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# Testing the context dependence of ant nutrient preference across habitat strata and trophic levels in Neotropical biomes

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## Abstract

Animals are integrated into the wider ecosystem via their foraging and behavior. The compensation hypothesis predicts that animals target their foraging efforts (i) toward nutrients that are scarce in the environment and (ii) toward nutrients that are not present in the usual diet of species, which varies across trophic levels. Understanding how foraging for resources varies locally, such as across habitat strata, and trophic levels will help to elucidate the links between the local environment and communities to the ecological functions that animals mediate. We examined whether the relative resource use of ants varies consistently along a habitat strata gradient and across trophic levels across Neotropical biomes. We placed 4500 baited tubes, each containing one of five liquid resources (sugar, amino acid, lipid, sodium, and distilled water) in one of three habitat strata (subterranean, epigeaic, and arboreal) across 60 transects in Amazon, Atlantic Forest, Caatinga, Cerrado, Pampa, and Pantanal biomes. We assessed the relative resource use of all ants across the habitat strata and among two different trophic groups across biomes. The relative preference for sugar increased from subterranean to arboreal strata in all biomes, while the relative preference for lipids decreased at

this gradient in five biomes. We also found that in general sugar-consuming ants foraged more for sugar and less for lipids than predatory ants across biomes. Conversely, we found no consistency across biomes in nutrient preference of amino acid and sodium across habitat strata or trophic levels. Overall, our results indicate sugar limitation in the arboreal stratum and lipid limitation on the ground across biomes and that the trophic level of ants strongly determines their foraging efforts—possibly because ants try to fix their dietary nutrient imbalances. Hence, our findings suggest strong local niche partitioning of sugar and lipid use across habitat strata and trophic levels and that other large spatial scale processes influence the local amino acid and sodium dynamics.

#### KEYWORDS

foraging activity, foraging behavior, habitat strata, niche partitioning, nutritional ecology, trophic ecology

## INTRODUCTION

Animals forage for different nutrients to meet their needs to grow, survive, and reproduce (Raubenheimer et al., 2009). These nutrients, however, are patchily distributed across space and time, and organisms at different trophic levels can access them differently (Hou et al., 2021; Raubenheimer et al., 2009). This differential spatial distribution of nutrients, and variation in the dietary requirements of species, may control animal nutrient preference in two ways described by the compensation hypothesis (Davidson, 2005; Kaspari et al., 2012). In terms of space, the hypothesis posits that animals tend to increase their foraging efforts for the rarest nutrient relative to demand in the environment. This effect is invariant among species and can drive animal foraging behavior at large (Kaspari et al., 2020; Lasmar et al., 2021) and small spatial scales (Yanoviak & Kaspari, 2000). On the other hand, in terms of trophic level, the hypothesis posits that species at different trophic levels are limited by different nutrients (Mayntz et al., 2005; Raubenheimer et al., 2009; Wilder et al., 2013), and that species tend to increase their foraging efforts for nutrients not fully supplied in their diet (Kaspari et al., 2008; Wilder et al., 2013). By foraging for nutrients, animals integrate themselves into the complex webs of interactions and functioning within ecosystems (Dudley et al., 2012; Maisey et al., 2021). Consequently, understanding how nutrient preference varies locally is a key step in linking variation in the local abiotic environment to local biotic composition and activity, and to the ecosystem functions that ecological communities mediate (Kaspari et al., 2012).

Following the compensation hypothesis concerning nutrient variation in space, at local scales, nutrient preference would be expected to vary among habitat strata

because different vertical habitats are dominated by different kinds of food webs (Yanoviak & Kaspari, 2000). Canopy or arboreal food webs are mainly based on living plant matter (i.e., green food web; Davidson et al., 2003), in which the food web presents carbon:nitrogen ratios of 40:1 or more (Yanoviak & Kaspari, 2000). Conversely, communities on the ground rely more on detritus-based food webs (i.e., brown food web; Kaspari & Yanoviak, 2009; Moore et al., 2004) in which the decomposition process increases nitrogen availability, resulting in a lower carbon:nitrogen ratio of 10:1. As both carbon and nitrogen are critical for life maintenance and body structure (Raubenheimer et al., 2009), animals in the arboreal stratum tend to increase their foraging for nitrogen-based food (invertebrates, Law & Parr, 2020; primates, Takahashi et al., 2019), while on the forest ground, they increase foraging for carbon-based food (Law & Parr, 2020; Yanoviak & Kaspari, 2000). In addition to these patterns of carbon and nitrogen, differences may also be found among habitat strata in relation to the availability of sodium, another important macronutrient for the physiological processes of animals (Kaspari, 2020). The ground surface receives sodium washed from the entire ecosystem, as well as from the decomposition process and from the excretion of animals (Clay et al., 2015; Kaspari, 2020). Much less sodium is available for consumers within the arboreal stratum compared with the ground (Kaspari, 2020) because plants use essential minerals other than sodium to maintain their osmotic equilibrium (Subbarao et al., 2003). This may lead to a greater foraging effort for sodium in the arboreal stratum than on the ground level (Kaspari et al., 2008). Consequently, fine-scale variation in nutrient availability can drive differences in animal nutrient preference and foraging behavior.

