

# Climate seasonality drives ant–plant–herbivore interactions via plant phenology in an extrafloral nectary-bearing plant community

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## Abstract

1. Interactions between ants and plants bearing extrafloral nectaries (EFNs) are among the most common mutualisms in Neotropical regions. Plants secrete extrafloral nectar, a carbohydrate-rich food that attracts ants, which in return protect plants against herbivores. This ant–plant mutualism is subjected to temporal variation, in which abiotic factors can drive the establishment and frequency of such mutualistic interaction. However, studies investigating how abiotic factors (e.g. climate) directly and indirectly influence ant–plant–herbivore interactions are incipient.
2. In this study, we investigated direct and indirect (via plant phenology) effects of temperature and rainfall on ant–plant–herbivore interactions. To address these goals, each month we estimated six plant phenophases (newly flushed leaves, fully expanded leaves, deciduousness, floral buds, flowers and fruits), the activity of EFNs and abundance of ants and herbivores in 18 EFN-bearing plant species growing in a markedly seasonal region (the Brazilian Cerrado) during a complete growing season.
3. Our results showed that (a) there were marked seasonal patterns in all plant phenophases, EFN activity and the abundance of ants and herbivores; (b) the peak of EFN activity and ant and herbivore abundance simultaneously occurred at the beginning of the rainy season, when new leaves flushed and (c) rainfall directly and indirectly (via changes in the production of new leaves) influenced EFN activity and this in turn provoked changes in ant abundance (but not on herbivores).
4. *Synthesis*. Overall, our results build towards a better understanding of how climate drives seasonal patterns in ant–plant–herbivore interactions, explicitly considering plant phenology over time.

## KEYWORDS

ant–plant interactions, biotic defence, Brazilian Cerrado, herbivory, indirect defence, mutualism, phenophases, seasonal environment

## 1 | INTRODUCTION

Mutualistic interactions are ubiquitous in nature, yet their outcomes are highly variable across time and space (Bronstein, 2015). Identifying the biotic and abiotic drivers of such spatial and temporal variation has been a central goal in ecology and evolutionary biology during last decades (Bronstein, Alarcón, & Geber, 2006; Thompson, 2005). Some studies have demonstrated that variability in biotic (e.g. species abundance, species identity, plant phenology; Bascompte, 2010; Belchior, Sendoya, & Del-Claro, 2016; Chamberlain, Kilpatrick, & Holland, 2010; Lange, Dáttilo, & Del-Claro, 2013) and abiotic (e.g. fire, temperature, rainfall; Alves-Silva & Del-Claro, 2013; Belchior et al., 2016; Dáttilo et al., 2015; Lange et al., 2013; Rico-Gray, Díaz-Castelazo, Ramírez-Hernández, Guimarães, & Holland, 2012) factors are fundamental drivers of spatiotemporal variation in mutualistic interactions. However, most research in this area has attempted to identify spatial drivers at different scales of variation (e.g. latitudinal, altitudinal, among populations, among individual plants; Bascompte, 2010; Chamberlain et al., 2010; Thebault & Fontaine, 2010), with a smaller area of emphasis dedicated to understanding the temporal dynamics (but see Nogueira et al., 2020; Vilela, Del-Claro, Torezan-Silingardi, & Del-Claro, 2018; Vilela, Torezan-Silingardi, & Del-Claro, 2014).

Plants bearing extrafloral nectaries (EFNs) and nectar-feeding ants are among the most common mutualistic interactions in the Neotropics (Bronstein, 1998; Del-Claro et al., 2018; Heil, 2015; Lange et al., 2013). Plants secrete extrafloral nectar, a carbohydrate-rich food that attracts ants. In return, ants aggressively patrol plants to repel or consume herbivores and consequently reduce plant damage and/or increase fruit production (Calixto, Lange, & Del-Claro, 2018; Del-Claro et al., 2016; Rosumek et al., 2009; Trager et al., 2010). This ant–plant mutualism is subjected to temporal variation, in which abiotic factors (e.g. climate) can directly drive the establishment and frequency of such mutualistic interactions. For instance, temporal patterns of rainfall in regions with a marked seasonal hydroperiod (dry and wet seasons) often result in variations in insect (e.g. ants, herbivores) population densities. In particular, insect abundance usually increases towards the rainy season (and with higher temperatures) because these climatic conditions enhance consumption rates and decrease the time of development (Abril, Oliveras, & Gómez, 2010; Ratte, 1985). Climate might also indirectly (via plant phenology) influence ant–plant mutualistic interaction (Heil, 2015). Plants have developed different adaptations to grow and develop, expressing their phenological events (called phenophases) at the most convenient period of the year (Van Loon, 2016). In regions with a well-marked fluctuation in seasonal rainfall, plants tend to produce new leaves at the beginning of the rainy season because these climatic conditions enhance soil moisture, and this would in turn result in improved nutrient absorption to produce new leaves (Silva, da Silva, de Carvalho, & Batalha, 2011). The production of new leaves is usually associated with an increased production and activity of EFNs which would in turn result in changes in the strength of ant–plant mutualistic interactions (Dáttilo et al., 2015; Lange et al., 2013).

In some insightful recent studies, Vilela et al. (2014, 2018) showed that changing climatic condition factors over a 10-year period directly influenced plant phenology (degree of overlap in flowering and resprouting among species) in a Malpighiaceae community (four EFN species) in the tropical savanna and this in turn had a direct impact on the outcomes of insect–plant interactions. Similarly, Nogueira et al. (2020) found that the ant–plant mutualism in 22 Bignoniaceae (a species-rich lineage of neotropical lianas) species in the Amazonian rainforest was indirectly and negatively related to precipitation, which was attributed to a decrease in the proportion of plants producing new EFN-bearing plant tissues during the wetter period. Although these studies have observed temporal patterns of ant–plant interactions, our study goes a step further and included 18 plant species with contrasting growth forms (lianas, shrubs and trees) from nine families, allowing us to make inferences at the community level. Likewise, we collected data over a year, which provides more accurate data about environmental variables, plant phenological events, and ant and herbivore abundance. Finally, we performed our experiment in a region with two well-defined seasons in a year (dry and rainy) which could result in stronger temporal patterns of ant–plant–herbivore interactions.

