

# Community richness is the main factor determining the structure of ant-plant mutualistic networks along environmental water gradients

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## Abstract

Many plant species rely upon ants to protect against herbivores. In arid environments, these plants often form stronger bonds with dominant ant species that, in turn, provide a better anti-herbivory defense than low aggressive subordinate ants. Dominant ants typically claim the plants producing more nutritious nectar, commonly leaving less valuable plants to be guarded by lower-quality bodyguards. As water scarcity increases, the value of the extrafloral nectar also increases, which can increase the control of the most valuable plants by dominant ants and of the displacement of outcompeted ants to less valuable plants, reducing niche overlap among ant species and, consequently, the generalization of ant-plant interactions at the community level. To evaluate this hypothesis, we crossed data from 63 empirical ant-plant networks with the mean precipitation rate of the sites and period in which the interactions were sampled. As the environment dries, ant-plant networks decreased in species richness but maintained other network properties. Surprisingly, the decline in the number of ant and plant species engaged in the mutualism along the precipitation gradient increased the interaction generalization in drier habitats. But this increased generalization is possible due to the increase in probability of interaction between all plant and ant species due to the lower richness. Hence, water availability primarily influences ant-plant interactions through its impact on ant and plant communities. This has significant eco-evolutionary consequences, as possibly increasing the persistence of this networks in drier environments, demonstrating a new pathway through which environmental factors can impact ecological interactions.

## INTRODUCTION

Mutualistic interactions often involve multiple, non-related species in a community (Bascompte and Jordano 2007b), potentially affecting the ecology and evolution of entire assemblages. However, mutualistic interactions are not distributed homogeneously across the assemblage of potentially interacting species (Vázquez et al. 2005). Rather, some species may interact with many potential partners available in the community, while others interact with only a few (Bascompte et al. 2003, Fagundes et al. 2017, Plowman et al. 2017, Guimarães 2020). This heterogeneous distribution of interactions across species can be influenced by the traits of the individuals and populations involved in the mutualism and their response to the local biotic and abiotic environment (e.g. Vázquez et al. 2005, Miller 2007, Albrecht et al. 2010, Dáttilo et al. 2013b, Maia et al. 2019). Whenever habitat conditions influence these mutualistic traits, it can indirectly affect the patterns of interaction in mutualistic networks and, consequently, the mechanisms shaping natural communities and determining species responses to environmental conditions (see Tylianakis and Morris 2017). Therefore, understand how interactions are distributed in response to environmental conditions can help us to understand the consequences to species interactions over global climatic changes.

Defensive mutualistic interactions between ants and plants with extrafloral nectaries (EFNs) are an example of mutualistic interaction in which ant and plant traits can be influenced by environmental factors (Kersch

& Fonseca 2005, Heil 2008, Pringle et al. 2013). Ant competitiveness determines their visitation patterns to plants with EFNs and their quality as bodyguards (Leal and Peixoto 2017, Leal et al. 2022). Dominant ants display a series of aggressive behaviors that ensures them a highly competitive ability (Stuble et al. 2017). This aggressiveness also makes dominant ants better mutualistic partners for plants with EFNs because they are more likely to behave aggressively towards herbivores, unlike subordinate ant species who do not typically exhibit aggression (Buckley and Gullan 1991, Xu and Chen 2010, Flores-Flores et al. 2018, but see Melati & Leal 2018). Like the ants, plant species also vary in their attractiveness to ant bodyguards, with those producing more concentrated and abundant nectar being more appealing to their partners (Blüthgen and Fiedler 2004a, c, Flores-Flores et al. 2018). Therefore, dominant ant species are likely to monopolize the most attractive plant species in the community, displacing subordinate species to less attractive plants (Blüthgen and Fiedler 2004c). At a broad scale, this kind of assortative pairing can predictably shape the patterns of ant-plant interaction along spatial gradients (Dáttilo et al. 2013b), especially along those in which the competitive pressure among ant species is also variable (Leal and Peixoto 2017, da Silva et al. 2019, Lasmar et al. 2021). In this case, ant-plant interactions should become less generalized and form groups of interacting species as the stronger competitive pressure among ant species, with dominant and subordinate ants interacting with a more dissimilar group of plants in the community.