In addition to spatial variation in nutrient preference, there can also be variation across trophic levels. Nutrients that limit the growth, survival, and reproduction of species can also vary across trophic levels (Behmer, 2009; Jensen et al., 2012). The plant-based diet of primary consumers and animal-based diet of predators, provide different amounts of essential nutrients: with increasing trophic levels, nutrient assimilation changes from a plant carbohydrate and fat-based diet to a nitrogen and sodium-based diet (Davidson et al., 2003; Kaspari, 2020; Wilder et al., 2013). Because of this, primary consumers can increase their foraging efforts for nitrogen and sodium sources to compensate for their nutrient imbalances caused by their typical diet (Hou et al., 2021; Kaspari, 2020). Conversely, predators may focus their foraging on sugar and lipids because energetic nutrients become increasingly limiting at higher trophic levels in both terrestrial (Kaspari et al., 2012; Wilder et al., 2013) and aquatic environments (Machovsky-Capuska & Raubenheimer, 2020). Thus, substantial differences in foraging behavior between low and high trophic levels influence how species interact with the abiotic and biotic environment.

Current knowledge of nutrient preference is highly localized, and we have a poor understanding of how general the relationship between habitat strata, trophic position, and foraging activity really is. For example, most studies have evaluated differences in foraging behavior between arboreal and epigeic strata (e.g., Law & Parr, 2020; Yanoviak & Kaspari, 2000), and we do not know how nutrient preference varies when accounting for subterranean stratum. The subterranean stratum is supported by the brown food web (Wardle et al., 2004; Zou et al., 2016), and it differs substantially from above-ground strata in terms of environmental conditions and the availability of resources (De Deyn & Van der Putten, 2005). This possibly leads organisms in the subterranean stratum to being even more reliant on the brown-food web than above-ground strata are. Since food webs and ecological processes from above and below-ground are connected (Scherber et al., 2010), assessing variation in foraging behavior across strata would refine our understanding of the functioning of local communities. Additionally, previous comparisons of resource use among strata evaluated differences between pairs of resources (e.g., using sugar and protein baits to represent carbon and nitrogen sources—Law & Parr, 2020; Yanoviak & Kaspari, 2000, but see Hou et al., 2021). Animals require a range of macro- and micronutrients; therefore, assessing more nutrients provides a more realistic picture of animal nutrient imbalances (Kaspari & Powers, 2016; Raubenheimer et al., 2009). Last, most studies that have evaluated patterns in resource use were conducted in a single region within the same vegetation type and under the same species pool influence. Therefore, we do not know

whether patterns of resource use are due to the context-dependence of the sampled region or if we may really infer general rules on ecological behavior. It is necessary to sample different ecoregions across large spatial scales covering a broader range of habitat strata and nutrient types to best understand foraging behavior.

Ants (Hymenoptera: Formicidae) are an excellent model for understanding nutrient preference among habitat strata and trophic levels. They are remarkably abundant in terrestrial habitats, from the subterranean stratum to the tree canopy (Hölldobler & Wilson, 2008; Schultheiss et al., 2022) and represent an enormous diversity of species that occupy several trophic levels (Baccaro et al., 2015). Therefore, here, we evaluated whether the nutrient preferences of ant assemblages across different biomes are consistent in the Neotropics. We assessed ant foraging behavior along a habitat strata gradient (subterranean, epigeic, and arboreal) to investigate the relative resource use of four resource types (sugar, lipids, amino acids, and sodium). Specifically, we asked: does the relative resource use of ants vary along a habitat strata gradient and across the trophic position of the ant species (contrasting specialized carbohydrate feeders with specialized predators)? If so, is that relative resource use variation consistent across different biomes? For this, according to the compensation hypothesis, we predict that (i) relative amino acid and sodium preference will increase from subterranean to arboreal strata, while relative sugar and lipid preference will decrease regardless of the biome. Moreover, we predict that (ii) in all biomes, primary consumers (the sugar-consuming ants) will forage more for both amino acid and sodium resources than predatory ants, while predatory ants will forage more for sugar and lipids than sugar-consuming ants—because these nutrients are rarer in the natural diets of these trophic groups.

