Research Article

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CAMBRIDGE
UNIVERSITY PRESS

Effect of ants on herbivory levels of *Inga laurina*: the interplay between space and time in an urban area

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Abstract

Extrafloral nectary plants not only occur in natural areas but also in urban parks. These areas are prone to edge effects, and plants face different microenvironmental conditions. We investigated the spatial variation of ant–plant interactions in an urban park, and we examined if plants with ants would show lower herbivory levels and if it depended on habitat type (interior or edges). Seedlings of *Inga laurina* were set in 200-m long transects (which covered both the west and east edges, and the interior) in an urban park and then experimentally assigned to be either ant-present or ant-excluded plants. Leaf herbivory was investigated throughout the wet season and was influenced by the interaction effect between ants and habitat type. Ants decreased the herbivory on the west edge, but on the east edge results were the opposite. The east edge had higher temperature and sunlight exposure in comparison to the other sites and was assumed to disrupt the stability of ant–plant interactions. In the interior of the fragment, herbivory depended on ant presence/absence and on the location of plants along the transect. Our study highlights how the outcomes of ant–plant interactions are spatially conditioned and affected by different types of habitats.

Introduction

In the neotropics, many plant species rely on ants for protection, and in exchange, plants provide ants with extrafloral nectar (EFN), a liquid that is rich in carbohydrates (Del-Claro *et al.* 2016). Despite the amount of literature showing that ants exert a positive effect on plant growth and biomass, reduction of herbivory, and increases in fitness (Rosumek *et al.* 2009, Pereira *et al.* 2020), we cannot rule out the fact that these interactions are conditional over time and space where they take place (Thompson 2005, Jones & Koptur 2015). The literature is filled with data showing the influence of ants on herbivory in punctual periods, usually at the end of the leaf flush season (Coley *et al.* 2005, Calixto *et al.* 2021). The main problem with this approach is that by examining herbivory in only one specific period, the data might mask important variations in leaf area loss during the leaf flush season (Monique *et al.* 2022). For instance, Nascimento and Del-Claro (2010) showed that plants with ants had consistently lower herbivory levels throughout the four months of observation; in contrast, Kelly (1986) revealed that plants with ants might show higher herbivory than plants without ants, depending on the census/sampling. In addition, Fuente & Marquis (1999) not only demonstrated that herbivory varies over time but also that it depends on the environment in which interactions occur (Del-Claro & Marquis 2015). In this context, to enrich the knowledge of ant–plant interactions, it might be wise to examine how herbivory varies along the whole leaf flush season and if it is affected by both ants and different environments.

The interplay between the environment and mutualistic ant-partners is a main factor controlling the outcomes in ant–plant interactions (Nogueira *et al.* 2015, Bächtold *et al.* 2016). Most of what we know about the spatiotemporal variation in ant–plant relationships comes from studies in natural areas; however, EFN plants also occur in fragments within urban settings, such as parks (Clarke *et al.* 2008, Silva *et al.* 2019). In a broad sense, urban parks are considered isolated forest habitats surrounded by an urban matrix without terrestrial connections/corridors with natural areas and are susceptible to anthropogenic disturbances (Pacheco & Vasconcelos 2007). In addition, these areas can be considered fragments and are consequently prone to edge effects, which create different microenvironmental conditions for species occurring on the edges and in the interior (Bolger *et al.* 2000).

For flying animals, parks are considered islands (McFrederick & LeBuhn 2006), but for terrestrial species, such as ants, parks can be regarded as refuges and encompass a subset of the species found in the forests (Santos *et al.* 2019). There is an assumption that the interactions between ants and plants in these places might be different from those in natural areas because the ant fauna of parks is restricted to a few species, most of which are non-specialist and

opportunistic (Pacheco & Vasconcelos 2007, Clarke *et al.* 2008). However, since EFN-drinking ants are generalists, interactions with plants are assumed to persist if EFNs are active (Silva *et al.* 2020).

In this study, we investigated the spatiotemporal variation of ant–plant interactions in a fragment located in an urban setting. Our experiment consisted of a three-factor design with 'time' (the period that corresponds to the whole leaf flush season), 'ants' (present or absent), and 'habitat type' (the transects that incorporate the interior and the edges of the fragment). We addressed the following question: do plants with ants show lower herbivory levels, and does it depend on habitat type (interior or edges) and time of sampling (different dates of sampling within the leaf flush season)?

Our study plant was *Inga laurina* (Sw.) Willd (Fabaceae), an EFN-bearing legume species. Plants in this genus have long been studied with regard to their interactions with ants (Koptur 1984, Bixenmann *et al.* 2013). Both the environment and ants seem to play an important role in the life history and herbivory of *Inga* species. For instance, habitat type and sunlight exposure are critical factors for the growth of *I. vera*; in addition, herbivory is greatly suppressed when ants are present (Kersch & Fonseca 2005). Thus, *Inga* species appear to be a good model for investigating how herbivory varies according to ants and the environment.

