UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

THE ROLE OF BIOGEOCHEMISTRY AND CLIMATE IN A NEOTROPICAL ANT COMMUNITY

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

Degree of

DOCTOR OF PHILOSOPHY

By

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Norman, Oklahoma
2017

THE ROLE OF BIOGEOCHEMISTRY AND CLIMATE IN A NEOTROPICAL ANT COMMUNITY

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF BIOLOGY

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Acknowledgements

First, I would like to thank my advisor Mike Kaspari for giving me this opportunity, for being supportive and believing in me, even after I decided to climb trees. I am grateful for his patience and guidance along the way, especially in improving my writing, as well as all the RA support he provided throughout the years. I thank my committee members for generous help and valuable advice throughout the process: Robert Cichewicz, Jeffrey Kelly, Michael Patten, and Lara Souza. I am grateful to Michael Patten for endless patience with my statistical dilemmas, for being prompt in providing feedback, and for his encouragement. Lara Souza for our community ecology discussions throughout the years, and for all her help in my professional development. Jeff Kelly, for all the stable isotope discussions, they changed my perspective on research.

Thanks to the staff of the Biology Department: Liz Cooley, Kaye Carter, Kyle Baker, George Davis, and Robbie Stinchcomb for providing valuable logistic support along the way, especially during months I was in the field, it was so much easier knowing I could rely on you. For the logistical help in Panama I thank Oris Acevedo, Belkys Jimenez, and Norisa Mercedes Soto Cadastre from the Smithsonian Tropical Research Institute.

I am grateful to Joe Wright for use of his field site and valuable insight on nutrient limitation in the tropics. Thanks to Steve Yanoviak for teaching me how to climb and introducing me to the natural history of Neotropical animals. I thank Eli Rodrigez, Stefan Brandel, Marko Maklewitz, Sara Neihaus, Eric Griffin, Dan Revillini,

Christie Riehl, Callum Kingwell and many more for their friendship; BCI would not be the same without you.

Big thanks to the less transient roommates of the Hardin House for their friendship, and all the fun, food and drinks we shared together. Emily Khazan, Alex (Bundy) Barnard, Daniel Curtis, Arthur Escalas and Camille Cosson, I can't imagine not knowing you. I thank Biology Graduate students Thayer Hallidayschult and Josh Cooper for all the real coffee breaks we shared over the years. I am grateful to the Kaspari lab members for their help: Brittany Benson for never being boring but always ready to draw Discothyreas if needed. Rebecca Prather for her friendship and support. Karl Roeder for all the discussions about ants and science, and for convincing me to accept that—this is fine. Mike Weiser for preparing me for the future in the realm of ecology with his valuable advice. A huge thanks to my former lab mates: Jon Shik, David Donoso, and Natalie Clay for their friendship and support, regardless of the geographic distance. Thanks to Jackson Helms for his linguistic help, and for his efforts to introduce me to Oklahoma.

Thank you, Marina and Luka, for keeping in touch after all these years, for numerous all-nighters that we pulled, for being positive, motivating, and always planning the next trip for 3 mudrice. Andreja, thank you for all the paper writing and discussions. Faja and Gaja, thank you for making it so easy to remember the good old times. Vedran, thank you for your love and support. Gligor, thank you for being a reality check.

Ana, there are no words to thank you for being an unimaginably amazing friend. I would never be able to finish if you were not a phone call away, somehow always knowing what to say. Both Marko and Ana, thank you for being my family away from family. I am grateful for the ridiculousness via Skype or in person, for tolerating never ending rants, enjoying good coffee, cooking weird dishes and supporting me every step of the way.

Finally, I thank my parents without which none of this would be possible, Mama, Tata, thank you for encouraging my curiosity and love of nature. I am grateful for always knowing I have a place to come back. Vlasta, thank you for being an older sister, and giving good advice. Thank you Majka for being proud of me and supportive of my choices, and for the never-ending supply of Griotte.

Lastly I thank many more of my friends I didn’t mention here, both old and new, for making it all worthwhile.

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Abstract

Human activities are rapidly changing nutrient availability and climate across the globe, and this trend is predicted to continue. The effect of biogeochemistry on consumer communities is underexplored in the world's most diverse ecosystems – the tropics. Nitrogen (N) and phosphorus (P) are leading candidates in limiting the fitness of arthropods because they are required in substantial quantities as the building blocks of numerous macromolecules, but are relatively scarce in most foods consumed by herbivorous insects. Thus, nutrient limitation of plants may negatively impact herbivores. In addition to changes in nutrient availability, climate is changing. These changes include global increases in temperature and alteration in precipitation regimes across the globe and Neotropical forests are predicted to suffer severe droughts. My work examines the role of both biogeochemistry and climate in Neotropical ants.

In Chapter One I investigate the effect of nutrient addition on the ant community of a tropical lowland forest in Panama. I used the Gigante fertilization experiment (GFP), which includes 32, 1600m^2 plots that have been fertilized annually with N, P, and potassium (K) since 1998. Plants in tropical lowland rainforests grow on old, weathered soils, and tropical plant growth is typically P limited. Plots fertilized with P fertilization had higher forging activity—number of baits occupied by ants—supporting the nutrient limitation hypothesis. The same plots, however, had lower genus level diversity, consistent with the *paradox of enrichment* frequently observed in plant communities. *Azteca* was the only ant genus whose activity was higher on P plots, a pattern largely driven by one species, *Azteca chartifex*.

Chapter Two investigates the potential of Neotropical ants to survive water loss by comparing ant communities in two habitats with different microclimates—the canopy and the litter. The Neotropical canopy was 1° C warmer, and 9 times drier than the litter, and canopy ants had 3 times higher desiccation resistance – the ability to reduce water loss. By exploring mechanisms for the observed difference, I found that smaller ants desiccated faster given their higher surface area to volume ratio, as desiccation resistance increased with ant mass, and canopy ants were, on average, 16% heavier than the understory ants. A second way to increase desiccation resistance is to carry more water. Although water content in canopy ants was on average 2.5% higher, it was not a good predictor of desiccation resistance. Animals experiencing dry conditions are likely experiencing warm conditions as well, so I examined if critical thermal maximum (CT_{max}), a measure of an ant's thermal tolerance positively co-varies with desiccation resistance. In canopy ants, desiccation resistance and CT_{max} were inversely related, suggesting a tradeoff, while the two were positively correlated in litter ants.

Chapter Three combines the results from the first two chapters to ask why is *A. chartifex* successful and dominant in this community, particularly on P plots, and yet has low desiccation resistance and CT_{max} for a canopy ant. The diet of *A. chartifex* largely includes honeydew and extra-floral nectar secretions. I predicted that workers of *A. chartifex* which had recently fed on this carbohydrate-rich diet, will use that energy to increase the upper limits of thermal tolerance. I used *A. chartifex* colonies from control and P plots from GFP where I had previously recorded higher foraging activity. As foraging activity can be governed by resource availability, I measured CT_{max} of field collected colonies and found that CT_{max} was 2° C higher in control plots than P. This difference disappeared when ants were starved. After providing colonies with a 10% sucrose solution, their CT_{max} increased by 5° C, supporting the hypothesis that carbohydrate nutrition allows higher thermal tolerance. This does not appear to be linked to colony trophic status, higher C:N ratios, or higher total body P. This short-term thermal plasticity linked to carbohydrate nutrition demonstrates the importance of diet in shaping physiological traits.

Chapter Four investigates the effect of biogeochemistry on *A. chartifex* nesting patterns across GFP. I found that N suppressed *Azteca* nest density, as plots which received N had 48% lower number of nests. Adding P did not affect colony abundance. The addition of both N and P significantly increased number of *A. chartifex* nests as well as the size of nests. The best predictor of colony size was tree size as larger trees supported larger nests. *Azteca chartifex* nests were non-randomly distributed, and certain tree species were preferred, despite their low frequency across the forest, while some abundant trees were avoided. Our study suggests that both nutrient availability and forest composition act in concert and help *A. chartifex* dominate this tropical forest.

My research finds that “bottom-up” forces shape this ant community by indirectly affecting the nest density and activity of a numerically and behaviorally dominant ant. In a rapidly warming world, carbohydrate availability and use may represent a fundamental predictor of the population and community responses of herbivorous insects in a changing world.

CHAPTER 1: Biogeochemical drivers of Neotropical ant activity and diversity

This chapter is published, with some modifications, as Bujan J, Wright JP, and Kaspari M. 2016. Biogeochemical drivers of Neotropical ant activity and diversity. *Ecosphere* 7(12).

Abstract

Human activities are rapidly changing biogeochemistry across the globe, yet little is known about biogeochemical impacts on higher-level consumers. In a Panamanian rainforest we measured the effects of chronic nitrogen, phosphorus and potassium fertilization on ants: hyper-abundant terrestrial arthropods and ecosystem engineers. We tested two compatible hypotheses: the nutrient limitation hypothesis – where adding a limiting nutrient increases ant activity and abundance; and the community homogenization hypothesis – where adding a limiting nutrient decreases ant diversity. Lowland tropical rainforests are expected to be phosphorus limited, so we predicted higher ant activity but lower diversity on phosphorus plots. In each fertilization plot we baited trees and lianas to attract both canopy and ground nesting ants.

After controlling for temperature, which accounted for roughly 20% of the variation in ant foraging activity, ant activity remained higher on phosphorus addition plots than on any other fertilization treatment. Genus level diversity was 16% lower on plots receiving phosphorus, than the control, consistent with the *paradox of enrichment* frequently observed in plant communities. This pattern, however, did not hold for species level diversity. The community-level response was largely driven by the most abundant genus, *Azteca* which increased foraging activity and abundance across phosphorus plots. The high activity and low diversity of ants on experimental phosphorus plots points to the potentially strong influence of biogeochemistry on these ubiquitous insects with potential ramifications for the forest food web.

This is, to our knowledge, the first study relating biogeochemistry of macronutrients to foraging activity, diversity and abundance of consumers, implicating strong bottom up structuring of the ant community in one of the world’s most diverse ecosystems.

Introduction

Biogeochemistry imposes an elemental template on populations and ecosystems (Redfield 1958; Williams and Fraústo da Silva 1996; Sterner and Elser 2002) and the 25 elements required for life are not equally distributed across the globe. Fertilization experiments complement comparative studies (Richardson et al. 2004; Elser et al. 2007) to test for biogeochemical limitation of abundance, diversity, and function of organisms (Gruner and Taylor 2006; Hillebrand et al. 2007). In terrestrial ecosystems most fertilization experiments have focused on primary producers (Tripler et al. 2006; Elser et al. 2007). Fertilization tends to reduce plant diversity (Hillebrand et al. 2007) while increasing plant biomass (Gruner et al. 2008) and plant nutrient content (Fox and Morrow 1992; Campo and Vasquez-Yanes 2004; Santiago et al. 2012). While nutrient limitation of plants may impact consumers (Oksanen et al. 1981; Power 1992; Schmitz 2010; Mooney et al. 2010), few studies explore how forest fertilization affects the abundance of herbivores and predators (Forkner and Hunter 2000; Gruner and Taylor 2006) and these have done so using indirect measures, such as plant damage (Hargrove et al. 1984; Fox and Morrow 1992; Campo and Dirzo 2003; Santiago et al. 2012).

The geography of nutrient availability arises from the balance of deposition and weathering (Sterner and Elser 2002). In recently glaciated soils from high latitudes mineral phosphorus (P) is often relatively abundant through the deposition of glacial dust (Vitousek and Sanford 1986). In tropical forests, most biologically available P and potassium (K) must be weathered directly from bedrock; leaching decreases their supply over time (Walker and Syers 1976). Nitrogen (N) supplies, in contrast, tend to increase with time, through atmospheric deposition and biofixation (Vitousek 2004). Thus, forests growing on young soils are often N limited (Vitousek and Howarth 1991), while forests growing on old weathered soils are often P limited (Tanner et al. 1998; Wardle et al. 2004, Alvarez-Clare et al. 2013).

There is growing evidence for a major role of P limitation in tropical forests. Phosphorus fertilization can double plant growth rate in tropical montane forests (Tanner et al. 1990) and increase herbivore and predator abundance (Campo and Dirzo 2003; Gruner 2004). Evidence for P limitation of terrestrial consumers remains incomplete. Fertilization experiments rarely exceed three years, which can underestimate effects on long-lived organisms and slow processes (Tilman and Wedin 1991; Tanner et al. 1990). Moreover, of the three studies that exceed three years of fertilization in lowland tropical forests, none addressed responses of higher trophic levels (Mirmanto et al. 1999; Wright et al. 2011; Alvarez-Clare et al. 2013).

The Gigante Fertilization Experiment (GFP) consists of 32 40×40 m plots of lowland moist forest that have been fertilized with N, P and K in a factorial block design for 16 years (Wright et al. 2011). The GFP offers a unique opportunity to explore how N, P, and K ramify through the forest's plants and limit the abundance, activity, and diversity of its consumers. Ants (Hymenoptera: Formicidae) are important consumers in most terrestrial ecosystems (Hölldobler and Wilson 1990). The ant populations of a tropical forest range from herbivores to specialized predators and scavengers, though the majority of ant species are omnivores, consuming plant, fungal, and animal tissue (Kaspari 2001; Blüthgen et al. 2003; Davidson 2005). Ant densities in the tropics can respond quickly (<1 month) to carbohydrate additions (Kaspari et al. 2012), Moreover, a colony's perennial and semi-sessile lifestyle (Keeler 1988; Gordon 1992) is suited to reflect long-term changes in biogeochemistry. Here we test two consumer-resource hypotheses that predict the relationship between nutrient availability and food web structure.

Nutrient limitation assumes that at least one chemical element is at suboptimal levels such that increasing its availability enhances the survival and reproduction of a population. Long-term fertilization on the GFP increased N, P and K concentration in soil (Wright et al. 2011), seedlings (Santiago et al. 2012), and abscised leaves (Kaspari et al. 2008). Fertilization has also increased consumer activity reflected as the decomposition rate of cellulose on P and K plots (Kaspari et al. 2008), and understory herbivory on P, K and PK plots (Santiago et al. 2012). This suggests that plants from P and K plots offer more palatable plant resources or that those plants are less defended (Coley et al. 1985). Moreover, these results suggest that P and K, but not N, are limiting in this forest. Thus, the positive effects of P and K fertilization on GFP herbivore and detritivore activity leads to the prediction that P and K plots should support higher ant activity (Blüthgen and Fiedler 2004a) than N or control plots.

Community homogenization assumes that 1) species have different nutrient requirements and 2) increasing the supply of the limiting nutrient enables species that excel at resource uptake to outcompete species that can survive and reproduce at lower levels (i.e., have higher nutrient use efficiency). Resource availability and diversity often have a unimodal relationship, where the addition of a limiting nutrient increases diversity up to a threshold followed by a steady decrease in diversity (Tilman 1987; Sterner and Elser 2002). Over time, eutrophication reduces niche space and can ultimately lead to competitive exclusion by the species with the lowest resource use efficiency (Tilman 1987; Tilman and Wedin 1991; Harpole and Tilman 2007). Community homogenization provides a mechanism for the *paradox of enrichment* (Rosenzweig 1971), where adding a limiting nutrient in excess reduces species richness (Sterner and Elser 2002). If tropical lowland forests are P limited (Tanner et al. 1998), then P addition should favor the subset of species with high P uptake and low P use efficiency and thereby reduce diversity.

We know of no studies examining how long term changes in biogeochemistry, the building blocks of resources, have impacted ant communities. We build upon previous studies that examined how these experimental gradients of N, P, and K impact producers and herbivory, to test predictions about how long term changes in biogeochemistry ramify through the food web to impact ant activity, abundance, and diversity. In doing so, we provide the first ecosystem level study exploring the consequences of long term fertilization with multiple macronutrients (and their interactions) for a consumer community in a Neotropical forest as well as for ant communities in any biome.

Materials and methods

We sampled ants during the rainy season in a lowland tropical forest on Gigante Peninsula within Barro Colorado Nature Monument (BCNM: 9°06’31’’N, 79°50’37’’W), Republic of Panama. Mean annual rainfall is c. 2600 mm and largely occurs during the rainy season from May-December, and mean annual temperature is 27° C (Leigh 1999). This old, secondary forest has been fertilized since 1998 with all factorial combinations of N, P and K. Eight treatments (+N, +P, +K, +NP, +NK, +PK, +NPK and control (CTR)) are replicated on four 40×40 m plots (Yavitt et al. 2009). Fertilizers are applied 4 times during the rainy season (for details see Wright et al. 2011). Within each replicate, the +N, +P, +K and +NPK treatments versus the control, +NP, +NK and +PK treatments form blocks to control for spatial variation in soils (Yavitt et al. 2009) and tree community composition (SJW, *unpublished data*) associated with a slight topographic gradient. This allowed us to further control for the potential effect of habitat heterogeneity on ant community composition.

To compare and quantify ant activity and species richness among fertilization treatments, we collected ants using bait transects. Baits are a standard sampling method for measuring ant activity (Bestelmeyer et al. 2000). We mixed two common baits—tuna and honey—to provide a bait that would 1) offer protein, lipids, carbohydrates, and salt, 2) be easily smeared on surfaces, and 3) could be made in quantity while maintaining consistent composition. In each fertilization plot, we set up two 30 m long and 1.6 m wide linear transects in cardinal directions crossing in the middle of the plot. Transects started and ended 5 m from plot edges. Along each transect, we baited every tree and liana stem at eye level, to attract both canopy and litter ants. At each fertilization treatment (4 plots in total) the number of baits ranged from 239 to 304 depending on the density of understory vegetation, which was independent of the treatment received. We baited ants between 8:30 and 17:00 h in June and re-sampled the same plots in July of 2013. We sampled during the rainy season because ant activity in these forests is 25% higher compared to the dry season (Kaspari and Weiser 2000). Furthermore, at the beginning of the rainy season most ants perform their mating flights (Kaspari et al. 2001), and colonies are expected to have higher nutrient requirements when taking care of the reproductive individuals. This is why we expected ants to be attracted by a rich resource provided as a bait more than they would be during a dry season. Sampling times were randomly assigned to the plots, the second time we sampled a plot we choose a different time of day, to minimize the temporal effects (e.g. if the plot was first sampled in the morning, re-sampling was done in the afternoon). We used the average of these two sampling events as a response variable in our activity analysis.

After one hour, we estimated the number of workers for each species on baits using a base 2 logarithmic scale (i.e. 1, 2, 4, 8, 16, 32, …). We identified distinctive, common ants by eye (e.g., *Cephalotes atratus, Ectatomma ruidum*), and for all others, we collected workers into 95% ethanol for identification in the lab using an online database (evergreen.edu/ants/antsofcostarica.html), supplemented with a reference collection of the senior author. A subset of problematic identifications were further checked with barcode analysis (Y. Basset *unpublished data*). Voucher specimens are deposited in the lab of the senior author and at the University of Panama. This allowed us to estimate activity and species richness of ants attracted to baits in each plot.

As ectotherms, ants are sensitive to changes in temperature; their activity is known to vary among species and increase at higher temperatures (Cerdá et al. 1998; Bestelmeyer 2000). Thus, we measured air temperature by aiming a portable IR thermometer (Fluke Corporation, Everett, WA; model 62) at eye level to a shaded spot > 10 m away. We measured the temperature at the beginning and end of the two transects as soon as the baits were placed, and one hour later, when they were collected. In total we took 8 temperature readings per one baiting trial and used the plot mean temperature as a covariate in analyses.

We used a spherical densiometer to measure canopy openness at the center of each plot facing each cardinal direction. We used the mean of these four measurements in our analysis. Canopy cover was dense (85.8 ± 5.5%), and did not vary among fertilization treatments (Kruskal Wallis: χ^2 (7) = 4.4, p = 0.73), so it is not included in our subsequent analyses.

Data analysis

We calculated foraging activity as the proportion of baits that were visited by ants. We used generalized linear mixed models (GLMMs) with binomial error distributions to evaluate relationships between ant activity, the factorial fertilization treatments (fixed effects) and temperature (a continuous covariate). The single random effect was comprised of spatial blocks. After accounting for slight overdispersion of our model by using a random intercept which contains the number of levels equal to the number of plots examined, we ran model selection based on an information theoretic approach. We dropped non-significant effects from the full model using probability values. We obtained p-values using the likelihood ratio tests of the full model against the model without the effect of interest (see Zuur et al. 2013 for details of model selection). After removing non-significant terms, we ran a model comparison based on ΔAIC values—the difference of the AIC of the *i*th model and the optimal model with the lowest AIC value. We repeated this analysis when testing the difference in ant activity of the four most active genera.

We tested the community homogenization hypothesis by examining the differences in Inverse Simpson diversity across nutrient additions, and the differences in genus and species richness using GLMMs. Inverse of Simpson diversity index allowed us to examine diversity on a uniform scale (Hill 1973). We used a polynomial regression to test the effect of abundance of the most active genus across the plots – *Azteca* on ant richness and diversity. All statistical analyses were performed in RStudio (R Core Team 2012) using the “fossil” package (Vavrek 2011), “lme4” package (Bates et al. 2012), and “nlme” package (Pinheiro et al. 2012).

Results

Responses of Ant Activity to Fertilization

Ant activity varied from 17% to 83% of baits occupied across our temperature range (Fig. A1). Air temperature varied 4.5 °C (24.3 to 28.8 °C) over the course of our observations. Temperature accounted for almost one fifth of the variation in foraging activity in both June (y = 0.26x - 6.80, $R^2 = 0.17$, $p < 0.001$) and July (y = 0.22x - 6.22, $R^2 = 0.19$, $p < 0.001$). Average activity increased 1.6-fold over this temperature range. As activity did not vary between June and July (paired t-test, d.f. = 31, p = 0.26), or across individual plots we used the mean activity per plot across the two months.

Three GLMM models without any interaction terms, and without N as a fixed effect, had the lowest ΔAIC values and were used to analyze the effects of nutrient and temperature on ant activity (Table A1). All together these three models would be expected to be optimal models in 69% of cases (AIC Weights, Table A1). Ant activity increased only on +P plots (i.e., +P, +NP, +PK, +NPK, n = 16, Fig. 1, Table A1); average activity was 9% higher on +P plots compared to plots that did not receive P (t = 2.1, d.f. = 25.4, p = 0.04).

The four most abundant genera were *Azteca, Dolichoderus, Ectatomma* and *Crematogaster* which accounted for 86% of total ant activity. Only *Azteca* foraging activity increased across all +P plots, and the same three models as for overall activity are optimal in 53% of the cases (GLMM, Table A2). Out of three added nutrients N was the worst, and P was the best predictor of *Azteca* activity, which was higher across +P plots by an average of 14% (t = 2.2, df = 28.0, p = 0.04). *Azteca* maximum activity was the highest on +P treatments, reaching up to 76% of all occupied baits on single P addition (Fig. 2A). *Dolichoderus*, the second most abundant genus, and in the same subfamily as *Azteca,* was not affected by +P, instead models including +N and +K treatments and their interactions best accounted for *Dolichoderus* activity in 41% of cases (GLMM, Table A3). Phosphorus addition had no discernible effect on *Ectatomma, Crematogaster* (analysis not shown here).

Responses of Diversity and Richness to Fertilization

We recorded a total of 82 ant species and 23 genera on the GFP (Table A4). Most of which were omnivorous ants from both canopy and the understory (Table A5). *Pheidole. Azteca*, *Camponotus,* and *Crematogaster* were the most species-rich genera, with 14, 9, 8 and 8 species. Given our activity data we focused on single term effects when testing for biogeochemical drivers of diversity.

Genus but not species diversity varied with fertilization treatment. Consistent with the paradox of enrichment we found lower genus level diversity across +P plots compared to -P plots (n = 16; Inverse Simpson Diversity Index: mean ± SE: D_P = 3.6 ± 0.4, vs. D_{noP} = 4.3 ± 0.3). Models with +P and +K as predictors are optimal models in 45% of the cases, while models containing only +P are optimal in ¼ of the cases (Table A6). Across the 7 treatments, genus diversity was highest on +N plots (n = 4; 5.3 ± 0.4), and lowest on the NPK plots (n = 4; 3.1 ± 0.4). In contrast, there was no difference in species diversity across +P plots compared -P (n = 16; D_P = 5.0 ± 0.4 vs. D_{noP} = 5.5 ± 0.5) or any other nutrient treatment, and the optimal models were indistinguishable from the model without any fixed effects (Table A7).

