LAILA FIETO RIBEIRO

EFFECTS OF FOOD RESOURCES ON ARBOREAL ANT COMMUNITIES IN BRAZILIAN SAVANNA

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Doctor Scientiae*.

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SUMÁRIO

RESUMO

RIBEIRO, Laila Fieto, D.Sc., Universidade Federal de Viçosa, fevereiro de 2017. **Effects of food resources on arboreal ant communities in Brazilian savanna.** Orientador: José Henrique Schoereder. Coorientadoras: Dalana Campos Muscardi e Tathiana Guerra Sobrinho.

A disponibilidade e qualidade de recursos alimentares estão entre os principais mecanismos que estruturam as comunidades. Um dos recursos mais importantes e explorados por formigas arborícolas são os nectários extraflorais (NEFs), porém, seu papel como mecanismo estruturador das comunidades ainda permanece não consolidado. Além disso, formigas arborícolas tendem a enfrentar um desequilíbrio nutricional por se alimentarem extensivamente de recursos ricos em carboidrato, carecendo de alimentos mais ricos em fontes proteicas. Dessa forma, a resposta da comunidade à composição nutricional dos recursos alimentares disponíveis no ambiente pode ser importante na previsão de padrões de diversidade e abundância. Esta tese teve como objetivo geral investigar a influência de recursos alimentares na estrutura da comunidade de formigas arborícolas no cerrado. No primeiro capítulo, nós investigamos o efeito da presença de NEFs na diversidade de formigas arborícolas em duas escalas, demonstrando como os padrões observados em árvores influenciam a comunidade em escala local, representada por parcelas. Formigas arborícolas foram amostradas em 32 parcelas de 20 x 20 m, nas quais a proporção de árvores com NEFs variou entre 0 a 60%. Nós observamos que árvores contendo NEFs possuem efeito na comunidade em escala local, influenciando a riqueza e composição de espécies de formigas, independentemente da densidade e riqueza de espécies de árvores. Nós também mostramos que, em escala local, a proporção de árvores contendo NEFs tem um efeito positivo na riqueza geral de formigas, primariamente devido a um aumento da ocorrência de espécies não centrais em redes de interação formiga-planta. No segundo capítulo, nós desenvolvemos um estudo manipulativo em ambiente natural, suplementando árvores com recursos ricos em proteína e carboidrato. Através deste experimento, visamos investigar se espécies de formigas com diferenças estequiométricas são atraídas por diferentes nutrientes, discutindo as implicações destas diferenças para a estrutura da comunidade. Considerando a comunidade como um todo, demonstramos que as formigas arborícolas foram mais fortemente atraídas pelos suplementos ricos em proteína, nutriente disponível em escassez no ambiente natural. Todavia, houve diferenças nas

respostas relacionadas à abundância, riqueza e composição de espécies de formigas entre os suplementos de proteína e carboidrato, sendo estas diferenças relacionadas à limitação de proteína das espécies. Por fim, nós também encontramos uma mudança na abundância de formigas forrageando nas árvores após a suplementação, sendo que as respostas a diferentes nutrientes também foram dependentes das diferenças estequiométricas entre espécies. Concluindo, nós demonstramos que árvores com NEFs representam um importante mecanismo na estruturação da comunidade local; e que devido às diferenças estequiométricas interespecíficas dentro da assembleia, o balanço de carboidrato e proteína presente nos recursos pode gerar padrões na distribuição e composição das espécies de formigas na comunidade arbórea.

ABSTRACT

RIBEIRO, Laila Fieto, D.Sc., Universidade Federal de Viçosa, February, 2017. **Effects of food resources on arboreal ant communities in Brazilian savanna.** Advisor: José Henrique Schoereder. Co-Advisors: Dalana Campos Muscardi and Tathiana Guerra Sobrinho.

The availability and quality of food resources are among the major mechanisms structuring animal communities. One of the most important and extensively explored food resources by arboreal ants are extrafloral nectaries (EFNs), but is contest their role as mechanism structuring communities and it is unclear how EFNs can affect the local diversity. Furthermore, arboreal ants tend to have high nutritional imbalanced in their diet due to feeding extensively on carbohydrate-rich resources and short supply of proteinaceous food. Therefore, the community response to the nutritional composition of food resources available in the environment might be important to predict patterns in diversity and abundance. This thesis aim to investigate the influence of food resources on arboreal ant community structure in Brazilian savanna, ecosystem that has an exceptional arboreal ant diversity and very high incidence of EFN-bearing trees. In the first chapter, we explored the effects of extrafloral nectaries on arboreal ant diversity at multiple scales, focusing on how patterns observed on individual trees influence ant communities at the site level. Arboreal ants were sampled in 32 plots, where the proportion of EFN-bearing trees ranged from 0 - 60%. We demonstrated that EFNs-bearing trees have a marked effect on communities of arboreal ants at the site scale, influencing species richness and composition, independently of overall tree density and richness. We also showed that the proportion of EFNs-bearing trees at site scale have a positive effect on overall ant species richness primarily through higher occurrence of non-core ant species in the ant-EFN network. In the second chapter, we performed a manipulative field experiment, supplementing trees with protein and CHO-rich resources. In this study, we investigated the variation in nutrient attractiveness among stoichiometrically contrasting arboreal ant species, and its implications for ant community structure. Considering the overall community, we demonstrated that arboreal ants were more attracted to supplements rich in protein, the scarcest nutrient on arboreal environment. However, we observed different responses in abundance, richness and composition of ant species between protein and CHO supplements, according to their discrepancies in N-limitation. Lastly, we also showed a change in abundance of

foraging ants after trees supplementation, but the responses to different nutrients were also dependent on stoichiometric discrepancies among species. In summary, we demonstrated that EFN-bearing trees represent an important mechanism to the structure of local arboreal ant community; and due to stoichiometric differences among ant species within the assembly, balances of carbohydrate and protein available in resources might generate patterns in distribution and composition of species in arboreal ant communities.

GENERAL INTRODUCTION

Ants dominate the invertebrate fauna in tropical canopies, being the most representative group in arboreal habitats and reaching up to 90% and 85% of abundance and biomass, respectively (Tobin 1995, Davidson and Patrell-Kim 1996, Floren and Linsenmair 2000). The striking abundance of canopy-dwelling ants is usually linked to the easy access to highly nutritious food resources, in addition to the predictability of such resources on arboreal habitat (Tobin 1991, 1994). These food resources are mainly represented by extrafloral nectaries (EFNs) and honeydew secreted by sap-feeding hemipterans, which are extensively consumed by arboreal ants, and in less extent, by exudates from lepidopteran larvae, plant wound secretions and pollen/fungal spores (Hölldobler and Wilson 1990, Baroni Urbani and de Andrade 1997, Davidson et al. 2004). All these plant-based resources are constituted predominantly by sugars, and have diluted concentrations of amino acids, lipids, phenols, alkaloids and volatile organic compounds (Blüthgen et al. 2004a, González-Teuber and Heil 2009). Therefore, the diet of arboreal ants is exceedingly biased to carbohydrate (CHO) intake, whereas they collect scant quantities of nitrogen from plant-derived resources and consequently experiencing a stoichiometric limitation of protein (Davidson 1997, Davidson et al. 2003).

While previous studies have documented the importance of nutrient ratios to ants' growth, metabolic process and reproduction (Grover et al. 2007, Kay et al. 2012, Wills et al. 2015), little attention has been given to the availability and composition of food resources to explain the distribution of arboreal ants (Gibb and Cunningham 2009, Powell et al. 2011). Traditionally, it has been considered that the major factors shaping arboreal ant community are cumulative outcomes of pairwise competitive interactions (Majer 1976, Blüthgen et al. 2004b, Blüthgen and Stork

2007, Dejean et al. 2010, Camarota et al. 2016), environmental attributes as vegetation complexity (Wang et al. 2001, Ribas et al. 2003, Lassau and Hochuli 2004, Powell et al. 2011, Leal et al. 2012, Klimes et al. 2015), or that ant community is randomly structured (Floren and Linsenmair 2000, Ribas and Schoereder 2002).

Furthermore, the particular role of EFNs as a mechanism driving arboreal ant distribution is still disputed. Some studies have reported that the availability of extra-floral nectar increases ant species richness (Lange et al. 2013, Belchior et al. 2016, Fagundes et al. 2016, Koch et al. 2016) and changes species composition (Camarota et al. 2015), while others have found a lack of response (Schoereder et al. 2010, Sendoya et al. 2016). Moreover, the studies that addressed this issue had focused on the effects of EFNs at the scale of an individual tree. Whether, and to what extent, effects on individual tree scale up to influence arboreal ant communities at larger spatial scales remains unknown.

On the other hand, a similar pattern has been reported with respect of a compartmentalization of ant species utilizing different types of food resources. Hemipteran honeydew resources are explored primarily by behaviourally dominant species, mainly exhibiting mass recruitment and foraging intensively to high-value food resources. Conversely, extra-floral nectar along with pollen, fungal spores and discarded honeydew are frequently consumed by ants species which forage solitarily and behave subordinately (Dejean et al. 2000, Blüthgen et al. 2004b, 2006, Gibb and Cunningham 2009, Fotso et al. 2015, Fagundes et al. 2016, Gadelha et al. 2016).

Beyond these related differences on diet and foraging behaviour, different arboreal species show discrepancies in their degree of protein limitation and adaptations to overcome the stoichiometric excess of CHO and shortage of protein in their diet (Blüthgen et al. 2003, Davidson et al. 2003, 2004). Such differences can be

expected to cause variation in preferences for protein over CHO among arboreal ant species, which consequently could induce niche partitioning within arboreal ant communities (Kay 2002, Blüthgen and Fiedler 2004). Considering that resources-ratio acquired might strongly influence ecological processes (Sterner and Elser 2002), a stoichiometric study encompassing experimental manipulation of different nutrients in a natural system might provide important insights about how different resources affect the structure of arboreal ant communities.

Thus, the focus of this thesis is to understand the influence of food resources on arboreal ant community structure in Brazilian savanna, ecosystem that has an exceptional arboreal ant diversity and very high incidence of EFN-bearing trees. Specifically, in the first chapter, we answer in what extent the occurrence of EFN-bearing trees drives arboreal ant diversity and composition at the site scale, independently of the vegetation structure. In the second chapter, we report on a manipulative field experiment using protein and CHO supplementation to investigate variation in nutrient attractiveness among stoichiometrically contrasting arboreal ant species, and its implications for ant community structure.

REFERENCES

Baroni Urbani, C., and M. L. de Andrade. 1997. Pollen Eating, Storing, and Spitting by Ants. Naturwissenschaften 84:256–258.

Belchior, C., S. F. Sendoya, and K. Del-Claro. 2016. Temporal variation in the abundance and richness of foliage-dwelling ants mediated by extrafloral nectar. PLoS ONE 11:1–17.

Blüthgen, N., and K. Fiedler. 2004. Competition for composition: Lessons from nectar-feeding ant communities Ecology 85:1479–1485.

Blüthgen, N., and Stork, N. E. 2007. Ant mosaics in a tropical rainforest in Australia and elsewhere: A critical review 32: 93–104.

Blüthgen, N., G. Gebauer, and K. Fiedler. 2003. Disentangling a rainforest food web using stable isotopes: Dietary diversity in a species-rich ant community. Oecologia 137:426–435.

Blüthgen, N., G. Gottsberger, and K. Fiedler. 2004a. Sugar and amino acid composition of ant-attended nectar and honeydew sources from an Australian rainforest. Austral Ecology 29:418–429.

Blüthgen, N., N. E. Stork, and K. Fiedler. 2004b. Bottom-up control and co-occurrence in complex communities: Honeydew and nectar determine a rainforest ant mosaic. Oikos 106:344–358.

Camarota, F., S. Powell, A. S. Melo, G. Priest, R. J. Marquis, and H. L. Vasconcelos. 2016. Co-occurrence patterns in a diverse arboreal ant community are explained more by competition than habitat requirements. Ecology and Evolution 6:8907–8918.

Camarota, F., S. Powell, H. L. Vasconcelos, G. Priest, and R. J. Marquis. 2015. Extrafloral nectaries have a limited effect on the structure of arboreal ant

communities in a neotropical savanna. Ecology 96:231–240.

Davidson, D. W. 1997. The role of resource imbalances in the evolutionary ecology of tropical arboreal ants. Biological Journal of the Linnean Society 61:153–181.

Davidson, D. W., S. C. Cook, and R. R. Snelling. 2004. Liquid-feeding performances of ants (Formicidae): Ecological and evolutionary implications. Oecologia 139:255–266.

Davidson, D. W., D. W. Davidson, S. C. Cook, R. R. Snelling, and T. H. Chua. 2003. Explaining the abundance of ants in lowland tropical rainforest canopies. Science 300:969–973.

Davidson, D. W., and L. Patrell-Kim. 1996. Tropical arboreal ants: Why so abundant? Neotropical biodiversity and conservation:127–140.

Dejean, A., B. L. Fisher, B. Corbara, R. Rarevohitra, R. Randrianaivo, B. Rajemison, and M. Leponce. 2010. Spatial distribution of dominant arboreal ants in a Malagasy coastal rainforest: Gaps and presence of an invasive species. PLoS ONE 5(2): e9319.

Dejean, A., D. McKey, M. Gibernau, and M. Belin. 2000. The arboreal ant mosaic in a Cameroonian rainforest (Hymenoptera: Formicidae). Sociobiology 35:403–420.

Fagundes, R., W. Dáttilo, S. P. Ribeiro, V. Rico-Gray, and K. Del-Claro. 2016. Food source availability and interspecific dominance as structural mechanisms of ant-plant-hemipteran multitrophic networks. Arthropod-Plant Interactions 10:207–220.

Floren, A., and K. E. Linsenmair. 2000. Do ant mosaics exist in pristine lowland rain forests? Oecologia 123:129–137.

Fotso, A. K., R. Hanna, M. Tindo, A. Doumtsop, and P. Nagel. 2015. How plants

and honeydew-producing hemipterans affect ant species richness and structure in a tropical forest zone. Insectes Sociaux 62:443–453.

Gadelha, Y. E. A., W. Dáttilo, O. Evangelista, and B. C. Lopes. 2016. Structure of mutualistic ant–treehopper interactions in the Brazilian Atlantic Forest. Journal of Tropical Ecology 32:250–259.

Gibb, H., and S. A. Cunningham. 2009. Does the availability of arboreal honeydew determine the prevalence of ecologically dominant ants in restored habitats? Insectes Sociaux 56:405–412.

González-Teuber, M., and M. Heil. 2009. The role of extrafloral nectar amino acids for the preferences of facultative and obligate ant mutualists. Journal of Chemical Ecology 35:459–468.

Grover, C. D., A. D. Kay, J. A. Monson, T. C. Marsh, and D. A. Holway. 2007. Linking nutrition and behavioural dominance: carbohydrate scarcity limits aggression and activity in Argentine ants. Proceedings. Biological sciences / The Royal Society 274:2951–2957.

Hölldobler, B., and E. O. Wilson. 1990. The ants. Belknap Press of Harvard University Press.

Kay, A. 2002. Applying Optimal Foraging Theory to Assess Nutrient Availability Ratios for Ants. Ecology 83:1935–1944.

Kay, A. D., J. Z. Shik, A. Van Alst, K. A. Miller, and M. Kaspari. 2012. Diet composition does not affect ant colony tempo. Functional Ecology 26:317–323.

Klimes, P., P. Fibich, C. Idigel, and M. Rimandai. 2015. Disentangling the diversity of arboreal ant communities in tropical forest trees. PLoS ONE 10:1–24.

Koch, E. B. A., F. Camarota, and H. L. Vasconcelos. 2016. Plant Ontogeny as a Conditionality Factor in the Protective Effect of Ants on a Neotropical Tree.

Biotropica 48:198–205.

Lange, D., W. Dáttilo, and K. Del-Claro. 2013. Influence of extrafloral nectary phenology on ant-plant mutualistic networks in a neotropical savanna. Ecological Entomology 38:463–469.

Lassau, S., and D. Hochuli. 2004. Effects of habitat complexity on ant assemblages. Ecography 2:157–164.

