Influence of the leaf-cutting ant Atta sexdens Linnaeus (1758) on the taxonomic and functional diversity of woody plants in Atlantic Forest fragments

Jéssica Magon Garcia  
Universidade Estadual de Londrina

Lya Carolina Pereira  
Universidade Estadual de Londrina

Heraldo Luís Vasconcelos  
Universidade Federal de Uberlândia

José Marcelo Domingues Torezan  
torezan@uel.br

Universidade Estadual de Londrina

Larissa Cerqueira Dias Rodrigues  
Universidade Estadual de Londrina

Research Article

Keywords: foraging, functional traits, herbivory, leaf-cutting ants, Seasonal Semideciduous Forest

Posted Date: July 6th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3128482/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Plant Ecology on May 10th, 2024. See the published version at https://doi.org/10.1007/s11258-024-01425-6.
Abstract

Leaf-cutting ants can act as a biotic filter, selecting plant traits according to foraging preferences, affecting plant community species composition. We evaluated the relationship between the floristic and functional diversity of woody plants in fragments of Semideciduous Seasonal Atlantic Forest (SAF) with the presence or absence of *Atta sexdens* (Linnaeus, 1758) nests. We expected that, in places where *A. sexdens* nests are present, floristic diversity is lower and the plant community is dominated by species characterized by more anti-herbivore defenses and lower nutritional content. We sampled nine SAF fragments in the northern Paraná state, Brazil. The diversity of woody regenerants (> 1m in height, <2cm diameter) was estimated and ten functional traits were evaluated: gap dependence, deciduousness, leaf nitrogen and carbon content, leaf C/N ratio, leaf trichomes, leaf thickness, specific leaf area, presence of latex and condensed tannins. Contrary to expectations, there were no differences in floristic diversity between sites with or without *A. sexdens*, and functional diversity was also similar, reflecting the floristic similarity. We suggest that the herbivory pressure by *A. sexdens* on the woody regenerants of SAF fragments is not strong enough to cause significant mortality in individual species and, consequently, affect plant diversity and composition. This may in part be related to the fact that most *A. sexdens* nests are established in the edges of the fragments and that ants from these nests also forage in the agricultural matrix. Longer-term studies are still needed to fully elucidate the effects of *A. sexdens* on Atlantic Forest fragments.

Introduction

Leaf-cutting ants (LCA), from the genera *Atta* and *Acromyrmex*, are among the herbivores that reportedly influence whole neotropical ecosystems, especially the plant community (Holldobler and Wilson 2011). This might be especially true in forest edge environments, where they can collect almost 15% of the leaf annual production (Urbas et al. 2007). These ants are considered generalists (Wirth et al. 2003), but are selective during foraging, and may prefer plants according to their ecological and functional traits, such as: 1) pioneer plants with a lower concentration of chemical defenses (Farji-Brener 2001), 2) leaves with few physical defenses (Howard 1988), and 3) individuals under water stress or with higher nutritional content (Howard 1987; Mundim et al. 2009; Ribeiro-Neto et al. 2012), among others. However, as the selection of plants by LCA is a complex process that involves the plants available in the foraging area and environmental factors, such as temperature and humidity, there is still no consensus on the plant traits that best determine the foraging patterns of these herbivores.

Most previous studies defined functional traits as any morphological, physiological, or phenological characteristics of organisms which impact performance indirectly through their effects on growth, survival, and reproduction, and they are subclassified by attributes, the specific value or modality of a functional trait (Violle et al. 2007). They can be selected through environmental filters, which are non-random factors that restrict the range of attributes in a local community (Díaz et al. 2007). In this context, LCA can act as biotic filters, since when selecting plants based on functional traits, they can negatively influence seed germination (Silva et al. 2007), seedling survival (Costa et al. 2016; Garcia et al.
2020), and even the reproductive success (Câmara et al. 2019) of the most preferred species. Thus, herbivory by LCA can potentially have profound effects on the plant community, altering the species composition, structure, and dynamics of plant communities, by modifying the recruitment of plant species (Costa et al. 2016; Rao et al. 2001; Vasconcelos and Cherrett 1997). Nevertheless, several lines of evidence indicate that herbivory by LCA is context-dependent, varying for instance according to the abundance of pioneer plant individuals (Farji-Brener 2001), the presence of cultivated crops (Blanton and Ewel 1985), the local density of nests (Rao et al. 2001), as well as among species of LCA (Vasconcelos 1990).

