

Ant brightness and size influence the seasonal vegetation use by ground-dwelling ants (Hymenoptera: Formicidae) in an Amazonia-Cerrado transition zone

Brilho e tamanho das formigas influenciam o uso sazonal da vegetação por formigas de solo (Hymenoptera: Formicidae) em uma zona de transição Amazônia-Cerrado

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Associate Editor: Thomas Parmentier

Abstract

1. Several factors influence species distribution within communities, with ecological filters playing a major role by shaping morphological, physiological and behavioural adaptations. Seasonal changes in environmental conditions—light incidence, temperature and humidity—can favour species capable of foraging under varying conditions or in different strata.
2. Ants are excellent models for studying how ecological filters structure communities throughout the year, as they exhibit traits that enable them to occupy a wide range of microhabitats. Two such traits that may reflect responses to environmental filters are body size and brightness. In this study, we investigated how ant distribution varies between soil and lower vegetation across two seasons, and how these patterns relate to ant morphology in an ecotonal area between the Amazon and Cerrado biomes.
3. Ants were sampled using pitfall traps placed on the ground and in the lower vegetation. We measured the size and brightness of representative individuals from each species. A total of 2659 occurrences were recorded, representing 237 species, 53 genera and 8 subfamilies. Species richness was higher during the dry season, especially in the soil. Species turnover was the main component of beta diversity, although this pattern varied with season. During the rainy season, many ground-dwelling ants shifted to the vegetation, increasing the nestedness component in cross-strata comparisons. Larger ants were more frequent in vegetation, while

smaller ants predominated in the soil. Darker individuals were more common in vegetation during the dry season; however, this seasonal migration of ground ants to the vegetation led to a lighter-coloured ant community on average during the rainy season.

4. These findings show that ant community composition in the soil and lower vegetation is not stable year-round. Seasonal climatic conditions influence foraging activity and species presence, highlighting the importance of seasonal dynamics and vertical stratification in shaping community structure.

KEYWORDS

adaptations, ants, colour, morphology, seasonality, stratification

INTRODUCTION

In community ecology, it is well understood that a local community represents a subset of the broader diversity observed at the regional scale (Jost, 2007; Whittaker, 1960). Understanding how a local community is assembled from the regionally available species pool is a complex task, as species distributions are shaped by multiple interacting factors that can work synergistically, antagonistically or neutrally (Djordjević & Tsiftsis, 2022; Henriques-Silva et al., 2013; Platts et al., 2019). For example, climatic characteristics can interact synergistically with plant structure, acting as ecological filters within a community by preventing species that are less well adapted from thriving in areas with specific microclimatic conditions (Da Cruz et al., 2021; De Arruda et al., 2021). At the same time, strong biotic filters can also function as ecological filters, working synergistically with the environment to locally exclude certain species, even those that are climatically adapted (Kraft et al., 2015).

The filtering pressure exerted by the environment is expected to be strong, as it drives morphological, seasonal or behavioural adaptations in species, enabling them to physiologically cope with conditions (Stevenson, 1985; Willmer & Unwin, 1981). UV-B radiation, for example, causes oxidative damage on ants and can act as a strong environmental filter, limiting species' occupation of more open habitats (Kaspari et al., 2015; Wang et al., 2008). Consequently, in areas with high microclimate variability at short geographical distances, as ecotones for example (E. Q. Marques et al., 2020), we can expect significant variation in community composition associated with the physical characteristics of species, along with seasonal changes and variations in vegetation structure (Araújo et al., 2020; Didham & Lawton, 1999; Stickley & Fraterrigo, 2021).

On the other hand, from an insect perspective, microclimatic conditions (and associated filters) can vary at small scales. A forest, for example, is not homogeneous; within its vertical strata, there are different habitats. When comparing the communities within these strata, the influence of environmental filters on species occurrence becomes evident (Longino & Nadkarni, 1990; Oliveira-Santos et al., 2022). From the ground to the treetops, there is significant variation in temperature and solar radiation (Zimmerman et al., 2007). As a habitat, forest soils tend to be much more stable and humid, buffering

atmospheric variations in temperature, humidity and solar radiation compared to vegetation (Kaspari et al., 2015).

The study of variations in diversity patterns influenced by environmental and biological filters benefits from the use of a taxon that responds to changes in habitat parameters, is locally abundant and rich in species: ants (Hymenoptera: Formicidae). Ants are recognised as habitat bioindicators, present in all strata of vegetation, from underground to the tree canopy, and they exhibit a high diversity of colours and sizes (Idec et al., 2023; Sosiak & Barden, 2021). The Formicidae family currently comprises approximately 17,000 species (Bolton, 2024) that play diverse roles in ecosystems. A significant advantage in understanding ant community assembly is that ants have a reproductive phase during which the queen actively selects a site for colony establishment (Wilson & Hunt, 1966), followed by, in most cases, a prolonged sedentary phase. In addition to eusociality and behavioural variations, which provide them with remarkable adaptive capabilities, ants also exhibit morphological and behavioural adaptations that enable them to thrive in various microhabitats (Hölldobler & Wilson, 1990). Ants also engage in intense competition, with dominant species acting as biological filters by preventing the occurrence or foraging of less competitive species (Vicente & Izzo, 2021; Yanoviak & Kaspari, 2000).

Seasonal changes intensify the differences between the strata by altering average temperatures, humidity, the number of sunny days and UV radiation. These environmental variations act as ecological filters, potentially reducing ant foraging and occurrence (Bishop et al., 2016). It is expected, then, that species living in vegetation will exhibit specific physiological, behavioural and morphological adaptations (Bishop et al., 2016; Yanoviak & Kaspari, 2000). These adaptations may not be necessary for those that live on the ground, especially when we talk about the Amazon, where we have high crowns with a closed canopy. When we observe this differentiation between strata due to abiotic variables, it suggests a temporally nested pattern, where a subset of species, particularly those in vegetation that have specific adaptations for thermal tolerance and desiccation resistance, can forage year-round and are competitively superior, while another part of the community forages only during the rainy season.

Ant size is closely related to water loss; in dry and hot environments, this is a critical trait, as larger organisms tend to lose less water

and maintain an optimal temperature for longer (Edney, 1957; Law et al., 2020). Because the surface-to-volume ratio of organisms regulates the rate of heat exchange with the environment, variation in the body size of animals is associated with larger organisms tending to be found in colder environments on average (Bishop et al., 2016). Colouration, particularly the darkness of the exoskeleton, is associated with protection against desiccation, thermoregulation and filtering harmful ultraviolet rays (Bishop et al., 2016; Kaspari et al., 2015; Law et al., 2020). Melanin accumulation is an evolutionary adaptation that provides resistance to environmental UV-B radiation by shielding against radiation and scavenging free radicals produced by UV rays (Wang et al., 2008). Colour and size are synergistically related, equipping ants with traits that enable them to inhabit various habitats that differ in temperature, light incidence and humidity, such as the different strata of a forest within and between seasons.

