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Research Article

Soil spiders (Arachnida: Araneae) in native and reforested Araucaria forests

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ABSTRACT: Spiders are part of the soil biodiversity, considered fundamental to the food chain hierarchy, directly and indirectly influencing several services in agricultural and forest ecosystems. The present study aimed to evaluate the biodiversity of soil spider families and identify which soil properties influence their presence, as well as proposing families as potential bioindicators. Native forest (NF) and reforested sites (RF) with Araucaria angustifolia (Bertol.) Kuntze were evaluated in three regions of the state São Paulo, both in the winter and summer. Fifteen soil samples were collected from each forest to evaluate the biological (spiders and microbiological), chemical and physical soil properties, in addition to properties of the litter (dry matter and C, N and S contents). For soil spiders, two sampling methods were used: pitfall traps and soil monoliths. In total, 591 individuals were collected, and distributed in 30 families, of which 306 individuals (22 families) came from pitfall traps and 285 individuals (26 families) from monoliths. Only samples obtained by the monolith method revealed seasonal differences in the mean density and richness of spiders between NF and RF. Canonical discriminant analysis showed the separation of these forests of Araucaria. Principal Component Analysis demonstrated the correlation of a number of spider families with certain soil properties (organic carbon, basal respiration, metabolic quotient, litter carbon, total porosity, bulk density and soil moisture). We identified 10 families (Anapidae, Corinnidae, Dipluridae, Hahniidae, Linyphiidae, Lycosidae, Nemesiidae, Palpimanidae, Salticidae, Scytodidae) that contributed most to separating native forest from the replanted forest, indicating the possibility of the spiders being used as bioindicators.

Keywords: spider fauna, soil biodiversity, soil properties, soil quality

Introduction

Spiders represent an extremely abundant group of terrestrial arthropod diversity, with more than 48,000 species described at global level so far, distributed over 117 families (World Spider Catalog, 2019). They represent a trophic level of consumers in the food chain and play a crucial role due to their predatory nature (Foelix, 2010; Jung et al., 2008a).

A number of authors have suggested that the structure of the environment may influence the abundance and richness of spider communities (Foelix, 2010). Their abundance and richness are strongly dependent on vegetation composition and structure (Oxbrough et al., 2007), landscape structure and disturbance (Rosa et al., 2018a, b; Schmidt et al., 2008), and soil properties (Rosa et al., 2018a, b). Thus, spiders have significant ecological importance for terrestrial, agricultural and forest ecosystems, where their role has been increasingly studied, evidencing edaphic factors that influence their presence and may provide useful information for monitoring environmental changes in most terrestrial ecosystems.

In Brazil, the devastation of forest areas has reached very significant proportions (Benati et al., 2005), requiring a joint effort for the collection and identification of terrestrial invertebrates in remaining areas. A. angustifolia, a native tree of the Mixed Ombrophilous forest from Brazil, is under the risk of extinction (Wrege et al., 2016). Inadequate exploration has led to a significant shrinkage of the original area and, currently, no more than 7 % of the original forest area remains (Wrege et al., 2015). Knowledge of terrestrial invertebrates in the *Araucaria* Forest has increased over the last few years (Baretta et al., 2007; Pereira et al., 2017), since this area concentrates a high diversity of soil organisms and represents an important biological reserve for the preservation of biodiversity.

Studies already published (Baretta et al., 2007; Jung et al., 2008a, b; Rosa et al., 2018a, b) lead to an understanding that the fauna of soil spiders can also be used as a quality indicator in natural and agricultural ecosystems. In our study we hypothesized that the Araucaria forest under anthropic impact might interfere with the abundance, diversity and environmental variables (soil chemical, physical and microbiological properties) and help explain the distribution of soil spider families. Thus, the present study aimed to evaluate, in native and reforested Araucaria forests, the abundance and diversity of soil spider families, in addition to identifying which soil properties influence their presence and indicate the most important families as potential biological indicators in the separation of these forests.

Materials and Methods

Description of studied forests

The study was carried out in three regions of the Mixed Ombrophilous forest, belonging to the Atlantic Forest Biome, in the state of São Paulo, Brazil. The climate is humid subtropical, with no dry season but the summers are mild (Cfb, according to Köppen's climate classification system) (Alvares et al., 2014). For all three zones, in the winter (Aug 2009), the mean temperature was 17 °C and rainfall was between 40-50 mm, whereas in the summer (Feb 2010), the mean temperature was between 22-25 °C and rainfall registered more than 300 mm. In each region, plots of approximately 1.0 ha were selected in native forest (NF) and reforestation (RF) with A. angustifolia (Bertol.) Kuntze. The native forests are more than 120 years old, while the reforested were planted between 20 and 45 years ago. The forests of each region were considered true replicates.

