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# The role of habitat simplification and seasonality in shaping interactions between ants, plants and herbivores in a neotropical savanna

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## Abstract

1. Protective interactions between ants and plants bearing extrafloral nectaries (EFNs) are highly conditional, and changes in abiotic conditions, including environmental seasonality, are known to influence their outcomes by changing plant phenology. Yet, there is still much to uncover, and despite living in the Anthropocene, we still know little about the effects of anthropogenic activities in shaping ant-plant interactions.
2. We evaluated the influence of chronic anthropogenic disturbances (CAD) and seasonality in shaping the outcomes of interactions between ants and EFN-bearing plants in the Brazilian cerrado. For this, we assessed the diversity of ants (total and arboreal ant fauna) and herbivorous insects in trees of *Qualea grandiflora* located in preserved cerrado and pasture areas. In addition, we assessed the activity of EFNs, the rate of leaf herbivory, and the number of leaves from different stages (sprouting, new, old) monthly for 1 year.
3. Ant diversity in the pasture increased during the dry season, while herbivore diversity increased in the rainy season, coinciding with higher EFN activity and leaf flushing. In the cerrado, EFN activity also peaked in the rainy season, and leaf herbivory levels were higher than in the pasture. Although ant abundance did not influence herbivore presence, greater arboreal ant diversity (especially in the pasture) was linked to lower herbivore richness but had no effect on leaf herbivory.
4. Our study shows that CAD interacts with seasonality to shape multiple aspects of ant-plant interactions, ranging from EFN secretion to leaf herbivory, with potential pervasive consequences for tropical ecosystems.

KEYWORDS
anthropization, ant-plant mutualism, climatic seasonality, extrafloral nectaries, herbivory, phenology

## INTRODUCTION

Ants and plants are involved in a plethora of mutualisms, ranging from non-protective including myrmecochory, pollination and protective, in which ants defend plants from enemies in exchange for food and nesting resources (Rico-Gray & Oliveira, 2007). However, given the ubiquity and complexity of ant-plant protective mutualisms, it is not surprising that, despite many previous efforts, researchers are only beginning to uncover the biotic and abiotic drivers of their outcomes (Calixto et al., 2018). To further complicate this issue, we are faced with an alarming rate of anthropogenic activities in natural habitats (Keck et al., 2025; Montràs-Janer et al., 2024), making the understanding of the role of land-use change and human-induced disturbances on the outcomes of ant-plant mutualisms paramount. In this scenario, there is still an emphasis on studies covering acute disturbances, such as fire and flooding (e.g., Costa et al., 2018; Del-Claro & Marquis, 2015; Fagundes et al., 2017; Vasconcelos et al., 2020). However, chronic anthropogenic disturbances (CADs), including the continuous removal of biomass from natural habitats for agriculture and livestock, are increasingly recognized as important drivers of ant-plant interaction outcomes (e.g., Neves et al., 2012; Oliveira, Câmara, et al., 2021; Oliveira-Lima et al., 2024). Indeed, CAD are putting increasing pressure on natural landscapes, especially in the tropics, which despite harboring the majority of ant-plant interactions is considered a frontier of agricultural expansion.

Interactions between ants and plants with extrafloral nectaries (EFNs) are among the most common mutualisms in the tropics (Bronstein, 1998; Heil, 2015). In these interactions, the ants protect their plant partners in exchange for extrafloral nectar, a liquid with a high concentration of carbohydrates (Heil, 2015). As with other ant-plant protective mutualisms, interactions between ants and EFN-bearing plants have highly variable outcomes, depending on changes in biotic and abiotic factors (Trager et al., 2010). Indeed, some previous studies have shown that CAD can play a critical role in shaping these interactions. First, CAD can strongly alter ant species composition, mostly through indirect effects due to changes in vegetation complexity, microclimatic parameters and soil conditions (Ribeiro-Neto et al., 2023), which reduces the availability of both food and nesting resources (Oliveira, Câmara, et al., 2021). Further, disturbance may reduce plant reward production by decreased resource availability under stressful conditions (Chavarro-Rodríguez et al., 2013; Oliveira, Câmara, et al., 2021), which in turn may reduce ant visitation (Díaz-Castelazo et al., 2017). However, many questions remain unanswered, and we must improve our knowledge of the influence of other factors in shaping the effects of CAD in ant-EFN interactions, including interspecific competition and climatic aspects, such as reduced rainfall and seasonality.

Regions with open vegetation, such as grasslands and savannas, are particularly prone to suffer from CAD, including intensive agricultural practices, because they do not require major land-use conversion for many anthropic activities, such as livestock and plantations (Correa et al., 2025). This is particularly true for the Brazilian Cerrado, which has become one of the last agricultural frontiers in Brazil and one of the most important regions for cattle ranching in South America (Alencar et al., 2020). In addition, the Cerrado is highly seasonal, with dry winters and rainy summers, and such climatic variations may directly and/or indirectly (through plant phenology) affect the dynamics of ant–EFN interactions (Costa et al., 2018). For instance, extrafloral nectar is usually offered in greater abundance during the rainy season due to the great availability of water and nutrients in the soil during this period (Calixto et al., 2021). Furthermore, nectar productivity is usually higher in young leaves because they are more susceptible to attack by herbivorous insects compared to old leaves (Queiroz et al., 2013). In general, higher quantity and quality of extrafloral nectar can enhance ant species richness, visitation frequency and the effectiveness of ant-mediated biotic defence (Lange et al., 2017; Pacelhe et al., 2019).

Despite being a particular target for agricultural expansion in South America, the Cerrado is extremely diverse and hosts a high number of ant species that engage in protective interactions with EFN-bearing plants (Oliveira & Freitas, 2004). Unsurprisingly, the majority of studies on interactions between ants and EFN-bearing plants have been conducted in this biome (Barbosa et al., 2025). Of critical importance, the Cerrado ant fauna is often strongly vertically stratified (Vasconcelos et al., 2023), and despite the relatively small height of the trees, it hosts a highly diverse arboreal ant fauna (Camacho & Vasconcelos, 2015; Camarota et al., 2016). This vertical stratification can have important consequences when assessing responses to disturbances and arboreal and ground-dwelling ants may suffer different effects from CAD and seasonality. Indeed, while ground-dwelling ants suffer only limited impacts of disturbances, arboreal ants can suffer strong effects, including direct (e.g., burning of nests) and indirect (drastic changes of vegetation structure) (Vasconcelos et al., 2017). Notably, arboreal ants are usually better plant defenders, and thus, a decrease or even an extirpation of arboreal ants can be detrimental to critical ecosystem services, such as herbivore control (Oliveira, Câmara, et al., 2021; Vasconcelos et al., 2020).

Here, we investigated the role of CAD in the outcomes of interactions between ants and EFN-bearing plants, with particular emphasis on the role of seasonal variation in shaping the responses of ants, herbivores and plants. Thus, we focused not only on the responses of ants and herbivores to CAD, but also on phenological aspects of the trees, including the proportion of active nectaries, and leaf stages (sprouting, new, old). More specifically, we tested the following hypotheses: (i) The amount of active EFNs will be greater in the rainy season than in the dry season, in new leaves than in old leaves, and in the cerrado area than in the pasture; (ii) The richness, abundance and diversity of ants (total and arboreal) will be greater in the rainy season than in the dry season, especially when there are new leaves and a greater proportion of active EFNs. Furthermore, we expect to find a greater diversity of ants in the cerrado than in the pasture; and (iii) The richness and abundance of herbivores, and consequently herbivory rates, will increase in the rainy season and in the preserved cerrado area, responding positively to the presence of new leaves, but negatively to the proportion of active EFNs and the occurrence of ants, especially arboreal ants.