Genus and species richness, the number of taxa observed, did not vary with fertilization. Genus richness trended lower on +P plots (n=16; +P = 8.3 ± 0.3 vs. -P = 9.3 ± 0.6), but did not significantly vary across the fertilization treatments (Table A8).

Across the 7 fertilization treatments, as with genus diversity, genus richness was highest on + N (9.8 ± 0.6), and lowest on + NPK (7.5 ± 0.5). A similar pattern held for species richness where +N and control had the highest richness and +NPK the lowest (+N = 13.3 ± 1.0; CTR = 13.3 ± 2.2; vs. +NPK = 10.8 ± 1.1; Table A9).

As the activity of *Azteca* increased on +P plots, and genus level diversity decreased on +P plots, we examined the effect of the most active and most abundant genus on overall genus and species richness and diversity. While there was no relationship of mean *Azteca* abundance and diversity, we found a unimodal relationship of *Azteca* abundance and genus richness ($F_{2,5} = 17.2$, $p = 0.006$, $R^2 = 0.87$, Fig. 3). *Azteca* abundance, however, was not a good predictor of species richness ($F_{2,5} = 3.1$, $p = 0.14$, $R^2 = 0.55$, Fig. 3).

We quantified measures of richness using raw counts of genera and species as well as Simpson's inverse diversity, an index that increases with richness and evenness. Genus richness was a strong predictor of species richness ($y = 1.4x + 0.8$, $R^2 = 0.91$, $p < 0.001$, Fig. A2), but accounted for less variability in the two measures that include evenness: genus diversity ($y = 0.4x + 0.4$, $R^2 = 0.78$, $p = 0.002$, Fig. A3A) and still less in species diversity ($y = 0.3x + 2$, $R^2 = 0.50$, $p = 0.04$, Fig. A3B). This is likely due to the frequency of genera (5, or 22%) and especially species (27, or 33%) that were recorded only once. Moreover, almost half of the species singletons were found in only three genera (*Pheidole* - 7% of the total species number, *Brachymyrmex* - 5% *Camponotus* - 4%).

Discussion

In a Panama forest, ant foraging intensity increased on plots after 16 years of P fertilization and genus diversity was lower on +P plots. *Azteca*, a numerically and behaviorally dominant ant genus (Davidson 2005), was implicated as a driver of these patterns. Three macronutrients (N, P and K) have long been shown to promote plant productivity (Vitousek and Sandford 1986; Davidson et al. 2004; Tripler et al. 2006) while lowering the plant diversity (Tilman and Wedin 1991; Hillebrand et al. 2007; Harpole and Suding 2011). Here we show that increasing the availability of P, an element whose biogeochemistry is changing due to its increased use in agriculture (Cordell et al. 2009), has similar effects on a dominant set of consumers, the ants.

The effects of biogeochemistry and temperature on consumer activity

The increase in ant foraging activity on +P plots is mostly attributable to *Azteca*, as foraging of other ant genera did not increase with +P. Phosphorous availability may influence *Azteca* activity in a number of ways. Phosphorous fertilization, when combined with N and carbohydrate addition can increase the densities of potential prey such as gamasids, collembolans and dermapterans (Jacquemin et al. 2012). Additionally, fertilization increased consumer activity since understory herbivory was higher on P, K and PK plots of GFP (Santiago et al. 2012). This may alleviate the increased demand for protein during the wet season when most tropical ants rear their brood (Kaspari et al. 2001). Beyond increasing access to protein, P may also increase access to carbohydrates if P fertilization increases plant exudate secretion, or the abundance and activity of hemipterans that excrete honeydew (Schade et al. 2003; Perkins et al. 2004). Higher carbohydrate production can increase both foraging activity and aggression in ant colonies (Grover et al. 2007; Kay et al. 2010). For example, adding sucrose, but not protein, to litter plots in a nearby Panamanian rainforest increased the abundance of *Wasmannia auropunctata*, an aggressive litter ant, while reducing the abundance of other ant species (Kaspari et al. 2012). Specific groups of herbivores and their impact across GFP have yet to be quantified, this would directly address the question of higher protein availability vs. higher honeydew availability. Finally, the increase in P concentration of plants on fertilized plots (Wright et al. 2011) may increase insect %P, and %RNA (Schade et al. 2003). This could allow ants to increase their growth rate and colony size resulting in higher ant abundance and activity. To test this mechanism P body content of ants from +P plots should be acquired, and our results suggest that focal species should be within the genus *Azteca*. We suggest these three mechanisms—an increased prey availability, higher availability of carbohydrate-rich food, and higher resource quality—act in concert to shape ant communities of tropical lowland rainforests.

Ectotherm activity is constrained by low temperatures (Huey and Kingsolver 1989). This appears to be true even in the narrow range of temperatures in tropical understories, where almost 20% of the observed variation in ant activity could be accounted for by an increase of just 4.5 degrees (24.3 to 28.8 °C). This temperature range is typically associated with high ant activity in a variety of ecosystems (Cerdá et al. 1998; Retana and Cerdá 2000; Bestelmeyer 2000). Within this range *Azteca* and *Dolichoderus* activity increased while *Ectatomma* and *Crematogaster* activity decreased (Fig. 2B). Contrary to these findings, in a xerophyllus subtropical forest, *Ectatomma* and *Crematogaster* increased their activity within that same temperature range (Bestelmeyer 2000). These differences may be attributable to local adaptation (Angilletta et al. 2007) to the cooler temperatures of the moist Panamanian forest. However, we suggest that part of this decrease in activity by *Ectatomma* and *Crematogaster* is avoidance of the dominant *Azteca* and to a lesser extent *Dolichoderus*. *Azteca*'s large colony size, high colony density, chemical weaponry and aggression allows them to exclude other ants from food resources (Davidson 2005; Dejean and Corbara 2007). Removing the large pendulous *Azteca* nests, and monitoring the responses of *Ectatomma* and *Crematogaster*, should allow us to evaluate these two hypotheses.

The effects of biogeochemistry and temperature on local diversity

We sampled ant activity and diversity on baits that simulate temporary resources. The diversity on baits should increase with the rate they are discovered and decrease with the rate that the subset of behavioral dominants—common in ant communities (Andersen 1992; Davidson 1998; Savolainen and Vepsäläinen 1988; Andersen 1997; Yanoviak and Kaspari 2000)—find and defend the baits.

Consistent with the paradox of enrichment (Rosenzweig 1971; Tilman 1987; Tilman and Pacala 1993), +P fertilized plots tended to have fewer ant genera, compared to the plots which did not receive P addition. Moreover, genus richness showed a unimodal relationship with the average abundance of *Azteca* across plots (Fig. 3), linking +P plots to diversity via aggression by this behaviorally dominant genus (Andersen 1992). The mechanism for this effect may lie in the role P plays in limiting growth rates of animals and plants (Tanner et al. 1990; Elser et al. 2000). If dominance behavior by *Azteca* ants drives down diversity, the high-tempo and abundant workers of *Azteca* may be promoted on +P plots at the expense of smaller colonies of slower growing, more heavily armored ants (Davidson 2005). This pattern has been previously recorded at a long-term N fertilization experiment of a temperate grassland, where diversity of consumers decreased, but their abundance increased at higher N concentrations (Haddad et al. 2000).

Given that tropical forests differ considerably in nutrient availability (Vitousek 1984; Kaspari and Yanoviak 2009), we predict that forests rich in P should show higher levels of interference competition by ants, and lower overall diversity than low P forests.

Caveats and next steps

Baiting is an efficient and easily repeatable method to estimate the abundance and activity of omnivores, which represent a large fraction of tropical ant fauna. Some ant species (e.g. fungus growers, specialist predators and subdominant ants) are rarely attracted to the baits, or possibly displaced from them are likely undersampled in this study (Bestelmeyer et al. 2000). Other collection methods that do not rely on attracting ants, like litter sifting or pitfalls (Agosti and Alonso 2000), could supplement and further test these results to better represent the total diversity of ants in this Panamanian forest.

We found genus, but not species estimates of diversity and richness responded significantly to fertilization and the abundance of *Azteca*. One likely reason is that species data, with their larger fraction of singletons found in only three genera, are inherently noisier and less conservative representations of community diversity and richness in the tropics given many genera remain poorly taxonomically resolved. Moreover, the higher number of singletons in species vs. genera, and their concentration in only three genera is one likely reason that the variability in genus richness (CV = 22.2%) was lower than that for species diversity (CV = 32.2%). Given that genus and species richness data are often correlated, genus diversity in the Neotropical ants has been proposed as an efficient way of exploring biodiversity responses (Groc et al. 2010).

Conclusions

The industrial revolution has increased the availability of ecosystem C, N and P (Mahowald et al. 2008; Cordell et al. 2009). Our data suggest the artificial enrichment of P may, through the paradox of enrichment, decrease ant diversity, and with it, the numerous ecosystem processes they provide (e.g. seed dispersal, scavenging, protection against herbivores, soil aeration, and nutrient turnover). What are the consequences of the increase in activity on +P plots—largely driven by *Azteca* compared to the variation due to diel temperature fluctuations? Understanding the mechanisms underlying P effects on *Azteca* activity, and its effects on other members of the ecosystem—plants and their secretions together with canopy and litter arthropods—will be instrumental when testing the mechanism behind the changes in consumer communities.

Acknowledgements

Field assistance with intense baiting was done with the help of David Fowler. Comments of Ana Ješovnik, David Donoso, Natalie Clay and Jackson Helms greatly improved the early versions of the manuscript. Oris Acevedo and Belkys Jimenez of Smithsonian Topical Research Institute provided logistical support in Panama. This study was funded by the Biology Department (OU), the College of Arts and Sciences (OU), Graduate Student Senate (OU), and National Science Foundation Macrosystem Grant to MK. The experiments in this study comply with current laws of the Republic of Panama.

Chapter 1 – figure legends

Figure 1. Differences in foraging activity - number of baits visited by ants across: control plots – CTR; no phosphorus plots (-P): CTR, N, K, NK; phosphorus addition plots (+P): P, PK, NP, NPK; no potassium plots (-K): CTR, N, P, NP; all potassium plots (+K): K, NK, PK, NPK; no nitrogen plots (-N): CTR, P, K, PK, all nitrogen plots (+N): N, NK, NP, NPK. The box and whisker plots are showing median of foraging activity, upper and lower quartiles, as well as the maximum values and outliers.

Figure 2. Relationships between maximum activity (%) – maximum number of baits visited by ants across different fertilization treatments (A) and mean temperatures (B) for the most abundant genera at baits.

Figure 3. The relationship between mean *Azteca* abundance at baits and species richness and genus richness. The relationship was significant for genus richness ($G = - 0.06x^2 + 2.4x – 35.6$, $R^2 = 0.87$, $p = 0.005$), and not significant for species richness ($S = - 0.02x^2 + 2.3x – 31.4$, $R^2 = 0.55$, $p = 0.14$).

Figure 1.

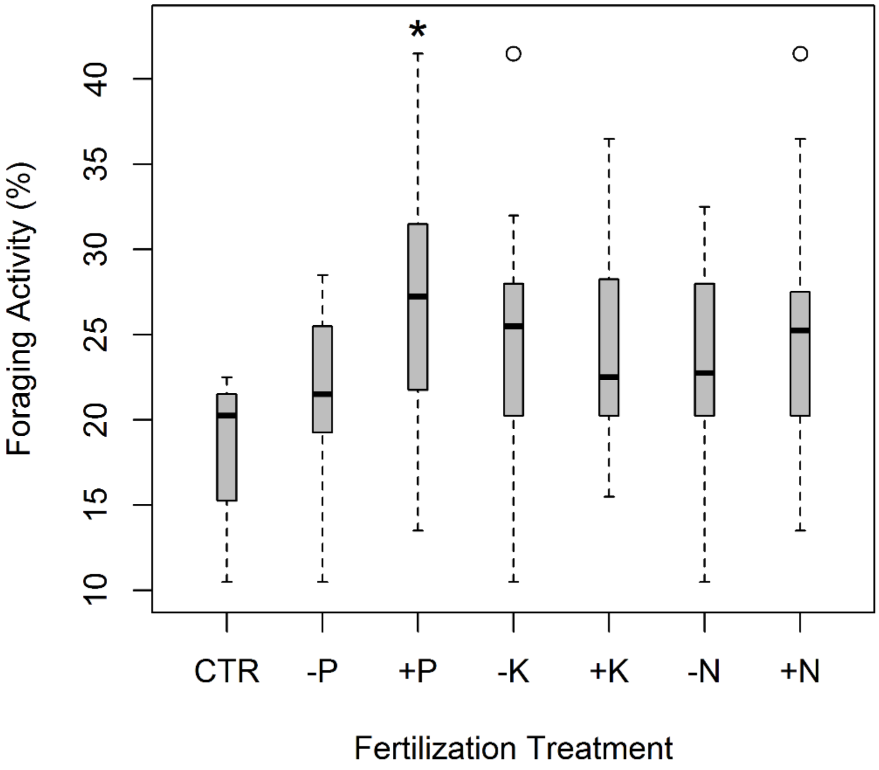

Foraging Activity (%)
10
15
20
25
30
35
40
*
CTR
-P
+P
-K
+K
-N
+N
Fertilization Treatment

Figure 2A

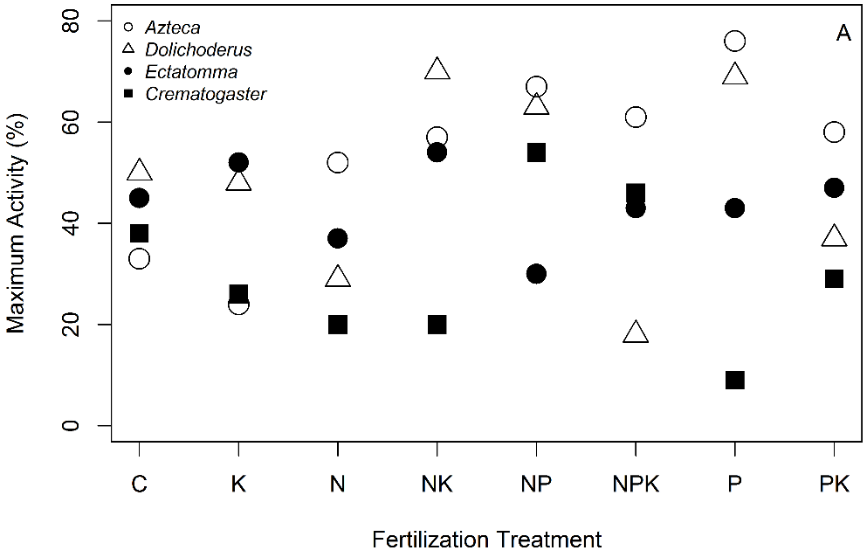

○ *Azteca*
△ *Dolichoderus*
● *Ectatomma*
■ *Crematogaster*
A
Maximum Activity (%)
0
20
40
60
80
C
K
N
NK
NP
NPK
P
PK
Fertilization Treatment

Figure 2B

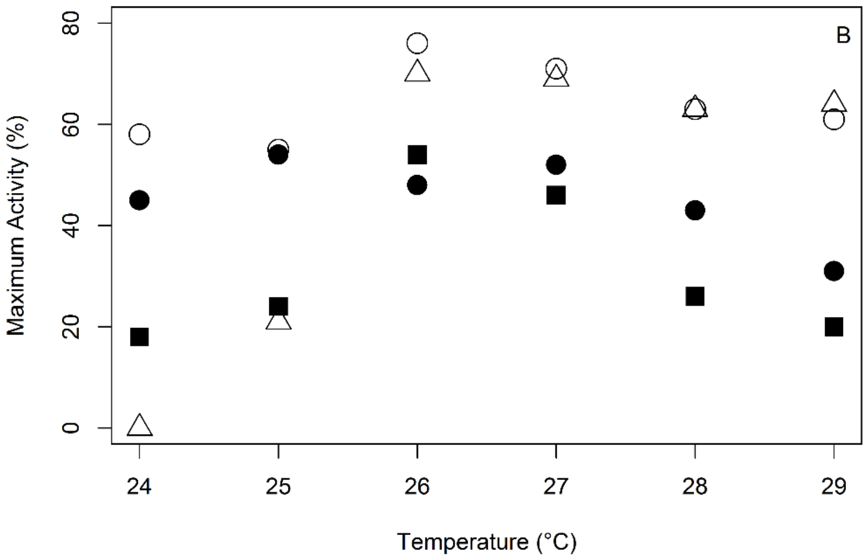
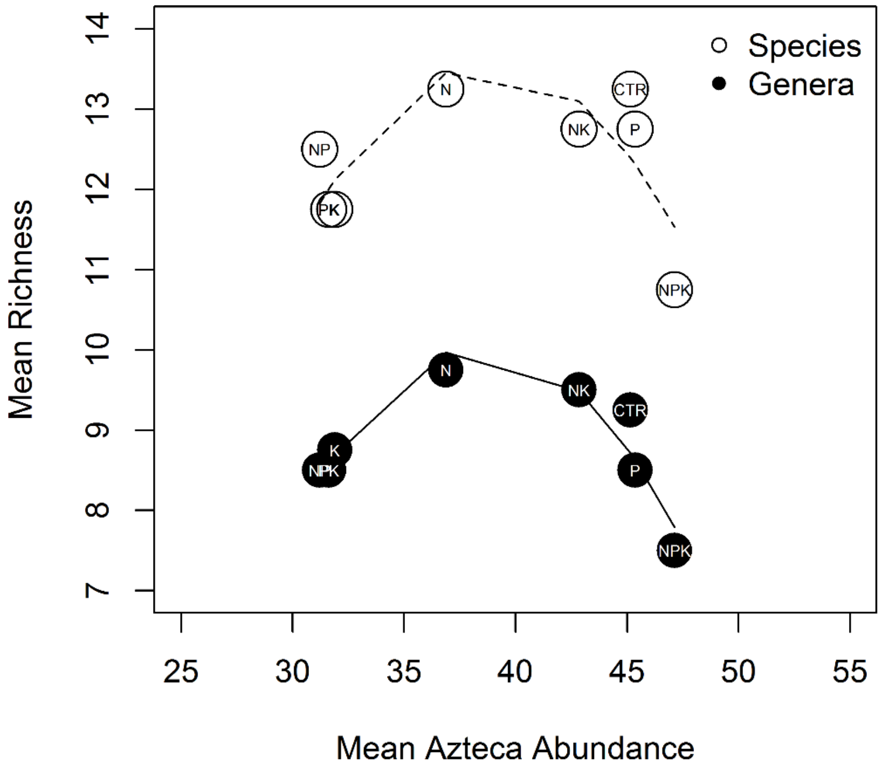


Figure 3.



Species
Genera
Mean Richness
Mean Azteca Abundance
7
8
9
10
11
12
13
14
25
30
35
40
45
50
55
N
CTR
NK
P
NP
PK
NPK
N
NK
CTR
K
NPK
P
NPK

CHAPTER 2: Desiccation resistance in tropical insects: causes and mechanisms underlying variability in a Panama ant community

This chapter is published, with some modifications, as Bujan J, Yanoviak SP and Kaspari M. 2016. Desiccation resistance in tropical insects: causes and mechanisms underlying variability in a Panama ant community *Ecology and Evolution* 6:6282-6291.

Abstract

Desiccation resistance, the ability of an organism to reduce water loss, is an essential trait in arid habitats. Drought frequency in tropical regions is predicted to increase with climate change, and small ectotherms are often under a strong desiccation risk. We tested hypotheses regarding the underexplored desiccation potential of tropical insects.

We measured desiccation resistance in 82 ant species from a Panama rainforest by recording the time ants can survive desiccation stress. Species' desiccation resistance ranged from 0.7 h to 97.9 h. We tested the Desiccation Adaptation Hypothesis, which predicts higher desiccation resistance in habitats with higher vapor pressure deficit (VPD) – the drying power of the air. In a Panama rainforest, canopy microclimates averaged a VPD of 0.43 kPa, compared to a VPD of 0.05 kPa in the understory. Canopy ants averaged desiccation resistances 2.8 times higher than the understory ants.

We tested a number of mechanisms to account for desiccation resistance. Smaller insects should desiccate faster given their higher surface area to volume ratio. Desiccation resistance increased with ant mass, and canopy ants averaged 16% heavier than the understory ants. A second way to increase desiccation resistance is to carry more water. Water content was on average 2.5% higher in canopy ants, but total water content was not a good predictor of ant desiccation resistance or critical thermal maximum (CT_{max}), a measure of an ant's thermal tolerance. In canopy ants, desiccation

resistance and CT_{max} were inversely related, suggesting a tradeoff, while the two were positively correlated in understory ants.

This is the first community level test of desiccation adaptation hypothesis in tropical insects. Tropical forests do contain desiccation resistant species, and while we cannot predict those simply based on their body size, high levels of desiccation resistance are always associated with the tropical canopy.

Introduction

Small ectotherms are often at risk of desiccation given their high surface area to volume ratio, proportionately low fat storage, and relatively high metabolic rate (Gibbs 2003; Harrison, Woods & Roberts 2012). Desiccation resistance— the ability for an organism to reduce water loss —is thus a useful trait in small ectotherms, especially in light of predicted increases in the frequency and severity of droughts (IPCC 2014). Tropical forests contain a large fraction of Earth's species, but, perhaps due to their high relative humidity, little attention has been given to the patterns of desiccation resistance in tropical arthropods (Stanley & Parsons 1981; Karan et al. 1998, Hoffmann et al. 2003; Lapinski & Tschapka 2014). Here we examine the patterns and mechanisms of desiccation resistance among 82 species of tropical ants from a diverse Panama rainforest community.

The most basic hypothesis for the distribution of desiccation resistance, which we call the Desiccation Adaptation Hypothesis, assumes that costs of desiccation resistance are balanced by benefits in arid environments. Ectothermic vertebrates and insects living in arid environments tend to be more desiccation resistant and lose water more slowly than their mesic counterparts (Eckstrand & Richardson 1981; Gibbs & Matzkin 2001; Tracy, Christian & Tracy 2010). Even at smaller scales, such as within a habitat, tiger beetle species with higher desiccation resistance use drier microhabitats (Schultz & Hadley 1987). The tropical rainforest canopy and understory have distinct microclimates: the air temperature experienced by insects in the canopy averages 1° C warmer than on the ground below; surface temperatures in the boundary layer—the air layer next to the surface—can average up to 10° C warmer (Oke 1978; Kaspari et al. 2015). Here we test the assumption that the vapor pressure deficit (VPD)—a measure of the drying power of the air—is higher in the canopy of a tropical forest, and contrast the desiccation resistance of canopy insects with litter insects.

Insects have a variety of mechanisms to reduce desiccation. First, larger insects tend to have lower surface area to volume ratio, more water storage, and more fat that can be converted to metabolic water (Hadley 1994). Within communities, larger species of fruit flies (Gibbs & Matzkin 2001), tiger beetles (Schultz & Hadley 1987) and ants (Hood & Tschinkel 1990) are more resistant to desiccation than their smaller congeners. Such examples largely come from the temperate zone. Here we test the Body Size Hypothesis in the tropics, where insects (e.g, *Drosophila* species) were found to have low desiccation resistance and low evolutionary potential for its increase (Hoffmann et al. 2003). Second, insects can also slow desiccation by simply having more water in their tissues (Hadley 1994). Canopy ants rely on more water-based food, such as honeydew, extra-floral nectar (Blüthgen et al. 2000) and are likely to have higher water content, and thus be pre-adapted to living in environments with high VPDs. Third, insects can actively slow water loss by, for example, closing spiracles, or increasing rectal water reabsorption (Harrison, Woods & Roberts 2012). A simple test for such active regulation compares the water loss of dead and living individuals. We predict that active water loss regulation will be more prevalent in the tropical canopy compared to the cooler, moister understory.

An individual's desiccation resistance may also be constrained by other adaptations to the warm canopy such as thermal tolerance, measured as critical thermal maximum (CT_{max}), the temperature at which animals lose the ability to control muscle contraction (Lutterschmidt & Hutchison 1997). We foresee two scenarios. First, if increased thermal tolerance and desiccation resistance require different costly investments then this can result in tradeoff between one investment over the other, causing a negative correlation between desiccation resistance and CT_{max}. For example, insects can prevent overheating through either passive (Lighton 1994) or active evaporative cooling (Heinrich 1980; Hadley, Toolson & Quinlan 1989). This allows them to tolerate higher temperatures but results in a high water loss (Renault, Vernon and Vannier 2005). Second, as temperature and VPD are often positively correlated (Parker 1995), the same traits that favor desiccation resistance may also favor thermal tolerance. For example, increased body size may allow an insect to better survive both thermal and desiccation stress. If so desiccation resistance and CT_{max} should be positively correlated.

