

## Article

# Nutrient Supplementation to Arboreal Ants: Effects on Trophic Position, Thermal Tolerance, Community Structure and the Interaction with the Host-Tree

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**Abstract:** Carbohydrates and proteins are essential to maintain the basic functions of animals. Over the course of one year we conducted a factorial experiment to determine the influence of carbohydrate (sucrose) and protein supplementation on the thermal tolerance, trophic position, overall abundance, species richness and composition, and on the strength of the protective effects of arboreal ants on their host tree (*Caryocar brasiliense*). Using *Azteca* ants as a model we found evidence of dietary and thermal plasticity among arboreal ants as colonies supplied with protein increased their trophic level relative to colonies that received no protein. Colonies that received sucrose increased their thermal tolerance on average by 1.5 °C over a six-month period, whereas those that did not receive sucrose did not change their thermal tolerance. Overall ant abundance was lower in control trees than in those that received any nutrient addition treatment. Species richness was also lower in control trees, but those receiving sucrose presented more species than those receiving only protein. There was greater similarity in species composition between the trees that received sucrose than between these and those receiving only protein or just water as control. Trees whose ant colonies received sucrose presented lower levels of leaf damage than those that did not. Overall, these results indicate that food resources can modulate the population and community ecology of arboreal ants as well as their interaction with the host trees. Interestingly, although arboreal ants are thought to be N-limited, it was the supplementation of sucrose—not protein—that elicited most of the responses.

**Keywords:** nutritional ecology; ecological stoichiometry; cerrado; formicidae; diet; nitrogen; sucrose



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## 1. Introduction

Nutrition influences every aspect of an animal's life, either directly by building the components of the organisms and affecting their performance, or indirectly by influencing the interaction between species and the environment at multiple scales [1]. Rather than simply maximizing food intake, consumers must regulate foraging in a way that matches the optimal mixture of macronutrients and micronutrients required for survival [2,3], and this becomes even more challenging when considering that nutrient availability varies between and within habitats [4]. Nutritional interactions between organisms and the environment might influence species physiology and behavior, working as a bottom-up force that alters species relations and the structure of biological communities [5–7].

Carbohydrates and proteins are essential compounds that act in a complementary way to maintain the basic functions of animals, such as growth, survival, and reproduction [8,9]. The availability of carbohydrates and protein depends on the type of food resource, which, in turn, varies temporally and spatially. For example, for organisms in the canopy of tropical forests there is often a greater availability of carbohydrates than of proteins [10]. According to the Compensation Hypothesis [11,12], the attractiveness and utility of a food resource to a given organism is conditional to its availability in the environment in question.

Thus, for consumers in the forest canopy, protein may be more attractive than the abundant carbohydrate [13,14].

Ants are one of the most ubiquitous and diverse groups of arthropods in the canopy of tropical forests and savannas [15]. In general, arboreal ants have a carbohydrate-based diet that follows the high availability of sugary plant and insect exudates in the canopy [16–18]. However, most arboreal ants are omnivorous [17,19,20], whose foraging behavior and trophic level can vary according to the availability of different food sources in the environment [21]. Although some studies suggest that ants are more constrained by protein sources in the canopy, and, therefore, more attracted to it [10,20], there is also evidence that, in fact, the most limiting resource for arboreal ants are carbohydrates [22]. It has been suggested that if selective pressures across ants' evolutionary history favored species that feed on the most abundant resource, then carbohydrates could be a limiting nutrient for arboreal ants, even considering its high abundance in the canopy [22].

Carbohydrates fuel the more energetically costly activities of the colonies and is related to the maintenance of the workers' body functions, whereas protein is fundamental for the growth and development of larvae and pupae [23]. In this sense, both nutrients act in complementary ways to ants' colony growth and survival, which ultimately could affect species interactions and the structure of communities [24,25]. Moreover, both carbohydrates and protein can interact and influence numerous other aspects of an ant's life. For example, carbohydrates can provide energy for thermoregulation [26] and increase evaporative cooling effects [27], while protein can influence the production of heat shock proteins, which are essential to cope with thermal stress [28,29]. Therefore, nutrition can alter the critical thermal maximum ( $CT_{max}$ ) of ant workers [6] which, in turn, can affect their foraging schedules, disrupt transitive hierarchies [30,31] and/or affect their mutualistic interactions with plants [32].

Many species of arboreal ants nest or forage on plants that have extrafloral nectaries (non-reproductive organs and plant tissues that produce carbohydrate-rich nectar, EFNs hereafter). This interaction can be considered mutualistic when ants prey on herbivores and, thus, increase plant fitness, while benefiting from nesting and food resources [18]. Several non-exclusive hypotheses have been proposed to explain why ants defend EFN-producing plants. The Fuel for Foraging Hypothesis [33–35] proposes that the ant visitors are fueled by carbohydrates provided in the EFNs, increasing ant's foraging and aggressiveness. The Ownership Hypothesis [36,37] proposes that a valuable resource elicits ownership behavior, so that ants would defend the plant against its natural enemies while protecting and dominating the food resource. Lastly, the Deficit Hypothesis [38] proposes that the nutritional imbalances of EFNs (high C:N) increases the ants' need for protein, increasing the chance that they attack an herbivore on the plant.

Although there are studies showing how the availability of carbohydrates alters the foraging behavior and aggressiveness of ants [39,40], ultimately benefiting the plant [41,42], none of these studies have explored how protein availability (and its interaction with carbohydrates) affects the outcome of the interaction between ants and plants. Furthermore, relatively few studies have evaluated how nutrient supplementation affects the thermal tolerances and trophic position of individual colonies, as well as the structure of the ant communities foraging on trees.

Here, we evaluated the influence of nutrient supplementation on the ecology of arboreal ants. For this, we performed a one-year long field experiment in which carbohydrate (sucrose) and protein were supplied to ants associated with an EFN-producing savanna tree species. We addressed four questions. At the level of individual ant colonies we asked: (1) What are the individual and combined effects of carbohydrate and protein supplementation on the thermal tolerance of arboreal ants? We expected that thermal tolerance would vary among colonies subject to different nutrient supplementation treatments, since carbohydrates and protein are known to influence the thermal tolerances of insects in different ways [28,29]. In addition, (2) we evaluated what are the individual and combined effects of carbohydrate and protein supplementation on the trophic position of arboreal

ants? According to the Compensation Hypothesis, which states that animals tend to forage in a way to correct nutritional imbalances [11,12], we expected that colonies supplemented with the most limiting resource would have the greatest change in their trophic positions.

At the community level we asked: (3) What are the individual and combined effects of carbohydrate and protein supplementation on the overall abundance, species richness, and composition of arboreal ants on trees? Given that competition over food resources is a strong structuring force among arboreal ant communities [43–46], we would expect that both protein and sucrose would generate an increase in the overall abundance and species richness of ants on trees. Furthermore, considering that the attractiveness of food resources varies between species [20], we also expected that species composition would be different across the nutrient supplementation treatments. Finally, we asked: (4) Does nutrient supplementation affect the protective effects of ants against the herbivores of their host tree (level of foliar damage)? We expected that, if the Ownership Hypothesis [34,36,37] is correct, then colonies receiving complementary food resources (i.e., both carbohydrate and protein) would better protect their host trees. On the other hand, if the Deficit Hypothesis [38] is correct, then the supplementation of protein should diminish the ants' need for protein and, therefore, their predatory activities. In this sense, the most protected trees would be those in which colonies receive carbohydrates only. Similarly, if the Fuel for Foraging Hypothesis [33–35] is correct, access to carbohydrates would fuel foraging and aggressive behavior of ants, increasing protection on trees supplemented with carbohydrates.

## 2. Materials and Methods

### 2.1. Study Area

This study was conducted at the Reserva Ecológica do Panga, a 404 ha reserve located 30 km south of Uberlândia, Minas Gerais, Brazil (19°10' S, 48°23' W). The mean annual temperature of the region is 22 °C and the mean annual rainfall is 1650 mm. All observations and experiments were performed on *Caryocar brasiliense* trees located within an area of approximately five hectares covered by the dominant vegetation type of the reserve (woodland savanna, locally known as *cerrado sensu stricto*), and which is characterized by a superior layer of trees and shrubs and a ground layer formed by grasses, herbs, and small shrubs [47]. *Caryocar brasiliense* is a common and widespread species in the savannas of central Brazil which produces EFNs on its leaves and inflorescences.