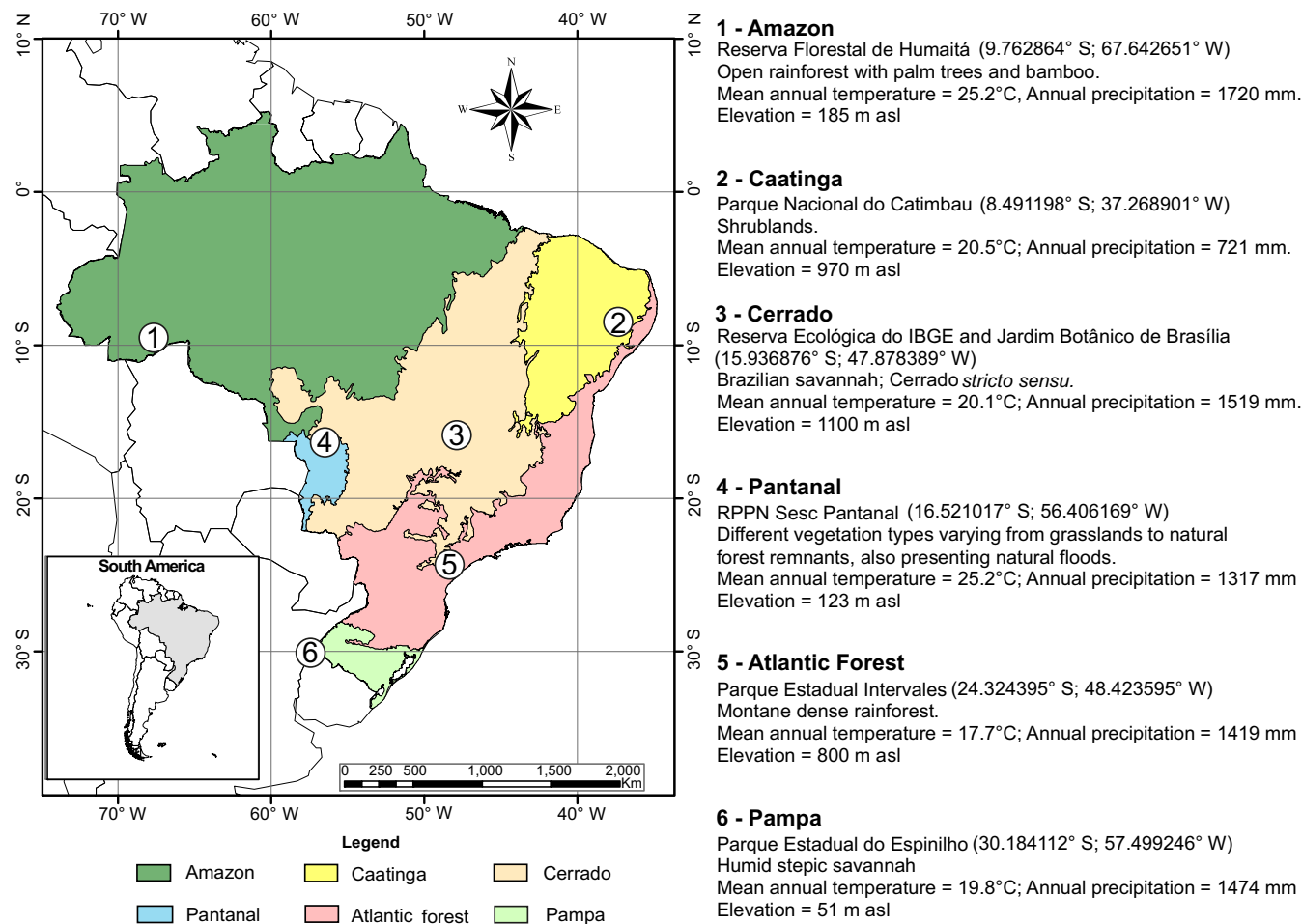
## MATERIALS AND METHODS

### Study area

We sampled in protected areas across six Brazilian biomes that differ substantially in terms of their vegetation types and climate (Figure 1; see also Lasmar et al., 2021). The sampled regions are separated by at least 900 km and at most by 3350 km. The sites are spread across 22° of latitude and 30.5° of longitude (Figure 1).

### Sampling of foraging ants

In each biome, we installed 10 transects as sampling units ( $6 \times 10 = 60$  transects in total) separated by at least



**FIGURE 1** Six Brazilian biomes and the specific location where samples were carried out. Details of sampling regions are described per biome in the right panel, indicating the protected area name and location, vegetation type, climate, and elevation. m asl, meters above sea level.

1 km on average. Each transect was 740 m long with 75 sampling points, each separated by 10 m. At each sampling point, we provided one of five liquid food resources into one of three habitat strata (subterranean, epigeaic, and arboreal). Hence, within each transect, each resource and strata combination ( $5 \times 3 = 15$  combinations) were repeated five times in sequence along each transect. Consequently, there were five pseudoreplicates of the 15 resource/habitat strata combinations per transect ( $5 \text{ pseudoreplicates} \times 5 \text{ resource types} \times 3 \text{ habitat strata} = 75 \text{ sampling points per transect}$ ), giving a total of 750 sampling points per biome ( $75 \text{ sampling points} \times 10 \text{ transects} = 750$ ) and 4500 sampling points across the study ( $750 \text{ points} \times 6 \text{ biomes} = 4500 \text{ points in total}$ ).

We placed the food resources in 50-mL Fisher Scientific polypropylene centrifuge tubes that had a 5 cm cotton ball containing 10 mL of solutions in distilled water for each resource type. The solutions were: 1% sodium (NaCl), 20% sugar (CHO, made with sucrose), 20% amino acids (AA, made with unflavored whey protein

isolate which contains L-glutamine and other branched-chain amino acids such as leucine, isoleucine, and valine), lipids (100% extra virgin olive oil, without water), and distilled water as a control. Analogous resource solutions have successfully been used in previous studies (e.g., Fowler et al., 2014; Guariento et al., 2021; Lasmar et al., 2021; Peters et al., 2014).

The placement of the tubes was different for each habitat strata. In the subterranean strata, we placed the tubes in a plastic box (4.5 cm high, 8 cm wide and 15 cm long) with an access hole c. 1.5 cm in diameter on each side. We buried the box c. 20 cm below the soil surface. In the epigeaic strata, we placed the baited tubes horizontally on the ground. For the arboreal strata, we fixed the tubes horizontally c. 2 m above the ground on the tree trunk closest to the sampling point, using a piece of adhesive and a string to keep the tube in place. The placement of the baited tubes began at 7:00 am for each transect except in the Caatinga biome. We delayed the baiting arrangement in Caatinga until 1:00 pm because of

morning rainfall that could potentially reduce ant activity. We restricted all of our sampling to take place during only sunny or partially cloudy days and never during rainfall events. We left tubes operating for 3 h. After that, we closed the tubes to sample the ants inside. We considered a tube as visited when there was at least one ant individual inside.

