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Recover and They’ll Come: Flower Visiting Bees Benefit from the Continuous of Micro-Environments Set by Regenerating Forest Fragments

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Abstract
Forest habitats are important sources of food and nesting resources for pollinators, primarily in urban areas and landscapes with intense agricultural activity. The forest fragmentation and environmental changes occurring in these green refuges are known to impose survival challenges to pollinating bees, leading to species loss. However, it is not well known how the species of bees that visit flowers are distributed in forest micro-environments. To fill this gap, we sampled flower visiting bees in a continuous forest matrix with micro-environments of two forest types (mature and regenerating forest). We examined how the local environmental changes and climatic conditions affect the composition and uniformity of bee communities in the different micro-environments. Our results indicated that both abundance and richness were similar between forest types studied here, however climatic conditions and plant flowering patterns affect the composition of bees. Thus, our results demonstrated that the continuous micro-environments can favor floral visits and the reintegration of bee communities, and still, that this strategy can be used to minimize the impacts of environmental changes at local scales.

Introduction
Changes in habitat caused by human activities (deforestation, fragmentation and loss of forests) impose environmental challenges for the survival of pollinator species and are the main drivers of the decline of pollinator populations (Ollerton, 2017; Wilson et al., 2018; Almeida et al., 2020). This important topic reminds us of the need to conserve natural habitats, since the pollination services performed by insects are not only essential to maintain biodiversity, but also for food production (Zermeño-Hernández et al., 2016; Stein et al., 2017), being considered of great economic value, since these environmental services represent US $577.00 billion annually and 35% of the food crops in the worldwide depend of the pollinating insects (Klein et al., 2007; Potts et al., 2016; Boscolo et al., 2017).

Bees are the most important pollinating insects in the world, since they visit a large number of flowers daily in natural, agricultural and urban landscapes (Hausmann et al., 2016; Stein et al., 2017; Winfree et al., 2018), being exclusive pollinators of many botanical groups (Scaven & Rafferty, 2013; Venjakob et al., 2016), and indispensable in increasing the production of agricultural crops (Stein et al., 2017; Blettler et al., 2018; Winfree et al., 2018). However, these insects receive little recognition for their services when the issue is intensive land use, which are among the main causes of habitat loss and the consequent decline in the richness and abundance of bees (Stangler et al., 2016; Wilson et al., 2018; Almeida et al., 2020). The effects of environmental changes on bee species at different scales and geographic regions seem to be consistent (Ferreira et al., 2015; Alaux et al., 2017; Wilson et al., 2018). However, the effects of environmental
changes on local landscapes cannot be excluded. In this regard, recent studies have shown that the landscapes that surround the forest matrix can influence the composition and distribution of bees (Marques et al., 2018; Rollin et al., 2019; Sobreiro et al., 2019), due to the permeability of these pollinating insects can be affected by the functional connectivity and heterogeneity of the landscape (Boscolo et al., 2017). Therefore, understanding the potential effects of local environmental changes on the diversity of bees can help to promote conservation strategies and improve the survival chances of these insects.

In South America, forest refuges cover an area of 842 million hectares, which represents 27% of the world’s forest cover (Fao, 2016), among them, the Atlantic Forest is one of the most threatened hotspots on the planet, with only 11.73% of its original cover, distributed in small (<50 ha) and isolated forest fragments (Ribeiro et al., 2009). These forest fragments of the biome are formed by different micro-habitats that are rarely considered in studies of bee diversity and flow, although recent results indicate the capacity of these pollinators to inhabit increasingly fragmented and anthropized forests (Neame et al., 2012; Botsch et al., 2017), therefore, these green patches are important sources of food and nesting resources for bees, and often serve as the only source in urban areas (Hausmann et al., 2016; Sobreiro et al., 2019; Odanaka & Rehan, 2020).

Studies show that the landscapes surrounding forests and climatic conditions influence the foraging flow of bees. According to Giannini et al. (2020), regional climatic changes can drastically reduce the richness of bees in forests, and this effect has a greater impact on species with medium or restricted geographical distribution, because they depend on more conserved forest habitats to survive. In addition, climatic variations within a landscape mosaic affect the dispersal of bees differently, the few authors who considered this factor in their research showed that warmer landscapes, with low humidity and water deficit seem to be especially related to mortality and lower abundance of species (Switanek et al., 2017; Hannah et al., 2017; Vanderplanck et al., 2019). In another study (Hamblin et al., 2018), the authors investigated the importance of urban green refuges and the effects of the heat islands (cities) on the bee community, they found that more than 1/3 of the abundance of bees decreased when the temperature increased, and that floral enrichment in warmer landscapes had little effect on the diversity of small bees. On the other hand, the successional diversification of the landscape can serve as stepping stones between fragments of mature forests, being used as a foraging reservoir for different species of bees (Sobreiro et al., 2019; Odanaka & Rehan, 2020). Thus, although these studies have investigated the flow of bees at different stages of the forest and geographical scales (β and γ), they show that environmental and climatic conditions are important factors in the distribution of bee species and community composition within forest fragments.

With this, the gaps that still need to be understood are: Can the forest regeneration close to remnants favor the flow of bees between different habitats? and Do the local microclimate conditions have a minor impact on the floral visits of bees in a continuous forest?

Despite their apparent importance in the distribution of bees, the role of different micro-environments in the floral visits of bees is not yet well understood, although previous studies have shown that these micro-habitats are able to offer different foraging resources and serve as a reservoir for the diversity of bees (Boscolo et al., 2017; Odanaka & Rehan, 2020). Observers of a recent research have shown that a continuous matrix of regenerating micro-environments can favor the reintegration of bee communities that are attracted by chemical traps (Sobreiro et al., 2019). However, we are not aware of studies that have compared bee visits to flowers in multiple micro-environments within a continuous of mature and regenerating forest.