Here we use a dominant, diverse tropical insect assemblage to test a basic desiccation adaptation hypothesis, and explore potential mechanisms for desiccation resistance.

Materials and methods

Study site

We conducted our sampling in a lowland tropical wet forest, during the rainy season on Barro Colorado Island (BCI; 9° 10' N, 79° 51' W), Republic of Panama. Mean annual temperature is 27° C, while mean annual rainfall is c. 2600 mm and largely occurs during the rainy season from May-December (Leigh 1999). So far 350 ant species are recorded for this forest (Donoso personal communication). We identified ant species in the lab using an online database (evergreen.edu/ants/antsofcostarica.html), supplemented with a reference collection of BCI ants of the senior author. Voucher specimens are deposited in the lab of the senior author.

Measuring microclimate

We contrasted the temperature and VPD of canopy and understory microclimates of six tree species: *Anacardium excelsum, Bombacopsis quinata, Ceiba pentandra, Dipteryx panamensis, Jacaranda copaia* and *Pseudobombax septenatum* that vary in their canopy architecture and their epiphyte load (Condit, Pérez & Daguerre 2010). We accessed the canopy using a single rope technique (Perry 1978). We placed HOBO Pro v2 (U23-002) Temp/Relative Humidity data loggers in the canopy and the understory. We tied the base of the logger with a zip tie and attached the probe directly on a branch or a liana with polyester twine. Understory loggers were placed either in the leaf litter or on the understory vegetation. As the sensor was 10 mm in diameter, it estimated relative humidity and temperature at 0-10 mm above the surface, still exceeding the size of the large ants we tested (e.g., *Cephalotes atratus*, Fig. 4). We collected the data after 2 weeks of logging temperature and relative humidity in 10-minute intervals. We calculated actual VPD by using our temperature and relative humidity measurements and formulae from Monteith & Unsworth (2007). We then calculated VPD as the difference between the saturation vapor pressure and actual vapor pressure, in kiloPascals (kPa). As VPD relies on both temperature and relative humidity it is more biologically relevant than relative humidity alone (Anderson 1936).

Measuring desiccation resistance

We measured desiccation resistance in 82 ant species from 8 subfamilies: 34 from the canopy and 48 from the understory (Table A10). We collected the ants with an aspirator and tested them the same day. We considered all ants nesting and foraging in the canopy as canopy ants, and ants nesting in the soil or litter as ground nesting ants regardless of their foraging preferences. Ants were collected from 1-10 colonies per species (median = 2), depending on species rarity. We placed 5 workers in glass Scintillation vials (1.5 cm in diameter) sealed with a mesh, next to which we attached a vial filled with fully dehydrated Drierite (W.A. Hammond Drierite Co. Ltd., Xenia, OH, USA). We drilled a 1 cm opening on the vial lids, which were then glued together with the mesh in between. Thus the relative humidity in the ant vial was approximately 0%. We used 10 mL glass vials for small ants, and 20 mL for larger ants (time to death does not change with the vial volume: Kruskal-Wallis, $\chi2 = 0.35$, df = 1, p = 0.56). We monitored worker condition hourly, recording the time to death for each of the 5 workers. As a control, ant vials were connected to an empty vial (i.e., no Drierite). Species level desiccation resistance was measured as the average time of death of 2nd and 3rd workers (i.e., LT_{50}).

Measuring hydration and water loss of ants

We tested the prediction that canopy ants were more hydrated, and lost water more slowly than understory ants, using 5 large-bodied common species from each habitat (larger species were easier to measure mass loss accurately). Foraging workers from the same colony were collected and weighed to 0.001 mg with a microbalance (Sartorius MC5), paired to be similar in weight, and one ant of the pair was killed by freezing at - 80° C. The pair was separately exposed to Drierite as above, the live ant checked every 30 min until it lost muscle control, and both ants were then weighed. Finally, both ants were dried in the oven at 60° C, and weighed to the nearest 0.001 mg to record their dry mass. Hydration of live ants is presented as a percent of water content at the outset of the experiment. Water loss is presented as the percent difference between wet mass at the outset and the end of desiccation trial for both live and dead ants.

Measuring CT_{max}

We measured the CT_{max} of each species with a digital dry bath (USA Scientific Thermal-Lok 2-position dry heat bath, advertised accuracy ± 0.2° C). We tested 5 workers from 3 different colonies for each species. Each worker was placed in an Eppendorf tube whose cap was filled with modeling clay, and then loaded in the dry bath. Starting at 36°C, we raised the temperature 2° C every 10 minutes, until all workers lost muscle control. We used the temperature at which 50% of workers lost muscle contraction as our CT_{max}. Ants used in these trials were oven dried at 60° C and then weighed to the nearest 0.001 mg with a microbalance.

Data Analysis

All analyses were conducted using R version 3.2.2 (R Core Team 2015). We used two-sample Wilcoxon tests to compare survival times of ants exposed to desiccant with the ants in control treatments, because data were non-normal. The same test was used to compare the differences in temperature and VPD between the canopy and litter. We checked the data for normality using the Shapiro Wilk W test. We used linear models to describe the relationship between the $\log_{10}$ –transformed LT_{50} and $\log_{10}$ transformed body mass (using *lm* function in the R package *stats*). Linear models were also used when testing the relationship of ant LT_{50} in air and 0% humidity, when testing for the relationship of CT_{max} and body mass, and to analyze the relationship of LT_{50} and CT_{max}. To test for the presence of outliers we used Grubbs' test in the R package *outliers*.

We used generalized linear models (GLMs) to test the effect of body mass, and habitat on ant desiccation resistance. We used an information-theoretic approach to remove non-significant effects from the full model using probability values (see Zuur et al. 2013 for model selection details). The model comparison was based on ΔAIC values—the difference of the AIC of the *i*th model and the optimal model with lowest AIC value.

Results

How does vapor pressure deficit vary between canopy and litter?

Daily temperature 10 mm above the branch surface in the canopy of the focal tree species during day hours (6:00 to 18:00 h) averaged 1.1° C higher than the temperature recorded in the understory (mean ± SD: 27.8° ± 2°C vs. 26.7° ± 1.5° C, Wilcoxon test $W = 8.0 \times 10^7$; $p < 0.001$). The difference reduced to 0.37° C at night (18:00 - 6:00 h, $W = 6.0 \times 10^7$; $p < 0.001$). These differences were consistent across canopies of different tree species and their accompanying litter (Bujan unpublished data). Daily VPD from the same sensor averaged 0.38 kPa higher in the canopy (0.43 ± 0.37 kPa, $W = 1.0 \times 10^8$, $p < 0.001$; Fig. A4A) than in the understory (0.05 ± 0.11 kPa). This difference decreased to 0.15 kPa during the night when the canopy was on average wetter than during the day 0.16 ± 0.19 kPa, as was the understory (0.012 ± 0.05 kPa, $W = 8.0 \times 10^7$, $p < 0.001$, Fig. A4B).

Testing the desiccation adaptation hypothesis

We tested desiccation resistance of 82 ant species ranging from 0.01 – 52.70 mg in dry weight. Canopy ants from 5 subfamilies and 10 genera exposed to a desiccant survived almost 3 times longer than understory ants from 7 subfamilies and 26 genera (LT_{50} = 32.2 ± 25.0 h vs. 11.5 ± 11, $W = 1316$, $p < 0.001$, Fig. 8). Canopy ants ranged from LT_{50} = 3.6 h (*Azteca chartifex* Emery, 1893) to 97.9 h (*Camponotus simillimus*

(Smith, 1862)) while understory ants survived desiccation stress from $LT_{50} = 0.7$ h (*Trachymyrmex isthmicus* Santschi, 1931) to 42.5 h (*Pachycondyla harpax* (Fabricius, 1804)). Control canopy ants survived 2.9 times longer in the air than when exposed to a desiccant (Fig. 8; W = 498, p = 0.01); understory ants survived twice as long (W = 205, p = 0.005). The increase between the difference of survival time in control and desiccation treatments increased with body mass (Fig. A5, $LT_{50control} - LT_{50dessicant} = 0.39mass + 1.19$, $R^2 = 0.24$, $p = 0.0005$).

Mechanism 1: body size enhances desiccation resistance

Canopy ants were on average 16% heavier than litter ants (KW: $\chi^2 = 4.9$, df = 1, p = 0.03). Desiccation resistance increased with body mass in both canopy and ground nesting ants (Fig. 6), and our linear model accounted for ca. ¼ of the variation ($LT_{50} = 0.27mass + 1.2$, $F_{1, 79} = 24.8$, $R^2 = 0.24$, $p < 0.001$). Body mass accounted for more variation in desiccation resistance of canopy ants ($LT_{50} = 0.26mass + 1.4$, $F_{1, 32} = 9.1$, $R^2 = 0.22$, $p = 0.005$), than for the understory ants ($LT_{50} = 0.20mass + 0.94$, $F_{1, 45} = 10.5$, $R^2 = 0.19$, $p = 0.002$). However, the slope and the variation in desiccation resistance explained by body mass in canopy and understory was not different from the values obtained at the community level. The optimal GLM model for explaining desiccation resistance includes both body mass and habitat as predictor variables and accounts for 42% of variation in desiccation resistance (Table A11).

There was a high variability in desiccation resistance within three genera with the most desiccation resistant species. Desiccation resistance of the genus *Camponotus* ranged from 10.9 - 97.9h, in genus *Neoponera* species ranged from 17.4 - 78.8 h, and in *Cephalotes* species range was 18.8 h - 66.5 h (Table A10). Some litter genera, however, consistently had low desiccation resistance eg. *Pheidole* (1.5 - 4.7 h), and fungus growing ants, such as *Cyrphomyrmex* (1.7 - 2 h) and *Apterostigma* (Table A10, 1.3 h). When we used GLMs with genus as a predictor variable, in addition to habitat and mass only the aforementioned fungus grower genera accounted for a portion of variation in desiccation resistance.

Mechanism 2: hydration enhances desiccation resistance

We studied the role of hydration in desiccation resistance in 10 common ant species—5 from each habitat—ranging in dry mass from 1.5 – 27.2 mg. These ants varied in % water content from 48% in *Eciton hamatum* (Fabricius, 1782) to 75% in *Camponotus sericeiventris* (Guérin-Méneville, 1838), but % water was not related to body mass ($F_{1, 8} = 0.004$, $p = 0.95$). Water content of canopy ants averaged 2.5% higher than the water content of understory ants (Fig. 7A, 61.3 ± 6.0% vs 58.8 ± 4.5%, W = 3280, p = 0.016). Water content, however, was not a good predictor of desiccation resistance ($F_{1, 8} = 0.41$, $p = 0.54$) or CT_{max} ($F_{1, 7} = 0.017$, $p = 0.90$).

Water loss was on average higher in the canopy than in the litter (Fig. 7B, 26.3 ± 10 vs. 22.1 ± 6.8, W = 3975, p = 0.01), but the average rate of ant water loss did not differ between the habitats (W = 4247, p = 0.54). Live and dead canopy ants did not differ in their total water loss under desiccation stress (W = 944, p = 0.17), nor did understory ants (W = 816, p = 0.56). Water loss rate of live canopy ants did not differ from their dead counterparts (W = 592, p = 0.70), same was true for the understory ants (W = 691, p = 0.90).

Is there a tradeoff between desiccation resistance and thermal tolerance?

We did not find a consistent tradeoff between LT_{50} and CT_{max} (Fig. 8). After using body mass as a covariate in our linear models, CT_{max} and desiccation resistance correlated in opposite ways in canopy and litter ants. Desiccation resistance in canopy ants decreased with CT_{max} ($F_{2,20} = 8.6$, $R^2 = 0.46$, $p = 0.002$). This relationship in canopy ants is even more pronounced when an outlier – *Azteca* cf. *chartifex,* was removed ($F_{2,19} = 25$, $p < 0.001$, $R^2 = 0.72$). Understory ants, however, show a positive relationship between desiccation resistance and CT_{max} ($F_{2,9} = 13$, $R^2 = 0.74$, $p = 0.002$).

Discussion

Here we show that desiccation can be a major challenge for small ectotherms even in a moist tropical forest. Daytime vapor pressure deficits were 9 times higher in the canopy than in the understory below, and canopy ants tolerated desiccation stress 3 times longer than understory ants. Desiccation resistant ants tended to be larger, although less than predicted by changes in their surface area to volume ratio. Moreover, canopy ants tend to contain more water than the understory ants, suggesting a possible role of water storage in postponing desiccation. Combined with an earlier study on thermal tolerance (Kaspari et al. 2015) these data point to large differences in both the microclimate between canopy and understory and the resulting traits of a dominant insect group.

Body size and desiccation resistance

Consistent with their lower surface area to volume ratios, larger insects typically have higher resistance to desiccation (Lighton, Quinlan, & Feener 1994; Chown & Nicholson 2004, Schilman, Lighton & Holway 2007, Harrison, Woods & Roberts 2012), but few studies include sufficient sample sizes to estimate the nature of this relationship. A notable exception is Hood and Tschinkel (1990), who found that across 25 ant species from a pine woodland and 11 ant desert species, desiccation resistance scaled to dry mass$^{0.55}$, which differed significantly from the expected value of dry mass$^{0.67}$. At the adjusted mass range we examined 64 species and overall found an even weaker relationship with body mass (Fig. A6, $b = 0.33$, $R^2 = 0.20$). When we examine this relationship at the habitat level both slopes were less steep, than predicted ($b_{\text{canopy}}=$ 0.35 and $b_{\text{litter}} = 0.32$).

Differences in surface area to volume ratios did not sufficiently account for variation in desiccation resistance in this community. A number of factors may reduce this constraint. Ants might be using behavioral adaptations to avoid overheating and desiccation stress, as small ectotherms are more susceptible to microclimate variability, specifically temperature changes (Woods, Dillon, & Pincebourde 2015). For example, activity of smaller ants was higher at lower VPD, while larger ants showed no preference for VPD levels in a lowland rainforest in Costa Rica (Kaspari 1993). In the tropical canopy epiphytes can provide a moister and cooler microclimate (Stuntz, Simon, & Zotz 2002) which might allow canopy ants to behaviorally avoid desiccating. Finally, larger ants might be less desiccation resistant than predicted by the surface area to volume ratio because of potential trade-offs between desiccation resistance and other traits like thermal performance (Kaspari et al. 2015, Baudier et al. 2015).

Overall, canopy ants of a subtropical pine woodland averaged 8 times higher desiccation resistance than the understory ants (Hood and Tschinkel 1990); this difference was 3 times lower in our tropical forest. The larger difference between two habitats at higher latitudes arises because of a higher VPD in the pine forest canopy. This pattern of lower desiccation resistance of insects in the tropical regions has been thoroughly studied in *Drosophila* species which have lower desiccation resistance in the tropics when compared to species from higher latitudes (Stanley & Parsons 1981; Karan et al. 1998; Hoffmann et al. 2003).

Water content is not a good predictor of desiccation resistance

In xeric habitats, large ants contain more water and have higher desiccation resistance than smaller workers from the same colony (Lighton, Quinlan, & Feener 1994; Johnson 2000). We found no relationship between ant water content and body mass. Habitat was a better predictor of the total water content than body mass as canopy ants, relying on a more water based diet, averaged 2.5% higher than ground nesting ants. The total water content of both canopy (61%) and understory ants (59%) is similar to the water content measured for desert ant workers 66% *Pogonomyrmex rugosus* Emery, 1895 (Lighton & Feener 1989), and 63% *Pogonomyrmex occidentalis* (Cresson, 1865), (Johnson 2000). Since water content was not a good predictor of CT_{max} or desiccation resistance, active evaporative cooling is likely not an efficient way of reducing body temperature in habitats with average relative humidity above 90%. Our results suggest that tropical ants do not use extra water to cope with desiccation or thermal stress. An absence of a water content - body mass relationship in the ants we studied could be due to mass range we used. We note that by testing ants heavier than 1.5 mg, we excluded a large proportion of small ants.

Ants did not differ in apparent ability to retain moisture

Contrary to our prediction, live canopy ants were not better at reducing water loss and had an overall 3.6% higher water loss rate than dead ants. Dead desert ants lose more water than live ants over time (Lighton et al. 1994), but this was not the case in any ants we tested. We found no significant differences in water loss between live and dead ants of either canopy or litter species (Fig. A7). Hood and Tschinkel (1990) also found no difference in water loss between live and dead ants in the higher latitude ant community. This suggests that canopy ants likely have other, passive, mechanisms for preventing water loss. For example, insects with less porous cuticles and those with more branched saturated lipids (Gibbs 2002) can reduce the cuticular respiration which accounts for more than 80% of the water loss in insects (Quinlan & Gibbs 2006).

Evidence for tradeoffs is complex

Our study shows the importance of examining the relationship between traits enabling survival in a set of coupled environmental conditions. Insect thermal sensitivity (Huey, Patridge & Fowler 1991; Hurlbert et al. 2008; Deutsch et al. 2008; Diamond et al. 2012) and desiccation resistance are frequently studied independently

(Hadley 1994; Chown 1993; Gibbs, Chippindale & Rose 1997; Schilman, Lighton & Holway 2007) despite their potential to interact (Renault, Vernon & Vannier 2005; Terblanche et al. 2006). Ectothermic vertebrates (Crowley 1987; Ladyman & Bradshaw 2003), and insects (Smith et al. 1999). often prefer lower temperatures under desiccation stress.

We found an increase in desiccation resistance with CT_{max} in the understory, while ant species of the tropical canopy showed the opposite pattern: decreased desiccation resistance as their CT_{max} increases. One possible solution to this puzzle lies in the cuticular lipids that coat the exoskeleton and inhibit water loss (Hood & Tschinkel 1990). As temperature increases, these lipids eventually change their consistency and increase cuticular permeability (Gibbs 2002; 2011). Our findings suggest that in canopy ants, which experience some of the most extreme temperatures in the tropical forests (Kaspari et al. 2015), more permeable cuticle increases evaporative water loss. This in turn, allows ants in the hottest environments to engage in passive evaporative cooling, and could be the reason why canopy ants with $CT_{max} > 46°$ C have lower desiccation resistances. If true, the composition, quantity and physics of cuticular hyrocarbons, may prove a useful functional trait in predicting the thermal ecology and water balance ability of small invertebrates.

Caveats

Our study quantifies the difference in microclimates during a tropical wet season. Dry season conditions of this tropical forest include higher VPD and higher temperatures than during the wet season (Leigh 1999). Ant activity in this forest is 25% lower during the dry season compared to the wet season (Kaspari & Weiser 2000). Furthermore, in drier conditions desiccation resistance has been shown to increase in a fruit fly species (Hoffmann, Shirriffs & Scott 2005). Our study may thus underestimate desiccation resistance in this assemblage, and its seasonality.

We measured total water loss gravimetrically at the time of death for each ant species. Measuring water loss with a flow-through or closed system respirometry would allow us to distinguish between excretory, respiratory and cuticular water loss (Harrison, Woods & Roberts 2012). Continuous monitoring of water loss in live and dead ants would further allow us to test if water loss regulation is present at the beginning of the exposure to the dry conditions in the canopy and litter ants.

Future Work

The Desiccation Adaptation Hypothesis remains a powerful and relatively untested tool in global change biology, and requires more validation across Earth's climates and invertebrate communities. Furthermore, the variety of mechanisms that can generate desiccation resistance, including fluidity of epicuticular lipids, deserve further study as a key functional trait in tiny ectotherms. Our works suggests that within any given ecosystem a variety of microclimates exist, and within any given community, a diversity of mechanisms can interact to generate the distribution of desiccation resistance among individuals and between populations. Against this backdrop of interesting complexity, ecologists are called upon to predict responses to a likely world of increasing seasonal and multi-annual drought in the subtropics and tropics (Fu 2015). One prediction arising from our work and that of Hood and Tschinkel (1990): the higher average desiccation resistance in canopy species suggests their increase at the expense of litter ants in a world of increasing droughts.

Acknowledgements

Lab work was done with the help of Brittany Benson. Michael Weiser designed the desiccators. Comments of Thayer Hallidayschult, Karl Roeder and Michael Weiser greatly improved the early versions of the manuscript. Oris Acevedo and Belkys Jimenez of Smithsonian Topical Research Institute provided logistical support in Panama. This study was funded by the National Science Foundation Macrosystem Grant EF-1065844 to MK and a Graduate Student Timmons Scholarship from the Department of Biology awarded to JB. The experiments in this study comply with current laws of the Republic of Panama.

Chapter 2 – figure legends

Figure 4. Worker of *Cephalotes atratus*, in a *Dipteryx panamensis* canopy next to the data loggers used for measuring the temperature and relative humidity. *Cephalotes atratus* was the second largest canopy ant we tested.

Figure 5. Log_{10} of lethal time (h) at which 50% of workers lost their muscle coordination (LT_{50}), after they have been exposed to air (white) and desiccant (gray). The box and whisker plots are showing median of $\log_{10}LT_{50}$, upper and lower quartiles, as well as the maximum values and outliers.

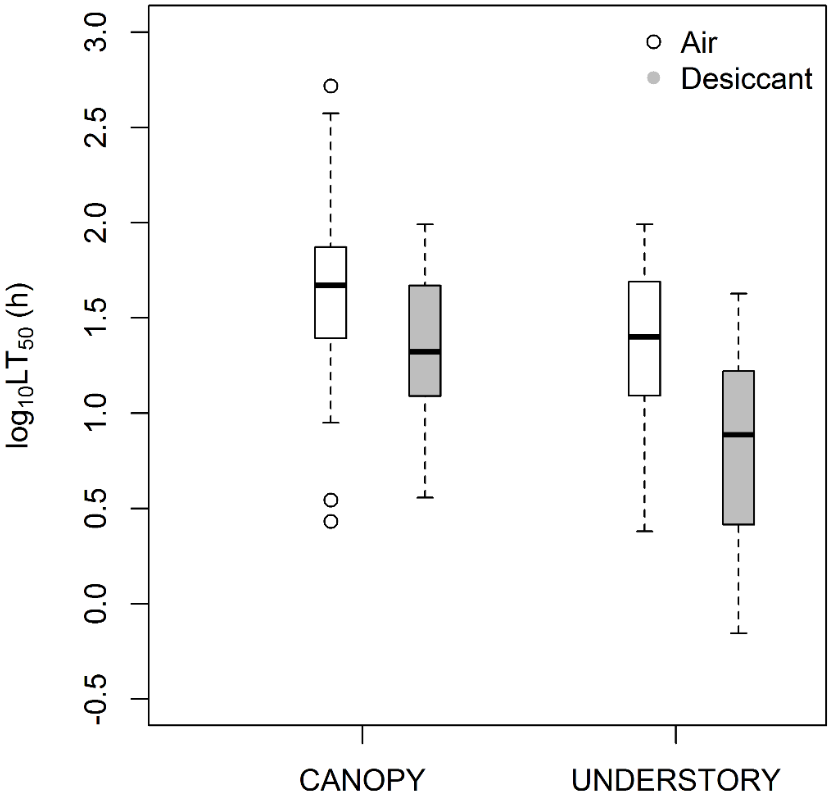
Figure 6. Relationship between species desiccation resistance (LT_{50}) and $\log_{10}$ body mass (mg) in canopy and understory ants. Both linear models for this relationship differ significantly from a slope of 0 (see text for details): canopy – gray line, understory – black line.

Figure 7. A) Total water content (%) of canopy and ground nesting ants. B) Total water loss (%) in canopy and ground nesting ants. The box and whisker plots are showing median of % water content (A) and % total water loss (B), upper and lower quartiles, as well as the maximum values and outliers.

Figure 8. Relationship between desiccation resistance (LT_{50}) and critical thermal maximum (CT_{max}) in the canopy and understory ants.

