicate statistical significance ($P = 0.05$).

taxa have an exclusively aquatic life cycle. Instead, these two groups of organisms are thought to disperse among freshwater habitats using phoretic hosts like anurans and reptiles (Lopez, Rodrigues & Rios 1999; Tiberti & Gentilli 2010; Sabagh & Rocha 2014). As *O. hastatus* predominantly forages at night (Camargo & Oliveira 2012), like anurans, we suggest that ants are inhibiting anurans from visiting bromeliads, reducing phoretic colonization by Ostracoda and *Helobdella* sp. Alternatively, direct effects of ants on ovipositing insects could alter the interaction networks between aquatic insect larvae and other aquatic animals in a way that benefits the latter. Such a mechanism is not supported by a previous study on the effects of spiders on bromeliad invertebrates, which found that when spiders reduce aquatic insects, ostracods showed a competitive release rather than a decrease (Romero & Srivastava 2010). The final explanation, that ants directly consume Ostracoda and *Helobdella* sp., seems implausible as these latter two taxa are truly pelagic and the ant is not able to enter the bromeliad tanks to catch aquatic prey.

Virtually all aquatic functional groups and terrestrial guilds were affected by *O. hastatus*, but to varying degrees. Interestingly, aquatic filter feeders (largely mosquitoes) were not significantly reduced by ants in any site, suggesting that ants may cause a shift from the detritus-detrivore energy channel in bromeliad food webs to the micro-organism-filter feeder energy channel (Trzcinski *et al.* 2016). Ants also led to a restructuring of the relative abundances of species in all sites except Cardoso, but the direction of this effect differed between sites. In broad terms, ants tended to result in more even abundance distributions (i.e. steeper SAC in Fig. 3) in Picinguaba, but less even distributions in Juréia. The site contingency of ant effects on abundance distributions, despite a similar depression of densities

(except the Cardoso terrestrial metazoans, as detailed next), suggests that the susceptibility of metazoans to ant predation depends on small differences between sites in their species pool or habitat context.

Although *O. hastatus* ants altered the overall composition and density of terrestrial metazoans, they did not affect the terrestrial metazoans at Cardoso. We suggest that this context-dependence is due to differences between sites in both statistical power and habitat. Only five of the sampled bromeliads contained terrestrial metazoans at Cardoso vs. nine or ten at the other sites, reducing the effective sample size in analyses of community composition for Cardoso. The reduced statistical power may explain why ants had marginal effects on terrestrial composition at Cardoso ($P = 0.067$) despite appearing to influence composition in multivariate space (Fig. 1). We were able to include empty bromeliads in analyses of terrestrial metazoan density, but ant effect sizes at Cardoso were then constrained by the overall low density of terrestrial metazoans at this site, which we suspect again resulted in a marginally insignificant effect of ants at this site ($P = 0.054$). As to the ultimate reasons why terrestrial metazoan densities are low at Cardoso, we can only speculate. This site difference cannot be related to differences in the size of bromeliads between sites, as we were scrupulous in sampling bromeliads of similar size. Cardoso is much more seasonal than the other two sites, and it is conceivable that phenological effects are responsible for the low densities in December. Cardoso, unlike the other sites, also has a dense carpet of large *Q. arvensis* bromeliads in the understorey, which contain many more aquatic insect larvae per hectare than the epiphytic *Vriesea*. It is possible that terrestrial adult insects originating from the understorey bromeliads support or include high densities of terrestrial predators, which exert top-down control on the terrestrial detritivores on *Vriesea*. Supporting this hypothesis, we note that the Cardoso terrestrial metazoans are heavily weighted to predators and omnivores, unlike those at Juréia and Picinguaba.