A meta-analysis found that, at a macroecological scale, plants with EFNs benefit more from ant attendance as the environment dries out, due to an increased probability of plant attendance by dominant ant species (Leal and Peixoto 2017). This is because the extrafloral nectar, a water source for ants (Heil 2011, 2015), becomes more valuable to ants when water is scarce, making plants with EFNs more attractive to ants (Ruffner and Clark 1986, Contreras et al. 2013, Leal and Peixoto 2017). However, it neglects that, even in drier habitats, plant species would not be equally valuable to ants. Then, dominant ant species might increase the monopolization of the best plant partners available in drier habitats, increasing the competitive exclusion of subordinate ants from the more valuable plant species. Consequently, it is possible that at a macroecological scale ant-plant interactions become more specialized and grouped as scarcer the water availability – something that can have relevant implications for the eco-evolutionary dynamic of this mutualism at a broad scale. For instance, increasing the contribution of direct effects on trait matching over indirect effects on interactions, accelerating the coevolutionary dynamics on interacting species with the group of interactors.

Here, we investigated how emergent patterns of interactions between ants and plants with EFNs (ant-plant interactions hereafter) at the community level vary over environmental water gradients and evaluated the role of ant competition for plant species secreting extrafloral nectar on the emergence of such patterns. For that, we hypothesized that (i) ant-plant interactions become more specialized and forming sub-groups of interaction partners as drier the environment and that (ii) these effects will be driven by a decline in the overlap of dominant and subordinate ant species in the use of these plants over the environmental water gradients. To evaluate our hypotheses, we used two different approaches based on the ecological network theory (Bascompte et al. 2003, Jordano et al. 2003, Bascompte and Jordano 2007b). In the first approach, we evaluated how the structure of ant-plant ecological networks and plant usage by all ant species varied along a macroecological gradient of water availability (first hypothesis). In the second approach, we evaluated the water availability effect on the patterns of plant usage specifically by dominant and subordinate ant species (second hypothesis).

## 2. METHODS

### 2.1. Networks studied

To evaluate our hypotheses, we built a dataset compiling data from empirical ant-plant networks available in published studies and representing interactions between ants and plants with EFNs in different communities worldwide (ant-plant networks hereafter). For that, we searched published studies at Science Direct, Web of Science platforms (Core library) and Google Scholar using the following key terms: “*ant-plant network*” (results without replicates: Science Direct = 16; Web of Science = 50; Google Scholar = 121) and “*ant-extrafloral nectar network*” (results: Science Direct = 67; Web of Science = 0; Google Scholar = 13). After this search, one of us (BM) read the title and abstract of all papers, checking if the studies reported ant-plant

networks based on interactions observed in the field. We did not consider papers reporting networks in which the ant-plant interactions were experimentally manipulated ( $N = 1$ ). After this first screening, we gathered 32 potentially suitable papers describing empirical ant-plant networks. Some of those papers investigated different questions using the same ecological networks. In such cases, we selected the oldest study from which we could extract the network’s information. Then, at the end of this first screening process, we gathered 22 papers suitable to our study.

## 2.2. Precipitation data

As a proxy for water availability in the habitat, we used the mean precipitation rate at the time and site in which the ant-plant interactions were sampled in each study (Konapala et al. 2020). This metric allowed us to estimate the precipitation rate experienced by the plants with EFNs and the ants when the interactions were observed, leading to more refined results about the influence of the local climatic conditions on the patterns of ant-plant interactions. We obtained this information from TRMM\_3B43 dataset (TRMM from now on), available at the NASA Giovanni web database, from NASA’S EOSDIS (NASA’S Earth Observing System Data Information System) (TRMM, 2001). In this database, the precipitation rate is measured as the amount of rainfall per area per unit of time (TRMM, 2001). TRMM compiles global climatic information for the period between 01/01/1998 to 31/12/2019, covering the period when most of the empirical networks in our database were collected ( $N = 21$ ).

To retrieve the precipitation data, we extracted from each paper the period and the geographical coordinates of the study site in which each network was sampled. This information was available in most of the papers included in our database. In two studies, the authors did not report the period or site in which the interactions were observed. Then, we excluded these studies from our dataset, which reduced our dataset to 19 studies. Among all networks described in these papers, only one network reported interactions collected over a period not covered by TRMM dataset (before 1998). In this case, we excluded this single network from our dataset but included the other two networks described in this study and collected after 1998 (Díaz-Castelazo et al. 2013).

In studies where the ant-plant interactions were sampled in an area equal to or inferior to one minute latitudinal and longitudinal scale, we used a one-minute scale as our standard spatial unit on TRMM ( $N = 43$  networks, 68.25%). Only two studies sampled the ant-plant networks in an area larger than a one-minute scale ( $N = 20$  networks, 31.75%). In these cases, we selected an area proportional to one minute at the center of the sampling area reported in the original paper. After setting the area, we informed the period of interest and extracted the precipitation information as a “time series of area-averaged values”. By doing that, we could obtain the monthly averaged mean precipitation rate in millimeters for the period in which each network included in our dataset was sampled.