Leal, I. R., B. K. C. Filgueiras, J. P. Gomes, L. Iannuzzi, and A. N. Andersen. 2012. Effects of habitat fragmentation on ant richness and functional composition in Brazilian Atlantic forest. Biodiversity and Conservation 21:1687–1701.

Majer, J. D. 1976. The Ant Mosaic in Ghana Cocoa Farms: Further Structural Considerations. The Journal of Applied Ecology 13:145.

Powell, S., A. N. Costa, C. T. Lopes, and H. L. Vasconcelos. 2011. Canopy connectivity and the availability of diverse nesting resources affect species coexistence in arboreal ants. Journal of Animal Ecology 80:352–360.

Ribas, C. R., and J. H. Schoereder. 2002. Are all ant mosaics caused by competition? Oecologia 131:606–611.

Ribas, C. R., J. H. Schoereder, M. Pic, and S. M. Soares. 2003. Tree heterogeneity, resource availability, and larger scale processes regulating arboreal ant species richness. Austral Ecology 28:305–314.

Schoereder, J. J., T. T. Sobrinho, M. M. Madureira, C. Ribas, and P. P. Oliveira. 2010. The arboreal ant community visiting extrafloral nectaries in the Neotropical cerrado savanna. Terrestrial Arthropod Reviews 3:3–27.

Sendoya, S. F., N. Blüthgen, J. Y. Tamashiro, F. Fernandez, and P. S. Oliveira. 2016. Foliage-dwelling ants in a neotropical savanna: effects of plant and insect exudates on ant communities. Arthropod-Plant Interactions 10:183–195.

Sterner, R. W., and J. J. Elser. 2002. Ecological stoichiometry : the biology of elements from molecules to the biosphere. Princeton University Press.

Tobin, J. E. 1991. A Neotropical rainforest canopy ant community: some ecological considerations. Pages 536-538 in Huxley, C. R., and Cutler, D. F., editors. Ant-plant interactions. Oxford University Press, Oxford.

Tobin, J. E. 1994. Ants as primary consumers: diet and abundance in the Formicidae. Pages 279-307 in Hunt, J. H., and Nalepa, C. A., editors. Nourishment and evolution in insect societies. Westview, Boulder.

Tobin, J. E. 1995. Ecology and diversity of tropical forest canopy ants. Pages 129-147 in Lowman, M. D., and Nadkarni, N. M., editors. Forest canopies. New York, Academic Press.

Wang, C., J. S. Strazanac, and L. Butler. 2001. Association Between Ants (Hymenoptera : Formicidae) and Habitat Characteristics in Oak-Dominated Mixed Forests 30:842–848.

Wills, B. D., C. D. Chong, S. M. Wilder, M. D. Eubanks, D. A. Holway, and A. V. Suarez. 2015. Effect of carbohydrate supplementation on investment into offspring number, size, and condition in a social insect. PLoS ONE 10:1–15.

CHAPTER ONE

EXTRA-FLORAL NECTAR AS A DRIVER OF ARBOREAL ANT COMMUNITIES AT THE SITE-SCALE IN BRAZILIAN SAVANNA

Extra-floral nectar as a driver of arboreal ant communities at the site-scale in Brazilian Savanna

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Abstract

Carbon-rich plant exudates such as those produced by extra-floral nectaries (EFNs) are key resources for arboreal ants. Compared with plants without EFNs, trees bearing EFNs often support a higher abundance and richness of ants. However, it is unclear if and to what extent such effects on individual trees scale up to influence arboreal ant communities at larger spatial scales. Here we address this issue in Brazilian Cerrado (savanna), which has a high abundance of EFN-bearing trees. We tested three predictions: (1) Arboreal ant richness is higher on trees bearing EFNs than on those without; (2) Arboreal ant species richness increases with the proportion of trees that bear EFNs at the site scale, due primarily to a higher likelihood of occurrence of non-core ant species; and (3) Ant species composition changes with the proportion of EFN-bearing trees at the site scale. We sampled arboreal ants in 32 plots (each 20 x 20 m), where the proportion of EFN-bearing trees ranged from 0 - 60%. We recorded a total of 72 ant species, ranging from 6-26 per plot. Seventeen ant species (mostly belonging to *Camponotus*, *Cephalotes* and *Crematogaster*) were identified as core species in at least one of the 32 plot networks. As predicted, ant species richness was significantly higher on EFN-bearing trees than on those without. We identified ant species that preferentially occurred on EFN-bearing trees, all of which were core partners in ant-EFN networks. In support of our second prediction, the proportion of EFN-bearing trees was a strong predictor of ant richness at the site scale, independently of overall tree density and richness, and this pattern was due to a higher occurrence of non-core ant species. Finally, species composition also varied with the proportion of EFN-bearing trees as we predicted, and we identify a group of specialist nectar-feeding ant species associated with plots having a high proportion of EFN-bearing trees. However, these specialist

nectar feeders do not appear to offer high-quality protection services because they were all non-core partners in the ant-plant networks, which suggests that the higher species richness is not likely to enhance protection services for EFN-bearing plants.

Key words: ant-plant interactions, arboreal ants, Cerrado, ecological networks, EFN, site-scale, tree density.

Introduction

Ants are a numerically and ecologically dominant taxon in terrestrial ecosystems, especially in the tropics (Hölldobler and Wilson 1990, Folgarait 1998). They are a particularly dominant component of the arboreal fauna of tropical forests (Latch et al. 2010, Davidson 1997), constituting a high proportion of arthropod biomass in canopy samples (Tobin 1995, Floren and Linsenmair 1997). A key factor driving arboreal ant richness and abundance is the presence of extra-floral nectaries (EFNs) (Koptur, 1992, Davidson et al. 2003, Blüthgen and Fiedler 2004a, Blüthgen and Fiedler 2004b, Blüthgen et al. 2004, Davidson 1997). The consumption of extra-floral nectar increases rates of ant foraging activity (Grover et al. 2007, Byk and Del-Claro 2011) and promotes colony growth and reproduction (Byk and Del-Claro 2011, Wills et al. 2015).

Some ant species are tightly associated with EFNs, and these are often core components of ant-EFN networks (Dáttilo et al. 2014b). Many of these core species (especially formicines and dolichoderines, along with the myrmicine genus *Crematogaster*) have digestive systems adapted to process large amounts of nectar, allowing prolonged and intense exploitation of such resources (Davidson 1997, Davidson et al. 2004). However, extrafloral nectar also attracts a wide range of generalist ant species (Oliveira and Brandão 1991; Schoereder et al. 2010), which results in a low fidelity between ant and plant partners (Blüthgen et al. 2000, Lange and Del-claro 2014, Camarota et al. 2015).

Despite the importance of EFNs to arboreal ant species, there is no consensus on their effects on the structure of arboreal ant communities. Some studies have reported that the availability of extra-floral nectar increases ant species richness (Lange et al. 2013, Belchior et al. 2016, Fagundes et al. 2016, Koch et al. 2016) and

changes species composition (Camarota et al. 2015), but others have found a lack of response (Schoereder et al. 2010, Sendoya et al. 2016). Moreover, these studies have focused on the effects of EFNs at the scale of an individual tree (Schoereder et al. 2010, Camarota et al. 2015, Belchior et al. 2016, Sendoya et al. 2016), and it is not clear whether and to what extent effects on individual trees scale up to influence arboreal ant communities at larger spatial scales. While several studies have examined the role of vegetation structure as a driver of patterns of arboreal ant diversity at a site scale (Wang et al. 2001b, Ribas et al. 2003, Lassau and Hochuli 2004, Powell et al. 2011, Leal et al. 2012, Klimes et al. 2015), little attention has been given to the role of EFNs as a mechanism underlying such relationships (but see Câmara et al. 2016).

Here we investigate the role of EFNs as a factor driving diversity and composition of arboreal ant communities at the site scale in Brazilian Cerrado. Cerrado has an exceptional arboreal ant diversity compared with tropical savannas elsewhere in the world (Campos et al. 2011, Camacho and Vasconcelos 2015), as reflects its evolution in association with expansions and contractions of rainforest, from which most of its arboreal ant fauna is derived (Campos et al. 2011). It also has a particularly high occurrence of EFN-bearing trees (Oliveira and Leitão-Filho, 1987), which can represent more than 20% of all woody plant species at a site (Boudouris and Queenborough 2013).

In this study, we ask to what extent does the occurrence of EFN-bearing trees drives arboreal ant diversity and composition at the site scale? Our hypothesis is that the diversity of arboreal ant species at the site scale increases with the abundance of EFN-bearing trees, primarily because it promotes the occurrence of non-core ant species in the ant-EFN network. We first identify the core ant species in our ant-EFN

network, and then test three predictions: (1) Trees bearing EFNs have higher arboreal ant richness than those without; (2) The richness of arboreal ants at the site scale increases not only with tree density, but also with the proportion of EFN-bearing trees, due primarily to a higher likelihood of occurrence of non-core ant species; and (3) Ant species composition varies with the proportion of EFN-bearing trees at a site.

Methods

Study site

The study was carried out in the Serra do Cipó National Park, situated in Minas Gerais state, Brazil. Cerrado is the dominant vegetation at lower elevations (800-1000 m) of the park (Giulietti et al., 1987), and sampling was conducted in an area of transition between two Cerrado phytophysiognomies (limits: 19°21'56"- 19°22'13" S and 43°36'57"- 43°37'6" W), "Cerrado *sensu stricto*" and "Campo Cerrado", with the latter having lower tree density (Coutinho 1990). The climate is tropical humid with the mean annual rainfall of 1,374 mm concentrated between November and January (Madeira and Fernandes 1999). Mean temperature shows little monthly variation, ranging from 20 °C to 22 °C (Madeira and Fernandes 1999).

Ant and plant sampling

We surveyed arboreal ants in 32 plots randomly set in the study site, each measuring 20 m X 20 m (400 m^2). The distance between nearest plots ranged from 20 – 50 m, and the most distant plots were separated from each other by 700 m. In each plot, we counted and identified all woody plants >8 cm in basal diameter, limit that includes most of tree species typically from Brazilian savanna (Felfili and Siva Júnior 1988) and categorized them according to the presence or absence of EFNs.

The site scale was represented by cumulative data sampled in all trees within each plot. Herbarium specimens of all tree species have been deposited in the Herbarium of the Natural History Museum of Federal University of Minas Gerais. During three minutes in each tree, we searched for honeydew-producing hemipterans that were tended by ants. We found a small proportion of trees (3% of total, two individuals bearing EFNs) in the study plots supporting trophobiose interaction and these trees were excluded from analyses so that our results were not confounded by the availability of alternative rich sources of liquid carbohydrate.

We sampled ants from all trees in each plot using three methods. First, we hand-collected all ants that we observed during a 3-minute period per tree. Second, we arbitrarily selected three distant branchlets that we beat 10 times and hand-collected the dislodged ants that fell onto an entomological umbrella. The hand-collecting and beat sampling were performed between 7:00 h and 17:00 h. Lastly, we secured three non-baited pitfall traps to each tree, and operated them for 48 hours. One of the traps was tied to the main trunk while the other two were tied to branches near foliage. The pitfall traps were plastic vials of 80 mL and 5 cm in diameter, containing water and detergent. The majority of trees were < 2 m in height, allowing easy access to the canopy using a small (1.3 m) ladder where necessary. All ant sampling was conducted between June and August 2014. Ants were sorted to species and identified in the laboratory to the lowest taxonomic level possible by comparing with the reference collection of the Community Ecology Laboratory at the Federal University of Viçosa, where voucher specimens for all species sampled are deposited. Whenever we could not confidently name a species, we assigned number codes that apply to this study only.

Statistical analysis

We examined associations between ant and tree species in each plot by building binary adjacency matrices based on the incidence (presence or absence) of each ant species on EFN-bearing trees. Ant species were classified as core and non-core within each plot network following the formula proposed by Dáttilo et al. (2013): $Gc = (k_i - k_{\text{mean}})/ \sigma k$, where k_i = mean number of links for a given ant species for the EFN-bearing species, k_{mean} = mean number of links for all ant species combined, and σk = standard deviation of the number of links for ant species. Core and non-core species were defined as those with $Gc > 1$ and $Gc < 1$ respectively.

We analyzed variation in ant species richness between individual trees with and without EFNs using generalized linear mixed models (GLMMs) with Poisson error. To account for spatial autocorrelation among trees located in the same plot, we treated individual trees as a random effect, controlling for pseudo-replication in GLMMs models.

To identify if there were ant species preferentially associated with EFN-bearing trees, we used Chi-square goodness of fit tests to compare the frequency of occurrence of common (occurring on at least 15 trees across all plots) ant species between tree species with and without EFNs.

We conducted hierarchical partitioning analysis (Mac Nally 2002) to calculate the relative independent effects of the proportion of EFNs, tree density and tree richness as predictors of total ant species richness, and also for core and non-core species richness at the plot level. We then tested the statistical significance of the independent contributions of each variable using 5000 permutations, with the significance was based on an upper confidence limit of 95% (Mac Nally 2002).

To assess whether ant species composition at the site level varied with the proportion of EFN-bearing trees, we used permutational multivariate analysis of variance (PERMANOVA; Anderson 2001), based on Jaccard dissimilarity with 999 permutations. Posteriorly, we tested the assumption of spatial homogeneity of variances (Anderson 2001) Non-metric multidimensional scaling (NMDS) was used to present the ordination of plot-level ant assemblages, with the size of points scaled in relation to the proportion of trees bearing EFNs.

Finally, we performed a multinomial species classification using the CLAM test (Chazdon et al. 2011) in order to classify ant species into four groups based on their frequency of occurrence in plots with low (<30%) versus high (>30%) proportions of trees bearing EFNs, with a specialization threshold of 60%. The groups were: (1) occurring preferentially in plots with a high proportion of EFN-bearing trees; (2) occurring preferentially in plots with a low proportion of EFN-bearing trees; (3) showing no preference; and (4) too rare to be assigned to a preference class.

All analyses were conducted using the software R (R Core Team 2016), with all models checked for the distribution of errors and over-dispersion in the data. We carried out the GLMMs using the lme4 v 1.1-11 package (Bates et al. 2015), hierarchical partitioning and associated randomization tests using hier.part v 1.0-4 (Walsh et al. 2013), and PERMANOVA and CLAM analysis using vegan v 2.4-0 (Oksanen et al. 2015).

Results

Across all plots, we sampled a total of 861 trees belonging to 46 species and 24 families (Appendix S1: Table S1). EFN-bearing trees represented 30% (14 species) of this total, and these contributed 33% of total tree abundance, varying from 0-60%

among plots. The most abundant tree species were *Hymenaea stignocarpa* (23.2% of all trees) and *Piptocarpha rotundifolia* (12.8%), species with and without EFNs respectively. Mean (±SE) plot species richness was 9.66 ± 1.7.

We recorded a total of 72 ant species, from 19 genera and 6 subfamilies (Appendix S1: Table S2). The richest genera were *Camponotus* (21 species) and *Pseudomyrmex* (13 species). Mean (± SE) ant species richness per plot was 15 ± 2.65, and ranged from six to 26.

Seventeen ant species were identified as core within one or more of the 32 plot networks. The species most frequently identified as core were *Camponotus crassus* and *Cephalotes betoi* (both core in 11 plots, or 34% of total), *Crematogaster stollii* (5 plots), and *Camponotus renggeri*, *Cephalotes pusillus* and *Crematogaster distans* (each 4 plots). The other species identified as core within a plot were *Camponotus bonariense*, *Camponotus melanoticus*, *Neoponera villosa* and *Pseudomyrmex* sp.8 (each in 2 plots), and *Azteca* sp.1, *Camponotus blandus*, *Cephalotes atratus*, *Dolichoderus diversus*, *Myrmelachista* sp. 3, *Pseudomyrmex* sp.1 and *Pseudomyrmex* sp. 4 (each in a single plot). Mean (±SE) richness of core species per plot was 1.9 ± 0.24.

Mean (±SE) ant species richness on trees bearing EFNs (2.13 ± 0.2) was significantly higher than on those without (1.59 ± 0.1; χ^2= 32.261; $p < 0.01$). Eighteen of the 72 ant species occurred on >15 trees, and 11 (61%) of these occurred more frequently on plants with EFNs than expect by chance (Table 1). All these 11 species were identified as core species in one or more plots (see above).