In this study, our aim was to understand the distribution patterns of ants present on the ground and in vegetation within a transition area between the Amazon and Cerrado, comparing the dry and rainy seasons. The Amazon–Cerrado transition, the largest ecotone area in the world, spans longitudinally over 6000 km (E. Q. Marques et al., 2020; Torello-Raventos et al., 2013). This ecotone, where the dense Amazon Rainforest meets the savanna-like vegetation of the Cerrado, has some of the highest deforestation rates worldwide (E. Q. Marques et al., 2020) and remains a major gap in biodiversity knowledge (Andrade-Silva et al., 2022; Morandi et al., 2016). To achieve this, we addressed the following questions: (1) Is there a difference in the number of ant species between ground and vegetation, and is this difference consistent between the dry and rainy seasons? (2) Are ants present during the dry season a subset of those present during the rainy season? (3) Is this subset composed of larger and darker ants? If so, (4) is the same pattern observed between ground and vegetation ants? We expect that the environmental conditions present during the dry season act as a strong enough environmental filter to select for a higher average of larger and darker ants, which becomes evident when comparing the soil and vegetation strata.

MATERIALS AND METHODS

Study area

The study was conducted in Xingu State Park (hereinafter referred to as PEX), located in the northeastern region of the state of Mato Grosso, in the municipality of Santa Cruz do Xingu, on the border with the state of Pará (Figure 1). The region is part of the Amazon Biome (sensu IBGE, 2019), situated within an ecological transition zone between the Amazon and Cerrado biomes. This area encompasses a mosaic of vegetation types, including seasonal forests, Cerrado formations, campinaranas, riparian forests, gallery forests and humid tropical forests (Córdova et al., 2021; Zappi et al., 2017). All the sample points were located in closed forest, which covers the major part of the regional phytophysiognomy. As a transition zone, the region exhibits marked seasonality, with a distinct dry season from May to October—

when monthly precipitation approaches 0 mm—and a rainy season from November to April, with monthly precipitation ranging from 200 to 450 mm and several rainy days (Ivanauskas et al., 2008).

Sampling was carried out in two periods: September 2022 (from the 14th to the 24th), during the dry season, and February 2023 (from the 6th to the 16th), during the rainy season. The sampling design followed the RAPELD (Rapid Assessments in Long-Term Ecological Research) methodology (Magnusson et al., 2005), as proposed by the Biodiversity Research Program (PPBio—<http://ppbio.inpa.gov.br>). Of the three main trails available in PEX, two were selected based on accessibility (Figure 1). In the first trail (9°46′43″ S, 52°24′60″ W), three fixed 250-m-long plots spaced 1 km apart were sampled. In the second trail (9°48′15″ S, 52°26′38″ W), seven plots of the same length and spacing were used, totalling 10 sampling plots.

Recent data extracted from the National Institute of Meteorology (INMET) meteorological station closest to PEX, located in the city of São José do Xingu-MT (−52.372500, −10.484167), showed significant variations in precipitation, temperature and solar radiation. Unfortunately, the INMET does not have data prior to September 2022 and February 2023, so equivalent data were obtained for September 2023, representing the dry season, and February 2024, representing the rainy season (Table S1).

Canopy openness measured across all sampling plots decreased from an average of 88.00% (range: 85.86%–90.86%) during the rainy season to 86.11% (range: 81.06%–90.26%) in the dry season, representing a reduction of 2.15%. Notably, higher canopy openness was recorded during periods of increased solar radiation (1587.54 KJ/m²), whereas lower openness coincided with reduced radiation levels (1404.94 KJ/m²). This direct relationship, combined with lower rainfall and elevated global temperatures (Table S1), likely intensified seasonal contrasts in microclimatic conditions, resulting in marked variation in light availability and temperature at both ground and understory levels.

Ant sampling

In each plot, both ground and arboreal pitfall traps were installed every 25 metres, resulting in 20 samples per plot (10 ground and 10 vegetation) and 200 samples in total (100 ground and 100 vegetation). For the ground pitfall traps, a 1000 mL container (15 cm in diameter) was buried with 1/3 water and a few drops of detergent to break the surface tension of the water. This method is widely used to estimate the composition of active species living on the ground within a given area (Bestelmeyer et al., 2000). A plastic cover was used to prevent debris and rain from entering. The arboreal pitfall traps consisted of a 300 mL plastic cup (8 cm in diameter) attached to a live tree in the understory, approximately 1.5 metres high (Figure 1). An aqueous solution containing urine (1/3) and a few drops of detergent was placed in these traps. Urine was used because vegetation tends to have a nitrogen and sodium shortage, making this type of solution highly attractive and partially selective to trap arboreal ants (Blanchard et al., 2024; Lasmar et al., 2023; Powell et al., 2011). Once

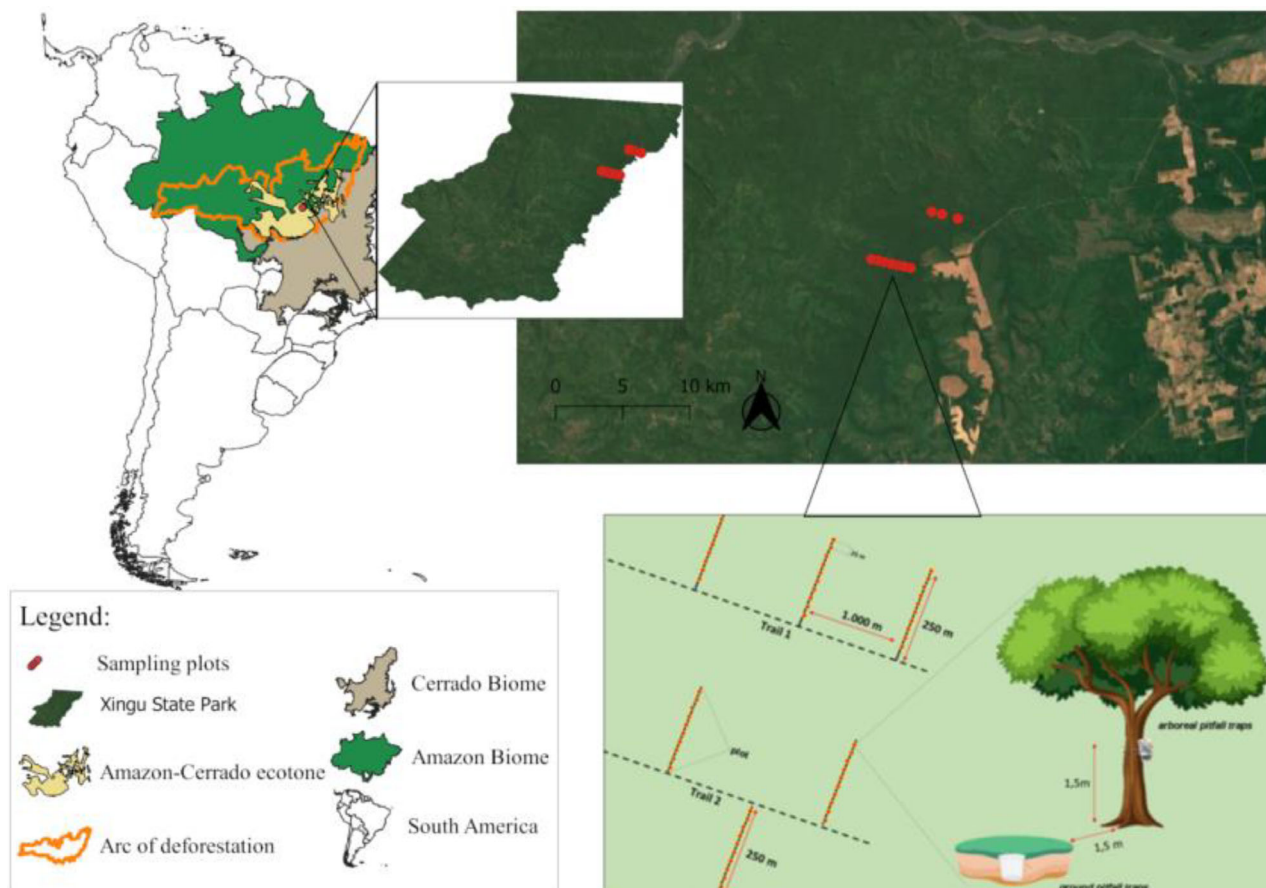


FIGURE 1 Map of the study site, indicating the sampling points. The map of South America (upper right corner) shows the Brazilian Amazon (in green), the Cerrado (in beige) and the transition zone (in yellow), where Xingu State Park is located. The ‘Arc of Deforestation’ is outlined in orange. In detail (upper left corner), the sampling sites are marked in red. At the bottom right, a schematic diagram illustrates how sampling plots were distributed along the main trails and how arboreal and ground pitfall traps were positioned within each fixed plot at Xingu State Park (PEX).