## 2.3. Hypothesis 1 – Effect of water availability on the structure of ant-plant networks

To evaluate if ant-plant interactions become more specialized and modular as drier the environment, we used binary bi-adjacency matrices describing the networks reported in each study. In these matrices, the ant-plant interactions were described as  $a_{ij} = 1$ , when the ant species  $i$  interacted with the plant species  $j$ , and  $a_{ij} = 0$  otherwise. Among the studies included in our database, 69% presented the frequency with which each ant and plant species interacted with each other (weighted networks). Since this quantitative information was not available in 31% of our networks and that weighted networks were not evenly distributed along our water gradient, we transformed the weighted networks into binary ones (i.e. presence/absence of interaction between each species pair), making all networks comparable among each other.

For each network, we extracted the following metrics: species richness (S), modularity (Q) (Newman 2006), connectance (C) and nestedness (NODF) (Almeida-Neto et al. 2008). The network S is the number of ant and plant species within the network and was calculated by summing the rows and columns of each binary matrix. The modularity (Q) estimates how grouped the interactions are within the networks. This modular pattern arises from the formation of cohesive groups of interacting species (modules) in which species within a given group are more densely connected to each other than to species from other groups

(Olesen et al. 2007). The connectance ( $C$ ) estimates the proportion of realized interactions from the total possible interactions within the network (Blüthgen and Menzel 2006, Blüthgen et al. 2008, Pellissier et al. 2018). The higher the connectance, the higher the proportion of realized interactions between the ant-plant species pairs and, consequently, the more generalized the interaction patterns (Blüthgen et al. 2008). Finally, the nestedness (NODF) describes a pattern in which species with fewer interactions often interact with a subset of the partners interacting with species with a larger number of interactions. Nested networks often show (1) interactions among generalists (species with multiple partners in the network), (2) asymmetries in the number of interactions, in which species with fewer interactions (specialists) often interact with generalists, (3) interactions among specialists are rare or absent (Bascompte et al. 2003, Guimaraes Jr et al. 2006, Bascompte and Jordano 2007a). In a perfect nested network, we expect a continuum in the degree of species specialization with no species segregation into modules of interacting species. Then, if the ant-plant interactions become more specialized and modular as drier the environment, we expect that the ant-plant networks exhibit lower connectivity and nestedness values and higher modularity levels as lower the mean precipitation of the habitat.

When the  $S$ ,  $Q$ , NODF, or  $C$  metrics were not directly reported in the original studies, we calculated them using the raw data reported by the authors in the supplementary material of each paper ( $n = 5$ ) or kindly sent to us by the authors of each study ( $n = 4$ ; see the acknowledgments). In the case of  $Q$  and NODF to obtain a normalized metric (see below), we re-analyze all networks but two networks for the nested metric in Luna et al. (2018). In one case, we could not retrieve all these metrics, but the paper reported the matrix of interaction as a figure (Dáttilo et al. 2014a). In this case, we recreated the matrix of interaction and used it to calculate our metrics of interest. We could not retrieve any focal metrics in six papers from our database. These papers were discarded, and our final database included 13 papers and 63 networks (see the list of all papers used in our analysis in Supporting information). When we retrieved some, but not all metrics of interest from the studies, we excluded the study only from the dataset used to analyze the specific absent metric. Because of it, we had datasets with different sample sizes for different metrics. In one paper, the metrics obtained corresponded to networks containing different types of interaction (e.g. networks containing ant-EFN plants and ant-aphides-plants – Blüthgen and Fiedler 2004a). In this case, we excluded the interactions not involving plants with EFNs and re-analyzed the network formed only by the interactions between plant species with EFNs and ant bodyguards.

When the  $C$  values were not directly reported in the studies, we calculated them using the raw data following:

$$C = \frac{I}{PA},$$

in which  $I$  is the number of realized interactions between ant-plant species pairs,  $P$  is the number of plant species, and  $A$  the number of ant species in the network. To calculate the NODF and the  $Q$  values, we used the ANINHADO (Guimarães and Guimarães 2006) and MODULAR software (Marquitti et al. 2013), respectively. To maximize the network’s modularity, we used the simulated-annealing algorithm (Guimerà and Amaral 2005) available at MODULAR (Marquitti et al. 2013).