## METHODS

### Study area

The study was carried out in areas of cerrado sensu stricto in the municipality of Montes Claros, Northern Minas Gerais, Brazil (Figure S1). The region is characterized by a dry tropical climate (Aw in the Köppen system), with well-marked rainy and dry seasons (Alvares et al., 2013). Specifically, in our study region, the month of April presents recurrent rains, while October has low rainfall. Therefore, we consider the period from May to October as the dry season and the period from November to April as the rainy season (Silveira & de Araújo, 2021). Two areas with different levels of land use were sampled. The first area is located in a preserved fragment of cerrado sensu stricto with native vegetation cover, high plant diversity and tree density (Freitas et al., 2020) (Figure S2a). The second area, located approximately 9 km from the first, is a rural property, originally covered by cerrado sensu stricto that was converted into pasture. Some scattered trees were maintained in the area and are found as isolated individuals in the landscape (Figure S2b).

We evaluated ant-plant-herbivore interactions in trees of *Qualea grandiflora* Mart. (Vochysiaceae), popularly known as *pau-terra* (Shimizu et al., 2020). This is an abundant native species in the Cerrado, where it is widely distributed in both more open and more forested vegetations, especially in the cerrado sensu stricto (Ratter et al., 2003). *Qualea grandiflora* has a pair of EFNs located on the petiole close to the insertion of each leaf (Oliveira et al., 1987) (Figure S2c,d). This species produces abundant nectar and has high concentrations of sugar (Lange et al., 2017), therefore it is frequently used by nectar-collecting ants (e.g., Camarota et al., 2015; Vidal et al., 2016) (Figure S2e). Notably, in the Cerrado, the effectiveness of ants against herbivores on plants carrying EFNs was tested first in *Q. grandiflora* (Oliveira et al., 1987; Oliveira & Freitas, 2004) and since then it has been widely included in studies that seek to evaluate ant-plant-herbivore interactions mediated by EFNs (Barbosa et al., 2025).

## Experimental design

For this study, 35 individuals of *Qualea grandiflora* were selected and marked, with 20 individuals sampled in the cerrado area and 15 individuals in the pasture area (where 20 individuals had also been initially selected, but five were accidentally knocked down), distanced from each other by at least 10 m. After being marked, all individuals were georeferenced using a GPS. Data collection was carried out monthly for 1 year, between May 2023 and April 2024, always in the morning. Ants and herbivorous insects associated with *Q. grandiflora* were sampled in all collections (Sá et al., 2025). The assessment of extrafloral nectar productivity was done only in the months when the resource was available. In this way, selected individuals were visually monitored to observe the activity of the EFNs, which were considered active when secreting nectar, evidenced by the presence of a droplet on the nectary (Figure S2c,d). Leaf herbivory levels were also recorded only in months when there were enough leaves to sample.

## EFNs activity

To measure the activity of EFNs, three apical branches were randomly selected from each tree (Sá et al., 2025). Each branch was isolated to allow quantification of active EFNs by preventing nectar removal by arthropods or dilution by rain and/or dew (Calixto et al., 2021; Heil et al., 2000). Isolation was performed as follows: First, a beating procedure was carried out in each branch to remove and collect arthropods that were foraging there. Then, to ensure that the nectar observed was related to the sampling period, all EFNs from the selected branches were washed with distilled water and dried with filter paper. Once this was done, we used fabric bags to wrap the branches. In addition, a layer of double-sided tape was applied close to the fabric at the base of each branch to reinforce the insulation (Heil et al., 2000; Lange et al., 2017). The branches were bagged for 48 hours. We defined the isolation period through pilot trials before official collections. We quantified for each of the three sampled branches the number of total EFNs and the number of active EFNs. From this quantification, we estimated the proportion of active nectaries per plant.

## Foliar phenology

Every month an observation and classification was made regarding the phenological state of the predominant foliage on the trees (Sá et al., 2025). We observed four stages of leaf development: 'Absent leaves', when the trees were completely defoliated; 'Leaves sprouting', when the plants began to work on issuing new leaves and only leaf buds were observed in the crowns; 'New leaves', when the trees had partially expanded leaves, but still tender and light green; and 'Old leaves', when the leaves were fully expanded, with a leathery appearance and dark green colour. Active EFNs were observed only in the "new" and "old" leaf stages. The EFNs on young leaves were green in colour and became brown as the leaves aged (Figure S2c,d). At the leaf sprouting stage, the leaf buds had not yet expanded, so neither the leaves nor the EFNs were actually developed at this stage. Likewise, leaves and consequently EFNs were absent when the canopy was completely defoliated.

## Ants and herbivores sampling

To measure the richness and abundance of ants and herbivores in individuals of *Q. grandiflora*, pitfall traps were installed (one per tree) consisting of 500-mL plastic cups (8 cm in height × 10 cm in diameter), without bait, containing a solution composed of water and detergent. The traps were tied to the trunk at a height of 1.30 m, and protected from rain using a plastic roof (Figure S2f), remaining in the field for 48 h (Ribas et al., 2003), simultaneously with the isolation of the branches. As already mentioned, the beating method was also carried out on three branches, which were struck 10 times each, with the help of a wooden stick, so that the insects that were foraging there fell onto the beating cloth, where they were captured with the aid of entomological tweezers (Freitas et al., 2023) (Figure S2g). All samples were labelled, stored in containers with 70% alcohol and taken to the laboratory for mounting and identification.

All ants were identified to the genus level according to the taxonomic keys of Baccaro et al. (2015) and then separated into morphospecies through the observation of individuals' morphological characteristics, except the *Cephalotes*, which were identified as species with the help of the taxonomic key by Oliveira, Powell, and Feitosa (2021), and *Pheidole gertrudae*, which was confirmed by the specialist Rodrigo Feitosa. Although our collection methods were aimed at capturing arboreal ants, we also recorded on the trees ants that forage and nest mostly in other substrates. Based on their habits, the collected ants were classified into two categories: (i) arboreal ants—including genera with strictly arboreal nesting and foraging habits (e.g., *Cephalotes* and *Pseudomyrmex*), as well as genera that, although some species are known or suspected to nest in the soil, forage extensively in trees (e.g., *Camponotus*); and (ii) non-arboreal ants – including genera predominantly associated with the soil for nesting and foraging, but that occasionally exploit resources in trees (e.g., *Dorymyrmex* and *Pheidole*) (Baccaro et al., 2015; Rosa et al., 2021). From this point onward, we will use the term "arboreal ants" (or arboreal ant richness/abundance) to refer exclusively to this category, while the term "total ants" (or total ant richness/abundance) will be employed to designate the entire set of sampled species, encompassing both categories, that is, both arboreal and non-arboreal ants.

Herbivorous insects were identified in families using the keys of Triplehorn and Johnson (2015) and Rafael et al. (2024) for adults and Costa et al. (2006) for the immatures. After this first identification, herbivores were separated into morphospecies through observation of the morphological characteristics of the individuals. We considered as herbivores the insects belonging to phytophagous families of the orders Coleoptera, Hemiptera, Lepidoptera, Orthoptera and Thysanoptera (Carrano-Moreira, 2014). All insects collected (ants and herbivores) are in the entomological collection of the Laboratory of Biodiversity and Ecological Interactions (LIEB) at Unimontes.