nitrogen is not limiting to soil ants, this method tends to capture just ants foraging on vegetation (Blanchard et al., 2024). Additionally, sodium is particularly attractive to Amazonian ants foraging in vegetation, but not to epigeic species (Lasmar et al., 2023). Ground pitfalls were left unbaited, as the soil disturbance during their installation—known as the digging-in effect—is inherently attractive to ants (Greenslade, 1973; Vasconcelos et al., 2014). Both types of traps were installed in the field for 48 h, after which all collected material was recovered and stored in 99% alcohol. All collected materials were transported to the Community Ecology Laboratory, LabECom, situated at the Universidade Federal de Mato Grosso (UFMT), Cuiabá. Subsequently, the specimens were sorted, and the ants were identified to the genus level using the dichotomous key by Baccaro et al. (2015). Following this initial identification, the genera were morphotyped by comparison with the reference collection, and certain genera were identified to the species level by specialists, utilising dichotomous keys (e.g., Camacho et al., 2022; de Albuquerque, 2014; Dias & Lattke, 2021; Feitosa & Prada-Achiardi, 2019; Fernández & Guerrero, 2019; Fernández & Serna, 2019; Guerrero, 2019; Mackay & Mackay, 2019; A. M. Oliveira et al., 2021; Palacio, 2019; Troya & Lattke, 2022; Ulyssa & Brando, 2021; Wild, 2005). The identity of the *Megalomyrmex* species was confirmed by specialist Dr. Lívia P. Prado.

Measuring the size of ants

The ants were photographed using a Leica EZ4HD magnifying glass attached to a computer, utilising the LAZ EZ application (Leica Application Suite version 3.4.0). All photographs were calibrated using a known scale. This calibration enabled the measurement of Weber's length, which was measured in the lateral view from the anterodorsal margin of the pronotum to the posteroventral margin of the mesosoma (Almeida et al., 2023; Parr et al., 2017). Weber's length serves as a proxy for the total body size of ant individuals (Weber, 1938) and is associated with several parameters related to ant physiology and metabolism (Parr et al., 2017), such as thermoregulation. The ImageJ programme (<http://imagej.nih.gov/ij>) was used to measure Weber length.

For this functional trait, six minor worker individuals were measured per species from different colonies depending on availability. It is recommended to measure six individuals per species to obtain an accurate average intraspecific variation among workers (Gaudard et al., 2019). In cases of polymorphic ants, we did not measure major workers (soldiers), as their inconsistent presence—especially in rare species—could bias the results, particularly when coloration differs between castes. Three measurements were taken per individual, and

the average value was calculated. The general average was calculated for each species.

Ant brightness data

To assess the brightness of the ants cuticles based on their integument colour, we utilised the colour palette proposed by Bishop et al. (2016) and Parr et al. (2017). Employing the palette, we categorically classified colours with the naked eye in comparison with the palette. We opted to exclusively measure the colour of the ants heads, as they are the organs most exposed to solar radiation and typically represent a substantial portion of the irradiated area (Klunk et al., 2022). Our focus was solely on measuring cuticle colour, disregarding any hair or debris present on the heads. Subsequently, we documented the predominant head colour of each ant species. Given the subjective nature of this measurement and potential interpretation discrepancies among observers, six people independently conducted the assessments, resulting in six colour measurements per observer, all following the same protocol. Finally, we computed the average (already converted, as will be elucidated later) among the observers for each species, intended for use in subsequent statistical analyses.

After each of the six observers defined the colour of every ant, we followed the methodology proposed by Bishop et al. (2016) to convert the colours into numeric variables. We assessed the RGB values (red, green and blue) of each colour in the palette using the ImageJ software. Utilising the obtained values, we employed the 'rgb2hsv' function from the grDevices package in the R programme to transform the RGB values into HSV values (hue, saturation and value). Within the HSV scale, hue (H) characterises the dominant wavelength, saturation (S) indicates the amount of hue present in the colour, and the value (V) denotes the brightness of the colour (referred to as brightness here), ranging from 0 to 1. In this spectrum, a value of 0 denotes an extremely dark tone and 1 denotes an extremely light tone. In this study, we exclusively utilised the value (V) from the HSV colour space.

Statistical analysis

In each plot (10 in total), we used the sum of the 10 sampling units (pitfalls) for each method (ground or arboreal) and season (rainy or dry) as replicates. This resulted in four co-dependent replicates per plot: arboreal/rainy, arboreal/dry, ground/rainy, ground/dry. Because we cannot rule out differences in sampling efficiency between soil and vegetation strata—especially considering potential biases in ant abundance due to differential attraction to urine—we first calculated the rarefied number of species by standardising the number of individuals per sample to the lowest individual count (14 individuals) recorded across all sampling points. To assess whether the rarefied ant species richness varied between seasons, stratum or their interaction, we used a generalised linear mixed model (GLMM) implemented with the glmmTMB package (Brooks et al., 2017), specifying 'plot

identity' nested within 'trail' as a random factor and assuming a Gaussian distribution. As complementary analysis, we also calculated the Hill numbers ($q = 1$) to assess the diversity independent of abundance variation. We then ran two additional models—one using the raw species count and another using Hill numbers—following the same structure as the rarefied species richness. All models were evaluated for overdispersion and the appropriate error distributions were employed (see Figure S1a,b). All models consistently showed the same results.

To assess the partitioning of spatio-temporal beta diversity among the ants, we constructed a dissimilarity matrix using the Sørensen index. From this matrix, we determined the percentage of dissimilarity explained by species turnover between sampling points (turnover) or variation in nesting (nestedness-resultant) using the beta.pair function from the Betapart package (Baselga et al., 2018). Additionally, we conducted non-metric multidimensional scaling (nMDS) analysis, condensing the total Sørensen dissimilarity matrix into two axes. The axes are plotted to visualise the patterns. Subsequently, we assessed the composition robustness of the different groups formed by season and strata in the nMDS through pairwise comparisons of plots using permutational multivariate analysis of variance (PERMANOVA) analysis with 999 randomisations, employing the function pairwise.adonis2.