Network’s modularity and nestedness can be affected by variation in the network  $S$  (Blüthgen et al. 2008). Because of it, direct comparisons of these metrics between different networks may lead to a misleading interpretation of the mechanisms driving the interactions between species pairs (Blüthgen et al. 2008). Hence, after gathering all metrics of interest, we estimated the  $Z$ -score of the  $Q$  and NODF values.  $Z$ -score reduces such bias by standardizing mean  $Q$  and NODF values per unit of the standard deviation of these metrics assuming a given theoretical benchmark ( $z$   $Q$  and  $z$  NODF from now on). To calculate the  $Z$ -scores, we calculated the mean and the standard deviation of  $Q$  and NODF values for 1000 simulated null networks based on each empirical network in our dataset. To generate the null networks, we used the null model type II (Bascompte et al. 2003), reported at ANINHADO as null model CE, and at MODULAR as null model 2. The null model type II states that the probability of interaction ( $P_{ij}$ ) between two species ( $i$  and  $j$ ) is proportional to the degree (number of interactions) of each species. Therefore, the type II null model of an incidence matrix of size  $P \times A$  (number of plant species  $\times$  number of ant species) could be depicted as:

$$P_{ij} = \frac{1}{2} \left( \frac{k_i}{A} + \frac{k_j}{P} \right),$$

in which  $k_i$  is the degree of each plant species and  $k_j$  is the degree of each ant species. Therefore, the Z-score of each metric of interest (NODF or Q) for each network was calculated as:

$$z_m = \frac{m_R - m_N}{\sigma_N^2},$$

in which  $m$  is the metric of interest (Q or NODF),  $m_R$  is the metric of interest from the real network,  $m_N$  is the mean value of the metric of interest generated by 1000 randomizations by type II null model and  $\sigma_N^2$  is the standard deviation of the metric of interest in the 1000 null matrices.

#### 2.4. Hypothesis 2 – Effect of water availability on the interaction patterns of dominant and subordinate ants with the plants

##### 2.4.1. Ant dominance classification

We obtained the identity of the ant species interacting with the plants with EFNs in nine of the 13 papers, representing 38 of the 65 networks in our dataset. We classified each ant species into categories of hierarchical dominance based on the literature (Andersen and Patel 1994, Andersen 1997, Cerdá et al. 2013, Stuble et al. 2017) (see Supporting information to a list of ants and their dominance hierarchies). This hierarchical classification tends to be relatively consistent across ant genera in different biogeographical regions (Cerdá et al. 2013). Because of it, this categorization has been successfully used to identify emergent macroecological patterns involving ant communities worldwide (Baccaro et al. 2011, Dáttilo et al. 2014b, Leal and Peixoto 2017, Leal et al. 2022).

In the literature, ant species exhibiting mass recruitment, overly aggressive behaviors, and a higher ability to monopolize resources and territories are classified as *dominants* (Cerdá et al. 2013). Ant species exhibiting mass recruitment (but less expressive than dominant species), a higher ability to monopolize food resources, but less frequent aggressive behavior are classified as *sub-dominants* (Andersen 1995, Arnan et al. 2011). When dominant ant species are absent in a community, sub-dominant ants could dominate, being functionally similar to dominant species (e.g. Andersen 1995). In such cases, sub-dominants have a similar ability to monopolize and defend plants with EFNs than the truly dominant ant species (Horvitz and Schemske 1984). Since we lack information regarding the composition of the ant community occurring in the areas where each study was conducted and that both dominant and sub-dominant ants can equally influence the behavior of subordinate ant species (Parr and Gibb 2010), we pooled dominant and sub-dominant ant species into the same group named as dominants hereafter (similar approach used by Leal and Peixoto 2017, Leal et al. 2022). Finally, we classified as *subordinates* the ant species that forage solitarily, not exhibiting mass recruitment and/or aggressive behaviors (Cerdá et al. 2013).

##### 2.4.2 Water effects on the patterns of dominant and subordinate visitation to the plants

To evaluate the role of water availability in the overlap of dominant and subordinate ant species visiting the plants with EFNs, we used the networks in which we had access to ant species identity ( $N = 37$ ). In three networks, no subordinate ant species interacted with the plants. These networks were excluded from all the analyses focusing on the subordinate species ( $N = 34$  networks) but maintained in the analyses focusing on the dominant ant species ( $N = 37$  networks). For each network, we calculated the proportion of dominant ant species. Additionally, we calculated three metrics to explore the patterns of plant use by dominant and subordinate ant species interaction: the Connectance, the Resource range (RR), and the Jaccard Index. These metrics are commonly used to describe the trophic specialization of species in the network (Jordano 1987, Poisot et al. 2012), which allowed us to evaluate the magnitude of dominant and subordinate trophic specialization and overlapping in the networks along the water gradients.