We hypothesized that the abundance and richness of bees in the flowers should increase with the diversity of micro-environments, because the variety of resources is higher in the mature forest, increasing foraging efficiency. In addition, flower visiting bees can benefit from the continuous forest matrix, because the impacts of the anthropic landscapes (monocultures and cities) surrounding forests can be lower. And also, the composition and uniformity of bee communities are affected by climatic conditions locally. Here, micro-environment is defined as heterogeneity of vegetation in each sampling site and continuous matrix as a set of micro-environments that form the mature and regenerating forest, according to environmental standards (Ibama, 1991; Brasil, 1994). Finally, our goal was to investigate which of these factors (diversity of micro-environments, continuous matrix and climatic conditions) can better explain the variation of the composition in the assemblage of bees and abundance of species in fragmented forests of the Atlantic Forest, in order to help to understand the responses of bees to local environmental changes.

Materials and Methods

Study area

The study was performed in twelve sampling sites selected in a continuous forest matrix with two types of forest: mature and regenerating forest. The sites (n = 12) were located in the Bela Vista Biological Refuge (RBV), municipality of Foz do Iguaçu (617.40 km²), western Paraná state, Brazil. The RBV unit has a conservation reserve that covers 1920 ha of Semi-deciduous Seasonal Forest, belonging to the Atlantic Forest biome (Ibama, 1991), under the protection of the Binational ITAIPU. The study region is surrounded by the Itaipu reservoir (17.20 km²), human occupation (<206 km²) and alternate crops of soybeans and corn (2.95 km²). This area includes several forest fragments with different stages of vegetation.
From these fragments, we selected two forests that covered vegetation with natural regeneration, reforested and forest remnant. In the fragments, we selected six sites located in the mature forest and another six in the regenerating forest, sampling the entire continuous forest matrix (Fig 1). The georeferencing information was analyzed and edited using QGIS version 2.18 (2016) software.

**Micro-environments characteristics and forest composition**

We used data sets from satellite imagery (Google Earth maps), Global Forest Change and OpenLayers in QGIS (http://www.openlayers.org) to select sampling sites in the two forest fragments and differentiate the vegetation levels from micro-environments in the forest matrix (Table 1; see Fig 1). The characteristics of the forest fragments and sites were made before starting the sampling. The size of forests and sites was measured using the satellite programs (Google Earth maps and QGIS). The size of each site was established in an interval between 0.29 and 0.51 hectares and was arranged in places with different stages of vegetation. The classification of vegetation is according to Brasil (1994), which divides the forests into primary and secondary (see Table 1).

The mature forest is a habitat with great biological diversity and minimal anthropic effects, where the composition and structure of the forest basically remains the same for centuries. Every plant community has tall trees (≥ 40 m), woody and flowering in the canopy. This forest structure is made up of seed banks, where old trees die, and young seedlings grow up to replace them (Brasil, 1994). The mature forest vegetation covers about 412 ha and it is composed of native forest (forest remnant and undisturbed by humans) and part reforested with approximately 40 years (Itaipu, 1978), whose native vegetation was lost due to agricultural practices. This type of vegetation is also known as primary forest or “climax”, it is a forest formation that has reached the highest stage of vegetative development, housing a great diversity of species, trees with variable height and diameter and formation of seed banks (Brasil, 1994).

The regenerating forest is a fragment that lost all forest cover and is recovering naturally (Brasil, 1994). This type of vegetation is usually young (<15 years), characterized by different successional stages of vegetation composition (initial, intermediate and advanced) (Itaipu, 1978; Brasil, 1994), with a high incidence of pioneer plants, generally exotic colonization species, forming small woodlands (Brasil, 1994).
In this study, the regenerating forest covers about 69 ha and is composed of secondary vegetation with three stages of natural regeneration, it has a high incidence of sunlight and young vegetation, many shrubs and small trees (Itaipu, 1978). The original forest was consumed, due to successive deforestation and agricultural practices and is recovering naturally through the process of natural regeneration.

**Sampling**

In the experimental design (Fig 2), twelve different sampling sites (n = 12) were selected, distributed in two forest fragments: mature and regenerating forest. In each forest, we delimited a 1500 m straight transect, covering six sampling sites with different stages of vegetation. The transect was walked sinuously in each forest, covering an area of 20 m by ± 250 m at each sampling site (see Fig 1). The sites were marked with spray paint and marking tape to standardize the sampling area throughout the year. Thus, we selected six sites in the mature forest and another six sites in the regenerating forest. The transect of each forest was traversed recursively throughout the sampling day. These two forests include sites at different stages of a continuous forest matrix (Table 1-characteristics of the micro-environments).

The bees were collected with an entomological net for 12 hours, from 6:00 am to 6 pm. In each forest studied here, the collections were made from October 2013 to September 2014, at intervals of 25-30 days, totaling 12 months and 144 hours of sampling (12 hours x 12 months). The two transects were traversed by different teams and the flowering plants were observed for 15 minutes, and all the bees that visited the plants were collected with an entomological net. The collected specimens were sacrificed in lethal chambers with ethyl acetate, separated in individualized paper envelopes for later assembly. In the laboratory, the bees were mounted on entomological pins, deposited in entomological boxes for their identified using dichotomous taxonomic keys and with the help of a specialist (R. B. Gonçalves, Federal University of Paraná). Subsequently, the bees were deposited in the Entomological Collection of the Beekeeping Laboratory of the Federal University of Grande Dourados (UFGD).

Samples of the plants visited by the bees were also taken to identify the species and estimate the floristic potential of the visits of the bees at each site. We limit the height of the flowering plants visited by the bees to 4.5 m to standardize the sampling between different micro-environments, since in the site with the initial stage of regeneration, the height...
of the flowering plants is a maximum of 2 m, while that in the mature forest sites, the height of flowering trees is greater than 40 m. In the laboratory, the plants were individualized into exsiccates, identified by specialists (Z. V. Pereira and A. Sciamarelli, Federal University of Grande Dourados), classified as: native, naturalized or exotic (see Table 2), according to REFLORA/CNPq-Virtual Herbarium Program and later deposited in the Herbarium of the Museum of Biodiversity (MuBio, FCBA-UFGD).