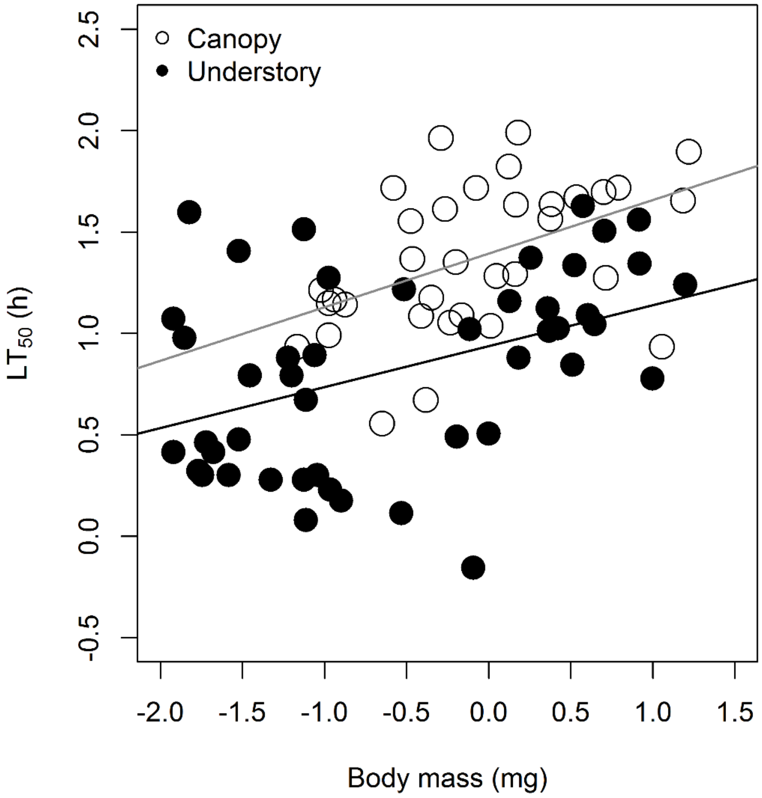
Figure 4.

Figure 5.



log$_{10}$LT$_{50}$ (h)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
○ Air
● Desiccant
CANOPY
UNDERSTORY

Figure 6.



○ Canopy
● Understory
LT50 (h)
-0.5
0.0
0.5
1.0
1.5
2.0
2.5
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
Body mass (mg)

Figure 7A

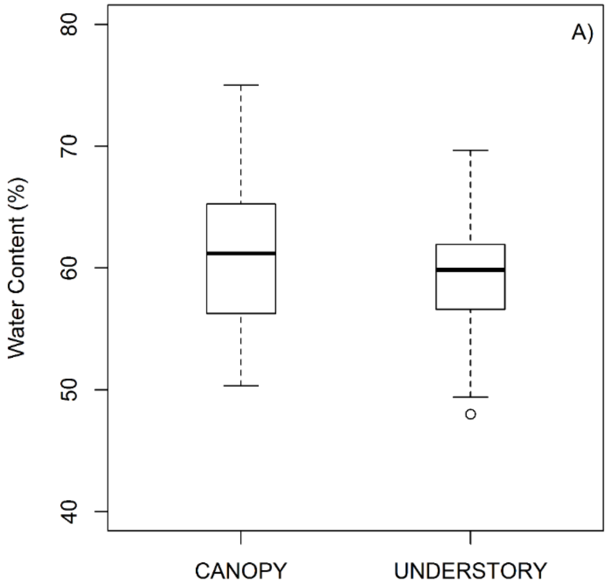


Figure 7B

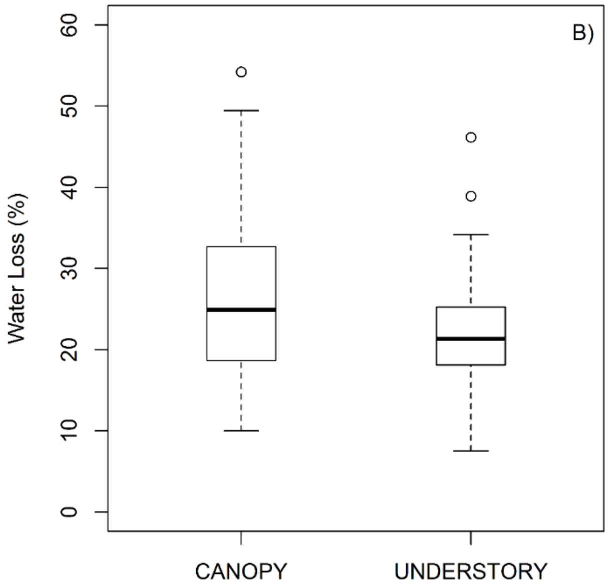
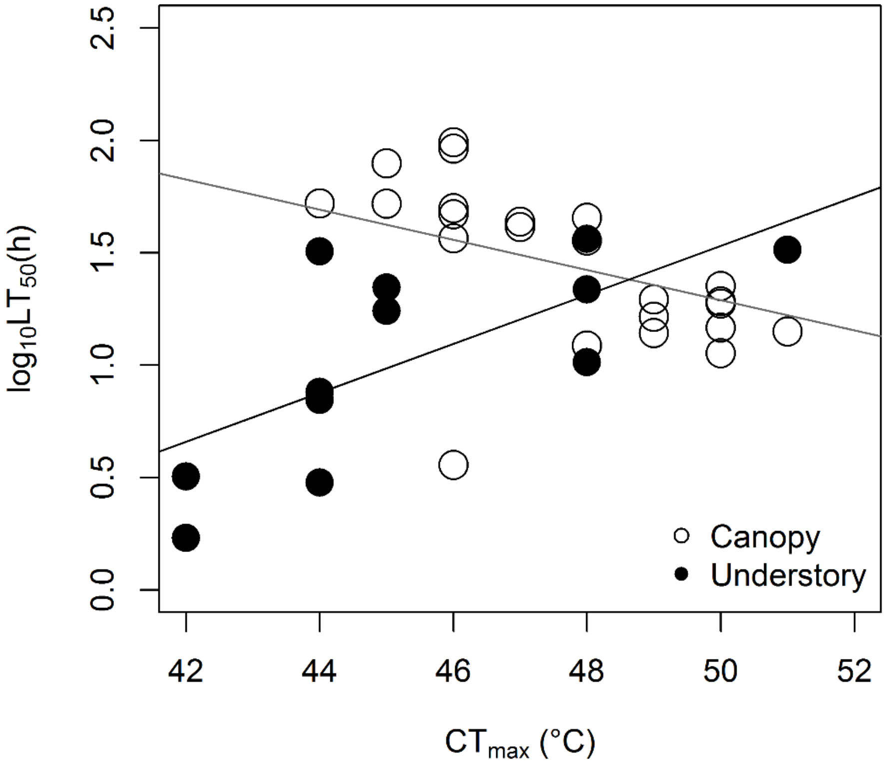


Figure 8.


2.5
2.0
1.5
1.0
0.5
0.0
$\log_{10}LT_{50}(h)$
42
44
46
48
50
52
CT_{max} (°C)
○ Canopy
● Understory

CHAPTER 3: Nutrition modifies critical thermal maximum of a dominant canopy ant

This chapter, with some modifications, is currently in review in

Journal of Insect Physiology

Abstract

While adaptive responses to climate gradients are increasingly documented, little is known about how individuals may alter their upper thermal tolerances. We explored how the nutritional state of *Azteca chartifex,* a canopy ant of the Panama rainforest whose diet largely includes honeydew and extra floral nectar secretions governed its CT_{max} – the temperature at which individuals lose their muscle control. We predicted that the workers of *Azteca chartifex* that recently fed on such carbohydrate-rich diet, will use that energy to increase its upper thermal tolerance. Moreover, if a carbohydrate-rich diet increases CT_{max}, then we predicted that ants from colonies with high CT_{max}s feed at a lower trophic level, and thus have a higher C/N ratio.

We used *A. chartifex* colonies from 40x40 m forest plots, in which plots fertilized with phosphorus showed higher *A. chartifex* foraging activity—number of baits occupied by ants—than controls. As foraging activity can be governed by resource availability, we first measured CT_{max} of field collected colonies. In freshly field-collected colonies, CT_{max} was 2° C higher in control plots. This difference disappeared when ants were provided with only water for 10 h. Ants were then provided *ad lib* 10% sucrose solution which increased CT_{max} by 5° C. We thus support the hypothesis that enhanced carbohydrate nutrition enables higher thermal tolerance, but this does not appear to be linked to colony trophic status, higher C/N ratios, or higher total body phosphorus.

This short-term thermal plasticity linked to carbohydrate nutrition demonstrates the importance of ant diet in shaping their physiological traits. It is especially relevant to

ant species that maintain high abundance by feeding on plant exudates. In a rapidly warming world, carbohydrate availability and use may represent a new element for predicting population and community responses of herbivorous insects.

Introduction

A well-established component of global climate change is an increase in average ambient temperature across many regions (IPCC 2014). How forecasted temperature increases will affect biodiversity has been studied extensively both spatially and temporally (Chen et al., 2011; Thackeray et al., 2016)—only two of the three axes of potential response (*sensu* Bellard et al. 2012). We know relatively little about the third axis, “self,” which refers to a species’ physiology (Calosi et al., 2008). Small cursorial insects are at a particularly high risk of overheating, and many studies recognize the importance of thermal acclimatization (Angilletta et al., 2007; Hoffmann et al., 2005; Kipyatkov and Lopatina, 2002). While biochemical adaptation studies reveal many mechanisms of adaptation to high temperature at cellular and molecular level (Denlinger and Yocum, 1998), we still lack a comprehensive understanding of how temperature affects metabolism of ectotherms (Schulte, 2015). The mechanisms behind physiological and behavioral adaptations that allow insects to regulate their tolerance of high temperatures are underexplored considering their global success and distribution (Angilletta 2009, Harrison et al. 2012). For example, few have explored how nutrition affects thermal tolerance of terrestrial insects, thermal maxima in particular (Nyamukondiwa and Terblanche, 2009). Those studies typically focus on larval stages (e.g., Stamp 1990, Andersen et al. 2010) rather than free-foraging adults that are exposed to a broader range of ambient temperatures (Jumbam et al., 2008). If nutrition can enable higher thermal tolerance, then the impact of climate change on animal populations might be mediated by dietary changes.

We studied ants because they are omnipresent ecosystem engineers and are vitally important to trophic dynamics (Hölldobler and Wilson 1990). Moreover, ant diversity peaks in the tropics (Fisher 2010), where climate variability is low, but CT_{max}s range from 40 - 56° C (Kaspari et al., 2015). Numerically and behaviorally dominant canopy ant—*Azteca* cf. *chartifex*— which feeds extensively on honeydew and plant secretions (Davidson, 2005), had low CT_{max} and the lowest desiccation resistance compared to other Neotropical canopy ants (Bujan et al. 2016a). We know that sugar availability is important in governing ant foraging patterns and feeding preferences in tropical species (Blüthgen and Fiedler, 2004a) , but we know almost nothing about how it affects thermal tolerance in ants.

Relationships between diet and thermal tolerance have focused on insects with sugar-based diets. For example, critical thermal maximum (CT_{max})— the temperature at which animals lose muscle control (Lutterschmidt and Hutchison, 1997)—increased in two fruit fly species (*Ceratis capitata* and *C. rosa*) and the silverleaf whitefly (*Bemisia argentifolii*), both of which tolerated higher temperatures when fed high concentrations of sucrose (Nyamukondiwa and Terblanche, 2009, Wolfe et al., 1998). Here we report on how sucrose availability influences the CT_{max} of a common rainforest ant.

We tested three complementary hypotheses regarding how carbohydrate availability modifies ant thermal maxima, and the mechanisms behind those changes.

First, we posited that higher carbohydrate availability enables *Azteca* to tolerate higher temperatures because sucrose provides an energy source used in thermoregulation (Chown and Nicholson 2002). While sugars stored in insect haemolymph can be used as metabolic fuel for energetically costly activities (Suarez et al., 1996), glycogen can have a dual role in heat tolerance—one as a source of ATP, and the other as a water source. Glycogen molecules bind 3-5 times more water than its own weight, so once glycogen is metabolized this water is released and can be used in evaporative cooling (Gibbs et al. 1997). Thus, we predict that ants from carbohydrate-rich patches of the forest will have higher CT_{max}, as will ants experimentally fed sucrose solution in the lab.

Second, we tested how a colony's CT_{max} would relate to its trophic level. We posited that if a diet rich in carbohydrates increased CT_{max}, then *Azteca* colonies with high CT_{max} should 1) feed at a lower trophic level, and 2) have higher carbon:nitrogen ratio (C/N) because those ants would have N-poor diet.

Third, we tested if phosphorus (P) promoted thermal tolerance. In an interspecific comparison of 26 Neotropical ant species, CT_{max} and whole body P content increased together (Kaspari et al., 2016). Moreover, carbohydrate availability can alter ant total body P (Kay et al., 2006). Finally, activity of *A. chartifex* increases with long term P fertilization in a tropical lowland forest (Bujan et al. 2016b), indicating that P can either directly or indirectly affect ant activity. We thus posited that the interspecific co-increase of CT_{max} and P would also hold intraspecifically among colonies from this Panama rainforest.

One of the mechanisms for increase in foraging activity of *A. chartifex* after P addition could be increase in carbohydrate-rich food across these plots, as aggression and activity of ants are enhanced by carbohydrates (Grover et al. 2007). Furthermore, understory herbivory was higher on P plots in this forest (Santiago et al., 2012), which suggest that plants across P plots provide a more palatable resource. As *A. chartifex* relies on high carbohydrate diet, and carbohydrate diet can increase ant activity and CT_{max}, we focused on P and control plots to test our hypotheses regarding the differences in a physiologically costly trait of CT_{max}.

Materials and Methods

Our study system was *Azteca* cf. *chartifex* colonies from a lowland moist forest within Barro Colorado Nature Monument, Republic of Panama. We sampled across the Gigante Fertilization Experiment (GFP: 9°06’31’’N, 79°50’37’’W) composed of 32 plots (40 × 40 m) fertilized since 1998 with nitrogen, P and potassium in a factorial block design (for details see Wright et al., 2011). Two species groups of *Azteca* build carton nest of chewed plant fiber: *Azteca* cf. *trigona* and *Azteca* cf. *chartifex*. When they occur in sympatry, as they do in Panama, their workers are morphologically indistinguishable (Longino, 2007). We thus used barcode analysis to identify the workers, and found that colonies belong to *Azteca chartifex* complex (Donoso *in prep*).

To assess how nutritional state influenced thermal tolerance, we collected 24 colonies on four control and four P plots. We focused our studies on control and P fertilization plots, as *A. chartifex* was previously found to be most abundant and active on P plots (Bujan et al. 2016b).

Each plot was represented by at least one colony (median = 3). Colonies were selected based on their accessibility. We could only use fragments of the colonies that were within the reach with a portable ladder. CT_{max} variation was not affected by plot identity (Kruskal-Wallis: $\chi^2 = 11.7$, df = 7, p = 0.11) which is why we don't include plot level analysis in our manuscript and focus only on the effect of fertilization. We collected workers by breaking off a piece of the carton nest from each accessible colony on the plot, sealing the piece in a plastic bag, and transporting the ants to the lab where they were placed in an open plastic container (20 × 30 × 12 cm) lined with Fluon™ to prevent escape. Two hours after collection we measured CT_{max} for five workers from each colony with a digital dry bath (USA Scientific Thermal-Lok 2-position dry heat bath, advertised accuracy ± 0.2° C). Each worker was placed in a 1.5 mL micro centrifuge tube whose cap was filled with modeling clay to prevent ants from hiding in the lid above the heating block. We used an established protocol from ant CT_{max} studies (Diamond et al., 2012; Kaspari et al., 2015): raising the temperature 2° C every 10 minutes, starting at 36° C, until reached their critical thermal maximum, CT_{max}, defined as the loss of ant righting response (Talbot, 1934). We used the average CT_{max} from these 5 workers as our response variable. Body size of ants does not affect their CT_{max} if thermal dry bath is used, because placing ants in the micro centrifuge tubes removes the boundary layer—the air layer next to the surface—and CTmax body size relationship disappears (Kaspari et al. 2015).

After measuring CT_{max} of field collected ants we provided colonies with only water *ad libitum* for 10 h, after which we measured their CT_{max}. Due to logistical constraints we used a total of 10 colonies (6 from controls and 4 from phosphorus plots) for these lab experiments. We then provided ants with 10% sucrose solution in a glass tube sealed with a cotton ball, and again measured CT_{max} after 10 h. Thus, all *A. chartifex* colonies generated three measures of CT_{max}: 1) after collection, 2) after food deprivation, and 3) after feeding *ad libitum* sucrose. Ants were kept at the same lab temperature during the experiments (22° C). Immediately after we provided colonies with water, and sucrose solution ants recruited to them in large numbers, and their gasters increased after drinking (*pers. obs.*). The volume of the sugar solution was lower after 10 h, sometimes even halved, suggesting the active uptake of the sugar solution. We did not reuse workers in these trials, but used a new set of 5 workers for each CT_{max} treatment.

To quantify how P fertilization influenced *A. chartifex* stoichiometry we froze some workers from each colony at -20° C immediately upon returning to the lab. Ants were dried at 60° C to a constant weight and ground ca. 100 mg (around 300 workers) into a fine powder with mortar and pestle. The samples were analyzed at the Smithsonian Tropical Research Institute Soil Lab with Inductively Coupled Plasma (ICP) for total organic P and 10 elements (Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, Zn).

We next used the remaining dried workers to determine how P fertilization influenced the relative trophic position and whole body C and N of these colonies, we quantified the stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$). We removed gasters and petioles from the workers, as the crop therein can contain undigested food, biasing an individual's isotopic signature based on its recent meal (Blüthgen et al., 2003; Tillberg et al., 2006). To set the autotroph baseline of our food web, we used healthy leaves from a common understory plant, *Alseis blackiana* (Rubiaceae) and to set the secondary consumer baseline we used a predatory ant *Pachycondyla harpax*. Samples were ground, and weighed in tin capsules to the nearest 0.001 mg on a microbalance (Cahn Instruments, Cerritos, CA). Stable isotope analysis and measurements of elemental C and N, were performed on a Finnigan Delta Plus mass spectrometer in the University of Georgia's Ecology Analytical Laboratory. Delta (δ) values are calculated as $((R_{sample}/R_{standard}) - 1) \times 1000$, where R is the ratio of heavy to light isotope ($^{15}N/^{14}N$) in the sample and in the standard. To calculate the trophic enrichment, we used the standard of 3.4‰ for each trophic level (Post, 2002).

To test for the differences between the fertilization treatments we used Wilcoxon rank sum test or t-test when comparing the differences between two groups. We decided on the analysis after testing our data for normality with Shapiro-Wilk test. All the analyses were run in R version 3.2.2 (R Core Team 2015).

Results

CT_{max} increases with dietary carbohydrates

Field collected ants from control plots averaged CT_{max}s 2.2° C higher than those of ants collected on P plots (Fig. 9; mean ± SE: 47.8 ± 0.7° C vs. 45.6 ± 0.7° C, $t_{22} = 2.3$, p =

0.03). This difference decreased by 30% and was not significant when the ants were then starved for 10 h and provided only with water (CTR = 46.2 ± 0.4° C vs. P = 44.5 ± 0.8° C, $t_8 = 2.1$, p = 0.07).

Once the same colonies were then fed a 10% sucrose solution, CT_{max} increased for both control and P plot colonies to a mean of ca. 51° C (CTR = 51.2 ± 0.4° C vs. P = 50.7 ± 0.8° C, $t_8 = 0.65$, p = 0.54). As the effect of fertilization treatment on CT_{max} disappeared after starving we pooled colonies from both controls and P plots receiving water (n = 10), and after the same colonies fed on sucrose solution (n = 10) to find a 5.0° C increase in CT_{max} after sugar feeding (Fig. 10; 51.0 ± 0.3° C; $t_{18} = 9.2$, $p < 0.001$). Regardless of the CT_{max} of a starved colony, all the colonies increased their CT_{max} after being fed sucrose (Fig. 10).

CT_{max} *is not affected by the relative trophic position*

We found no difference in $\delta^{13}C$ of *Azteca chartifex* collected at control and P plots (Fig. 11; mean ± SE: CTR = - 28.6 ± 0.1‰ vs. P = - 28.7 ± 0.3‰; Wilcoxon test: W = 34, p = 0.46). The same was true for $\delta^{15}N$ tested workers (CTR = 3.78 ± 0.23‰ vs. P = 3.98 ± 0.23‰; W = 20, p = 0.46). The range of $\delta^{15}N$ recorded at control and P plots did not suggest a shift in trophic level (CTR = 3.1 - 4.8‰ vs. P = 2.6‰ - 4.6‰).

Effect of fertilization on elemental chemistry

To test if fertilization influenced the overall stoichiometry of ant workers we compared whole body chemistry of a total of 23 colonies from control and P plots (Table A12).

We found no significant difference across fertilization treatments for any of the 11 elements examined, including P. The elements recorded in highest concentrations were K (overall mean ± SD = 9.98 ± 1.62%), and P (7.46 ± 0.76%). Furthermore, there was no difference in %C (Fig. A8; W = 19, p = 0.39) or %N (W = 14.5, p = 0.16) between control and P plots. There was no difference in C/N ratios of *A. chartifex* across studied plots (Fig. A9; W = 35, p = 0.39). The same was true when whole ground-up ants were tested for the %C, %N and C/N ratios.

Discussion

Sucrose availability increases CT_{max}

Azteca chartifex workers supplemented with sucrose could tolerate 5° C higher temperatures than fasted workers. Such a large increase in thermal maximum would allow this dominant canopy ant to expand its thermal niche and match the CT_{max} of the most thermally tolerant canopy ants in this forest (Kaspari et al. 2015). Our experiments showed that dietary carbohydrates enabled this ant to tolerate previously lethal temperatures. We thus found support for the hypothesis that CT_{max} varies with diet. By contrast, we did not find support for the hypothesis that higher CT_{max} is associated with a shift in trophic position. Even though CT_{max} of field collected ants from control plots was 2° C higher than in ants from P plots, we found no difference in the trophic position, C/N ratios or P concentration of those colonies, suggesting that CT_{max} plasticity is regulated by short-term dietary choices.

There are several ways in which individuals can use carbohydrates to increase their thermal maxima. In insects, ingested sucrose can be stored as the disaccharide trehalose in haemolymph (Thompson, 2003) or glycogen in muscle and fat bodies (Sacktor, 1970). Sugars from insect haemolymph can then be used to quickly generate ATP (Suarez et al., 1996), as increase in thermal stress increases ATP demand (Sokolova, 2013). Sufficient ATP availability is highly correlated with the ability to tolerate high temperatures (Pörtner, 2001). One of the roles of ATP in fighting thermal stress is enabling the synthesis of heat shock proteins (HSPs). In ants exposed to high temperatures HSP70 is commonly found (Gehring and Wehner, 1995; Maisov et al., 2007) and its' synthesis is ATP-dependent (King and MacRae, 2015). Glycogen storage can contribute to heat tolerance not only trough generating ATP and metabolic water, but also trough releasing bound water which can then be used for evaporative cooling (Gibbs et al., 1997). Higher glycogen content was recorded in flying females of ants (Keller and Passera, 1990), and a comparable pattern has been reported in bees (Neukirch, 1982). The first step to test how easily accessible energy in form of glycogen facilitates higher CT_{max} would be to measure glycogen content in fasted ants and ants provided with sucrose. We predict that ants capable of raising their CT_{max} will contain a higher concentration of glycogen, and that CT_{max} would not increase in the same way in ants fed protein instead of carbohydrates.

Trophic position and CT_{max}

Azteca is typically considered an herbivore and the most N-limited Neotropical ant genus (Davidson, 2005). We found *A. chartifex* to be omnivorous, a result that did not vary between fertilization treatments (Fig. 3). A dominant Paleotropical canopy ant, *Oecophylla smaragdina* which also relies on honeydew secretions was found to have gasters with lower $\delta^{13}C$ and $\delta^{15}N$ than the remaining body (Blüthgen et al., 2003; Feldhaar et al., 2009). In this particular case, when short-term dietary choices are changing a physiological trait, to accurately record differences in $\delta^{13}C$ and $\delta^{15}N$ and C/N ratio prior and after ingestion of the last meal, only gasters of starved and sucrose fed ants should be analyzed.