The connectance of each hierarchical group ( $C_d$  and  $C_s$ ) described the proportion of all interactions reported in each network performed by dominant or subordinate ant species to the total possible interactions within the networks. We calculated  $C_s$  and  $C_d$  as a subset of the general network connectance as follows:

$$C_d = \frac{I_d}{PA_d}, C_s = \frac{I_s}{PA_s},$$

where  $C_d$  and  $C_s$  is the connectance of dominant and subordinate ant species, respectively.  $I_d$  ( $I_s$ ) is the number of interactions performed by dominant (subordinate) ant species,  $P$  is the number of plant species, and  $A_d$  ( $A_s$ ) is the number of dominant (subordinate) ant species in each network. If the decline in water availability reduces dominant and subordinate overlap in using plants with EFNs,  $C_d$  and  $C_s$  values will decline when the mean precipitation rate also declines.

Resource Range (RR) is a binary metric resulting from the normalization of the total number of interactions realized by the species within the network (Poisot et al. 2012). In our case, it will describe the proportion of plant species used by dominant and subordinate ant species within each community. To calculate RR, we first counted the number of interactions performed by each ant species within the networks. Then, we estimated the mean RR of ants from each hierarchical group ( $RR_d$  and  $RR_s$ ) as follows:

$$RR_d = \frac{1}{A_d} \sum_{i=1}^{A_d} \frac{P - k_i}{P - 1}, RR_s = \frac{1}{A_s} \sum_{j=1}^{A_s} \frac{P - k_j}{P - 1}$$

In which  $k_i$  ( $k_j$ ) is the number of interactions the species of dominant (subordinate) ant species  $i$  ( $j$ ). This metric ranges from 0 to 1, with 0 indicating the use of all plant species within the networks by ants from each group (i.e. complete generalization) and 1, the use of only one plant species by all ants from each group (i.e. complete specialization). Then, if decreasing in water availability increase the monopolization of more valuable plants by dominant ants and the competitive exclusion of the subordinate one to less valuable plants, we expect that the  $RR_d$  and  $RR_s$  values increase as the mean precipitation rate decreases.

Finally, we used the Jaccard Index ( $J$ ) as a proxy for the overlapping degree of dominant and subordinate ant species interacting with the plants in each network. To calculate it, we created  $P \times 2$  binary matrices describing the patterns of dominant and subordinate ant interaction with the plants in the networks. In this case, we were not interested in when each ant species from each group interacted with each plant species. Instead, we focused on when each plant species interacted with any dominant or subordinate ant in that specific community (1 when any dominant and/or subordinate ant species visited the plant and 0 otherwise). Once we built the matrices for each network, we calculated the Jaccard index, using the *vegdist* function at *vegan* package in the R program. At *vegdist*, we used the method “jaccard” which estimates the dissimilarity between the patterns of dominant and subordinate ants with the plants. To make it more intuitive, we subtract one from the dissimilarity index (1 – dissimilarity) to obtain the Jaccard similarity index. Therefore, the lower the Jaccard similarity index for a network, the lower the overlap among the plant species used by dominant and subordinate ants. Therefore, if the decline in water availability improves the monopolization of the more valuable plants in the community by dominant ants and the displacement of subordinate ants to less valuable plants, we expect that the Jaccard index will decrease as the mean precipitation decreases.

## 2.5 Statistical analysis

Initially, we created a series of linear mixed regression models (LMM) to investigate all our predictions, assuming that the residuals of our data were normally distributed and heteroscedastic. However, once we ran the models, we observed that these assumptions were not true for almost all LMM models. Because of that, we built different models according to the nature of the dataset used for each analysis. For models that broke the normality assumption (see Supporting information), we fitted a GLMM (Generalized Linear Mixed Model), using the error distribution with the best fit to the data. If the LMM model broke the assumption of variance homogeneity (see Supporting information), we fitted a GLS (Generalized Least Square) regression. We used the GLS because it allowed us to adjust the residual variance while keeping the raw data, avoiding the use of non-parametric tests or data transformation (Zuur et al. 2009). In all models described below, we used the mean precipitation rate and the paper identity as predictors and random factors, respectively.

To evaluate if ant-plant interactions become more specialized and modular as drier the environment (hypothesis 1), we first evaluated the effects of the mean precipitation on the S of the networks. For that, we built a GLS model using the of 53 networks as our continuous response variable. Then, to evaluate the effects of

the mean precipitation rate on the specialization and modularity of the networks, we built three models, one for each of the following response variables: connectance (C; N = 51 networks), zNODF (nestdness; N = 63), and zQ (modularity; N = 51). For the connectance model, we built a GLMM with a Gamma distribution. Because the connectance can be influenced by the size of the networks (Yodzis 1980, Blüthgen and Menzel 2006), we included the S of each network as a weight in this model. For the zNODF and zQ, we build GLS models using each metric as a response variable.