Samples were performed on the same day in both forest fragments by different groups. During the sampling, measurements of the climatic conditions were also performed, for each hour of the day, in both forest fragments. The parameters used were: air temperature (°C – Digital thermohygrometer) which is a measure of heat trapped in the air, measured here in degrees Celsius; Wind speed (m/s - Digital anemometer) that measures the displacement speed of the air flow in meters (m) per second (s) (Baede et al., 2001; Hunt et al., 2018). Solar intensity (lx – Digital Luxmeter) measures the solar radiation per square meter (lx/m²). The relative humidity (UR – Thermohygrometer) of the air was measured as the ratio between the amount of water in the air (absolute humidity) and the maximum amount that could be at the same temperature (saturation point) given in units of gram per kilogram (g/kg) (Baede et al., 2001; Marcilio-Silva et al., 2017). Finally, precipitation (mm – rain gauge) is a measure in millimeters (mm), resulting from the sum of the amount of water in the same place and period of time (Baede et al., 2001).

Data analysis

The diversity of bees at the twelve sites was calculated using the Shannon-Wiener index (H’). To compare the dominance of species at all sampled sites (n = 12), we used Berger-Parker (d) and Simpson (D-1) index (Melo, 2008). And the uniformity of the bee assemblage composition at the sites was analyzed using Evenness (J’), with a range between 0.1 and 1, where 1 represents the maximum diversity (Magurran, 2004).

The circular graphics of richness and abundance of bees were used to compare the 12-month samplings in both forests. Circular statistics are commonly applied to analyze the periodic behavior of sampled data arranged around a circle (Gu, 2014). Each column in the circle represents one month of bee sampling, with 12 hours of bee collections each month, adding up to 144 hours of sampling (12 hours x 12 months) at the six sites in each forest (mature and regenerating forest). The Principal Component Analysis (PCA) was used to analyze the species composition in the different micro-environments. The PCA is a statistical method of multivariate analysis suitable for identifying the correlation between a dependent variable and independent parameters (Abdi & Williams, 2010). In the PCA, the first axis represents the species composition and the second axis, the sites (n = 12). The circular graphics were made with the vegan package (Oksanen et al., 2018), and the PCA graphics with the ggplot2 package (Wickham et al., 2020), both built with R software version 3.4.1 (R Development Core Team, 2017).

An Analysis of Variance (ANOVA) was performed to determine significant differences in the following parameters: (i) richness of bees per month; (ii) richness of bees among forests; (iii) richness of bees between sites; (iv) species of flowering plants per month; (v) species of flowering plants among forests; (vi) species of flowering plants between sites. ANOVA is a test that is used to determine if the means of two or more groups are significantly different (Magurran, 2004),
it was also used here to determine if the abundance of bees was significantly different by month, forest and sites. This test was performed using R software.

In order to test the correspondence between the composition of the assemblages of bees and the heterogeneity of the studied sites, we performed an analysis of hierarchical clustering for the similarity matrix using the Jaccard index. The similarity was considered representative when the similarity average was greater than 0.5. In addition, we made the Mantel test to verify the influence of the spatial distances between the sampled sites and the composition of the assemblages of bees. This test analyzes the correlation between the two matrices (spatial distance and composition of assemblages). To perform the analysis of hierarchical clustering and the Jaccard and Mantel tests, we use the vegan package in the R software.

Finally, based on our hypotheses, we investigated the responses of the bee assemblage to climatic and temporal conditions in the sites of the two forests (mature and regenerating forest) using Non-Metric Multidimensional Scaling (NMDS). In the NMDS, the first axis represents the assemblage of bees in the sites and the second axis represents the climatic and temporal conditions, being that the climatic conditions are represented by the relative humidity of the air (UR), precipitation (mm), solar intensity (lx), air temperature (°C), wind speed (m/s), and temporal conditions are represented by hours and months. We used the Jaccard similarity index to measure the relationships between the assemblage of bees and each variable related to climatic and temporal conditions. The similarity was considered representative when the p-value was equal or less than 0.05. To perform the NMDS, we also used the vegan package in the R software.

Results

We collected 203 flower visiting bees, belonging to five families, 26 genera and 40 species. Of these, 141 specimens were sampled in the mature forest and 62 specimens in the regenerating forest. The abundance differed significantly by month (df = 11, F = 2.6722, p = 0.0039), although it did not change between types of forest (df = 1, F = 3.8553, p = 0.06235). The same 17 species of bees were sampled in the two forest fragments, with 18 species found exclusively in the mature forest and 5 species were exclusive in the regenerating forest. The highest abundance of bees in the mature forest was at the MF6 site (n = 35 individuals) and in the regenerating forest was at the RF5 site (n = 14 individuals), while the lowest abundance at the sites was MF5 (n = 15 individuals) and RF1 (n = 7 individuals) in the mature and regenerating forest, respectively. Furthermore, ANOVA indicated that the abundance did not differ significantly between sites (df = 11, F = 1.2997, p = 0.2424) (Appendix 1).

The circular distribution pattern of bees per month varied throughout the year in the mature forest, representing the highest richness and abundance for all months, except December 2013 and June 2014, where the richness and abundance of bees were higher in the regenerating forest, representing 11.29% (n = 7 individuals, 5 spp.) and 1.61% (n = 1 individual, 1 spp.), respectively. The month with the highest abundance was January 2014 in both forests, showing 14.28% (n = 29 individuals) in the mature forest and 12.31% (n = 25 individuals) in the regenerating forest. *Apis mellifera* Linnaeus was the only species with a total occurrence of 11 months in both forests, registering 27.09% (n = 55 individuals) of the total number of bees collected. Followed by *Augochlora* sp.1 with occurrence during 6 months representing 4.92% (n = 10 individuals) in both forests and *Exomalopsis auropilosa* Spinola with occurrence of 3 months representing 5.41% (n = 11 individuals) in the regenerating forest. The richness of species was statistically different per month (df = 11, F = 12.384, p = 1.055e-12), but there were no changes between forest types (df = 1, F = 3.6635, p = 0.06872) and between sites (df = 11, F = 1.3627, p = 0.2095) (Fig 3).