The first location for sampling NF and RF was subdivided into area 1 for native forest (1,185 m; 22°48′26″ S, 44°21′59″ W) and area 2 for reforestation (1,126 m; 22°48′24″ S, 44°22′27″ W) (Pereira et al., 2013). The areas belong to the municipality of Bananal. The soil in NF (area 1) and RF (area 2) was classified as Dystric Haplic Cambisol (FAO, 2015).

The second region studied (area 3, NF; 678 m; 23°50'27" S, 49°08'40" W) is in the municipality of Itaberá, SP. In this region (area 4, RF; 740 m; 24°04'19" S, 49°04'10" W) in the municipality of Itapeva, SP, there was a predominance of *Araucaria* and understory species. The soil in NF (area 3) and RF (area 4) were classified as Alumic Vetic Ferralsol and Eutric Ferralic Nitisol, respectively (FAO, 2015).

The third region is in the municipality of Barra do Chapéu (area 5, NF; 785 m; 24°28'40" S, 49°01'07" W) and the municipality of Iporanga (area 6, RF; 932 m; 24°20'14" S, 48°36'14" W). The soil in NF (area 5) and RF (area 6) was classified as Dystric Haplic Cambisol (FAO, 2015). More detailed descriptions regarding flora, physical and chemical soil properties in all areas can be found in Pereira et al. (2013).

Experimental design and sampling

The collections were carried out in the winter (Aug 2009) and the summer (Feb 2010). In each forest, 15 *Araucaria* trees with a minimum spacing of 20 m were chosen at random. Fifteen soil samples were collected to analyze the community of soil spiders, in addition to the microbiological, chemical and physical parameters, as well as the dry matter and chemical parameters of the litter. The samples were extracted from the forest floor at a distance of 2 m from the trunk of each tree.

For the evaluation of microbiological, chemical and physical properties, five subsamples were collected at each sampling point (tree), using a Dutch auger,

in the 0-20 cm layer, making a total of 15 composite samples. The litter was sampled at the same point, in a 25 \times 25 cm area. For microbiological analysis, the samples were packed in polyethylene bags, refrigerated and transported to the laboratory in an ice-cooled box. Thereafter, the soil samples were sieved (2-mm mesh), ground and stored in a refrigerator (4 °C) until analysis. For chemical analysis, the samples were air-dried, sieved (2-mm mesh) and stored at room temperature. For physical analyses, both disturbed and undisturbed samples were collected. Disturbed soil samples were air-dried, sieved (2-mm mesh) and stored. In the winter, at the same sampling points, undisturbed samples were collected using stainless-steel rings 5 cm in diameter and the samples were kept intact and sealed (to avoid moisture loss) until the analyses.

Sampling of soil spiders

To avoid any underestimation of the density and diversity of the community of soil spiders and thus conduct a more accurate analysis of this community (Baretta et al., 2007; Brennan et al., 2005), and since the adoption of only one method may be ineffective, the following two sampling methods were used: pitfall traps and soil monoliths $(25 \times 25 \text{ cm})$ in the layer from 0 to 20 cm (Baretta et al., 2007). Each trap consisting of one glass container 6 cm in diameter and 12 cm in height was buried in the soil, with its upper part open and level with the surface, and kept in place for three days. Each trap received 200 mL of neutral detergent solution at 2.5 %. The samples of the pitfall traps were successively passed through sieves of different mesh thicknesses (0.2, 0.15 and 0.1 mm). Before removing the soil monolith, the litter was sampled in the same area. The monoliths were collected using a galvanized iron implement (Baretta et al., 2007). The samples were manually screened to separate spiders from plant and soil fragments. The spiders collected by both methods were preserved in 75 % alcohol solution until identification. These spider collection methods (monoliths and pitfall traps) have already been discussed in studies on spider ecology (Baretta et al., 2007; Brennan et al., 2005).

Since the identification of spider species is based on the presence of genital organs, which are developed only in adults, this study was carried out at family level only, in order to make use of the maximum possible number of individuals (Baretta et al., 2007). After identification, the individuals were labeled and preserved in 80 % ethyl alcohol. All the material was deposited in the Arachnological collection of the Butantan Institute (A.D. Brescovit, curator). After identification, the following parameters were determined: abundance (individuals per trap, ind. trap⁻¹) for pitfall traps, density (individuals per square meter, ind. m^{-2}) for monoliths, richness, Shannon's diversity index (H) and Simpson's dominance index (Is). The indices were calculated using the PAST program v2.17c.