## Foliar herbivory levels

To evaluate leaf herbivory rates, on each plant, as long as there were enough leaves, a random branch containing at least 10 leaves was collected monthly. This collection took place after removing the isolation carried out in the tree branches; therefore, it did not influence the productivity of the EFNs. Soon after collection, the leaves were taken to the laboratory and scanned to create digital images, with the aid of a Canon Lide 300 Scanner. These images were calibrated at a 1-cm scale and used to calculate the leaf area removed by herbivorous insects (chewing insects), and the total leaf area using imageJ v1.53 software (Schneider et al., 2012). Subsequently, the percentage of area removed was calculated for each leaf, using the following formula: (leaf area consumed / total leaf area) * 100 (Ramos et al., 2022). The average percentage of leaf area removed was estimated for each plant individual.

## Data analyses

All analyses were conducted at the tree level, using each tree as an independent sampling unit. Mean values per tree were used for richness, abundance and herbivory metrics to standardize comparisons between cerrado ($n = 20$) and pasture ($n = 15$) areas. Using the *lme4* (Bates et al., 2015) and *glmmTMB* packages (Brooks et al., 2022), we constructed generalized linear mixed-effect models (GLMM) to test all of our hypotheses. In our models, *Q. grandiflora* individuals were treated as random variables. In all models, we used the model simplification criterion, where all non-significant explanatory variables were removed from the complete model (Crawley, 2012). All analyses were performed using R software version 4.4.1 (R Core Team, 2024).

We first tested the effects of seasonality, leaf phenology and environment on the productivity of EFNs (hypothesis 1). In this case, the proportion of active EFNs was the response variable, and the seasons (dry and rainy), leaf stage (old or new), environments (cerrado and pasture) and the interaction between environment and season were the explanatory variables, using Beta error distribution (logit link).

Regarding ants, we first carried out analyses considering the entire diversity of collected ants (total ant richness and total ant abundance). Later, we repeated the same analyses, considering only the arboreal ant community (arboreal ant richness and arboreal ant abundance). Specifically, we evaluated the effects of the explanatory variables season, leaf stages (absent, budding, new and old), proportion of active EFNs, type of environment and interaction between environment and season, on the response variables total ant richness and abundance, and on the richness and abundance of arboreal ants, using Poisson error distribution (hypothesis 2). Significant differences in ant richness and abundance (total and arboreal) between leaf stages were compared using post hoc testing, using the *phia* package (De Rosario-Martinez et al., 2015).

The same procedure was repeated to evaluate how these same factors influence the richness and abundance of herbivorous insects. Furthermore, we evaluated how herbivores are affected by the ant community (hypothesis 3). In this case, the total richness and abundance of ants and the richness and abundance of arboreal ants were used as explanatory variables. The richness and abundance of herbivores were the response variables. Additionally, we also tested whether seasonality, environment type, EFN productivity and the presence of arboreal ants influence foliar herbivory levels (hypothesis 3). The explanatory variables included season, environment type, number of active EFNs and arboreal ant diversity, while the percentage of leaf area removed by herbivores was used as the response variable. For these analyses, we fitted a Binomial with link logit error distribution to the response.

To ensure that observed differences in ant diversity were not influenced by extremely abundant species, we also calculated Hill numbers ($q = 0, 1, 2$) for each sampled tree. These metrics quantify species diversity while weighting species differently according to their relative abundance ($q = 0$ corresponds to species richness, $q = 1$ to the exponential of Shannon diversity, and $q = 2$ to the inverse

Simpson index). Analyses were performed using the diversity function in the *vegan* package (Oksanen et al., 2025). We additionally verified that similar patterns emerged when using presence–absence (frequency) data, confirming that our conclusions were not driven by a single hyperdominant taxon.

Additionally, to elucidate the patterns of ant species composition between environments and seasons, we performed a permutational multivariate analysis of variance (PERMANOVA) based on Bray–Curtis distances (999 permutations) using the *adonis2* function in the *vegan* package (Oksanen et al., 2025). Prior to analysis, species abundance data were transformed using the Hellinger method to reduce the influence of dominant species. To verify the assumption of homogeneity of dispersion, we used the *betadisper* function, also in *vegan*. To visualize compositional differences, we performed a non-metric multidimensional scaling (NMDS) ordination with two dimensions using the same distance matrix. In addition, we conducted an indicator species analysis (function *multipatt*, package *indicspecies*; de Caceres et al., 2010) to identify species significantly associated with each environment. The strength of association between each species and environment was measured using the *r.g* statistic, and significance was assessed with 999 permutations.

# RESULTS

## Ant and herbivore diversity

A total of 31,451 ants were collected, distributed across six subfamilies, 21 genera and 60 species/morphospecies (Table S1). We recorded a total of 31 ant species ($N = 1536$ individuals) using the beating method, and all 60 species ($N = 29{,}915$) with pitfall traps. Myrmicinae was the richest subfamily in terms of genera (seven), followed by Dolichoderinae (five), Formicinae (four), Dorylinae and Ectatomminae (two genera each), and finally Pseudomyrmecinae with only one genus. Among the 21 genera, seven correspond to ants with predominantly arboreal habits: *Camponotus*, *Cephalotes*, *Crematogaster*, *Dolichoderus*, *Myrmelachista*, *Nesomyrmex* and *Pseudomyrmex*.

Even though a smaller number of plants was sampled (i.e., 15 plants compared to 20 in the cerrado), the pasture stood out for its richness and abundance of ants, totalling 28,651 specimens sampled (91% of the total sampled ants), distributed in 18 genera and 41 species/morphospecies. Considering all the diversity of ants collected in this area, *Pheidole*, with only three morphospecies, was the most abundant genus, totalling 25,090 individuals (with emphasis on *Pheidole gertrudae*, which alone totals 25,077 individuals), followed by *Camponotus* (1466 individuals and seven morphospecies) and *Dorymyrmex* (1293 individuals and three morphospecies). Considering only the diversity of arboreal ants, seven genera were sampled in the pasture, with *Camponotus* being the most diverse, followed by *Pseudomyrmex* (227 individuals and seven morphospecies) and *Cephalotes* (112 individuals and five morphospecies). In the cerrado, 14 genera of ants were sampled, distributed in 36 morphospecies, which together represent an abundance of 2800 individuals (9% of the total sampled ants). The most diverse genus in this area was *Cephalotes* (with 1010 individuals and six morphospecies), then *Camponotus* (775 individuals and six morphospecies) and *Crematogaster* (453 individuals and three morphospecies). Six of the seven genera of arboreal ants were present in the cerrado, including the three mentioned here.