### 2.2. Supplementation Experiment

We selected and marked 67 medium sized (3–4 m in height) *C. brasiliense* trees that were separated from each other by at least 12 m. This distance was large enough to ensure that ants from a given experimental tree were not foraging in another tree. In fact, foraging by predominantly arboreal species was largely restricted to the host tree. This was because the savanna where our experiment took place was relatively open and, therefore, there was very little connectivity between the crowns of different trees [48]. Each of the selected trees received one of the following nutrient supplementation treatments, (1) carbohydrate only (20% water solution of sucrose) (n = 17 trees); (2) protein only (20% water solution containing equal parts of isolate whey protein, calcium caseinate and egg white powder) (n = 16); (3) carbohydrate and protein (mixture containing equal parts of the carbohydrate and protein solutions) (n = 19); and (4) control trees (water only) (n = 15). The amounts of carbohydrate and protein added to the water solutions are similar to those used in previous studies [20,25] and were chosen because they mimic the concentration of these two elements in natural food sources [49,50]. A total of six sealed plastic cups (6 cm high, 5 cm in diameter), with access holes (five 6 mm diameter holes, drilled on the sides of each cup), were wired onto the branches of each experimental tree. Each cup was filled with 60 mL of water, sugar, protein, or sugar and protein solution. A piece of folded tissue paper in the cup served both as a feeding platform and a structure that ants could use to escape the liquid if they fell in. Solutions and tissue platforms were changed weekly. The experiment had a duration of one year (August 2020–July 2021).

### 2.3. Critical Thermal Maximum ( $CT_{max}$ )

To evaluate the influence of the nutrient supplementation treatments on ant's resistance to heat, we measured the  $CT_{max}$  of 21 colonies of an unidentified *Azteca* species (hereafter *Azteca*) just prior to and again six months after the beginning of the experiment. The  $CT_{max}$  of the ants was determined using dry bath equipment (Kasvi Dry Bath model K80-S01/02). In each test, 20 workers of the same colony were placed individually in Eppendorf tubes sealed by cotton and placed randomly in the dry bath equipment. The initial temperature of the test was 36 °C, which was increased by two degrees every 10 min of exposure until death or permanent loss of muscle coordination in the workers. Tests were carried out within a maximum period of up to four hours after the collection of the ants in the field. We considered the  $CT_{max}$  of the species as the average temperature of death or permanent loss of muscle coordination of the 20 workers.

### 2.4. Stable Isotope Analyses

We determined the isotopic signature ( $\delta^{15}N$ ) and the carbon and nitrogen ratios (C:N ratio) of ants from 18 *Azteca* colonies. For this, we collected approximately 20 workers from each colony 10 months after the beginning of the experiment. We removed the gaster of each worker during sample preparation to avoid the effect from recently ingested food items on the analysis [16,51]. Ant samples were dried in an oven at 60 °C for 48 h and then crushed with an agate mortar and pestle. The dried samples were put into small tin capsules in precisely weighed amounts (1.25–1.5 mg) then molded into a spherical shape, put on ELISE dishes, and sent to the University of California Stable Isotope Facility, in Davis, CA, USA, for analysis. The results were expressed in delta notation per thousand, with an internationally acknowledged standard as reference.

### 2.5. Sampling of the Ant Fauna

To estimate the abundance and diversity of arboreal ants in the experimental trees ( $n = 67$ ), two observers counted the number and determined the identity of the ants foraging on the main trunk and branches of each tree for a period of two minutes during the morning (7:00 a.m.–9:30 a.m.), and again in the afternoon (12:00 a.m.–2:30 p.m.), and evening (7:00 p.m.–9:30 p.m.) of the same day. This was done once in May 2021, and again one month later by the same two observers. When necessary, ant specimens were collected and stored in alcohol for later identification in the laboratory. Voucher of all species collected are deposited at the Zoological Collection from the Federal University of Uberlândia (UFU) in Uberlândia, Brazil.

### 2.6. Artificial Nests

We also assessed the effect of nutrient supplementation on the occupation of artificial wooden nests by arboreal ants. For this we wired six nests to the branches of about half of the experimental trees (32 of the 67 trees). The nests were made of bamboo (~100 mm in length and 10 mm in diameter). Three of the bamboo nests had an opening of 6 mm<sup>2</sup> and the other three an opening of 8 mm<sup>2</sup>, which were the most used nest entrance sizes in an experiment conducted earlier [48]. The bamboo nests were installed in October 2020 and removed in August 2021. Each nest was sealed with adhesive tape and transported to the laboratory, where they were opened and the identity and number of ants was recorded, as well as the presence of eggs, larvae, pupae, and alates.

### 2.7. Herbivory Measurements

We estimated the level of leaf damage on 59 of the 67 experimental trees. For this, one branch from each tree was randomly selected and 10–14 newly initiated leaves (with no signs of damage) from this branch were marked using plastic-coated wires placed around the leaf petiole. This was done in September 2020 when *C. brasiliense* was flushing new leaves. After 90 days, we collected the marked leaves and produced a scanned image

of each one. The area damaged by chewing insects and the total area of each leaf was determined using the ImageJ software [52].

## 2.8. Statistical Analyses

We analyzed the individual and interactive effects of the nutrient supplementation treatments on the isotopic signature ( $\delta^{15}\text{N}$ ) and on the carbon to nitrogen ratio (C:N) of *Azteca* ant workers using two-way ANOVAs, in which the predictor variables were carbohydrate (with or without) and protein (with or without). The same model was used to evaluate the magnitude of change (the effect size) of the  $\text{CT}_{\text{max}}$  of the *Azteca* ants. The magnitude of change was calculated as the logarithm of the response ratio using the formula  $\log(\text{CT}_{\text{max}} \text{ after supplementation} / \text{CT}_{\text{max}} \text{ before supplementation})$ . Similarly, two-way ANOVAs were employed to evaluate the effects of nutrient supplementation on the species richness and overall abundance (i.e., the abundance of all species combined) of ants foraging on trees, as well on the proportion of artificial nests colonized by ants and the abundance of ants in nests. Finally, a two-way Anova was run to evaluate effects on leaf herbivore damage in *C. brasiliense* trees. When a significant interaction between the effects of carbohydrate supplementation and protein supplementation was detected, we performed a posteriori, pairwise multiple comparisons among individual treatments using the Tukey method.