Soil chemical, physical and microbiological properties

The soil chemical properties analyzed were pH $(CaCl_2)$, exchangeable cations $(Al^{3+}, Ca^{2+}, Mg^{2+} \text{ and } K^+)$, P, H+Al and organic carbon content (C-org) (van Raij et al., 2001). In disturbed samples, the following soil properties were analyzed: particle size (Gee and Or, 2002) and soil moisture (Moist) (Embrapa, 2011). Undisturbed samples were analyzed for soil bulk density (Bd) (Blake and Hartge, 1986), total porosity (TP), microporosity (Mi) and macroporosity (Ma) (Embrapa, 2011).

For microbiological analyses, 100-g soil samples with the moisture content corrected to 60 % of maximum water retention capacity were used to quantify the microbial biomass carbon (MBC) (Vance et al., 1987), using the value of 0.33 as the correction factor in the calculations. Microbial activity was evaluated based on the determination of basal respiration (C-CO₂ released) (Alef and Nannipieri, 1995). The metabolic quotient (qCO_2) was calculated by dividing the C-CO₂ released by MBC, whereas the microbial quotient (qMic) was calculated by the expression (MBC/C-org)/10 (Anderson and Domsch, 1993).

The enzyme dehydrogenase (DH) is very active in living cells and reflects the oxidative capacity of the soil microbial biomass. Dehydrogenase activity was quantified in soil samples (5 g at natural moisture), which received 5 mL of 1 % triphenyl tetrazolium chloride (TTC) solution and were incubated at 37 °C for 24 h. The TTC used as electron acceptor is enzymatically reduced to triphenyl tetrazolium formazan (TTF) and was extracted with 10 mL of methanol. Enzyme activity was measured in a spectrophotometer at 485 nm (Casida Jr. et al., 1964).

Litter dry matter and C, N and S contents

Litter dry matter (DM-Lt) was determined at 55 °C until constant weight. Then, the litter was ground, passed through a 100-mesh sieve and its contents of C (C-Lt), N (N-Lt) and S (S-Lt) were determined by dry combustion in a simultaneous C, N, and S analyzer (Vario EL cube - Elementar).

Statistical analysis

Analysis of variance (ANOVA) of the main effects was used to compare the abundance (ind. trap⁻¹), density (ind. m⁻²), richness and the H and Is indices between native and reforested forests, using the STATISTICA program v7.0. Data were transformed (\sqrt{x} + 1), when needed, to meet the normal distribution and homoscedasticity of error variances and were compared by LSD test ($p \le 0.05$). The LSD test was performed to compare the forests in each season and collection methods.

Data of density and abundance of soil spider families were subjected to Principal Components Analysis (PCA) for the periods studied (winter and summer), using the CANOCO 4.0 program. Only the significant ($p \le 0.05$) chemical (P and C-org), physical (Bd, TP, Moist) and microbiological properties of the soil (MBC, C-CO₂ and qCO₂) and of the litter (C-Lt and DM-Lt) were used as passive explanatory environmental variables, so as to increase the robustness of the analysis.

Additionally, the main families of soil spiders and their main properties were subjected to Canonical Discriminant Analysis (CDA) to identify which were the most relevant to the separation of the forests, using the parallel discriminant ratio (Pereira et al., 2017) in the SAS program, version 8.2.

Both analyses were used because they present different ideas. PCA was performed to verify the association of taxonomic fauna groups with the studied forests, subsequently using an indirect gradient, and the explanatory environmental variables (only those significant at 0.05 probability level) to help explain the reason for the preferential presence of the organisms in the different forests. On the other hand, CDA was carried out to separate the systems studied and confirm (through an associated statistical test) whether any separation between the systems could be detected, in addition to verifying which families contributed most to the separation. The use of PCA and CDA has already been published, in connection with other soil use systems and organisms (Pereira et al., 2017).

Results

Univariate analysis

A total of 591 individuals were collected, distributed over 30 families. Of these, 306 individuals, from 22 families, were collected in pitfall traps and 285 (4560 m⁻²) individuals, from 26 families, in monoliths. Considering both sampling methods and regardless of the period, the most frequent families relative to the total (591) were Linyphidae (199 ind., 34 %), Theridiidae (70 ind., 12 %), Dipluridae (50 ind., 9 %), Oonopidae (44 ind., 8 %) and Corinnidae (34 ind., 6 %), representing 67 % of the individuals collected (Table 1).

As per the pitfall trap method, none of the attributes of Araneae families differed (p > 0.05) between the forests in the two sampling periods. By contrast, as per the soil monolith method, there was a difference ($p \le 0.05$) between the forests in the summer, with mean densities of 52 ind. m⁻² in NF and 26 ind. m⁻² in RF. The same occurred for the mean richness, with 2 families in NF and 1 in RF (Table 1). Although there were no differences (p > 0.05) in the values of richness of families and H, there was always a slight increase in the values found in NF, compared to RF, for each season and collection method (Table 1).