Here, we investigated direct and indirect (via plant phenology) effects of climate on ant–plant–herbivore interactions in 18 EFN-bearing plant species of nine sympatric families of the Brazilian Cerrado, an ecosystem marked by a seasonal hydroperiod. In particular, we investigated whether: (a) there was seasonal variation in ant–plant–herbivore interactions; (b) the peak of the activity of EFNs, ants and herbivores simultaneously occurred during the same season and (c) there was a direct and indirect influence of climatic variables (temperature, rainfall) on ant–plant–herbivore interactions. To address these goals, each month we estimated six plant phenophases (newly flushed leaves, fully expanded leaves, deciduousness, floral buds, flowers and fruits), the activity of EFNs and the abundance of ants and herbivores. Our main hypothesis was that due to the strong climate seasonality of the Brazilian Cerrado, plants produce active EFNs at the beginning of the rainy season, when new leaves flush, resulting in the highest abundance of nectar-feeding ants and herbivores (i.e. temporal variation of ant–plant–herbivore interactions). Overall, this study will improve our understanding of how climate drives seasonal patterns in ant–plant–herbivore interactions, explicitly considering plant phenology over time.

## 2 | MATERIALS AND METHODS

### 2.1 | Study area

We performed this study in the Ecological Reserve of the Clube de Caça e Pesca Itororó de Uberlândia (CCPIU: 48°18'01"W; 18°59'05"S, Uberlândia, Brazil). The study area had ~200 ha of cerrado sensu stricto vegetation (Oliveira & Marquis, 2002). Open areas consisted of shrubs and small trees, whereas more enclosed

areas consisted of trees reaching up to 15 m in height (Del-Claro, Rodriguez-Morales, Calixto, Martins, & Torezan-Silingardi, 2019). The climate is markedly seasonal with the rainy season from October to March, and the dry season from April to September. The annual mean temperature varies from 18 to 28°C and the rainfall from 800 to 2,000 mm (Alvares, Stape, Sentelhas, De Moraes Gonçalves, & Sparovek, 2013).

## 2.2 | Data collection

For this study, we systematically established ten 50-m transects at least 50 m apart from each other. Transects started perpendicular to a reserve trail (2.5-m wide) going through the vegetation. In order to diminish edge effects, we set transects separated at least 10 m from the trail. All transects were parallel to each other and soil characteristics, light availability and humidity of each transect were similar.

We selected the 18 most common and abundant plant species (Appolinario & Schiavini, 2002; Lange, Calixto, & Del-Claro, 2017; Lange & Del-Claro, 2014) bearing EFNs in our field site: *Banisteriopsis malifolia* (Nees & Mart.) B. Gates (Malpighiaceae), *Banisteriopsis stellaris* (Griseb.) B. Gates (Malpighiaceae), *Bauhinia rufa* (Bong.) Steud. (Fabaceae), *Bionia coriacea* (Nees & Mart.) Benth. (Fabaceae), *Caryocar brasiliense* Cambess. (Caryocaraceae), *Eriotheca gracilipes* (K.Schum.) A.Robyns (Malvaceae), *Heteropterys pteropetala* A. Juss. (Malpighiaceae), *Lafoensia pacari* A.St.-Hil. (Lythraceae), *Manihot caerulescens* Pohl (Euphorbiaceae), *Ouratea hexasperma* (A.St.-Hil.) Baill. (Ochnaceae), *Ouratea spectabilis* (Mart.) Engl. (Ochnaceae), *Qualea grandiflora* Mart. (Vochysiaceae), *Qualea multiflora* Mart. (Vochysiaceae), *Qualea parviflora* Mart. (Vochysiaceae), *Senna rugosa* (G.Don) H.S.Irwin & Barneby (Fabaceae), *Senna velutina* (Vogel) H.S.Irwin & Barneby (Fabaceae), *Smilax polyantha* Griseb. (Smilacaceae), and *Stryphnodendron polyphyllum* Mart. (Fabaceae). In total, we tagged 180 plant individuals equally divided among the 10 transects. Each transect included one individual of each species. We selected these individuals based on similar phenological characteristics within each species (e.g. total height and number of branches).

Each month, we collected data of plant phenology for each tagged individual of all species from March 2017 to February 2018. In particular, we evaluated six phenophases: newly flushed leaves, fully expanded leaves, deciduousness, floral buds, flowers and fruits. For each phenophase, we used the following semi-quantitative scale: 0 = absence of plant phenological event; 1 = sporadic event (1%–25% of the branches); 2 = infrequent event (26%–50% of the branches); 3 = frequent event (51%–75% of the branches); and 4 = very frequent event (76%–100% of branches; Fournier, 1974). For each plant, we also evaluated EFN activity by estimating the number of plants with active EFN monthly. We considered that EFNs were active when they were bright and light in colour, and generally producing extrafloral nectar. By contrast, we considered that EFNs were inactive when they were dark with a necrotic aspect and did not produce nectar (Calixto, Lange, & Del-Claro, 2015). The main reason to choose EFN activity instead of other EFN characteristics

(e.g. volume, concentration) was that the circular statistics used for all plant phenological events (see details in Section 2.4) work with presence or absence of the specific event over time. Finally, to quantify the number of ants and herbivores we conducted field observations on two consecutive days per month during the same period as the plant phenology observations. Each day we censused half of transects (five transects) in both daytime (08.00–12.00 hr) and night-time (07.00–1.00 hr). In each plant individual, we first collected ants and other insects without touching the plant. Next, using a white inverted umbrella placed under the plant, we shook the branches and collected those insects that fell on the umbrella. We subsequently stored all insects in 70% ethanol. Then we identified insect herbivores by using insect field guides. At night, we used flashlights covered with red cellophane to lessen the deterrence of insects.