All individuals are wild bees, except *Apis mellifera*, which represented 27.09% (n = 55 individuals) of the total number of bees sampled. *Apis mellifera* was the most abundant species in both forests, representing 67.27% (n = 37 individuals) in the mature forest and 32.73 (n = 18 individuals) in the regenerating forest. Among the wild bees, *Exomalopsis auropilosa* Spinola was the most abundant species in the mature forest with 6.40% (n = 13 individuals) and *Bombus morio* (Swederus) in the regenerating forest with 5.91% (n = 12 individuals). The lowest abundance of bees was sampled for 13 species with 1 specimen each, which represented 6.40% (n = 13 individuals) (see Appendix 1). Interestingly, the honey bee (*A. mellifera*) was not collected for six months (November, December, May, June, July and September) in the regenerating forest.

The Shannon-Wiener index (H′) indicated that the diversity was higher in the mature forest (H′ = 2.20) than in the regenerating forest (H′ = 1.74). For the sites, the highest diversity was at the MF2 (H′ = 2.295) and the lowest at the RF4 (H′ = 0.693). For the Berger-Parker index (d), the dominance was higher in the regenerating forest (d = 0.39) than in the mature forest (d = 0.20). For the sampled sites, the Berger-Parker at the MF4 and MF5 showed the highest dominance (d = 0.50), while at the RF2 it presented the lowest dominance (d = 0.16). The Simpson index indicated that the dominance was higher in the regenerating forest (D = 0.75) than in the mature forest (D = 0.87). Among the sites, the MF2 site obtained the maximum dominance (D = 0.89) and the RF4 site reached the lowest dominance value (D = 0.5). Finally, the evenness was higher in the regenerating forest (J = 0.84) than in the mature forest (J = 0.81). Among the sites, the highest evenness was recorded at the RF4 site (J ′ = 1), while the lowest evenness was found at the RF5 site (J ′ = 0.79) (Appendix 1- Diversity indeces).

We identified 21 species of flowering plants visited by bees, belonging to 11 families and 20 genera (Table 2).
Of these, 14 plant species were collected in the mature forest and 17 species in the regenerating forest. Only one individual was sampled for the genus *Ludwigia* L. and it was not identified to the species level. The plant species (Table 2, Classification) collected were classified according to the REFLORA/CNPq-Virtual Herbarium program, being 15 native plants, 4 naturalized plants and 2 exotic plants introduced in Brazil. The same 10 plant species were sampled in the two forest fragments, with 7 plant species found exclusively in the mature forest and 4 plant species were exclusive in the regenerating forest.

*Centratherum punctatum* Cass. with 36.94% (75 bee specimens, 24 bee species), followed by *Hedychium coronarium* J. Koenig with 13.30% (27 bee specimens, 12 bee species) and *Leonurus sibiricus* L. with 7.88% (13 bee specimens, 7 bee species) were the plant species that attracted the higher abundance of bees. The least visited plant species attracted the highest richness of bees, corresponding to 55.65% of the total (Table 2, Bees'). In addition, ANOVA indicated that the plant species visited by bees varied significantly from one month to the next (df = 11, F = 8.4381, p = 0.0006872), although did not change between forest types (df = 1, F = 0.9197, p = 0.3584505) and among sites (df = 11, F = 0.6577, p = 0.773). And yet, the Jaccard similarity index revealed that the assemblage of bees is influenced by the richness of plants (df = 20, F = 1.31, p = 0.02).

**Fig 3.** Circular distribution of the abundance and richness of bees, represented by the total number of bees sampled per month in the two forest fragments (mature and regenerating forest) of the Atlantic Forest, Paraná, southern Brazil. From October 2013 to September 2014.
Principal Component Analysis (PCA) revealed differences in the bee assemblage ordination among sampling sites (Fig 4). The MF4 and MF6 sites are grouped by the similar abundance of the same species, whereas the diversity and evenness pattern of species grouped the RF1, RF2, RF5 and MF5 sites. We observed that the regenerating forest sites (RF1, RF2, RF3, RF4 and RF5) are grouped on the upper left side of the ordination and the mature forest sites (MF1, MF2, MF3, MF4 and MF6) are grouped on the other sides of the ordination, except MF5 that is grouped with the regenerating forest sites. Furthermore, Exomalopsis auropilosa is apparently closer to the mature forest on the right-central side of the ordination, as well as Augochlorella ephyra (Schrottky, 1910) is the species positioned closer to the regenerating forest on the upper left side of the ordination. Apis mellifera is the only species that was sampled at all sites and it is grouped among geographically closest sites (MF2, MF3 and MF4) of the mature forest. Whereas Coelioxys sp1, Thectochlora basiata (Strand, 1910), Pseudaugochlora graminia (Fabricius, 1804) and Schwarziana quadripunctata (Lepeletier, 1836) are isolated on the upper right of the ordination closer to MF2 site of the mature forest with high degree of micro-environment conservation (see Table 1).

The role of climatic and temporal conditions

The dendrogram showed that the micro-environments of the mature and regenerating forests are similar in structure, with the exception of some sites of the regenerating forest (RF1, RF4 and RF6) (Fig 5). Despite this, the Jaccard index indicated a low similarity of the assemblage of bees between forests (mean of similarity = 0.46) and between sites (mean of similarity = 0.22). The Mantel index revealed that the spatial distances (rM = 0.3883, significance = 0.009) and the environmental heterogeneity (rM = 0.2582, significance = 0.039) between the sites present a low correlation, but with significant influence on the composition of the assemblages.