Principal Component Analysis (PCA)

According to PCA, it is possible to verify that the factors that best explain the differences are the higher C content of the litter in NF, in addition to higher soil moisture, porosity, P, MBC and C-CO₂ in RF. On the

Table 1 -	Number of individuals colle	ected by pitfall traps a	and soil monoliths,	in the winter	and summer,	in each family of	soil spiders in	native (NF)
and refe	prested (RF) Araucaria fores	sts, in addition to cer	tain attributes of th	he families of	f Araneae.			

			Pitfa	ll traps			Soil m	onoliths	
		Wi	nter	Sun	nmer	Winter		Summer	
Families		NF	RF	NF	RF	NF	RF	NF	RF
Amaurobiidae	Ama	0	0	0	0	32	0	0	0
Anapidae	Ana	1	1	0	1	48	0	0	0
Anyphaenidae	Any	0	0	0	0	0	16	0	0
Araneidae	Ara	0	1	2	1	16	16	16	16
Caponiidae	Сар	0	0	0	0	0	0	16	0
Corinnidae	Cor	3	0	12	7	16	16	144	16
Ctenidae	Cte	3	1	6	1	16	0	80	32
Dipluridae	Dip	2	1	0	0	0	0	624	128
Gnaphosidae - Gnaphosinae	Gna	0	0	2	1	0	0	128	0
Gnaphosidae - Prodidominae	Prod	0	2	0	0	32	0	192	64
Hahniidae	Hah	5	4	0	11	0	32	16	16
Idiopidae	ldio	0	0	0	0	16	0	0	16
Linyphiidae	Liny	44	47	22	38	192	64	352	160
Liocranidae	Lio	1	0	0	0	0	0	32	32
Lycosidae	Lyc	0	3	1	6	0	0	0	0
Mysmenidae	Mys	0	0	2	0	0	0	0	0
Nemesiidae	Nem	0	2	0	0	0	144	32	128
Oonopidae	Oon	3	1	8	4	64	16	208	160
Palpimanidae	Palp	1	0	0	0	0	0	16	0
Pholcidae	Phol	0	0	1	0	0	0	16	0
Pisauridae	Pisa	0	0	0	0	0	0	16	0
Salticidae	Salt	0	2	5	2	48	0	112	112
Scytodidae	Scy	0	1	0	0	16	0	0	16
Segestriidae	Sege	0	0	0	0	0	0	16	0
Sparassidae	Spar	0	0	0	1	0	0	0	16
Tetragnathidae	Tetra	1	0	2	2	0	0	0	0
Theraphosidae	Thera	0	0	0	0	16	0	80	32
Theridiidae	Ther	11	8	10	6	64	48	224	224
Theridiosomatidae	Theri	1	0	1	0	0	0	0	0
Thomisidae	Thom	0	0	0	1	0	48	0	16
Zodariidae	Zod	0	0	0	0	0	0	16	64
Total of individuals		76	74	74	82	576	400	2336	1248
Mean of individuals*		2 ± 2	2 ± 2	2 ± 1	2 ± 2	13 ± 22	9 ± 14	52 a ± 49	26 b ± 29
Total richness		12	13	13	14	13	9	19	18
Mean richness		1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	0.5 ± 1	2 a ± 2	1 b ± 1
Shannon's diversity (H)		0.22 ± 0.39	0.19 ± 0.34	0.36 ± 0.47	0.26 ± 0.43	0.09 ± 0.24	0.06 ± 0.19	0.53 ± 0.58	3 0.30 ± 0.47
Simpson's dominance (Is)		0.20 ± 0.36	0.21 ± 0.37	0.37 ± 0.46	0.25 ± 0.40	0.10 ± 0.26	0.07 ± 0.25	0.43 ± 0.43	$3\ 0.31 \pm 0.44$

Mean \pm standard deviation (n = 45). *Refers to abundance (ind. trap⁻¹) when collected by soil traps and density (ind. m⁻²) when collected by soil monoliths. Boldface values indicate a significant difference by LSD test (p < 0.05).

other hand, certain of the parameters studied (pH, Al^{3+} , Ca^{2+} , Mg^{2+} , K^+ , H+Al, particle size, Mi, Ma, *q*Mic, DH, N-Lt and S-Lt) in this forest, showed no relation with soil spider families. This is important, because in future research studies there will be no need to evaluate these parameters, which will save time, material and human resources.

The PCA separated the NF from the RF collected by pitfall traps. In the winter, the principal component 1 (PC1) represented 24 % and the principal component 2 (PC2) represented 11 %. There was greater abundance of spiders in the NF (Figure 1A), where the soil is covered with litter containing higher levels of C, which was found mainly in individuals from the Araneidae, Corinnidae, Hahniidae, Linyphiidae, Liocranidae, Palpimanidae, Theridiidae and Theridiosomatidae. The Anapidae, Oonopidae, Dipluridae and Nemesiidae families showed less association with NF, and the RF was more associated with Salticidae, Scytodidae and Gnaphosidae - Prodidominae, which are related to soils with higher moisture contents. The Lycosidae, Ctenidae and Tetragnathidae families showed no specific tendency for any of these forests and were observed at sites with higher litter mass.