Forest edges and secondary forests are known to have a predominance of pioneer species (Laurance et al. 2006). Consequently, some studies have shown that colonies in forest edges have smaller foraging areas (Silva et al. 2013), leading to an increase in the density of nests (Rao et al. 2001; Silva et al. 2009). This may also be related to the decrease in predator and/or parasitoid populations, negatively impacted by habitat fragmentation (Terborgh et al. 2001; Palmeirim et al. 2021). These factors positively affect LCAs, resulting in higher herbivory rates per colony (Urbas et al. 2007). Thus, in landscapes with high levels of human disturbance, such as Seasonal Atlantic Forests (SAF) (Carlucci et al. 2021), it is relevant to investigate whether LCA foraging activity acts as a possible ecological filter, affecting the dynamics of plant communities.

There are studies on the importance of plant functional traits for the selection of plant substrate by these ants in various habitats (Costa et al. 2016; Garcia et al. 2020; Howard 1987, 1988; Silva et al. 2013; Vasconcelos and Cherrett 1997); however, most of the research on this topic is experimental and does not assess if environmental conditions can interfere with the choice of plant material. It is known that in nature these herbivores modulate foraging to obtain the best cost-benefit ratio (Kost et al. 2005). In addition, there are no studies evaluating whether the preference for certain plant attributes affects the floristic and functional diversity of the studied ecosystems, that is, the top-down control of herbivory on the vegetation.

Therefore, the objective of the current study was to evaluate the relationship between the floristic and functional diversity of woody plants in fragments of SAF with the presence or absence of *Atta sexdens* (Linnaeus, 1758) nests. In places where *A. sexdens* nests are present, we expected 1) lower floristic diversity and 2) the plant community to be dominated by species characterized by greater investment in anti-herbivore defenses and lower nutritional content.

**Materials and Methods**

**Study sites**

Nine forest fragments (hereafter, sites) were selected (Fig. 1; Fig. S1; Table S2), in the northern region of Parana state, in southern Brazil, some of which belong to the Long-Term Ecological Research Program "Mata Atlântica do Norte do Paraná" (PELD-MANP). Forest fragments were selected to maximize size
variation (in hectares), and level of isolation from other fragments. The nine fragments are surrounded by an agricultural matrix (Garcia 2022).

The climate in the region is Cfa according to the Köppen classification, a subtropical climate. The mean annual temperature ranges from 21ºC to 23ºC. The average temperature in the coldest month is below 17ºC, and in the hottest month above 26ºC. Mean annual precipitation ranges from 1200 to 1800 mm (Nitsche et al. 2019). Rainfall is concentrated in the summer months, but there is no defined dry season (Nitsche et al. 2019). Frosts are infrequent.

The soils in the region are classified as eutrophic dark red oxisol and eutroferric nitosol (Bhering et al. 2007), of basaltic origin, and are highly fertile. The vegetation belongs to the Atlantic Forest domain (IBGE 2012) covering an inland region with less influence from the Atlantic Ocean; the Semideciduous Seasonal Forest is one of the most threatened Atlantic Forest types (Carlucci et al. 2021). The studied landscape is dominated by soybean and maize plantations. The forest cover ranges from 8 to 12% of the landscape, including remnants of mature and regenerating forests.

Figure 1

The occurrence of A. sexdens nests was determined by surveying belt transects that were 250 m long and 20 m wide (0.5 hectare per sampling unit) each. All transects were positioned perpendicular to the forest edge and were also used for placing the vegetation plots (see below). One transect was established for every 100 hectares of forest fragment area, with a minimum of one and a maximum of five transects per fragment. A minimum distance of 500 m was adopted between the transects established in the same forest fragment. The arrangement of transects perpendicular to the edges is part of another study (Garcia 2022) which evaluated the variation in nest density in relation to the distance from the edge. Ribeiro et al. (2009) and Tabarelli et al. (2010) suggest that the extent of edge effects on the Atlantic Forest vegetation is around 100 m. Therefore, we decided to extrapolate this value and use 250 m long transects, which were measured from the forest border with agriculture (0 m).