To investigate if the structure of the ant-plant networks along the water gradient will be driven by changes in the patterns of dominant and subordinate ant interaction with the plants with EFNs (hypothesis 2), we first evaluated the effects of the mean precipitation rate on the proportion of interactions performed by dominant and subordinate ants on the networks. For that, we used a GLMM with a Gamma distribution, including the proportion of dominant ant species in 37 networks as our response variables. To investigate if water availability affected the connectance of dominant and subordinate ant species in the networks, we built two GLMMs with Gamma distribution. For each model, we used the  $C_d$  (N = 37) and the  $C_s$  (N = 34) values as our response variables. To investigate if the mean precipitation rate affected the RR of dominant and subordinate ant species, we built two GLMs, including the mean RR of dominant (N = 37) and subordinate (N = 34) ants as our response variables in each model. To investigate the overlapping in the plants used by dominant and subordinate species, we used a GLS model including the Jaccard values of each network as our response variable (N = 34). We built and analyzed the GLMM and GLS models described above using the “nlme” (Bates and Pinheiro 2000) and the “lme4” package (Bates et al. 2015) in R software, respectively (R Core Team 2022).

### 3. RESULTS

The networks in our dataset were sampled in sites where the precipitation rate ranged from 15.53 to 433.82 mm. Our networks presented an average S of  $27.83 \pm 18.5$  species (mean  $\pm$  SD), involving an average  $14.83 \pm 10.48$  ant species and  $13.41 \pm 10.00$  plant species with EFNs. In the subset of networks where plant or ant species were identified, there was a large asymmetry in the number of plant and ant genera interacting in the networks (plants: 191 genera, ants = 44 genera in total). Regarding the ants, the most common genera interacting with the plants with EFNs were *Camponotus* (Formicinae) and *Pseudomyrmex* (Pseudomyrmecinae) (17% and 12%, respectively). Plants genera, in turn, was more variable than ants in the interactions, with the most common genera being *Cylindropuntia* (Cactaceae) and *Acacia* (Fabaceae) (5% and 4% respectively).

#### 3.1 Effect of water availability on the structure of ant-plant networks

The networks were less rich as lower the mean precipitation rate (GLS;  $F_{1,51} = 14.813$ ,  $p < 0.001$ ), losing almost one species at every 100 millimeters decrease in the mean precipitation rate (Coefficient value = 0.08; Fig. 1a.). The average connectance of the networks was  $0.32 \pm 0.16$ . Conversely to the network species richness, the network connectance was not affected by the variation in the mean precipitation rate (mm) (GLMM;  $\chi^2 = 0.5811$ ,  $df = 1$ ,  $p = 0.445$ ; Fig. 1b). Similarly, the mean z NODF were not affected by variations in the mean precipitation rate ( $2.43 \pm 2.57$ ; GLS;  $F_{1,51} = 1.72$ ,  $p = 0.20$ ; Fig. 1c). Regarding the modularity, the networks are often less modular than expected by the null model, presenting  $-0.16 \pm 1.65$  z Q and, like zNODF, z Q was not affected by the mean precipitation rate (GLS;  $F_{1,49} = 0.379$ ,  $p = 0.540$ . Fig. 1d).

#### 3.2 The role of ant competitive hierarchy on network structure

Dominant ants realized, on average,  $3.12 \pm 2.02$  (mean  $\pm$  SD) interaction and subordinate ants realized  $2.47 \pm 1.79$  interactions with plants along the networks. Dominant ants represented, on average,  $71.62\% \pm 18.1\%$  of all the ant species in the networks, and this proportion was not affected by variation in the mean precipitation rate (GLMM;  $\chi^2 = 1.743$ ,  $df = 1$ ,  $p = 0.186$ ). However, the connectance of dominant ( $C_d$ ) and subordinate ( $C_s$ ) ant species varied along the precipitation gradient. Contrary to our expectations, both increased as the precipitation rate decreased ( $C_d$ :  $0.38 \pm 0.16$ ; LMM;  $\chi^2 = 16.209$ .  $df = 1$ ,  $p < 0.001$ ; Estimate = 1; Fig. 3a;  $C_s$ :  $0.32 \pm 0.22$ ; LMM;  $\chi^2 = 10.441$ ,  $df = 1$ ,  $p < 0.001$ ; Estimate = 0.99; Fig. 3b).