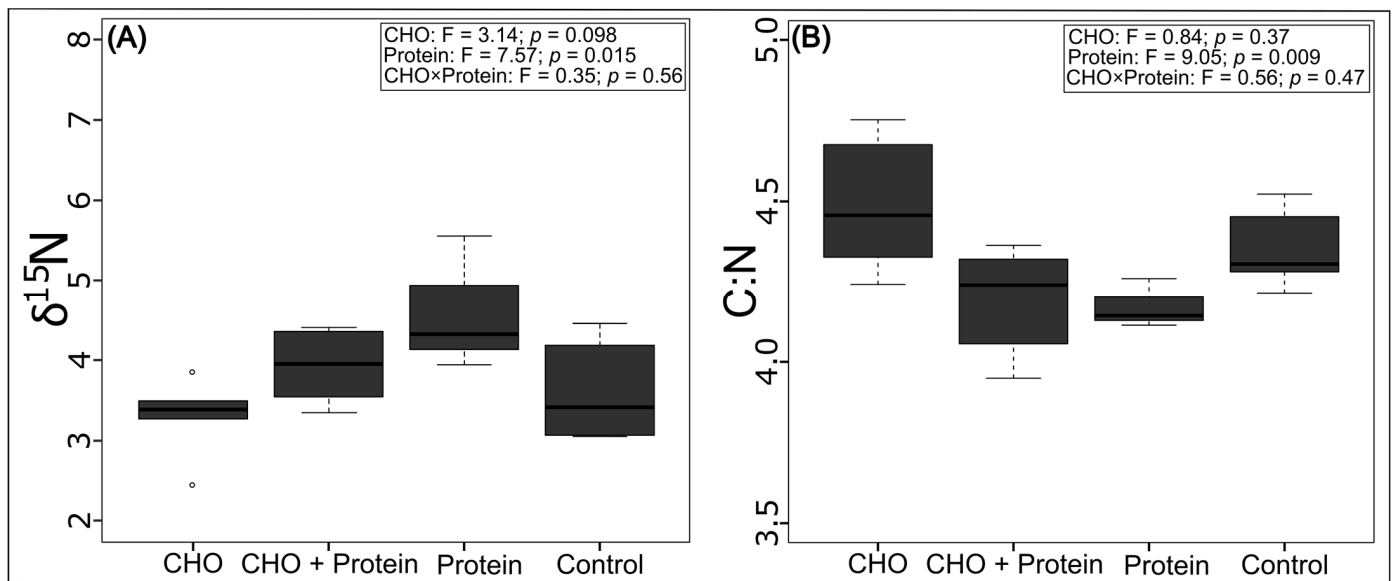
Data on ant abundance were  $\log(x + 1)$  transformed prior to the analyses to meet the assumption of data normality and homoscedasticity. Similarly, data on leaf herbivory was arcsine square root transformed prior to the analysis. A Gaussian error distribution was assumed in most models, except the one on species richness in which a quasi-Poisson model was run, and the one on the proportion of artificial nests colonized by ants in which a binomial distribution was assumed. Model assumptions were checked by evaluating the plot of the residuals against the fitted values and the normal probability plot. Analyses were performed in R v.4.1.1 [53], using the packages “car” [54] and “emmeans” [55].

To evaluate the differences in ant species composition among trees subject to different nutrient addition treatments we performed a two-way cluster analysis. For this, we first built a matrix containing information about the total number of trees (from each nutrient addition treatment) in which each ant species was recorded. The analysis was run in PCORD 7.0 (MJM Software Design, Gleneden Beach, OR, USA) using the Bray–Curtis index of similarity (with individual species data relativized by the species total) and the group average linkage method [56]. In this analysis, we used only data for those species that occurred in at least three of the 67 trees sampled, since the occurrence of a rare species in a giving treatment could be more related to its low abundance in the community than to its nutritional requirements.

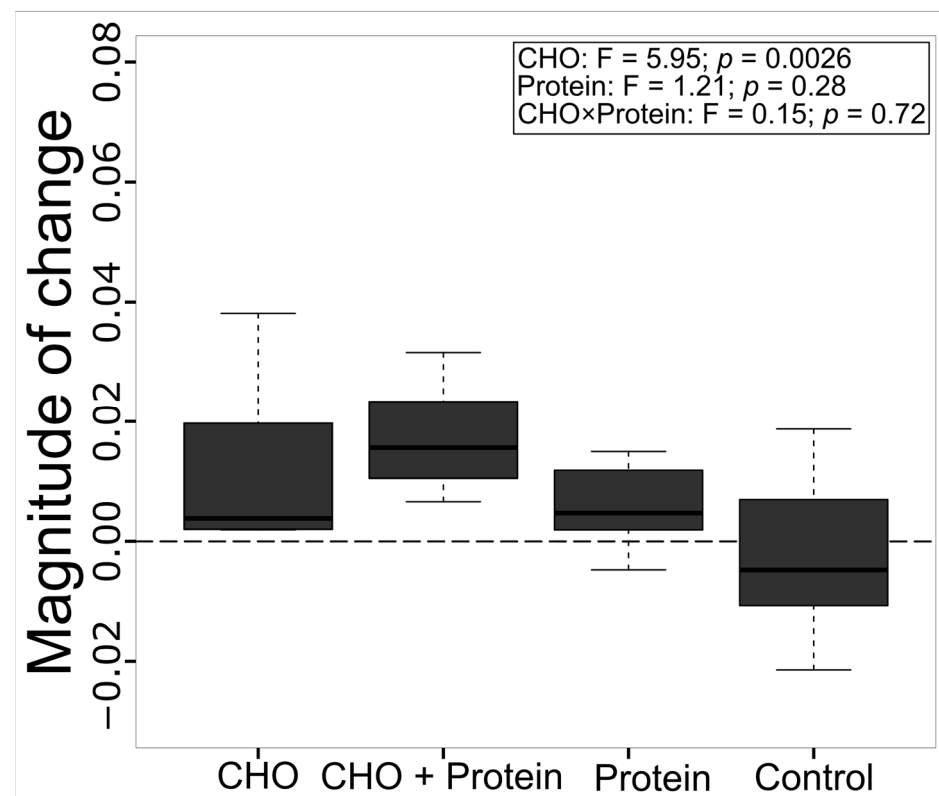
## 3. Results

### 3.1. Effects on the Trophic Position and Thermal Tolerance of *Azteca*

Protein supplementation presented a significant effect on the  $\delta^{15}\text{N}$  and C:N ratio of the *Azteca* colonies, whereas the supplementation of carbohydrate (sucrose) did not have an effect (Figure 1). Colonies that received protein (i.e., only protein or protein together with carbohydrate) presented, on average, a  $\delta^{15}\text{N}$  4% greater and a C:N ratio 7.2% lower than those that did not. Conversely, while sucrose supplementation affected the critical thermal maxima ( $\text{CT}_{\text{max}}$ ) of *Azteca*, protein did not have an effect (Figure 2). On average, colonies receiving sucrose (sucrose only or sucrose together with protein) increased their  $\text{CT}_{\text{max}}$  in 1.59 °C, whereas the mean absolute change in the  $\text{CT}_{\text{max}}$  of the colonies that did not receive sucrose was close to zero (−0.022 °C).



**Figure 1.** Effects of carbohydrate and protein supplementation on the  $\delta^{15}\text{N}$  (A) and the carbon to nitrogen ratio (B) of *Azteca* sp. colonies. Circles represent outlier values.



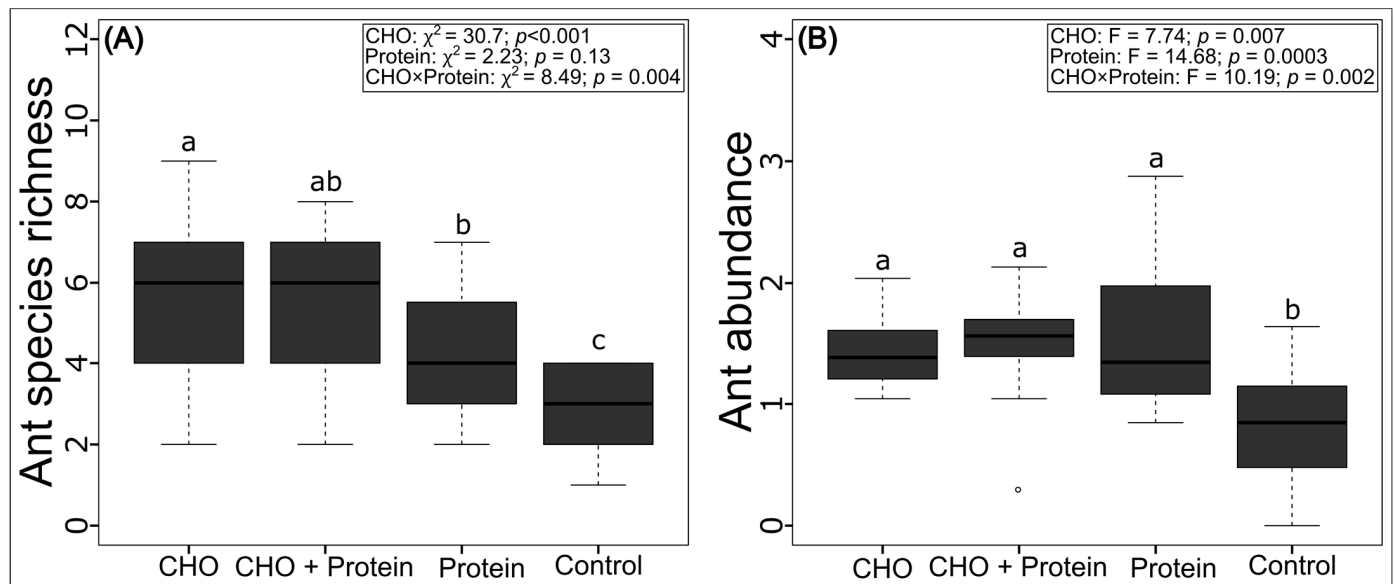
**Figure 2.** Effect of carbohydrate and protein supplementation on the thermal tolerance of *Azteca* sp. Values represent the magnitude of the difference (effect size) in the  $\text{CT}_{\text{max}}$  of ant workers prior and six months after the beginning of the experiment. The dashed line represents the null expectation.