## 2.3 | Climatic data

We obtained climatic data (namely daily averages of rainfall, humidity and temperature) of our fieldwork season from Climate Station of the Instituto de Geografia da Universidade Federal de Uberlândia. For statistical analyses, we calculated mean humidity and temperature, and total rainfall for each month.

## 2.4 | Statistical analyses

We performed all statistical analyses in R software 4.0.0 (R Core Team, 2020). Packages used are detailed in each analysis below.

First, we investigated whether there was seasonal variation (i.e. peaks in activity or abundance) in plant phenophases (newly flushed leaves, fully expanded leaves, deciduousness, floral buds, flowers and fruits), EFN activity, ant and herbivore abundance throughout the year. For this, we used circular statistical analysis (Morellato, Alberti, & Hudson, 2010; Novaes et al., 2020). Circular statistics evaluate recurring biological events associated with climatic factors, where specific units of time (e.g. months) are converted in angles (Morellato et al., 2010; Novaes et al., 2020). Basically, it uses the presence/absence or abundance of a specific event over time, identifying peaks of activity (events concentrated in specific times). We converted months into angles, and used the abundance of each factor to calculate the mean vector ( $\mu$ ), mean vector length ( $r$ ), median, standard deviation, Rayleigh test  $Z$  and Rayleigh test  $p$ . Rayleigh test  $Z$  with significant values ( $p < 0.05$ ) and mean vectors ( $r$ ) close to 1 indicate seasonality of the data, that is, phenological activity was concentrated around one single date or mean angle (Morellato et al., 2010; Novaes et al., 2020). We conducted circular analyses with the package `CIRCULAR` (Agostinelli & Lund, 2017). We performed the Watson's goodness of fit test to examine unimodality before conducting circular analyses. In the case of EFN activity and abundance of ants and herbivores, we used the total number of plants with active EFNs, ants and herbivores per month for analysis at the community level.

Second, we investigated whether the peak in the activity of EFNs, and the abundance of ants and herbivores simultaneously occurred in the same period and when plants produce new leaves. To corroborate our hypothesis, the peak (mean vector) of EFNs, ants and herbivores should occur at the same time (or shortly after the appearance of leaf flushing), always before any other phenophase, and should not overlap with other phenophase mean vectors. Since there is a sequence of plant phenological events over time, we expected that EFNs peak when newly flushed leaves appear, and not during other plant phenological events. For this, we used two statistical approaches: circular statistics and overlap analysis. With these two statistical approaches, we were able to test for differences between peak activity of plant phenological events (e.g. EFN activity and newly flushed leaves) and also to analyse the overlap of event occurrence over time. We firstly carried out Watson's two-sample test of homogeneity to test whether peaks were different, and compared the mean vector in the circular diagrams of EFNs activity with newly flushed leaves, fully expanded leaves, deciduousness, floral buds, flowers, fruits, ants and herbivores. We secondly conducted niche overlap analyses by using temporal abundance matrices, which were analysed with the coefficient of overlapping (Ridout & Linkie, 2009) and niche overlap null models. The coefficient of overlapping uses Kernel density estimates fitted to the data to approximate two density functions, that is, it represents a quantitative measure of overlap (0 = no overlap, 1 = identical peak). We used the estimator Dhat1, since it is the best option for small samples sizes (Ridout & Linkie, 2009). In the case of the niche overlap null model, we used two randomization algorithms (1,000 simulated null assemblies), RA3 and RA4, and Pianka's metric with the package EcoSimR (Gotelli, Hart, & Ellison, 2015). RA3 rearranges the rows and RA4 rearranges the non-zero row values; both retain 'niche breadth' of each species.

Finally, we investigated whether climatic variables directly and indirectly influenced the ant-plant-herbivore interaction. For this, we carried out a structural equation modelling (SEM) based on monthly data at plant individual level using the LAVAAN package

(Rosseel, 2012). SEM is a multivariate statistical analysis that estimates direct and indirect relationships among variables. Specifically, we investigated direct associations among climatic factors (temperature and rainfall), newly flushed leaves, EFN activity and the abundance of ants and herbivores, as well as the indirect associations between climate and the abundance of ants (via newly flushed leaves and EFN activity) and herbivores (via newly flushed leaves, EFN activity and ants). We only chose newly flushed leaves as plant phenophase for these analyses because their peak simultaneously occurred with the peak in ant and herbivore abundance (see Section 3). The hierarchical order of the variables included in the SEM (i.e. EFNs > Ants > Herbivores) was chosen because EFNs attract ants and these in turn prey on herbivores. We estimated direct effects in the SEM as standardized partial regression coefficients, whereas we obtained indirect effects by combining the specified coefficients for direct effects on both the predictor and the response. We assessed the significance of direct and indirect coefficients with z-tests. We evaluated the goodness of fit of the general model using the Chi-square statistic, comparative fit index (CFI), the root mean square error of approximation (RMSEA) and the standardized root mean square residual (SRMR).