Table 2. Plant species: native, naturalized and exotic sampled in the mature and regenerating forest fragment of the Atlantic Forest, Paraná, southern Brazil.

| Plant species               | Family            | Species                              | In | Forest | Classification | Bees |
|-----------------------------|-------------------|--------------------------------------|----|--------|----------------|------|
| Lista                       | Family            | Species                              |    |        |                |      |
| 1  | Asteraceae        | Bidens pilosa L. | 1  | M      | Naturalized  | 1    |
| 2  | Centratherum punctatum Cass. | 49  | M, R | Native        | 75   |
| 3  | Elephantopus mollis Kuntz | 3   | M    | Native       | 4    |
| 4  | Emilia sonchifolia (L.) DC. ex Wight | 6   | M, R | Native       | 7    |
| 5  | Porophyllum ruderale (Jacq.) Cass. | 2   | R    | Native       | 4    |
| 6  | Tridax procumbens L. | 1   | R    | Native       | 1    |
| 7  | Vernonia polyanthes (Spreng.) Less. | 10  | M, R | Native       | 15   |
| 8  | Vernonia scorpioides (Lam.) Pers. | 1   | R    | Native       | 1    |
| 9  | Boraginaceae      | Heliotropium nicotianaefolium A. DC. | 2   | M      | Native       | 3    |
| 10 | Commelinaceae     | Commelina nudiflora L. | 9   | M, R | Native       | 11   |
| 11 | Euphorbiaceae     | Croton glandulosus L. | 4   | M, R | Native       | 4    |
| 12 | Lamiaceae         | Leonotis nepetifolia (L.) R. Br. | 4   | M      | Naturalized  | 8    |
| 13 | Leonurus sibiricus L. | 13  | M, R | Exotic     | 16   |
| 14 | Nectandra megapotamica (Spreng.) Mez | 2   | M, R | Native       | 2    |
| 15 | Onagraceae        | Ludwigia sp1 L. | 2   | M, R | Native       | 4    |
| 16 | Poaceae           | Brachiaria brizantha (Hochst. ex A. Rich.) Stapf | 3   | R      | Exotic       | 4    |
| 17 | Rhamnaceae        | Ziziphus joazeiro Mart. | 1   | R      | Native       | 1    |
| 18 | Solanaceae        | Solanum sisymbriifolium Lam. | 5   | M, R | Native       | 7    |
| 19 | Verbenaceae       | Lantana trifolia L. | 4   | R    | Native       | 6    |
| 20 | Zingiberaceae     | Hedychium coronarium J. Koenig | 10  | M, R | Naturalized  | 27   |

Total of individuals M:90, R:43 133 203 115
e= Some species were sampled in both forests. M = Mature forest. R = Regenerating forest. In = number of individuals.
The Non-metric multidimensional scaling (NMDS) revealed a cluster of the assemblage of bees for all the sites studied here, with the exception of the RF4 site (Fig 6). When we tested the Jaccard similarity index, we found that the composition of the assemblages of bees was significantly influenced by the climatic conditions: air temperature (df = 20, $F = 1.666$, $p = 0.001$), air relative humidity (df = 20, $F = 1.6084$, $p = 0.001$), wind speed (df = 18, $F = 1.6556$, $p = 0.001$), precipitation (df = 11, $F = 1.7867$, $p = 0.001$), and solar intensity (df = 19, $F = 1.4946$, $p = 0.001$). Furthermore, the temporal conditions influenced the assemblage of bees in the forests: months (df = 11, $F = 1.4376$, $p = 0.001$) and hours (df = 136, $F = 1.1417$, $p = 0.007$). In both forests (mature and regenerating forest), the lowest air temperature and the highest air relative humidity were recorded at sunrise (between 6 am to 7 am), while wind speed and precipitation varied throughout the day.

**Discussion**

Our results showed that the richness and abundance of bees in the flowers did not differ between the mature and regenerating forests, however, the climatic conditions of the micro-environments seem to influence the assemblage of bees in the continuous forest matrix. In this regard, Boscolo et al. (2017) investigated the responses of the flow of bees to the heterogeneity of the landscape and the functional connectivity, they found that the reduced functional connectivity affects the
communities of bees at the landscape level. Although studies that investigate flower visiting bees among several forest types are not rare (Fowler et al., 2016; Boscolo et al., 2017; Hass et al., 2018; Jauker et al., 2019), very little is known about the responses of bees to local environmental changes, especially comparing micro-environments within forests, as well as the effects of microclimates conditions on the composition of the bee species in space (mature and regenerating forest) and time (as shown by the circular statistics, Fig. 3).

Studies indicate that flower visiting bees suffer negative effects of the habitat fragmentation (Schleuning et al., 2011; Aguiar et al., 2018; Jauker et al., 2019). However, our results seem to show that the natural regeneration in fragmented habitats can favor the floral visits and the reintegration of the bee community in these fragments. This fact can be evidenced by the occurrence of the same 17 species of bees in the mature and regenerating forest (see appendix 1). It is well known that the habitat fragmentation affects the abundance and richness of bees, as well as the biological flows between landscapes (Ferreira et al., 2015; Boscolo et al., 2017; Sobreiro et al., 2019). However, our results indicate that a large part of the bee species recorded here are less sensitive to the more fragmented habitat (regenerating forest). Possibly the reason for this result may be the high connectivity among micro-environmental units that may be increasing the permeability of the bees between forest fragments, in addition to providing a greater amount of foraging resources, because different landscapes can provide various types of feeding and nesting resources that the bees need (Boscolo et al., 2017), thus increasing the foraging efficiency.