The negative effects of *O. hastatus* ants on both aquatic and terrestrial metazoans may have implications for the functioning of both ecosystems. In Costa Rica, bromeliad-associated ants are responsible for reductions in leaf herbivory (Hammill, Corvalan & Srivastava 2014). Consumption of terrestrial detritivores, especially isopods, by predatory ants may also have conspicuous consequences. Terrestrial isopods are known to increase microbial activity and litter decomposition, leading to rapid mineralization of litter (Hassall, Turner & Rands 1987; Hättenschwiler & Bretscher 2001; Zimmer 2002; Bastow 2011). Similarly, reductions in aquatic detritivores may have cascading effects on litter decomposition (Srivastava & Bell 2009). Finally, since bromeliads obtain nutrients from aquatic and terrestrial decomposition (Ngai & Srivastava 2006), any reduction in decomposition may affect bromeliad nutrition. However, *O. hastatus* also contributes directly to bromeliad nutrition by

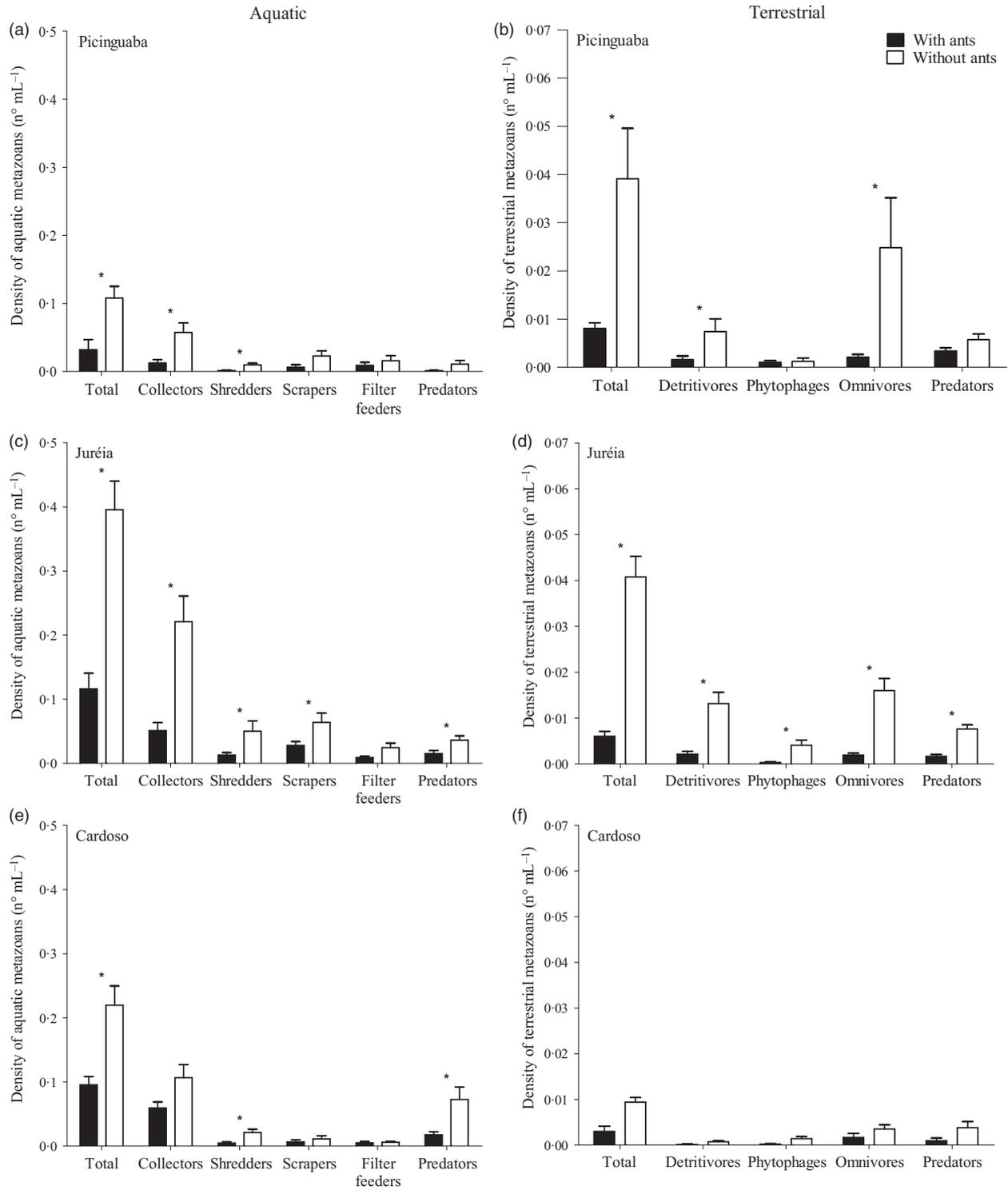
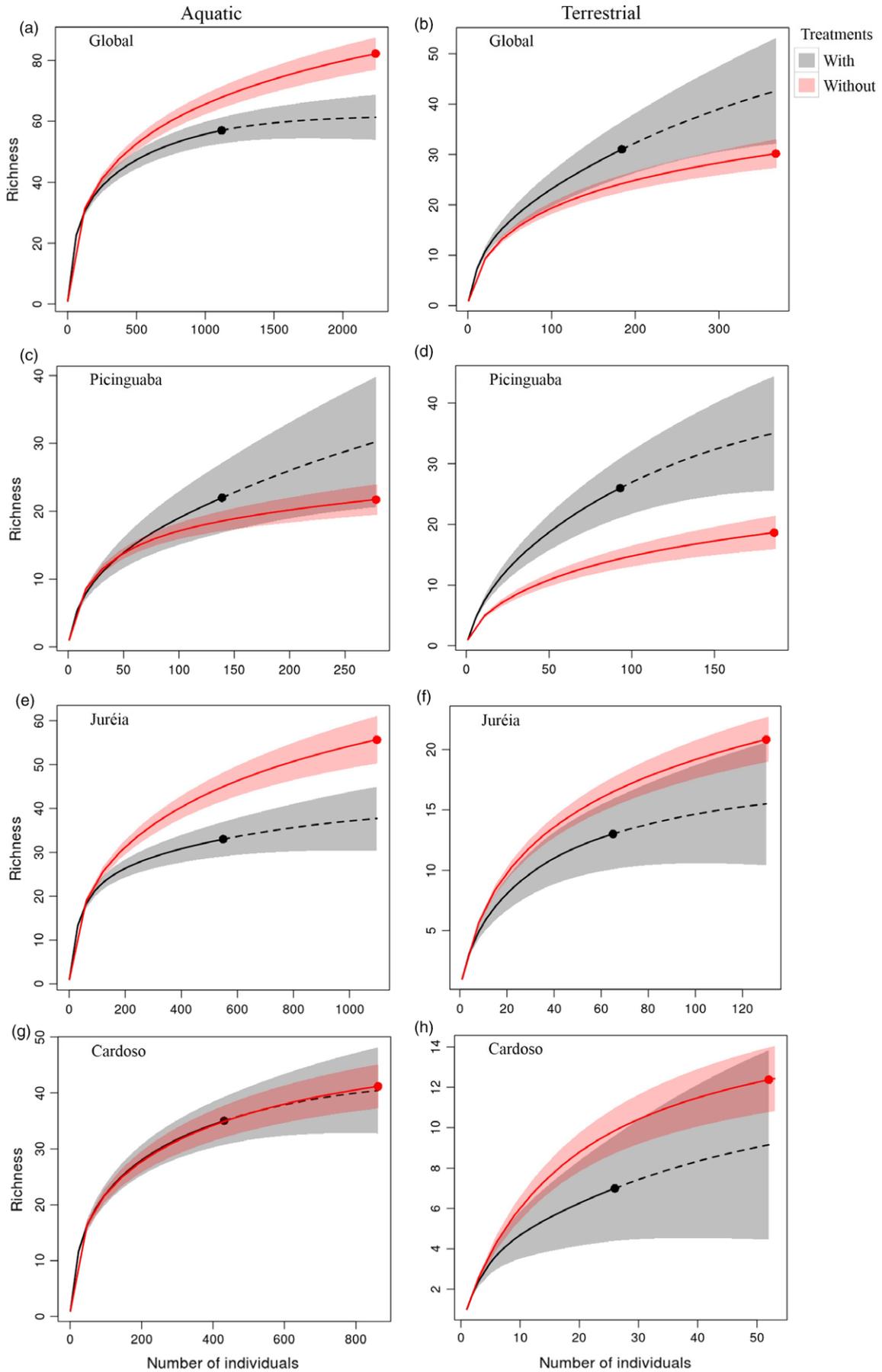