Thermal plasticity was not the result of an ant's relative trophic position but was affected by a quickly metabolized energy source. Both field-caught ants and lab-fed ants altered their thermal tolerances depending on the ingested food. If sucrose availability increases ant CT_{max}, then ants from control plots with higher CT_{max} either have more available carbohydrates, or those colonies have higher carbohydrate requirements compared to ants on P plots. Differences in carbohydrate content can be hard to detect by measuring elemental ratios (Anderson et al., 2004), thus field differences in carbohydrate availability remain a likely explanation for observed differences in CT_{max} of ants from control and P plots. In general, fertilization increases plant biomass (Gruner et al., 2008) and plant nutrient content (Campo and Vazquez-Yanes, 2004; Fox and Morrow, 1992), which can then lead to increases in herbivory (Gruner et al., 2008; Santiago et al., 2012) and predation (Gruner, 2004; Gruner and Taylor, 2006). Common eudicot tree species across P plots have increased foliar P concentration, resulting in decrease of their N:P ratio (Mayor et al., 2009), which suggests that these plants likely have reduced C:P ratio. This difference compared to control plots could result in lower production of EFN and reduced carbohydrate availability for ants. Testing bait preference across the plots—providing ants with a choice of sugar and protein baits—would reveal if ants on P plots have limited protein or carbohydrate availability.

Worker stoichiometry is consistent across fertilizations

Elemental body chemistry of workers did not vary across focal nutrient treatments. Unlike lab studies, which have shown a direct or indirect effect of diet on stoichiometry (Cease et al., 2016; Kay et al., 2006; Perkins et al., 2004; Schade et al., 2003; Woods et al., 2002) we found no relationship between plot fertilization and worker elemental body composition. Perhaps, our result is not surprising as *A. chartifex* colonies can have large territories, which may be greater than 20 m in diameter (Adams, 1994). Depending on the nest position in these 40 x 40-m plots, the territory of *Azteca* could span a fertilization treatment as well as the buffer zone between plots. Thus, in a forest, where ants are exposed to a heterogeneous environment, they are averaging across a plethora of available resources. Consistent body stoichiometry across fertilizations suggests *A. chartifex* colonies are successful at reaching their intake target (Dussutour and Simpson 2008). The potential consumer level differences caused by fertilizations might be noticeable at the colony size and/or reproductive output, instead of alterations in elemental chemistry or trophic position.

Conclusions

Carbohydrates can increase foraging activity, aggression (Grover et al. 2007; Kay et al. 2010), and, in the case of *A. chartifex*, CT_{max}. Here we examined the plasticity of critical thermal maxima in field collected and lab fed ants. Field collected ants from control plots had 2° C higher CT_{max} compared to ants from P fertilizations, likely due to differences in carbohydrate availability. We found short-term variability in CT_{max} of *A. chartifex* which can be raised by 5° C when fed a carbohydrate-rich diet. This ability has not been previously explored in ants, and as such our results demonstrate the importance of insect nutrition in shaping functional traits associated with physiology. Our study species is dominant, fast to recruit at baits, and aggressive in defending them. Thermal plasticity could be one of the traits that enables this canopy species to outcompete other ants. These findings suggest that ants that feed preferentially on carbohydrates have the ability to broaden their thermal niche and withstand elevated ambient temperatures. Importantly it may be that insects whose diet is chiefly carbohydrate based are the ones most likely to thrive in a rapidly warming world.

Acknowledgements

Comments of Katie Marshall, Michael Patten, Karl Roeder, and Michael D. Weiser greatly improved the early versions of the manuscript. Oris Acevedo and Belkys Jimenez of Smithsonian Topical Research Institute provided logistical support in Panama. The experiments in this study comply with current laws of the Republic of Panama. This study was funded by the Biology Department (OU), the Graduate Student Senate (OU), and Frankopan Fund Fellowship awarded to JB, and National Science Foundation Macrosystem Grant EF-1065844 awarded to MK.

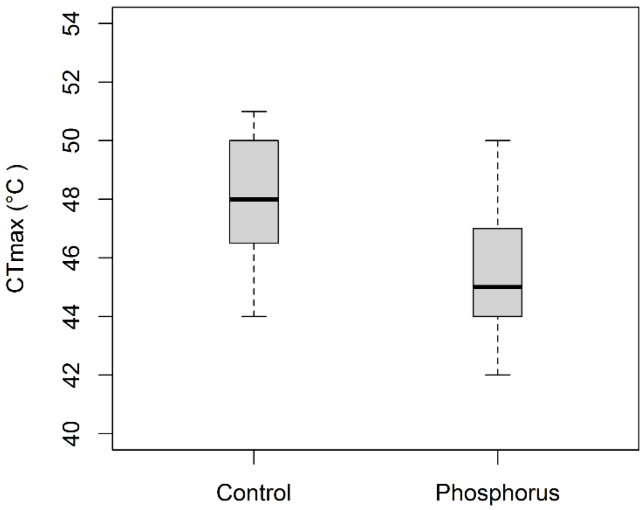
Chapter 3 – figure legends

Figure 9. Difference in CT_{max} between *Azteca chartifex* workers from control (CTR) and phosphorus (P) fertilization plots. We tested 12 colonies from each treatment.

Figure 10. Critical thermal maximum (CT_{max}) of each *Azteca chartifex* colonies tested after provided only with water and after being fed 10% sucrose solution. CT_{max} was significantly higher after ants received the sucrose diet. Colonies from control plots (black) are connected with a solid line; from P plots (gray) connected with a dashed line.

Figure 11. Trophic position of *Azteca chartifex* workers in δ-space. Each dashed line marks the beginning of the next trophic level. The first dashed line delimits the baseline of our food web with the $\delta^{15}N$ of autotrophs (white). Workers of *A. chartifex* from different plots are shown in black (control) and orange (P), and *Pachycondyla harpax* is shown in gray. Error bars represent standard error of the mean.

Figure 9.



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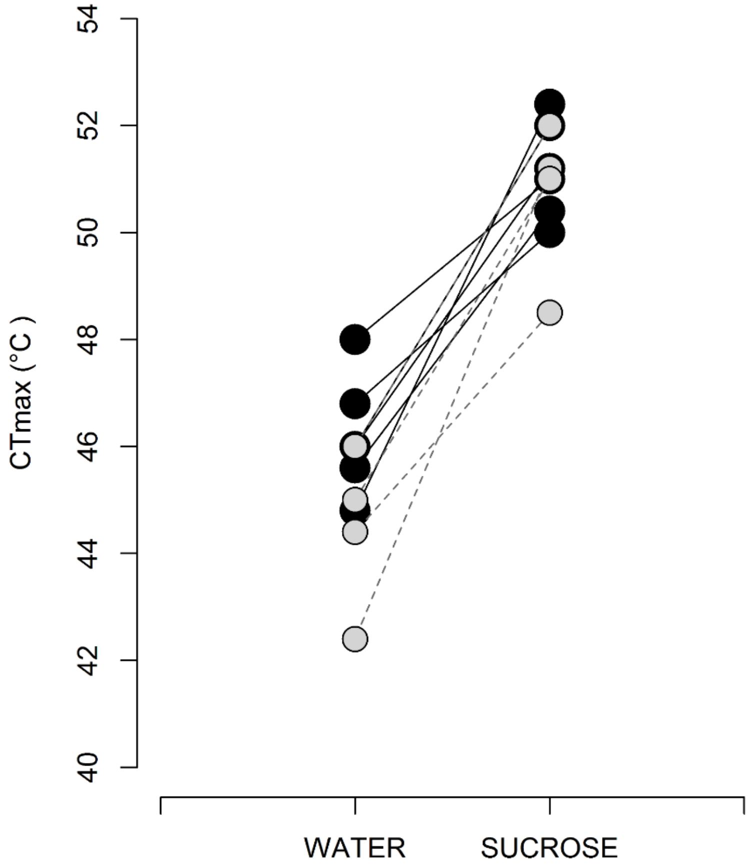

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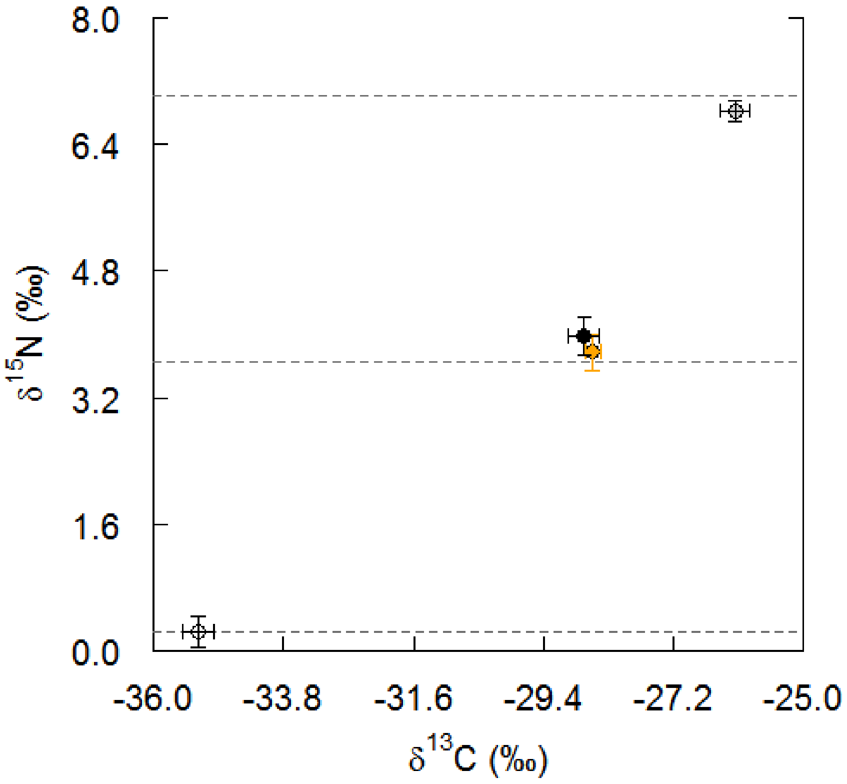

CHAPTER 4: Biogeochemistry and forest composition shape nesting preference of a dominant canopy ant

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Abstract

Biogeochemistry across the globe is altered by human activities, however we know little about how these alterations affect activity and distributions of higher level consumers. We tested how chronic fertilization of a tropical lowland forest, governs nesting patterns of a numerically and behaviorally dominant ant *Azteca chartifex*. We used a fertilization experiment in a Panamanian forest where nitrogen (N), phosphorus (P), and potassium (K) were added in a factorial design for almost two decades. We test the nutrient limitation hypothesis which predicts that previously recorded increase in foraging activity of *A. chartifex* on P plots is a result of higher nest abundance. In contrast, fertilization with N—a nutrient plentiful across this forest—is predicted to reduce the number of nests. We found partial support for our hypothesis, as plots which received N had 48% lower nest abundance. Nest size followed the same pattern. Adding P did not affect colony abundance, however the addition of both N and P significantly increased number of *A. chartifex* nests. The best predictor of colony size, was tree size, as larger trees supported larger nests. Tree size accounted for 45% of variation in nest height, and larger trees contained higher nests.

Tree specificity hypothesis predicts that *A. chartifex* preferentially nests on trees rich in extrafloral nectaries, honeydew secretions and lianas. *Azteca chartifex* nests were indeed non-randomly distributed, but trees with extrafloral nectaries were not preferred. Despite their low frequency across the forest certain tree species were selected, while some abundant species (e.g. palms) were avoided. Lastly we examined *A. chartifex*

recruitment and abundance at baits and found that within 5 m from the host tree nest height was the best predictor of both. This suggests that colonies placed lower at plots such as N and PK rely more on the understory resources, while others, e.g. NP plots are foraging almost exclusively in the canopy. Our study suggests that both nutrient availability and forest composition act in concert to govern *A. chartifex* nesting patterns, which then enable this dominant ant to outcompete other ants by fast colonization of the resources.

Introduction

Little is known about how biogeochemistry affects community structure and relative abundance of herbivores in the world's most diverse ecosystems, the tropics (Campo and Dirzo, 2003; Gruner and Taylor, 2006). Nitrogen (N) and phosphorus (P) are building blocks of macromolecules and thus are required in substantial quantities, yet N and P are scarce in most foods consumed by herbivorous insects, and foliage typically averages N and P concentrations 5–10 fold lower than in herbivore tissue (Elser et al., 2000). In tropical lowland rainforests, plants grow on old, weathered soils that typically are P limited (Alvarez-Clare et al., 2013; Vitousek and Sanford, 1986), so it may be that nutrient limitation of producers negatively affects consumers (Power, 1992).

Ants are important terrestrial consumers (Hölldobler and Wilson 1990) with diets that range from strictly herbivorous to predatory; hence, they experience ranges of low NP to high NP food. A colony’s long life-span and semi-sessile lifestyle (Gordon, 1992; Keeler, 1988) allow ants, like plants, to react and respond to long-term environmental change. For example, chronic P addition increased ant activity and decreased genus level diversity in a tropical moist forest in Panama (Bujan et al., 2016). This community-level response was driven largely by the most abundant species in genus *Azteca*—*Azteca* cf. *chartifex*. Furthermore, in this same tropical forest, chronic N addition decreased arthropod abundance and richness (Kaspari et al. in review). We explored mechanisms behind the numerical and behavioral dominance of *A. chartifex* in

Neotropical lowlands via three non-mutually exclusive hypotheses, two regarding the effect of biogeochemistry and one regarding forest composition.

We used a long-term fertilization experiment on the Gigante Fertilization Plots (GFP) in Panama where fertilization increased N, P, and potassium (K) concentrations in the soil (Wright et al., 2011), seedlings (Santiago et al., 2012), and abscised leaves (Kaspari et al., 2008). Fertilization with P and K also increased rates of leaf herbivory (Santiago et al., 2012). Plants from P and K plots offer either more palatable plant resources, or those plants are defended less (Coley et al., 1985). Moreover, these results suggest that P and K, but not N, limit nutrient turnover via decomposition (Kaspari et al., 2008) and govern herbivory patterns (Santiago et al., 2012). Generally, increase in food quality enhances ant colony size and the production of the reproductive caste (Aron et al., 2001; Kay et al., 2010; Portha et al., 2002). Thus, the nutrient limitation hypothesis posits that if higher ant activity in an area is caused by higher colony density and reproductive investment, then nest density or nest size should increase on +P plots. In contrast, by adding a plentiful nutrient, such as N, and further saturating the system, we predict depressed nest density and size as a result of eutrophication.

Resource availability can govern not only nest density, but also foraging patterns of canopy ants, as they are more likely to forage in the understory during long droughts, when resource availability in the canopy is low (Hahn and Wheeler, 2002). Numerical dominance and bait recruitment may foster territoriality by ant species and in turn lead to resource monopolization (Blüthgen and Fiedler, 2004). Thus, the resource dominance hypothesis predicts that if +N plots contain lower resource availability in the canopy, ant recruitment and numerical dominance at understory baits will be higher. Furthermore, resource-deprived ants are predicted to recruit to larger distances from the nest, so colonies from N plots are predicted to have wider foraging ranges. We predict the opposite for colonies found on +P plots.

Responses to fertilization treatments may be affected by ant behavior, in that many *Azteca* species engage in ant-plant mutualisms where a plant, for example *Cecropia* spp., provides nesting sites and produce food rich in sugar and lipids for their ant inhabitants, and in return ants defend the plants from herbivores and vines (Agrawal and Dubin-Thaler, 1999; Ferguson et al., 1995; Janzen, 1973; Perfecto and Vandermeer, 2006). *Azteca* species that do not engage these strong mutualisms, such as *Azteca chartifex*, still rely heavily on secretions from extra floral nectaries (EFN) and honeydew (Blüthgen et al., 2000). Because polydomous species of ants—species with colonies whose nests are spatially dispersed—form their nests close to a stable resource (Holway and Case, 2000; Lanan et al., 2011), we predict *Azteca* will preferentially nest on trees that provide more food either in form of EFN or honeydew. Additionally, because lianas are used as ant walkways (Clay et al., 2010), we predict that trees linked by lianas to other canopies will host more nests of behaviorally dominant and territorial *Azteca* (Adams, 1990). Likewise, we predict that *Azteca* will avoid nesting on resource-poor trees or trees disconnected from the rest of the forest (e.g., low liana load).

To test how biogeochemistry and tree composition act in concert to shape the mosaic of *Azteca* nests and activity in the lowland tropics we used a long-term fertilization experiment in Panama. By affecting the most dominant canopy consumer we might be altering the strength of trophic interactions across the forest, which is why it is important to understand the effect of biogeochemical makeup of the forest on its' consumers. These are not only affecting ant community structure, but are likely to ramify through the food web affecting both higher trophic levels, e.g. predation by large mammals, or lower trophic levels by altering herbivore abundance. Focusing solely on the effects of resource quality and availability on plant productivity and diversity is not enough to fully understand ecosystem responses to nutrient alterations.

Materials and Methods

We conducted *Azteca* cf. *chartifex* nest census in a lowland moist forest We conducted *Azteca* cf. *chartifex* nest census in a lowland moist forest of Gigante Fertilization Experiment (GFP: 9°06'31''N, 79°50'37''W) within Barro Colorado Nature Monument, Republic of Panama. During July 2015 we recorded each nest across 32 plots (40 × 40 m) fertilized with N, P and K in a factorial design since 1998. Each of the eight treatments (+N, +P, +K, +NK, +NP, +PK, +NPK) is replicated 4 times, including the controls.

In Panamanian forests occur two species groups of *Azteca* which build pendulous carton nest of chewed plant fiber: *Azteca* cf. *chartifex* and *Azteca* cf. *trigona*. When these two complexes occur in sympatry their workers are difficult to distinguish (Longino, 2007). Thus, we used barcode analysis of the workers and found that our colonies belong to *Azteca chartifex* complex (D. Donoso *in prep*) henceforth *A. chartifex*.

Nest density

To test the differences in nest density across fertilization treatments we visually inspected the canopy of each plot for the presence of *A. chartifex* nests. If the canopy was particularly dense we used binoculars. We started at the south edge of the plot, and walked in a straight line to the north edge stopping every 5 meters to inspect the canopy above. After reaching the plot edge we moved 5 meters east and applied the same procedure walking southwards. In total we had 81 inspection points at each plot. When we located a nest, we marked its' location with a GPS (Garmin GPSMAP 64s) and assigned a unique identifying number. We used the total number of nests recorded at each plot as our response variable. We first analyzed nest density data using generalized linear models (GLM) with N, P, K, their interactions, and replicate as fixed factors. Each of the 4 replicates includes all 8 treatments, and accounts for spatial variation in soils (Yavitt et al. 2009) and tree composition associated with a slight topographic gradient (SJW, unpublished data).

Nest size

We measured nest length and height using a Laser Distance Meter (Leica Disto D5) affixed on a tripod. We recorded three distances (Fig. 1A): first, to the top of the nest (a), bottom of the nest (b), and the distance to the ground (d). We also recorded two angles: one between the nest top and bottom (α), and the other between the nest top the ground (β). This allowed us to calculate nest length (l) and the height of the nest in the canopy (h). Lastly, we took a nest photo at the same location where all the measurements were taken.

We calculated the nest length and height from our nest measurements (Fig. 1A), as the side in an irregular triangle: $l^2 = a^2 + b^2 - 2a \times b \times \cos(\alpha)$. Using the photo of the nest and Image J software, we measured the nest length in pixels which allowed us to calculate the diameter of the nest and, to calculate the approximate nest volume. We sorted the nests in 3 shape categories: prolate spheroid (Fig. 1B), cone (Fig. 1C), cylinder (Fig. 1D) and we used appropriate formulae to calculate the volumes of these shapes (Hillebrand et al., 1999; Sun and Liu, 2003).

Recruitment and dominance at baits

To study *Azteca chartifex* recruitment and dominance at baits we focused on 4 treatment plots: CTR, N, P. We selected N as it was found to be important in depressing invertebrate abundance and diversity (Kaspari et al. in review), while P was found to increase *A. chartifex* activity (Bujan et al., 2016). To test if *A. chartifex* recruitment is affected by the vicinity of the colony as well as colony’s size and height, we used a set of 15 baits. We started our baiting transect with the tree hosting the nest, and baited understory vegetation every 0.5 - 1 m at the eye level. We choose the direction of the densest vegetation to set up the transect, as these ants are known to use lianas as walkways (Adams et al., 2016). We used mixed tuna with honey as our bait. We recorded number of ants using a base 2 logarithmic scale (i.e. 1, 2, 4, 8, 16, 32, …), and the number of ants present at baits after 5 min (recruitment) and 1 h (dominance) with respect to the vicinity of the colony. We baited 12 colonies from control, and +P plots, and 7 colonies from +N treatments as the number of colonies was low on N plots. At the beginning and the end of our baiting trial we measured air temperature by aiming an IR thermometer (Fluke Corporation, Everett, WA; model 62) at eye level to a shaded spot > 10 m away.

We examined recruitment rate and numerical dominance at baits placed at 3 different distances from the nest: 1) on the host tree bait, 2) all the baits within 5 m (sum of ant abundance at baits 1-5) and 3) between 6-10 m (sum of ant abundance at baits 6-10 m) from the host tree. For both recruitment and dominance at baits we used full ANCOVA models using nutrient treatments: N, P, average temperature, nest characteristics (height and volume), and plot identity to control for spatial variation.

Tree specificity

We recorded the size of the plant hosting the nest and the type (tree or liana). If the tree was >10 cm in DBH then we used the identifications and measurements from the 2013 tree census from the plots (Wright et al., 2011). To test for tree nesting preference of *Azteca* we first calculated the actual frequency of each tree species across all plots, and then we compared those with the observed nest frequencies using a Chi-square test, a frequently used approach in testing nesting preferences (Thomas and Taylor, 1990). All analyses were performed in R version 3.3.2 (R Core Team 2016).

Results

Nutrient limitation hypothesis: effect of fertilization on nest density

We recorded the lowest number of colonies on N plots (Fig 12., 2.5 ± 1.04) while the colonies were on average most abundant on control plots (11.0 ± 2.04). Overall N addition lowered nest density compared to the plots which did not receive N (Table1).

Three GLM had the lowest AIC values (within 2 AIC differences) and were used to analyze the effects of nutrients on *Azteca* colony density (Table 1). All together these three models would be expected to be optimal models in 75% of cases (AIC Weights, Table 1). Nest density was on average 48% lower on +N plots (i.e. +N, +NK, +NP, +NPK; n = 16; 4.3 ± 0.9; Fig. 13, Table 1) compared to the plots which didn't receive N (i.e. CTR, +P, +K, +PK; n =16; 8.2 ± 0.8). However, number of colonies was significantly higher when N was added together with P (i.e. +NP, +NPK; Table 1; n = 8; 6.0 ± 1.25).

Nutrient limitation hypothesis: effect of fertilization on nest size

Overall nest length ranged from 0.21 - 2.61 m (mean ± SE: 0.93 ± 0.04 m), and nest volume ranged from 0.5 - 289.0 L (mean ± SE: 54.3 ± 3.9 L). The optimal models explaining variation in nest size contained DBH and replicate, while N was the only nutrient which contributed to these two top models (Table 2). Larger trees, and trees located in the northern, less palmy part of GFP on average contained larger nests (Fig. 14).

Azteca *recruitment rate and dominance*

There was no significant difference in ant recruitment at baits located on nest tree across treatments. Same was true for baits which were 5 - 10 m away from the nest tree, while within 5 m closest to the host tree ants were faster at recruiting if their nest was lower in the canopy, abundance at baits was positively correlated with nest height (ANCOVA: F = 5.3, p = 0.03). Same was true when testing the dominance of ants at baits, after one hour, as the best predictor of abundance at baits within 5 m was the nest height in the canopy (ANCOVA: $F = 26.6$, $p = 0.006$). Recruitment rate or dominance were not significantly affected by the two nutrients treatments examined (N and P), nest volume, plot they were collected at or the temperature at which ants were foraging.

Are nesting sites tree specific?

We recorded a total of 249 tree species across the fertilization plots, and *Azteca* nested on 41 species (17%). We found a non-random distribution of *Azteca chartifex* nests on tree species (χ^2 (1) = 2437.8, $p < 0.001$). The highest number of nests was recorded on *Heisteria cocinea* (12%) and *Tetragastris panamensis* (11%). Certain tree species were preferred as nest sites despite their low frequency across the plots: *Prioria copaifera*, *Drypetes standleyi*, and *Trattinnickia guianense*. Others, such as palm *Oenocarpus mapora*, was avoided despite being the third most abundant tree across fertilization plots, and only 1% of nests were recorded in its canopy.

Among the total species pool *Azteca* showed no preference for choosing trees with EFN for its nesting sites. In fact, when we examine tree species which hosted ≥5% of the nests we find that none of these species contains EFN, and together they hold 43% of the nests. And while the EFN by themselves are not a guarantee to attract *Azteca*, we found that more nests are placed on large trees with EFN (Table A13).