The frequency and abundance of almost all bee species were higher in the mature forest, while only 10 bee species recorded higher sampling in the regenerating forest. A factor that can be determining in the frequency and abundance of bee species is the configuration of the landscape (Alaux et al., 2017; Boscolo et al., 2017; Sobreiro et al., 2019), because the plant species distribution and the vegetation density among open areas, woodland and forests are different, and this influence other factors, as wind speed, air temperature and flowering plant diversity (Fischer et al., 2016; Aleixo et al., 2017; Sobreiro et al., 2019). An interesting result found here is that the greatest flow of bees in the regenerating forest was restricted to the summer months, revealing a clear seasonality of the abundance of bees in the fragment. In fact, some studies have already shown that the abundance of bees is higher during the summer season (Junior et al., 2010; Fischer et al., 2016; Aleixo et al., 2017; Roberts et al., 2017), and this seasonality seems to be associated with the spatial distribution of flowering plants (Aleixo et al., 2017; Boscolo et al., 2017; Roberts et al., 2017), because forage bees are constantly in search of flowers with more resources. Furthermore, in the summer, some species of creeping flowering plants are more abundant at sites with higher sunlight incidence (Lowenstein et al., 2014; Marcilio-Silva et al., 2017), as in the regenerating forest, where solar intensity and air temperature were higher than in the mature forest. Also, some plant species that flourish for a longer period may be more common in altered landscapes (Roberts et al., 2017; Marcilio-Silva et al., 2017; Rotchés-Ribalta et al., 2018), thus increasing the bee abundance at these sites. Another important factor affecting the bee floral visits in fragments with natural regeneration is the climatic condition of each micro-environment, because such condition can vary during the annual seasons (Gostinski et al., 2016; Matos et al., 2016), altering the bee assemblage composition. Nonetheless, empirical verification about flow of flower visiting bees in the forests among annual seasons is still needed.
Studies have shown a high diversity of bees in small fragments of tropical forests (Abrahamczyk et al., 2011; Neame et al., 2012; Stangler et al., 2016; Botsch et al., 2017; Junqueira & Augusto, 2017; Sobreiro et al., 2019). Controversially, in our study we sampled a low richness and abundance of bees compared to other forest studies of similar size (Stangler et al., 2016; Boscolo et al., 2017; Botsch et al., 2017). We presume that this may occur due to the standardization of the height of flowering plants (4.5 m). It should be considered that most of the species that form mature vegetation flourish near the canopy (Ramalho, 2004; Oliveira & Oliveira, 2016), and this could affect the attractiveness of the flowers in vertical stratification (Frankie & Coville, 1979; Ulyshen et al., 2010; Stangler et al., 2016), because many bees prefer canopy flowers in primary forests (Ramalho, 2004; Stangler et al., 2016; Nakamura et al., 2017).

So, in order to obtain a comparative pattern between mature and regenerating forest sites, we set a flowering plant height limit to collect the floral visitors, because it is evident that the diversity of flowering plants is much higher in mature forests (remnants) than in areas with initial regeneration stage, such as the RF6 sampling site (see Table 1) (Boscolo et al., 2017; Roberts et al., 2017; Rotchés-Ribalta et al., 2018).

Additionally, some studies investigated the foraging networks of bees and found that some species forage habitats with different landscapes to satisfy their specific feeding and nesting needs (Viana et al., 2012; Sobreiro et al., 2019; Boscolo et al., 2017). Since in highly connected landscapes, the accessibility of bees to different (micro) environments appears to be higher (Hass et al., 2018; Boscolo et al., 2017; Kratschmer et al., 2018; Marques et al., 2018). Here we can consider that in a continuous matrix of micro-environments, the bees in search of floral resources would be more dispersed in the mosaic, looking for the flowers with the greatest amount of resources and therefore the richness and abundance of local bees would be less, as suggested by Boscolo et al. (2017). This fact may better explain the low numbers of individuals found in our study, since it does not mean that the bee communities in both types of forest fragments are less abundant and diverse, but rather the bees are possibly more distributed among micro-environments that form mature and regenerating forests.

Flowers are fundamental resources for the survival of bee communities and they are commonly used as indicators of the diversity of floral visitors (Neame et al., 2012; Fowler et al., 2016; Stangler et al., 2016; Boscolo et al., 2017). Interestingly, the results found here display that few flowering plant species attracted the highest bee abundance (58.12%), while many flowering plant species attracted the highest bee richness (55.65%) (see Table 2, Beeα). Since in this case, the flowers are competing for bees, this result suggests that some flowering plant species with greater amount of resources and floral abundance in the micro-environments possibly are more visited by pollinators (Heinrich, 1979; Fowler et al., 2016).

And also, factors such as type and variety of floral resources can influence the bee richness and abundance (Fowler et al., 2016; Aleixo et al., 2017; Roberts et al., 2017), because in periods with greater amount of floral resources, the bees collect proportionally less pollen types, frequently visiting flowers with more advantageous resources (Aleixo et al., 2013; Aleixo et al., 2017). In addition, the degree of specialization of bees that visit flowers may be another factor to consider here, since many flowering plant species attracted most bee species, registering few visitors of each bee species per flower. About this, recently Jauker et al. (2019) investigated changes in the plant-pollinator network after habitat loss, and they observed that specialization degree between plants and pollinators can represent a filter in the reintegration process of the insect communities in altered landscapes, once that the specialists are more susceptible to changes in local environment and habitat quality. The association between specialization and change/disturbance intensity results in faster or slower community reintegration. It is worth emphasizing that studies dedicated to investigate the generalist and specialist bees that visit flowers in recovered and regenerating habitats are scarce (Jauker et al., 2019; Thomson, 2019) and this knowledge gap still needs to be filled in.