The transect method for ant nest sampling was adapted from that described by Jaffe and Vilela (1989) and Wirth et al. (2003). On each transect, nests were located visually or through evidence of foraging activity (e.g. foraging trails or ant activity) and georeferenced with GNSS receivers. The nests were classified as active or inactive; in the case of active nests, they were also classified as new or juvenile/adult. Colonies with a mound of earth up to 2 m² that did not have a defense caste (soldiers) were considered new; the others were considered juvenile/adult colonies (Autuori 1941). New nests are vulnerable to several biotic (predation by anteaters and armadillos, infection of the fungus garden by invasive microorganisms) and abiotic (heavy rainfall) factors that cause high colony mortality (Meyer et al. 2009; Vieira-Neto and Vasconcelos 2010). Therefore, only established (juvenile/adult) colonies were included in the analysis, as commonly adopted when studying Atta colonies (Meyer et al. 2009; Silva et al. 2009; Wirth et al. 2003).
Ten workers, preferably soldiers, were collected from each nest sampled in the transects for species-level identification. All collected material was placed in vials with 70% alcohol. All ant samples were identified to species level with the help of an ant taxonomist.

In a previous work (Garcia 2022) it was found that from the edge to 50 m there was a total of seven active nests, from 50 to 100 m three, and from 100 to 150 m two. No active nests were observed in any forest fragment in the distances from 150 to 250 m. Furthermore, two concurrent nests were never recorded in the same 50 m transect segment; the minimum distance between two nests exceeded 50 m. Thus, in the current study, we chose to consider the presence or absence of *A. sexdens* according to the vegetation sampling plots (hereafter, plots; see below). When the nest, foraging trails, or scouts of *A. sexdens* occurred inside the vegetation plot or within the 10 m field of view of the vegetation sampling plot, it was considered as the presence of *Attta*, and the reverse as the absence. Thus, to compare the floristic composition and functional traits between plots with or without *A. sexdens*, only data from the three plots per transect, at distances from 0 to 150 m were considered. As there was a discrepancy in the number of plots with the presence (21 plots) and absence of nests (59 plots), 21 plots without ant nests were randomly excluded, in order to balance the sample.

Regenerating woody plant sampling

We determined the species richness and abundance of woody plants within each transect, in six 5 x 5 m permanent plots, allocated at a distance of approximately 50 m from each other. In each plot, all woody individuals ≥ 1 m in height and diameter at breast height (1.30 m from the soil surface) ≤ 2 cm were counted and measured. This criterion of inclusion was adopted in order to avoid both seedlings (known to be a transitory, highly dynamic group), and adult individuals (that were probably established in the study sites before the establishment of the LCA nests).

Species-level identification of the sampled plant individuals was carried out in the field whenever possible, but samples were taken and confirmed or identified in the Herbarium of the State University of Londrina (UEL) – FUEL, where vouchers of all species collected were also deposited.

A total of 122 species belonging to 82 genera and 37 families, and two undetermined species were sampled (Garcia 2022). Those containing 11 or more individuals, comprising a list of 28 species belonging to 20 genera and 16 families of plants, representing 78.5% of all individuals sampled in the plots were selected for the functional trait analyses.

Functional traits

Ten leaves of at least 5 different individuals were collected from the 28 most abundant species, alternating between young and old leaves, and between leaves exposed to the sun or in the shade. The collection was made without excluding the petioles and giving priority to leaves without any type of damage (herbivory, fungal infection, water stress, etc.). Transport and storage of the leaves were carried out under refrigerated and humid conditions (Pérez-Harguindeguy et al. 2013).
For all species, ten physical, ecological, nutritional, and chemical functional traits commonly associated with herbivore attraction or inhibition were measured (Table S3). The factors considered, hereafter called functional traits were; specific leaf area (SLA), leaf thickness (LTh), presence of leaf trichomes (LTr), gap dependence (GAP), deciduousness (DEC), leaf nitrogen (N) and carbon (C) mass percentage, leaf C/N ratio, presence of latex (LX), and foliar concentration of condensed tannins (CT).

The LTh and SLA traits were measured based on Pérez-Harguindeguy et al. (2013) recommendations. Information about CT, LTr, LX, GAP, and DEC were retrieved from scientific publications and databases (see information in Table S4). N and C leaf content were measured at the EMBRAPA facilities (the Brazilian Agricultural Research Corporation) using a TOC device analyzer Elementar, model Vario TOC Cube (Fontana and Bianchi 2017). The concentration of condensed tannins (CT) was determined using the protein precipitation method of Hagerman and Butler (1978).