To understand whether our baits were representatively sampling from the wider ant community, we also sampled with pitfall traps, which is the most representative sampling method of ants (Schmidt et al., 2022). The pitfall trap data act as a proxy of ant abundance for all 60 of the baiting transects across the six biomes. By comparing ant abundance in pitfall traps and the ant foraging activity recorded by the baited tubes, we would know: (i) whether ants did not visit a bait because they did not occur in this habitat or (ii) whether baits did not attract ants due to methodological issues; (e.g., high ant abundance in pitfall traps but low ant foraging in baiting tubes, possibly indicating a problem with our baiting experiment). Thus, we started the pitfall sampling transects after the baiting experiment to avoid possible ant population depletions caused by pitfall traps (Lasmar et al., 2017). Pitfall transects were placed 20 m away in parallel from the corresponding baiting transects, totaling 60 pitfall transects across the entire study. Pitfall transects were 200 m long and had five sampling points separated by 50 m. At each sampling point, we collected ants at three habitat strata using arboreal, epigeaic, and subterranean pitfall traps. Arboreal traps were installed at 1.5 m above the ground, tied in the middle of a tree trunk. Epigeaic traps were installed at the ground level and subterranean traps were buried at 20 cm under the ground. Pitfall traps were 8 cm in diameter and 12 cm in depth, and contained a 200 mL solution of water, salt (0.4%) and liquid soap (0.6%). Arboreal and epigeaic traps had a lid to cover and protect them against rain and sunlight. Subterranean traps were closed with lids and had four lateral holes of *c.* 1.5 cm in diameter on the sides. All pitfall traps remained in the field operating for 48 h.

We counted all ant worker individuals from both baited tubes and pitfall traps in the laboratory. For baited tubes, we also identified all collected ant workers to genera following Baccaro et al. (2015), and whenever possible to species using the relevant literature and through comparison with the ant reference collection at the “Laboratório de Ecologia de Formigas” of the Universidade Federal de Lavras (UFLA) and Entomological Collection Padre Jesus Santiago Moure of Universidade Federal do Paraná (DZUP). Sources for species-level identification of the ant genera collected are described in Lasmar (2022) Dataset. All voucher specimens are deposited in the reference collection of DZUP

(query details: <https://figshare.com/s/bd1082fbaa07a6f5ae5d>; [Lasmar et al. Ecology—Data.xlsx]).

We classified the ants of baited tubes into two trophic groups: sugar-consuming and predatory ants to represent low and high trophic levels based on relevant literature (see Lasmar, 2022 Dataset). Sugar-consuming were ants that predominantly directly feed on plant sugar sources such as floral and extrafloral nectaries and indirectly by trophobiosis. Predatory ants were ants that predominantly feed on other living animals.

## Statistical analyses

### Validating the baiting experiments

We considered ant foraging activity as the proportion of baited tubes that were visited by ants per habitat strata and per transect. This is highly correlated with the number of individual ant workers captured in each of the baited tubes (see Lasmar et al., 2021). We also used the mean number of worker ants captured in pitfall traps per habitat strata per transect as another proxy of abundance. To estimate the relationship between ant foraging activity from baited tubes and our proxy of total ant foragers from the pitfall traps, we ran a generalized linear mixed model (GLMM) using the *lmer* function from the “lme4” package in R (Bates et al., 2014). Ant foraging activity from baited tubes was the response variable and was logit-transformed to meet Gaussian assumptions (Warton & Hui, 2011). The explanatory variables were the interaction of mean of ant worker individuals from pitfall traps and habitat strata and the interaction of ant worker individuals<sup>2</sup> and habitat strata to also represent a unimodal function. Each datapoint was the estimate of ant abundance or activity per habitat strata per transect. We set each transect as the random variable since all three habitat strata were sampled in the same transect.

### Does the relative resource use of ants vary along a habitat strata gradient, and is this variation consistent across different biomes?

To answer these questions, we calculated a measure of relative resource use as a response variable. We calculated this across all ants as the number of baited tubes of a given resource that were visited by ants, divided by the total number of visited baited tubes of all resource types (including control tubes) per habitat strata and per transect. Consequently, this was done for each resource type ( $n = 5$ , including control), each stratum ( $n = 3$ ), each transect ( $n = 60$ ), for a total of 900 datapoints.

This metric measures how popular a given resource was in a particular stratum and transect relative to how much foraging occurred at that location (Lasmar et al., 2021).

To test our first prediction related to whether ants change their preference for different resources along a habitat strata gradient and if these changes are consistent across different biomes, we performed two GLMMs. In the first model, we used relative resource use (logit-transformed) for all ants as the response variable. To evaluate relative resource use across a habitat strata gradient, we transformed the habitat strata into a continuous variable to represent a habitat strata gradient by attributing levels for each stratum (0 to subterranean stratum, 0.5 to epigaeic stratum, and 1 to arboreal stratum). Thus, the interaction of resource type, habitat strata and biome were used as explanatory variables (fixed effects). We used transect as a random variable to account for pseudoreplication of different strata within the same transect. We also compared the results of the previous model that used habitat strata as a continuous variable to a second model in which we kept the habitat strata as a categorical variable. Thus, in the second model, we also used relative resource use (logit-transformed) for all ants as the response variable, however, we had the interaction of resource type, habitat strata (categorical variable), and biome as explanatory variables (fixed effects), and transect as a random variable. If the interaction factor of the second model was significant (i.e.,  $p \leq 0.05$ ), we performed a contrast analysis per resource type across our three habitat strata and per biome using the *emmeans* function in “emmeans” package in R (Lenth, 2019) to further understand the differences between the relative use of different food resources. Contrast analysis output are in the Appendix S1: Figure S3 and Tables S3–S8.