Hierarchical partitioning indicated that the proportion of trees with EFNs was the strongest independent predictor of ant species richness at the site scale (explained variance = 47%), ahead of overall tree density (33%) and tree richness (20%; Table

2). This was also the case for the richness of non-core ant species, whereas none of the variables independently predicted variation in the richness of core ant species (Table 2).

Ant species composition at the site scale varied significantly with the proportion of trees bearing EFNs (Permanova; $F_{1,30} = 2.27$; $p = 0.002$; Fig. 1)**.** Of the 72 ant species recorded, CLAM classified 22 (31%) as having no preference for plots based on proportion of trees bearing EFNs, and 44 species (61%) were indicated as "too rare" to be assigned to a preference class (Fig. 2). Of the remaining six species, four (6%; *Cephalotes pusillus*, *Dolichoderus diversus*; *Myrmelachista* sp. 3 and *Pseudomyrmex* sp. 8) occurred preferentially in plots with a high proportion of EFN-bearing trees) and two (3%; *Crematogaster distans* and *Pseudomyrmex kuenckeli*) occurred preferentially in plots with a low proportion of EFN-bearing trees.

Discussion

In this study, we explored the effects of extrafloral nectaries on arboreal ant diversity at multiple scales, focusing on how patterns observed on individual trees scale up to influence ant communities at the site level. Based on one of the most intensive surveys of arboreal ants reported for Brazilian Cerrado, we found support for our hypothesis that EFN-bearing trees have a positive effect on the diversity of arboreal ant species at the site scale, and that this occurs primarily through the increased occurrence of non-core ant species in the ant-EFN network.

Given that we found a high number of species interacting with EFN-bearing trees in our study system, we agree with the assertion that such ant-EFN interactions should be characterized as being highly generalist, as is typical of ant-EFN interactions (Oliveira and Brandão 1991, Jordano et al. 2003). However, a limited

subset of ant species occurred preferentially on trees with EFNs, and all of these were identified as core partners in ant-EFN interactions. The most common core species belonged to the genera *Camponotus*, *Cephalotes* and *Crematogaster*, which is typically the case in ant-EFN networks in Brazilian Cerrado (Lange et al. 2013, Dáttilo et al. 2014a, 2014b, Lange and Del-Claro 2014, Sendoya et al. 2016). Species of these genera possess specializations for feeding extensively on liquid resources (Davidson et al. 2003). Species of *Camponotus* have a proventriculus that is morphologically adapted to a diet heavily based on plant exudates (Davidson 1997). Species of *Cephalotes* have bacterial gut symbionts whose atmospheric nitrogen fixation complements a nitrogen-poor diet (Russell et al. 2009) based on high nectar exploitation (Byk and Del-Claro 2011). Nestmates of *Crematogaster* species frequently perform trophalaxis (sharing of liquid food), which is an unusual trait among myrmicines (Davidson 1997) and indicates a diet relying on plant exudates (Davidson et al. 2003, 2004, Ness et al. 2009). The only core ant species that did not show a preference for EFN-bearing trees was *Crematogaster distans*. This species performs the vast majority of trophobiotic interactions with honeydew-producing hemipterans in our study system (L. Ribeiro, personal observations), and honeydew is a more nutritious and predictable resource than is nectar (Blüthgen et al. 2000, Blüthgen and Fiedler 2004a). Thus, we suggest that *C. distans* is more closely associated with the presence of hemipterans than with EFN-bearing trees.

We confirmed our prediction that ant species richness would be higher on trees bearing EFNs than on those without, underpinning the importance of this food resource as a driver of arboreal ant diversity (Davidson et al. 2003). Extrafloral nectar contains essential amino acids in addition to mono and disaccharides (Blüthgen et al. 2004a, Nepi et al. 2012), and thus represents a high quality food

reward (Marazzi et al. 2013). However, not all previous studies found differences in arboreal ant richness between trees with and without EFNs (Schoereder et al. 2010, Sendoya et al. 2016). This may reflect seasonal or other temporal variation in nectar production (Belchior et al. 2016), or possibly limitations of sampling methodology: in both the above studies ants were surveyed through pitfall traps baited with sardine and honey, which could bias the sampling towards behaviourally dominant, recruit-foraging species that might actively exclude others (Agosti et al. 2000,Wang et al. 2001a).

Consistent with our second prediction, the richness of arboreal ants at the plot level increased with the proportion of EFN-bearing trees. As has been documented in previous studies of arboreal ants (Ribas et al. 2003, Powell et al. 2011, Klimes et al. 2012), we found a positive correlation between ant diversity and tree density. However, we found that the proportion of trees bearing EFNs was a stronger independent predictor of ant diversity than was tree density. The proportion of EFN-bearing trees was a strong independent predictor of the richness of non-core ant species, but not core species, which supports our expectation that the promotion of overall species richness is due primarily to a higher likelihood of occurrence of non-core ant species.

We also found a positive relationship between ant species richness and tree richness, a pattern also found for arboreal ants in tropical forests (Klimes et al. 2012). This can be explained by higher tree richness translating to a greater variety of resources for ants (Armbrecht et al. 2004, Haddad et al. 2011) through increased habitat heterogeneity (Riedel et al. 2014). However, the relationship was relatively weak, which reflects the high degree of generalism of EFN-attending ant species (Blüthgen et al. 2007, Rico-Gray and Oliveira 2007).

Finally, as we also predicted, the species composition of arboreal ant communities varied with the proportion of EFN-bearing trees, as would be expected from an increased likelihood of occurrence of non-core ant species with an increasing proportion of EFN-bearing trees. The ant species that we identified as preferentially occurring in plots with a high proportion of EFN-bearing trees belonged to the genera *Cephalotes*, *Dolichoderus*, *Pseudomyrmex* and *Myrmelachista*. All these genera are specialist leaf-foragers (Blüthgen et al. 2003), with plant exudates constituting a substantial portion of their diets (Davidson 1997, Davidson et al. 2003). Câmara et al. (2016) similarly found that the richness of *Dolichoderus* ants was explained by the density of EFN-bearing trees in Atlantic forest. We also identified two ant species, *Crematogaster distans* and *Pseudomyrmex kuenckeli,* associated with plots having a low proportion of EFN-bearing trees. We have previously noted that *C. distans* was strongly associated with trophobionts in our study system, and *P. kuenckeli* also was commonly observed interacting with sap-feeding hemipterans (L. Ribeiro, personal observations). Thus there is a negative spatial relationship between ant species specializing on EFNs and those on trophobionts in our study system, which warrants further exploration.

To our knowledge, this is the first study to have demonstrated that EFN-bearing trees have a marked effect on communities of arboreal ants at the site scale, influencing both species richness and composition. Therefore the presence of EFNs not only influences arboreal ants on individual trees, but also has a substantial effect on the ant-EFN network of the broader community. What are the implications of this for the provision of protection services by ants to EFN-bearing plants? As is typically the case for a generalized mutualism (Bascompte and Jordano 2007), the strength of interaction varied markedly among the EFN-tending ants in our system. The most

common species associated with EFNs belonged to genera that have trait specializations allowing extensive foraging on liquid resources; such specialization is suggestive of a co-evolutionary history of association between these ants and EFN-bearing trees (Guimarães et al. 2011), and consequently, these species are likely to provide the strongest benefits to their mutualistic partners (Vázquez et al. 2005, Mello et al. 2015). The observed increase in ant diversity with an increase in the proportion of EFN-bearing trees was due to an increased likelihood of non-core ant species, whose plant-protection services are likely to be relatively weak (Bascompte and Jordano 2007). Moreover, although four core ant species were positively associated with an increase in the proportion of EFN-bearing trees, three of these occurred in only a few plots, and the only widespread species (*Cephalotes pusillus*) has been shown in a previous study to provide little benefit to its host plants (Byk and Del-Claro 2010). Therefore, we suggest that the EFN-driven increases in ant diversity at the site scale are unlikely to result in substantially enhanced protection services for EFN-bearing plants.

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References

Agosti, D., J. D. Majer, L. E. Alonso, and T. R. Schultz. 2000. Ants: Standard methods for measuring and monitoring biodiversity. Smithsonian Institution Press.

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26:32–46.

Armbrecht, I., I. Perfecto, and J. Vandermeer. 2004. Enigmatic Biodiversity Correlations: Ant Diversity Responds to Diverse Resources. Science 304:284–286.

Bascompte, J., and P. Jordano. 2007. Plant-Animal Mutualistic Networks: The Architecture of Biodiversity. Annual Review of Ecology, Evolution, and Systematics 38:567–593.

Bates, D., M. Maechler, B. Bolker, S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 67:1–48.

Belchior, C., S. F. Sendoya, and K. Del-Claro. 2016. Temporal variation in the abundance and richness of foliage-dwelling ants mediated by extrafloral nectar. PLoS ONE 11:1–17.

Blüthgen, N., and K. Fiedler. 2004a. Competition for composition: Lessons from nectar-feeding ant communities. Ecology 85:1479–1485.

Blüthgen, N., and K. Fiedler. 2004b. Preferences for sugars and amino acids and their conditionality in a diverse nectar-feeding ant community. Journal of Animal Ecology 73:155–166.

Blüthgen, N., G. Gebauer, and K. Fiedler. 2003. Disentangling a rainforest food web using stable isotopes: Dietary diversity in a species-rich ant community. Oecologia 137:426–435.

Blüthgen, N., G. Gottsberger, and K. Fiedler. 2004a. Sugar and amino acid composition of ant-attended nectar and honeydew sources from an Australian rainforest. Austral Ecology 29:418–429.

Blüthgen, N., F. Menzel, T. Hovestadt, B. Fiala, and N. Blüthgen. 2007. Specialization, Constraints, and Conflicting Interests in Mutualistic Networks. Current Biology 17:341–346.

Blüthgen, N., N. E. Stork, and K. Fiedler. 2004b. Bottom-up control and co-occurrence in complex communities: Honeydew and nectar determine a rainforest ant mosaic. Oikos 106:344–358.

Blüthgen, N., M. Verhaagh, W. Goitía, K. Jaffé;, W. Morawetz, and W. Barthlott. 2000. How plants shape the ant community in the Amazonian rainforest canopy: the key role of extrafloral nectaries and homopteran honeydew. Oecologia 125:229–240.

Boudouris, J., and S. a Queenborough. 2013. Diversity and distribution of extra-floral nectaries in the cerrado savanna vegetation of Brazil. PeerJ 1:1–13.

Byk, J., and K. Del-Claro. 2010. Nectar- and pollen-gathering *Cephalotes* ants provide no protection against herbivory: A new manipulative experiment to test ant protective capabilities. Acta Ethologica 13:33–38.

Byk, J., and K. Del-Claro. 2011. Ant-plant interaction in the Neotropical savanna: Direct beneficial effects of extrafloral nectar on ant colony fitness. Population Ecology 53:327–332.

Camacho, G. P., and H. L. Vasconcelos. 2015. Ants of the Panga Ecological Station, a Cerrado Reserve in Central Brazil. Sociobiology 62:281–295.

Câmara, T., W. R. Almeida, M. Tabarelli, A. N. Andersen, and I. R. Leal. 2016. Habitat fragmentation, EFN-bearing trees and ant communities: Ecological

cascades in Atlantic Forest of northeastern Brazil. Austral Ecology 42: 31–39.

Camarota, F., S. Powell, H. L. Vasconcelos, G. Priest, and R. J. Marquis. 2015. Extrafloral nectaries have a limited effect on the structure of arboreal ant communities in a neotropical savanna. Ecology 96:231–240.

Campos, R. I., H. L. Vasconcelos, A. N. Andersen, T. L. M. Frizzo, and K. C. Spena. 2011. Multi-scale ant diversity in savanna woodlands: An intercontinental comparison. Austral Ecology 36:983–992.

Chazdon, R. L., A. Chao, R. K. Colwell, S.-Y. Lin, N. Norden, S. G. Letcher, D. B. Clark, B. Finegan, and J. P. Arroyo. 2011. A novel statistical method for classifying habitat generalists and specialists. Ecology 92:1332-1343.

Coutinho, L. M. 1990. Fire in the ecology of Brazilian cerrado. Pages 82–105 in Goldammer, J.G., editor. Fire in the tropical biota. Springer-Verlag, Berlin.

Dáttilo, W., R. Fagundes, C. A. Q. Gurka, M. S. A. Silva, M. C. L. Vieira, T. J. Izzo, C. Díaz-Castelazo, K. Del-Claro, and V. Rico-Gray. 2014a. Individual-based ant-plant networks: Diurnal-nocturnal structure and species-area relationship. PLoS ONE 9(6): e99838.

Dáttilo, W., P. R. Guimarães, and T. J. Izzo. 2013. Spatial structure of ant-plant mutualistic networks. Oikos 122:1643–1648.

Dáttilo, W., F. M. D. Marquitti, P. R. Guimarães, and T. J. Izzo. 2014b. The structure of ant – plant ecological networks : Is abundance enough ? Ecology 95:475–485.

Davidson, D. W. 1997. The role of resource imbalances in the evolutionary ecology of tropical arboreal ants. Biological Journal of the Linnean Society 61:153–181.

Davidson, D. W., S. C. Cook, and R. R. Snelling. 2004. Liquid-feeding performances

of ants (Formicidae): Ecological and evolutionary implications. Oecologia 139:255–266.

Davidson, D. W., D. W. Davidson, S. C. Cook, R. R. Snelling, and T. H. Chua. 2003. Explaining the abundance of ants in lowland tropical rainforest canopies. Science 300:969–973.

Fagundes, R., W. Dáttilo, S. P. Ribeiro, V. Rico-Gray, and K. Del-Claro. 2016. Food source availability and interspecific dominance as structural mechanisms of ant-plant-hemipteran multitrophic networks. Arthropod-Plant Interactions 10:207–220.

Felfili, J. M.; and Silva-Júnior, M. C. 1988. Distribuição dos diâmetros numa faixa de cerrado na Fazenda Água Limpa (FAL) – DF. Acta Botanica Brasilica 2: 85-104.

Floren, A., and K. E. Linsenmaier. 1997. Diversity and recolonisation dynamics of selected arthropod groups on different tree species in a lowland rain forest in Sabah, Malaysia. Pages. 344–381 in N. E. Stork, J. Adis, and R. K. Didham, editors. Canopy Arthropods. Chapman and Hall, London.

Folgarait, P. J. 1998. Ant biodiversity and its relationship to ecosystem functioning: a review. Biodiversity and Conservation 7: 1221-1244.

Grover, C. D., A. D. Kay, J. A. Monson, T. C. Marsh, and D. A. Holway. 2007. Linking nutrition and behavioural dominance: carbohydrate scarcity limits aggression and activity in Argentine ants. Proceedings of the Royal Society B 274:2951–2957.

Guimarães, P. R., P. Jordano, and J. N. Thompson. 2011. Evolution and coevolution in mutualistic networks. Ecology Letters 14:877–885.

Haddad, N. M., G. M. Crutsinger, K. Gross, J. Haarstad, and D. Tilman. 2011. Plant

diversity and the stability of foodwebs. Ecology Letters 14:42–46.

Hölldobler, B., and E. O. Wilson. 1990. The ants. Belknap Press of Harvard University Press.

Jordano, P., J. Bascompte, and M. J. Olesen. 2003. Invariant Properties in Coevolutionary Networks of Plant – Animal Interactions. Ecology Letters 6:69–81.

Klimes, P., P. Fibich, C. Idigel, and M. Rimandai. 2015. Disentangling the diversity of arboreal ant communities in tropical forest trees. PLoS ONE 10:1–24.

Klimes, P., C. Idigel, M. Rimandai, T. M. Fayle, M. Janda, G. D. Weiblen, and V. Novotny. 2012. Why are there more arboreal ant species in primary than in secondary tropical forests? Journal of Animal Ecology 81:1103–1112.

Koch, E. B. A., F. Camarota, and H. L. Vasconcelos. 2016. Plant Ontogeny as a Conditionality Factor in the Protective Effect of Ants on a Neotropical Tree. Biotropica 48:198–205.

Koptur, S. 1992. Extrafloral nectary-mediated interactions between insects and plants. Pages 81–129 in Bernays, E. A., editor. Insect–plant interactions. Vol. 4. CRC Press. Boca Raton.