Fig. 2. The effect of *Odontomachus hastatus* colonies on the total density of aquatic and terrestrial metazoans, and the density of metazoans within each aquatic functional group or terrestrial guild (number per mL of the bromeliad tank) within *Vriesea procera* bromeliads at the three field sites (see Fig. 1 for abbreviations). Bars indicate SE and asterisks (*) indicate statistical differences between treatments (ANOVA/Tukey HSD *post-hoc* test, $\alpha = 0.05$).

Fig. 3. Rarefaction-based species accumulation curves for aquatic and terrestrial metazoans of *Vriesea procera* bromeliads, with and without *Odontomachus hastatus* ant colonies. Plots show species accumulation for the three field sites separately (see Fig. 1 for site abbreviations) as well as combined (global analyses). Curves were constructed based on interpolation and extrapolation approaches (see Materials and methods). Solid curves are the interpolated rarefaction curve while dashed curves were the extrapolated curve. Dots represent the sample number.



enriching the soil around the roots (Gonçalves *et al.* 2016). The ecosystem level effects of *O. hastatus* colonies in bromeliads remain an intriguing subject for future study.

Many studies have now highlighted the connections between the aquatic and terrestrial ecosystems in terms of flows of organisms, energy and nutrients, and most of these studies reveal how allochthonous subsidies connect terrestrial and aquatic environments (e.g. Roth *et al.* 2007; Bartels *et al.* 2012). Nonetheless, only a few studies have reported a link between ecosystems in terms of predators transmitting top-down effects through multiple trophic levels (Borer *et al.* 2008). For example, Knight *et al.* (2005) showed that the effect of aquatic predators on aquatic larvae can ultimately affect terrestrial plants, while Romero & Srivastava (2010) suggested that the effects of predatory spiders on flying insects can ultimately affect aquatic insect larvae in bromeliads. Here, our results indicate that the cross-ecosystem effects of *O. hastatus* on the aquatic organisms are at least as strong as its effects on the terrestrial metazoans.

In conclusion, our results demonstrate that *O. hastatus* changed the community structure of aquatic and terrestrial metazoans in food webs of *V. procera*, and that its effects on the aquatic food web were at least as strong as its effects on the terrestrial food web. The most likely explanation for this strong effect on aquatic ecosystems is a direct consumptive or trait-mediated effect of ants on some life stage of the aquatic organisms. Specifically, we suggest that *O. hastatus* might have a direct consumptive effect on other ant species, spiders, lepidopterans and isopods, a consumptive and/or a trait-mediated indirect effect on flying adults inhibiting oviposition in bromeliads and a trait-mediated indirect effect on frogs inhibiting phoresy of Ostracoda and *Helobdella* sp. Future studies are needed to show the consequences of predatory ants for the ecosystem functioning of bromeliad food webs.

Author's contributions

A.Z.G., D.S.S., P.S.O. and G.Q.R. conceived the ideas and designed the methodology; A.Z.G. collected the data; A.Z.G., D.S.S., P.S.O. and G.Q.R. analysed the data; A.Z.G., D.S.S., P.S.O. and G.Q.R. wrote the paper. All authors contributed critically to the drafts and gave final approval for publication.

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Data accessibility

Data available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.1v244> (Gonçalves *et al.* 2017).

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Supporting Information

Details of electronic Supporting Information are provided below.

Table S1. ANOVA summarizing the effects of the presence of *Odontomachus hastatus* ants on 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 Jurúia-Itatins (Jurúia) and Parque Estadual da Ilha do Cardoso (Cardoso), São Paulo State, Brazil.