The mean RR for dominant ant species was  $0.72 \pm 0.16$ , and it was not influenced by the variation in the mean precipitation rate (GLM;  $\chi^2 = 0.093$ ,  $df = 1$ ,  $p = 0.173$ ; Fig. 2c). In the same way, the mean RR of subordinate ants ( $0.79 \pm 0.21$ ) was similar to the RR of dominant ants and it was also not affected by variation in the mean precipitation rate (GLM;  $\chi^2 = 0.149$ ,  $df = 1$ ,  $p = 0.149$ ; Fig. 2d). We also observed no variation in Jaccard index along the precipitation gradient ( $0.40 \pm 0.31$ ; GLS;  $F_{1,34} = 0.244$ ,  $p = 0.624$ ), indicating that the dominant-subordinate overlap in the plant usage is not influenced by water availability.

We observed a data gap at intermediary levels of precipitation rate (254.11 to 433.82 mm – see Fig.1). Because of this gap, data from the wettest networks in our dataset (representing one paper with 12 networks from the same location, all with mean precipitation rate of 433.82 mm) could behave as outliers, compromising the fit of our models. To evaluate this bias, we removed these networks from our dataset and performed again all analyses described in the *Statistical analysis* section. In all cases, there was no qualitative change in any results described above, indicating that our results were not biased by the asymmetric distribution of the networks along the precipitation gradient (see Support information).

## DISCUSSION

Our results showed that as the habitats become drier, the number of ant and plant species interacting with each other decreases, resulting in smaller networks. However, the mean precipitation rate did not affect any other metric describing the structure of the ant-plant networks. Also different from our expectations, the connectance of dominant and subordinate ants increased as the mean precipitation rate declined. Considering that connectance is a metric that intuitively accounts for the probability that any pair of species interact in the network (Landi et al. 2018), it indicates that the decline in water availability at the broader scales increases the generalization of ant-plant interactions. In turn, it increases the chances of both dominant and subordinate ant’s species interact with species of EFN-bearing plants. Interestingly, the mean precipitation rate did not affect the  $RR_d$ ,  $RR_s$ , or Jaccard similarity index. This lack of effect suggests that changes in the connectance of dominant and subordinate ant species are not due to changes in competitive ant behavior towards the plants but rather a consequence of processes related to the variation in species richness of the network along the precipitation gradient.

Several studies have shown that ant and EFN-plant communities’ richness declines along precipitation gradients (Dunn et al. 2009, Luo et al. 2022, Queiroz et al. 2022). For this reason, it is not surprising that the species richness of ant-plant networks declined with the decline of the mean precipitation rate. However, we observed no effect of the mean precipitation rate on any other descriptors of the ant-plant networks. This is an unexpected result since species richness of networks network is a crucial trait shaping the structure of ecological networks (Boccaletti et al. 2006, Minoarivelo and Hui 2016, Mariani et al. 2019) and, the influence of the mean precipitation rate on it would lead to modifications in other aspects of the network. However, we controlled, in our analysis, the effects of species richness on the metrics describing the network structure (connectance, nestedness, and modularity), either by using normalized metrics (as  $zQ$  and  $zNODF$ ) or the species richness as a weighted factor in our models. For this reason, the lack of precipitation effect on the network structure indicates that variation in water availability had no additional effect on the network structure other than those deriving from the variation in the species richness. This finding directly contrasts with the results from studies evaluating the role of precipitation in ant-plant networks at the local scale (Rico-gray et al. 1998, Rico-Gray et al. 2012, Câmara et al. 2018). At local scale, variation in climatic conditions across the sampled habitats tends to be relatively smaller, resulting in the observation of ant-plant interactions along a relatively narrower precipitation gradient than ours. Therefore, it is possible that the role of water availability in shaping the patterns of ant-plant interactions at the community level is relatively stronger at the local scale, with effects at a broader scale being likely an indirect consequence of its effects on the diversity of ant and plant assemblages across communities.

Similar to the metrics describing the structure of the ant-plant networks, the progressive generalization of ant-plant interactions along our macroecological water gradient is likely a consequence of the negative effects of water scarcity on the number of ant and plant species interacting with each other along the precipitation gradient. Two mechanisms can drive this increased generalization. First, it is possible that the decline of the

richness of plant species available in the drier environment increases the relative value of any plant partner to ants, regardless of its quality. In this scenario, interacting with the maximum of plant species available can be as or more advantageous to dominant ants than monopolizing the few high-quality plant species available in drier habitats. By reducing the monopolization strength of high-quality plants, dominant ants allow the visitation of the subordinate ones to more plant species explaining why both dominant and subordinate ants increased their connectance with the decline in the mean precipitation rate. Alternatively, it is possible that, although less rich, plant species with EFNs are more abundant in drier habitats. Since ants and plants are sessile organisms, their interaction depends on how close the ant nests and plants are (Dáttilo et al. 2013c), and this proximity should increase as more abundant ant and plant species populations are. In this case, any increase in the abundance of plants with EFNs in a community may increase the probability of all plant and ant species interacting, leading to a more generalized pattern of ant-plant interaction.