**Does the relative resource use of ants vary across ant trophic position, and is this variation consistent across different biomes?**

We calculated the relative resource use for each ant trophic group (sugar-consuming or predatory ants) in a similar way as above but subsetting the data by trophic group. For this analysis, we excluded ants that were omnivorous (i.e., neither predatory nor sugar-consuming) because omnivorous ants change their trophic position in response to environmental resource availability (Gibb & Cunningham, 2011). Consequently, the calculation of relative resource use per trophic group was done for each resource type ( $n = 5$ ), each stratum ( $n = 3$ ), each transect ( $n = 60$ ), and for the two trophic groups separately ( $n = 2$ ), with a total  $n = 1800$ .

To test our second prediction related to the differences in resource use between sugar-consuming and predatory ants across different biomes, we also performed a GLMM. We modeled relative resource use logit-transformed as the response variable and the interaction between resource type, trophic group, and biome as the explanatory variables (fixed effects). We used habitat strata nested in transect as a random variable. We included the habitat strata as a random effect to control for differences in resource use among habitat strata. Again, once the interaction factor was significant, we ran a contrast analysis for each resource use between two trophic groups and per biome using *emmeans* function in “emmeans” package in R (Lenth, 2019). Contrast analysis output are in the Appendix S1: Figure S4 and Table S10. To facilitate the data visualization, we also transformed the trophic position of ants into continuous variables by attributing levels for each stratum (0 to predatory ants and 1 to sugar-consuming ants). We performed all the analyses in R version 4.1.0 (R Core Team, 2021).

## RESULTS

In total, we captured 18,411 ant workers in pitfall traps. Additionally, 26,752 ant workers belonging to 255 ant species and 40 genera visited our baited tubes across the study (see Lasmar, 2022). Across biomes, 5% to 13% of ant species occurred in baits in all three habitat strata, 3.6% to 15% occurred in both epigaeic and arboreal strata, 8.3% to 21.4% occurred in both subterranean to epigaeic strata, and 0% to 3.6% occurred in both subterranean and arboreal strata (Appendix S1: Figure S1). Furthermore, we found a considerable number of habitat specialist ants across biomes as 10.7% to 20.2% of ant species occurred only at arboreal baits, 20.31% to 60% occurred only at epigaeic baits, and 5% to 23.4% occurred only at subterranean baits (Appendix S1: Figure S1).

### Validating the baiting experiments

Ant foraging activity, as estimated from the baited tubes, was not affected by the interaction of habitat strata and mean ant worker individuals ( $F_{2,145.6} = 2.63$ ,  $p = 0.074$ ) or by the interaction of habitat strata habitat strata and mean ant worker individuals<sup>2</sup> ( $F_{2,151.2} = 0.92$ ,  $p = 0.398$ ) and neither by the single variable ant worker individuals<sup>2</sup> ( $F_{1,169.6} = 0.02$ ,  $p = 0.867$ ). However, ant foraging activity was affected by habitat strata ( $F_{2,133.8} = 6.08$ ,  $p = 0.002$ ) and was positively correlated with mean ant worker individuals from the pitfall traps ( $F_{1,166.5} = 4.55$ ,  $p = 0.034$ , Appendix S1: Figure S2, marginal  $R^2 = 0.53$ , conditional

$R^2 = 0.63$ ). Consequently, baits in transects that presented low ant visitation rates were probably caused by low ant abundance within the environment, rather than due to any methodological issues with the baits themselves. The greatest foraging activity was in the epigeaic stratum, followed by the arboreal and subterranean strata (Appendix S1: Figure S2).

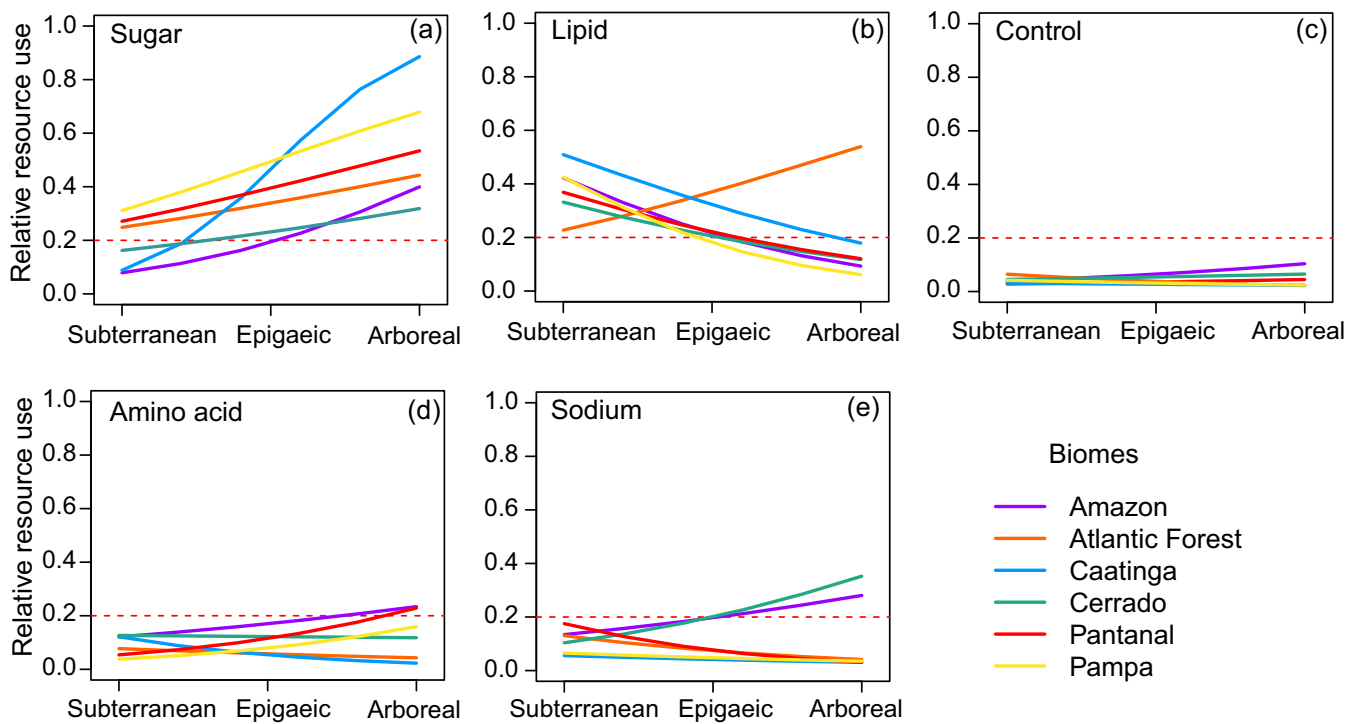
### Does the relative resource use of ants vary along a habitat strata gradient, and is this variation consistent across different biomes?