Tree size (DBH) was strongly positively correlated with nest height, as nests were placed higher in the canopy of larger trees (Fig. A10; Height = 0.02DBH + 6.4, $F_{1, 163} = 132.2$, $R^2 = 0.45$, $p < 0.001$). Nests were found between 1.8 – 29.6 m in the canopy (mean ± SE: 10.47 ± 0.38 m). Therefore, when analyzing effect of nutrients on nest height, we controlled for DBH. Nests were the highest in the canopies of trees on plots receiving +NP fertilization (Table A14; 12.33 ± 0.95 m) and +K (Table A14; 11.2 m), while they were placed low at +PK plots (p = 0.02; 10.5 ± 0.82 m). Plots receiving only +N addition were on average placed the lowest of all fertilization additions as nests were on average at only (8.8 ± 1.34). These 4 nutrient additions NP, PK, K and N, together with tree size are the best at explaining nest height in the canopy.

Discussion

Biogeochemistry can impact consumers by altering their food quality and quantity, Biogeochemistry can impact consumers by altering their food quality and quantity, resulting in increase in abundance of herbivores and predators (Gruner, 2004; Haddad et al., 2000). Here we use a large-scale fertilization experiment to explore biogeochemical effects on the nest density, nest size, and foraging activity of a dominant ant, *Azteca chartifex*. Only nest density on these N, P K factorial fertilization plots changed, but there was no evidence for enhanced number of nests with any treatment. Instead, N additions decreased nest density for 48%. This difference is more striking when only N addition and control plots are compared, as +N plots have 4 times lower number of nests than controls. In contrast, colony size and activity were invariant across the biogeochemical template. We are the first to test the nesting preferences of *A. chartifex*, and found that despite of low occurrence of some tree species across the forest they are preferred nest sites, while others are avoided such as the most abundant palm. Our study suggests that *A. chartifex* nesting preferences are driven by nutrient availability and forest composition acting in concert and helping *Azteca's* dominance in this forest.

Nutrient limitation hypothesis

Across fertilization treatments there was a clear pattern of lower colonization on +N Across our fertilization treatments there was a clear pattern of lower colonization on +N plots likely caused by lower resource availability on those plots. Addition of N, in N-limited ecosystems, increases the number of phloem feeders, which results in an increase of ants tending them (Strauss, 1987). This tropical N-rich forest harbors low arthropod abundance and richness on +N plots (Kaspari et al. in review). Thus, lack of arthropod prey, and consequently honeydew, could be the reason why N plots are avoided by *A. chartifex*, in favor of more resource rich parts of the forest. Additionally, nests on N plots were placed lower in the canopy, further supporting the notion that those canopies are poor in resources. As polydomous species are known to construct their nests close to a stable resource (Lanan et al., 2011), *A. chartifex* nest placement suggests these ants, on N plots, are more dependent of the understory resources. *Azteca* ants which engage in mutualisms defend their host tree from herbivores (Jutsum et al., 1981; Oliveira et al., 2015), and in return they feed on plant secretions. Although these *Azteca* are not mutualistic, plants on N plots might not be able to provide enough secretions to support their ant bodyguards. Furthermore, if herbivory in the canopy is lower on N plots, same as in the understory (Santiago et al., 2012), resources in form of herbivores and their secretions are also less abundant. Therefore, the sole addition of N is likely saturating the system, and maybe even becoming toxic for plants which might be one of the reasons for such stark reduction in nest density.

In contrast, we found an increase in nest density on +NP. This forest experiences a co-limitation with N and P (Kaspari et al., 2008), as addition of +NP in this forest increased seedling height (Santiago 2012), litterfall and decomposition (Kaspari et al. 2008). This dominant consumer is thus preferentially choosing plots with faster nutrient turnover, following the limitation of the system. Average nest size was also lower on N plots, a pattern clearly driven by lower number of nests across this fertilization. Majority of the nests recorded are up to 50 L in size. Larger sizes might be selected against, as large nests could be more instable, or more likely under tamandua predation which reduces numbers of *Azteca* nest sizes in these forests (Hirsch et al., 2014).

Recruitment and Dominance

Azteca recruitment is not affected by the two single addition nutrients N and P which is likely due to the territoriality of *Azteca* which places guardian workers across its territory (Adams 1990). Here we predicted that higher ant abundance at baits on P plots (Bujan et al. 2016b) could be driven by colony level requirements – more resources needed so ants will: 1) recruit to the baits faster; 2) forage further away from the colony, and 3) dominate the resources (higher abundance). What we found is that the nest height is the driver of ant activity near the colony. Thus, phosphorus plots might have higher activity across the baits because they are overall lower than other treatments, and contains more nests compared to nitrogen which hosts the lowest nests.

Nesting sites are tree specific

We found no preference toward species bearing EFN species. This is likely because tree species from genera that produce EFN such as *Inga* (Bixenmann et al. 2011) are abundant across the forest, but will never host *A. chartifex* nests, due to their small size. Inga was the most specious genus found across the plots represented with 9 species, all of which contain EFN (Croat 1978, Goitia and Jaffe 2009, Bixenmann et al. 2011) and none of which hosted a nest. Three tree species were preferred by *A. chartifex* despite their low frequency: *Prioria copaifera*, *Drypetes standleyi*, and *Trattinnickia guianense*. None of these species are recorded to produce extra floral nectar in Panama (Schupp and Feener 1991), so these trees are not actively attracting the ants. However, all are particularly high canopy trees with average heights 28 - 30 m (Wright, unpublished data). This is likely the reason why are they being preferred as nesting sites, regardless of their low frequency in the forest. Other species, such as palm *Oenocarpus mapora* are being avoided. Palms in this forest are generally lower trees with small canopy and weak wood, and consequently they are rarely colonized by lianas. *Azteca*, and other canopy ants, rely on resources in the canopy of the host tree, and they use lianas walkways (Adams et al., 2016) for access to other canopies, and as a source of EFNs. So, palm canopies could be too isolated, and not large enough to provide sufficient resources for *A. chartifex* colonies. We observed only 2 colonies (3 nests) on palms, and they were generally smaller nests (mean ± SE = 14.8 ± 10.1 L). Some species in the genus *Azteca* nest in palms, for example, coconut palm plantations, but these species don't build large pendulous carton nests and instead they chose the basis of spathes for their nest placement (Way and Bolton 1997). We found that larger trees had nests much higher, and those canopies could support larger nests. One of the reasons for this pattern is the heavy reliance on the resources in the crown such as sugar secretions provided by the tree itself, and honeydew secretions of Hemiptera. Thus, it is less energy demanding to place a nest closer to the crown resources, than placing them always at a certain height and then forage further up.

Caveats

We were unable to measure smallest nests not measured (usually smaller than 1 L), however these were usually satellite nests around the main nest. Addition of these smaller nests is unlikely to significantly change the colony size. Due to the logistical constraints, it would be almost impossible to measure plant secretions across the canopy of the fertilization plots and the abundance of phloem feeders. Thus, we did not directly test for the differences in resource availability across the plots. Targeted canopy fogging would be one of the possible ways to answer if the abundance of Hemiptera increased with fertilization, and how this affects *A. chartifex* nesting and foraging patterns.

Conclusions

To our knowledge this is the first study addressing the effect of biogeochemistry and forest composition on nesting patterns of non-mutualistic *Azteca*. We show that the addition of N, in a N rich ecosystem, halves the number of *A. chartifex* nests. This suggests that biogeochemistry impacts habitat usage of a dominant canopy consumer, and it remains to be tested if this bottom up effect scales up to other arthropods. We also found that nest selection is non-random, but *A. chartifex* prefers tall canopy trees and its' presence will likely shape canopy arthropod communities in those trees. Nest density *of A. chartifex* and nest position in the canopy likely have ramifying effects in both canopy and understory by altering herbivore abundance. Additionally, predation patterns might change with altered biogeochemistry, as *A. chartifex* larvae are common food for tamanduas, so they might be targeting areas with high nest densities. Because of the effects of biogeochemistry on nesting patterns and foraging activity on dominant consumers such as *A. chartifex*, to fully understand ecosystem responses to nutrient alterations we need more studies focusing on response of higher level consumers. Anthropogenic alterations of nutrient availability will likely affect consumer communities across the globe, and we just begun exploring these effects.

Acknowledgements

Comments of Michael Patten greatly improved the early versions of the manuscript. Oris Acevedo and Belkys Jimenez of Smithsonian Topical Research Institute provided logistical support in Panama. This study was funded by the Biology Department (OU), the Graduate Student Senate (OU), and Frankopan Fund Fellowship awarded to JB, and National Science Foundation Macrosystem Grant EF-1065844 awarded to MK. The experiments in this study comply with current laws of the Republic of Panama.

Chapter 4 – figure legends

Figure 12. Diagram of measuring *Azteca chartifex* nest height and length (A). We measured three distances: (a) to the top of the nest, (b) to the bottom of the nest, and (d) the distance to the ground. We measured two angles: (α) between the nest top and bottom, and (β) between the nest top the ground. This allowed us to calculate nest length (l) and nest height in the canopy (h). Nest images are examples of three shape categories we used: prolate spheroid (B), cone (C), and cylinder (D).

Figure 13. *Azteca chartifex* nest abundance across control plots (CTR) and plots that received nitrogen addition +N (+N, +NK, +NP, +NPK); phosphorus addition +P (+P, +PK, +NP, +NPK), and both nitrogen and phosphorus addition +NP (+NP, +NPK).

Figure 14. Nest size across different parts of the forest, on trees of different diameter at breast height (DBH) across the plots receiving N addition (blue circles), and without receiving N (orange circles). Circle size corresponds to the nest volume, nest size estimate. A categorical variable replicate (1-4) accounted for differences in topography and vegetation composition. Maximum difference was 36 m, as there is a gentle slope going from north-east corner of the plots, also less palm covered (4) to lower, more palm-rich plots in the south-west (1).

Figure 12.

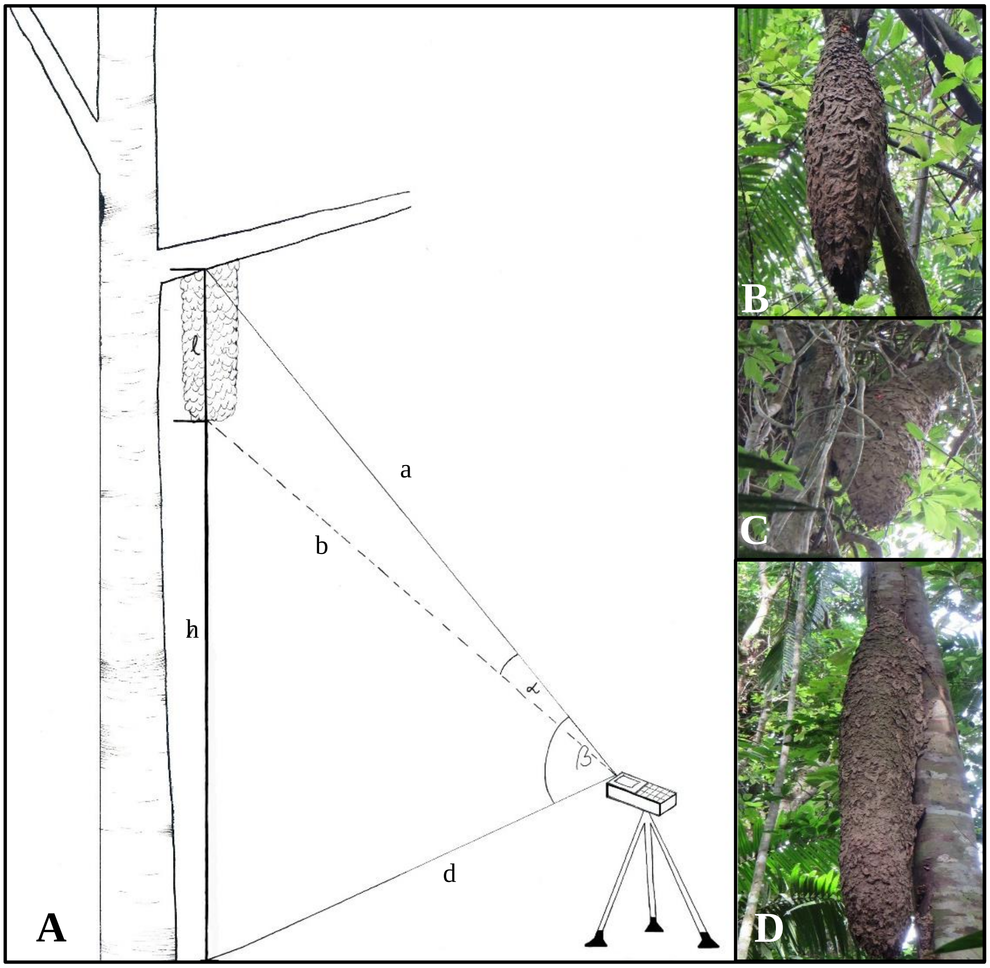

Figure 13.

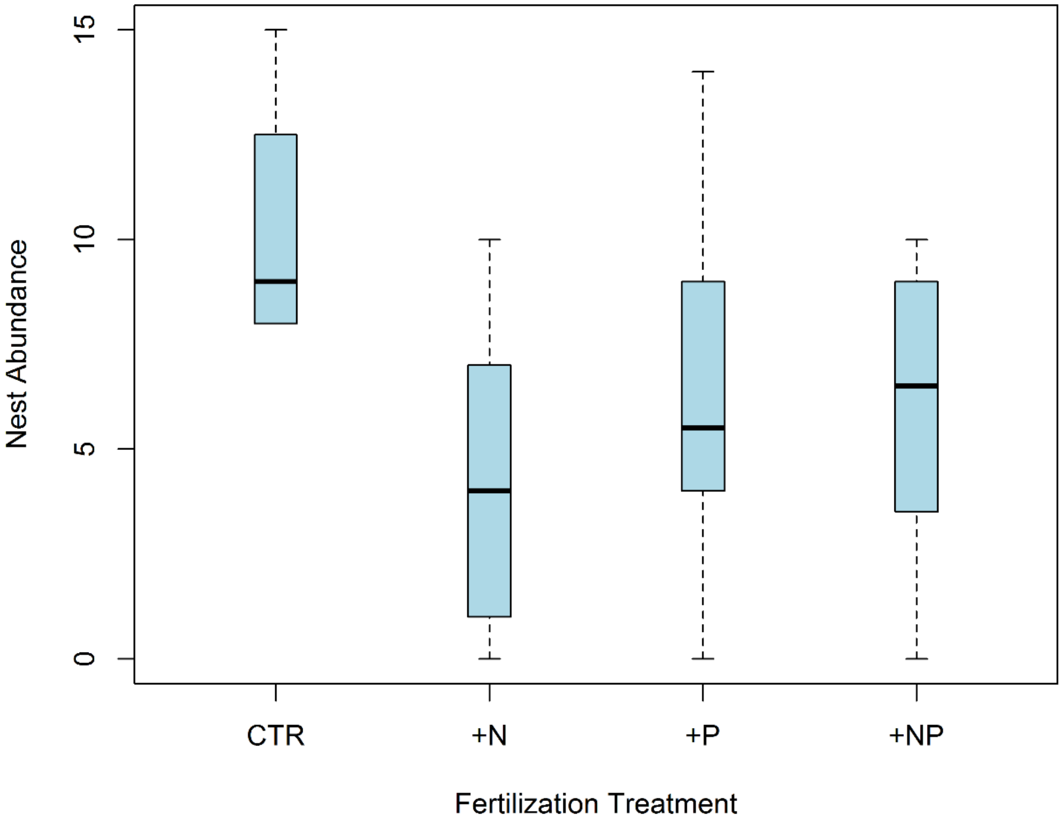

Figure 14.

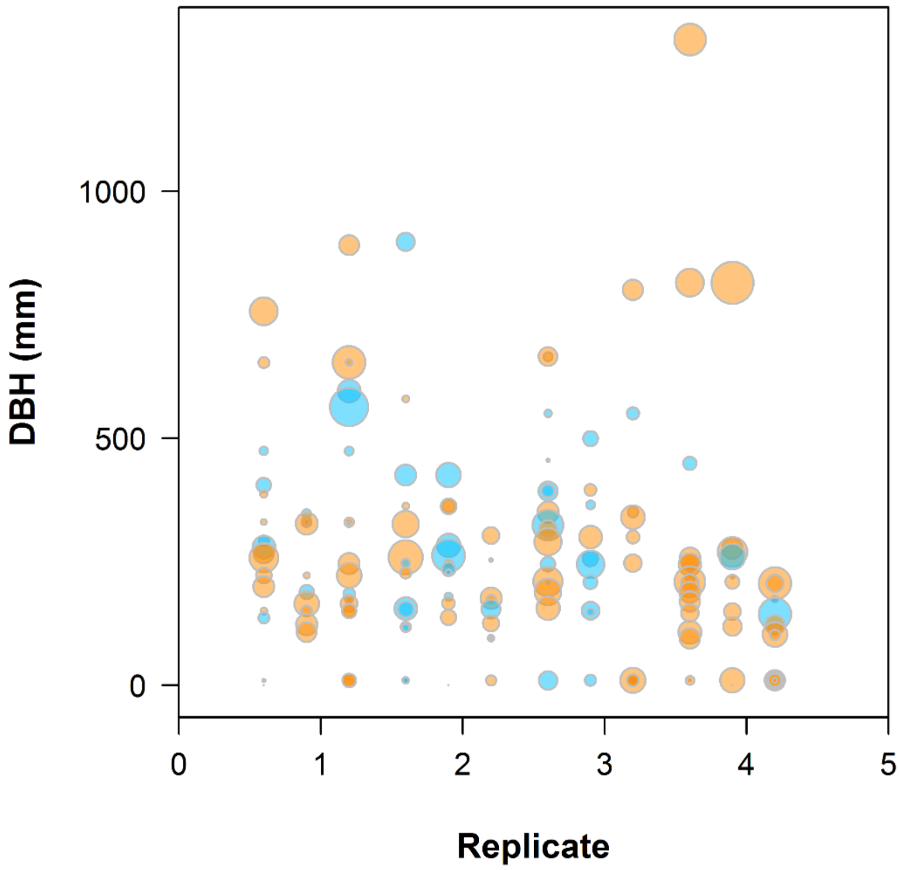

Table 1.

Generalized linear models used to test nest abundance across fertilization plots. For each model degrees of freedom, AIC values, ΔAIC (difference between the *i*th model and the model with the lowest AIC), and AIC weights are listed. Model terms include all the terms present in a particular model. Bold are models within 2 AIC from the optimal model with the lowest AIC value. Treatments in italic indicate significance $p < 0.05$.

MODELterms	DF	AIC	ΔAIC	AIC Weights
N, NP	**4**	**170.047**	**0**	**0.371**
***N, NP*, REPL**	**5**	**170.886**	**0.839**	**0.244**
***N*, P, *NP*, REPL**	**6**	**172.028**	**1.981**	**0.138**
N	3	172.942	2.895	0.087
N, P, *NP*, PK, REPL	7	173.302	3.254	0.073
N, P, K, *NP*, NK, PK, REPL	9	174.27	4.223	0.045
N, P, K, *NP*, PK, REPL	8	174.464	4.416	0.041
Null	2	180.76	10.712	0.002

Table 2.

Generalized linear models used to test variation in nest volume across the fertilization plots. For each model degrees of freedom, AIC values, ΔAIC (difference between the *i*th model and the model with the lowest AIC), and AIC weights are listed. Model terms include all the terms present in a particular model. Bold are models within 2AIC from the optimal model with the lowest AIC value. Treatments in italic indicate significance $p < 0.05$.

MODELterms	DF	AIC	ΔAIC	AIC Weights
REPL, DBH	**4**	**1683.758**	**0**	**0.458**
N, *REPL, DBH*	**5**	**1684.679**	**0.92**	**0.289**
N, NP, REPL, DBH	6	1686.574	2.816	0.112
N, P, NP, REPL, DBH	7	1688.351	4.592	0.046
N, P, K, NP, NK, PK, REPL, DBH	10	1689.309	5.551	0.029
N, P, NP, PK, REPL, DBH	8	1689.391	5.633	0.027
N, P, K, NP, PK, REPL, DBH	9	1689.542	5.784	0.025
DBH	3	1690.986	7.228	0.012
Null	2	1880.841	197.083	0

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Appendix

Chapter 1 - Supplemental Material

Table A1. Generalized linear mixed models fit by the Laplace approximation used to test the differences of total ant foraging activity. Fixed effects: N, P, K and temperature (T); random effect is block, an 8 level categorical variable. For each model degrees of freedom, AIC values, ΔAIC (difference between the *i*th model and the model with the lowest AIC), and AIC weights are listed. Model terms include all the terms present in a particular model. Bold are models within 2AIC from the optimal model with the lowest AIC value.

MODEL TERMS	Df	AIC	ΔAIC	AIC Weights
T, N, P, K, TN, TP, TK, NP, NK, PK, TNP, TNK, TPK	16	99.5	14.6	0.00
T, N, P, K, TP, TK, NP, NK, PK, TNP, TNK, TPK	15	97.5	12.6	0.00
T, P, K, TN, TP, TK, NP, NK, PK, TNP, TNK, TPK	15	97.5	12.6	0.00
T, P, K, TP, TK, NP, NK, PK, TNP, TNK, TPK	14	96.9	12.0	0.00
T, P, K TP, TK, NP, NK, PK, TNP, TNK	13	95.1	10.2	0.00
T, P, K, TP, TK, NP, NK, PK, TNP, TPK	13	96.8	11.9	0.00
T, P, K, TP, TK, NP, NK, PK, TNK, TPK	13	100.4	15.5	0.00
T, P, K, TP, TK, NP, NK, PK, TNP	12	94.9	9.9	0.00
T, P, K, TP, TK, NP, NK, PK, TNK	12	99.0	14.1	0.00
T, P, K, TP, TK, NP, NK, PK, TPK	12	98.5	13.6	0.00
T, P, K, TP, TK, NP, NK, PK	11	97.4	12.5	0.00
T, P, K, TP, TK, NP, NK	10	95.4	10.5	0.00
T, P, K, TP, TK, NP, PK	10	95.7	10.7	0.00
T, P, K, TP, TK, NK, PK	10	95.9	10.9	0.00
T, P, K, TP, TK, NK	9	93.9	8.9	0.00
T, P, K, TP, TK, PK	9	93.9	9.0	0.00
T, P, K, TP, TK, NP	9	93.7	8.8	0.00
T, P, K, TP, TK	8	91.9	7.0	0.01
T, P, K, TP	7	89.9	5.0	0.03
T, P, K, TK	7	89.9	5.0	0.03
T, P, K	6	87.9	3.0	0.08
T, P	5	86.0	1.1	0.21
T, K	5	89.9	5.0	0.03
P, K	**5**	**86.9**	**2.0**	**0.13**
T	4	88.0	3.1	0.08
P	**4**	**84.9**	**0.0**	**0.35**
K	4	89.5	4.6	0.04

Table A2 Generalized linear mixed models fit by the Laplace approximation used to test the differences of *Azteca* foraging activity. Fixed effects: N, P, K and temperature (T); random effect is block, an 8 level categorical variable. For each model degrees of freedom, AIC values, ΔAIC (difference between the *i*th model and the model with the lowest AIC), and AIC weights are listed. Bold are models within 2AIC from the optimal model with the lowest AIC value.