Materials and methods

Study area

The study was conducted between November 2019 and May 2020 at the Parque Ambiental Macambira (16°44′26.20″ S, 49°19′11.10″ W, 827 m above sea level) in the city of Goiânia, Brazil. The park is used for recreation and educational purposes and lacks large-scale human disturbances, such as mowing, fires, and logging. The park contains a typical and conserved Cerradão with trees up to 10 m tall. The climate has two well-defined seasons: a dry winter from May to September and a rainy summer from October to April.

The park is an urban fragment with approximately 25 ha and is surrounded by avenues and houses all around its borders. The park itself is asymmetric; one part resembles an arrow, and the other a square, both of which are linked by a boomerang-shaped area of vegetation. The so-called west edge (16°44'26.20"S, 49°19'10.64"W) is limited by a matrix formed by deforested land containing a few shrubs and grasses; this matrix extends up to 150 m until the nearest street (Supplementary material 1). The so-called east edge (16°44'23.07"S, 49°19'6.63"W) ends in an avenue. The interior of the park is well preserved with large trees with canopies covering the understory and does not sustain perturbations other than storms that once in a while may provoke the fall of trees and branches. The most frequent plant species are *Hymenaea courbaril* L. (Fabaceae), *Tapirira guianensis* Aubl. (Anacardiaceae), *Xylopia sericea* A. St.-Hil. (Annonaceae), *Schefflera morototoni* (Aubl.) Maguire, Steyerm. & Frodin (Araliaceae), *Mauritia flexuosa* L. f. (Arecaceae), and *Anadenanthera colubrina* (Vell.) Brenan (Fabaceae).

Study plant

The genus *Inga* is restricted to the neotropics and contains approximately 300 species (Kursar *et al.* 2009). The study plant, *I. laurina*, grows up to 20 m tall. The EFNs occur at the base of the opposite leaflets. The leaf flush is related to the onset of the rainy period (October), and the production of extrafloral nectar is continuous throughout the dry season, which lasts until April (end of the rainy season). From May to September, the plant does not produce any leaves, and the EFNs are non-functional. Several species of *Inga* are known to be visited by EFN-drinking ants, and studies have examined the potential of ants as antiherbivore defences in *Inga*, as well as the performance of plants in different environments (Koptur 1985, Kersch & Fonseca 2005, Bixenmann *et al.* 2013). Two species of *Inga* are present in the park (*I. alba* (Sw.) Willd and *I. nobilis* Willd.), and together with *I. laurina*, these legumes are part of the park's management plan.

Experimental design

We established a study design with 4 rows per 11 column-transects in the park; each row contained 22 plants and each column had eight plants. The largest linear transects were 200 m long (Figure 1) and were set from one edge to the other of the park (Supplementary material 1). A total of 88 seedlings of *I. laurina* were purchased from a commercial greenhouse located in Goiânia city, Brazil, transported to the park and transplanted into established transects. At this point, the seedlings were between 15 and 20 cm tall, and there was no significant difference in height, number of leaves, and number of EFNs between plants ($p > 0.05$ in all cases, details not shown here).

Each linear transect was 200 m long and was spaced 10 m from each other. A total of 22 seedlings of *I. laurina* were planted in pairs (one ant-present and another ant-excluded seedling) in each transect; each pair was 20 m distant from the other pair, and each plant from each pair was 1 m distant from the other (Figure 1).

Our sample size was equal to 88 seedlings (11 plant pairs *times* 4 transects = 88 seedlings), which were divided into ant-present and ant-excluded individuals ($n = 44$ plants in each group). The restriction of ant access to plants was made with the use of nontoxic wax (Tanglefoot ©), which was applied to the foot of each 'ant-excluded' seedling. The 'ant-present' plants were left undisturbed, except by a thin layer of wax which was applied to a portion of the main stem to control for the effect of the wax; this did not restrain the access of ants to plant parts. Leaves and stems from each seedling were clipped to avoid the formation of vegetation bridges between plants.

In many ant-plant studies, scientists tag single individual plants and divide their bushes into experimental units where one bush receives the resin to avoid ants and a neighbour bush is assigned as a control unit (Alves-Silva *et al.* 2014, Aristizábal & Metzger 2019). The advantage of this approach is that herbivores can rapidly assess the ant-free areas and forage accordingly. In addition, insects that whenever are harassed by ants in control bushes can rapidly fly to the nearest ant-excluded plant parts to keep feeding. In our study, the *I. laurina* plants were rather small to be divided into experimental bushes, so we preferred to grow two seedlings, one with and the other without ants, close to each other (1-m space), which covers the distance between experimental plant pairs in the study by Mody & Linsenmair (2004).