In the model we used, habitat strata as a continuous variable, the relative resource use of foraging ants depended on the interaction between resource type, habitat strata gradient, and biome ( $F_{20,790} = 4.08$ ;  $p < 0.001$ ; both marginal and conditional  $R^2 = 0.40$ , Appendix S1: Table S1). We also obtained similar results in the model that we used habitat strata as a categorical variable, in which the relative resource use of foraging ants also depended on the interaction between resource type, habitat strata gradient, and biome ( $F_{40,760} = 2.63$ ;  $p < 0.001$ ; both marginal and conditional  $R^2 = 0.42$ , Appendix S1: Table S2).

In general, moving from the subterranean to the arboreal stratum, the relative use of sugar increased consistently across biomes (Figure 2a), and the relative use of lipids declined in all biomes, except in Atlantic Forest where the relative use of lipids increased along the strata gradient (Figure 2b). The relative use of amino acid and sodium was more complex. Moving from the subterranean to the arboreal stratum the relative use of amino acid increased in Amazon, Pampa, and Pantanal biomes, but decreased in the other biomes (Figure 2d). From the subterranean to the arboreal stratum, the relative use of sodium increased in Amazon and Cerrado biomes but decreased in the other biomes (Figure 2e).

### Does the relative resource use of ants vary across ant trophic position, and is this variation consistent across different biomes?

The relative resource use of ants from the two trophic groups depended on the interaction between trophic group, resource type, and biome ( $F_{20,555} = 4.08$ ,  $p < 0.001$ , both marginal and conditional  $R^2 = 0.34$ , Appendix S1: Table S9). The only consistent pattern found across biomes



**FIGURE 2** Relative resource use of foraging ants at three habitat strata in six Brazilian biomes. In each panel, we demonstrated the ant relative resource use of sugar (a), lipid (b), amino acid (d), sodium (e), and control (c) across subterranean, epigeaic, and arboreal strata. Each habitat was transformed into continuous variable by attributing levels for each stratum (0 to subterranean stratum, 0.5 to epigeaic stratum, and 1 to arboreal stratum). Different colored lines represent the function of one of six biomes. Red dashed lines represent the expectation where five resources are equally used (i.e., relative use 0.2).

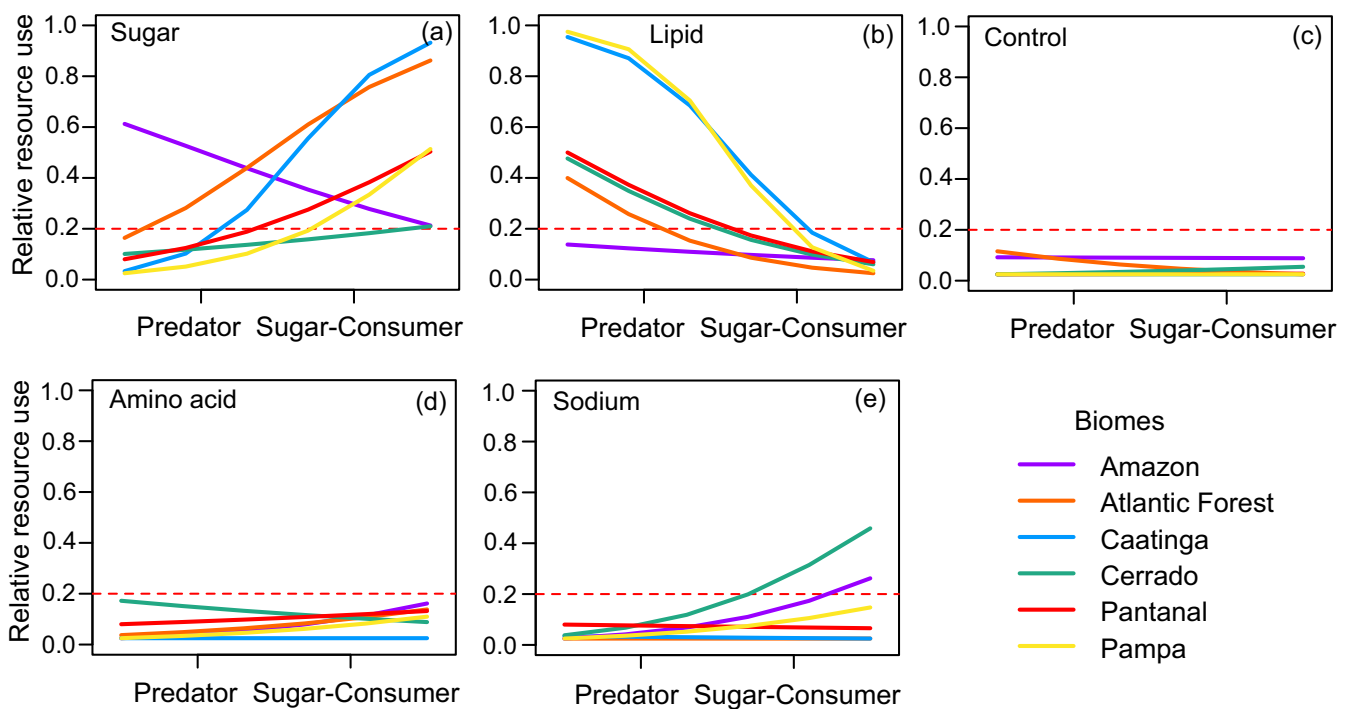
was that predatory ants showed higher foraging effort for lipids compared with sugar-consuming ants (Figure 3b). Sugar-consuming ants showed greater foraging effort for sugar than predatory ants in five biomes (except in the Amazon biome; Figure 3a). Sugar-consuming ants showed greater foraging effort for amino acid than predatory ants in Amazon, Atlantic Forest, Pampa, and Pantanal but showed lower foraging effort than predatory ants in Cerrado and no difference in Caatinga (Figure 3d). Sodium patterns were also complex. Sugar-consuming ants showed greater foraging effort for sodium than predatory ants in Amazon, Cerrado, and Pampa biomes. However, sugar-consuming ants showed lower foraging effort for sodium than predatory ants in Pantanal and no difference in Atlantic Forest and Caatinga biomes (Figure 3e).