PERMANOVA provides an overview of which communities (across space, strata and season) are more or less similar to each other. It does not reveal the magnitude of the dissimilarity or what mechanisms are causing it. In this study, we assume that ants do not disperse between sampling plots, though migration between strata is possible. We also assume there are no significant colonisation or extinction events between our collection periods and that the observed differences rely on local migration and differential foraging. Our aim is to determine how local communities change between strata and how the local community within each stratum varies across seasons. To achieve this, we recalculated the spatio-temporal dissimilarity, partitioning it into pairwise comparisons for each plot. For each plot, we obtained two dissimilarity component values (nestedness and turnover) for: (a) the comparison between vegetation and soil within each season; (b) the comparison between seasons for each stratum; and (c) the cross-comparison between soil ants collected during the wet season and vegetation ants collected during the dry season, as well as between soil ants collected during the dry season and vegetation ants collected during the wet season (Figure 2). To carry out the statistical comparisons implied by these strata and season comparisons, we then performed three distinct generalised linear mixed models (GLMM), using both turnover and nested beta dissimilarity values as response variables. The focal comparison (a: strata; b: season and c: cross comparison strata*season) and type of dissimilarity were included as explanatory variables. Since both partitioned measures of dissimilarity are co-dependent, we included plot identity nested within trail identity as a random variable to control for pseudoreplication.

Finally, we calculated the weighted averages for both the size and brightness of the species in each plot, considering the number of occurrences of each species (Community-Weighted Mean—CWM). To

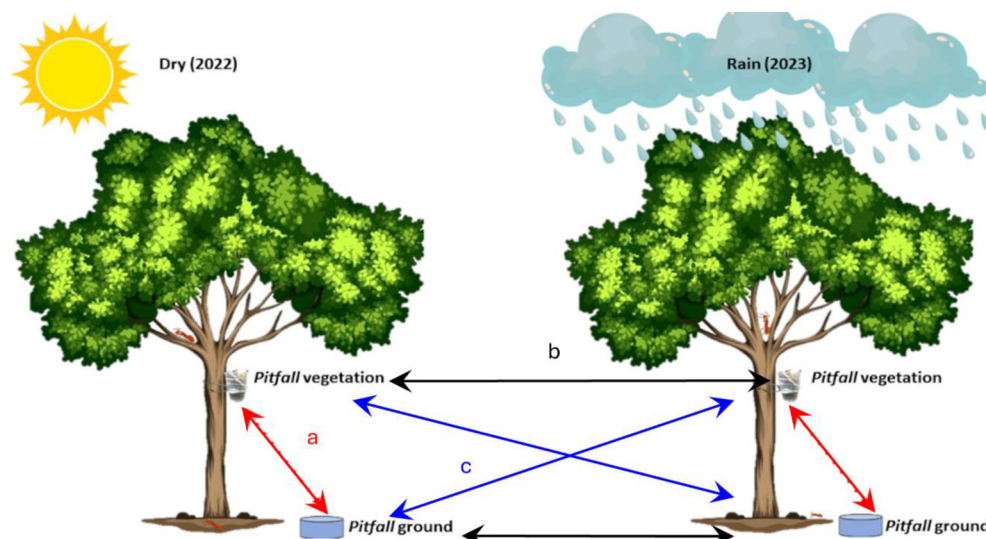


FIGURE 2 Schematic figures demonstrating the statistical comparison of the analysis of diversity components. (a) The red arrow refers to the comparison of different components of beta diversity (nestedness or turnover) between ground and vegetation communities independent of the season; (b) the black arrows refer to the comparison of the components of beta diversity between stations independent of the strata; and (c) the blue arrows refer to a cross-comparison of diversity components between ground and vegetation strata and between the rainy and dry seasons, comparing the composition of different strata between seasons.

determine whether the size and colour of individuals vary between strata and seasons, we conducted GLMM tests. We used the Gaussian family for size and the beta family for colour. In all GLMM analyses, the distribution was fitted to the appropriate error model, and over-dispersion was assessed using the DHARMa package (Hartig, 2022). Statistical analyses were performed in the R programme (version 4.3.2, R Core Team, 2024), and graphs were generated using the ggplot2 package (Wickham et al., 2016).

RESULTS

Species richness patterns

We sampled 2659 ants from 237 species, belonging to 53 genera and eight subfamilies (Table S2, Figure S2). The genera with the highest richness were *Pheidole* (25 species), *Crematogaster* (20 species), *Camponotus* (18 species) and *Solenopsis* (13 species). When evaluated by season, we found 85 species exclusive to the dry season, 32 exclusives to the rainy season, and 120 occurring in both seasons. Regarding the vertical strata, we identified 135 species exclusive to the ground, 33 exclusives to vegetation and 69 species foraging in both strata.

Overall, we sampled more ant species on the ground ($n = 204$) than on the vegetation ($n = 102$, see Table S1 and Figure S2). We observed a difference in rarefied ant species richness per plot between strata, particularly in ground-dwelling ants, where the number of species tended to be significantly higher (GLMM $\chi^2_{1,19} = 43.16$, $p < 0.001$). However, we did not observe any significant differences in the rarefied ant richness of vegetation ants between seasons ($\chi^2_{1,19} = 0.006$, $p = 0.94$) or interaction ($\chi^2_{1,19} = 2.56$, $p = 0.11$) (Figure 3).

The most frequently occurring species on both seasons were *Ectatomma lugens* (136 occurrences, 34%), *Neoponera verenae* (109, 27.25%), *Pheidole* sp5 (103, 25.75%), *Pachycondyla harpax* (98, 24.5%), *Pheidole* sp1 (94, 23.5%). The most frequently occurring species exclusively in the dry season were *Dolichoderus bispinosus*, *Dolichoderus decollatus*, *Pseudomyrmex* sp5 (eight occurrences each, 4% each) and *Camponotus rectangularis* and *Pseudomyrmex* sp2 (seven each, 3.5% each). The most frequent species in the rainy season was *Cyphomyrmex* sp. n. A (10 occurrences, 5%), *Nylanderia* sp7 (eight, 4%), *Carebara urichi*, *Pseudoponera* sp2 (five each, 2.5% each), *Paratrachymyrmex* sp2 (four, 2%).

Regardless of season, the most frequently occurring species on both strata were *Pheidole* sp5 (103 occurrences, 25.75%), *P. harpax* (98, 24.5%), *Pheidole* sp1 (94, 23.5%), *Pachycondyla crassinoda* (89, 22.25%) and *Crematogaster carinata* (84, 21%). Even though they occur on both the ground and vegetation, the four most frequently occurring species on both strata tend to preferentially occur on the ground, with over 80% of the records on this stratum. The most frequently occurring exclusive species on the ground were *E. lugens* (136, 68%), *N. verenae* (109, 54.5%), *Nylanderia* sp1 (42, 21%), *Pheidole* sp3 (34, 17%), *Pheidole* sp15 (28, 14%). On vegetation, the most frequently occurring exclusive species were *Crematogaster erecta*, *Pseudomyrmex* sp2 (seven each, 3.5% each), *Camponotus burtoni*, *Camponotus* PEX1 (four each, 2% each) and *Dolichoderus bidens* (three, 1.5%).