On the other hand, evidence demonstrates that the plant diversity loss is directly related to bee diversity decline (Steffan-Dewenter & Westphal, 2008; Abrahamczyk et al., 2011; Boscolo et al., 2017), and the fragmentation and alteration of forests seem to be among the main causes of reduction in native plant populations (Stangler et al., 2016; Sobreiro et al., 2019). These findings reinforce concerns about the severe threat to pollination services (Steffan-Dewenter & Westphal, 2008; Schweiger et al., 2010; Neame et al., 2012), since natural and semi-natural habitats strongly influence the health of bees through diversified supply in pollen diet, and other feeding and nesting resources (Neame et al., 2012; Alaux et al., 2017; Aleixo et al., 2017). However, we may have found evidence that continuous micro-environments can mitigate the loss of plant diversity and favor the reintegration of bee communities in the naturally regenerating forest close to mature forest. One possible evidence for this is that most of the plant species sampled here are native species from the Atlantic Forest and attracted a higher abundance and richness of bees than exotic or naturalized plant species, as also observed by Morandin & Kremen (2013) and Salisbury et al. (2015). Another important point is that the highest native plant species richness visited by bees was sampled in the regenerating forest, and this low density of invasive plants (naturalized and exotic) may mean that the fragment is in an advanced recovery process (see Table 2). Furthermore, the occurrence of bees in these environments may indicate the participation of these pollinators in the regeneration of the forest. Within this scenario, the continuous micro-environments seem to be a crucial factor in sustaining the diversity of bees in the flowers of the different forest mosaic units.
As old forests are being increasingly reduced to fragmented landscapes (Alaux et al., 2017; Boscolo et al., 2017; Jauker et al., 2019; Sobreiro et al., 2019), the expected effect is the decline of the diversity of plants and pollinators (Auguiar et al., 2018; Marques et al., 2018; Sobreiro et al., 2019). However, we can interpret here that occurrence of bees in different micro-environments is possibly facilitating the flow of pollen between plants at different sites, and this seems to mitigate the impacts of forest reduction on the responses of plant-flower visitors. The reduction of native vegetation causes changes in the structure of the forests, reducing the availability of feeding and nesting resources, in addition to impacting the water cycle and the climatic conditions of the micro-environments that make up the forests (Aleixo et al., 2017; Switanek et al., 2017; Kamper et al., 2017).

Our results demonstrate that the bee assemblages are similar between forest fragments studied here, which means that natural regeneration surrounding forest remnant can be an efficient alternative in preventing species loss, since the forest matrix among habitats can be a fundamental factor to determine the effects forest reduction on bee communities (Boscolo et al., 2017; Hass et al., 2018). Thus, a continuous of forest types or physiognomies seems to be able to increase the biological flux of species among micro-environments in at least intermediate and advanced process of regeneration. Based on our results, we can infer that the plant and bee diversity found here were not different between fragments, because this continuous may be contributing to the increase of species that colonize forests in secondary growth (regeneration) (Stangler et al., 2016; Hass et al., 2018; Sobreiro et al., 2019). But this should be interpreted with caution considering the small spatial distance between forests, and that even small bees have a flight range which can vary between 250 to 1100 m (Zurbuchen et al., 2010; Wright et al., 2015; Hausmann et al., 2016), being able to fly all micro-environments studied here. Therefore, more research is needed to understand the floral visits of bees in a gradient of micro-environments, comparing alpha (within fragment), beta (between fragment) and gamma (regional) diversity, since the scale of observation affects the community diversity metrics in habitat gradients (Tyliaanakis et al., 2006).

Despite the information on population stability in heterogeneous landscapes (Oliver et al., 2010; Alaux et al., 2017; Botsch et al., 2017), for the pollinating insects, such as bees, the balance of communities in a matrix of landscape appears to depend on the proximity to the forest remnant (Boscolo et al., 2017; Papanikolaou et al., 2017; Sobreiro et al., 2019). However, our results show that the composition of bee assemblages in forests with secondary growth (regenerating forests) can be affected by climatic variations of micro-environments, even in fragments close to forest remnants, and this fact can be evidenced by the important influence of the climatic conditions in the composition of the bee communities studied here. About this issue, we can highlight that in the regenerating forest, the climatic variations were more prominent than in the mature forest with higher air temperatures, wind speed and sunlight, and even despite the significant effects of microclimates on the composition of bee assemblages between forests, most species appear to forage in all micro-environments, as the same 17 species of bees were sampled from both forests (see Appendix 1, Fig 6), no restrictions on microclimate variation, and this may indicate that some wild bee taxa can better cope with local climatic changes and disturbances (Switanek et al., 2017; Hunt et al., 2018; Dew et al., 2019; Sobreiro et al., 2019). In addition, other species of bees have indicated restrictions to more conserved or altered sites, being that 18 species of bees were exclusively sampled in the mature forest, while 5 species were exclusive in the regenerating forest (see Appendix 1). This result shows that the microclimates of the continuous forest affect the composition of bee taxa in a different way and consequently the flow of floral visits. Therefore, we strengthen the argument that the conservation of forest remnants and the connectivity between green areas are fundamental to mitigate the loss of bee species at a local scale (Boscolo et al., 2017; Kratschmer et al., 2018; Sobreiro et al., 2019).