The diversity of herbivores included a total of 778 insects, distributed across five orders, 32 families and 180 morphospecies (Table S2). The majority (629 individuals and 97 morphospecies) belong to the Hemiptera order. The second most diverse order of herbivores was Coleoptera (with 103 individuals and 57 morphospecies), followed by Orthoptera (23 individuals and 17 morphospecies), Thysanoptera (21 individuals and 8 morphospecies) and Lepidoptera (with only two individuals of different morphospecies). The pasture area also presented a higher diversity of herbivores, with 568 individuals collected (73% of the total), distributed in 28 families and 120 morphospecies. Cicadellidae was the most diverse family, with 334 individuals from 28 morphospecies, followed by Alydidae (45 individuals and six morphospecies), Chrysomelidae (13 morphospecies) and Psyllidae (seven morphospecies), with 30 individuals each. Meanwhile, the cerrado presented 27% of the total herbivorous insects sampled, with 210 specimens collected and a richness of 21 families and 85 morphospecies. The most diverse herbivore families in the cerrado were the same as those cited for pasture (Alydidae with 70 individuals and five morphospecies; Cicadellidae with 54 individuals and 15 morphospecies; and Chrysomelidae with 23 individuals and 16 morphospecies).

## Factors affecting EFN activity

The proportion of active EFNs in *Q. grandiflora* was almost four times higher in the rainy season (mean 0.19 ± SD 0.01) compared to the dry season (0.05 ± 0.01) (Table 1; Figure 1a) and twice as high in young leaves (0.23 ± 0.02; Table 1; Figure 1b) than in old leaves (0.12 ± 0.01). However, there were no significant differences in the proportion of active EFNs between cerrado and pasture.

## Factors affecting ant diversity

The total richness and total abundance of ants also showed seasonal variations, being higher in the dry season (Table 2; Figure S3a,b). More specifically, there was a slight reduction in ant richness during the rainy season (3.28 ± 0.25) compared to that observed for the dry season (3.54 ± 0.25). Seasonal differences were more pronounced for total abundance, with an average of 117 (± 33.2) individuals sampled in the dry season and 32.76 (± 33.2) individuals sampled in the rainy season. Arboreal ants also showed greater richness in the dry season (2.65 ± 0.14) than in the rainy season (2.45 ± 0.14; Table 2; Figure S3c). However, their abundance was not affected by the season.

The total richness of ants was greater in the period in which the trees had old leaves (3.9 ± 0.22; Table 2; Figure S4a), with a reduction

**TABLE 1** Result of the statistical model (GLMM) that evaluated the effects of the season (dry and rainy), leaf stage (new and old), environment (cerrado and pasture) and the interaction between environment and season on the proportion of active EFNs.

| Response variables | Explanatory variables | $\chi^2$ | GL | *p* |
|---|---|---|---|---|
| Proportion of active EFNs | Season | 4.844 | 1 | 0.027 |
| | Leaf stage | 14.487 | 1 | <0.001 |

*Note*: As we followed the model simplification criterion, only explanatory variables with significance ($p < 0.05$) appear in the final model. Model fit: $R^2$ (marginal) = 0.399.

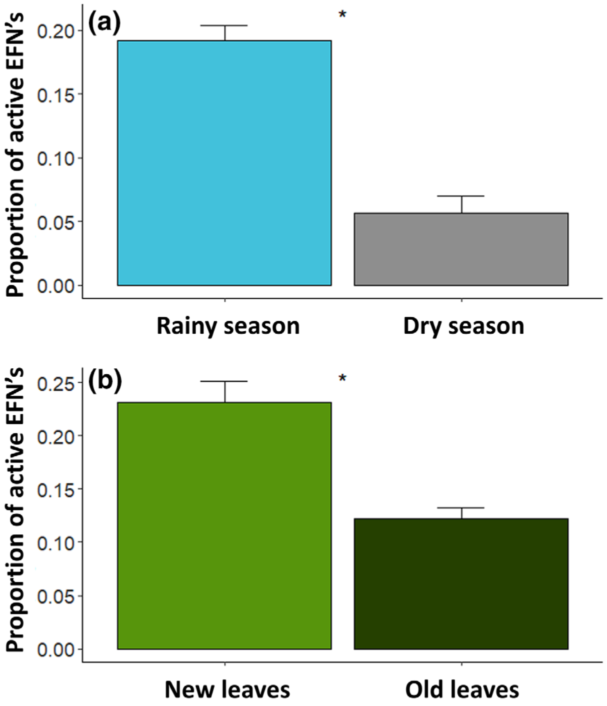

**FIGURE 1** Proportion of extrafloral nectaries (EFNs) of *Q. grandiflora* active (a) in different seasons (dry and rainy) and (b) and between different leaf stages (new leaves and old leaves). Bars show mean ± SE. Asterisks (*) indicate significant differences ($p < 0.05$).

when the leaves were new (3.07 ± 0.31), and further reducing in the period in which the crowns were defoliated and the leaves were absent (2.04 ± 0.28). The richness of arboreal ants followed the same pattern, presenting an average richness of 2.93 (± 0.13; Table 2; Figure S4b) for plants with old leaves, 2.21 (± 0.21) for plants with new leaves, and 1.53 (±0.19;) for plants with missing leaves. Ant abundance (total and arboreal) was not affected by foliage phenological variations.

Regarding the effects of the environment type on ant diversity, we found significant differences in total richness and total abundance between cerrado and pasture (Table 2). More specifically, the pasture had a higher total ant richness (4.11 ± 0.30) than the cerrado (2.88 ± 0.27). The difference was even greater when it came to the abundance, and the pasture had 13 times more ants (159.17 ± 41.07) than the cerrado (11.67 ± 35.56). The richness and abundance of arboreal ants did not differ between cerrado and pasture. We found that the percentage of active EFNs positively affected both total ant richness and arboreal ant richness (Table 2).

Although ant richness was higher in the pasture, diversity metrics that account for species abundances revealed an opposite pattern. Shannon diversity (Hill $q = 1$) was significantly lower in the pasture compared to the cerrado (Estimate = −1.33 ± 0.40, $p = 0.001$) and higher in the rainy season compared to the dry season (Estimate = +1.06 ± 0.39, $p = 0.008$; $R^2 = 0.21$, $F_{2,68} = 9.26$, $p < 0.001$). This indicates that, despite containing more species, ant assemblages in the pasture are strongly dominated by a few highly abundant taxa, mainly *Pheidole gertrudae*.

Analysing the interaction between environment and season, we saw that only in the pasture there was significant variation in the ant community between seasons, with higher richness (4.77 ± 0.34; Table 2; Figure 2a) and abundance (258.54 ± 46.92; Table 2; Figure 2b) in the dry season than in the rainy season (3.45 ± 0.34 for richness and 59.8 ± 46.92 for abundance). Despite a trend for higher richness in the rainy season (3.14 ± 0.29) compared to the dry season (2.61 ± 0.29), the cerrado did not show significant differences in ant richness or abundance between seasons. The interaction between environment and season was not significant for either the richness or the abundance of arboreal ants.

The PERMANOVA revealed significant differences in ant species composition between environments and seasons ($F = 17.24$, $R^2 = 0.436$, $p = 0.001$; Figure S5). No evidence of heterogeneity in multivariate dispersion was detected among environments ($F = 0.14$, $p = 0.712$) or seasons ($F = 0.20$, $p = 0.656$). Indicator species analysis identified 23 ant species with significant associations ($p < 0.05$): 11 species were indicative of the cerrado and 12 of the pasture (Table S3). Cerrado indicators included *Cephalotes pavonii*, *C. minutus* and *Pseudomyrmex* sp. 2, while pasture indicators comprised *Camponotus* sp. 1, *Pseudomyrmex* sp. 5 and *Dorymyrmex* sp. 3.