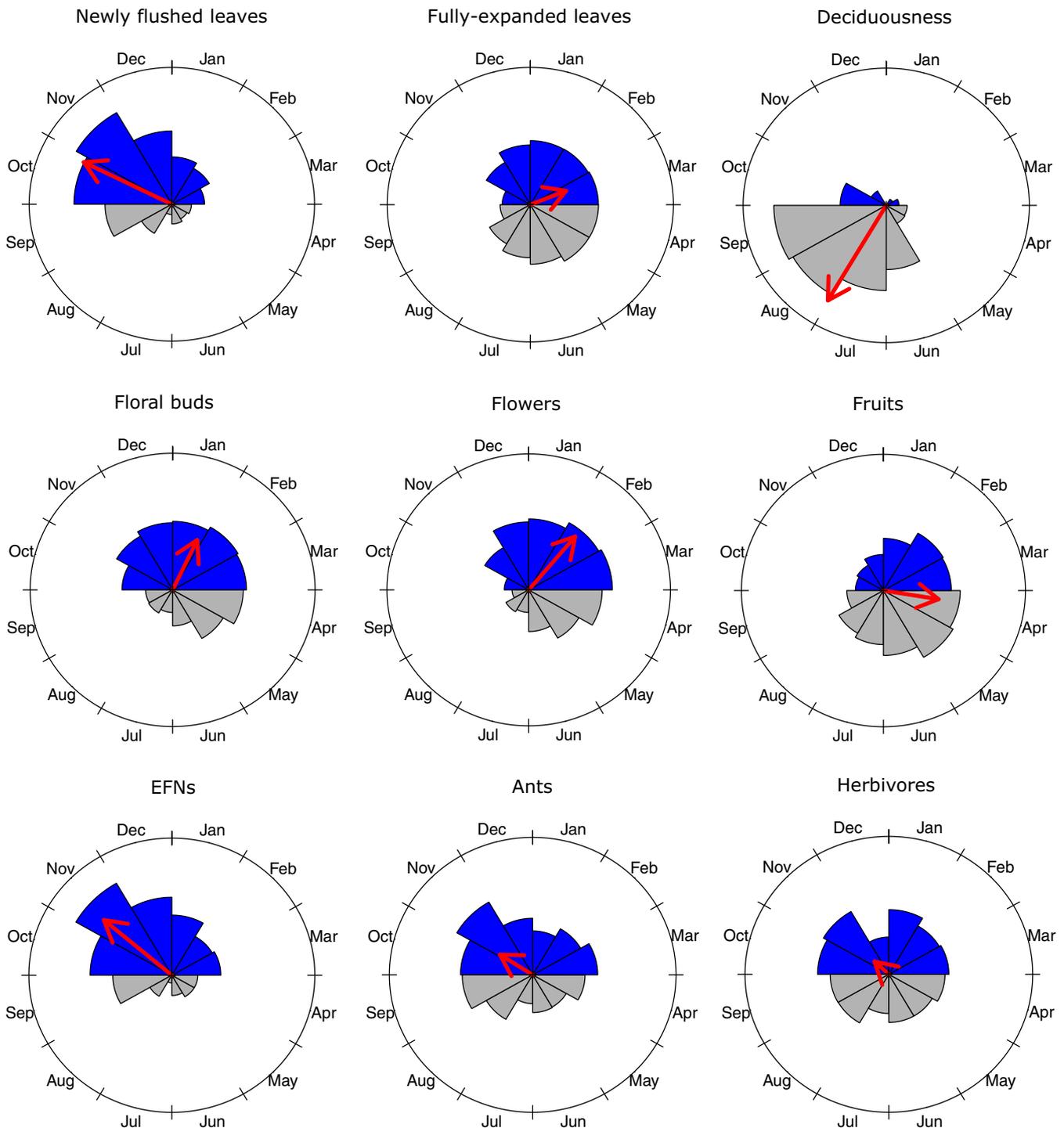
### 3 | RESULTS

#### 3.1 | Seasonal variation in plant phenology, EFN activity and ant and herbivore abundance

There was significant seasonal variation in all plant phenophases, EFN activity and ant and herbivore abundance (Table 1). Leaves flushed at the beginning of the rainy season, peaking in October (Table 1; Figure 1; Figure S1). Abundance of fully expanded leaves peaked in March (Table 1; Figure 1; Figure S1). Leaves dropped (i.e. deciduousness) at the end of the dry season, peaking in August (Table 1; Figure 1; Figure S1). Abundance of floral buds and flowers peaked in the rainy season, in January and February

**TABLE 1** Circular statistical analyses testing for seasonal variation in newly flushed leaves, fully expanded leaves, deciduousness, floral buds, flowers, fruits, extrafloral nectaries (EFNs), ants and herbivores of community. Significant ( $p < 0.05$ ) effects are in bold

Analysed factors	Mean vector ( $\mu$ )	Month	Length of mean vector ( $r$ )	Median	Circular standard deviation	Rayleigh test ( $Z$ )	Rayleigh test ( $p$ )
Newly flushed leaves	296.457	October	0.69	300.984	49.465	0.689	<0.001
Fully expanded leaves	680.693	March	0.27	61.022	92.788	0.269	<0.001
Deciduousness	210.511	August	0.80	210.969	37.891	0.804	<0.001
Floral buds	25.485	January	0.40	31.004	77.226	0.403	<0.001
Flowers	39.902	February	0.51	31.004	66.345	0.512	<0.001
Fruits	99.297	April	0.39	90.988	78.308	0.393	<0.001
EFNs	310.278	November	0.62	300.984	55.739	0.623	<0.001
Ants	302.731	November	0.27	300.984	92.438	0.272	<0.001
Herbivores	312.993	November	0.14	300.984	114.589	0.135	<0.001



**FIGURE 1** Annual peaks in six plant phenophases, the activity of extrafloral nectaries (EFNs), and the abundance of ants and herbivores in 18 EFN-bearing plant species in the Brazilian Cerrado. Arrow position represents the mean angle (mean month) where arrow length represents the length of mean vector ( $r$ ). Rainy season (in blue)—October–March; Dry season (in gray)—April–September. Statistical results are depicted in Table 1

respectively (Figure 1; Table 1; Figure S2). Fruit production mostly occurred during the dry season, peaking in April (Table 1; Figure 1; Figure S2). EFN activity and the abundance of ants and herbivores peaked at the beginning of the rainy season (November; Figure 1; Table 1; Figure S1). Results at species level are shown in Table S1; Figures S1 and S2.