Lange, D., W. Dáttilo, and K. Del-Claro. 2013. Influence of extrafloral nectary phenology on ant-plant mutualistic networks in a neotropical savanna. Ecological Entomology 38:463–469.

Lange, D., and K. Del-Claro. 2014. Ant-Plant Interaction in a Tropical Savanna : May the Network Structure Vary over Time and Influence on the Outcomes of Associations? PLoS ONE 9(8):e105574.

Lassau, S., and D. Hochuli. 2004. Effects of habitat complexity on ant assemblages. Ecography 2:157–164.

Lach, L., L. C. Parr, and K. L. Abbott. 2010. Ant Ecology. Oxford University Press, Oxford.

Leal, I. R., B. K. C. Filgueiras, J. P. Gomes, L. Iannuzzi, and A. N. Andersen. 2012. Effects of habitat fragmentation on ant richness and functional composition in Brazilian Atlantic forest. Biodiversity and Conservation 21:1687–1701.

Madeira, J. A., and G. W. Fernandes. 1999. Reproductive phenology of sympatric taxa of *Chamaecrista* (Leguminosae) in Serra do Cipó, Brazil. Journal of Tropical Ecology 15:463–479.

Marazzi, B., J. L. Bronstein, and S. Koptur. 2013. The diversity, ecology and evolution of extrafloral nectaries: Current perspectives and future challenges. Annals of Botany 111:1243–1250.

Mello, M. A. R., F. A. Rodrigues, L. da F. Costa, W. D. Kissling, Ç. H. Şekercioğlu, F. M. D. Marquitti, and E. K. V. Kalko. 2015. Keystone species in seed dispersal networks are mainly determined by dietary specialization. Oikos 124:1031–1039.

Mac Nally, R. 2002. Multiple regression and inference in conservation biology and ecology: Further comments on identifying important predictor variables. Biodiversity and Conservation 11:1397–1401.

Nepi, M., C. Soligo, D. Nocentini, M. Abate, M. Guarnieri, G. Cai, L. Bini, M. Puglia, L. Bianchi, and E. Pacini. 2012. Amino acids and protein profile in floral nectar: Much more than a simple reward. Flora: Morphology, Distribution, Functional Ecology of Plants 207:475–481.

Ness, J. H., W. F. Morris, and J. L. Bronstein. 2009. For ant-protected plants, the best defense is a hungry offense. Ecology 90:2823–2831.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G.

L. Simpson, P.Solymos, M. H. H. Stevens, and H. Wagner. 2015. vegan: Community Ecology Package. R package version 2.4-0., http://CRAN.R-project.org/package=vegan.

Oliveira, P. S., and C. R. S. Brandão. 1991. The ant community associated with extrafloral nectaries in the Brazilian cerrado. Pages 198–212 in C. R. Huxley, and D. F. Cutler, editors. Ant-plant interactions. Oxford University Press. Oxford.

Oliveira, P. S., and H. F. Leitão-Filho. 1987. Extrafloral nectaries: their taxonomic distribution and abundance in the woody flora of Cerrado vegetation in southeast Brazil. Biotropica 19:140–148.

Powell, S., A. N. Costa, C. T. Lopes, and H. L. Vasconcelos. 2011. Canopy connectivity and the availability of diverse nesting resources affect species coexistence in arboreal ants. Journal of Animal Ecology 80:352–360.

R Development Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ribas, C. R., J. H. Schoereder, M. Pic, and S. M. Soares. 2003. Tree heterogeneity, resource availability, and larger scale processes regulating arboreal ant species richness. Austral Ecology 28:305–314.

Rico-Gray, V., and P. S. Oliveira. 2007. The ecology and evolution of ant– plant interactions. University of Chicago Press, Chicago, USA.

Riedel, J., S. Dorn, and K. Mody. 2014. Assemblage composition of ants (Hymenoptera: Formicidae) affected by tree diversity and density in native timber tree plantations on former tropical pasture. Myrmecological News 20:113–127.

Russell, J. a, C. S. Moreau, B. Goldman-Huertas, M. Fujiwara, D. J. Lohman, and N.

E. Pierce. 2009. Bacterial gut symbionts are tightly linked with the evolution of herbivory in ants. Proceedings of the National Academy of Sciences of the United States of America 106:21236–21241.

Schoereder, J. J., T. T. Sobrinho, M. M. Madureira, C. Ribas, and P. P. Oliveira. 2010. The arboreal ant community visiting extrafloral nectaries in the Neotropical cerrado savanna. Terrestrial Arthropod Reviews 3:3–27.

Sendoya, S. F., N. Blüthgen, J. Y. Tamashiro, F. Fernandez, and P. S. Oliveira. 2016. Foliage-dwelling ants in a neotropical savanna: effects of plant and insect exudates on ant communities. Arthropod-Plant Interactions 10:183–195.

Tobin, J. E. 1995. Ecology and diversity of tropical forest canopy ants. Pages 129–147 in Lowman, M.D., and N. M. Nadkarni, editors. Forest Canopies. Academic Press, New York.

Vázquez, D. P., W. F. Morris, and P. Jordano. 2005. Interaction frequency as a surrogate for the total effect of animal mutualists on plants. Ecology Letters 8:1088–1094.

Walsh, C. J., and R. Mac Nally. 2013. hier.part: hierarchical partitioning. R package version 1.0-4.,http://CRAN.R-project.org/package=hier.part.

Wang, C., J. Strazanac, and L. Butler. 2001a. A comparison of pitfall traps with bait traps for studying leaf litter ant communities. Journal of Economic Entomology 94:761–765.

Wang, C., J. S. Strazanac, and L. Butler. 2001b. Association Between Ants (Hymenoptera : Formicidae) and Habitat Characteristics in Oak-Dominated Mixed Forests. Environmental Entomology 30:842–848.

Wills, B. D., C. D. Chong, S. M. Wilder, M. D. Eubanks, D. A. Holway, and A. V. Suarez. 2015. Effect of carbohydrate supplementation on investment into

offspring number, size, and condition in a social insect. PLoS ONE 10:1–15.

Table legends

Table 1. Ant species occurring on ≥ 15 trees, and results of Chi-square analysis testing if they occur more frequently on trees bearing EFNs that expected by chance.

Table 2**.** Independent effects (percentage of explained variance) of each variable for total ant species richness, and core and non-core ant species richness (results from hierarchical partitioning).

Tables

Table 1

Ant species	Total number of trees	Occurrence (%)		Chi	*p*-value
		EFN *(n = 287)*	non-EFN *(n = 574)*		
*Camponotus crassus**	181	25.4	18.8	5.05	0.02
Crematogaster distans	125	14.6	14.5	0.005	0.94
*Camponotus bonariense**	96	14.6	9.4	25.53	<0.01
*Cephalotes pusillus**	82	15.7	6.4	18.93	< 0.01
*Camponotus melanoticus**	69	13.9	5.1	20.49	< 0.01
*Crematogaster stollii**	65	10.1	6.3	4.03	0.04
*Cephalotes betoi**	59	10.1	5.2	7.13	<0.01
*Neoponera villosa**	41	7.3	3.5	6.20	0.01
Camponotus renggeri	34	4.9	3.7	0.98	0.39
Camponotus sp. 10	33	3.5	4.0	0.14	0.71
Myrmelachista sp.3*	29	5.2	2.4	4.57	0.03
*Dolichoderus diversus**	27	4.5	2.4	3.62	0.05
Azteca sp.1 *	26	5.6	1.7	9.60	<0.01
Camponotus blandus	26	3.1	3.0	0.02	0.89
Pseudomyrmex sp.1	19	2.4	2.1	0.11	0.74
Camponotus rufipes	18	2.4	1.9	0.26	0.61
*Cephalotes atratus**	18	3.5	1.4	4.09	0.04
Pseudomyrmex sp.3	17	2.1	1.9	2.80	0.30

* Ant species that occur more frequently on EFNs-bearing trees that expected by chance.

Table 2.

Variable	Independent effect	Significance
Total species richness		
Proportion of EFNs	47%	< 0.001*
Tree density	33%	< 0.001*
Tree richness	20%	0.005*
Non-core species richness		
Proportion of EFNs	56%	< 0.001*
Tree density	25%	< 0.001*
Tree richness	19%	0.003*
Core species richness		
Proportion of EFNs	47%	0.22
Tree density	28%	0.45
Tree richness	25%	0.48

*Significant values after 5000 randomizations.

Figure Legends

Figure 1. NMDS plot of ant species composition at the site scale, according to the proportion of trees bearing EFNs (indicated by point sizes), highlighting the classes of low and high proportion of EFNs (point colours; stress = 0.07). PERMANOVA indicated that species composition varied significantly (P = 0.002) among the proportion classes.

Figure 2. CLAM analysis classifying the 72 ant species into four classes according to their occurrence in plots with low vs high proportion of trees bearing EFNs: no preference (black circles); too rare to be assigned specificity preference (blue circles); preferring plots with a high proportion of EFN-bearing trees (High preference; red triangles: *M*2 = *Myrmelachista* sp. 2; *Dd* = *Dolichoderus diversus*; *P*3 = *Pseudomyrmex* sp. 3; *Cp* = *Cephalotes pusillus)*; preferring plots with a low proportion of EFN-bearing trees (Low preference; (green diamonds: *Cd* = *Crematogaster distans*; *Pk* = *Pseudomyrmex kuenckeli).*

Figures

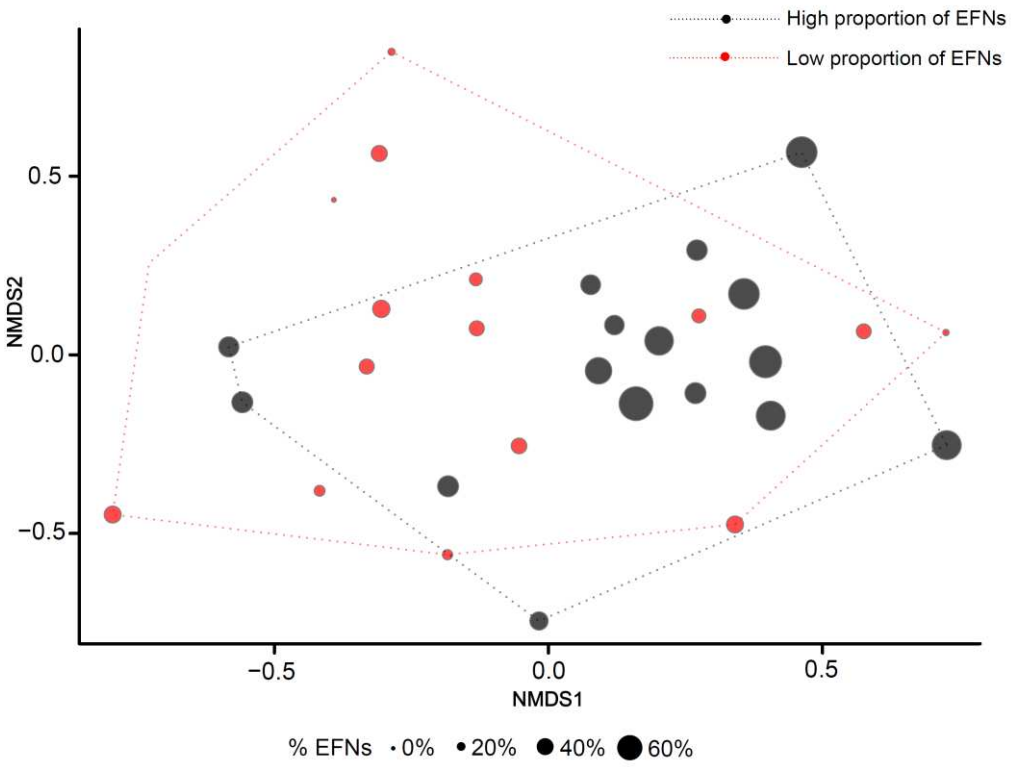

Figure 1

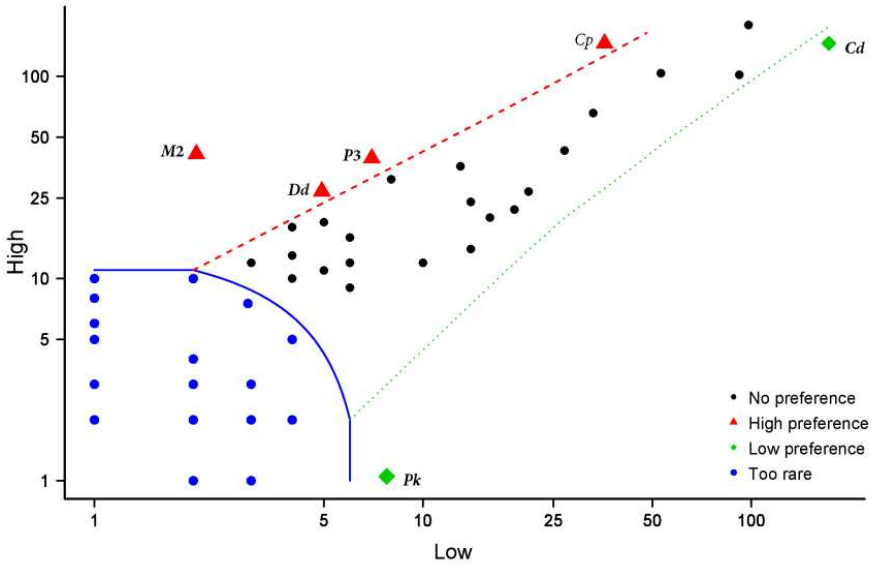
Cp
Cd
M2
P3
Dd
Pk
High
Low
100
50
25
10
5
1
1
5
10
25
50
100
No preference
High preference
Low preference
Too rare

Figure 2

Appendix

Appendix S1. Table S1. Plant species sampled in Serra do Cipó National Park (PNSC), Minas Gerais, Brazil. Plant species with EFNs are indicated by an asterisk.

Family	Plant species
ANONNACEAE	*Annona crassifolia* Mart.
APOCYNACEAE	*Aspidosperma tomentosum Mart.*
	Ditassa sp.
ARALIACEAE	*Schefflera macrocarpa* (Cham. & Schltdl.) Frodin
	Schefflera morototoni (Aubl.) Maguire et al.
ASTERACEAE	*Eremanthus crotonoides* (DC.) Sch.Bip.
	Piptocarpha rotundifolia (Less.) Baker
BIGNONIACEAE	**Cybistax antisyphilitica* (Mart.) Mart.
	**Handroanthus ochraceus* (Cham.) Mattos
	**Zeyheria* sp.
	**Zeyheria tuberculosa* (Vell.) Bureau ex Verl
CALOPHYLLACEAE	*Kielmeyera coriacea* (Spreng.) Mart.
	Kielmeyera petiolares Mart. & Zucc.
CARYOCARACEAE	**Caryocar brasiliense* Camb.
CONNARACEAE	*Rourea* sp. Aubl.
CLETHRACEAE	*Clethra scabra* Pers.
EBENACEAE	*Diospyros hispida* A.DC.
ERYTHROXYLACEAE	*Erythroxylum suberosum* A. St.-Hil.
	Erythroxylum tortuosum Mart.
FABACEAE	*Dalbergia miscolobium* Benth.
	**Enterolobium gummiferum* (Mart.) MacBryde
	**Hymenaea stigonocarpa* Mart. ex Hayne
	Leptolobium dasycarpum Vogel
	**Plathymenia reticulata* Benth.
	**Stryphnodendron adstringens* (Mart.) Coville
	**Stryphnodendron rotundifolium* Mart.
LOGANIACEAE	*Antonia ovata* Pohl
	Strychnos pseudoquina A. St.-Hil.