### 3.2. Effects on Overall Ant Abundance, Species Richness and Composition

There was a significant interaction between the effects of carbohydrate and protein supplementation on ant species richness per tree. Control trees had fewer ant species than those that received carbohydrate, protein, or both (Tukey test,  $p \leq 0.008$ ). In addition, there was a difference between those that received only carbohydrate or only protein, with the



former presenting more species than the latter (Tukey test,  $p = 0.042$ ) (Figure 3). Similarly, there was a significant interaction between the effects of carbohydrate and protein on the overall abundance of foraging ants per tree. Overall ant abundance in control trees was significantly lower than in trees from all the remaining treatments (Tukey test,  $p \leq 0.006$ ) (Figure 3).

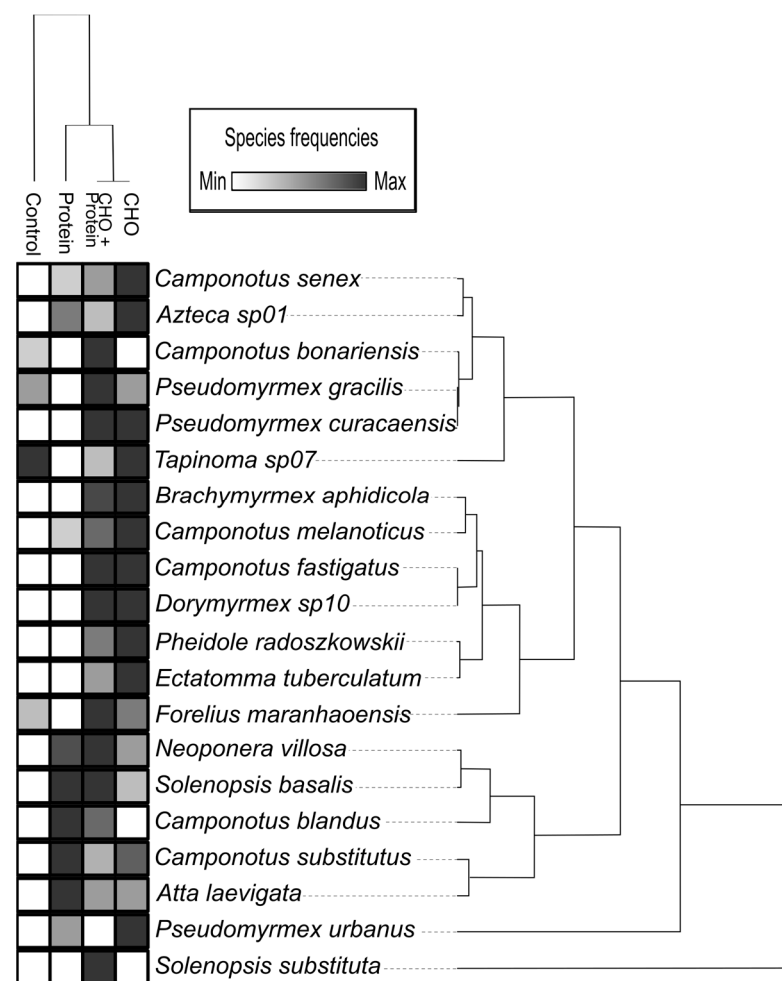


**Figure 3.** Effects of carbohydrate and protein supplementation on the species richness (A) and overall abundance (B) of ants (log transformed) foraging in *Caryocar brasiliense* trees. Since there was a significant interaction between the main effects, a multiple comparison test was performed. Different letters above box plots indicate significant differences among mean values.

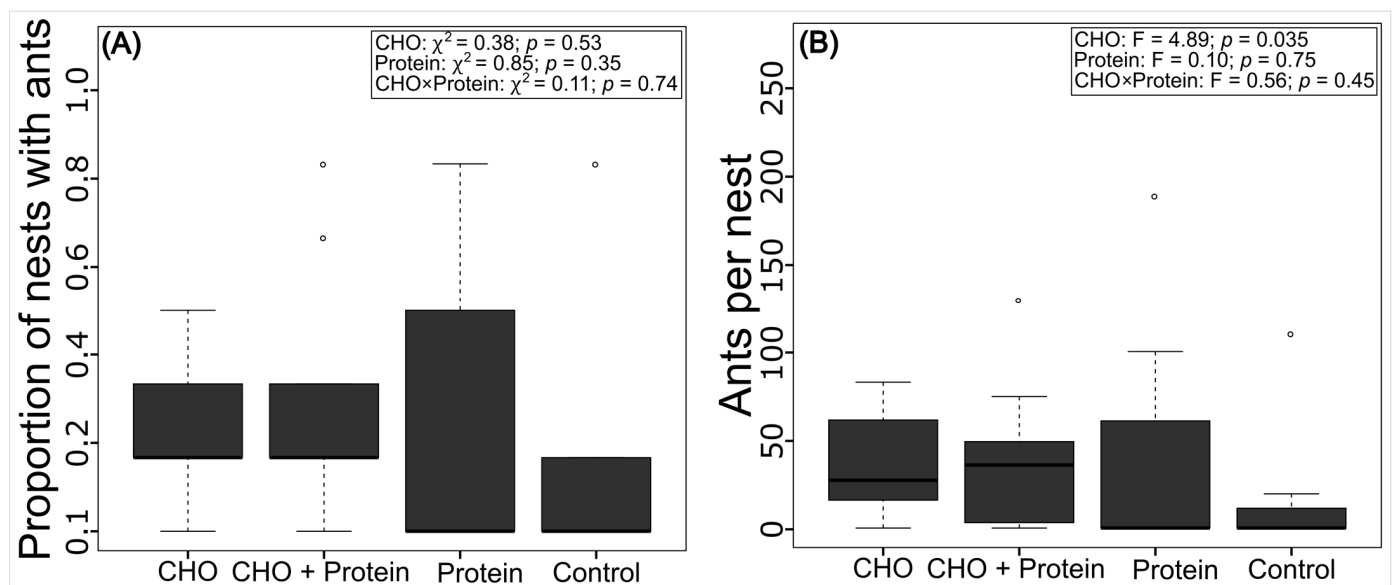
We recorded a total of 43 ant species from 19 genera in the 67 experimental trees (Table S1). In total, 23 species were rare being recorded in just one or two trees. Excluding the rare species, we found that the similarity in species composition was comparatively greater between trees that received sucrose only and those that received sucrose and protein than between these two and those that received protein only or the control trees (Figure 4). Five species (*Azteca* sp. 1, *Camponotus senex*, *C. bonariensis*, *Pseudomyrmex gracilis*, *P. curacaensis* and *Tapinoma* sp. 7) were relatively widespread being found in trees from any treatment. *Brachymyrmex* nr. *aphidicola*, *Camponotus melanoticus*, *C. fastigatus*, *Dorymyrmex* sp. 10, *Ectatomma tuberculatum* and *Pheidole radoszkowskii*, were found mainly in trees receiving sucrose (alone or in combination with protein) whereas *Atta laevigata*, *Camponotus blandus*, *Camponotus substitutus*, *Neoponera villosa* and *Solenopsis basalis* in trees that received protein (alone or in combination with sucrose) (Figure 4).