MODEL TERMS	Df	AIC	ΔAIC	AIC Weights
T, N, P, K, TN, TP, TK, NP, NK, PK, TNP, TNK, TPK	16	130.6	10.7	0.00
T, N, P, K, TP, TK, NP, NK, PK, TNP, TNK, TPK	15	128.8	8.9	0.00
T, P, K, TN, TP, TK, NP, NK, PK, TNP, TNK, TPK	15	128.8	8.9	0.00
T, P, K, TP, TK, NP, NK, PK, TNP, TNK, TPK	14	127.0	7.1	0.01
T, P, K, TP, TK, NP, NK, PK, TNP, TNK	13	127.5	7.6	0.01
T, P, K, TP, TK, NP, NK, PK, TNP, TPK	13	128.8	8.9	0.00
T, P, K, TP, TK, NP, NK, PK, TNK, TPK	13	130.2	10.3	0.00
T, P, K, TP, TK, NP, NK, PK, TNP	12	127.4	7.5	0.01
T, P, K, TP, TK, NP, NK, PK, TNK	12	128.6	8.6	0.00
T, P, K, TP, TK, NP, NK, PK, TPK	12	129.2	9.3	0.00
T, P, K, TP, TK, NP, NK, PK	11	127.3	7.4	0.01
T, P, K, TP, TK, NP, NK	10	125.4	5.4	0.02
T, P, K, TP, TK, NP, PK	10	127.0	7.1	0.01
T, P, K, TP, TK, NK, PK	10	126.6	6.7	0.01
T, P, K, TP, TK, NK	9	124.8	4.9	0.02
T, P, K, TP, TK, PK	9	125.3	5.4	0.02
T, P, K, TP, TK, NP	9	125.0	5.1	0.02
T, P, K, TP, TK	8	123.4	3.5	0.05
T, P, K, TP	7	122.6	2.7	0.07
T, P, K, TK	7	123.8	3.8	0.04
T, P, K	6	123.0	3.1	0.06
T, P	**5**	**121.0**	**1.1**	**0.15**
T, K	5	124.9	5.0	0.02
P, K	**5**	**121.7**	**1.8**	**0.11**
T	4	122.9	3.0	0.06
P	**4**	**119.9**	**0.0**	**0.27**
K	4	124.0	4.1	0.03

Table A3. Generalized linear mixed models fit by the Laplace approximation used to test the differences of *Dolichoderus* foraging activity. Fixed effects: N, P, K and temperature (T); random effect is block, an 8 level categorical variable. For each model degrees of freedom, AIC values, ΔAIC (difference between the *i*th model and the model with the lowest AIC), and AIC weights are listed. Model terms include all the terms present in a particular model. Bold are models within 2AIC from the optimal model with the lowest AIC value.

MODEL TERMS	Df	AIC	ΔAIC	AIC Weights
T, N, P, K, TN, TP, TK, NP, NK, PK, TNP, TNK, TPK	16	123.0	5.0	0.02
T, N, K, TN, TP, TK, NP, NK, PK, TNP, TNK, TPK	15	121.3	3.3	0.05
T, N, P, K, TN, TK, NP, NK, PK, TNP, TNK, TPK	15	121.3	3.3	0.05
T, N, K, TN, TK, NP, NK, PK, TNP, TNK, TPK	**14**	**119.4**	**1.4**	**0.12**
T, N, K, TN, TK, NP, NK, PK, TNK, TPK	13	121.5	3.5	0.04
T, N, K, TN, TK, NK, PK, TNP, TNK, TPK	13	121.5	3.5	0.04
T, N, K, TN, TK, NK, PK, TNK, TPK	**12**	**119.6**	**1.6**	**0.11**
T, N, K, TN, TK, NK, TNK, TPK	**11**	**119.9**	**1.9**	**0.09**
T, N, K, TN, TK, NK, PK, TNK	**11**	**119.9**	**1.9**	**0.09**
T, N, K, TN, TK, NK, TNK	**10**	**118.0**	**0.0**	**0.23**
T, N, K, TN, NK, TNK	9	120.8	2.8	0.06
T, N, TN, TK, NK, TNK	9	120.9	2.9	0.05
T, N, TN, NK, TNK	8	120.9	2.9	0.05
N, TN, NK, TNK	7	125.0	7.0	0.01
TN, NK, TNK	6	127.6	9.6	0.00
N, NK, TNK	6	127.6	9.6	0.00
NK, TNK	5	125.6	7.6	0.01
N, TN, TNK	6	131.5	13.5	0.00
N, TN, NK	6	131.4	13.4	0.00
TNK	4	127.5	9.5	0.00
NK	4	127.4	9.4	0.00
N	4	129.4	11.4	0.00

Table A4. List of ant species recorded across different fertilization treatments. Morphospecies are bracketed by "_".

SPECIES NAME	C	K	N	NK	NP	NPK	P	PK
Acromyrmex octospinosus	1	0	0	0	0	0	0	0
Acromyrmex volcanus	1	1	1	0	1	0	0	1
Apterostigma dentigerum	0	0	0	0	0	0	1	0
Azteca _sp.3_ (Azteca cf. instabilis)	1	1	1	1	1	1	1	1
Azteca brevis	0	0	0	1	0	0	1	1
Azteca cf charitfex	1	1	1	1	1	1	1	1
Azteca flavigaster	0	0	0	1	1	0	0	0
Azteca gnava	0	0	0	1	0	0	0	0
Azteca instabilis	0	0	0	0	1	0	0	0
Azteca nigra	0	0	0	1	0	0	0	0
Azteca pilosa	0	0	1	0	1	0	1	0
Azteca tondusi	0	0	0	0	1	0	0	0
Brachimyrmex longicornis	0	0	0	1	0	0	0	0
*Brachymyrmex _JTL007_*	0	0	1	1	0	1	0	1
Brachymyrmex coactus	0	0	0	0	1	0	0	0
Brachymyrmex heerii	0	0	0	0	0	0	0	1
*Brachymyrmwx _JTL002_*	0	0	0	1	0	0	0	1
*Camponotus _JTL056_*	0	0	0	0	1	0	0	0
Camponotus brevis	1	1	1	1	0	0	1	1
Camponotus cuneidorsus	0	0	0	1	0	0	0	0
Camponotus excisus	0	1	0	1	0	1	0	0
Camponotus linnaei	0	0	0	0	0	0	1	0
Camponotus nitidor	0	1	1	0	1	0	1	0
Camponotus novogranadensis	1	1	0	0	1	0	0	0
Camponotus sanctaefidei	0	0	0	0	0	1	0	0
Cephalotes atratus	1	1	1	0	1	1	1	0
Cephalotes basalis	0	0	0	0	0	0	0	1
Cephalotes minutus	0	0	0	0	0	0	1	0
Cephalotes umbraculatus	0	0	0	1	1	1	1	0
*Crematogaster _sp4_*	0	0	0	0	1	0	1	0
Crematogaster acuta	1	1	1	0	0	0	0	0
Crematogaster brasiliensis	1	0	0	0	0	0	1	0
Crematogaster carinata	1	1	1	1	1	1	1	1
Crematogaster flavosensitiva	0	0	0	0	1	0	0	0
Crematogaster limata	1	1	1	1	0	1	0	1
Crematogaster sumichrasti	0	0	1	0	0	0	0	0

Crematogaster tenuicula	1	1	1	1	1	1	1	1
Dolichoderus bispinosus	1	1	1	1	1	1	1	1
Dolichoderus debilis	1	1	1	1	1	1	1	1
Dolichoderus laminatus	0	0	0	1	0	0	0	0
Ectatomma ruidum	1	1	1	1	1	1	1	1
Ectatomma tuberculatum	1	1	1	1	0	1	0	1
Hypoponera _sp._	0	0	0	0	0	1	0	0
Neoponera carinulata	1	0	1	0	1	0	0	1
Neoponera striatinodis	1	0	0	0	0	0	1	1
Neoponera unidentata	1	1	0	1	1	1	1	1
Neoponera villosa	0	0	1	1	0	0	1	1
*Nylanderia _JTL006_*	0	0	0	0	0	1	0	0
Nylanderia steinheili	1	1	1	1	0	1	1	1
Odontomachus bauri	0	1	0	1	0	0	0	1
Pachycondyla harpax	0	0	0	0	0	0	0	1
Paraponera clavata	1	0	0	0	0	0	0	0
*Pheidoe _sp1_*	0	0	1	0	0	0	0	0
*Pheidole _cnp_*	1	0	0	0	0	0	0	0
*Pheidole _dasybrown_*	1	0	0	0	0	0	0	0
*Pheidole _lash4_*	0	0	0	0	1	0	0	0
*Pheidole _lash9_*	1	1	0	1	0	0	1	1
*Pheidole _shikii_*	0	0	0	0	0	0	0	0
Pheidole _sp._	0	1	0	0	0	0	0	0
*Pheidole _sp2_*	0	0	0	0	0	0	1	0
Pheidole caltrop	1	0	0	0	0	0	0	0
Pheidole cocciphaga	1	1	1	1	1	1	1	1
Pheidole dasypyx	1	0	0	1	0	0	1	1
Pheidole harrisonfordi	0	1	1	1	0	0	0	1
Pheidole rugiceps	0	0	0	0	1	0	0	0
Pheidole sensitiva	1	0	0	0	0	1	0	0
Procryptocerus belti	0	0	0	1	1	0	0	0
*Pseudomyrmex _black_*	0	0	1	0	0	0	0	0
Pseudomyrmex boopis	1	1	1	1	1	1	1	1
Pseudomyrmex gracilis	0	0	0	1	0	0	0	0
Pseudomyrmex oki	1	1	1	1	1	1	1	1
Rogeria _sp._	0	0	0	0	1	0	0	0
Rogeria blanda	0	0	0	0	1	0	0	0
Sericomyrmex amabilis	0	0	0	0	0	1	0	0
Solenopsis _sp._	0	0	1	1	1	1	1	0
*Solenopsis _sp.1_*	0	0	1	0	1	0	0	0
*Solenopsis _sp.2_*	1	1	1	1	1	0	1	1

*Solenopsis _sp.3_*	0	0	0	0	0	1	1	0
Solenopsis geminata	1	0	0	0	1	0	0	0
Solenopsis terricola	0	0	1	0	0	0	0	0
Tapinoma melanocephalum	1	0	0	0	0	0	0	0
Wasmannia auropunctata	1	1	1	1	1	1	1	1

Table A5. List of ant species and their accompanying subfamilies, from two studied habitats attracted to baits across fertilization plots.

SUBFAMILY	SPECIES	HABITAT
Myrmicinae	*Acromyrmex octospinosus*	canopy
Myrmicinae	*Acromyrmex volcanus*	canopy
Myrmicinae	*Apterostigma dentigerum*	understory
Dolichoderinae	*Azteca _sp.3_ (Azteca cf. instabilis)*	canopy
Dolichoderinae	*Azteca brevis*	canopy
Dolichoderinae	*Azteca cf charitfex*	canopy
Dolichoderinae	*Azteca flavigaster*	canopy
Dolichoderinae	*Azteca gnava*	canopy
Dolichoderinae	*Azteca instabilis*	canopy
Dolichoderinae	*Azteca nigra*	canopy
Dolichoderinae	*Azteca pilosa*	canopy
Dolichoderinae	*Azteca tondusi*	canopy
Formicinae	*Brachimyrmex longicornis*	canopy
Formicinae	*Brachymyrmex _JTL007_*	understory
Formicinae	*Brachymyrmex coactus*	understory
Formicinae	*Brachymyrmex heerii*	understory
Formicinae	*Brachymyrmwx _JTL002_*	canopy
Formicinae	*Camponotus _JTL056_*	canopy
Formicinae	*Camponotus brevis*	canopy
Formicinae	*Camponotus cuneidorsus*	canopy
Formicinae	*Camponotus excisus*	canopy
Formicinae	*Camponotus linnaei*	canopy
Formicinae	*Camponotus nitidor*	canopy
Formicinae	*Camponotus novogranadensis*	canopy
Formicinae	*Camponotus sanctaefidei*	canopy
Myrmicinae	*Cephalotes atratus*	canopy
Myrmicinae	*Cephalotes basalis*	canopy
Myrmicinae	*Cephalotes minutus*	canopy
Myrmicinae	*Cephalotes umbraculatus*	canopy
Myrmicinae	*Crematogaster _sp4_*	.
Myrmicinae	*Crematogaster acuta*	understory
Myrmicinae	*Crematogaster brasiliensis*	canopy
Myrmicinae	*Crematogaster carinata*	canopy
Myrmicinae	*Crematogaster flavosensitiva*	understory
Myrmicinae	*Crematogaster limata*	canopy
Myrmicinae	*Crematogaster sumichrasti*	canopy

Myrmicinae	*Crematogaster tenuicula*	canopy
Dolichoderinae	*Dolichoderus bispinosus*	canopy
Dolichoderinae	*Dolichoderus debilis*	canopy
Dolichoderinae	*Dolichoderus laminatus*	canopy
Ectatomminae	*Ectatomma ruidum*	understory
Ectatomminae	*Ectatomma tuberculatum*	understory
Ponerinae	*Hypoponera _sp._*	understory
Ponerinae	*Neoponera carinulata*	canopy
Ponerinae	*Neoponera striatinodis*	canopy
Ponerinae	*Neoponera unidentata*	canopy
Ponerinae	*Neoponera villosa*	understory
Formicinae	*Nylanderia _JTL006_*	understory
Formicinae	*Nylanderia steinheili*	understory
Ponerinae	*Odontomachus bauri*	understory
Ponerinae	*Pachycondyla harpax*	understory
Paraponerinae	*Paraponera clavata*	understory
Myrmicinae	*Pheidoe _sp1_*	understory
Myrmicinae	*Pheidole _cnp_*	understory
Myrmicinae	*Pheidole _dasybrown_*	understory
Myrmicinae	*Pheidole _lash4_*	understory
Myrmicinae	*Pheidole _lash9_*	understory
Myrmicinae	*Pheidole _shikii_*	understory
Myrmicinae	*Pheidole _sp._*	understory
Myrmicinae	*Pheidole _sp2_*	understory
Myrmicinae	*Pheidole caltrop*	understory
Myrmicinae	*Pheidole cocciphaga*	understory
Myrmicinae	*Pheidole dasypyx*	understory
Myrmicinae	*Pheidole harrisonfordi*	understory
Myrmicinae	*Pheidole rugiceps*	understory
Myrmicinae	*Pheidole sensitiva*	understory
Myrmicinae	*Procryptocerus belti*	canopy
Pseudomyrmecinae	*Pseudomyrmex _black_*	canopy
Pseudomyrmecinae	*Pseudomyrmex boopis*	understory
Pseudomyrmecinae	*Pseudomyrmex gracilis*	canopy
Pseudomyrmecinae	*Pseudomyrmex oki*	canopy
Myrmicinae	*Rogeria _sp._*	understory
Myrmicinae	*Rogeria blanda*	understory
Myrmicinae	*Sericomyrmex amabilis*	understory
Myrmicinae	*Solenopsis _sp._*	understory
Myrmicinae	*Solenopsis _sp.1_*	understory
Myrmicinae	*Solenopsis _sp.2_*	understory

Myrmicinae	*Solenopsis _sp.3_*	understory
Myrmicinae	*Solenopsis geminata*	understory
Myrmicinae	*Solenopsis terricola*	understory
Dolichoderinae	*Tapinoma melanocephalum*	understory
Myrmicinae	*Wasmannia auropunctata*	understory

Table A6. Linear mixed-effects models used to test the differences in genus diversity across treatments were fitted by the restricted maximum likelihood. Fixed effects: N, P, K and temperature (T), random effect is block, an 8 level categorical variable. For each model degrees of freedom, AIC values, ΔAIC and AIC weights are listed. Model terms include all the terms present in the model in question. Bold are models within 2AIC from the optimal model with the lowest AIC value.

MODEL TERMS	Df	AIC	ΔAIC	AIC Weights
T, N, P, K	7	109.7	4.5	0.05
T, P, K	6	107.7	2.5	0.12
P, K	**5**	**105.2**	**0.0**	**0.45**
P	**4**	**106.3**	**1.2**	**0.25**
Null	3	107.6	2.4	0.13

Table A7. Linear mixed-effects models used to test the differences in species diversity across treatments were fitted by the restricted maximum likelihood. Fixed effects: N, P, K and temperature (T), random effect is block, an 8 level categorical variable. For each model degrees of freedom, AIC values, ΔAIC and AIC weights are listed. Model terms include all the terms present in the model in question.

MODEL TERMS	Df	AIC	ΔAIC	AIC Weights
T, N, P, K	7	131.7	3.2	0.07
T, P, K	6	130.5	2.0	0.13
P, K	5	129.1	0.5	0.26
K	4	128.5	0.0	0.34
Null	3	129.5	0.9	0.21

Table A8. Linear mixed-effects models used to test the differences in genus richness across treatments were fitted by the restricted maximum likelihood. Fixed effects: N, P, K and temperature (T), random effect is block, an 8 level categorical variable. For each model degrees of freedom, AIC values, ΔAIC and AIC weights are listed. Model terms include all the terms present in the model in question.

MODEL TERMS	Df	AIC	ΔAIC	AIC Weights
T, N, P, K	7	138.1	3.2	0.09
T, P, K	6	136.9	2.0	0.16
T, P	5	136.1	0.5	0.33
P	4	135.0	0.0	0.43
Null	3	1.95	2.0	0.15

Table A9. Linear mixed-effects models fit by the restricted maximum likelihood used to test the differences in species richness across treatments. Fixed effects: N, P, K and temperature (T); random effect is block, an 8 level categorical variable. For each model degrees of freedom, AIC values, ΔAIC and AIC weights are listed. Model terms include all the terms present in the model in question.

MODEL TERMS	Df	AIC	ΔAIC	AIC Weights
T, N, P, K	7	168.2	0.2	0.23
FAT, P, K	6	168.2	0.2	0.22
P, K	5	167.9	0.0	0.25
K	4	168.5	0.5	0.19
Null	3	169.7	1.8	0.11

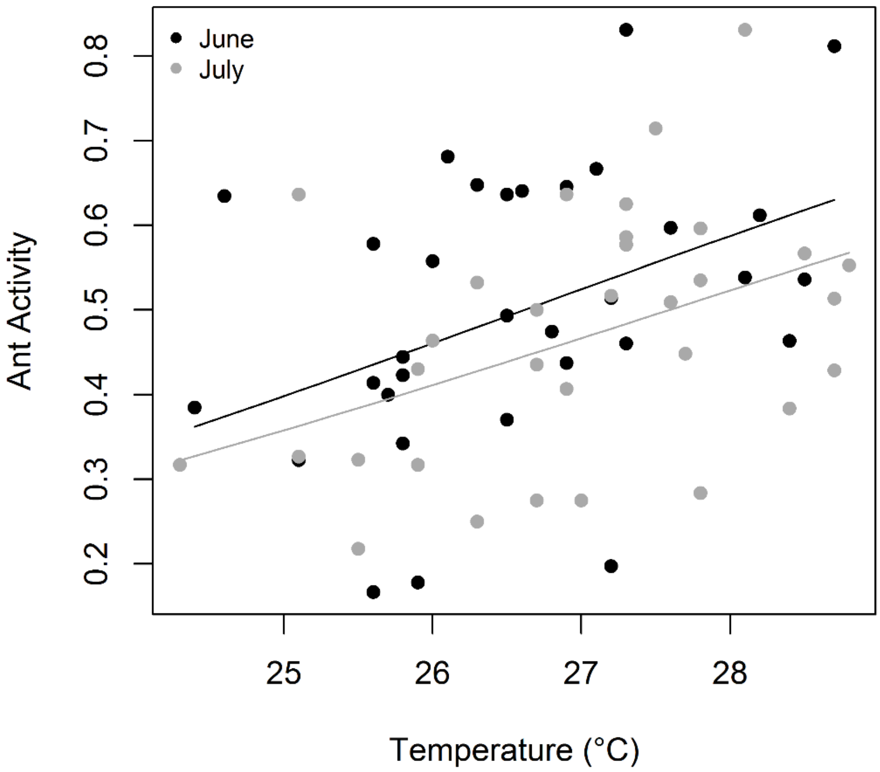
Chapter 1 - Supplement Figure Legends

Figure A1. The relationship between temperature and the ant activity – proportion of baits visited by ants. Data for both June (black dots) and July (gray dots) are shown. GLMs show a significant effect of temperature on proportion of foraging activity in both months ($p < 0.001$).

Figure A2. The relationship between genus richness and mean species richness across all genera collected on fertilization plots. Error bars represent standard deviation from the mean.

Figure A3. The relationship between genus richness and mean inverse Simpson diversity index for genera (A), and species (B). Error bars represent standard deviation from the mean.

Figure A1.



● June
● July
Ant Activity
0.2
0.3
0.4
0.5
0.6
0.7
0.8
25
26
27
28
Temperature (°C)

Figure A2.

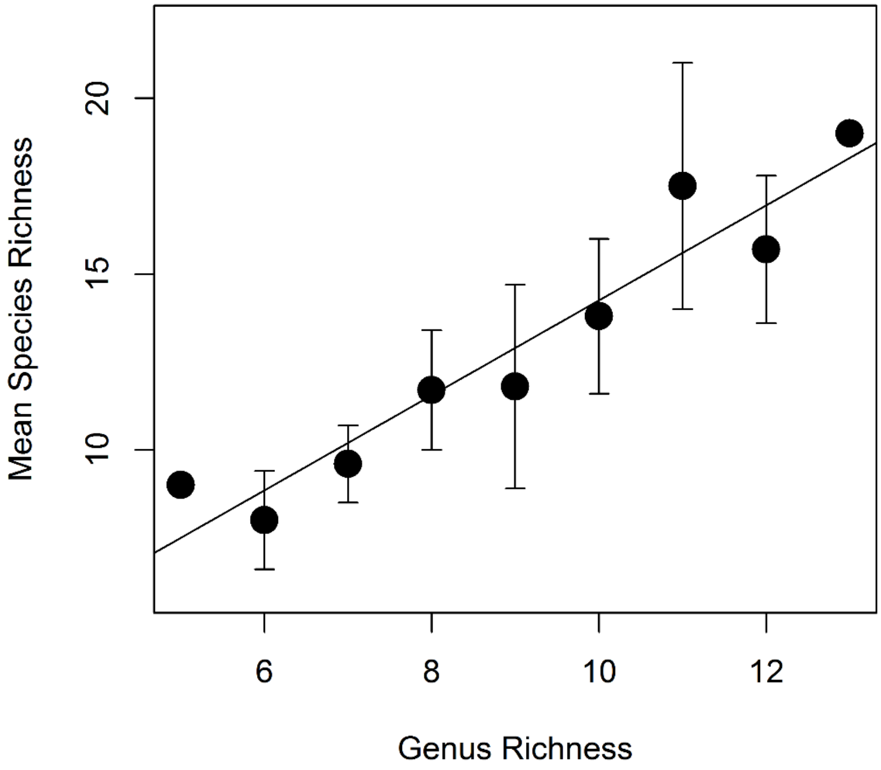


Figure A3A.

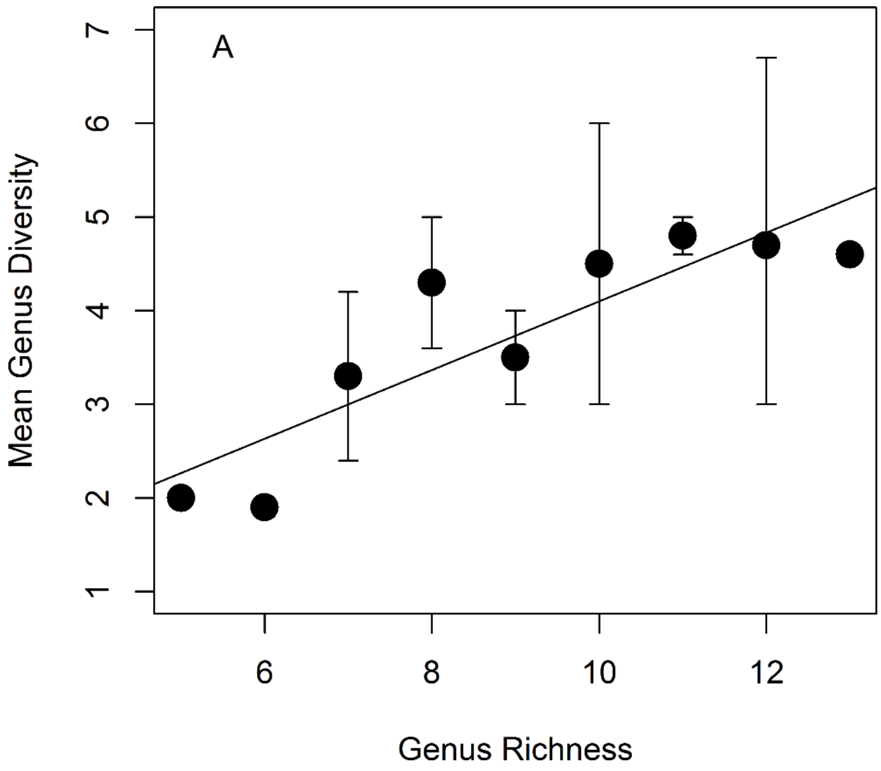
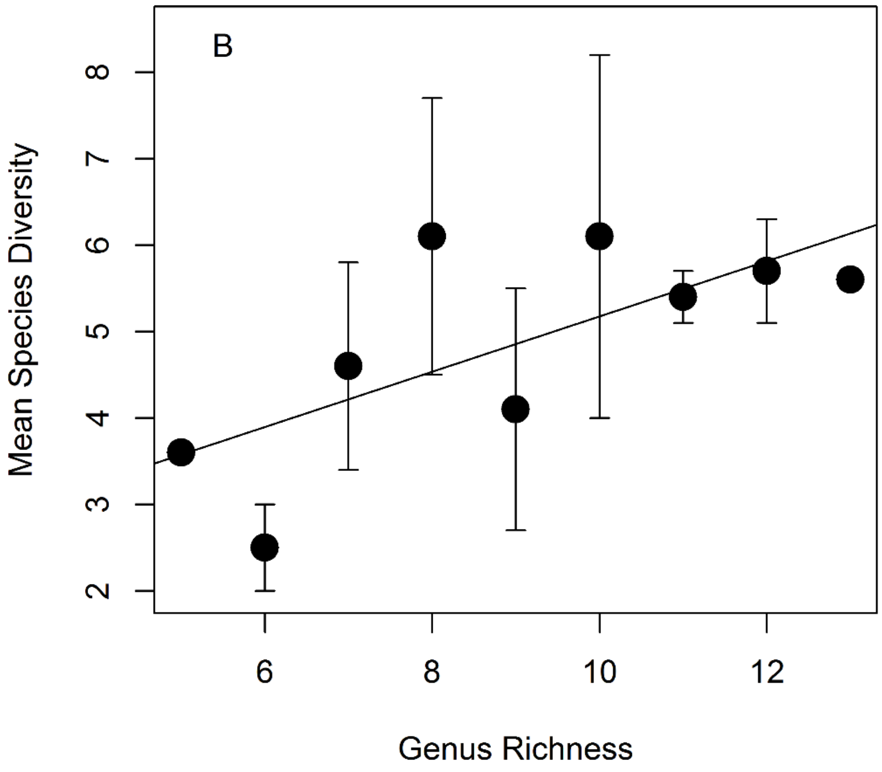


Figure A3B.