### 3.2 | Simultaneous occurrence of peaks in EFN activity and ant and herbivore abundance

We found significant differences between the peak in EFN activity and the peak in all other factors analysed (Table 2—Watson two-test). The peak in the EFN activity, and the abundance of ants

**TABLE 2** Circular statistical analyses and niche overlapping analyses comparing EFNs activity patterns in 18 EFN-bearing plant species from Brazilian Cerrado

Phenophase	Watson two-test		Coefficient overlap		Null model (RA3)			Null model (RA4)			
	$U^2$	p-value	Dhat1	Observed index	Simulated index	p-value	SES (standardized effect size)	Observed index	Simulated index	p-value	SES (standardized effect size)
EFNs × Newly flushed leaves	2.568	<0.001	0.89	0.976	0.459	0.001	3.097	0.976	0.456	0.001	3.203
EFNs × Fully expanded leaves	34.753	<0.001	0.49	0.544	0.636	0.842	-1.089	0.544	0.639	0.872	-1.202
EFNs × Deciduousness	36.192	<0.001	0.22	0.223	0.406	0.847	-1.06	0.223	0.403	0.836	-1.024
EFNs × Floral buds	12.317	<0.001	0.59	0.702	0.597	0.182	0.977	0.702	0.601	0.180	0.941
EFNs × Flowers	17.364	<0.001	0.49	0.55	0.559	0.515	-0.071	0.55	0.556	0.506	-0.045
EFNs × Fruits	33.459	<0.001	0.37	0.346	0.602	0.999	-2.417	0.346	0.598	0.998	-2.335
EFNs × Ants	8.77	<0.001	0.73	0.903	0.607	0.001	2.892	0.903	0.605	0.001	2.929
EFNs × Herbivores	10.157	<0.001	0.66	0.805	0.649	0.021	2.11	0.805	0.654	0.019	2.051

and herbivores overlapped and occurred shortly after leaf flushing and did not overlap with other phenophase mean vector (Figure 1; Table 1). Based on niche overlap analyses, the highest EFN activity was synchronized with ant and herbivore abundance and newly flushed leaves (October–November, Figure 2a–c), presenting 66%–89% niche overlap over the year (Table 2). For the other factors such as fully expanded leaves, deciduousness, floral buds, flowers and fruits, we did not find significant differences in their peaks either based on coefficient overlap or niche overlap null models (Table 2).

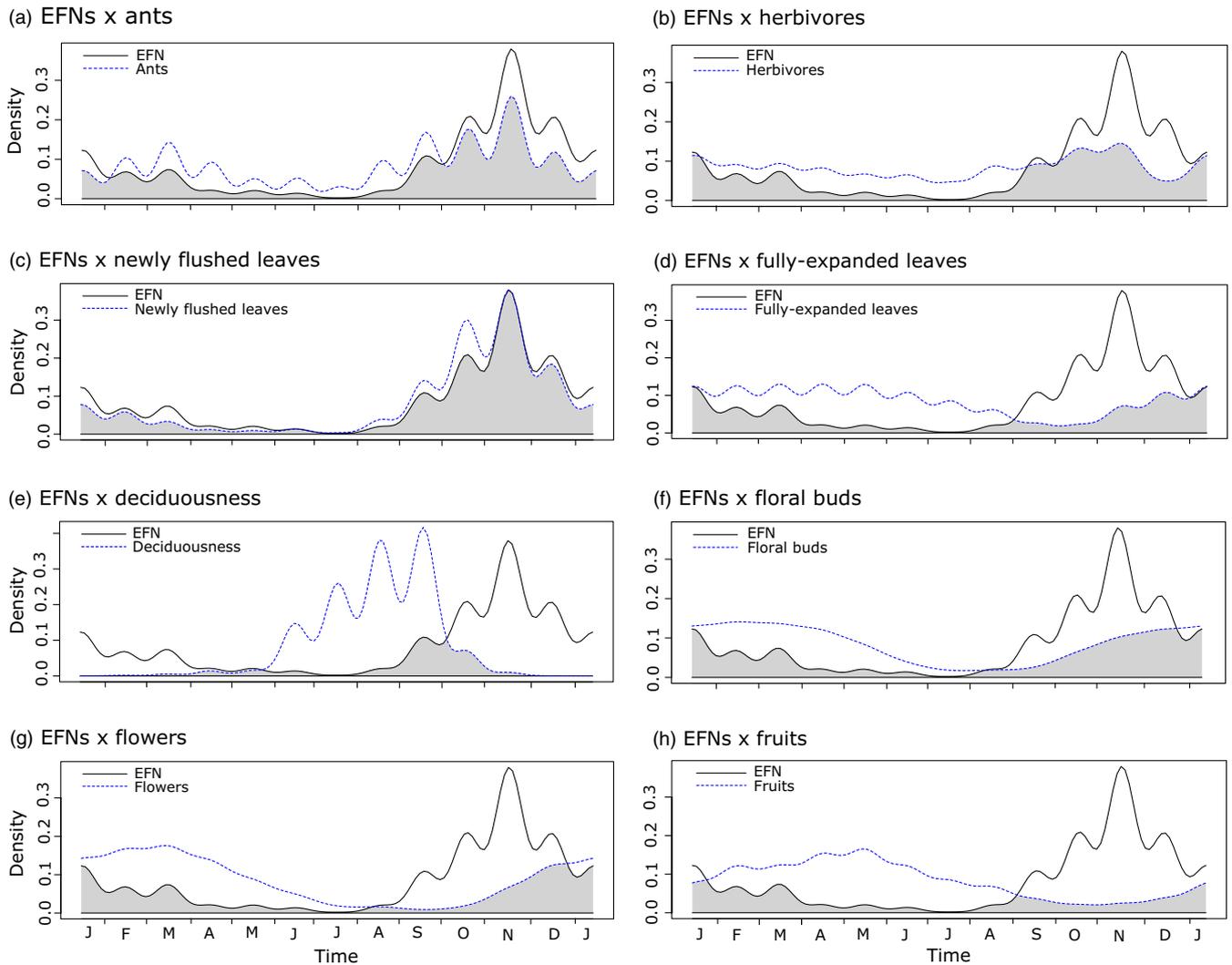
### 3.3 | Direct and indirect effects of climatic variables on ant–plant–herbivore interactions