LAMIACEAE	**Aegiphila integrifolia* (Jacq.) Moldenke
	Hyptidendron canum (Pohl ex Benth.) Harley
MALPIGHIACEAE	*Byrsonima verbascifolia* (L.) DC.
	**Peixotoa tomentosa* A.Juss.
MYRTACEAE	*Eugenia aurata* O.Berg
	Eugenia dysenterica (Mart.) DC.
	unidentified
NYCTAGINACEAE	*Guapira noxia* (Netto) Lund
OCHNACEAE	**Ouratea hexasperma* (A. St.-Hil.) Baill.
PROTEACEAE	*Roupala montana* Aubl.
RUBIACEAE	*Palicourea rigida* Kunth
	**Tocoyena formosa* (Cham. & Schltdl.) K. Schum.
SAPOTACEAE	*Pouteria ramiflora* (Mart.) Radlk.
	Pouteria torta (Mart.) Radlk.
SAPINDACEAE	*Matayba marginata* Radlk.
SOLANACEAE	*Solanum lycocarpum* A.St.-Hil.
VOCHYSIACEAE	**Qualea grandiflora* Mart.
	Vochysia elliptica Mart.

Appendix S1. Table S2. Arboreal ant species recorded in study plots in Serra do Cipó National Park (PNSC), Minas Gerais, Brazil.

Subfamily	Species
Dolichoderinae	*Azteca* sp. 1
	Dolichoderus diversus Emery, 1894
	Dorymyrmex sp. 1
	Dorymyrmex sp. 2
	Forelius sp. 1
	Linepithema neotropicum Wild, 2007
	Tapinoma cf. *amazonae*
Ectatomminae	*Ectatomma planidens* Borgmeier, 1939
	E. tuberculatum Olivier, 1792
Formicinae	*Brachymyrmex* sp. 1
	Brachymyrmex sp. 2
	Brachymyrmex sp. 3
	Camponotus blandus (F. Smith, 1858)
	C. bonariensis Mayr, 1868
	C. crassus Mayr, 1862
	C. leydigi Forel, 1886
	C. melanoticus Emery, 1894
	C. personatus Emery, 1894
	C. nr. *senex*
	C. renggeri Emery, 1894
	C. rufipes Fabricius, 1775
	C. senex F. Smith, 1858
	Camponotus sp. 1
	Camponotus sp. 4
	Camponotus sp. 9
	Camponotus sp. 10
	Camponotus sp. 12
	Camponotus sp. 13
	Camponotus sp. 17
	Camponotus sp. 18

	Camponotus sp. 19
	Myrmelachista nr. *gallicola* Mayr, 1887
	M. nodigera Mayr, 1887
	Myrmelachista sp. 3
Myrmicinae	*Atta* sp. 1
	Atta sp. 2
	Cephalotes atratus Linnaeus, 1758
	C. betoi Andrade & Baroni Urbani, 1999
	C. depressus (Klug, 1824)
	C. grandinosus (Smith, F., 1860)
	C. nr. *persimilis* De Andrade, 1999
	C. pusillus Klug, 1824
	Crematogaster distans (Mayr, 1870)
	C. stolli Forel, 1885
	Crematogaster sp.2 (crinosa group)
	Crematogaster sp. 4
	Crematogaster sp.6
	Nesomyrmex spininodis (Mayr, 1887)
	Nesomyrmex sp. 1
	Ochetomyrmex semipolitus Mayr, 1878
	Pheidole oxyops Forel, 1908
	Pheidole sp. 1
	Pheidole sp. 3
	Pheidole sp. 5
	Solenopsis invicta Buren, 1972
	S. tridens Forel, 1911
	Solenopsis sp. 1
	Solenopsis sp. 4
	Solenopsis sp. 7
Ponerinae	*Neoponera villosa* (Fabricius, 1804)
Pseudomyrmecinae	*Pseudomyrmex kuenckeli* (Emery, 1890)
	P. schuppi (Forel, 1901)
	P. termitarius (Smith, F., 1855)
	Pseudomyrmex sp. 1
	Pseudomyrmex sp. 2
	Pseudomyrmex sp. 3

Pseudomyrmex sp. 4

Pseudomyrmex sp. 8

Pseudomyrmex sp. 10

Pseudomyrmex sp. 11

Pseudomyrmex sp. 12

Pseudomyrmex sp.13

CHAPTER TWO

NUTRIENT AVAILABILITY AS A DRIVER OF ARBOREAL ANT COMMUNITIES: DIFFERENTIAL RESPONSES TO NUTRIENT SUPPLEMENTATION

Nutrient availability as a driver of arboreal ant communities: differential responses to nutrient supplementation

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Abstract

Resource-ratio theory predicts that consumers aim to target optimal ratios of complementary nutrients. Accordingly, arboreal ants, which have a surplus of carbohydrate-rich food resources and a protein-stressed diet, should be attracted to the most limited resource in the environment (protein). However, different functional groups of arboreal ant have stoichiometric discrepancies among them, ranging from high N-limited (trophobiont tenders = TT), intermediate N-limited (leaf foragers = LF) and least N-limited (predators = PD), which may promote interspecific differences in nutritional requirements within the assembly. Here we report results from a manipulative field experiment using protein and carbohydrate (CHO) supplementation on trees to answer: 1) Do different functional groups show different responses to protein and CHO supplementation according to differences in the extent to which they are N-limited? 2) Does the addition of macronutrients affect the general foraging activity of species attracted to them, and how does this affect the composition of the overall ant foraging community? Protein supplements were more attractive to the overall ant community, with strikingly higher ant abundance and higher richness than in carbohydrate supplements. The same pattern was found for TT, which is the most N-limited functional group. In contrast, functional groups less N-limited showed higher species richness (LF) and abundance (PD) in CHO-rich supplements. The different functional group responses were reflected in differences in overall species composition among supplements. Moreover, we also observed an increase in total abundance of ant foraging on trees after addition of protein-rich supplements. The foraging abundance of TT increased with addition of protein and carbohydrate supplements, but with a more pronounced response at protein. Protein supplementation led to a decreased abundance of LF. Considering that TT are

behaviorally dominant species and have large colonies, interference competition from them could have influenced LF responses. This study highlights the nutrient availability as a driver of communities of arboreal ants. We suggest that differences in taxon-specific nutritional needs determine patterns of resources utilization and species assembly in arboreal ant communities.

Key words: community structure, ecological stoichiometry, nutritional ecology, Formicidae, Cerrado.

Introduction

Protein and carbohydrate (CHO) are key nutrients sustaining metabolic processes, growth and reproduction in animals (Simpson and Raubenheimer 1993). These nutrients have very different chemistries, but have complementary functions that act synergistically to enhance animal fitness (Tilman 1982). The availability of protein and carbohydrate varies considerably among food resources, and one of the major challenges for consumers is to acquire an appropriate balance to target their nutritional needs (Feldhaar 2014), rather than simply maximizing food intake (Simpson and Raubenheimer 2000). Ecological stoichiometry explores the ecological effects of such constraints, investigating how different nutrient ratios can drive the abundance and organization of biological communities (sensu Sterner and Elser, 2002). The specific nutrient requirements of organisms are a fundamental driver of species interactions with their environment, and consequently affect many ecological processes (Elser and Urabe 1999, Kay 2002, Feldhaar 2014).

In ecological stoichiometry, resource-ratio theory predicts that consumers are more attracted to the nutrient that is relatively less available in the environment, because it is metabolically limiting (Tilman 1982, Galef 1991, Simpson and Raubenheimer 1993, Kay 2004). For example, arboreal and litter ants have contrasting dietary preferences that reflect the different availability of nutrients in their respective habitats (Kaspari and Yanoviak 2001, Ness et al. 2009). Whereas litter ants have a protein-biased diet (Kaspari et al. 2012), the main resources consumed by arboreal ants are plant-based exudates (honeydew and extra-floral nectar) that have extremely high sugar content (Rico-Gray and Oliveira 1997, Blüthgen et al. 2004a, Nepi et al. 2012). Arboreal ants therefore face a stoichiometric limitation of protein, and would be highly attracted to protein-rich resources

according to resource-ratio theory (Kaspari and Yanoviak 2001, Ness et al. 2009). Moreover, the carbohydrate-based diets of arboreal ants can fuel high rates of worker activity and consequently promote foraging for protein resources (Davidson 1998, Grover et al. 2007, Ness et al. 2009, Rudolph and Palmer 2013).

Most exudate-feeding ants can be classified into one of two functional groups based on diet and foraging behaviour (Davidson et al. 2003, 2004): "trophobiont tenders", which specialize on tending hemipterans for honeydew, and "leaf foragers", which forage for extra-floral nectar along with pollen, fungal spores and discarded honeydew. Trophobiont tenders typically are behaviourally dominant species, occur in very large colonies and exhibit mass recruitment, foraging intensively in high-value food resources. In contrast, leaf foragers have small to medium-sized colonies, forage solitarily and behave subordinately (Blüthgen et al. 2003). Trophobiont tenders monopolize honeydew because it is the most valuable exudate resource. It occurs in higher volumes, is more predictable in space and time, and often is more N-enriched than other exudate sources (Blüthgen and Fiedler 2004a).

Trophobiont tenders and leaf foragers also possess different adaptations for overcoming an N-deprived diet. Trophobiont tenders use a strategy of high exudate input combined with substantial carnivory. Most (belonging to the subfamilies Formicinae and Dolichoderinae, and myrmicine genus *Crematogaster*) possess a specialized proventriculus adapted for processing and storing large volumes of fluid, and allowing for the sharing of ingested food with nestmates (Davidson 1997). On the other hand, leaf foragers (mostly within the subfamilies Pseudomyrmecinae and the myrmicine tribe Cephalotini, along with some camponotines) overcome N-deprivation through the metabolic activity of endosymbiotic bacteria resident in their guts.

Trophobiont tenders and leaf foragers have different nutrient-consumption ratios, which are also different in comparison to predator ants that also feed on plant exudates (Blüthgen et al. 2003, Davidson et al. 2003). Predator ants have the highest $\delta^{15}N$ ratios (a higher ratio reflects a higher trophic position in the food web; Fry 2006), and leaf foragers the lowest, with the latter being considered functionally herbivorous because of the low fraction of nitrogen in their diets (Davidson et al. 2003, Davidson 2005). Such differences in nitrogen assimilation are reflected in differences in N-limitation. Davidson (2005) found that predator ants exhibited the smallest values of N-limitation, and that although trophobiont tenders have higher $\delta^{15}N$ ratios than leaf foragers (Blüthgen et al. 2003), they are intrinsically more N-limited, and thus are more likely to pursue prey. Such differences can be expected to lead to marked variation in preferences for protein over CHO among arboreal ant species, which consequently promotes niche partitioning within arboreal ant communities (Kay 2002, Blüthgen and Fiedler 2004a).

Here we report a manipulative field experiment that uses protein and CHO supplementation to investigate variation in food attractiveness and resource access among stoichiometrically contrasting arboreal ant species, and its implications for ant community structure, in a Neotropical savanna. Our study addresses two questions: 1) Do different functional groups show unequal responses to protein and CHO supplementation according to differences in the extent to which they are N-limited? 2) Does the addition of macronutrients affect the general foraging activity of species attracted to them, and how does this affect the composition of the overall ant foraging community?

We first hypothesize that the abundance, richness and composition of ant species vary among protein and CHO supplements according to variation among species in

their dependence on N-rich resources. We predict that overall community will be more attracted to protein supplements, the nutrient with shortest supply in the environment. We also predict that trophobiont tenders will be most strongly attracted to protein supplements because they are most N-limited. In contrast, we predict that leaf foragers will be more attracted to CHO rather than protein supplements, because they are less N-limited and have strong reliance on endosymbiont bacteria to target their nitrogen ratios. We also predict that predator species will be more attracted to CHO supplements due to their N-biased diet.

Secondly, we hypothesize that nutrient supplementation affect the community of foraging ants and the effect is more pronounced on trees supplemented with protein-rich supplements. We predict that both protein and CHO supplements will lead to an increase in the general foraging abundance of trophobiont tenders, due to a consequent stimulation of foraging activity of their large colonies, but this effect will be greater for protein-rich supplements.

Methods

Study site

We conducted the study in the Serra do Cipó National Park, Minas Gerais, Brazil, in a transition between two Brazilian savanna phytophysiognomies "Cerrado *sensu stricto*" and "Campo Cerrado" (Coutinho 1990). The vegetation is characterized by scattered and low (mostly 3-5 m) trees, with low canopy connectivity (limits of study area: 19°21'56"- 19°22'13" S and 43°36'57"- 43°37'6" W). The tree layer is dominated by species of Fabaceae (33.3% of all trees in the study plots), Asteraceae (12%) and Sapotaceae (8.4%). The region is characterized by a tropical humid climate with a winter (May to September) dry season and

summer (October to April) wet season, and a mean annual rainfall of 1,374 mm (Madeira and Fernandes 1999). Mean temperature shows little monthly variation, ranging from 20 °C to 22 °C (Madeira and Fernandes 1999).

Supplementation treatments

We established 32 (20 X 20)m plots and marked all woody plants > 8 cm in basal diameter within each plot, limit that includes most of tree species typically from Brazilian savanna (Felfili and Silva Júnior 1988). The distance between nearest plots ranged from 20 – 50 m, and the most distant plots were separated from each other by 700 m. Plots were randomly assigned one of four supplement treatments (8 plots per treatment, with all trees within a plot receiving the same supplement) consisting of synthetic foods dissolved in water following Dussutour and Simpson (2008) and Kaspari et al. (2012): 1) protein, consisting of a 20% solution containing equal parts of whey protein isolate, calcium caseinate, and egg white powder (Protein); 2) carbohydrate, containing 20% sucrose (CHO); 3) protein and carbohydrate, consisting of an equal mixture of 1 and 2 (Protein+CHO); 4) distilled water, as a control (Water).

In each case, cotton wool was soaked in 40 ml of the supplement, placed in an 80-ml plastic vial, and tied to trees using a flexible wire. Each tree received three vials, with one tied to the main trunk and the others tied to branches near foliage. The plastic vials were covered by lids to reduce evaporation, and five holes (4.5 mm diameter) were drilled in each lid to allow ant access. We placed a wooden stick in each entrance hole so that ants could readily move in and out, preventing the vials from acting as traps. Supplements were placed in trees for a 7-day period during March (wet season) and June (dry season) 2014.

Ant sampling

We sampled ants on supplements as a measure of the relative attractiveness of the different nutrient types, and ants elsewhere on trees as a measure of the influence of different supplements on the general foraging activity of different species. We collected ants on supplements between 7:00 h and 17:00 h on the second and seventh day of supplementation. Vials were detached from trees, placed into a deep tray, and specimens were collected using an aspirator tube and transferred to a vial filled with ethanol (70%). Vials were then either returned to the trees (day 2) or removed (day 7).

We surveyed foraging ants on trees a week before and again on the first day of supplementation, using three non-baited pitfall traps wired to each tree. Traps were 5-cm diameter (80 ml) plastic vials containing water and detergent, and operated for 48 hrs. One of the traps was tied to the main trunk and the others to branches near foliage. Traps were located at least 0.5 m from supplements in order to minimize the capture of workers recruiting to them.

Ant functional groups

We classified ant species into one of three functional groups based on the feeding biology of the genus to which they belong, following Davidson et al. (2003) and Davidson (2005), and considering all genera recorded from at least 5% (43) of total trees. Species of *Camponotus*, *Cephalotes*, *Myrmelachista*, *Ochetomyrmex* and *Pseudomyrmex* were classified as leaf foragers (LF), and species of *Azteca*, *Crematogaster* and *Dolichoderus* as trophobiont tenders (TT). Exceptions were *Camponotus rupifes*, *C. renggeri*, *Cephalotes atratus* and *Pseudomyrmex kuenckeli*,

which we classified as TT based on the literature (Dejean et al. 1999, Del-Claro and Oliveira 1999, Blüthgen et al. 2000, Oliveira and Freitas 2004) and our own field observations. We classified species of *Ectatomma* and *Neoponera* as predators (PD), although they also forage extensively on plant resources (Oliveira and Freitas 2004, Davidson 2005, Koch et al. 2016).

Data Analysis

In order to analyse variation in ant richness and abundance on supplements, we fitted generalized linear mixed models (GLMMs) with the different supplements as explanatory variables. We performed a similar analysis for ants sampled in pitfall traps, but with time (before and during supplementation) as the explanatory variable, fitting a separate model for each type of supplement. In this analysis, we excluded 13 trees which we recorded extremely high ant abundance of *Myrmelachista* species in pitfall traps that could otherwise distort the results. To account for spatial autocorrelation among trees located in the same plot, we treated individual trees as a random effect, controlling for pseudo-replication in GLMMs models. We conducted the analysis for all species combined, and for each functional group.