## DISCUSSION

Here, we investigated the nutrient preference of ants along a habitat strata gradient and across trophic guilds using different nutrient baits in six biomes in the Neotropics. We report, for the first time, that ants in our study system mostly differed in their nutrient preference along the vertical habitat strata gradient and across

trophic levels, with sugar and lipid patterns most consistent across biomes and context dependent for amino acid and sodium patterns. By sampling extensively across habitat strata at local scales across a range of environments and using various resource types, our findings can help to understand the functioning of local communities in the Neotropics.

Our study system, covering a large spatial extent, reveals a mostly consistent pattern of relative sugar and lipid use across biomes. Our data on sugar and lipids use are in accord with the findings of Fowler et al. (2014) in North American temperate forests—sugar use is highest in the trees, and lipid use was highest on the ground. Even accounting that the use of lipids increased from subterranean to arboreal strata in the Atlantic Forest biome, lipid use was always higher than the expectation of 20% (Figure 2b), which indicates that lipids may be limiting in all habitat strata in this biome. If animals show high foraging effort for the most limiting nutrient, as the compensation hypothesis predicts, our results indicate that sugar is the most limiting nutrient in the arboreal environment, even though it is still likely to be naturally abundant in these areas. Although there might not be any sugar sources where we placed the baits on the tree trunk in the Amazon and Atlantic Forest biomes,



**FIGURE 3** Relative resource use of foraging ants from two trophic groups in six Brazilian biomes. In each panel, we demonstrated the predatory and sugar-consuming ants relative resource use of sugar (a), lipid (b), amino acid (d), sodium (e), and control (c). Both the categories of predatory and sugar-consuming ants were transformed into continuous variable by attributing levels for each trophic levels (0 to predator and 1 to sugar-consumer). Different colored lines represent the function the relative resource use of one of six biomes. Red dashed lines represent the expectation where five resources are equally used (i.e., relative use 0.2).



those arboreal ants possibly easily access sugar in the canopy, where they likely nest. Thus, we suspect that even amino acids could also be limiting in the arboreal stratum at some level (Davidson, 2005), sugar is the most limiting resource on the green food webs, and lipids might be limiting on the brown food webs across biomes.

The contrasting patterns between the relative sugar and lipid use across biomes may also reflect different selective pressures on ants depending on the strata that they occupy. As the sugar and lipid preferences found here were high exactly where such resources are relatively abundant in the environment, selective pressures could favor ants that feed on the most available resource in each stratum. Importantly, it seems that these selective pressures have been present throughout the evolution of the ants. Ants most likely originated within the subterranean stratum, and then they spread to the surface and diversified in the Cretaceous (Lucky et al., 2013). Once above ground, ants were able to access new and different plant resources (Lucky et al., 2013; Nelsen et al., 2018). This novelty was responsible for the evolution of various ant-plant interactions, including mutualisms involving different plant resources (Nelsen et al., 2018). Thus, it is possible that the contrasting patterns of sugar and lipid use reflect different past and current selective pressures across habitat strata for three reasons. First, both past and present subterranean ants are mostly predators (Lucky et al., 2013; Nelsen et al., 2018; Wong & Guénard, 2017), and they usually prefer lipids instead of other resources (Wilder et al., 2013 and discussed below). Second, selective pressures on mutualistic interactions between ants and plants could also conserve the lipid preference habits from evolutionary ancient predatory ants in the epigeic stratum (Nelsen et al., 2018). For example, the elaiosome of seeds is lipid-rich (Fischer et al., 2008), which mimics the constitution of invertebrate prey and attracts high-quality dispersers such as predatory and scavenging ants (Giladi, 2006; Hughes et al., 1994; Leal et al., 2014). Third, because of past selective pressures of extrafloral nectaries on ants, the arboreal stratum is now dominated by ants who have a close relationship with plants and their sugar resources (Rico-Gray & Oliveira, 2007). All these three aspects support increased sugar preference from the subterranean to the arboreal strata in all biomes and with the decreasing lipid preference along this gradient in almost all biomes, except in Atlantic Forest. In this sense, it seems that when looking for general patterns of nutrient preference in several regions, patterns of resource use across habitat strata also reflect the dietary niche conservatism of ants.