We might also have chosen to not distribute the *I. laurina* in experimental pairs, but rather into ant-present and ant-excluded plants that were distributed evenly in the park and distant from each other. However, we were afraid that some plants might perish, which would affect the even sample size between experimental plants. In our current approach, if one plant from the pair perished, its counterpart was going to be excluded from the study as well; luckily this did not happen. To conclude, the decision to keep plant pairs 1-m spaced from each other was based on a logistic reason, since it permitted a quick evaluation of plants by one researcher in the field.
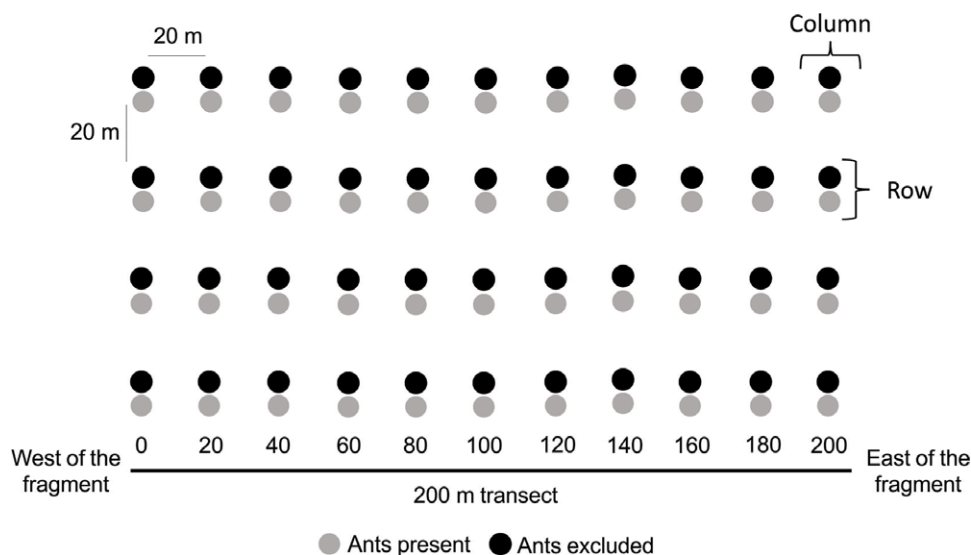
Figure 1. Representation of the transects established in the park to grow the individuals of *Inga laurina*. Each row sustained 22 seedlings, which were assigned as ant-present or ant-excluded treatments. Rows started from the very west edge and ended at the east edge (the limit between the park and the surrounding non-forested matrix). A clear delimitation of the extension of edges and the interior was made later, based on microclimatic data and statistical analyses (see Results section) (see also Supplementary material 1).

Data collection

Data collection commenced 21 days after plant acclimation in the area. The plants were scanned six times, from mid-December to late March, which corresponds to the wet season (see results for the dates). Field trips were carried out on December 16 (2019) and in the following dates of 2020: January 6, January 27, February 17, March 9, and March 30. In each field trip, the 'ant-present' seedlings were scanned for the presence of ants, and the ant-excluded plants were also checked to guarantee that no ants trespassed the wax barrier. Ants visiting the control plants were photographed to permit an initial classification to the genus level (this is possible for common genera, such as *Camponotus*, *Cephalotes*, and *Crematogaster*) (Supplementary material 1). At the end of the fieldwork, the ants were collected.

Our measure of herbivory was the ratio between the number of leaves and the number of leaves with herbivory (leaf area loss) in each seedling (González-Teuber *et al.* 2012). First, we aimed to measure herbivory as the leaf area loss (% and mm² per leaf), using digital images. However, leaves usually had most of their areas and margins attacked by beetles and caterpillars, thus restraining us from measuring leaf area loss with accuracy (Supplementary material 1). Our measure of herbivory might not have been the most appropriate (i.e., damaged leaves divided by the total number of leaves; Alves-Silva & Del-Claro 2014; González-Teuber *et al.* 2012), but it was the only possible method to assess the herbivory levels in *I. laurina*. Nonetheless, Burger *et al.* (2021) did not notice differences between this method and the measure of leaf area loss. Thus, we believe our results are trustworthy.