Chapter 2 - Supplemental Material

Table A10. List of ant species from two studied habitats used to measure workers' critical thermal maximum (CT_{max}) and lethal time when exposed to the desiccant (LT_{50}). The number of colonies (N_{col}) refers to the number of colonies used to measure LT_{50}.

Subfamily	Genus	Species	HABITAT	LT_{50} (h)	N_{col}	CT_{max}
Dolichoderinae	*Azteca*	*chartifex*	canopy	3.6	3	46
Dolichoderinae	*Azteca*	*_sp3_*	canopy	12.2	10	48
Dolichoderinae	*Azteca*	*pilosula*	canopy	12.3	1	-
Dolichoderinae	*Dolichoderus*	*bispinosus*	canopy	19.6	4	49
Dolichoderinae	*Dolichoderus*	*Debilis*	canopy	22.5	5	50
Dolichoderinae	*Dolichoderus*	*laminatus*	canopy	43.1	1	-
Formicinae	*Brachymyrmex*	*longicornis*	canopy	8.6	1	-
Formicinae	*Camponotus*	*_JTL056_*	canopy	10.9	1	-
Formicinae	*Camponotus*	*_JTL044_*	canopy	35.8	2	-
Formicinae	*Camponotus*	*novogranadensis*	canopy	41.1	4	-
Formicinae	*Camponotus*	*sericeiventris*	canopy	49.8	1	-
Formicinae	*Camponotus*	*sanctaefidei*	canopy	52.1	1	-
Formicinae	*Camponotus*	*linnaei*	canopy	52.2	1	45
Formicinae	*Camponotus*	*brevis*	canopy	91.9	3	46
Formicinae	*Camponotus*	*simillimus*	canopy	97.9	4	46
Myrmicinae	*Acromyrmex*	*volcanus*	canopy	8.6	2	-
Myrmicinae	*Cephalotes*	*umbraculatus*	canopy	18.8	2	50
Myrmicinae	*Cephalotes*	*atratus*	canopy	45.2	4	48
Myrmicinae	*Cephalotes*	*minutus*	canopy	66.5	2	-
Myrmicinae	*Crematogaster*	*stollii*	canopy	4.7	1	-
Myrmicinae	*Crematogaster*	*tenuicula*	canopy	13.9	6	49
Myrmicinae	*Crematogaster*	*carinata*	canopy	14.1	10	51
Myrmicinae	*Crematogaster*	*brasiliensis*	canopy	14.7	1	50
Myrmicinae	*Crematogaster*	*limata*	canopy	16.4	4	49
Myrmicinae	*Procryptocerus*	*belti*	canopy	11.3	2	50
Ponerinae	*Neoponera*	*carinulata*	canopy	36.7	2	46
Ponerinae	*Neoponera*	*unidentata*	canopy	43.4	5	47
Ponerinae	*Neoponera*	*striatinodis*	canopy	46.9	9	46
Ponerinae	*Neoponera*	*_jtl13_*	canopy	52.4	1	44
Ponerinae	*Neoponera*	*bugabensis*	canopy	78.8	5	45
Pseudomyrmecinae	*Pseudomyrmex*	*_spPSW01_*	canopy	9.8	1	-

Pseudomyrmecinae	*Pseudomyrmex*	*viduus*	canopy	15	2	-
Pseudomyrmecinae	*Pseudomyrmex*	*boopis*	canopy	19.2	1	50
Pseudomyrmecinae	*Pseudomyrmex*	*oki*	canopy	23.3	6	-
Dolichoderinae	*Tapinoma*	*melanocephalum*	litter	6.2	3	-
Dolichoderinae	*Technomyrmex*	*fulvus*	litter	18.8	1	-
Ecitoninae	*Eciton*	*hamatum*	litter	7	2	44
Ecitoninae	*Eciton*	*dulcium*	litter	10.6	2	-
Ecitoninae	*Eciton*	*burchellii*	litter	11.1	1	-
Ecitoninae	*Labidus*	*praedator*	litter	3.2	1	42
Ecitoninae	*Nomamyrmex*	*esenbeckii*	litter	6	2	-
Ectatomminae	*Ectatomma*	*ruidum*	litter	21.7	3	48
Ectatomminae	*Ectatomma*	*tuberculatum*	litter	36.4	2	48
Ectatomminae	*Gnamptogenys*	*regularis*	litter	14.4	1	-
Formicinae	*Brachymyrmex*	*coactus*	litter	16.7	1	-
Formicinae	*Brachymyrmex*	*heeri*	litter	39.6	1	-
Formicinae	*Camponotus*	*_JTL004_*	litter	22.1	1	45
Formicinae	*Paratrechina*	*guatemalensis*	litter	1.2	1	-
Formicinae	*Paratrechina*	*longicornis*	litter	7.8	3	-
Myrmicinae	*Apterostigma*	*_JTL015_*	litter	1.3	1	-
Myrmicinae	*Atta*	*cephalotes*	litter	7.6	2	-
Myrmicinae	*Atta*	*colombica*	litter	10.3	1	48
Myrmicinae	*Crematogaster*	*flavosensitiva*	litter	32.6	2	51
Myrmicinae	*Cyphomyrmex*	*rimosus*	litter	1.7	7	42
Myrmicinae	*Cyphomyrmex*	*costatus*	litter	1.9	1	-
Myrmicinae	*Cyphomyrmex*	*major*	litter	2	2	-
Myrmicinae	*Pheidole*	*_lash9_*	litter	1.5	1	-
Myrmicinae	*Pheidole*	*_sp2_*	litter	1.9	1	-
Myrmicinae	*Pheidole*	*multispina*	litter	2	2	-
Myrmicinae	*Pheidole*	*_sp1_*	litter	2.1	1	-
Myrmicinae	*Pheidole*	*mendicula*	litter	2.6	1	-
Myrmicinae	*Pheidole*	*harrisonfordi*	litter	2.9	4	-
Myrmicinae	*Pheidole*	*rugiceps*	litter	3	3	44
Myrmicinae	*Pheidole*	*bicornis*	litter	4.7	1	-
Myrmicinae	*Sericomyrmex*	*amabilis*	litter	3.1	1	-
Myrmicinae	*Solenopsis*	*_lash5_*	litter	2.6	1	-
Myrmicinae	*Solenopsis*	*_JTL002_*	litter	9.5	2	-
Myrmicinae	*Solenopsis*	*terricola*	litter	11.8	7	-
Myrmicinae	*Strumigenys*	*_sp2_*	litter	2	1	-
Myrmicinae	*Strumigenys*	*_sp1_*	litter	6.2	1	-
Myrmicinae	*Strumigenys*	*gundlachi*	litter	25.5	1	-
Myrmicinae	*Tetramorium*	*bicarinatum*	litter	16.5	1	-

Myrmicinae	*Trachymyrmex*	*isthmicus*	litter	0.7	1	-
Myrmicinae	*Wasmannia*	*auropunctata*	litter	7.6	6	44
Paraponerinae	*Paraponera*	*clavata*	litter	14.5	2	-
Ponerinae	*Hypoponera*	*_JTL002_*	litter	10.5	1	-
Ponerinae	*Leptogenys*	*_JTL007_*	litter	12.3	1	-
Ponerinae	*Leptogenys*	*_jelSUL_*	litter	13.3	1	-
Ponerinae	*Leptogenys*	*punctaticeps*	litter	23.6	2	-
Ponerinae	*Odontomachus*	*bauri*	litter	32	4	44
Ponerinae	*Neoponera*	*villosa*	litter	17.4	1	45
Ponerinae	*Pachycondyla*	*harpax*	litter	42.5	1	-

Table A11. Generalized linear models used to test the differences in desiccation resistance among 82 ant species. Habitat and body mass were used as predictor variables. For each model degrees of freedom, AIC values, ΔAIC and AIC weights are listed. Model terms include all the terms present in the model in question.

MODEL TERMS	Df	AIC	ΔAIC	AIC Weights
Mass + Habitat	4	82.3	0	1
Mass	3	102.2	19.9	0
Habitat	3	98.9	16.6	0
Null	2	122.9	40.6	0

Chapter 2 - Supplement Figure Legends

Figure A4. The difference in vapor pressure deficit (VPD) in the canopy and the litter during the day (A) and night (B).

Figure A5. Relationship between the difference of ant lethal time in the air and when exposed to the desiccant with respect to body mass. All the values were $\log_{10}$ transformed.

Figure A6. Relationship between desiccation resistance (LT_{50}) and body mass on a log scale from our study (solid lines) compared to ants studied by Hood and Tschinkel (1990) – dashed lines. Canopy – dashed gray: $LT_{50} = 0.61\text{mass} + 0.89$, $R^2 = 0.66$, and understory – dashed black: $LT_{50} = 0.45\text{mass} + 1.68$, $R^2=0.63$. At the adjusted mass range, in our community, canopy ants – gray solid: $LT_{50} = 0.35\text{mass} + 1.43$, $R^2 = 0.28$, $p = 0.002$, and understory ants – black solid: $LT_{50}= 0.32\text{mass} + 0.92$, $R^2 = 0.27$, $p = 0.001$).

Figure A7. Water loss (%) in different ant species examined. Canopy species are shown in blue – light blue represents water loss in live ants, and dark blue in dead ants. Litter species are shown in gray: light – live ants, dark – dead ants. The box and whisker plots are showing median of % total water loss, upper and lower quartiles, as well as the maximum values and outliers. We tested the following species: *Pseudomyrmex gracilis* (PSEUgrac), *Dolichoderus bispinosus* (DOLIbisp), *Cephalotes atratus* (CEPHatra), *Cephalotes umbraculatus* (CEPHumbr), *Camponotus sericeiventris* (CAMPseri), *Eciton hamatum* (ECIThama), *Odontomachus bauri* (ODONbaur), *Ectatomma tuberculatum* (ECTAtube), *Ectatomma ruidum* (ECTAruid), *Atta colombica* (ATTAcolo).

Figure A4A

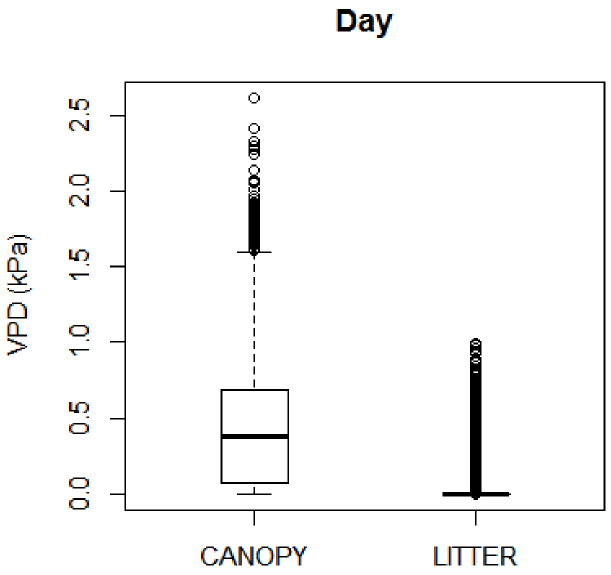


Figure A4B

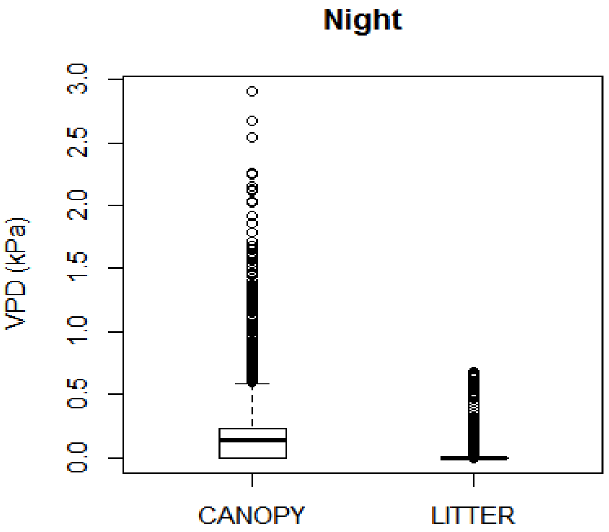


Figure A5

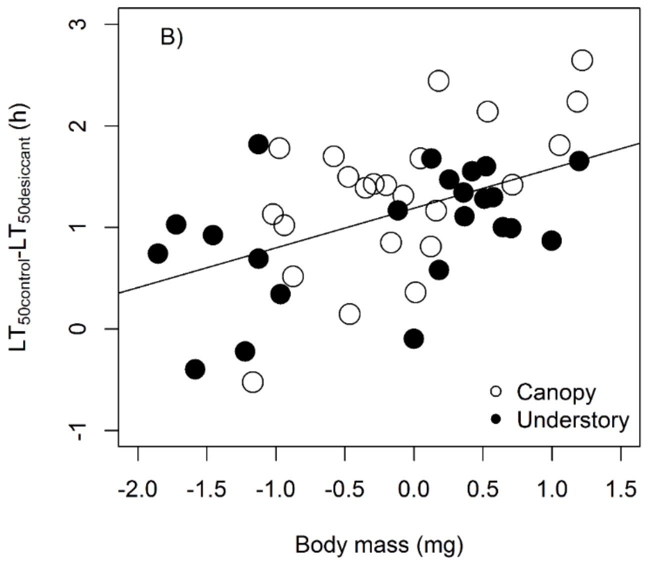

B)
$LT_{50control}-LT_{50desiccant}$ (h)
3
2
1
0
-1
Body mass (mg)
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
○ Canopy
● Understory

Figure A6

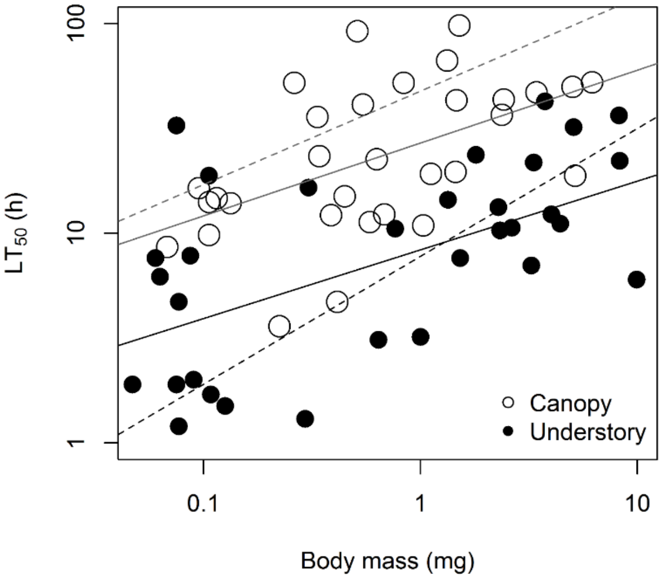
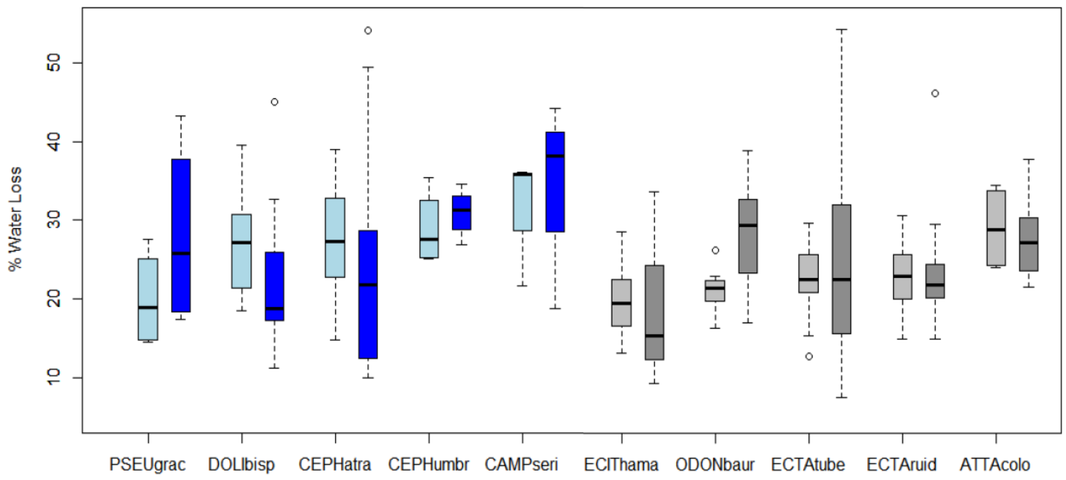


Figure A7


% Water Loss
10
20
30
40
50
PSEUgrac
DOLIbisp
CEPHatra
CEPHumbr
CAMPseri
ECIThama
ODONbaur
ECTAtube
ECTAruid
ATTAcolo

Chapter 3 - Supplemental Material

Table A12. Concentration (ppm) of 10 different chemical elements in *Azteca chartifex* workers collected across control and phosphorus plots.

Element	Control	Phosphorus	χ^2	p-value
Al	245.4 ± 207.7	476 ± 946.8	0.08	0.78
Ca	1,276.2 ± 341.5	1,172 ± 299.8	0.08	0.78
Cu	15.4 ± 5.2	16 ± 5.2	0.08	0.77
Fe	246.9 ± 175.3	363 ± 569.2	0.0	1.00
K	10,323.8 ± 1403.5	9,120 ± 1755.6	2.40	0.12
Mg	1,237.7 ± 139.2	1,244 ± 295.3	0.60	0.44
Mn	146.9 ± 58.8	163 ± 30.2	0.28	0.60
Na	2,313.8 ± 320.3	2,221 ± 468.5	0.02	0.88
P	7,443.1 ± 178.1	7,278 ± 1299.8	0.42	0.51
Zn	165.4 ± 13.3	164 ± 28.4	0.41	0.52
n(colonies)	13	10		

Chapter 3 - Supplement Figure Legends

Figure A8. A) Amount of carbon (%C) and B) nitrogen (%N) in worker ants collected on control and phosphorus treatments.

Figure A9. Carbon:nitrogen (C/N) ratio of ant workers from control and phosphorus plots of *Azteca chartifex* workers.

Figure A8.

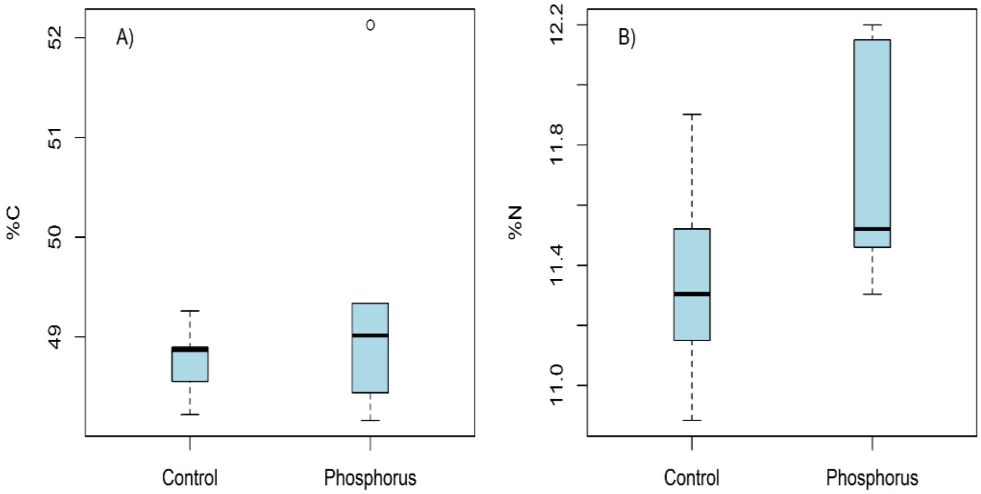
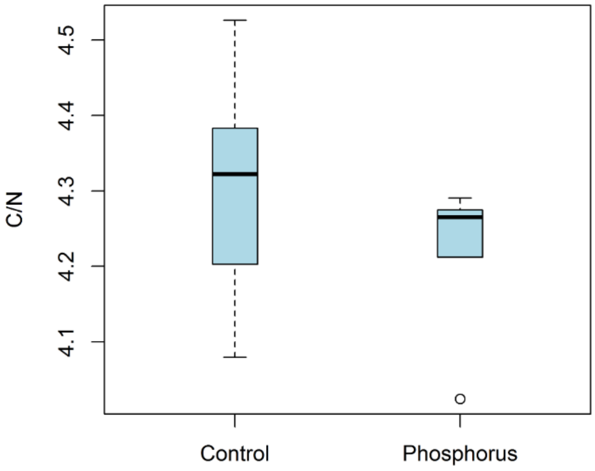

A)
%C
52
51
50
49
Control
Phosphorus
B)
%N
12.2
11.8
11.4
11.0
Control
Phosphorus

Figure A9.



Chapter 4 - Supplemental Material

Table A13. Generalized linear models used to test variation in nest density related to the presence of extra-floral nectaries (EFN) and tree height. Height used in this model selection is the average height of each tree species. For each model degrees of freedom, AIC values, ΔAIC (difference between the *i*th model and the model with the lowest AIC), and AIC weights are listed. Model terms include all the terms present in a model. Bold is the optimal model with the lowest AIC value. Treatments in italic indicate significance $p < 0.05$.

MODELterms	DF	AIC	ΔAIC	AIC Weights
EFN, *HEIGHT*	**4**	**867.637**	**0**	**0.999**
HEIGHT	3	882.22	14.583	0.001
EFN	3	949.437	81.801	0
Null	2	1045.581	177.944	0

Table A14. Generalized linear models used to test variation in nest height across the fertilization plots. For each model degrees of freedom, AIC values, ΔAIC (difference between the ith model and the model with the lowest AIC), and AIC weights are listed. Model terms include all the terms present in a particular model. Bold is the optimal model with the lowest AIC value. Treatments in italic indicate significance $p < 0.05$.

MODELterms	DF	AIC	ΔAIC	AIC Weights
N, K, NP, PK, DBH	**7**	**953.97**	**0**	**0.314**
N, K, NP, PK,* REPL, *DBH	**8**	**954.606**	**0.636**	**0.228**
K, NP, PK, DBH	6	956.023	2.053	0.112
N, P, K, NP, PK, REPL, DBH	9	956.21	2.24	0.102
NP, DBH	4	956.536	2.566	0.087
N, P, K, NP, NK, PK, REPL, DBH	10	957.133	3.163	0.065
DBH	3	957.765	3.795	0.047
K, NP, DBH	5	957.864	3.895	0.045
Null	2	1140.604	186.634	0

Figure A10. Relationship between nest height and accompanying tree size measured as diameter at breast height (DBH).