### Data analysis

To assess whether the occurrence of *A. sexdens* influenced the diversity of plants present in forest fragments (see the list of species in Table S7), linear mixed models (LMMs) were performed (Zuur et al. 2009) with data on the richness and abundance of woody regenerating plants and the presence or absence of *A. sexdens* (explanatory variable), using plots as sampling units; study site identity and plot number were included as random variables. The study site identity was included to consider the natural variability of environmental characteristics at the local scale, and the plots because of the spatial dependence (neighborhood) among them. For the LMMs, the *lmer* function of the ‘lmer4’ package (Bates et al. 2014) was used because the residuals presented normal distribution and homoscedasticity. The maximum likelihood method was used to select the best model.

Subsequently, to analyze the influence of the occurrence of *A. sexdens* on the floristic composition, a Non-Metric Multidimensional Scaling (NMDS; Legendre and Legendre 1998) was performed, using the plot-level abundances of woody regenerating plants, and labeling plots with or without *A. sexdens*. Bray-Curtis dissimilarity was used and abundance data transformation was performed using log (x + 1) (Zar 2010). To confirm the graphic results of the NMDS, a similarity analysis (ANOSIM) was used to compare the floristic composition between the plot groups with or without *A. sexdens* (Clarke and Warwick 2000). Complementarily, the *metaMDS* function of the ‘vegan’ package (Oksanen et al. 2018) and the Bray-Curtis coefficient were used in the rankings produced to adjust the variable presence or absence of *A. sexdens*, through the *envfit* function of the ‘vegan’ package (Oksanen et al. 2018). This procedure evaluates the relationship between the occurrence of *A. sexdens* and the composition of the plant communities studied.

In order to compare the plant functional traits between plots with or without *A. sexdens*, data from the 28 most abundant species were used. For the quantitative traits, the mean of the attributes was used, weighted by the abundance of the species for each plot, whereas for categorical attributes (LTr, LX, DEC, and GAP) the proportion of occurrence was used (number of individuals with the functional attribute per
plot/number of individuals occurring in the plot). For the attribute GAP, the proportion of gap-specialist individuals was used in the analysis, and for DEC, the proportions of deciduous and semi-deciduous individuals were used. We first built a Spearman correlation coefficient matrix (Zar 2010) with the ten functional traits to find those that were strongly correlated with each other ($r \geq 0.70$; Table S5; Petchey and Gaston 2002). Based on the results, the following functional traits were excluded from the analysis: C/N ratio; SLA and gap dependence.

The remaining traits were used to calculate the functional diversity index using the “FD” package (Laliberte and Legendre 2010). The analysis used data on the abundance of each species in the plots with and without *A. sexdens*, and data on the functional attributes of each species. Distance matrices were then produced using the `gowdis` function from the “FD” package. The values of functional richness (FRic), functional diversity (FDiv), and functional equity (FEve) were analyzed from the “dbFD” function between sites with and without ant nests. The Wilcoxon test (Zar 2010) was used to compare the results of functional diversity indices obtained in sites with and without ant nests.

Still using the “FD” package, the CWM (Community-level Weighted Means) was calculated (Lavorel et al. 2008), then the data were transformed into a matrix by the `vegdist` function of the “vegan” package. To evaluate possible differences between the functional attributes of sites with or without *A. sexdens*, an ANOSIM-type similarity analysis was performed using the “vegan” package. Presence or absence data, such as trichomes and deciduousness, were transformed before analysis using the `functcom` function. The CWM values of each trait were also compared between sites with and without *A. sexdens* using the Wilcoxon test.

All analyses were performed with the functional traits as a whole, individually, and also organized in groups: ecological (DEC), nutritional (C and N), and defense (LTh, LTr, LX and CT) traits. In the case of the ecological group, it was not possible to calculate the results of FRic, FDiv, and FEve of the “FD” package, because a single trait (DEC) does not generate a distance matrix. Results were considered significant when $\alpha < 0.05$, and all statistical analyses were performed in R 4.1.0 (R Core Team 2020).

**Results**

The presence of *A. sexdens* did not have a significant influence on the richness ($\chi^2 = 0.97$, DF = 1, p = 0.33) and abundance ($\chi^2 = 0.84$, DF = 1, p = 0.36) of woody regenerants.