We are presenting the results on the responses of flower visiting bees to continuous micro-environments, emphasizing the effects of local environmental changes on species composition. Although previous studies reveal the effects of forest changes on regional and global bee diversity (Morante-filho et al., 2016; Marques et al., 2018; Almeida et al., 2020), little is known about the responses of these floral visitors to local characteristics. Here, we present that continuous micro-environments can favor floral visits and promote the reintegration of bee communities into regenerating forests close to remnants. And yet, the least conserved micro-environments do not appear to deplete their species richness of bees compared to mature ones, as long as they form a continuous matrix involving a mature forest fragment. Furthermore, the seasonality of bees in the regenerating forest during the summer reinforces the importance of preserving forest remnants that provide foraging resources throughout the year. However, the climatic conditions and the flowering patterns of the plants seem to be decisive in the occurrence of bees in both mature and regenerating forests. In this scenario, the Atlantic forest fragments are becoming smaller and more isolated (Ribeiro et al., 2009), compromising the diversity of bees and the provision of pollination services (Boscolo et al., 2017; Winfree et al., 2018; Sobreiro et al., 2019), and the continuous forest can be a way to mitigate the effects of local environmental changes, because it favors floral visits and the reintegration of bee communities in fragmented habitats. Therefore, we hope that these facts can be considered in future actions to minimize the human impact on the diversity of bees and improve the conservation strategies of this threatened ecosystem.
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Authors’ contribution

AI Sobreiro: conceptualization, methodology, investigation, formal analysis, georeferencing and writing. LLS Peres: conceptualization, methodology, investigation and writing. VV Alves-Junior: conceptualization, methodology, investigation and writing. JA Henrique: writing. RM Mussury: writing.

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Appendix 1. Bees sampled in twelve Atlantic Forest forest fragments (six mature and six regenerating forest sites), Paraná, southern Brazil.

| Species (genus or tribe) | Mature forest | Regenerating forest | List of plants (List)* |
|-------------------------|---------------|---------------------|-----------------------|
| MF1 | MF2 | MF3 | MF4 | MF5 | MF6 | RF1 | RF2 | RF3 | RF4 | RF5 | RF6 | Total |
| **Andrenidae** | | | | | | | | | | | | |
| Arhyiosagre sp1 Brèthes, 1922 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| **Apidae** | | | | | | | | | | | | |
| Apis mellifera Linnaeus, 1758 | 3 | 8 | 7 | 9 | 5 | 5 | 3 | 2 | 3 | 1 | 6 | 3 |
| Bombus brasilensis Lepeltier, 1836 | 1 | 1 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| Bombus morio (Swerdus, 1877) | 0 | 1 | 0 | 3 | 1 | 2 | 1 | 1 | 0 | 2 | 1 | 12 |
| Melipona sp1 Illiger, 1806 | 1 | 2 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 7 |
| Trigona spinipes (Fabricius, 1793) | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 3 |
| Plebeia droryana (Friese, 1900) | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Schwarziana quadrupunctata (Lepeltier, 1836) | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 2 |
| Tetragonisca angustula (Latreille, 1811) | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Emphorini sp1 Robertson, 1904 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 |
| Melissopha richardiae Bertoni and Schrottky, 1910 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 3 |
| Gaestischia sp1 Michener, LaBerge and Moure, 1955 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Exomalopsis analis Spinola, 1853 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 1 | 1 | 5 |
| Exomalopsis aureopila Spinola, 1853 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 2 | 1 | 0 | 4 | 11 |
| Exomalopsis sp1 Spinola, 1853 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Exomalopsis sp2 Spinola, 1853 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Exomalopsis sp3 Spinola, 1853 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
| Exomalopsis sp4 Spinola, 1853 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lophopedia minor Aguiar, 2009 | 0 | 1 | 0 | 1 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 6 |
| Paratetrapedia fervida (Smith, 1879) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Tetrapedia diversipes Klug, 1810 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 3 |
| Ceratina sp1 Latreille, 1802 | 0 | 2 | 0 | 3 | 1 | 4 | 0 | 1 | 1 | 0 | 0 | 13 |
| **Colletidae** | | | | | | | | | | | | |
| Protolooalpa sp1 Smith, 1853 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| **Halictidae** | | | | | | | | | | | | |
| Augochlora iphigenia Holmberg, 1886 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 2 |
| Augochlora sp1 Smith, 1853 | 2 | 1 | 1 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 1 | 1 |
| Augochlora sp2 Smith, 1853 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| Augochlora sp3 Smith, 1853 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 |
| Augochlora lechlera ephrya (Schrotthyk, 1910) | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Augochloropsis sp1 Cockerell, 1897 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 | 2 | 1 | 0 | 8 |
| Lasioglossum cunipracumos (Pérez, 1903) | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| Pseudaugochlora erythrogaster Almeida, 2008 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 3 |
| Pseudaugochlora graminea (Fabricius, 1804) | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Thectochaeta basiatra (Strand, 1910) | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Agapostemon chapadensis Cockerell, 1900 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 |
| Agapostemon seminellas Cockerell, 1900 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Megachilidae** | | | | | | | | | | | | |
| Anthidium mancicatum (Linnaeus, 1758) | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| Hypanthidium divarccatum (Smith, 1854) | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Coelioxys sp1 Latreille, 1809 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Megachile sp1 Latreille, 1802 | 2 | 1 | 0 | 3 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 9 |
| Total of individuals | 15 | 30 | 17 | 29 | 15 | 35 | 7 | 13 | 14 | 6 | 14 | 203 |
| Total of species | 9 | 17 | 9 | 14 | 9 | 19 | 5 | 11 | 11 | 5 | 7 | 5 | 121 |

**Diversity indexes**

| | Shannon-Wiener (H′) | Simpson (D-1) | Berger-Parker (d) | Evenness (J) |
| | | | | |
| --- | --- | --- | --- | --- |
| | 2.133 | 0.875 | 0.187 | 0.970 |
| | 2.295 | 0.891 | 0.166 | 0.957 |
| | 1.975 | 0.837 | 0.235 | 0.904 |
| | 1.802 | 0.834 | 0.275 | 0.904 |
| | 1.999 | 0.808 | 0.335 | 0.926 |
| | 1.277 | 0.845 | 0.428 | 0.926 |
| | 1.605 | 0.693 | 0.307 | 0.912 |
| | 1.778 | 0.769 | 0.307 | 0.912 |
| | 0.693 | 0.804 | 0.307 | 0.912 |
| | 1.431 | 0.683 | 0.307 | 0.912 |
| | 1.494 | 0.75 | 0.307 | 0.912 |

* = List with number referring to the sampled plants (Table 2). ---- = there is no result.