### 3.3. Colonization of the Artificial Nests

From 192 artificial nests, 44 (22.9%) were colonized by a total of four species of ants, *Camponotus senex*, *Camponotus melanoticus*, *Camponotus bonariensis* and *Pseudomyrmex gracilis*. From these 44 occupied nests, 6 had only ant workers, and 38 contained workers and ant brood (eggs, larvae and/or pupae). Neither carbohydrate nor protein supplementation affected the proportion of artificial nests colonized by ants (Figure 5). However, nests in tree supplemented with carbohydrates had significantly more ant workers per nest than those in trees without carbohydrates, whereas protein supplementation did not affect the abundance of ants in the artificial nests (Figure 5).



**Figure 4.** Two-way cluster dendrogram showing the relative frequency of different ant species in trees subject to different nutrient supplementation treatments.

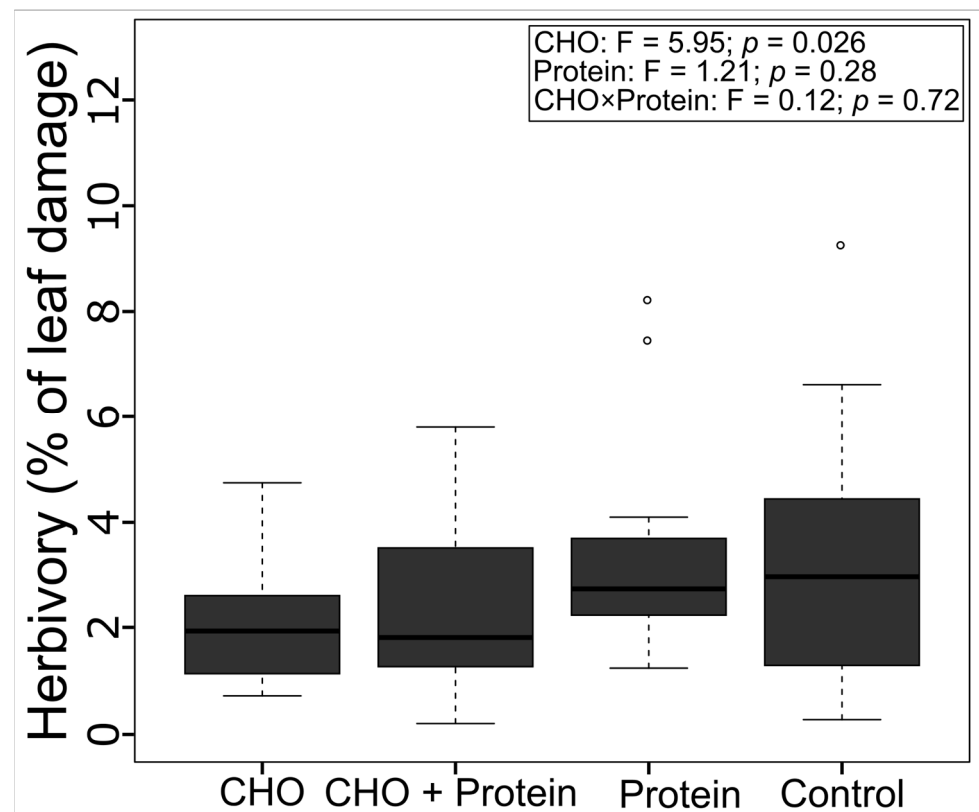


**Figure 5.** Effects of carbohydrate and protein supplementation on the proportion of artificial nests colonized by ants (A) and the overall abundance of ants in the colonized nests (B). Circles represent outlier values.



### 3.4. Leaf Herbivory

Damage by leaf chewing insects was 1.5 times lower in trees that received carbohydrates than in those that did not, whereas leaf damage in trees receiving protein was not significantly different from those that did not (Figure 6).



**Figure 6.** Indirect effect of carbohydrate and protein supplementation on the amount of leaf damage by chewing insects in *Caryocar brasiliense*. Circles represent outlier values.

## 4. Discussion

### 4.1. Colony Level Effects

The results of this study showed that colonies of *Azteca* that received protein had a lower C:N ratio and a higher  $\delta^{15}\text{N}$  than those that were not supplemented with protein. These findings reinforce the notion that omnivorous ants, such as *Azteca*, have some degree of dietary plasticity, depending on the relative availability of different nutrients in the environment [21,57]. Ants of the genus *Azteca* are numerically and behaviorally dominant, with polydomous nests that commonly are distributed through numerous branches of the same plant [58–60]. They are usually associated with coccoid Hemipterans in the vegetation from which they obtain carbohydrate-rich honeydew [17,61], which may help to explain why only the experimental supplementation of protein—but not that of sucrose—affected the trophic level of *Azteca*.

In addition, we found evidence of thermal plasticity in *Azteca* since colonies increased their  $\text{CT}_{\text{max}}$  after receiving sucrose for six months. These results support earlier findings with laboratory colonies of *Azteca chartifex* [6], which showed that *Azteca* support higher temperatures when fed with carbohydrates. The observed increase in the  $\text{CT}_{\text{max}}$  of *Azteca* colonies can be explained because, in insects, carbohydrates are stored in the hemolymph as disaccharides [62], and thus can be used to generate ATP [63], which is essential to cope with thermal stress [64]. In addition, carbohydrates can also be stored as glycogen in muscle and fat bodies [65], and this can generate ATP and metabolic water, and increase evaporative cooling effects through the releasing of bound water, thus improving desicca-

tion resistance [27]. Irrespective of the exact mechanism, it is clear that a carbohydrate-rich diet can help arboreal ants to expand their thermal niche and tolerate higher temperatures, potentially increasing foraging performance and competitive ability [cf. 6]. On the other hand, although protein is necessary to the production of heat shock proteins [28,29], a higher content of protein over carbohydrates on an insect diet is also related to reduced performance and increased mortality [66–69], since the elimination of nitrogenous waste products is highly costly [66,69] and can increase physiological stress [70].

#### 4.2. Community Level Effects

Relative to control trees, trees receiving carbohydrate and/or protein had more ant species and more ant workers foraging on their leaves and branches. Surprisingly however, and in contrast to a similar study that involved the supplementation of these same nutrients to arboreal ants over the short term (7 days) [20], we found that trees receiving only sucrose had more species than those receiving only protein. Nevertheless, in agreement with this same study [20], we found significant variation in species composition between trees subject to different nutrient addition treatments, reinforcing the view that different ant species present different levels of attractiveness to carbohydrates or protein sources. In fact, predatory arboreal ants like *Neoponera villosa* and *Solenopsis basalis* [71] were more frequent in trees supplemented with protein than in those receiving sucrose only.

Contrary to expected, the nutrient addition treatments did not affect the rate of occupancy of the artificial nests placed in the experimental trees. However, nests in trees that were supplemented with carbohydrates had more ants than those that did not receive carbohydrates. This result is in line with a laboratory study in which *Cephalotes pusillus* colonies provided with a diet rich in extrafloral nectar had five times more individuals per colony as compared to control colonies [72]. One possibility is that the increased availability of carbohydrates on trees has reduced the mortality of ant workers [35,69], ultimately increasing colony size. The fact that protein supplementation did not elicit a similar response can perhaps be explained because all the species (three *Camponotus* and one *Pseudomyrmex*) that colonized the artificial nests can overcome their N-deficiencies by means of endosymbiotic bacteria [73,74].