Microclimatic data (temperature, humidity, and sunlight exposure) were collected to permit the classification of the edges and the interior of the fragment, because differences in temperature and humidity, for instance, are strikingly different on the edges and the interior, permitting its recognition and use in statistical analyses (Christianini & Oliveira 2012, Mendonça *et al.* 2015). Temperature and humidity data were recorded in the mornings (08:00 to 09:00 to avoid daily variation; Wang *et al.* 2009) of sunny days using a digital thermo hygrometer (MTH-1300 Minipa). The device was placed between each pair of seedlings, and the data were recorded. The light incidence was measured with Google's smartphone app Science Journal for Android OS (V. 3.5.329666436 from 2020; the app was discontinued by Google). By using the app, a single smartphone (model Samsung S9) was consistently used throughout the study, and it was held above and between the pairs of *I. laurina*. The camera then registered the luminosity in lux units.

Statistical analyses

The microclimatic data (light, temperature, and humidity) were visualized in different figures, and data were not linear, but rather varied depending on the location of the transects (Figure 2a, b, c). For instance, the last group of tagged plants (locate at a distance of 200 m along the transect – Figure 1), located on a border of the park (on the east), had the highest temperature and luminosity, and the lowest humidity. In contrast, some transects in the interior of the fragment had opposite values.

A non-linear regression was made for each microclimatic variable, yielding polynomial models for temperature (intercept $= 25.24$, $x = -0.74$, $x^2 = 0.08$); humidity (intercept $= 76.84$, $x = 4.72$, $x^2 = -0.44$) and luminosity (intercept $= 4930.0$, $x = -2086$, $x^2 = 201.5$). We fit an adjusted curve along the fragment's transects which demonstrated a parabolic trend of temperature, humidity, and luminosity. Regardless of the microclimatic variable, all of them showed that the inner part of the fragment (the interior) had different conditions in comparison with the borders. In fact, the adjusted parabolic curve allowed us to recognize three distinct areas within the fragment, which were labelled as 'west edge', 'interior', and 'east edge' (Figure 2a, b, c). The visual estimation and classification of edges and the interior were appropriate in our study as figures, and the polynomial adjusted regression curves were used to quantify how physical variables varied along the transects.

A more traditional approach would be to pairwise-compare each part of the transect to classify them onto edges and interior; however, this is not feasible due to the characteristics of the fragment. For instance, the temperature in the 20 m and 180 m transects was similar (Figure 2a) and one might assume that both parts of the transect should be put in the same categorical factor; nonetheless, they are 160 m away from each other and are separated by parts with lower temperatures. The 180 m transect is also very different (in terms of temperature, humidity and luminosity) from the subsequent transect (200 m) (Figure 2a). The adjusted parabolic curve revealed that the edges had increased values of
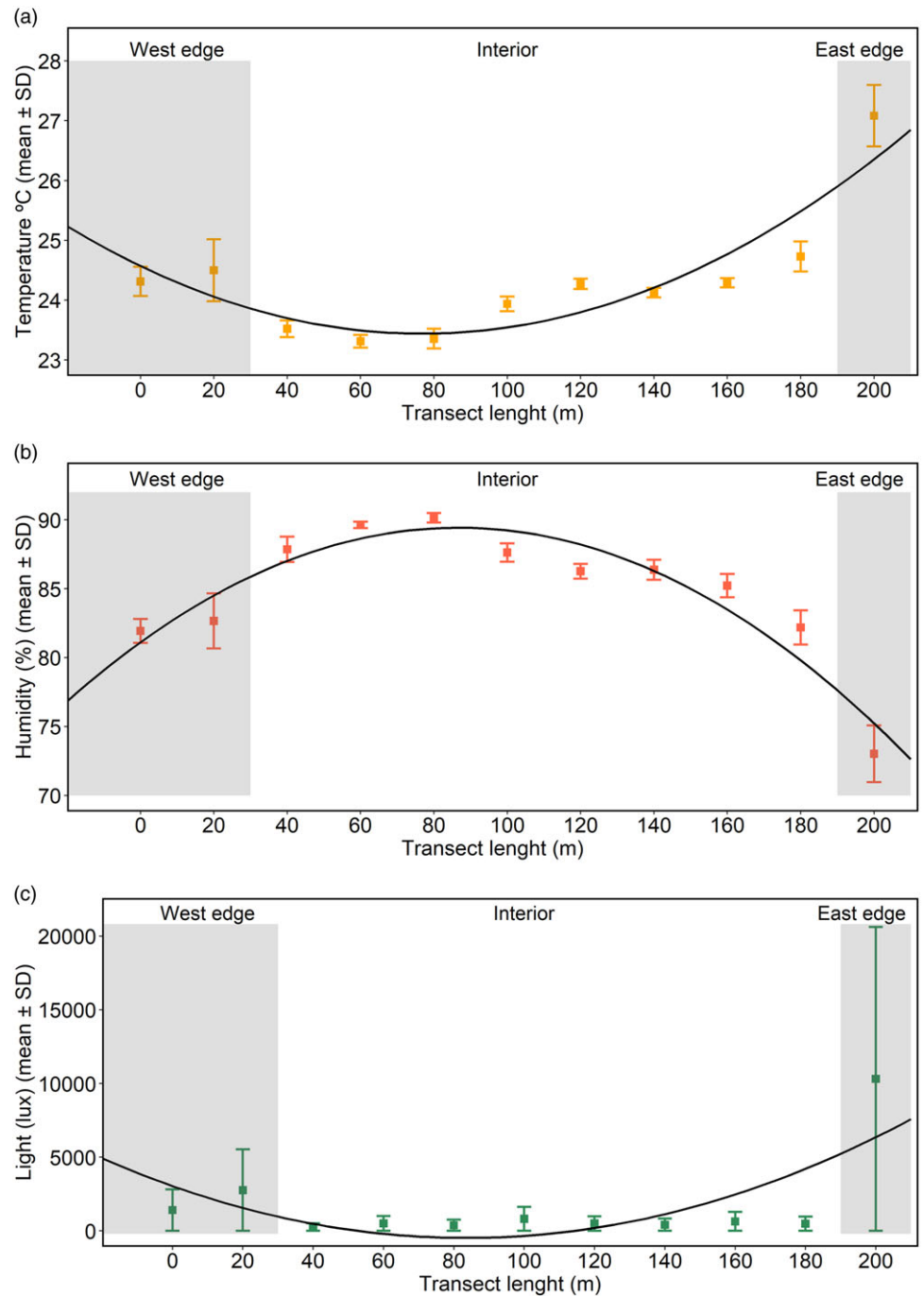
Figure 2. Variation of (a) temperature, (b) humidity, and (c) luminosity along the 200 m long transect within the park. In those places regarded as 'edges,' we noticed a visible contrast of environmental factors in comparison to the interior. The figure shows the mean and standard deviation.