To ensure that our results were not confounded by the natural occurrence of large sources of carbohydrate-rich resources, we excluded from analysis the very small proportion (2%) of trees with honeydew-producing hemipterans, and included the presence of extrafloral nectaries as a co-variable in the analyses. The presence of EFNs was associated with higher ant species richness in both supplement and pitfall samples. However, it did not influence the pattern of ant responses to supplements and thus, we did not showed the effect of EFNs in the results.

To assess variation in ant species composition in relation to supplementation, we performed permutational multivariate ANOVA (PERMANOVA) based on Bray-Curtis dissimilarity with 5000 permutations, using square root transformed abundance data. We tested the assumption of spatial homogeneity of variances (Anderson 2001) and ran multiple pairwise comparisons among treatments using Bonferroni corrected *P*-values.

We computed indicator values for each species, considering both their specificity and fidelity and based on a species abundance matrix (IndVal, Dufrêne and Legendre 1997), to identify species showing strong associations with particular supplements. The significance of IndVal measures for each species was tested using 10.000 Monte Carlo permutations.

We conducted all analysis using the software R (R Core Team 2016), and analyzed the residuals to check for data normality and homoscedasticity in all models. We performed pairwise contrast analyses to evaluate differences among treatments, combining the most similar levels and comparing models (Crawley 2012). We used poisson error distribution and corrected for over-dispersion when required with negative binomial distribution. We carried out GLMMs using the *lme4* v 1.1-11 package (Bates et al. 2015), IndVal using *labdsv* v 1.8-0, and PERMANOVA using *vegan* v 2.4-0 (Oksanen et al. 2015).

Results

The ant fauna

We sampled a total of 860 trees belonging to 46 species and 24 families. The mean (±SE) number of trees per treatment was 214 ± 15. In total, we recorded 88 ant species (65 on supplements, and 83 in pitfall traps) from 29 genera, with the richest

genera being *Camponotus* (22 species), *Pseudomyrmex* (15), *Crematogaster* (6), and *Cephalotes* (5) (Table S1). Most of the 18 species recorded in traps but not at supplements were very uncommon, occurring on < 3 trees. Exceptions were *Camponotus* sp. 12 (10 trees), *Camponotus* sp. 22 (19 trees) and *Pheidole* sp. 1 (8 trees). It is possible that they are strictly nocturnal, and therefore not recorded by our protocol for sampling supplements. The most common species overall were *Camponotus crassus* (occurring in 48% of trees), *Crematogaster distans* (34%), and *Cephalotes betoi* (20%). A total of 51 species were classified according to their feeding biology, 36 as leaf foragers, 12 as trophobiont tenders, and four as predators (Table S1).

Ants attracted to supplements

Mean overall ant abundance was higher on Protein and Protein+CHO than CHO for total ants (χ^2= 398.490, $P < 0.001$) and trophobiont tenders ($\chi^2 = 202.65$, $P < 0.001$), and higher on CHO and Protein+CHO than Protein for predators ($\chi^2 = 18.807$, $P < 0.001$; Table 1, Fig. 1). Mean abundance of leaf foragers did not vary significantly among supplements. The proportional contribution to total ant abundance of trophobiont tenders was substantially higher on Protein+CHO and Protein than on CHO supplements (91% in Protein+CHO and Protein; 52% in CHO).

Total ant species richness varied significantly among supplements (χ^2= 250.277, $P < 0.001$, Table 1), with mean richness at Protein and Protein+CHO both about 20% higher than at CHO (Fig. 2). Mean species richness also varied significantly among supplements for trophobiont tenders ($\chi^2 = 73.678$, $P < 0.001$) and leaf foragers ($\chi^2 = 108.249$, $P < 0.001$); highest richness was on Protein for the former, but CHO and Protein+CHO for latter (Fig. 2). Species richness of predators

varied significantly only between supplements and the control (χ^2 = 20.785, P < 0.001, Fig. 2).

The composition of ant species attracted to Protein was similar to that at Protein+CHO ($F_{1,705}$ = 1.034; P-adjusted = 1.000), but this was significantly different to that attracted to CHO supplements (PERMANOVA – $F_{3,1095}$ = 10.03; P = 0.01). Using IndVal, we identified four TT species significantly associated with Protein supplements, and two LF, one PD, and one TT species significantly associated with CHO (Table 2).

Foraging ant community

There was no change in ant abundance in pitfall traps in control trees after the addition of water (Table 1). On trees supplemented with CHO, the only change in ant abundance during supplementation was an increase in trophobiont tenders (χ^2 = 2.049, P = 0.15, Fig. 3). On trees supplemented with Protein, there was an increase in abundance of trophobiont tenders (χ^2 = 8.259, P < 0.01), but also an increase in total ant abundance (χ^2 =20.689, P < 0.001) and a decrease in leaf foragers (χ^2 = 12.846, P < 0.001, Fig. 4). The same pattern was found on trees after Protein+CHO supplementation (Total ants: χ^2 = 5.985, P = 0.01; TT: χ^2 = 6.971, P < 0.01; LF: χ^2 = 19.102, P < 0.001, Fig 5). There were no differences in ant species richness during supplementation compared with beforehand, whether considering all ants or individual functional groups (Table 1). There were also no significant differences in foraging ant species composition after supplementation in any of the treatments: Water (PERMANOVA: $F_{1,270}$ = 1.17, P = 0.27), CHO ($F_{1,340}$ = 1.05, P = 0.37), Protein+CHO ($F_{1,309}$ = 1.31, P = 0.20), and Protein ($F_{1,376}$= 1.21, P = 0.24).

Discussion

Ants attracted to supplements

Our manipulative field experiment examined differential responses of stoichiometrically contrasting arboreal ant species to protein and carbohydrate supplementation, and its implications for the structure of arboreal ant communities. We first hypothesized that the abundance, richness and composition of ant species will vary among protein and CHO supplements according to their dependence on N-rich resources. As we predicted, supplements rich in protein (i.e. Protein and Protein+CHO) were more attractive to overall community than those without protein, with ant abundance at protein baits being three-fold higher than at carbohydrate baits, and mean richness approximately 20% higher. Similar results were reported by Yanoviak and Kaspari (2000), who found higher activity of arboreal ants on N-rich baits than sugar baits (although these results were confounded by higher levels of salt and fats in the N-rich baits). This contrasts with the results from a study of litter ants, where Kaspari et al. (2012) observed more individuals on CHO than protein baits. Such contrasting responses from arboreal and litter ants are consistent with resource ratio theory, which predicts that consumers are more attracted to the less-available nutrient in their environment (Tilman 1982) in order to maintain elemental homeostasis (Kay et al. 2006).

As also predicted, trophobiont tenders were most strongly attracted to protein-rich supplements. They represented 91% of total individuals on protein baits, compared with 52% on carbohydrate baits. Among all individuals sampled on proteinaceous supplements, 88.9% belonged to trophobiont tenders genera *Azteca* and *Crematogaster*. This particular high occurrence can be explained according to the extraordinary high levels of N-limitation already recorded for both genera

(Davidson 2005), which can be translated that those taxa are stimulated to pursue of protein-rich resources. Together with their intrinsic characteristic of protein starvation, both genera have large colonies and recruit massively to resources (Wheeler 1986a, Longino 2003), which reflected in very high abundance on valuable resource and outnumbered other ants in protein supplements.

As also predicted, more leaf foragers species were attracted to carbohydrate than protein supplements. The majority of leaf forager species (32 of 36) were camponotines, cephalotines and pseudomyrmecines, which present co-evolutionary histories with endosymbiotic bacteria that provide nitrogen to support their stoichiometrically imbalanced diets (Eilmus and Heil 2009, Russell et al. 2009, Anderson et al. 2012). Unexpectedly, we did not observed higher abundance of leaf foragers in CHO-rich supplements than in protein-rich ones. This can be at least partly explained by their solitary-foraging behavior, collecting and leaving supplements and therefore avoiding being censured.

Predator species are the least N-limited exudate-feeding ants, and as predicted they occurred in higher abundance on carbohydrate than protein supplements. The most common predator species were *Ectatomma tuberculatum* and *Neoponera villosa*, which collectively represented 91% of all predator individuals. Both species forage extensively both on the ground and on trees, preying on small arthropods and also collecting extrafloral nectar (Wheeler 1986b, Blüthgen et al. 2000, Byk and Del-Claro 2010, Dáttilo et al. 2014). Both species have $\delta^{15}N$ ratios that are typical of carnivorous insects (Davidson et al. 2003), and use their high nitrogen intake to produce proteinaceous venom (Morgan et al. 2003). Therefore, in contrast to trophobiont tenders and leaf foragers, predators are somewhat CHO-limited (Davidson 2005) and hence were more attracted to carbohydrate supplements.

Furthermore, the lack of response in predators' richness could be due to the very low number of species recorded on supplements (four species), in addition to the high frequency of only two predator species, as already pointed above.

The different functional group responses outlined above were reflected in differences in overall species composition among supplements, in addition to a significant association of more N-limited species to protein supplements and less N-limited species to CHO supplements. The only exception was the trophobiont tender *Crematogaster* sp. 6, which, unlike other members of the genus, was most abundant at carbohydrate supplements. Its feeding biology and associated nutrient limitation is therefore likely to be very different to that typical of its congeners. The degree of N-limitation follows taxonomic lines, but some ecological variation surely exists within groups (Blüthgen et al. 2003).

Notably, we found no additive effect of supplements when they were combined, as in all cases combined supplements produced the same results as either one of the single supplements. A similar result was found for neotropical litter ants (Kaspari et al. 2012) and these findings reinforce the idea that ants are notable limited by the nutrient scarce in the environment, since their attractiveness by supplements containing blend of high-quality nutrients was not as strong as the attraction by the single supplement.

Foraging ant community

Our second hypothesis was that protein and CHO supplementation would change the community of foraging ants, with a more pronounced effect on trees supplemented with protein. As predicted, we found a numerical response in overall community and functional groups with supplement addition on trees; however,

richness and composition did not change. Probably, this can be explained by the fact that the species pool on trees was unlikely to change during the short duration (7 days) of supplementation. A previous supplementation experiment likewise did not find change in the richness of litter ants at supplements (Kaspari et al. 2012).

Considering all species, we unexpectedly observed an increase of foraging abundance exclusively during protein-rich supplementation, whereas the addition of carbohydrate did not affect the overall community foraging on trees. As we predicted, the abundance of trophobiont tenders increased with addition of both protein and carbohydrate supplements, but with a more pronounced response at protein. We pointed out that the composition of CHO supplements were purely sucrose, which is the most attractive sugar for arboreal ant species (Blüthgen and Fiedler 2004b). Therefore, the massive supply of high-quality resources, such as the CHO and protein supplements, stimulated the foraging and possibly the patrolling activity of populous trophobiont tenders colonies on trees.

In contrast to trophobiont tenders response, we did not detect a increasing of predators and leaf foragers abundance on trees supplemented with the most limited resource for these functional groups (i.e., CHO). Moreover, while protein-rich supplements led to an increase in the foraging abundance of trophobiont tenders, they led to a decreased abundance of leaf foragers. One possible explanation for this result is the overlap of foraging space between trophobiont tenders and leaf foragers, in consequence of the addition of high-quality food resources. Considering that trophobiont tenders have substantially larger colonies than leaf foragers (Davidson et al. 2004), the increased in trophobiont tenders foragers could have limited the foraging space available for leaf foragers. Interspecific encounters should affect foraging activity and ants might avoid foraging at intense encounter patches (Gordon

and Kulig 1996, Brown and Gordon 2000). Decreasing the rate of encounters in areas with high density of individuals, leaf foragers could minimized lost in foraging time or reduced the effects of exploitative competition (Gordon and Kulig 1996).

Another reasonably explanation for the decrease in leaf foragers abundance on trees supplemented with protein-rich resources is their exclusion by aggressive trophobiont tenders. Changes in foraging abundance of dominant species linked to changes in resource availability potentially influence the total number of species foraging (Andersen 1992). We could infer that the great availability of a protein-rich resource induced agonistic behavior of trophobiont tenders against subordinate leaf foragers, in order to prevent access of other ants to this high-quality food. As the abundance of dominant ants increased, we should expect a consequent reduction in others species abundance and richness via competitive exclusion (Andersen 1992, Parr et al. 2005).

Furthermore, competitive interaction could also have interfered in the segregation of species among different resources. That way, leaf foragers and predators could not showed high attractiveness by protein supplements because trophobiont tenders have monopolized them and excluded them (regulation of momentary diversity – Andersen 1992). Nevertheless, our results indicate that attractiveness by the most limited nutrient is the main mechanism behind the compartmentalization observed among functional groups. If interference competition was strong acting, we should expect a decrease in species richness and abundance of leaf foragers, in supplements highly attractive for trophobiont tenders. Instead, we observed that both functional groups showed higher attractiveness for the supplement consisted by the combination of nutrients (i.e., Protein+CHO), along with the supplement constituted purely with their respective most limited nutrient. We

highlight that the extent to how competitive interactions influence in arboreal ant species attractiveness by complementary nutrients can be seen as a valuable focus for future work.

Conclusion

We showed a higher attractiveness of overall arboreal ant community for protein over carbohydrate, whereas different functional groups were attracted to different nutrients, in accordance with their specific diet requirements. We suggest that differences in taxa-specific nutritional needs in arboreal ant assembly may determine patterns of resources utilization in such communities. Our results have important implications for dietary niche differentiation among arboreal ant species with a high dependence on plant exudates. Plant exudates present marked variation in chemical composition and ratios of protein and carbohydrates (Yeoh et al. 1992) and it is known that arboreal ant species have different gustatory preferences for particular types of amino acids and sugars (Blüthgen and Fiedler 2004a, 2004b). Such variation in chemical composition of resources potentially plays an important role shaping the distribution of arboreal ant species in the canopy, but this has received little attention. We also point out that interference competition from behaviourally dominant ants could influence the patterns of nutrient attractiveness by subordinate species, as already showed for Australian ant communities. However, such indirect effects could vary between continents as reflecting biogeographic differences in the importance of behaviourally dominant ant species. For example, the overall abundance of arboreal ants is far higher in Australian compared with Brazilian savannas, due largely to an extremely high abundance of behaviourally dominant species of *Iridomyrmex* (Campos et al. 2011). We point out the importance

in more stoichiometric studies to be developed in distinct regions, such as neotropical savanna, in order to improve our understanding about mechanisms driving arboreal ant distribution.

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References

Andersen, A. N. 1992. Regulation of "momentary" diversity by dominant species in exceptionally rich ant communities of the Australian seasonal tropics. The American naturalist 140:401–420.

Anderson, K. E., J. A. Russell, C. S. Moreau, S. Kautz, K. E. Sullam, Y. Hu, U. Basinger, B. M. Mott, N. Buck, and D. E. Wheeler. 2012. Highly similar microbial communities are shared among related and trophically similar ant species. Molecular Ecology 21:2282–2296.

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology:32–46.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 67:1–48.

Blüthgen, N., and K. Fiedler. 2004a. Competition for composition: Lessons from nectar-feeding ant communities. Ecology 85:1479–1485.

Blüthgen, N., and K. Fiedler. 2004b. Preferences for sugars and amino acids and their conditionality in a diverse nectar-feeding ant community. Journal of Animal Ecology 73:155–166.

Blüthgen, N., G. Gebauer, and K. Fiedler. 2003. Disentangling a rainforest food web using stable isotopes: Dietary diversity in a species-rich ant community. Oecologia 137:426–435.

Blüthgen, N., G. Gottsberger, and K. Fiedler. 2004. Sugar and amino acid composition of ant-attended nectar and honeydew sources from an Australian rainforest. Austral Ecology 29:418–429.

Blüthgen, N., M. Verhaagh, W. Goitía, K. Jaffé;, W. Morawetz, and W. Barthlott. 2000. How plants shape the ant community in the Amazonian rainforest

canopy: the key role of extrafloral nectaries and homopteran honeydew. Oecologia 125:229–240.

Byk, J., and K. Del-Claro. 2010. Nectar- and pollen-gathering Cephalotes ants provide no protection against herbivory: A new manipulative experiment to test ant protective capabilities. Acta Ethologica 13:33–38.