#### 4.3. Effect on the Host Tree

We detected significant lower levels of leaf damage in trees that received sucrose, whereas protein supplementation did not have an effect. These results lend some support to the Fuel for Foraging Hypothesis, since, according to this hypothesis, carbohydrates can fuel more metabolically expensive behavior, increasing aggression and improving foraging performance by ants, which ultimately can lead to an increase in the protection of the host plants [33,35]. Carbohydrate supplementation also increased the number of ant workers occupying the artificial nests, and thus this may well have enhanced the level of protection provided by ants, given that larger colonies are more effective in defending their partner plants against herbivores than smaller ones [75]. Similarly, it is relatively well known that some ant species are more aggressive towards insect herbivores than others (e.g., [76,77]), and in this sense the larger number of ant species foraging in trees supplemented with carbohydrates may have increased the chances that the more aggressive ants were present in these trees [78]. It is also possible that carbohydrate supplementation, by increasing the  $CT_{max}$  of arboreal ants, may have allowed these ants to forage in hotter periods (when, otherwise, they would probably not be active), thus increasing the chances of encountering potential herbivores and ultimately benefiting the host plant [32]. Finally, it is important to mention that herbivory levels were very low among all surveyed trees, and, in this sense, it is likely that the differences in leaf damage we detected may not have a significant influence on plant fitness. However, considering that herbivory pressure can vary temporally and spatially [79,80] and that ant–plant mutualisms are highly facultative [81], the relevance of carbohydrate availability to the strength of ant–plant interactions may be more important

in other contexts, like just after an intense fire [80] or across the different ontogenetic stages of the host-plants [57].

## 5. Conclusions

Overall, the results of this study indicate that the availability of food resources can modulate the population and community ecology of arboreal ants as well as the mutualistic interaction between these ants and their host trees. The supplementation of carbohydrates as well as of protein increased the number of ant workers and the number of ant species foraging on trees. However, and although arboreal ants are thought to be N-limited [18,82,83], it was the supplementation of sucrose—not protein—that elicited most of the responses. Only sucrose-supplemented colonies had an increase in their thermal tolerance, and trees with more carbohydrate available were visited by more ant species, had larger colonies, and were better protected than those that did not receive carbohydrates. In this sense, our results give further support for the idea that carbohydrates can be more important than protein for arboreal ants despite the higher availability of sources of carbohydrate than of protein in the tree canopy [22].

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d15060786/s1>, Table S1: List of the ant species recorded in this study in trees supplemented or not with carbohydrate (CHO) or protein. Numbers represent the number of trees in which the species was recorded.

**Author Contributions:** L.A.Z. and H.L.V. conceived the study and wrote the first draft. L.A.Z., R.E.O.S.L. and A.Q. performed the field work. K.C.N. and R.E.O.S.L. helped with the data analysis and ant species identifications. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** The data that support the findings of this research are available from the authors upon request.

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## References

1. Simpson, S.J.; Raubenheimer, D. The nature of nutrition: A unifying framework. *Aust. J. Zool.* **2012**, *59*, 350–368. [\[CrossRef\]](#)
2. Simpson, S.J.; Raubenheimer, D. The hungry locust. *Adv. Study Behav.* **2000**, *29*, 1–44. [\[CrossRef\]](#)
3. Feldhaar, H. Ant Nutritional Ecology: Linking the Nutritional Niche Plasticity on Individual and Colony-Level to Community Ecology. *Curr. Opin. Insect Sci.* **2014**, *5*, 25–30. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Elser, J.J.; Sterner, R. *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*; Princeton University Press: Princeton, UK, 2002; pp. 262–369.
5. Raubenheimer, D.; Simpson, S.J.; Mayntz, D. Nutrition, Ecology and Nutritional Ecology: Toward an Integrated Framework. *Funct. Ecol.* **2009**, *23*, 4–16. [\[CrossRef\]](#)
6. Bujan, J.; Kaspari, M. Nutrition Modifies Critical Thermal Maximum of a Dominant Canopy Ant. *J. Insect Physiol.* **2017**, *102*, 1–6. [\[CrossRef\]](#)
7. Raubenheimer, D.; Simpson, S.J. Nutritional Ecology and Foraging Theory. *Curr. Opin. Insect Sci.* **2018**, *27*, 38–45. [\[CrossRef\]](#)
8. Tilman, D.; Kilham, S.S.; Kilham, P. Phytoplankton Community Ecology: The Role of Limiting Nutrients. *Annu. Rev. Ecol. Syst.* **1982**, *13*, 349–372. [\[CrossRef\]](#)

9. Simpson, S.J.; Raubenheimer, D. A multi-level analysis of feeding behaviour: The geometry of nutritional decisions. *Philos. Trans. R. Soc. London Ser. B Biol. Sci.* **1993**, *342*, 381–402. [\[CrossRef\]](#)
10. Yanoviak, S.P.; Kaspari, M. Community Structure and the Habitat Template: Ants in the Tropical Forest Canopy and Litter. *Oikos* **2000**, *89*, 259–266. [\[CrossRef\]](#)
11. Kaspari, M.; Yanoviak, S.P. Bait Use in Tropical Litter and Canopy Ants—Evidence of Differences in Nutrient Limitation. *Biotropica* **2001**, *33*, 207–211. [\[CrossRef\]](#)
12. Davidson, D.W. Ecological Stoichiometry of Ants in a New World Rain Forest. *Oecologia* **2005**, *142*, 221–231. [\[CrossRef\]](#)
13. Takahashi, M.Q.; Rothman, J.M.; Raubenheimer, D.; Cords, M. Dietary Generalists and Nutritional Specialists: Feeding Strategies of Adult Female Blue Monkeys (*Cercopithecus mitis*) in the Kakamega Forest, Kenya. *Am. J. Primatol.* **2019**, *81*, e23016. [\[CrossRef\]](#)
14. Law, S.J.; Parr, C. Numerically Dominant Species Drive Patterns in Resource Use along a Vertical Gradient in Tropical Ant Assemblages. *Biotropica* **2020**, *52*, 101–112. [\[CrossRef\]](#)
15. Hölldobler, B.; Wilson, E.O. *The Ants*; Harvard University Press: Cambridge, UK, 1990. [\[CrossRef\]](#)
16. Blüthgen, N.; Gebauer, G.; Fiedler, K. Disentangling a Rainforest Food Web Using Stable Isotopes: Dietary Diversity in a Species-Rich Ant Community. *Oecologia* **2003**, *137*, 426–435. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Davidson, D.W.; Cook, S.C.; Snelling, R.R.; Chua, T.H. Explaining the Abundance of Ants in Lowland Tropical Rainforest Canopies. *Science* **2003**, *300*, 969–972. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Rico-Gray, V.; Oliveira, P.S. *The Ecology and Evolution of Ant-Plant Interactions*; University of Chicago Press: Chicago, IL, USA, 2007; pp. 99–193.
19. Davidson, D.W.; Cook, S.C.; Snelling, R.R. Liquid-Feeding Performances of Ants (Formicidae): Ecological and Evolutionary Implications. *Oecologia* **2004**, *139*, 255–266. [\[CrossRef\]](#)
20. Ribeiro, L.F.; Solar, R.R.C.; Sobrinho, T.G.; Muscardi, D.C.; Schoederer, J.H.; Andersen, A.N. Different Trophic Groups of Arboreal Ants Show Differential Responses to Resource Supplementation in a Neotropical Savanna. *Oecologia* **2019**, *190*, 433–443. [\[CrossRef\]](#)
21. Tsang, T.P.N.; Guénard, B.; Bonebrake, T.C. Omnivorous Ants Are Less Carnivorous and More Protein-limited in Exotic Plantations. *J. Anim. Ecol.* **2020**, *89*, 1941–1951. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Lasmar, C.J.; Bishop, T.R.; Parr, C.L.; Queiroz, A.C.M.; Wilker, I.; Feitosa, R.M.; Schmidt, F.A.; Ribas, C.R. Testing the Context Dependence of Ant Nutrient Preference across Habitat Strata and Trophic Levels in Neotropical Biomes. *Ecology* **2023**, *104*, e3975. [\[CrossRef\]](#)
23. Blüthgen, N.; Feldhaar, H. Food and shelter: How resources influence ant ecology. In *Ant Ecology*; Lach, L., Parr, C., Abbott, K., Eds.; Oxford University Press: Oxford, UK, 2010. [\[CrossRef\]](#)
24. Rowles, A.D.; Silverman, J. Carbohydrate Supply Limits Invasion of Natural Communities by Argentine Ants. *Oecologia* **2009**, *161*, 161–171. [\[CrossRef\]](#)
25. Kaspari, M.; Donoso, D.; Lucas, J.A.; Zumbusch, T.; Kay, A.D. Using Nutritional Ecology to Predict Community Structure: A Field Test in Neotropical Ants. *Ecosphere* **2012**, *3*, art93. [\[CrossRef\]](#)
26. Chown, S.L.; Nicholson, S.W. *Insect Physiological Ecology: Mechanisms and Patterns*, 1st ed.; Oxford University Press: Oxford, UK, 2004; pp. 115–153. [\[CrossRef\]](#)
27. Gibbs, A.G.; Chippindale, A.K.; Rose, M.R. Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. *J. Exp. Biol.* **1997**, *200*, 1821–1832. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Andersen, L.H.; Kristensen, T.N.; Loeschcke, V.; Toft, S.; Mayntz, D. Protein and Carbohydrate Composition of Larval Food Affects Tolerance to Thermal Stress and Desiccation in Adult *Drosophila Melanogaster*. *J. Insect Physiol.* **2010**, *56*, 336–340. [\[CrossRef\]](#) [\[PubMed\]](#)
29. King, A.M.; MacRae, T.H. Insect Heat Shock Proteins During Stress and Diapause. *Annu. Rev. Entomol.* **2015**, *60*, 59–75. [\[CrossRef\]](#)
30. Cerdá, X.; Retana, J.; Cros, S. Thermal Disruption of Transitive Hierarchies in Mediterranean Ant Communities. *J. Anim. Ecol.* **1997**, *66*, 363. [\[CrossRef\]](#)
31. Cerdá, X.; Retana, J.; Manzaneda, A. The Role of Competition by Dominants and Temperature in the Foraging of Subordinate Species in Mediterranean Ant Communities. *Oecologia* **1998**, *117*, 404–412. [\[CrossRef\]](#)
32. Fitzpatrick, G.; Lanan, M.C.; Bronstein, J.L. Thermal Tolerance Affects Mutualist Attendance in an Ant–Plant Protection Mutualism. *Oecologia* **2014**, *176*, 129–138. [\[CrossRef\]](#)
33. Carroll, C.R.; Janzen, D.H. Ecology of foraging by ants. *Annu. Rev. Ecol. Syst.* **1973**, *4*, 231–257. [\[CrossRef\]](#)
34. Davidson, D.W. Resource Discovery versus Resource Domination in Ants: A Functional Mechanism for Breaking the Trade-off: Discovery/Dominance Trade-off in Ants. *Ecological Entomology* **1998**, *23*, 484–490. [\[CrossRef\]](#)
35. Grover, C.D.; Kay, A.D.; Monson, J.A.; Marsh, T.C.; Holway, D.A. Linking Nutrition and Behavioural Dominance: Carbohydrate Scarcity Limits Aggression and Activity in Argentine Ants. *Proc. R. Soc. B* **2007**, *274*, 2951–2957. [\[CrossRef\]](#)
36. Janzen, D.H. Allelopathy by Myrmecophytes: The Ant Azteca as an Allelopathic Agent of Cecropia. *Ecology* **1969**, *50*, 147–153. [\[CrossRef\]](#)
37. Davidson, D.W.; Longino, J.T.; Snelling, R.R. Pruning of Host Plant Neighbors by Ants: An Experimental Approach. *Ecology* **1988**, *69*, 801–808. [\[CrossRef\]](#)
38. Ness, J.H.; Morris, W.F.; Bronstein, J.L. For Ant-Protected Plants, the Best Defense Is a Hungry Offense. *Ecology* **2009**, *90*, 2823–2831. [\[CrossRef\]](#)