We found direct, significant positive effects of rainfall on newly flushed leaves (standardized parameter coefficient:  $\beta = 0.48$ ), EFN activity ( $\beta = 0.34$ ), and ant abundance ( $\beta = 0.43$ ; Figure 3; Table 3). Temperature had direct, significant positive effect on ant abundance ( $\beta = 0.29$ ). Newly flushed leaves ( $\beta = 0.78$ ) had direct, significant positive effect on EFNs, which had direct, significant positive effect on ants ( $\beta = 1.06$ ). We also found that rainfall indirectly and positively affected ant abundance (via increases in the production of newly flushed leaves and EFN activity; Figure 3). Finally, we found no direct or indirect effects of climate, newly flushed leaves, EFN activity and ants on herbivore abundance (Figure 3; Table 3).

## 4 | DISCUSSION

### 4.1 | Seasonal variation in plant phenology, EFN activity and ant and herbivore abundance

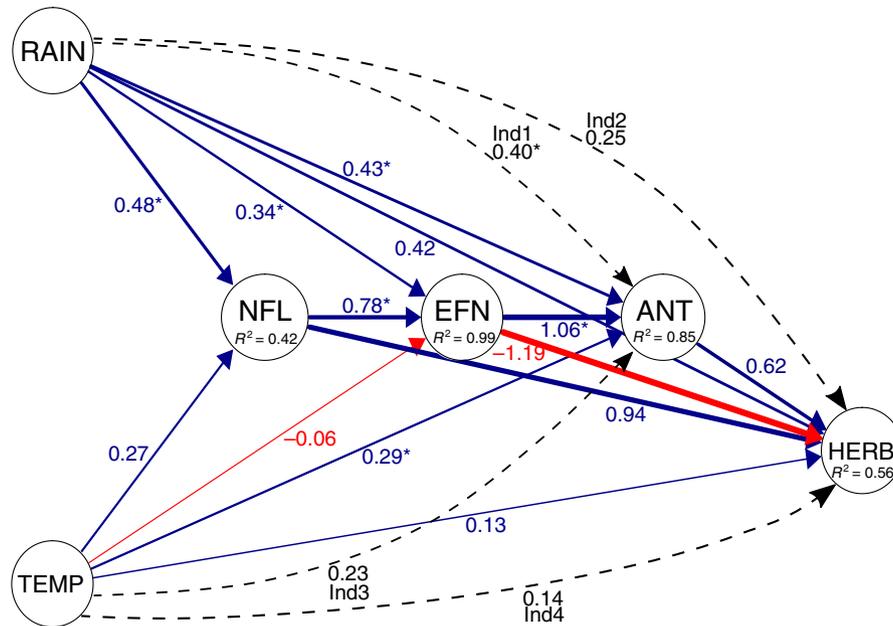
We found seasonal variation in plant phenological patterns (peaks in plant life cycle events), the activity of EFNs and the abundance of ants and herbivores. In strong seasonal environments such as the Brazilian Cerrado, rainfall and higher/lower temperatures are concentrated at specific times of the year (October–March). These temporal climate patterns directly influence plant phenology, since in these environments, plants adjust timing of phenophase expression in the most suitable period of the year (Novaes et al., 2020; Silva et al., 2011; Wright, Calderón, & Muller-Landau, 2019). New leaves were mainly produced at the beginning of the rainy season (September–October, Figure 1), as shown by Novaes et al. (2020) for other Cerrado species. At this time, rainfall increases soil moisture enhancing roots nutrients absorption, which is demanding for growth and differentiation of new plant tissues such as leaves (Silva et al., 2011). Immediately after leaf flushing, leaves expanded showing a peak of activity in March (Figure 1), but remained present on plants for almost the entire year. Then, plants produced buds, flowers and fruits which were present on plants until the beginning of the next rainy



**FIGURE 2** Kernel density functions showing patterns of overlapping of extrafloral nectaries (EFNs) and ants, herbivores, newly flushed leaves, fully expanded leaves, deciduousness, floral buds, flowers and fruits, throughout the year in 18 different EFN-bearing plant species in the Brazilian Cerrado. Shaded areas correspond to the coefficient of overlap. Statistical results are shown in Table 2

season, when they dispersed (e.g. Novaes et al., 2020; Vilela et al., 2014). To produce and maintain these reproductive structures, plants need a continuous supply of nutrients, which are produced in leaves (Taiz & Zeiger, 2009). Deciduousness occurred at the end of the dry season (August, Figure 1) to avoid drought, which can compromise photosynthesis (Tomlinson et al., 2013). Our plant species flowered throughout the rainy season and at the beginning of dry season (February, Figure 1), when rainfall increases soil moisture (a limiting factor) and when pollinator activity is higher in order to increase cross-pollination, and consequently fruit set (Silva et al., 2011; Wolda & Roubik, 1986). Finally, fruits were mainly produced at the end of the rainy season and along dry season (April, Figure 1) probably to time seed germination to take place when there was greater availability of soil moisture, that is, in the rainy season (Ranal, Santana, & Schiavini, 2009). Overall, each plant phenophase showed a peak during the year as a means of optimization due to the seasonal climatic patterns of the Cerrado.