The changes in nutrient preference across habitat strata found in our study system were not consistent across biomes for amino acid and sodium use. The relative amino acid use increased from subterranean to

arboreal strata in Amazon, Pampa, and Pantanal biomes, but subtly decreased in Atlantic Forest, Caatinga, and Cerrado. This difference may be due to differences in amino acid limitation across biomes. For example, Atlantic Forest, Caatinga, and Cerrado biomes had lower amino acid use than would be expected if all resources were used equally at random (i.e., 20%) in all strata. Accordingly, the Caatinga and Cerrado biomes are known for high levels of amino acids in extrafloral nectaries (Leal et al., 2017; Oliveira & Marquis, 2002; Schoereder et al., 2010), which may reduce amino acid limitation in the vegetation and explain the difference in amino acid use compared with other biomes.

The context dependence patterns across biomes for sodium along a habitat strata gradient, could be related to differences in sodium input and output in the ecosystems. Sites further from the sea have less sodium input in the ecosystem, and the more resource limiting it becomes (Kaspari et al., 2008). The high foraging effort for sodium in Amazon and Cerrado may therefore be because these biomes are far from the sea (Figure 1). In addition, both biomes have high levels of precipitation that can increase sodium limitation (Lasmar et al., 2021). Although Pantanal is also far from the sea, this biome is also seasonally flooded, which likely dilutes sodium in the soil, explaining the high use of sodium in the subterranean stratum.

In terms of the relative resource use of ants occupying different trophic positions, we also found mostly consistent patterns across biomes for sugar and lipid use but not for amino acid and sodium use. Sugar-consuming ants largely showed greater foraging effort for amino acids and sodium than predatory ants—as predicted from theory. However, in the Cerrado and Caatinga biomes, this was not the case in relation to the amino acid use and nor in the Atlantic Forest, Caatinga, and Pampa biomes in relation to sodium use. We think the different dynamics of amino acid and sodium in the ecosystem may also explain this context dependence. For example, the sugar-consuming ants from Cerrado and Caatinga probably face high amino acid sources from extrafloral nectaries in those biomes, as discussed above. This could explain why sugar-consuming ants showed low foraging effort for amino acid than predatory ants in Cerrado and no difference in Caatinga. Additionally, in Atlantic Forest and Caatinga, the closest biomes to the sea, it is probably that both trophic levels easily access sodium sources, which may explain the lack of difference between trophic levels. On the other hand, we found that the relative sugar use is high for sugar-consuming ants compared with predatory ones in all biomes, except in the Amazon. This suggests that sugar-consuming ants are more limited by sugar than predatory ants in most of the biomes. Conversely, predatory ants showed higher foraging effort for lipids than

sugar-consuming ants in all biomes, as predicted. The diet of predatory arthropods is more reliant on protein from their prey and obtaining energy from protein is costly and presents a lower net energetic gain relative to sugar and lipids resources (Raubenheimer et al., 2009; Wilder et al., 2013). Accordingly, previous studies have reported that arthropods of higher trophic levels are lipid-limited, in which the lipid intake can prevent these animals from starving and increases their reproductive rate (Jensen et al., 2012; Wilder et al., 2013)—this may explain why predatory ants focus their foraging efforts for lipids in all biomes. In this sense, the trophic level of ant species likely largely determines their foraging efforts possibly because they try to fix their dietary nutrient imbalances from their typical diet.

There are some methodological caveats in our study. First, our arboreal sampling focused on ants on tree trunks rather than in the high canopy in Amazon and Atlantic Forest biomes. This may obscure strata differences in foraging preferences. However, the overlap in the ant fauna between our epigaeic and arboreal samples was similar to other studies in the Neotropics (e.g., 10% to 50% of ants forage at both on the ground and at the canopy in Antoniazzi et al., 2020; Leponce et al., 2021; Neves et al., 2021). Second, we only used liquid food resources, and these might not always reflect the real food items that ants forage for.

## CONCLUSION

Here, we advance understanding of the functioning of local communities through ant nutrient preference. Nutrient preference variation of amino acid and sodium along a habitat strata gradient and across ant trophic levels are context dependent on the biomes. This indicates that local nutrient dynamics are also influenced by other processes at larger spatial scales. On the other hand, nutrient preference of sugar and lipid are mostly consistent across biomes, presenting contrasting patterns across both habitat strata and trophic levels. Sugar and lipid are the main energetic resources used by ants in this and other studies from different regions and continents (e.g., Fowler et al., 2014; Peters et al., 2014). Thus, contrasting patterns of sugar and lipid show strong niche differentiation across space and trophic levels, which might locally promote the coexistence of hyperdiverse ant assemblages in the Neotropics and possibly in other biogeographic regions.

## AUTHOR CONTRIBUTIONS

Chaim J. Lasmar, Tom R. Bishop, and Catherine L. Parr originally formulated the idea. Chaim J. Lasmar and

Icaro Wilker conducted the field and laboratory work. Chaim J. Lasmar and Rodrigo M. Feitosa conducted the species identification. Chaim J. Lasmar analyzed the data. Chaim J. Lasmar led the writing with substantial collaboration from all co-authors.

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## CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.


## DATA AVAILABILITY STATEMENT

Data (Lasmar, 2022) are available in Figshare at <https://doi.org/10.6084/m9.figshare.20360034>, with voucher ant specimens deposited in DZUP (<https://dzupufpr.wixsite.com/colecao>) as detailed in our Figshare repository.

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