Campos, R. I., H. L. Vasconcelos, A. N. Andersen, T. L. M. Frizzo, and K. C. Spena. 2011. Multi-scale ant diversity in savanna woodlands: An intercontinental comparison. Austral Ecology 36:983–992.

Coutinho, L. M. 1990. Fire in the Ecology of the Brazilian Cerrado. Pages 82–105. Springer Berlin Heidelberg.

Crawley, M. J. 2012. The R book, Second Edition edn. John Wiley & Sons, Chichester.

Dáttilo, W., F. M. D. Marquitti, P. R. Guimarães, and T. J. Izzo. 2014. The structure of ant – plant ecological networks: Is abundance enough? Ecology 95:475–485.

Davidson, D. W. 1997. The role of resource imbalances in the evolutionary ecology of tropical arboreal ants. Biological Journal of the Linnean Society 61:153–181.

Davidson, D. W. 2005. Ecological stoichiometry of ants in a New World rain forest. Oecologia 142:221–231.

Davidson, D. W., S. C. Cook, and R. R. Snelling. 2004. Liquid-feeding performances of ants (Formicidae): Ecological and evolutionary implications. Oecologia 139:255–266.

Davidson, D. W., D. W. Davidson, S. C. Cook, R. R. Snelling, and T. H. Chua. 2003. Explaining the abundance of ants in lowland tropical rainforest canopies. Science 300:969–973.

Dejean, A., B. Corbara, and J. Orivel. 1999. The Arboreal Ant Mosaic in Two Atlantic Rain Forests 20:133–145.

Del-Claro, K., and P. S. Oliveira. 1999. Ant-Homoptera interactions in a neotropical savanna: The honeydew-producing treehopper, *Guayaquila xiphias* (Membracidae), and its associated ant fauna on *Didymopanax vinosum* (Araliaceae). Biotropica 31:135–144.

Dufrêne, M., and P. Legendre. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs 67:345–366.

Dussutour, A., and S. J. Simpson. 2008. Description of a simple synthetic diet for studying nutritional responses in ants. Insectes Sociaux 55:329–333.

Eilmus, S., and M. Heil. 2009. Bacterial associates of arboreal ants and their putative functions in an obligate ant-plant mutualism. Applied and Environmental Microbiology 75:4324–4332.

Feldhaar, H. 2014. Ant nutritional ecology: Linking the nutritional niche plasticity on individual and colony-level to community ecology. Current Opinion in Insect Science 5:25–30.

Felfili, J., and M. C. da Silva Júnior. 1988. Distribuição dos diâmetros numa faixa de cerrado na Fazenda Água Limpa (FAL), em Brasilia-DF. Acta Botanica Brasilica 2:85–105.

Fry, B. 2006. Stable Isotope Ecology. Springer New York, New York, NY.

Galef, B. G. 1991. A contrarian view of the wisdom of the body as it relates to dietary self-selection. Psychological Review 98:218–223.

Gordon, D. M., and A. W. Kulig. 1996. Founding, foraging, and fighting: colony size and the spatial distribution of harvester ant nests'. Ecology 77:2393–2409.

Grover, C. D., A. D. Kay, J. A. Monson, T. C. Marsh, and D. A. Holway. 2007. Linking nutrition and behavioural dominance: carbohydrate scarcity limits aggression and activity in Argentine ants. Proceedings. Biological sciences / The Royal Society 274:2951–2957.

Kaspari, M., D. Donoso, J. A. Lucas, T. Zumbusch, and A. D. Kay. 2012. Using nutritional ecology to predict community structure: a field test in Neotropical ants. Ecosphere 3:1–15.

Kaspari, M., and S. P. Yanoviak. 2001. Bait use in tropical litter and canopy ants - evidence of differences in nutrient limitation. Biotropica 33:207–211.

Kay, A. 2002. Applying Optimal Foraging Theory to Assess Nutrient Availability Ratios for Ants. Ecology 83:1935–1944.

Kay, A. 2004. The relative availabilities of complementary resources affect the feeding preferences of ant colonies. Behavioral Ecology 15:63–70.

Kay, A. D., S. Rostampour, and R. W. Sterner. 2006. Ant stoichiometry: Elemental homeostasis in stage-structured colonies. Functional Ecology 20:1037–1044.

Koch, E. B. A., F. Camarota, and H. L. Vasconcelos. 2016. Plant Ontogeny as a Conditionality Factor in the Protective Effect of Ants on a Neotropical Tree. Biotropica 48:198–205.

Longino, J. T. 2003. The Crematogaster (Hymenoptera, Formicidae, Myrmicinae) of Costa Rica. Zootaxa 151:1–150.

Madeira, J. a., and G. W. Fernandes. 1999. Reproductive phenology of sympatric taxa of *Chamaecrista* (Leguminosae) in Serra do Cipó, Brazil. Journal of Tropical Ecology 15:463–479.

Morgan, E. D., H. Jungnickel, S. J. Keegans, R. R. do Nascimento, J. Billen, B. Gobin, and F. Ito. 2003. Comparative survey of abdominal gland secretions of the ant subfamily Ponerinae. Journal of chemical ecology 29:95–114.

Nepi, M., C. Soligo, D. Nocentini, M. Abate, M. Guarnieri, G. Cai, L. Bini, M. Puglia, L. Bianchi, and E. Pacini. 2012. Amino acids and protein profile in floral nectar: Much more than a simple reward. Flora: Morphology, Distribution, Functional Ecology of Plants 207:475–481.

Ness, J. H., W. F. Morris, and J. L. Bronstein. 2009. For ant-protected plants, the best defense is a hungry offense. Ecology 90:2823–2831.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P.Solymos, M. H. H. Stevens, and H. Wagner. 2015. vegan: Community Ecology Package. R package version 2.4-0.

Oliveira, P. S., and A. V. L. Freitas. 2004. Ant-plant-herbivore interactions in the neotropical cerrado savanna. Naturwissenschaften 91:557–570.

Parr, C. L., B. J. Sinclair, A. N. Andersen, K. J. Gaston, and S. L. Chown. 2005. Constraint and competition in assemblages: a cross-continental and modeling approach for ants. The American naturalist 165:481–494.

R Development Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rico-Gray, V., and P. S. Oliveira. 2007. The ecology and evolution of ant– plant interactions. University of Chicago Press, Chicago, USA.

Rudolph, K. P., and T. M. Palmer. 2013. Carbohydrate as fuel for foraging, resource defense and colony growth - a long-term experiment with the plant-ant *Crematogaster nigriceps*. Biotropica 45:620–627.

Russell, J. a, C. S. Moreau, B. Goldman-Huertas, M. Fujiwara, D. J. Lohman, and N. E. Pierce. 2009. Bacterial gut symbionts are tightly linked with the evolution of herbivory in ants. Proceedings of the National Academy of Sciences of the United States of America 106:21236–21241.

Simpson, S. J., and D. Raubenheimer. 1993. A Multi-Level Analysis of Feeding Behaviour: The Geometry of Nutritional Decisions. Philosophical Transactions of the Royal Society B: Biological Sciences 342:381–402.

Simpson, S. J., and D. Raubenheimer. 2000. The Hungry Locust. Advances in the Study of Behavior 29:1–44.

Sterner, R. W., and J. J. Elser. 2002. Ecological stoichiometry : the biology of elements from molecules to the biosphere. Princeton University Press.

Tilman, D. 1982. Resource Competition and Community Structure. Princeton University Press, Princeton, New Jersey.

Wheeler, D. E. 1986a. Polymorphism and Division of Labor in *Azteca chartifex* laticeps (Hymenoptera: Formicidae). Journal of the Kansas Entomological Society 59:542–548.

Wheeler, D. E. 1986b. Ectatomma tuberculatum: Foraging Biology and Association with *Crematogaster* (Hymenoptera: Formicidae). Annals of the Entomological Society of America 79:300–303.

Yanoviak, S. P., and M. Kaspari. 2000. Community structure and the habitat templet: ants in the tropical forest canopy and litter. Oikos 89:259–266.

Yeoh, H. H., Y. C. Wee, and L. Watson. 1992. Leaf protein contents and amino acid patterns of dicotyledonous plants. Biochemical Systematics and Ecology 20:657–663.

Table legends

Table 1. Results of linear mixed models fitted with supplement and time as explanatory variables and total/functional group richness and abundance as response variables. *Total* = all ants sampled; *TT* = trophobiont tenders, *LF* = leaf foragers and *PD* = predators. Asterisks indicated significant results.

Table 2. List of ant species significantly associated with a specific supplement. IndVal indices and their significance were also indicated.

Tables

Table 1.

Explanatory variable		Response variable					
		Species richness			Abundance		
		df	χ^2	*P*-value	*df*	χ^2	*P*-value
Supplement	* *Total*	3,1713	250.277	< 0.001	*Total	3,1712	398.490	< 0.001
	**TT*	3,1713	73.678	< 0.001	**TT*	3,1712	202.65	< 0.001
	**LF*	3,1713	108.249	< 0.001	**LF*	3,1712	153.601	< 0.001
	**PD*	3,1713	20.785	< 0.001	**PD*	3,1712	18.807	< 0.001
Time	***Water***				***Water***			
	Total	1,418	1.046	0.31	*Total*	1,417	0.163	0.68
	TT	1,418	0.033	0.86	*TT*	1,417	0.006	0.93
	LF	1,418	0.325	0.57	*LF*	1,417	1.962	0.16
	PD	1,418	3.345	0.07	*PD*	1,417	0.525	0.47
	CHO				***CHO***			
	Total	1,464	1.769	0.18	*Total*	1,458	2.049	0.15
	TT	1,464	0.02	0.89	**TT*	1,458	6.326	0.01
	LF	1,464	2.593	0.11	*LF*	1,458	2.824	0.10
	PD	1,464	2.909	0.09	*PD*	1,458	1.864	0.17
	Protein+CHO				***Protein+CHO***			
	Total	1,388	1.518	0.21	**Total*	1,384	5.985	0.01
	TT	1,388	0.159	0.69	**TT*	1,384	6.971	< 0.01
	LF	1,388	3.14	0.08	**LF*	1,384	19.102	< 0.001
	PD	1,388	0.059	0.81	*PD*	1,384	3.045	0.08
	Protein				***Protein***			
	Total	1,434	0.528	0.46	**Total*	1,428	20.689	< 0.001
	TT	1,434	2.578	0.11	**TT*	1,428	8.259	< 0.01
	LF	1,434	0.354	0.55	**LF*	1,428	12.846	< 0.001
	PD	1,434	0.495	0.48	*PD*	1,428	1.430	0.23

Table 2.

Supplement	Species	Functional group	IndVal	*P*-value
CHO	*Cephalotes pusillus*	Leaf foragers	0.11	0.002
	Brachymyrmex sp. 3	Leaf foragers	0.06	0.008
	Neoponera villosa	Predators	0.04	0.002
	Crematogaster sp. 6	Trophobiont tenders	0.04	0.008
Protein	*Crematogaster distans*	Trophobiont tenders	0.16	0.003
	Azteca sp. 1	Trophobiont tenders	0.12	0.001
	Cephalotes atratus	Trophobiont tenders	0.04	0.02
	Pseudomyrmex kuenckeli	Trophobiont tenders	0.03	0.01

Figure legends

Fig. 1 Variation in (a) all ants; (b) trophobiont tenders; (c) leaf foragers and (d) predators species abundance among supplements. *Bars* represent SE and *Different letters* indicate significant differences among treatments.

Fig. 2 Variation in (a) all ants; (b) trophobiont tenders; (c) leaf foragers and (d) predators richness among supplements. *Bars* represent SE and *Different letters* indicate significant differences among treatments.

Fig. 3. Mean abundance of (a) all ants; (b) trophobiont tenders; (c) leaf foragers and (d) predators on trees after and during CHO supplementation. *Bars* represent SE and *Different letters* indicate significant differences among treatments.

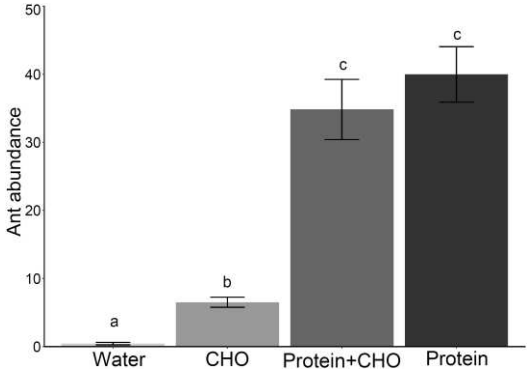
Fig. 4. Mean abundance of (a) all ants; (b) trophobiont tenders; (c) leaf foragers and (d) predators on trees after and during Protein+CHO supplementation. *Bars* represent SE and *Different letters* indicate significant differences among treatments.

Fig. 5. Mean abundance of (a) all ants; (b) trophobiont tenders; (c) leaf foragers and (d) predators on trees after and during Protein supplementation. *Bars* represent SE and *Different letters* indicate significant differences among treatments.