We also found seasonal variation in the activity of EFNs and the abundance of ants and herbivores (peak in November, Figure 1). EFNs mainly occurred in young leaves which have a large amount of nutrients. Therefore, greater activity of these protective structures (Calixto et al., 2015) is expected when new plant tissues are produced (i.e. at the beginning of the rainy season; Calixto, Lange, Bronstein, Torezan-Silingardi, & Del-Claro, 2020; Calixto et al., 2018; Del-Claro et al., 2016; Del-Claro, Stefani, Nahas, & Torezan-Silingardi, 2017; Sousa-Lopes, Alves-da-Silva, Alves-Martins, & Del-Claro, 2019). Seasonal variation in the abundance of ants and herbivores were also related to seasonal climatic conditions of the Brazilian Cerrado (Vilela et al., 2014, 2018). The seasonality of insect abundance would reflect the temporal patterns of rainfall in regions with a marked seasonality, where the number of insects decreases during the dry season and increases during the rainy season (Abril et al., 2010; Coley & Barone, 1996; Ratte, 1985). However, these relationships can be also strongly influenced by plant phenology and sequential flowering in the Brazilian Cerrado (Vilela et al., 2014).



**FIGURE 3** Structural Equation Modelling diagram showing direct and indirect effects of climatic variables (rainfall and temperature) on newly flushed leaves, extrafloral nectaries (EFN) activity and the abundance of ants and herbivores. Values outside circles represent the standardized parameter coefficient ( $\beta$ ), while values inside the circles represent proportion explained by explanatory variables ( $R^2$ ). Asterisks represent significant effect ( $p < 0.05$ ). RAIN-rainfall, TEM-temperature, NFL-newly flushed leaves, EFN-Extrafloral nectaries, ANT-ants, HERB-herbivores. Dashed line represents indirect effects. Ind1—effects of rainfall on ants via newly flushed leaves and EFN activity; Ind2—effects of rainfall on herbivores via newly flushed leaves, EFN activity and ants; Ind3—effects of temperature on ants via newly flushed leaves and EFN activity; Ind4—effects of temperature on herbivores via newly flushed leaves, EFN activity and ants. Analyses were based on monthly data at plant individual level. Parameters of the model:  $\chi^2 = 2.770$ ,  $p = 0.096$ ; CFI = 0.979; RMSEA = 0.384; SRMR = 0.010. CFI values range from 0 to 1, where values close to 1 indicate a better fit model. RMSEA and SRMR values also range from 0 to 1, where values close to 0 indicate a better fit model

#### 4.2 | Simultaneous occurrence of peaks in EFN activity and ant and herbivore abundance

The peak in the activity of EFNs, and the abundance of ants and herbivores simultaneously occurred at the beginning of the rainy season, when new leaves flushed. As a response to the evolutionary pressures, several plants concentrate on different defensive strategies along leaf ontogeny (Boege & Marquis, 2005; Calixto et al., 2015; Coley & Barone, 1996; Endara et al., 2017; Godschalx, Stady, Watzig, & Ballhorn, 2016) and allocate defences according to the tissue value and the probability of herbivore attack (Optimal Defence Theory; Calixto, Lange, et al., 2020; McKey, 1979; Rhoades, 1979). Due to the high concentration of nutrients present in young leaves, they are an attractive resource for herbivores, thus requiring a high allocation of resources for defences. Therefore, EFNs should be more active in newly flushed leaves than in fully expanded leaves in order to increase the attraction of protective ants. As a highly predictable carbohydrate-rich food resource, extrafloral nectar can influence ant abundance, attracting more number of ants when such food rewards are active.

In addition, increases in ant abundance might be a response to herbivore availability through the season. Extrafloral nectar is a liquid with a high concentration of sugar, but with low amounts of amino acid (González-Teuber & Heil, 2009; Koptur, 1994). Due to this high difference in the carbohydrate-protein ratio, foraging ants need

to collect high amounts of nectar or look for resources present on plants that have a high proportion of proteins, that is, herbivorous insects (Blüthgen & Feldhaar, 2010; Davidson, 1997). Thus, some ants can change their foraging patterns according to the availability of resources in the environment (Belchior, Del-Claro, & Oliveira, 2012; Whitford & Jackson, 2007). For instance, Anjos, Luna, Borges, Dáttilo, and Del-Claro (2019) showed that granivorous ants can change their foraging behaviour due to the seasonality of the environment, which results in changes in resource availability (also see Donoso, Johnston, & Kaspari, 2010). Therefore, in our region we observe that biotic and abiotic factors lead to the simultaneous occurrence of EFNs, ants and herbivores, and the production of new leaves.

#### 4.3 | Direct and indirect effects of climate on ant-plant-herbivore interactions

We found that climatic variables directly influenced the ant-plant-herbivore interaction. In particular, we found that rainfall had positive direct effects on ant abundance. Rainfall is an important factor in dry ecosystems that triggers animal activity (Wolda, 1978). Some groups of insects are strictly controlled in their seasonality and their abundance by rainfall patterns, being more active and abundant in periods of increased rainfall (Lange et al., 2015; Munique & Calixto, 2018; Wolda, 1978). For instance,

**TABLE 3** Structural equation modelling results for direct and indirect influences of abiotic variables (rainfall and temperature) on newly flushed leaves, extrafloral nectaries (EFN) activity, and abundance of ants and herbivores. Analyses were based on monthly data at plant individual level monthly values. Ind1—effects of rainfall on ants via newly flushed leaves and EFN activity; Ind2—effects of rainfall on herbivores via newly flushed leaves, EFN activity and ants; Ind3—effects of temperature on ants via newly flushed leaves and EFN activity; Ind4—effects of temperature on herbivores via newly flushed leaves, EFN activity and ants