Ant species composition

Ant composition differed between strata (ground and vegetation) and seasons (dry and rainy) (Figure 4). The partitioning of ant biodiversity

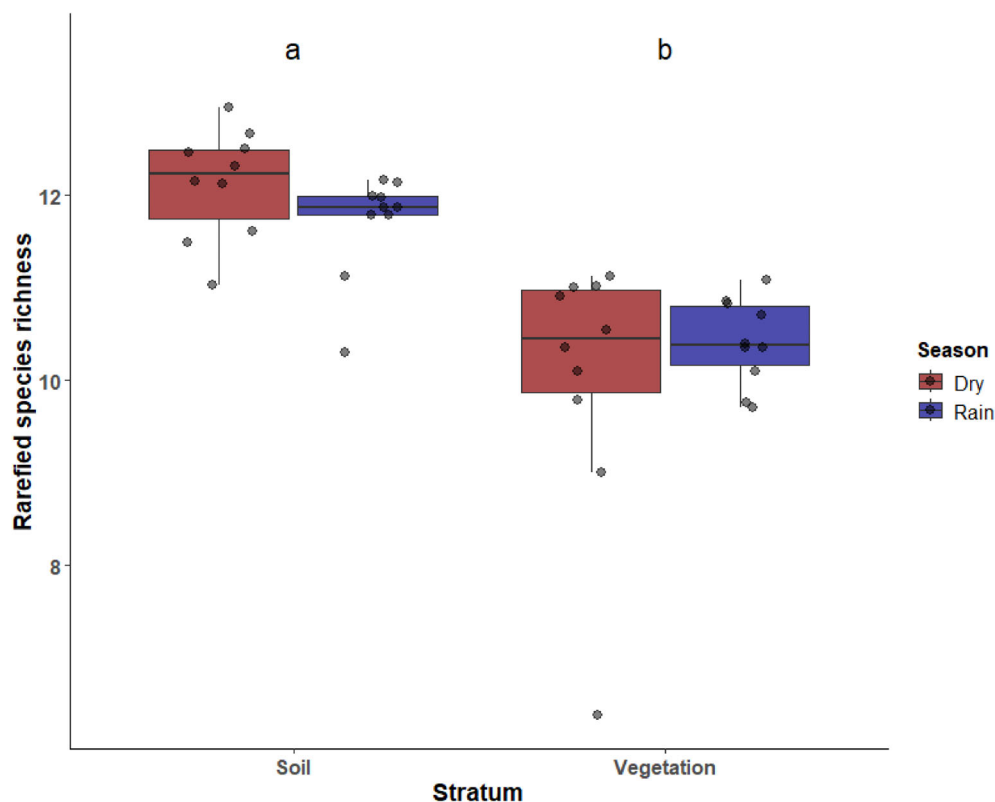


FIGURE 3 Rarefied number of ants species collected per plot in Xingu State Park (PEX) during the dry and rainy seasons in the soil and vegetation strata. The different letter above the boxes indicates statistically significant differences ($p < 0.05$).

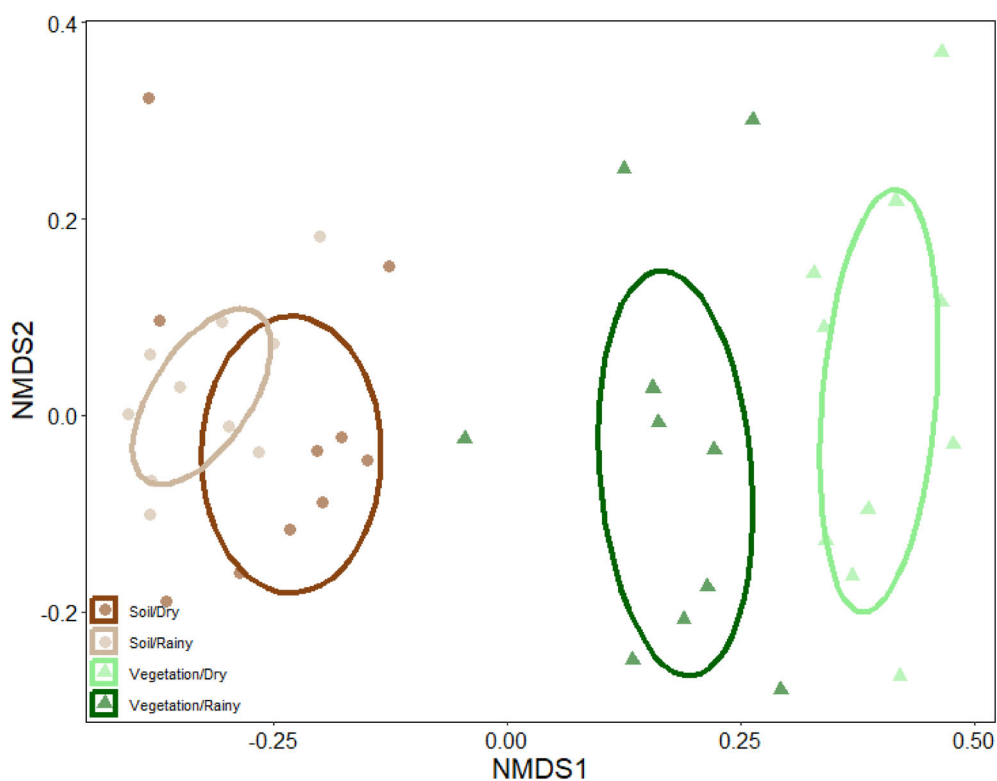


FIGURE 4 Non-metric Multidimensional Scaling (NMDS) ordination in two axes of ant composition between strata and seasons in Xingu State Park, MT, Brazil. The round symbols represent the ground ant community and the triangular symbols represent the vegetation ant community (stress: 0.1464).

TABLE 1 Summary of results from permutational multivariate analysis of variance analyses paired between strata and season in Xingu State Park, MT, Brazil.

Factor	df	SS	R^2	Pseudo F	p
Soil-rainy × Soil-dry	1	0.2849	0.09606	1.9128	<0.001
Soil-rainy × Vegetation-rainy	1	1.5642	0.35333	9.8351	<0.001
Soil-rainy × Vegetation-dry	1	2.4491	0.4527	14.889	<0.001
Soil-dry × Vegetation-rainy	1	1.2834	0.28371	7.1296	<0.001
Soil-dry × Vegetation-dry	1	1.8613	0.35797	10.036	<0.001
Vegetation-rainy × Vegetation-dry	1	0.5780	0.14104	2.9555	<0.001

Note: df is the degrees of freedom of the sample, SS is the SumOfSqs, which is the sum of squares, R^2 is the coefficient of determination, and the p -value was obtained through 999 permutations.

demonstrated that dissimilarity between sampling points was primarily due to species replacement, explaining 81.30% of beta diversity, whereas nesting accounted for only 18.70%. In paired comparisons, it was evident that all strata and seasons were distinct from one another (Table 1).

Variations in dissimilarity components across seasons and strata

When evaluating the components of beta diversity, we observed that the species replacement (turnover) components were consistently greater than the nested components in all comparisons, regardless of strata or season. In fact, the turnover was the main component in the comparison across seasons (e.g., ground from the dry season with ground from the rainy season) (GLMM $\chi^2_{1,39} = 90.88$, $p < 0.001$) but did not differ between seasons ($\chi^2_{1,19} = 0.007$, $p = 0.93$), and there was no interaction between the factors ($\chi^2_{1,39} = 0.61$, $p = 0.433$). The same pattern was observed when comparing the strata, where there was no difference between the strata ($\chi^2_{1,39} = 3.13$, $p = 0.077$) and no interaction ($\chi^2_{1,39} = 0.178$, $p = 0.673$). When comparing ant composition across strata in a crosswise manner between the rainy and dry seasons, we also did not observe significant differences ($\chi^2_{1,39} = 0.96$, $p = 0.327$). Surprisingly, however, even though species replacement continued to be the largest component of total beta diversity ($\chi^2_{1,39} = 86.54$, $p < 0.001$), it was clear that the composition of vegetation ants in the rainy season was significantly more nested than the composition of ground ants in the dry season (Figure 5c), as evidenced by the interaction ($\chi^2_{1,39} = 14.04$, $p < 0.001$). This implies that a portion of the ground ant species forages on vegetation in the rainy season, but not in the dry season, where the difference between strata is primarily determined by species replacement.