temperature and light in comparison to the interior; in contrast, the humidity was conceivably lower on the edges. According to our classification, the west edge accounted for two transects (0 to 20 m, $n = 16$ plants), the interior for eight transects (40 to 180 m, $n = 64$ plants) and the east edge for one transect (200 m, $n = 8$ plants) (Figure 2a, b, c).

In order to statistically compare the microclimatic data among these three habitat types indicated by Figure 2, we performed Kruskal–Wallis test (non-parametric data) followed by Dunn's test (a posteriori) for the values of temperature, humidity and sunlight exposure in each habitat type. Once we realized that the physical data of these three sites were statistically different (see Results section), the west edge, the east edge, and the interior of the

fragment were incorporated into the subsequent analyses as categorical data.

General linear mixed model tests (GLMM) were used to examine the influence of plant groups (with or without ants), the location of plants in the transects, and dates of sampling (employed as a fixed and also as the random factor) on herbivory (% of attacked leaves per plant). Tukey's *post hoc* tests were made whenever necessary to investigate in detail results that were statistically significant.

To verify if the herbivory data were spatially correlated, which might influence the results, we made Moran's and Mantel tests, as well as a linear mixed-effects regression with spatial data of plants. None of the analyses were significant (results not shown); thus, we

Table 1. Variation of leaf herbivory in *Inga laurina* according to ants (presence and absence), date of sampling (six occasions), and transect length (200 m) in a tropical urban park. The interaction effect between ants*transect length*samplings was not conducted as it decreased the fit of the GLMM model.

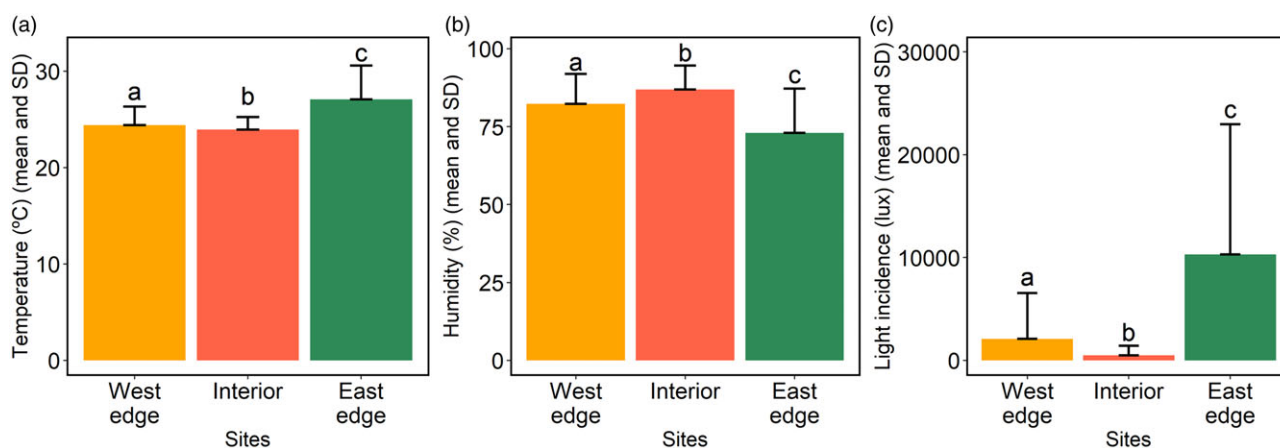| Variables	Wald χ^2	Df	*P*-values
Ants	23.3	1	<0.0001
Sampling	389.1	5	<0.0001
Transect length	474.73	10	<0.0001
Ants*transect length	600.14	10	<0.0001
Ants*sampling	34.5	5	<0.0001
Sampling*transect length	380.54	50	<0.0001