	Estimate	SE	z-value	$p(> z )$	Standardized latent variable coefficient	Standardized coefficient
<b>Direct effects</b>						
Herbivores~						
Newly flushed leaves	0.438	0.658	0.665	0.506	0.438	0.937
EFN	-0.511	0.788	-0.648	0.517	-0.511	-1.192
Ants	0.137	0.107	1.280	0.201	0.137	0.616
Rainfall	0.178	0.291	0.614	0.539	0.178	0.417
Temperature	3.526	7.628	0.462	0.644	3.526	0.131
Newly flushed leaves~						
Rainfall	0.441	0.224	1.975	0.048	0.441	0.482
Temperature	15.690	14.083	1.114	0.265	15.690	0.272
EFN~						
Rainfall	0.340	0.039	8.763	0.000	0.340	0.340
Temperature	-4.035	2.229	-1.810	0.070	-4.035	-0.064
Newly flushed leaves	0.855	0.043	19.663	0.000	0.855	0.785
Ants~						
Rainfall	0.834	0.349	2.390	0.017	0.834	0.433
Temperature	35.771	15.399	2.323	0.020	35.771	0.295
EFN	2.041	0.355	5.750	0.000	2.041	1.057
<b>Indirect effects</b>						
Ind1	0.770	0.414	1.859	0.043	0.770	0.400
Ind2	0.105	0.100	1.054	0.292	0.105	0.246
Ind3	27.385	25.075	1.092	0.275	27.385	0.226
Ind4	3.746	4.510	0.831	0.406	3.746	0.139

Lange, Calixto, Rosa, Sales, and Del-Claro (2019) showed that abundance of one of the most common foliage-dwelling ant, *Camponotus crassus*, in a cerrado sensu stricto vegetation was greater during the months with greater rainfall. Similarly, we also found that temperature had positive direct effects on ant abundance, probably through increases in consumption rates and body size, and decreases in developmental time of ants (Abril et al., 2010; Ratte, 1985). For instance, Abril et al. (2010) showed that temperature affected the complete brood development of the Argentinian ant *Linepithema humile*, in which the higher the temperature, the shorter the development times.

We also found that rainfall indirectly (via changes in plant phenology and EFN activity) influenced ant abundance. In particular, we found that increased rainfall enhanced the production of new leaves, and consequently EFN activity, resulting in turn in increased abundance of ants. The appearance of new young tissues at the beginning of the rainy season is associated with a higher activity of EFNs, which attracts a greater number of ants. In agreement with our findings, Korndörfer and Del-Claro (2006) also showed that EFNs of a Cerrado plant, *L. pacari* St. Hil. (Lythraceae), are active

in new leaves, remaining active until full leaf expansion. Similarly, Nogueira et al. (2020) showed that ant attendance of Bignoniaceae in Brazilian Amazonia was influenced by new leaves production, which is affected by environmental conditions.

In addition, we found that climate did not directly or indirectly (via plant phenology, EFN activity and ant abundance) influence herbivore abundance. One plausible explanation of these findings is that, although the abundance of herbivores showed a peak in November, they were feeding on plants for almost every month of the year (Figure 1), independently of the plant phenophase. Herbivores can feed on different plant tissues (e.g. leaves, flowers, fruits) throughout the growing season (niche differentiation through resource partitioning; Dyer et al., 2007; Novotny et al., 2010), consequently maintaining their abundance over time (Marques, Price, & Cobb, 2000). It is also expected that in a set of 18 plant species of nine families, variation among individuals in phenological events exist. Although we observed strong seasonality in herbivore abundance, herbivores can be present in Cerrado the whole growing season, surpassing the adversities by moving from plant to plant depending on resources availability (Vilela et al., 2014).

Finally, although we found a positive relationship between ant and herbivore abundance, such relationship was not significant. One plausible explanation for this unexpected result could be that due to the high availability of plant-based resources (i.e. extrafloral nectar), ants do not protect plants and forage looking for extrafloral nectar only since it is a nutrient-rich predictable resource (Bixenmann, Coley, & Kursar, 2011; González-Teuber & Heil, 2009). In a previous study, Bixenmann et al. (2011) showed that ant visitation increased as extrafloral nectar production increased. However, when they provided very high concentrations of artificial nectar, ants tended not to patrol other plant parts, staying only on those with EFNs. A potential limitation is that our study was correlational. Therefore, further studies should include ant exclusion treatments in order to make stronger conclusions about the benefits of ant defence.

#### 4.4 | Final remarks

In this study, we assessed an important factor in structuring ecological interactions, namely seasonal climate patterns. In these seasonal environments, plants have evolved adaptations to express key life-history events during the most convenient periods of time (Wright et al., 2019). Current changes in abiotic conditions, especially in rainfall and temperature, can change seasonal patterns and consequently shift plant phenophases occurrence over time (Vilela & Del-Claro, 2018; Visser & Holleman, 2001). Hypothetically, this phenophase displacement can lead to a collapse of multitrophic interactions that plants are engaging, since there will be an absence of overlap between the peaks of activity of plant phenophases and the peaks of abundance of ants and herbivores (Visser & Holleman, 2001). Thus, current climate changes may disrupt ant–plant–herbivore interactions in seasonal environments, as the Brazilian Cerrado, through phenological mismatches (e.g. Valiente-Banuet et al., 2015), especially between newly flushed leaves, EFN activity, and ant and herbivore abundance. Studies evaluating the impacts of biotic and abiotic effects on insect–plant interactions are paramount to understanding how these relationships are shaped in a spatiotemporal scale. In order to increase our knowledge about different ecological and evolutionary processes that structure these mutualistic interactions, further studies should evaluate different potential factors that could be involved, such as the impacts of biotic and abiotic variables at different spatiotemporal scales.

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#### AUTHORS' CONTRIBUTIONS

E.S.C., D.L. and K.D.-C. conceived the ideas and designed methodology; E.S.C. collected the data; E.S.C., L.R.N. and D.F.B.d.S. analysed the data; E.S.C., L.R.N., D.F.B.d.S., D.L., X.M. and K.D.-C. interpreted the results; E.S.C. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

#### PEER REVIEW

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#### DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.sbccc2fr45> (Calixto, Novaes, et al., 2020).

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#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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