During the summer (Figure 1B), PC1 explained 24 % and PC2 explained 10 % of the data variation, and



Figure 1 – Relationships between the principal components 1 (PC 1) and 2 (PC 2), separating native (NF) and reforested (RF) Araucaria forests, families of soil spiders (in italics) and explanatory environmental variables (in red) sampled with pitfall traps in the winter (A) and summer (B). Ana = Anapidae; Ara = Araneidae; Cor = Corinnidae; Cte = Ctenidae; Dip = Dipluridae; Gna = Gnaphosidae – Gnaphosinae; Prod = Gnaphosidae – Prodidominae; Hah = Hahniidae; Liny = Linyphiidae; Lio = Liocranidae; Lyc = Lycosidae; Mys = Mysmenidae; Nem = Nemesiidae; Oon = Oonopidae; Palp = Palpimanidae; Phol = Pholcidae; Salt = Salticidae; Scy = Scytodidae; Spar = Sparassidae; Tetra = Tetragnathidae; Ther = Theridiidae; Theri = Theridiosomatidae; Thom = Thomisidae. C-Lt = Litter carbon contents; DM-Lt = Litter dry matter; Moist = Soil moisture. PC1 = Principal Component 1; PC2 = Principal Component 2.

the highest number of families was more associated with the RF. Mysmenidae, Pholcidae, Salticidae, Tetragnathidae and Theridiosomatidae were associated with NF, while Anapidae, Araneidae, Corinnidae, Gnaphosidae - Gnaphosinae, Hahniidae, Linyphiidae, Lycosidae, Oonopidae, Sparassidae, Theridiidae and Thomisidae were associated with RF. Ctenidae did not show any specific association with the forests, but was very influenced by the litter C content (C-Lt). Similarly, Anapidae, Lycosidae and Sparassidae were greatly influenced by C-Lt, while Araneidae, Corinnidae, Gnaphosidae - Gnaphosinae, Oonopidae, Theridiidae and Thomisidae were influenced by soil moisture.

For spiders sampled by the soil monolith method, the PCA detected a separation between NF and RF and a greater association of certain spider families with one kind of forest or the other. In the winter (Figure 2A), PC1 represented 21 % and PC2 10 %. The Anapidae, Corinnidae, Ctenidae, Linyphiidae, Salticidae and Scytodidae families were associated with NF, with no correlation with the environmental variables, whereas Araneidae, Anyphaenidae, Hahniidae, Idiopidae, Amaurobiidae, Nemesiidae, Oonopidae, Gnaphosidae Prodidominae, Theraphosidae, Theridiidae and Thomisidae were correlated with RF, and their presence was favored in soil with higher contents of P, C-org and MBC. Other favorable factors were higher moisture content and total porosity, combined with greater soil respiration.

For the sampling with soil monoliths in the summer (Figure 2B), PC1 explained 19 % and PC 8 % of variations in the data. Caponiidae, Corinnidae, Ctenidae, Dipluridae, Gnaphosidae - Gnaphosinae, Hahnidae, Pholcidae, Pisauridae, Salticidae, Segestriidae, Theridiidae and Thomisidae were more associated with NF, being influenced by soil density, whereas Araneidae, Idiopidae, Linyphiidae, Liocranidae, Nemesiidae, Oonopidae, Palpimanidae, Gnaphosidae - Prodidominae, Scytodidae, Sparassidae, Theraphosidae and Zodariidae were associated with RF and their presence was favored by soil moisture, C contents of soil and litter, and soil respiration.

Canonical Discriminant Analysis (CDA)

Differences between RF and NF were evaluated using canonical discriminant analysis, considering the attributes of environmental variables (chemical, physical and microbiological properties of soil and chemical properties of litter), and the presence of soil spider families. The Wilks' Lambda test for spider fauna attributes and environmental variables indicated discrepancies between winter and summer and types of forest (NF and RF) ($p \le 0.0001$) by the canonical discriminant functions 1 (CDF1) and 2 (CDF2). Therefore, a CDA was performed for each sampling period (Figure 3). In addition, there were high canonical correlations in the pitfall trap method in both the winter (0.94) and summer (0.92), and in the soil monolith method, between winter (0.93) and summer (0.91). Thus, the CDA model used explained a great part of the variability present in the forests. The polarized distribution of Araucaria forests (native and reforested) indicates high dissimilarity between the forests studied, where CDF1 separated the NF from the RF (Figure 3).