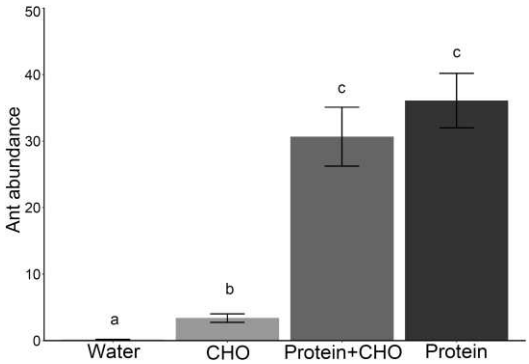
Figures

Fig. 1

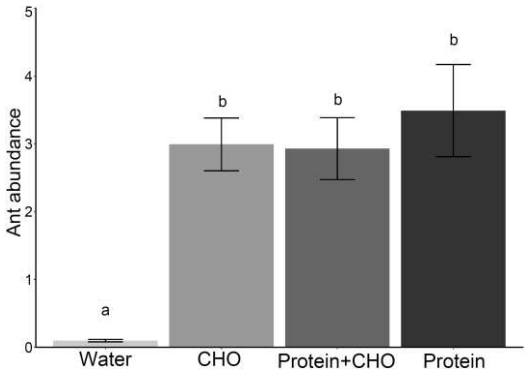
(a) All species



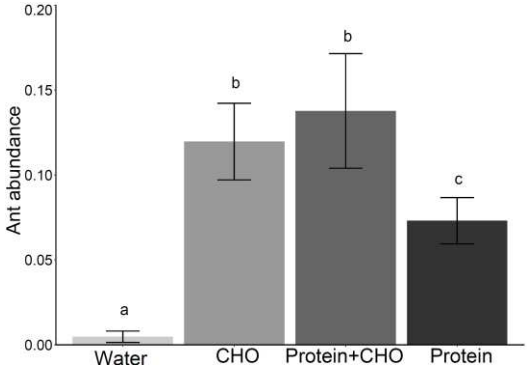
(b) Trophobiont tenders



(c) Leaf foragers



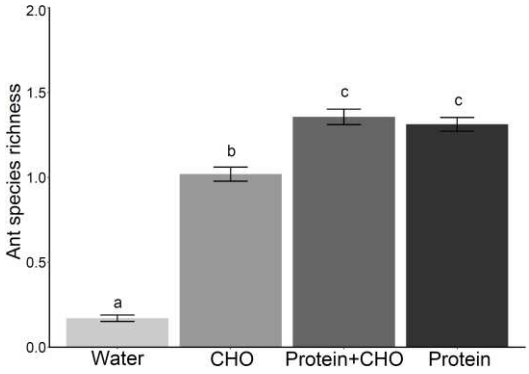
(d) Predators



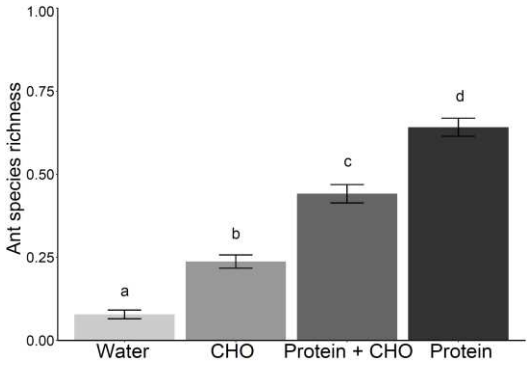
Supplements

Fig. 2

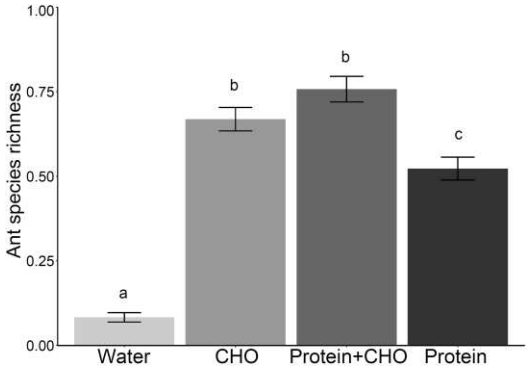
(a) All species



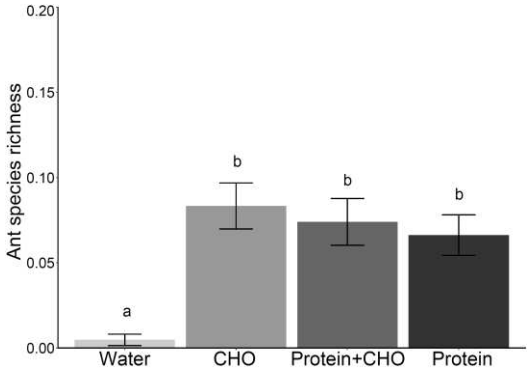
(b) Trophobiont tenders



(c) Leaf foragers



(d) Predators

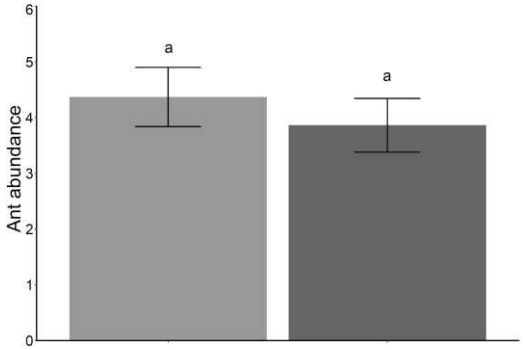


Supplements

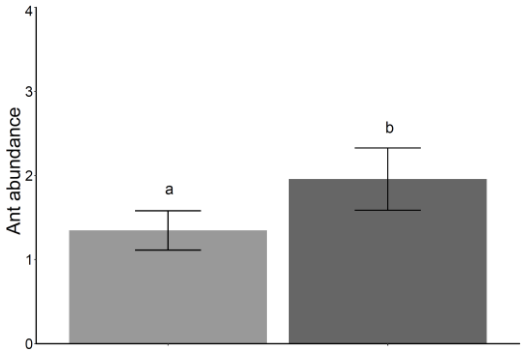
Fig. 3

CHO

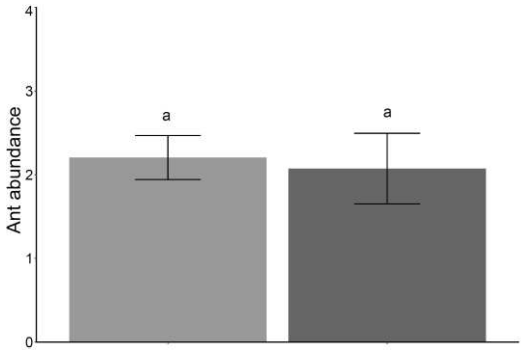
(a) All species



(b)Trophobiont tenders



(c) Leaf foragers



(d) Predators

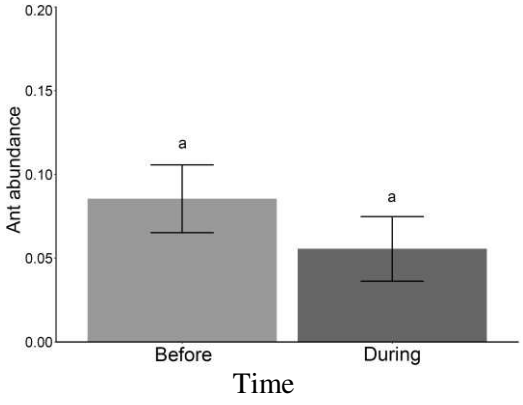
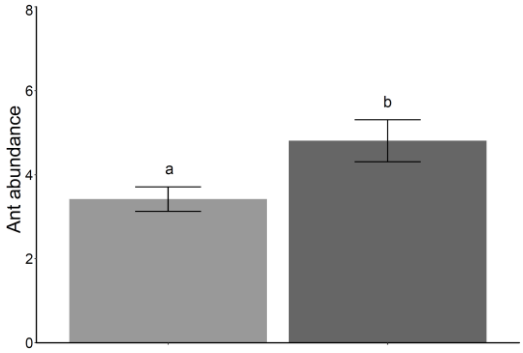


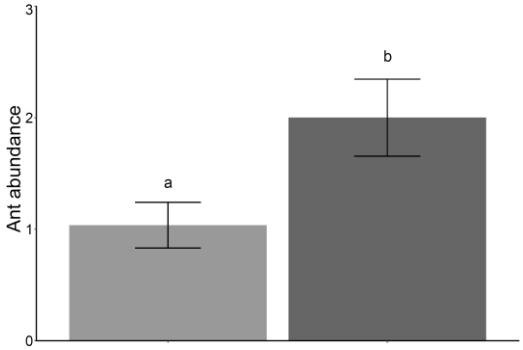
Fig. 4

Protein+CHO

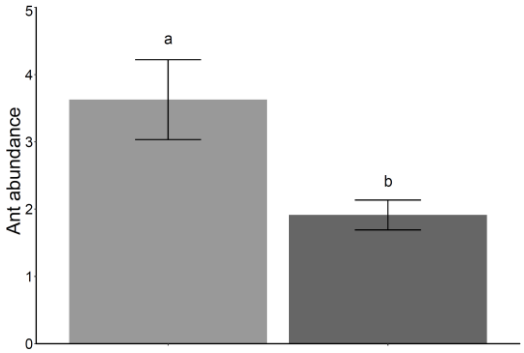
(a) All species



(b) Trophobiont tenders



(c) Leaf foragers



(d) Predators

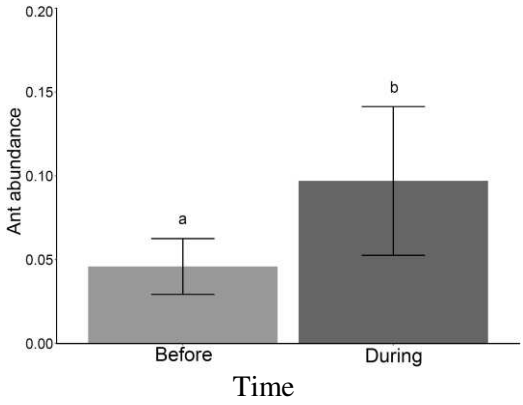
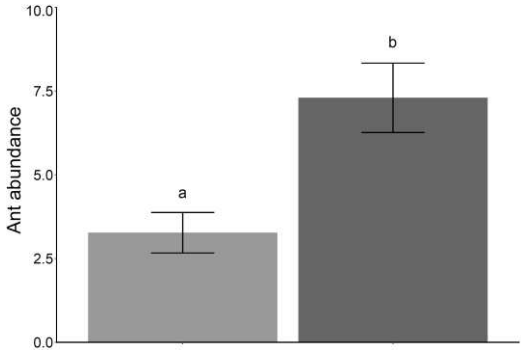


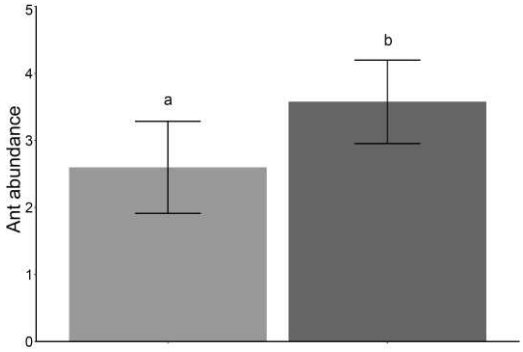
Fig. 5

Protein

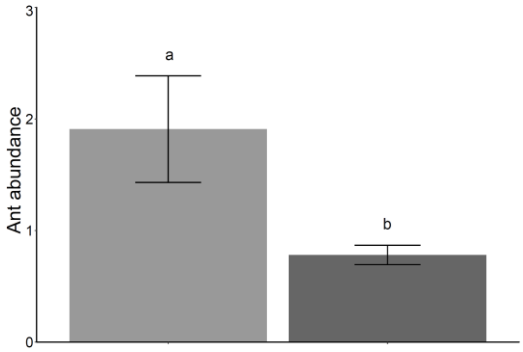
(a) All species



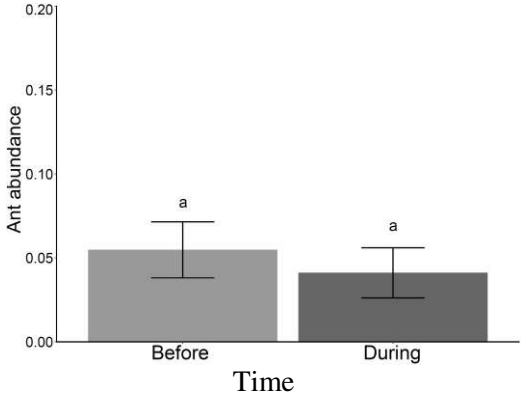
(b) Trophobiont tenders



(c) Leaf foragers



(d) Predators



Appendix

Table S1. List of ant species sampled and their classification into functional groups. TT = trophobiont tenders; LF = leaf foragers; PD = predators.

Subfamily	Species	Functional group
Dorylinae	*Labidus praedator* (Smith, F., 1858)	**-**
	Neivamyrmex pseudops (Forel, 1909)	**-**
	Neivamyrmex sp. 1	**-**
Dolichoderinae	*Azteca* sp. 1	**TT**
	Dolichoderus diversus Emery, 1894	**TT**
	Dorymyrmex sp. 1	**-**
	Dorymyrmex sp. 2	**-**
	Dorymyrmex sp. 3	**-**
	Forelius sp. 1	**-**
	Linepithema neotropicum Wild, 2007	**-**
	Tapinoma cf. *amazonae*	**-**
Ectatomminae	*Ectatomma permagnum* Forel, 1908	**PD**
	E. planidens Borgmeier, 1939	**PD**
	E. tuberculatum Olivier, 1792	**PD**
	Gnamptogenys sulcata (Smith, F., 1858)	**-**
Formicinae	*Brachymyrmex* sp. 1	**-**
	Brachymyrmex sp. 3	**-**
	Camponotus blandus (F. Smith, 1858)	**-**
	C. bonariensis Mayr, 1868	**-**
	C. crassus Mayr, 1862	**-**
	C. leydigi Forel, 1886	**-**
	C. melanoticus Emery, 1894	**-**
	C. personatus Emery, 1894	**-**
	C. renggeri Emery, 1894	**TT**
	C. rufipes Fabricius, 1775	**TT**
	C. senex F. Smith, 1858	**LF**
	C. vittatus Forel, 1904	**LF**
	Camponotus sp. 1	**LF**
	Camponotus sp. 4	**LF**
	Camponotus sp. 9	**LF**

	Camponotus sp. 10	**LF**
	Camponotus sp. 12	**LF**
	Camponotus sp. 17	**LF**
	Camponotus sp. 18	**LF**
	Camponotus sp. 19	**LF**
	Camponotus sp. 21	**LF**
	Camponotus sp. 22	**LF**
	Camponotus sp. 23	**LF**
	Myrmelachista nr. *gallicola* Mayr, 1887	**LF**
	M. nodigera Mayr, 1887	**LF**
	Myrmelachista sp. 3	**LF**
	Nylanderia sp. 1	**-**
Myrmicinae	*Atta* sp. 1	**-**
	Atta sp. 2	**-**
	Cephalotes atratus Linnaeus, 1758	**TT**
	C. betoi Andrade & Baroni Urbani, 1999	**LF**
	C. depressus (Klug, 1824)	**LF**
	C. grandinosus (Smith, F., 1860)	**LF**
	C. nr. *persimilis* De Andrade, 1999	**LF**
	C. pusillus Klug, 1824	**LF**
	Crematogaster distans (Mayr, 1870)	**TT**
	C. evallans Forel, 1907	**TT**
	C. stolli Forel, 1885	**TT**
	Crematogaster sp.2 (crinosa group)	**TT**
	Crematogaster sp. 4	**TT**
	Crematogaster sp.6	**TT**
	Nesomyrmex spininodis (Mayr, 1887)	**-**
	Nesomyrmex sp. 1	**-**
	Ochetomyrmex semipolitus Mayr, 1878	**LF**
	Pheidole gertrudae Forel, 1886	**-**
	P. oxyops Forel, 1908	**-**
	Pheidole sp. 1	**-**
	Pheidole sp. 3	**-**
	Pheidole sp. 4	**-**
	Pheidole sp. 5	**-**
	Sericomyrmex sp. 1	**-**

	Solenopsis invicta Buren, 1972	**-**
	S. tridens Forel, 1911	**-**
	Solenopsis sp. 1	**-**
	Solenopsis sp. 4	**-**
	Solenopsis sp. 7	**-**
	Wasmannia auropunctata (Roger, 1863)	**-**
Ponerinae	*Neoponera villosa* (Fabricius, 1804)	**PD**
Pseudomyrmecinae	*Pseudomyrmex kuenckeli* (Emery, 1890)	**TT**
	P. schuppi (Forel, 1901)	**LF**
	P. termitarius (Smith, F., 1855)	**LF**
	Pseudomyrmex sp. 1	**LF**
	Pseudomyrmex sp. 2	**LF**
	Pseudomyrmex sp. 3	**LF**
	Pseudomyrmex sp. 4	**LF**
	Pseudomyrmex sp. 8	**LF**
	Pseudomyrmex sp. 9	**LF**
	Pseudomyrmex sp. 10	**LF**
	Pseudomyrmex sp. 11	**LF**
	Pseudomyrmex sp. 12	**LF**
	Pseudomyrmex sp.13	**LF**
	Pseudomyrmex sp.14	**LF**
	Pseudomyrmex sp.16	**LF**

GENERAL CONCLUSIONS

In this thesis we aim to investigate the effect of food resources as mechanism driving arboreal ant communities. In the first chapter, we showed that arboreal ant diversity at site scale is under positive influence of extrafloral nectaries (EFNs), independently of vegetation complexity. We demonstrated that ant species richness was higher on trees bearing EFNs than on those without and most common ant species occurred preferentially on plants with EFNs. We also showed that these observed patterns on individual trees scale up to influence ant communities at the site level: we observed a change in species composition and an increase in species richness with the proportion of EFN-bearing trees at the plot level. Interactions between plants bearing extrafloral nectaries and ants are common and widespread, particularly in tropical regions of the world. Therefore, our data advance the broad understanding of the community-wide influences of EFN on arboreal ants. We highlight that future ecological studies should take in account the availability of extrafloral nectaries as a potential mechanism determining arboreal ant communities at local scale.

In the second chapter, we showed different functional group responses to protein and CHO supplementation, in accordance with their specific diet requirements. Moreover, we demonstrated that the addition of different nutrients on trees shift the community of foraging ants, with a positive or negative influence in their abundance depending on the functional group. We suggest that differences in taxa-specific nutritional needs in arboreal ant assembly may determine patterns of resources utilization and dietary niche differentiation in such communities. Ecological stoichiometric studies potentially have much to say about the structure and nature of interactions in arboreal ant communities. However, this approach is

still in infancy and we call attention to experimental manipulations which are very fruitful approach to advancing our understanding about how the nutritional composition of food resources affects the niche differentiation and species distribution in the arboreal ant assembly.

We suggest that the mechanisms based on resource availability (first chapter) and quality (second chapter) described in this thesis, have a great potential to explain the arboreal ant community structure. Moreover, we highlight that species-specific biological aspects, such as feeding behaviour and nutritional adaptations, could be essential for a better comprehension about the process behind the diversity patterns of arboreal ant communities.