Figure 3. Comparison of (a) temperature, (b) humidity, and (c) light incidence in the sites classified as edges and interior of the urban fragment. Lowercase letters upon bars indicate statistical differences based on the Kruskal–Wallis test and on Dunn's test (a posteriori).

rule out the spatial location of plants as a variable of influence in our study.

Statistical procedures were performed using R statistical software, version 4.2.2.

Results

Plants up to 20 m from the west edge and the plants at the opposite site, at the very end of the transect (200 m), were considered to be on the edges (see Figure 1 for details on plant distribution). Figure 2 shows that the temperature, humidity, and light incidence at these sites were different from the so-called interior. The adjusted curve in the figure shows decreases in temperature and luminosity from the edges to the interior and an increase in humidity. The comparison among the edges and the interior yielded significant results (*P* < 0.0001 in all cases) (Figure 3). The east edge had the highest temperature, lowest humidity, and highest luminosity (Figure 3). The interior had the highest humidity, but the temperature was milder, and luminosity was the lowest in comparison to the edges (Figure 3). The physical variables were all related (Spearman's correlation values: temperature and humidity = −0.94; temperature and luminosity = 0.91; humidity and luminosity = −0.86).

Leaf herbivory was significantly influenced by the presence of ants (plants with ants had on average 10% less herbivory than plants without ants), the time of sampling, the location of plants along the transects (herbivory was higher on the edges and lower

in the interior of the fragment), and by the interaction effects among the variables examined (Table 1). The interaction effect between ants and transect length indicates that not only herbivory depended on ant presence/absence but also on the location of plants along the transect (Figure 4). In fact, a significant effect of ant presence was noted for plants at the beginning of the transect (0 metres from the west edge); in contrast, at the end of the transect results were just the opposite (Figure 4). In the interior of the fragment, results varied as ants either had or had not a positive effect (i.e., reduction) on herbivory levels (Figure 4).

Further comparisons considering each side of the park (west and east edges, and the interior of the fragment) showed that on the west edge, the plants without ants had an almost 2-fold higher herbivory rate, on average, than plants with ants (35% and 18%, respectively). The herbivory was similar between plant groups in the interior of the fragment, and plants with ants on the east edge showed higher herbivory (on average 25% more). The interaction effect of ants and dates of sampling occurred because herbivory varied throughout the leaf flush season in plants with and without ants (Supplementary material 2).

The plants did not sustain one single ant species consistently, as during fieldwork, we noticed shifts of ants in each seedling. Therefore, it was not possible to examine the effect of each ant on herbivory, and we considered the community of ants as a whole. We came across ants belonging to genera *Camponotous*, *Crematogaster*, *Cephalotes*, *Solenopsis*, and *Ectatomma*, and all of them visited the EFNs of *I. laurina*.
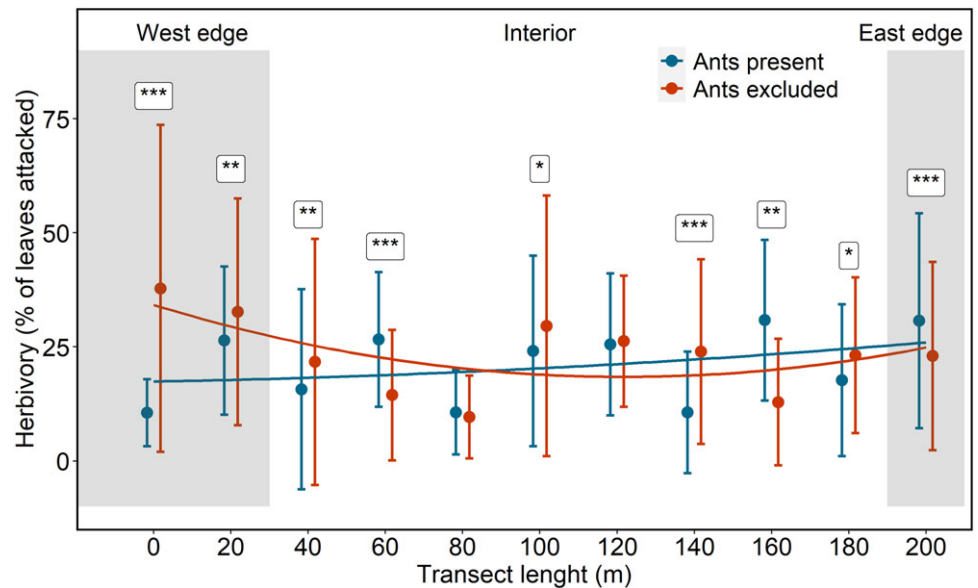
Figure 4. Variation in the herbivory levels of *Inga laurina* according to transect length and plant groups (presence or absence of ants). The bars indicate the mean and SD values. Superscript asterisks indicate statistically significant differences according to Tukey's post hoc tests.