Values of Parallel Discriminant Ratio (PDR) referring to the CDA (Table 2), which demonstrates the potential of each attribute of the families of soil spiders to discriminate soil quality, because they contributed more to the separation between the forests studied.



Figure 2 - Relationships between the principal components 1 (PC 1) and 2 (PC 2), separating native (NF) and reforested (RF) Araucaria forests, families of soil spiders (in italics) and explanatory environmental variables (in red) sampled with soil monoliths (25 \times 25 cm) in the winter (a) and summer (b). Ama = Amaurobiidae; Ana = Anapidae; Any = Anyphaenidae; Ara = Araneidae; Cap = Caponiidae; Cor = Corinnidae; Cte = Ctenidae; Dip = Dipluridae; Gna = Gnaphosidae - Gnaphosinae; Prod = Gnaphosidae – Prodidominae: Hah = Hahniidae: Idio = Idiopidae: Liny = Linyphiidae; Lio = Liocranidae; Nem = Nemesiidae; Oon = Oonopidae; Palp = Palpimanidae; Phol = Pholcidae; Pisa = Pisauridae; Salt = Salticidae; Scy = Scytodidae; Sege = Segestriidae; Spar = Sparassidae; Thera = Theraphosidae; Ther = Theridiidae; Thom = Thomisidae; Zod = Zodariidae. Bd = Bulk density; C-CO2 = Basal respiration; C-Lt = Litter carbon contents: C-org = organic carbon content; MBC = Microbial biomass carbon; Moist = Soil moisture; qC02 = Metabolic quotient; P = Phosphorus; TP = Total porosity. PC1 = Principal Component 1; PC2 = Principal Component 2.

In general, other studies (Pereira et al., 2017) have shown good values, above 0.1, to discriminate forests. However, the present study considered values above 0.02 only to discriminate the forests, because of the low number of individuals per family. Thus, Hahniidae (0.064), Lycosidae (0.025), Shannon's diversity (0.100) and Simpson's dominance (0.098) contributed to the



Figure 3 – Standardized canonical coefficients (SCC) of the canonical discriminant functions 1 and 2 (CDF1 and CDF2), discriminating native (NF) and reforested (RF) *Araucaria* forests, considering the properties of the environmental variables (chemical, physical and microbiological properties of soil and chemical properties of litter) and families of soil spiders collected with pitfall traps, in the winter (A) and summer (B), and with soil monoliths (25 × 25 cm), in winter (C) and summer (D). Black symbols represent the mean value of SCC for each forest (n = 45).

separation of forests through the pitfall trap method, all in the summer. As per the soil monolith method, Anapidae (0.084), Linyphiidae (0.085), Salticidae (0.033), Scytodidae (0.035) and richness (0.112) contributed to the separation of forests in the winter, while in summer they were Corinnidae (0.029), Dipluridae (0.036), Nemesiidae (0.054), Palpimanidae (0.051), richness (0.066) and Simpson's dominance (0.020) contributed in the summer.

Discussion

Diversity of spider families

Twenty six percent of the total families described at global level were collected in this study. Since there are no historical records for these forests in relation to the true local diversity, these families can serve as indicators for discriminating environments (Figure 3 and Table 2) (Jung et al., 2008a, b). The band of families found with the highest frequency in this study is also among the most abundant group of spiders in most Brazilian soil ecosystems, thus corroborating their representativeness (Benati et al., 2005; Brescovit et al., 2004; Rosa et al., 2018a).

The forest ecosystem conservation status had little influence on the richness and diversity of spider families. However, environmental stability was a determinant factor for the presence of Linyphiidae, when different soil use systems were evaluated in a gradient of anthropic intervention (Rosa et al., 2018a, b), corroborating all these studies. Other studies have reported not only higher abundance, but also a higher

Table 2 – Values of Parallel Discriminant Ratio (PDR) referring to
the Canonical Discriminant Analysis (CDA) for the attributes of the
families of soil spiders in the winter and summer, regardless of the
forest evaluated.