Our results related to the mean  $RR_d$ ,  $RR_s$ , and the Jaccard index supports the mechanism stating the increase in probability of interaction due to abundance and spatial distribution of ants and plants. The resource range is a normalization of how many links a given species makes, being not influenced by the network connectance or species richness (Poisot et al. 2012). Then, if the generalization in the patterns of ant attendance to EFN plants in drier environments would be driven by changes in the competitive behavior of ants, we could expect an increase in the mean RR for both dominant and subordinate ants. Additionally, if both dominant and subordinate ants use more plant species available as drier the habitat, we may expect an increase in dominant-subordinate overlap as the mean precipitation rate decreases. However, we observe no effect of the mean precipitation rate on the RR values or the degree of dominant-subordinate overlapping along the gradient. It indicates that ants and plants interact similarly along the water gradient, and the generalization in the patterns of ant-plant interaction may be just a consequence of an increased probability of ant-plant species interaction in poorer drier communities.

The preponderant role of the species richness on the generalization of ant-plant interactions may have two significant ecological implications for the dynamic of these interactions across habitats. First, theoretical models have shown that connectance tends to beget stability and persistence of mutualistic networks in space and time (e.g. Thébault and Fontaine 2010, Sauve et al. 2014). Therefore, more connected ant-plant networks may be more stable (but see Allesina and Tang 2012) and persistent than the ones from wetter habitats. In this case, by increasing the overall connectance and the connectance of subordinate and dominant ant species in the networks, the decline in water availability may indirectly increase ant-plant interaction persistence, including its persistence in the face of environmental disturbances. In the face of the current biodiversity crisis, it suggests that, at broader scale, the decline in water availability may be associated with a decline in the susceptibility of ant-plant interactions to environmental disturbance. Like the ones driven by human activities and climate change.

Second, although water availability had no effect on the competitive behavior of dominant ant species, it is likely that they are the main ones driving the direct and indirect effects among ant and plant species with EFNs and the network structure along water gradients. Due to their numerical and behavioral dominance, dominant ant species are commonly the most connected species in ant-plant networks worldwide, holding a higher number of interactions with the plant species available (Dáttilo et al. 2014b, Costa et al. 2016). Although both dominant and subordinate ants became more connected to the plants as the precipitation declined, water availability did not affect the degree of the network nestedness, suggesting the maintenance of the role of dominant ant species in regulating this mutualism, regardless of water availability. Finally, it is important to highlight that the significant role of community richness in shaping mutualistic networks along environmental macroecological gradients has already been reported in other studies using other mutualistic systems as a model, like pollination (e.g. Devoto et al. 2005, Lance et al. 2017). It suggests that the role of community diversity in shaping mutualistic networks at a broader scale is not restricted to ant-plant interactions, being more general than previously expected.

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## FIGURES LEGENDS:

**Figure 1** . Effect of the mean precipitation rate on the (a) species richness, (b) connectance, (c) nestedness, and (d) modularity of mutualistic networks representing interactions between ants and plants bearing extrafloral nectaries. The species richness of networks decreased with the decrease in the mean precipitation rate in the habitat (mm), but no other predictor of the network structure varied along the gradient. Each point represents one network, and the size of each point indicates the species richness ( $S$ ) of the respective network.

**Figure 2** . Effect of the mean precipitation rate ( $m$ ) on the connectance ( $C$ ) and resource range ( $RR$ ) of dominant (a and c, respectively), and subordinate ant species (b and d, respectively).  $C_d$  and  $C_s$  increased with the decrease in the habitat' mean precipitation rate (mm), while  $RR_d$  and  $RR_s$  did not vary along the water gradient. Each point represents one network, and the size is the richness ( $S$ ) of each network.

**Figure 3**. Relationship between the mean precipitation rate (mm) and the similarity in the patterns of dominant and subordinate ant species interaction with plant species with extrafloral nectaries (Jaccard

index of similarity). Precipitation rate did not affect the overlap between dominant and subordinate ant species visiting the plants with EFNs. Each point represents one network, and the size is the richness  $S$  of each network.

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