Species characteristics between seasons and strata

The weighted average size of the species comprising the local ant assemblage ranged from 1341 to 2785 mm. We did not observe any

difference in ant size between the seasons (GLMM $\chi^2_{1,39} = 0.006$, $p = 0.936$). In fact, communities in the dry season had an average size of 2.087 mm (± 0.345 standard deviation), whereas those in the rainy season had an average size of 2.048 mm (± 0.438 standard deviation) (Figure 6a). However, vegetation ants were, on average, larger ($\chi^2_{1,39} = 23.76$, $p < 0.001$). There was no interaction between the season and stratum ($\chi^2_{1,39} = 0.3$, $p = 0.579$).

The average brightness of the ant cuticle per plot ranged from 0.245 to 0.518. We observed that ants in vegetation during the rainy season were clearly lighter, measuring 0.391 (± 0.055 —standard deviation), whereas those in vegetation during the dry season were darker, measuring 0.319 (± 0.054). In contrast, ground ants showed no difference between the dry season (0.381 ± 0.049) and the rainy season (0.365 ± 0.044 standard deviation). These differences in cuticle brightness did not vary between strata ($\chi^2_{1,39} = 1.56$, $p = 0.211$) but tended to be greater in the dry season ($\chi^2_{1,39} = 3.65$, $p = 0.056$). Furthermore, a strong interaction between the two factors was detected ($\chi^2_{1,39} = 9.3$, $p = 0.002$), indicating that the brightness of ground ants did not vary between seasons, but vegetation ants were clearly darker only in the dry season (Figure 6b). When we individually observed the species contributing to the average brightness in the vegetation, we noticed that many ants, typical of the ground or with a high frequency of occurrence in the ground between seasons, such as some species of the genera *Pheidole* and *Solenopsis*, as well as species such as *Wasmannia auropunctata*, *Crematogaster limata* and *Dolichoderus imitator*, among others, totalling 276 records, foraged in the vegetation. These ants originating from the ground had a weighted average of 0.3978, contributing to a shift in the average towards greater brightness in the vegetation community. This foraging of ground ants on vegetation is almost entirely restricted to the rainy season, so that in the dry season, almost exclusively typical species of vegetation are found in this stratum.

DISCUSSION

Our study demonstrates how variations in seasonality and vertical stratification interact to shape the ant assemblage in the Amazon–Cerrado transition zone. Contrary to our expectations, we observed a

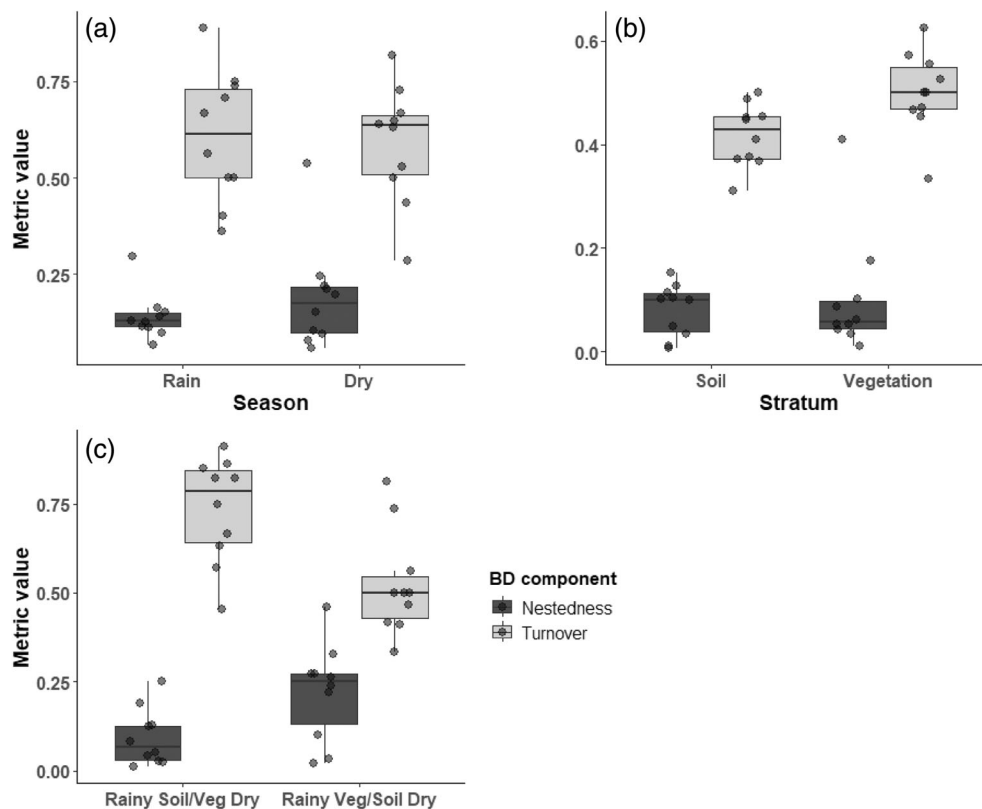


FIGURE 5 Comparison of the contributions of beta diversity components (nestedness and turnover) at paired sampling sites: (a) between seasons, regardless of stratum; (b) between strata, regardless of season; and (c) cross-comparisons between ground in the dry season and vegetation in the rainy season, and vice versa. All comparisons among nestedness and turnover were statistically different.