	Parallel Discriminant Ratio					
	Pitfall traps Soil monoliths					
	Winter	Summer	Winter	Summer		
Families of soil spiders						
Amaurobiidae	-	-	0.000	-		
Anapidae	0.000*	-0.001	0.084	-		
Anyphaenidae	_* *	-	0.000	-		
Araneidae	0.003	0.004	-0.002	0.000		
Caponiidae	-	-	-	0.018		
Corinnidae	0.015	0.012	0.000	0.029		
Ctenidae	0.008	-0.001	0.000	0.015		
Dipluridae	0.001	-	-	0.036		
Gnaphosidae - Gnaphosinae	-	-0.005	-	-0.041		
Gnaphosidae - Prodidominae	-0.007	-	0.000	0.011		
Hahniidae	0.004	0.064	0.000	0.000		
Idiopidae	-	-	0.000	-0.003		
Linyphiidae	-0.001	-0.001	0.085	-0.038		
Liocranidae	-0.013	-	-	0.000		
Lycosidae	-0.003	0.025	-	-		
Mysmenidae	-	-0.015	-	-		
Nemesiidae	-0.006		-0.139	0.054		
Oonopidae	0.016	0.005	-0.006	-0.011		
Palpimanidae	0.014	-	-	0.051		
Pholcidae	-	-0.009	-	0.017		
Pisauridae	-	-	-	0.011		
Salticidae	-0.007	0.010	0.033	0.000		
Scytodidae	0.007	-	0.035	-0.012		
Segestriidae	-	-	-	0.007		
Sparassidae	-	0.000	-	-0.014		
Tetragnathidae	-0.007	0.000	-	-		
Theraphosidae	-	-	0.000	-0.013		
Theridiidae	0.014	-0.008	-0.007	0.000		
Theridiosomatidae	0.000	0.011	-	-		
Thomisidae	-	-0.011	-0.075	-0.003		
Zodariidae	-	-	-	0.005		
Attributes of spider families						
Organisms***	-0.001	0.000	0.000	0.000		
Richness	-0.003	-0.025	0.112	0.066		
Shannon's diversity (H)	0.004	0.100	0.003	-0.074		
Simpson's dominance (Is)	-0.002	0.098	0.000	0.020		

*Positive values indicate the effects of separation between the native and reforested forests, whereas negative values indicate similarities. Boldface values represent the most important attributes for discriminating the forests studied (n = 45). **Indicates that there were no individuals collected. ***Refers to abundance when collected by soil traps and density when collected by soil monoliths.

richness of spiders in environments with greater stability, as is the case of native areas (Malumbres-Olarte et al., 2013; Pommeresche et al., 2013; Rodriguez-Artigas et al., 2016). In our survey, however, it was not possible to find results demonstrating such divergence in the mean abundance and total diversity of families. Additionally, on occasion considerable diversity can be found in more recently disturbed environments (Jung et al., 2008b). Other factors can be influencing the composition of soil spider families, such as climatic condition (Mantyka-Pringle et al., 2015), floristic composition and diversity in the area (Jung et al., 2008b; Sabais et al., 2011), as well as the soil community as a whole, and are directly related to the spiders, either through the presence of food (such as springtails, mites and dipterans, which are important prey) (Gravesen, 2008; Pommeresche et al., 2013), or by the absence or presence of predators (Rosa et al., 2018a).

Community distribution of soil spider families and their relationships with soil properties

The structure and composition of the flora as a primordial factor modulates the composition of the spider community, but the differences in environmental conditions in the litter and soil have a substantial effect on the composition of the spider families (Oxbrough et al., 2007).

Only C-Lt had influence on the composition of the families associated with the NF for the winter. Therefore, Corinnidae was described as a family established in forests where temporal stability (over the periods) is greater (Rosa et al., 2018a) under different soil use systems. Here these spiders were found in the forest due to their habit of actively searching for prey (Lo-Man-Hung et al., 2011). Organic matter is very important to the biological complexity in various ecological niches. The favoring of accumulation of surface litter in quantity and quality maintains the microclimate and moisture, as well as the diversity of microhabitats at this site and this is favorable to various soil organisms that are attracted by the diversity of plant residues, becoming potential prey for spiders (Malumbres-Olarte et al., 2013; Rosa et al., 2018a). Thus, the C-Lt, C-org and other parameter linkage to organic matter, become an indirect conditioner for the abundance of spider families.

On the other hand, although the frequency of Linyphiidae has been reported in native areas (Almada and Sarquis, 2017; DiCarlo and DeBano, 2019), it has also been associated with higher contents of organic material in these areas (Rosa et al., 2018a), a consequence of higher C-Lt, pointing to a close association between the presence of these two indicators in NF. In addition, Rosa et al. (2018b) also reported higher frequency of organisms of this family in more stable areas (native pasture), compared to no-tillage areas. The relationship between NF and Palpimanidae is a consequence of the feeding behavior of this family, in which several species are known for their reduced movement in the search for food (Cerveira and Jackson, 2005) and they are more associated with the soil and superficial litter (Rosa et al., 2018a), which results in their greater presence in native areas, being more easily captured by the methods used here. Associated with NF, Corinnidae, Linyphiidae and Palpimanidae were highly sensitive to the environmental conditions or changes, being responsible for the

separation of NF and RF (Figure 3) through the values of parallel discriminant ratios (Table 2).

In the winter, soil moisture was relevant to the separation of the forest types (Figures 1A and 2A), and this factor reflects on the presence of soil spider families. Increase in the moisture gradient favored the density of spiders in peatlands compared to improved grasslands (Kajak et al., 2000). In addition, soil moisture may indirectly affect spiders through its influence on vegetation development (Cattin et al., 2003), favoring the formation of microhabitats with great humidity that are favorable to spiders and their prey.