## Factors affecting herbivore diversity

The richness and abundance of herbivorous insects also showed significant variations between seasons (Table 3). In terms of richness, more herbivore species were recorded during the rainy season (1.46 ± 0.13; Figure S6a) compared to the dry season (0.84 ± 0.13). Abundance followed the same pattern, with the rainy season having more than twice the number of herbivores (2.63 ± 0.28; Figure S6b) found in the dry season (1.08 ± 0.28).

**TABLE 2** Results of statistical models (GLMM's) evaluating the effects of season (dry and rainy), leaf stage (absent, sprouting, new and old), proportion of active EFNs, environment (cerrado and pasture) and the interaction between environment and season on the richness and abundance of ants (total and arboreal).

| Response variables | Explanatory variables | $\chi^2$ | GL | $p$ |
|---|---|---|---|---|
| Total ant richness | Season | 27.235 | 1 | <0.001 |
| | Leaf stage | 4.490 | 1 | 0.034 |
| | Prop. of active EFNs | 4.181 | 1 | 0.041 |
| | Environment | 4.524 | 1 | 0.033 |
| | Environment × Season | 8.458 | 1 | 0.004 |
| Total ant abundance | Season | 8.043 | 1 | 0.004 |
| | Environment | 7.372 | 1 | 0.007 |
| | Environment × Season | 11.139 | 1 | <0.001 |
| Arboreal ant richness | Season | 26.933 | 1 | <0.001 |
| | Leaf stage | 5.572 | 1 | 0.018 |
| | Prop. of active EFNs | 4.047 | 1 | 0.044 |

*Note*: As we followed the model simplification criterion, only explanatory variables with significance ($p < 0.05$) appear in the final model. The abundance of arboreal ants does not appear in the table due to the lack of relationship with any explanatory variable. Model fit (Total ant richness): $R^2$ (marginal) = 0.566; Model fit (Total ant abundance): $R^2$ (marginal) = 0.183; Model fit (Arboreal ant richness): $R^2$ (marginal) = 0.328.

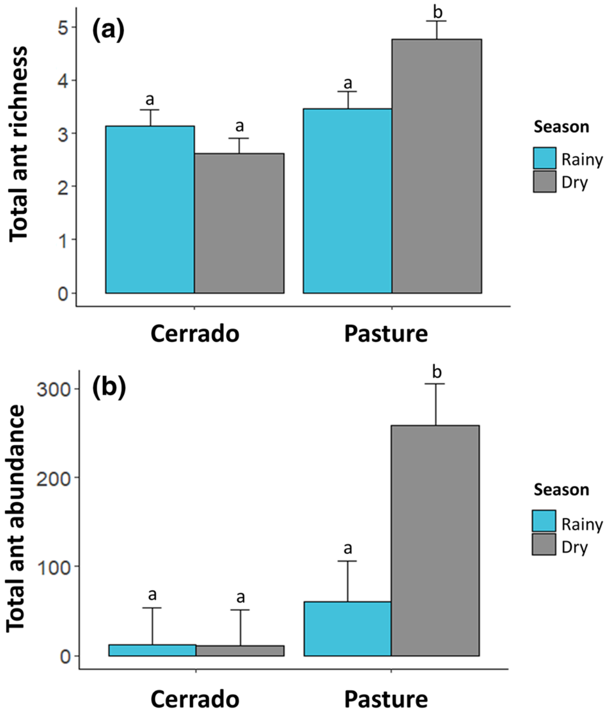

**FIGURE 2** Comparison of (a) total ant richness and (b) total abundance of ants sampled from *Q. grandiflora* trees between seasons (dry and rainy) in different environments (cerrado and pasture). Bars show mean ± SE. Letters indicate significant differences ($p < 0.05$).

Furthermore, we observed significant differences between herbivore richness and different leaf stages (Table 3; Figure S6c). More herbivore species were recorded when trees had new leaves (1.91 ± 0.20) than when they had old leaves (1.05 ± 0.12) or defoliated crowns (0.96 ± 0.18). The abundance of herbivorous insects was not influenced by leaf phenology. Furthermore, herbivore richness and abundance were not affected by the proportion of active EFNs.

We found significant differences in the richness and abundance of herbivores between cerrado and pasture (Table 3). In the pasture, the average number of insect herbivore species was 1.80 (± 0.10), almost three times greater than the number recorded for the cerrado, which was 0.65 (± 0.09). Similarly, the abundance of herbivores in the pasture was more than three times higher than in the cerrado, with averages of 3.15 (± 0.23) and 0.87 (± 0.20), respectively.

Analysing the interaction between environment and season, we found that, in the cerrado, the herbivore community did not show significant variations in richness or abundance between seasons. However, for the pasture, we observed marked differences. In this environment, herbivores were at least twice as rich (2.53 ± 0.14; Figure 3a) and were almost four times more abundant (5.0 ± 0.31; Figure 3b) in the rainy season, than in the dry season (1.07 ± 0.14 for richness; 1.31 ± 0.31 for abundance) (Table 3).

The total abundance of ants, and also the abundance of arboreal ants, had no significant impact on the richness or abundance of herbivorous insects. However, total ant richness and arboreal ant richness appear to influence the herbivore community in different ways. While total ant richness was associated with an increase in the presence of herbivores, with positive effects on both richness (Table 3; Figure 4a) and abundance (Table 3; Figure 4b), arboreal ant richness had opposite effects, being associated with a decrease in richness (Table 3; Figure 4c), but an increase in abundance (Table 3; Figure 4d) of herbivores.

## Factors affecting leaf herbivory

Foliar herbivory rates were significantly higher during the rainy season (0.20 ± 0.24; Table 3; Figure 5) compared to the dry season (0.15 ± 0.15). Additionally, we observed higher levels of herbivory in the cerrado (0.27 ± 0.26; Table 3; Figure 5) than in the pasture area (0.11 ± 0.13). We also identified a negative relationship between the

**TABLE 3** Results of statistical models (GLMMs) evaluating the effects of season, leaf stage, proportion of active EFNs, environment, interaction between environment and season and ant richness and abundance (total and arboreal) on the herbivore insect richness, herbivore insect abundance and leaf herbivory.

| Response variables | Explanatory variables | $\chi^2$ | *p* |
|---|---|---|---|
| Herbivore insect richness | Season | 17.351 | <0.001 |
| | Leaf stage | 9.306 | 0.025 |
| | Environment | 79.486 | <0.001 |
| | Environment × Season | 26.475 | <0.001 |
| | Ants total richness | 23.705 | <0.001 |
| | Arboreal ant richness | 28.329 | <0.001 |
| Herbivore insect abundance | Season | 34.577 | <0.001 |
| | Environment | 54.764 | <0.001 |
| | Environment × Season | 49.12 | <0.001 |
| | Ants total richness | 19.315 | <0.001 |
| | Arboreal ant richness | 18.559 | <0.001 |
| Leaf herbivory | Season | 2.580 | <0.05 |
| | Environment | 4.670 | <0.001 |
| | Prop. active EFNs | 3.050 | <0.05 |

*Note*: As we followed the model simplification criterion, only explanatory variables with significance ($p < 0.05$) appear in the final model. Model fit (Herbivore insect richness): $R^2$ (marginal) = 0.383; Model fit (Herbivore insect abundance): $R^2$ (marginal) = 0.318; Model fit (Leaf herbivory): $R^2$ (marginal) = 0.557.