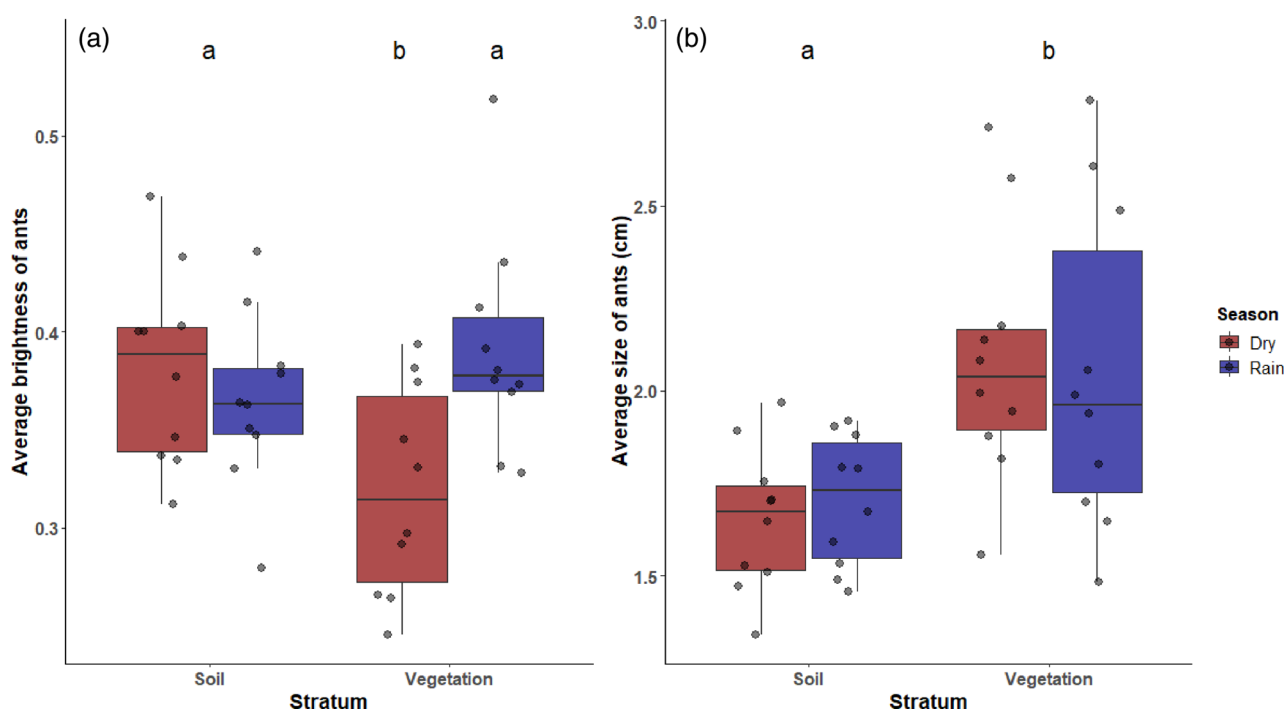


FIGURE 6 (a) Community-weighted means (CWM) for ant size by stratum (soil and vegetation) and season (dry and rainy) (Y-axis: cm) and (b) for mean ant cuticle brightness across stratum (soil and vegetation) and seasons (rainy and dry) (Y-axis: closest to 0 black—closest to 1 light). The different letter above the boxes indicates statistically significant differences ($p < 0.05$).

greater number of species in the dry season, particularly on the ground. Because the ancestral habitat of ants is the ground, a greater number of species occupying this stratum is expected (Brühl et al., 1998). Furthermore, ant composition showed a high species turnover between the strata and seasons. However, we observed a greater contribution of nestedness when comparing the dry season ground ant assemblage with the rainy season vegetation ant assemblage. This implies that the typical stratification (or turnover) observed between ground and vegetation communities, as noted in several studies (Almeida et al., 2023; Brühl et al., 1998; De Arruda et al., 2021; B. F. Oliveira & Scheffers, 2019; Vasconcelos & Vilhena, 2006), is apparently not completely stable across seasons and this difference may be influenced by species loss, not just replacement. In fact, during the rainy season, some ant species sampled on the ground forage in the vegetation, replacing some species typical of this stratum. These invading species were absent from the vegetation during the dry season. Although they are similar in size to typical vegetation ants, they are lighter in colour. We cannot rule out the presence of a phylogenetic signal, with more closely related ants being better able to access the vegetation during the dry season. However, the increased occurrence of lighter species suggests a seasonal relaxation of environmental filtering (e.g., UV radiation) during the rainy season, which may enhance the role of biological filtering (e.g., competition).

Ground and vegetation ants tend to have completely different organisations, exhibiting distinct feeding behaviours and physiological restrictions. Ant species associated with vegetation are typically exposed to higher temperatures and greater incidence of UV-B radiation (Kaspari et al., 2015). The ant community in vegetation has a high availability of sugary liquids for food originating from extrafloral nectaries and sugary drops ('honeydew') from Hemiptera (Campos & Camacho, 2014; Davidson et al., 2003; Guindani et al., 2017; Marques et al., 2015). However, protein and salt availability are typically low in arboreal ants (Kaspari et al., 2008; Kaspari & Yanoviak, 2001). In contrast, ground ants inhabit environments with little or no direct light, experience more stable and moderate temperatures and have high protein but restricted carbohydrate availability (Kaspari et al., 2012; Silvestre & da Silva, 2001). These ecological differences explain the strong observed stratification in the composition of ground and vegetation-dwelling ants (Basset et al., 2003; De Arruda et al., 2021; Kaspari & Yanoviak, 2001; Oliveira-Santos et al., 2022; Vicente et al., 2016). Indeed, we observed strong species turnover in species composition among strata and seasons. However, the scenario changed when we cross-evaluated the diversity of ants in the ground during the dry season with the diversity of ants in the vegetation during the rainy season. We observed that a portion of the typical ground ant community migrated to the vegetation during the rainy season, replacing local species usually found on vegetation in the dry season. This migration led to an increase in nestedness, explaining the dissimilarity among sampling points in the crossed comparison, but a high turnover when comparing just the vegetation ant community between seasons. Additionally, this suggests that the environmental and biological filters determining beta diversity between strata are not temporally stable.

In fact, our results suggest that environmental and biological filters interact in the assembly of vegetation composition (Cadotte & Tucker, 2017). We cannot affirm that ground-dwelling ants competitively replace vegetation-dwelling ants in foraging within the lower vegetation. However, since ant species richness in the vegetation does not differ between seasons, we can suggest that ground ants may seasonally prevent some vegetation specialists from foraging locally, probably pushing them upwards into higher vegetation strata. In a site where ants typical of savanna-like and closed forest habitats are present in the regional pool, a specific environmental filter does not limit occupation but instead temporarily restricts the foraging of some ground-dwelling ant species on vegetation. When the environmental filter, such as UV-B radiation, weakens, these ground ants likely displace some low-vegetation ant species, following a seasonal expansion-retreat foraging pattern and maintaining high alpha and beta diversity. This hypothesis is supported by our results regarding the morphological characteristics of ants, including average size and brightness, across seasons and strata.

As expected, we observed differences in the mean size of ants between ground and vegetation communities, and this difference remained stable across seasons. Worker size is closely related to ant metabolism and may be shaped by microclimatic variations (Baudier et al., 2015). Canopy ants face thermal foraging restrictions to prevent overheating and desiccation of trees, as temperatures can reach 50°C in the canopy (Kaspari et al., 2015; Spicer et al., 2017). Although our study did not access ants present in the canopies, but rather in the understory, we observed that the ants foraging in this stratum were larger in size, which may be related to the higher temperatures filtering out smaller species. The dominance of larger ants in vegetation, as observed also in other studies (Almeida et al., 2023; Law et al., 2020; Yanoviak & Kaspari, 2000). Arboreal and ground-dwelling ants often represent groups with different evolutionary histories (Moreau et al., 2006), suggesting that the observed patterns could result from closely related species accessing the vegetation layer. Although we cannot entirely rule out the presence of a phylogenetic signal in the community we studied here, a study conducted in a region near our study area indicates that the larger body size observed in arboreal ants lacks strong phylogenetic support within regional communities (Almeida et al., 2023). Instead, the observed pattern may be better explained by the fact that larger individuals reach their equilibrium temperature more slowly (Edney, 1957; Hadley et al., 1989; Law et al., 2020), enhancing their ability to tolerate extreme or fluctuating heat, such as in sun-exposed environments within the vegetation layer (Law et al., 2020).

We also found darker ants present in the vegetation, especially in the dry season. Although previous studies did not detect a significant signal for darkness across the ant phylogeny (Nelsen et al., 2018), including among vegetation-dwelling groups (Law et al., 2020), we cannot entirely dismiss this hypothesis for the studied community. Nonetheless, in addition to size, cuticle darkening represents another morphological adaptation that insects possess for resistance to desiccation, thermoregulation and mainly UV-B protection, as demonstrated by several authors across different taxa (Bishop et al., 2016;

Kalmus, 1941; Law et al., 2020; Lopez et al., 2021). Ants with darker cuticles have advantages due to the low permeability of the cuticle combined with melanin (Hadley et al., 1989; Kalmus, 1941), making this characteristic advantageous for retaining water in dry, especially warmer environments. Another crucial point to highlight is protection against ultraviolet rays; high exposure to this type of radiation can have deleterious effects on the life expectancy and reproduction of these animals (Wang et al., 2008). Therefore, darker animals with high levels of melanin have advantages in environments with high UV-B irradiation (Bishop et al., 2016), making this type of radiation an environmental filter in the selection of species that can forage in vegetation. However, when we analysed the average brightness of ants in vegetation during the rainy season, we found that this parameter was almost the same as that of ants present on the ground. During the rainy season, there are noticeably more cloudy days, the trees have more leaves, and the light incident on the understory is lower, which synergistically represents a significant decrease in the incidence of UV-B rays. This implies a decrease in the pressure exerted by this microclimatic filter during the rainy season, allowing 'non-adapted' ants to occupy the understory.