39. Kay, A.D.; Zumbusch, T.; Heinen, J.L.; Marsh, T.C.; Holway, D.A. Nutrition and Interference Competition Have Interactive Effects on the Behavior and Performance of Argentine Ants. *Ecology* **2010**, *91*, 57–64. [\[CrossRef\]](#)
40. Pacelhe, F.T.; Costa, F.V.; Neves, F.S.; Bronstein, J.; Mello, M.A.R. Nectar Quality Affects Ant Aggressiveness and Biotic Defense Provided to Plants. *Biotropica* **2019**, *51*, 196–204. [\[CrossRef\]](#)
41. Kost, C.; Heil, M. Increased Availability of Extrafloral Nectar Reduces Herbivory in Lima Bean Plants (*Phaseolus Lunatus*, Fabaceae). *Basic Appl. Ecol.* **2005**, *6*, 237–248. [\[CrossRef\]](#)
42. González-Teuber, M.; Silva Bueno, J.C.; Heil, M.; Boland, W. Increased Host Investment in Extrafloral Nectar (EFN) Improves the Efficiency of a Mutualistic Defensive Service. *PLoS ONE* **2012**, *7*, e46598. [\[CrossRef\]](#)
43. Savolainen, R.; Vepsäläinen, K.; Vepsäläinen, K. A Competition Hierarchy among Boreal Ants: Impact on Resource Partitioning and Community Structure. *Oikos* **1988**, *51*, 135. [\[CrossRef\]](#)
44. Parr, C.L.; Gibb, H. Competition and the role of dominant ants. In *Ant Ecology*; Lach, L., Parr, C., Abbott, K., Eds.; Oxford University Press: Oxford, UK, 2010; pp. 77–96.
45. Cerda, X.; Arnan, X.; Retana, J. Is competition a significant hallmark of ant (Hymenoptera: Formicidae) ecology. *Myrmecol. News* **2013**, *18*, 131–147.
46. Camarota, F.; Vasconcelos, H.L.; Koch, E.B.A.; Powell, S. Discovery and Defense Define the Social Foraging Strategy of Neotropical Arboreal Ants. *Behav. Ecol. Sociobiol.* **2018**, *72*, 110. [\[CrossRef\]](#)
47. Eiten, G. The cerrado vegetation of Brazil. *Bot. Rev.* **1972**, *38*, 201–341. [\[CrossRef\]](#)
48. Powell, S.; Costa, A.N.; Lopes, C.T.; Vasconcelos, H.L. Canopy Connectivity and the Availability of Diverse Nesting Resources Affect Species Coexistence in Arboreal Ants: Species Coexistence in Arboreal Ants. *J. Anim. Ecol.* **2011**, *80*, 352–360. [\[CrossRef\]](#)
49. Bluthgen, N.; Gottsberger, G.; Fiedler, K. Sugar and Amino Acid Composition of Ant-Attended Nectar and Honeydew Sources from an Australian Rainforest. *Austral. Ecol.* **2004**, *29*, 418–429. [\[CrossRef\]](#)
50. Dussutour, A.; Simpson, S.J. Description of a Simple Synthetic Diet for Studying Nutritional Responses in Ants. *Insect. Soc.* **2008**, *55*, 329–333. [\[CrossRef\]](#)
51. Tillberg, C.V.; McCarthy, D.P.; Dolezal, A.G.; Suarez, A.V. Measuring the Trophic Ecology of Ants Using Stable Isotopes. *Insect. Soc.* **2006**, *53*, 65–69. [\[CrossRef\]](#)
52. Rasband, W. Image J Documentation. 2013. Available online: <http://rsb.info.nih.gov/ij/docs/index.html> (accessed on 12 January 2021).
53. R Core Team. R: A Language and Environment for Statistical Computing. 2022. Available online: <https://www.R-project.org/> (accessed on 20 January 2021).
54. Fox, J.; Weisberg, S. Using car and effects Functions in Other Functions. *Using Car Eff. Funct. Other Funct.* **2020**, *3*, 1–5.
55. Russell, V.L. Emmeans: Estimated Marginal Means, aka Least-Squares Means. R Package Version 1.6.2-1. 2021. Available online: <https://CRAN.R-project.org/package=emmeans> (accessed on 20 January 2021).
56. Peck, J.E. *Multivariate Analysis for Community Ecologists: Step-by-Step Using PC-ORD*; MJM Software Design: Gleneden Beach, OR, USA, 2010; pp. 1–162.
57. Duyck, P.-F.; Lavigne, A.; Vinatier, F.; Achard, R.; Okolle, J.N.; Tixier, P. Addition of a New Resource in Agroecosystems: Do Cover Crops Alter the Trophic Positions of Generalist Predators? *Basic Appl. Ecol.* **2011**, *12*, 47–55. [\[CrossRef\]](#)
58. Dejean, A.; Grangier, J.; Leroy, C.; Orivel, J. Predation and Aggressiveness in Host Plant Protection: A Generalization Using Ants from the Genus *Azteca*. *Naturwissenschaften* **2009**, *96*, 57–63. [\[CrossRef\]](#)
59. Koch, E.B.A.; Camarota, F.; Vasconcelos, H.L. Plant Ontogeny as a Conditionality Factor in the Protective Effect of Ants on a Neotropical Tree. *Biotropica* **2016**, *48*, 198–205. [\[CrossRef\]](#)
60. Dejean, A.; Orivel, J.; Leponce, M.; Compin, A.; Delabie, J.H.C.; Azémar, F.; Corbara, B. Ant–Plant Relationships in the Canopy of an Amazonian Rainforest: The Presence of an Ant Mosaic. *Biol. J. Linn. Soc.* **2018**, *125*, 344–354. [\[CrossRef\]](#)
61. Johnson, C.; Agosti, D.; Delabie, J.H.; Dumpert, K.; Williams, D.J.; Tschirnhaus, M.V.; Maschwitz, U. Acropyga and Azteca Ants (Hymenoptera: Formicidae) with Scale Insects (Sternorrhyncha: Coccoidea): 20 Million Years of Intimate Symbiosis. *Am. Mus. Novit.* **2001**, *3335*, 1–18. [\[CrossRef\]](#)
62. Thompson, S.N. Trehalose—The insect ‘blood’ sugar. *Adv. Insect Physiol.* **2003**, *31*, 205–285. [\[CrossRef\]](#)
63. Suarez, R.K.; Lighton, J.R.; Joos, B.; Roberts, S.P.; Harrison, J.F. Energy Metabolism, Enzymatic Flux Capacities, and Metabolic Flux Rates in Flying Honeybees. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 12616–12620. [\[CrossRef\]](#)
64. Sokolova, I.M. Energy-Limited Tolerance to Stress as a Conceptual Framework to Integrate the Effects of Multiple Stressors. *Integr. Comp. Biol.* **2013**, *53*, 597–608. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Sacktor, B. Regulation of Intermediary Metabolism, with Special Reference to the Control Mechanisms in Insect Flight Muscle. In *Advances in Insect Physiology*; Elsevier: Amsterdam, The Netherlands, 1970; Volume 7, pp. 267–347, ISBN 978-0-12-024207-8. [\[CrossRef\]](#)
66. Lee, K.P.; Simpson, S.J.; Clissold, F.J.; Brooks, R.; Ballard, J.W.O.; Taylor, P.W.; Soran, N.; Raubenheimer, D. Lifespan and Reproduction in *Drosophila*: New Insights from Nutritional Geometry. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 2498–2503. [\[CrossRef\]](#)
67. Makalkov, A.A.; Simpson, S.J.; Zajitschek, F.; Hall, M.; Dessman, J.; Clissold, F.J.; Raubenheimer, D.; Bonduriansky, R.; Brooks, R.C. Sex specific fitness effects of nutrient intake on reproduction and lifespan. *Curr. Biol.* **2008**, *14*, 1062–1066. [\[CrossRef\]](#)
68. Dussutour, A.; Simpson, S.J. Communal Nutrition in Ants. *Curr. Biol.* **2009**, *19*, 740–744. [\[CrossRef\]](#)