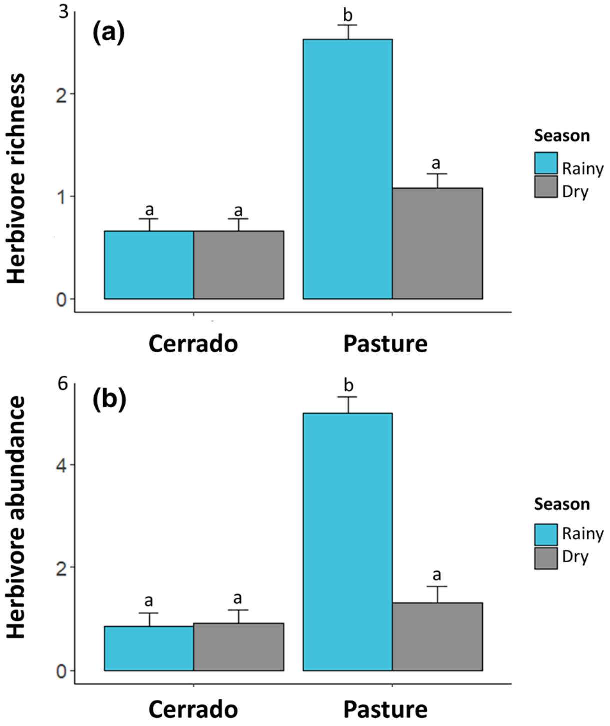

**FIGURE 3** Comparison of (a) richness of herbivore insects (b) abundance of herbivore insects sampled from *Q. grandiflora* trees between seasons (dry and rainy) in different environments (cerrado and pasture). Bars show mean ± SE. Letters indicate significant differences ($p < 0.05$).

proportion of active EFNs and foliar herbivory levels (Table 3; Figure 6). However, we found no evidence of a relationship between foliar herbivory and the presence of arboreal ants.

# DISCUSSION

Here, we investigated the role of CADs in the outcomes of interactions between ants and EFNs, with particular emphasis on the role of seasonal variation in shaping the responses of ants, herbivores, and plants. All three main hypothesis were partially corroborated, although with important exceptions. We found that the amount of active EFNs was higher in the rainy season when *Q. grandiflora* was flushing its leaves, although with no effect of land use on EFN activity. While there were no seasonal effects on the diversity of ants and herbivores on the cerrado, there were opposite effects of seasonality for ants and herbivores in the pasture: whereas ant diversity increased in the dry season, the diversity of herbivores increased in the rainy season. Concordantly, we found a negative effect of EFN activity on leaf herbivory, which was higher in the cerrado during the rainy season. The abundance of ants had no effect on the presence of herbivores on trees, while the diversity of arboreal ants decreased the richness of these insects, possibly due to the presence of abundant and/ or aggressive ant species. Further, despite having a detrimental effect on the richness of herbivores, arboreal ants had no effects on leaf herbivory, which is perhaps related to an unnoticed increase in sap-sucking herbivores. Our study did not quantify the rate of direct interactions between ants and EFNs, and thus our inferences are based on indirect outcomes derived from EFN activity, ant diversity and herbivory patterns. Future studies incorporating direct observations of EFN visitation frequency and duration would provide valuable insights into how the behavioural dynamics of ants mediate the ecological consequences of these mutualisms across environments and seasons.

As expected, we found that the amount of active EFNs was higher in the rainy season and in new leaves. This is a recurrent

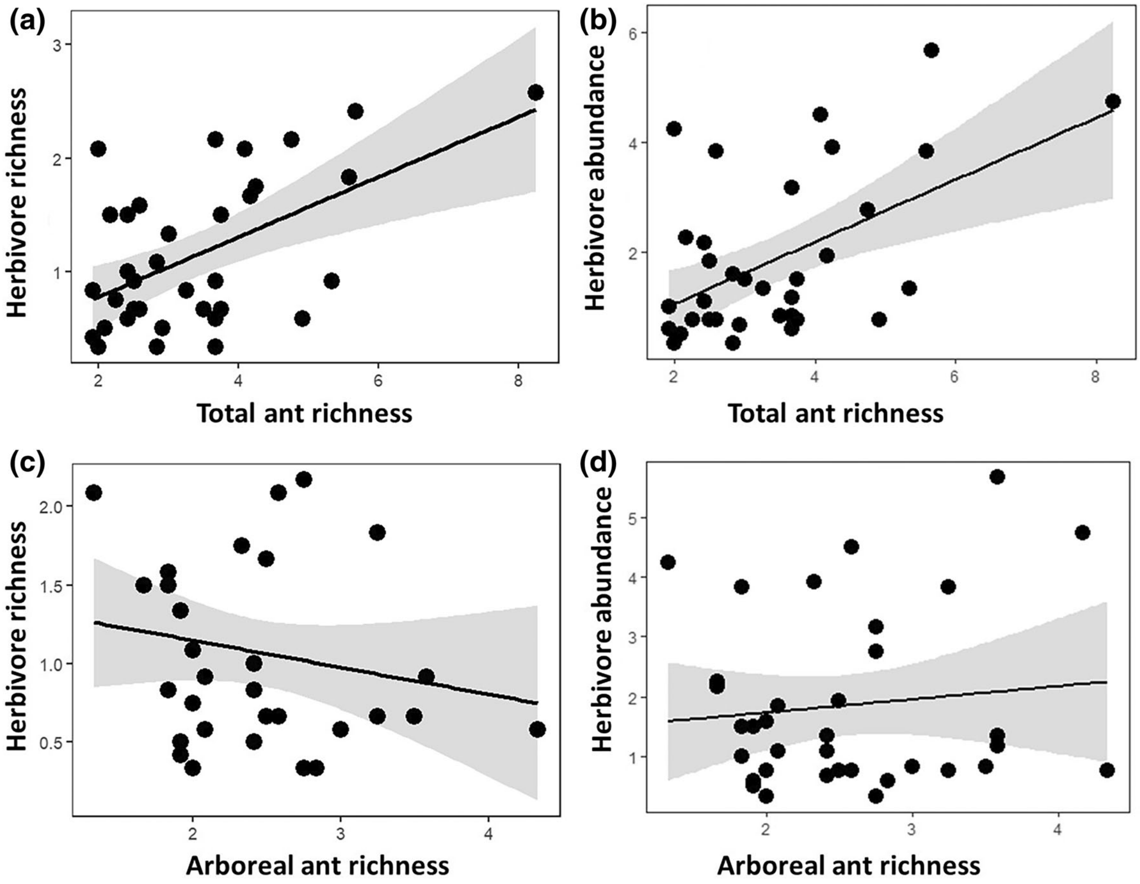

**FIGURE 4** Effects of ant diversity on the diversity of herbivorous insects on *Q. grandiflora* trees. (a) Relationship between total ant richness and herbivorous insect richness. (b) Relationship between total ant richness and herbivorous insect abundance. (c) Relationship between arboreal ant richness and herbivorous insect richness. (d) Relationship between arboreal ant richness and herbivorous insect abundance. Shaded areas represent 95% confidence intervals.