Discussion

Here, we found evidence of different microenvironments within the study area. Data on temperature, humidity, and luminosity revealed three distinct sites, two edges, and the interior. These sites, together with ants, were responsible for contrasting herbivory levels in *I. laurina*. On the west edge, plants with ants had the lowest herbivory levels, a result expected according to ant–plant interaction models of mutualism, because EFN plants deprived of ant-partners may experience increased herbivory levels (Kost & Heil 2005, Katayama & Suzuki 2011). Studies have shown that the production of extrafloral nectar is higher in EFN plants susceptible to direct sunlight exposure (Millán-Cañongo et al. 2014) and embedded in an environment with other plants (Alves-Silva & Del-Claro 2013); thus, it is expected that ants will be more frequent in such unshaded plants and defend the plant from herbivores (Alves-Silva & Del-Claro 2013, Bächtold et al. 2016). This situation was observed on the west edge.

On the east edge, results were the opposite, as plants with ants had higher herbivory than their counterparts. The east edge receives a huge influence from the surrounding non-forested matrix; in fact, the very east edge faces a main city avenue. Conversely, the west edge has no such abrupt change in the environment, as the matrix is formed by a deforested area with grasses and seedlings, which belongs to the park's limit of occupation. Therefore, the east edge can be assumed to be severely disturbed in comparison to the west edge. The east edge had the highest temperature, luminosity, and lowest humidity in comparison to the other sites. Plants in highly stressed environments, such as the east edge, may be more susceptible to herbivore attack (García-Jain et al. 2021). This occurs because plants cannot maintain their homeostasis when they are subjected to harsh conditions; in this case, their tolerance surpasses the limits of their phenotypic plasticity, incurring several changes, both physiological and morphological (Nikiforou & Manetas 2017, Aguilar-Peralta et al. 2020).

The study plant, *I. laurina*, is not a pioneer species, but rather a secondary and late-successional species (Uriarte et al. 2004, Polli et al. 2020). Thus, by being exposed to such a harsh habitat as the east edge (in comparison to the other sites), both the plants

and the ants might have faced detrimental effects (Kersch & Fonseca 2005, Yamawo et al. 2014). Insect herbivores benefit from highly stressed plants, both by the plant's reduced levels of defences and because of the disruption of top-down control, as the composition of the natural enemies of herbivores varies on spatial scales (Wirth et al. 2008, Santos et al. 2013, Pereyra et al. 2015). In the interior, a shaded habitat, ants reduced the herbivory in some plants, but not in others; nonetheless, the pooled results revealed no difference in herbivory according to plant groups. Usually, shade-tolerant and late-successional plants (such as *I. laurina*; Uriarte et al. 2004; Polli et al. 2020) may have increased secondary compound defences in the shade, which is their ideal environment; this prevents herbivore attack (Coley 1988, Dudt & Shure 1994).

The two edges of the park showed contrasting results in terms of herbivory according to ant presence/absence. We may conclude that ant-plant interactions were not affected by a mild stress (west edge), and in fact, gaps may strengthen the ant visitation to plants and the reduction of herbivory rates (Alves-Silva & Del-Claro 2013). Nonetheless, a harsh environment such as the east edge might have disrupted the interactions between ants and the non-pioneer species *I. laurina*. In this site, ants might have changed their foraging behaviour and abandoned the plants during some periods, or the plants might have been visited by species of ants that offered no protection from herbivores (Palmer & Brody 2007, Nogueira et al. 2012, Leal et al. 2015, Andersen 2019). Some plants that are located in harsh environments (e.g., edges, proximity to roads) and are subjected to full sunlight exposure face severe water stress which in turn is a detrimental factor for extrafloral nectar production (Newman & Wagner 2013, Kunert et al. 2015, Cruz Rocha et al. 2019). Thus, ants might have had no benefit in visiting the plants, making them susceptible to herbivory attack. The ant-plant specificity could unravel some of these assumptions, but *I. laurina* was visited by several ant species, thus limiting our knowledge.