The NMDS (stress = 0.23) combined with ANOSIM ($R = 0.02$; $p = 0.22$) enabled the comparison of the floristic composition between sites with or without *A. sexdens* and indicated no differences between groups (Fig. 2). In the same way, in the ordinations produced there was no influence of the presence of *A. sexdens* on the floristic compositions of woody regenerants ($R^2 = 0.03$, $p = 0.60$).

Figure 2
In general, functional traits were similar between plots with and without *A. sexdens* (see Table S6), reflecting the similarity of floristic diversity. Functional richness (FRic), functional diversity (FDiv), and functional equity (FEve) between the groups with and without *A. sexdens* also did not present significant differences (Table 1). The two sets of functional traits for the communities (defense and nutritional), again comparing the sites with and without *A. sexdens*, also showed no differences in FRic, DDiv, and FEve (Table 1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>With <em>A. sexdens</em></th>
<th>Without <em>A. sexdens</em></th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Standard deviation</td>
<td>Mean</td>
</tr>
<tr>
<td>FRic (all traits)</td>
<td>0.36</td>
<td>0.25</td>
<td>0.36</td>
</tr>
<tr>
<td>FDiv (all traits)</td>
<td>0.75</td>
<td>0.10</td>
<td>0.72</td>
</tr>
<tr>
<td>FEve (all traits)</td>
<td>0.69</td>
<td>0.18</td>
<td>0.72</td>
</tr>
<tr>
<td>FRic (defense)</td>
<td>0.35</td>
<td>0.27</td>
<td>0.30</td>
</tr>
<tr>
<td>FDiv (defense)</td>
<td>0.70</td>
<td>0.13</td>
<td>0.70</td>
</tr>
<tr>
<td>FEve (defense)</td>
<td>0.52</td>
<td>0.16</td>
<td>0.56</td>
</tr>
<tr>
<td>FRic (nutritional)</td>
<td>0.34</td>
<td>0.24</td>
<td>0.37</td>
</tr>
<tr>
<td>FDiv (nutritional)</td>
<td>0.79</td>
<td>0.09</td>
<td>0.80</td>
</tr>
<tr>
<td>FEve (nutritional)</td>
<td>0.66</td>
<td>0.13</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Similar results were found by CWM followed by ANOSIM, comparing the group of all functional traits together (R = 0.02, p = 0.19), and ecological (R = 0.03, p = 0.17), nutritional (R = 0.01, p = 0.33), and defense traits (R = 0.03, p = 0.17), showing no significant differences between sites with and without *A. sexdens*. The same occurred with the functional traits individually (Table 2), none showed differences between the evaluated sites.
### Table 2

Mean values and standard deviation of the Community-level Weighted Means (CWM) at sites with or without *Atta sexdens*, in nine fragments of Semideciduous Seasonal Atlantic Forest. Mean and standard deviation values are presented only for continuous variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>With $A. \text{sexdens}$</th>
<th>Without $A. \text{sexdens}$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Standard deviation</td>
<td>Mean</td>
</tr>
<tr>
<td>Deciduousness</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Leaf carbon</td>
<td>45.96</td>
<td>1.63</td>
<td>46.88</td>
</tr>
<tr>
<td>Leaf nitrogen</td>
<td>2.32</td>
<td>0.63</td>
<td>2.32</td>
</tr>
<tr>
<td>Leaf thickness</td>
<td>0.22</td>
<td>0.03</td>
<td>0.21</td>
</tr>
<tr>
<td>Leaf trichomes</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Leaf latex</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Condensed tannins</td>
<td>38.29</td>
<td>27.45</td>
<td>40.06</td>
</tr>
</tbody>
</table>

### DISCUSSION

This study is the first to evaluate the influence of leaf-cutting ants *A. sexdens* on the taxonomic and functional diversity of woody plants (> 1m and < 2 cm in DBH) in fragments of Semideciduous Seasonal Atlantic Forest. We hypothesized that sites with *A. sexdens* would have a comparatively higher frequency of species characterized by leaves with greater investment in anti-herbivore defenses and of lower nutritional value than sites without *A. sexdens*. Contrary to our expectations, plant communities with *A. sexdens* presented, on average, similar leaf concentrations of condensed tannins, carbon, nitrogen, and trichomes, as well as a similar proportion of latex-producing individuals, to communities without *A. sexdens*. In addition, we found no significant differences in the overall abundance or in the taxonomic and functional diversity of woody plants.