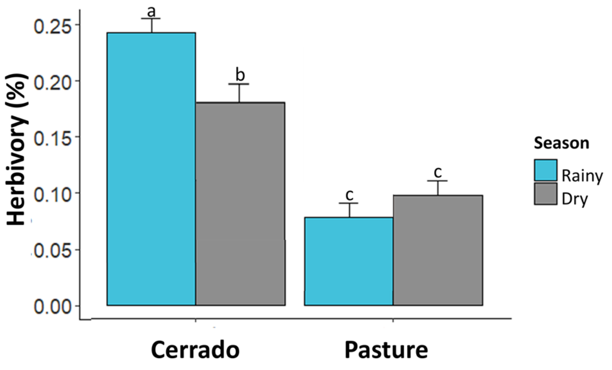

**FIGURE 5** Comparison of foliar herbivory rates recorded for *Q. grandiflora* (a) across different seasons (dry and rainy) and (b) environments (cerrado and pasture). Bars show mean ± SE of observed herbivory rates per tree. Letters indicate significant differences ($p < 0.05$).

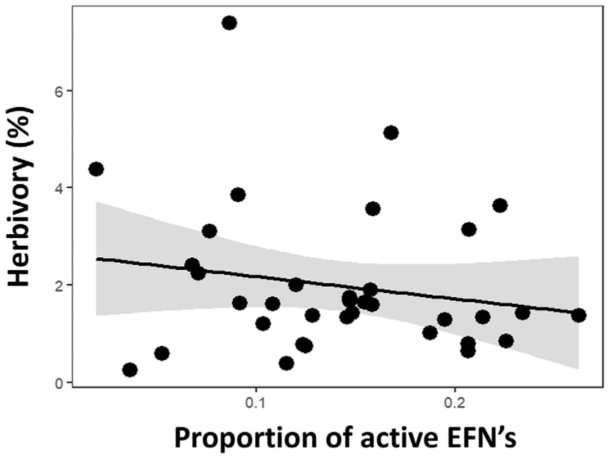

**FIGURE 6** Effect of the proportion of active extrafloral nectaries (EFNs) on foliar herbivory rate in *Q. grandiflora* trees. Line represents model-predicted herbivory rates (partial effects), with shaded areas indicating 95% confidence intervals.

pattern in seasonal habitats and is related to a higher amount of resources in the soil for the plants (Queiroz et al., 2013), including water (Silva et al., 2011). However, contrary to what we expected, we did not find differences in the amount of active nectaries between the preserved (cerrado) and anthropized (pasture) areas. Nevertheless, we did not measure the sugar concentration of the extrafloral nectar, so the lack of differences in active nectaries may not reflect potential variation in nectar quality (sugar concentration or volume) between cerrado and pasture areas (Heil, 2015; Pacelhe et al., 2019). This issue is not straightforward, and while there is often variation in nectar quality under stressful conditions (Heil, 2015; Oliveira, Câmara, et al., 2021), there is no consensus on whether CAD has impacts on the quality of extrafloral nectar (Chavarro-Rodríguez et al., 2013; Díaz-Castelazo et al., 2017).

Most studies have shown that ant activity in the vegetation increases in the rainy season, as there is a higher availability of extrafloral nectar (e.g., Calixto et al., 2021; Lange et al., 2013). However, contrary to expected, we did not detect changes in ant species richness and abundance between seasons in the preserved cerrado. Notably, when assessing the environment-season interaction, we saw that the pasture had a greater diversity of ants in the dry season, indicating that the effects of seasonality were more pronounced in the pasture. Previous studies have shown that ants face a decrease in resource diversity under harsher conditions, with a decrease in the supply of seeds and insect prey (Chinarelli et al., 2022), making EFN-bearing trees a hub of critical resources for ants. Indeed, the supply of nectar can also play an important role in the survival of ants in the dry season, as at this time of high water stress, the water in nectar, in addition to sugar, constitutes an essential resource for ants (Valdez-Ojeda et al., 2025). Whereas we did not detect any effect of season per se, this nectar supply may have proven essential under the increased stressful conditions due to CAD.

While overall ant diversity increased during the dry season on pasture trees, this pattern was not observed for arboreal ants on cerrado trees. The explanation relies on multiple factors. Arboreal ants depend on limited nesting sites and rarely relocate colonies when vegetation connectivity is low (Adams et al., 2023). Because the arboreal stratum is already a stressful environment, species adapted to high temperature and desiccation (Law et al., 2020) are likely less affected by CAD and seasonal drought. In contrast, ground-dwelling ants such as *Dorymyrmex*, *Forelius* and *Pheidole* increased on pasture trees during the dry season, likely exploiting EFNs opportunistically when other resources were scarce. Thus, our results support previous findings which indicate that scattered trees in agricultural landscapes attract a high number of both arboreal and ground-nesting ant species in the cerrado (Frizzo & Vasconcelos, 2013).

Despite higher species richness in the pasture, Hill diversity revealed lower evenness, indicating that ant assemblages were dominated by a few generalist species, mainly *Pheidole gertrudae*. This pattern suggests that habitat simplification favours tolerant taxa at the expense of a balanced community structure. In contrast, the cerrado supported more even assemblages with a greater representation of less abundant arboreal species. Overall, these results show that land-use change affects not only species richness but also the evenness and functional structure of ant communities.

Our analysis also revealed differences in ant species composition between environments and seasons. The pasture and cerrado harboured distinct assemblages, with 23 indicator species showing clear environmental associations. While arboreal taxa such as *Cephalotes* and *Pseudomyrmex* were characteristic of the cerrado, the pasture was dominated by generalist and thermophilic species of *Camponotus*, *Dorymyrmex* and *Forelius*. These compositional shifts indicate that land-use change promotes the replacement of specialized arboreal ants by ground-dwelling generalists tolerant to higher temperatures and resource scarcity. Such turnover may explain the patterns observed in EFN activity and ant diversity, as altered species pools can change the intensity and effectiveness of ant-plant interactions (e.g., Vasconcelos et al., 2020). Similar patterns of taxonomic turnover linked to habitat disturbance have been reported in other Neotropical savannas (e.g., Oliveira-Lima et al., 2024), emphasizing that species composition, rather than richness alone, plays a major role in determining ecosystem functioning under anthropogenic pressure.

Opposed to ants, herbivores increased their diversity in the rainy season in pasture trees. This was highly expected as the increased water availability in the soil favours the vegetative growth of plants and, consequently, the supply of food resources for these organisms (Novaes et al., 2020). In addition, there is a higher proportion of younger leaves in the rainy season, which are more palatable and nutritious (Calixto et al., 2021; Queiroz et al., 2013). However, as the leaves mature, they become more resistant and rich in phenolic compounds, becoming less palatable to these insects (Calixto et al., 2021; Queiroz et al., 2013). In addition, the higher presence of herbivores can simply reflect a decrease in the activity of natural enemies (Altieri & Letourneau, 1982), including ants (Neves et al., 2012), which were more abundant in the pasture during the dry season. Thus, once the individuals of *Q. grandiflora* offer a hub of resources for ants under harsher conditions, herbivores may avoid such plants, as they could be easily preyed upon by these ants, which are mostly generalist omnivores.