It is important to emphasise that, although a portion of the terrestrial community has access to vegetation in the dry season, these ants do not interfere with the average size of the community. In fact, the average size of the component species does not vary depending on seasonality in any of the strata, contradicting our initial hypothesis. This stability in the average size between seasons suggests that the filtering exerted by temperature acts independently of seasonality in both strata. However, we cannot rule out that only large ground ants could access vegetation in the rainy season due to physiological and mobility limitations of small ants in accessing and foraging in vegetation (Almeida et al., 2023; Baudier et al., 2015; Kaspari et al., 2015). Also, biological filters may be acting in conjunction with environmental filters, as small ants may present a disadvantage in competition/aggression with larger vegetation ants (Mottl et al., 2021). These environmental and biological filters are not mutually exclusive; they apparently interact seasonally in the assembly of local communities. This interaction becomes evident through successive assessments of community patterns under different climatic conditions. As observed in our study, a snapshot assessment in each season can lead to false conclusions. Although we did not investigate the phylogenetic relationships among species in this manuscript, the ground-dwelling ants accessing the vegetation may also constitute a group of closely related species. Therefore, we suggest that future studies consider phylogenetic relationships and the seasonal dynamics of environmental and biological filtering processes to better understand the determinants of ant community composition.

CONCLUSION

Our results demonstrate clear differences in the number and composition of species between seasons and strata, primarily driven by species turnover rather than nestedness. This turnover reflects shifts in

foraging activity associated with ant body size, with smaller species predominating on the ground and larger species more common in the vegetation. Seasonal changes in ground-dwelling ant composition are not linked to variation in body coloration (brightness), but in vegetation strata, ants tend to be darker during the dry season—possibly as an adaptation to thermal stress and increased UV-B radiation. The contribution of nesting to the difference between communities becomes more relevant only when comparing vegetation in the rainy season with ground strata in the dry season: large, pale-coloured ground ants access the sub-canopy and partially replace typical vegetation-dwelling species. This pattern suggests a seasonal weakening of environmental filtering, enabling ground ants to exploit higher strata, likely limiting the foraging of some vegetation ants. Overall, our findings emphasise that snapshot observations of ant communities may lead to contrasting patterns depending on the season, underscoring the importance of considering temporal dynamics in ecological assessments.

AUTHOR CONTRIBUTIONS

Willian Schornobay Bochenki: Investigation; writing – original draft; visualization; writing – review and editing; conceptualization. **Ricardo Eduardo Vicente:** Writing – original draft; investigation; writing – review and editing; data curation. **Tom Rhys Bishop:** Writing – original draft; writing – review and editing; methodology. **Thiago Junqueira Izzo:** Conceptualization; investigation; methodology; validation; writing – review and editing; writing – original draft; formal analysis.

ACKNOWLEDGEMENTS

The authors thank the team at the Secretaria de Estado de Meio Ambiente (SEMA-MT), for their logistical support under Cooperation Agreement (0323/2021/SEMA/MT). Our gratitude extends to all collaborators who participated in the two material collection and sorting campaigns: Moisés Zappani, Reynaldo da Silva Santos, Paulo R. Milhomem da Silva, Mariana C. Pereira, Marcos Penhacek, Giselle M. Lourenço, Wilson França, Maeda, Gabriel Almeida, Ivanildo Castro, Milton O. Córdova, Rogério Santos, Letícia Correia, Camila Volff, and Diego Rodrigues. We especially thank Miriã Ferraz e Souza for designing the map and assisting with material sorting. We also acknowledge institutional support from the Amazon Protected Areas Program (ARPA), the Biodiversity Research Program (PPBio; Proc. 441228/2023-2 and 441260/2023-3), and the National Institute of Science and Technology for Amazonian Biodiversity (INCT-CENBAM; Proc. 406474/2022-2). TJI is supported by the CNPq-Brazil productivity grant (Process 312684/2021-5). Finally, author WSB thanks the Coordination for the Improvement of Higher Education Personnel (CAPES; Proc. 23108.017500/2022-00) for the master's scholarship.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad at <https://doi.org/10.5061/dryad.r2280gbp8>.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Data of filtered environmental variables from September 2023 and February 2024 available at the National Institute of Meteorology (INMET) of the meteorological station in São José do Xingu-MT, Brazil. Available at: <https://portal.inmet.gov.br/> [Accessed 25th March 2024].

Table S2. Records of ant species (Hymenoptera: Formicidae) collected at Xingu State Park across two seasons (dry and rainy) and two strata (ground and low vegetation). The numbers for each species represent their frequency (number of sampling points where they were present) in each treatment.

Figure S1. Raw number of species (on the left) and Hills diversity ($q = 1$) (on the right) of ants collected per plot in Xingu State Park (PEX) in the Dry and Rainy seasons in the terrestrial and vegetation strata. The number of species is higher on the soil (Negative Binomial GLMM $\chi^2_{1,19} = 158.16$, $p < 0.001$) but did not differ between seasons (GLMM $\chi^2_{1,19} = 0.7$, $p = 0.4$) and there is no interaction between strata and seasons (GLMM $\chi^2_{1,19} = 2.15$, $p = 0.14$). We employed here hill number to attenuate the effect of differences in the number of occurrences (abundance) of ants, however the results are virtually the same of the found on real number of species: The is Hills diversity of ants per plot was higher on ground strata (GLMM $\chi^2_{1,19} = 150.16$, $p < 0.001$), but, we did not observe any significant differences in the richness of vegetation ants between seasons ($\chi^2_{1,19} = 1.7$, $p = 0.19$) or interaction ($\chi^2_{1,19} = 2.78$, $p = 0.10$). The different letters above the boxes indicate statistically significant differences ($p < 0.05$).

Figure S2. Rarefaction curves (solid lines) with extrapolation to 1034 individuals (dashed lines), representing the maximum sample size observed (Soil/Dry season). The overall community pattern reinforces the differences in species richness per plot observed in Figure S1a,b, showing consistent differences between strata. However, the rarefaction curves reveal an emergent pattern not detected at the plot level. While vegetation strata exhibit stable species richness across seasons (Vegetation/Dry $\approx$ Vegetation/Rainy), the soil strata show higher overall richness in the dry season than in the rainy season. This difference is observable in the median number of species in the raw data (Figure S1a), but it is not significant, as there is natural variance among plots.

How to cite this article: Bochenki, W.S., Vicente, R.E., Bishop, T.R. & Izzo, T.J. (2025) Ant brightness and size influence the seasonal vegetation use by ground-dwelling ants (Hymenoptera: Formicidae) in an Amazonia-Cerrado transition zone. *Ecological Entomology*, 50(6), 1082–1095. Available from: <https://doi.org/10.1111/een.70000>