In our study, we quantified herbivory in *I. laurina* throughout the leaf flush season, and we registered the periodical variations in herbivory and whether they varied according to site (edges and interior) and ant presence/absence. This approach is uncommon in ant–plant studies, with results either showing that the effects of ants are consistent or not depending on the census (Kelly

1986, Nascimento & Del-Claro 2010). In *I. laurina,* we covered the whole leaf flush season and examined in different periods how plants were performing with and without ants. In the end, we found a significant interaction effect between ants and sampling date, indicating that herbivory was higher or lower depending on the presence of ants and the date of sampling. This result is in accordance with Monique *et al.* (2022) who show that plants with ants experienced large variations in flower production during the reproductive season. Thus, it is clear that the true effect of ants on plants could only be examined if one takes into consideration how plants with ants experience higher o lesser performance in a given period of its phenology, especially when ants are supposed to patrol the plants (i.e., when extrafloral nectaries are active).

Urban parks have been regarded as unimportant places for ant biodiversity, both because of their relatively low areas, and the existence of only a subset of species that occur in natural areas (Menke *et al.* 2011). Nonetheless, we showed that the same mechanisms that influence ant-plant interactions in natural areas, such as plant phenology, microclimatic gradients, ant presence, and spatial scales (Nogueira *et al.* 2020, Calixto *et al.* 2021, Monique *et al.* 2022), are also present in the study park. In this context, not only parks are important to increase our understanding of ant-plant interactions, but it is also surprising that these relationships in such (so-called) disturbed habitats do persist, revealing potential micro hotspots of ant-plant interactions. Our understanding of the biodiversity in tropical urban parks in the tropics is scarce (< 10% of studies in parks) (Nielsen *et al.* 2014). In Brazil, most investigations in urban areas evaluate the abundance and community composition of epigaeic ants; in addition, most studies were conducted in the Atlantic Forest biome (Munhae *et al.* 2009, 2015, Melo *et al.* 2022). To the best of our knowledge, ours is the first study in Brazil to evaluate how herbivory in an urban park (with the matrix surrounded by a city) is affected by ant-partners, space and time.

One potential limitation of the study was the lack of ant–plant specificity, as more than one ant species was observed visiting the same individual plant during the study; this prevented us from examining the role of each species in plant protection. Nonetheless, this was a characteristic of the study, and since the interaction between EFN plants and ants is facultative, plants can be visited by several ant species (Anjos *et al.* 2017). Moreover, the stability of ant-plant mutualism in the tropics depends on only a few arboreal species of ants (Lange *et al.* 2013, Fagundes *et al.* 2017, Câmara *et al.* 2018), which coincidentally are species that occur in parks (Aranda *et al.* 2022). In this context, the authors might consider the effect of the ant community on plants rather than analysing each ant species separately (Franco & Cogni 2013, Raupp *et al.* 2020). Another potential limitation of the study was the sampling of only one urban area, and this is justified by logistics and the period of fieldwork, that was time-consuming, as we conducted an experimental approach based on ant presence/absence covering a whole plant phenophase, which is the same approach used in natural areas to investigate plant-ant interactions (Fagundes *et al.* 2017, Monique *et al.* 2022). The sampling of ants in one or few areas is not uncommon for urban habitats (Ribeiro *et al.* 2012, Cantone 2018, Souza-Campana *et al.* 2020), and besides, most of what we know about ant-plant interactions in natural areas comes from studies conducted in single areas as well. Nonetheless, the interpretation of our results should consider the conditions of the study park, and we expect that further comparable studies could enhance our knowledge of plant-ant interactions in urban areas.

The outcomes of the interplay between the spatiotemporal effect of ants on herbivory and the concomitant effect of the environment (fragmentation, edge effects) and time are difficult to generalize, given the variability of possible results (e.g., the interactions among all variables can lead to various different scenarios) (Yamawo *et al.* 2014, Pereyra *et al.* 2015, Câmara *et al.* 2017). Here, we provide further information on this system by showing that leaf herbivory in an urban park depends both on space and on time. There is not much literature on edge effects in the Cerrado biome, let alone investigations of edge effects and ant–plant interactions in urban settings (Santos *et al.* 2019). Our study highlights how the outcomes of ant–plant interactions are conditional. In the study park, a 200 m spatial variation was enough to display variations in the effectiveness of ants as plant guards of *I. laurina.*

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