The total ant richness and arboreal ant richness influenced the herbivore community in opposite ways. Whereas the richness and abundance of herbivores were favoured by the diversity of ants in general, increased arboreal ant richness was linked to a decrease in herbivore richness (but not in their abundance). This indicates that only arboreal ants actually established a mutualistic relationship with *Q. grandiflora* (and possibly with trophobiont herbivores) as detected before for other tree species (Oliveira et al., 2019; Vasconcelos et al., 2020). Presumably, the presence of some behaviourally dominant and aggressive arboreal ant species acted like a filter for herbivorous insects, allowing the presence of only those species that are less likely to be preyed upon by ants. But we must point out that we detected only an effect of arboreal ant species richness and not abundance. Importantly, most interactions between ants and EFN-bearing plants are diffuse, and while a few species can successfully defend the plants, most species are rather opportunistic (Fagundes et al., 2017; Flores-Flores et al., 2018). Thus, increasing the abundance of opportunistic ants would have little or no effect on herbivores. However, a higher diversity of ants indicates an increased possibility of the presence of good ant partner species in the trees (Del-Claro & Marquis, 2015; Moura & Del-Claro, 2023). Usually, these good partners are those aggressive species (e.g., *Camponotus* and *Crematogaster*) that recruit a relatively large number of foragers (Fagundes et al., 2017; Flores-Flores et al., 2018), but abundance does not equal good defensive abilities, and even species with low recruitment can effectively capture herbivores, such as those from the genera *Pseudomyrmex* and *Ectatomma* (Souza et al., 2024).

We found that herbivory was higher in the rainy season and in the cerrado. The explanation for this is quite simple, as there were more herbivores in the rainy season, when there was a higher amount of new leaves, which are more palatable and nutritious. In addition, there was a higher diversity of ants in the pasture, which had a negative influence on the presence of herbivores. Although herbivore diversity was also higher in the pasture, this occurred only in the rainy season, whereas ant diversity peaked in the dry season, indicating a temporal separation between both groups that may have influenced herbivory on *Q. grandiflora*. Herbivory decreased with increasing numbers of active EFNs, which were more abundant in the rainy season, when ant diversity was lower. However, as most ant visitors are opportunistic, a higher species number does not necessarily ensure better plant protection. Attracting effective partners is more important than species richness (Del-Claro & Marquis, 2015; Miller, 2007). Moreover, nectar abundance can enhance ant aggressiveness, potentially strengthening plant defence (Pacelhe et al., 2019).

Despite having significant impacts on the presence of herbivores on the trees, increasing their abundance and decreasing their richness, arboreal ants did not exert influence on the herbivory rates of *Q. grandiflora*. The lack of correspondence between arboreal ant richness effects on herbivory rates may reflect the limitations of our herbivory metric, which captures only leaf-chewing damage. Many recorded herbivores were sap-sucking hemipterans, showing weak correlation with leaf area loss (Ferreira et al., 2022). Because herbivores also exploit other plant tissues (Pereira et al., 2020), total impacts on plant fitness may be underestimated. Moreover, ant aggressiveness varies with the type of herbivore damage; sucking damage can increase nectar sugar and trigger stronger ant responses, whereas chewing damage mainly affects ant abundance (Raupp et al., 2020). Detecting such subtle effects, however, is challenging given the inconspicuous damage caused by sap-sucking insects (Vidal et al., 2016).

## CONCLUSIONS

Our study highlights the complexity of the mutualism between ants and plants with EFNs, such as *Q. grandiflora*, indicating how seasonality and land use can influence the supply of resources by plants and, consequently, the structure of ant communities, herbivorous insects and herbivory. Based on our results, we hypothesize that habitat simplification can intensify the effects of climatic seasonality on ant and herbivore communities, and also favour the presence of opportunistic ants, which do not establish a protective mutualism with *Q. grandiflora*. However, the protective services attributed to different ant species were inferred from the literature and preliminary observations and thus require rigorous empirical testing in future studies. Since our results were mostly based on correlations, we claim that future studies assessing the role of CADs on ant-plant interactions should take an experimental approach, controlling the presence of ants and herbivores on the trees. It is also important to assess the effects of different land uses on ant-plant-herbivore interactions, including agriculture and distinct levels of urbanization.

## AUTHOR CONTRIBUTIONS

**Edvânia Costa de Oliveira Sá:** Data curation; investigation; methodology; visualization; writing – original draft; writing – review and editing. **Flávio Camarota:** Validation; visualization; writing – original draft; writing – review and editing. **Poliane Neres Morais:** Investigation; methodology; writing – review and editing. **Érica Vanessa Durães de Freitas:** Investigation; methodology; writing – review and editing. **Frederico Neves:** Validation; visualization; writing – review and editing. **Tatianne Gizelle Marques Silva:** Conceptualization; validation; visualization; writing – review and editing. **Walter Santos de Araújo:** Conceptualization; data curation; formal analysis; project administration; resources; validation; visualization; writing – original draft; writing – review and editing.

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## CONFLICT OF INTEREST STATEMENT

The authors have no relevant financial or non-financial interests to disclose.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad Digital Repository at https://doi.org/10.5061/dryad.02v6wwqhk.

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## SUPPORTING INFORMATION

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

**Figure S1.** Location of the two study areas located in the municipality of Montes Claros, north of Minas Gerais, Brazil. The anthropized area (pasture) is represented in the lower left corner, and the yellow circles indicate the 15 *Q. grandiflora* trees sampled in that location. The lower right corner represents the preserved area (a fragment of cerrado *sensu stricto* with native vegetation cover), and the green circles indicate the 20 trees sampled at the site.

**Figure S2.** (a) preserved area (cerrado) during the rainy season; (b) anthropized area (pasture) during the rainy season; (c) active extrafloral nectaries on young leaves of *Qualea grandiflora*; (d) active extrafloral nectaries on old leaves of *Q. grandiflora*; (e) ant of the genus Crematogaster visiting an extrafloral nectary of *Q. grandiflora*; (f) pitfall trap installed on the trunk of each *Q. grandiflora* tree for collecting ants and herbivorous insects; (g) beating procedure performed on three branches of each *Q. grandiflora* tree for collecting ants and herbivorous insects.

**Figure S3.** Comparison of (a) total ant richness, (b) total ant abundance and (c) richness of arboreal ants sampled on *Q. grandiflora* trees between the different seasons (dry and rainy). Bars show mean ± SE. Asterisks (*) indicate significant differences ($p < 0.05$).

**Figure S4.** Comparison of (a) total ant richness and (b) arboreal ant richness between different leaf stages (absent, budding, new and old) on *Q. grandiflora* trees. Bars show mean ± SE. Letters indicate significant differences ($p < 0.05$).

**Figure S5.** NMDS ordination showing ant species composition across environments and seasons. Ellipses represent 95% confidence intervals around centroids.

**Figure S6.** Comparison of (a) richness of herbivore insects and (b) abundance of herbivore insects sampled from *Q. grandiflora* trees between different seasons (dry and rainy); and (c) comparison of herbivorous insect richness between different leaf stages (absent, budding, new and old) in *Q. grandiflora* trees. Bars show mean ± SE. Asterisks (*) and letters indicate significant differences ($p < 0.05$).

**Table S1:** List of morphospecies/species and abundance of ants sampled on *Q. grandiflora* trees during the dry and rainy seasons in the two study areas (preserved and anthropized) located in the municipality of Montes Claros, northern Minas Gerais. The trees indicate genera of predominantly arboreal ants.

**Table S2:** List of morphospecies/species and abundance of herbivorous insects sampled on *Q. grandiflora* trees during the dry and rainy seasons in the two study areas (preserved and anthropized) located in the municipality of Montes Claros, northern Minas Gerais.

**Table S3.** Indicator species of ants significantly associated with each environment. Results of the indicator species analysis (function multipatt, 999 permutations) using the r.g association function. Only significant species ($p < 0.05$) are shown.

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