One potential caveat of our study is that we do not have information about the ages of the nests found, and therefore for how long the plants from the sites we sampled were subject to LCA herbivory. Young nests (< 3 years of age; Forti et al. 2011) are likely to have a lesser impact on regenerating woody plant individuals than adult nests (> 3 and up to 8 years in age, the mean longevity of *Atta* queens in the field – Boer et al. 2009), as young nests harvest a comparatively much smaller amount of vegetation than adult nests (Costa et al. 2008). Furthermore, although the plants we sampled were relatively small, they were not seedlings, and thus much more likely to tolerate a single or a few repeated events of herbivory by LCA (Mundim et al. 2012).
Another factor to consider is that most of the nests we found were less than 50 m from the edge of the fragments and thus could have had access to plant resources in the agricultural matrix. In fact, during the fieldwork, foraging trails were frequently observed leaving the forest to harvest leaves, flowers, fruits, and seeds in the agricultural matrix. This can be considered an example of complementary resource use, which has been defined as situations in which the critical resources required for an organism to complete its life cycle are not contained in a single habitat type, but in two or more (Dunning et al. 1992).

Poutasse (2010) and Blanton and Ewel (1985) also observed complementary foraging areas for *Atta* species, which suggests that this may be a common behavior for the genus, made possible by its high capacity to generate networks of highly flexible and long trails, spatially adjusted according to the optimal foraging (Silva et al. 2013). For LCA, cultivated plants may represent an advantageous substrate, as they have fewer structural and chemical defenses and higher nutritional value when compared to forest species (Berish 1986; Massad et al. 2011). However, it is important to highlight two negative aspects of plants cultivated in the agricultural matrix as a substrate for LCA, namely, that they are available only on a seasonal basis and the contamination by pesticides.

In conclusion we found no significant effects of *A. sexdens* on the floristic diversity or functional diversity of woody plants, at least not on plants of the size class we evaluated. We attribute this lack of effect, at least in part, to the generalist and opportunistic foraging behavior of *A. sexdens*, particularly among those nests located near the border of the fragments, which foraged not only on forest plants, but also extensively on plants from the agricultural matrix. Future studies that seek to determine the potential impacts of LCA herbivory on native plant communities should consider the continuous and long-term monitoring of both the plant community and the ant colonies.

**Declarations**

**Acknowledgements**: Authors are grateful to Dr. Marco Nogueira, Dr. Nilton Syogo Arakawa, Ms. Ricardo Luis Nascimento de Matos and Dr. Halley Caixeta de Oliveira who allowed the authors to use their laboratories and helped with foliar chemical analyses. The authors also thank Alba Cavalheiro, Odair do Carmo Pavão and Norival Soares do Cabo for their help in the field and in laboratory activities, and ant taxonomist Jacques Hubert Charles Delabie for identifying ant samples.

**Funding**: The Long-Term Ecological Research Site Mata Atlântica do Norte do Paraná (PELD-MANP) is supported by CNPq (grants 441540/2016-3 and 441510/2020-5) and Fundação Araucária (41872.434.40722.27092013). CNPq provided research grants for the JMDT (309244/2015-3). CAPES provided PhD scholarship to JMG (“Financial Code 001”) and a Scientific Initiation scholarship to LCDR (88887.505837/2020-00).

**Competing interests**: The authors declare no competing interests.

**Author contributions**: All authors agree to submission of the manuscript. JMG, JMDT and LCDR conceived the research and wrote the text; JMG and LCDR did the field and laboratory work; JMG,
LCDR and LCSMP performed statistical analysis; JMG, JMDT, HLV, LCDR and LCSMP edited the manuscript.

References


18. Hagerman AE, Butler LG (1978) Protein precipitation method for the quantitative determination of tannins. Journal of Agricultural and Food Chemistry 26: 809-812. https://doi.org/10.1021/jf60218a027


Figures
Figure 1

Location of the nine of the Semideciduous Seasonal Atlantic Forest fragments in the north of Paraná state, Brazil. The nine forest fragments selected are in red, other forest fragments in light green; light yellow to white colors are agriculture, pastures, urban sites, and other open areas.
Figure 2

Non-Metric Multidimensional Scaling (nMDS) plot comparing the floristic composition of plots with or without *Atta sexdens* in nine Semideciduous Seasonal Atlantic Forest fragments.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryInformation.doc