69. Kay, A.D.; Shik, J.Z.; Van Alst, A.; Miller, K.A.; Kaspari, M. Diet Composition Does Not Affect Ant Colony Tempo: Diet Composition Does Not Affect Ant Colony Tempo. *Funct. Ecol.* **2012**, *26*, 317–323. [[CrossRef](#)]
70. O'Donnell, M. Insect excretory mechanisms. *Adv. Insect Physiol.* **2008**, *35*, 1–122. [[CrossRef](#)]
71. Zuanon, L.A. Niche partitioning and Thermal tolerance of arboreal ants in a Neotropical savanna. Master's Thesis, Universidade Federal de Uberlândia, Uberlândia, Brazil, 2018.
72. Byk, J.; Del-Claro, K. Ant–Plant Interaction in the Neotropical Savanna: Direct Beneficial Effects of Extrafloral Nectar on Ant Colony Fitness. *Popul. Ecol.* **2011**, *53*, 327–332. [[CrossRef](#)]
73. Russell, J.A.; Moreau, C.S.; Goldman-Huertas, B.; Fujiwara, M.; Lohman, D.J.; Pierce, N.E. Bacterial Gut Symbionts Are Tightly Linked with the Evolution of Herbivory in Ants. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 21236–21241. [[CrossRef](#)]
74. Hu, Y.; Sanders, J.G.; Łukasik, P.; D'Amelio, C.L.; Millar, J.S.; Vann, D.R.; Lan, Y.; Newton, J.A.; Schotanus, M.; Kronauer, D.J.C.; et al. Herbivorous Turtle Ants Obtain Essential Nutrients from a Conserved Nitrogen-Recycling Gut Microbiome. *Nat. Commun.* **2018**, *9*, 964. [[CrossRef](#)] [[PubMed](#)]
75. Rocha, C.F.D.; Bergallo, H.G. Bigger Ant Colonies Reduce Herbivory and Herbivore Residence Time on Leaves of an Ant-Plant: Azteca Muelleri vs. Coelomera Ruficornis on Cecropia Pachystachya. *Oecologia* **1992**, *91*, 249–252. [[CrossRef](#)] [[PubMed](#)]
76. Rico-Gray, V.; Thien, L.B. Effect of Different Ant Species on Reproductive Fitness of Schomburgkia Tibicinis (Orchidaceae). *Oecologia* **1989**, *81*, 487–489. [[CrossRef](#)]
77. Del-Claro, K.; Marquis, R.J. Ant Species Identity Has a Greater Effect than Fire on the Outcome of an Ant Protection System in Brazilian Cerrado. *Biotropica* **2015**, *47*, 459–467. [[CrossRef](#)]
78. Yachi, S.; Loreau, M. Biodiversity and Ecosystem Productivity in a Fluctuating Environment: The Insurance Hypothesis. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 1463–1468. [[CrossRef](#)]
79. Fagundes, R.; Dáttilo, W.; Ribeiro, S.P.; Rico-Gray, V.; Jordano, P.; Del-Claro, K. Differences among Ant Species in Plant Protection Are Related to Production of Extrafloral Nectar and Degree of Leaf Herbivory. *Biol. J. Linn. Soc.* **2017**, *122*, 71–83. [[CrossRef](#)]
80. Vasconcelos, H.L.; Koch, E.B.A.; Camarota, F.; Tito, R.; Zuanon, L.A.; Maravalhas, J. Severe Fires Alter the Outcome of the Mutualism between Ants and a Neotropical Savanna Tree. *Biol. J. Linn. Soc.* **2020**, *131*, 476–486. [[CrossRef](#)]
81. Bronstein, J.L.; Alarcón, R.; Geber, M. The evolution of plant–insect mutualisms. *New Phytol.* **2006**, *172*, 412–428. [[CrossRef](#)]
82. Blüthgen, N.; Fiedler, K. Competition for composition: Lessons from nectar-feeding ant communities. *Ecology* **2004**, *85*, 1479–1485. [[CrossRef](#)]
83. Nepi, M.; Soligo, C.; Nocentini, D.; Abate, M.; Guarnieri, M.; Cai, G.; Bini, L.; Puglia, M.; Bianchi, L.; Pacini, E. Amino Acids and Protein Profile in Floral Nectar: Much More than a Simple Reward. *Flora Morphol. Distrib. Funct. Ecol. Plants* **2012**, *207*, 475–481. [[CrossRef](#)]

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