Spider families influenced by other properties and, in turn, associated with RF, as in the case of Gnaphosidae - Prodidominae, have also been reported as being present in native Araucaria forest with reduced human intervention in the state of São Paulo, Brazil (Baretta et al., 2007), although little is known about this family (Rosa et al., 2018a). Idiopidae was reported in a review by Rix et al. (2017) as common in stable areas of natural reserves, as in smaller or remaining reserves close to urban areas in Australia, despite being very rare. This fact seems to indicate that Araucaria reforestation fragments are already in an advanced stage of regeneration, thus enabling the emergence of families that seek more stabilized ecosystems. The same is valid for Oonopidae, which is associated with forest areas (Battirola et al., 2010), in addition to these spiders' close relationship with litter, where they search for food, and this is considered a developed environment (Rosa et al., 2018b).

The correlation between the different families and the forests was also influenced by the sampling period, since Linyphiidae was related to NF in the winter and RF in the summer. Such discrepancies in a different sampling period, for spider families has also been evidenced by other authors (Indicatti et al., 2008; Rosa et al., 2018b). In the cases of Nemesiidae, according to Rosa et al. (2018b), and Araneidae, according to Marc et al. (1999), associated with RF, their representativeness occurred in the summer due to the reproduction cycle, because their eggs hatch in spring. Araneidae are considered generalists that do not undergo alterations in their community under different managements systems (organic and conventional) (Picchi et al., 2016), indicating their low capacity to be environmental indicators due to their high adaptability (Benati et al., 2005). The correlation of Palpimanidae with the forests was also influenced by the sampling period and, in the summer, they were more associated with RF, as has already been described, probably due to their low mobility in search for food, making them more present in forests where litter permits greater presence of prey.

In both seasons, the moisture content, C-org and $qCO_{2^{\prime}}$ in addition to C-Lt, were more associated with RF, separating it from NF. This indicates that floristic quality and composition were important in this result, evidencing that the interactions of the spider fauna with

the soil fauna in general are important determinants of the carbon cycle (Morriën et al., 2017). Anapidae are present in stable forests, but it is possible to note a strong association with C-Lt, since they were present in the forests where this attribute was significant for the separation, as determined by the PCA. Lycosidae, also influenced by C-Lt, has direct association with organic matter content and positive correlation with the soil cover (Birkhofer et al., 2018), being abundant in Eucalyptus reforestation (Rosa et al., 2018a). In southern Brazil, spiders of this family have been reported as syntropic organisms (Rosa et al., 2018b), generalists (Almada and Sarquis, 2017) and as inhabitants of degraded and regenerating environments (DiCarlo and DeBano, 2019). The wide distribution of these families and their capacity to be influenced by the characteristics of the soil environment indicate the possibility of their use as indicators of environmental changes in native or reforested Araucaria.

On the other hand, bulk density showed a strong association with NF, an area that in turn showed higher abundance of spiders in this period. Physical variables are important for spider communities (Yang et al., 2017) and, in addition, variables such as soil density affect all trophic groups as well as spiders (Kajak et al., 2000; Rosa et al., 2018a). In the present study, soil density was determinant for the presence of Ctenidae in NF in the summer. This result demonstrates the importance of more stable environments throughout the year in the maintenance of soil organisms and spider fauna, since climatic changes can determine modifications in the composition of the spider fauna (Mantyka-Pringle et al., 2015).

Families associated with NF in general have a lower capacity for adapting to alterations and, therefore, are established in more stable environments, as in the case of Salticidae (DiCarlo and DeBano, 2019; Rosa et al., 2018a) and Caponiidae (Rosa et al., 2018a), and where the physical complexity of the areas favors the formation of webs such as for Tetragnathidae (Almada and Sarquis, 2017) or traps for Dipluridae (Brescovit et al., 2004). Certain spiders, such as Pholcidae, have the habit of actively seeking prey, and these are found in higher density in native areas (Malumbres-Olarte et al., 2013). Pholcidae was found exclusively in agriculturally unexplored areas when compared to grazing areas in southern Brazil (Silva and Ott, 2017) and genera of this family were found in Restinga areas at different stages of environmental regeneration (primary, medium and advanced) (Benati et al., 2005). Only four species of this family are found in areas under high anthropic intervention (Simonneau et al., 2016), which brings us back to the fact that these can be used as indicators of regeneration that point to differences even in advanced stages of recovery of reforested areas.

The greater association of qualitative factors with RF could indicate that these fragments already have good stability and high plant complexity, thus favoring the ecological relationships related to energy flows in the ecosystem (Rosa et al., 2018b) and the diversity of spiders. However, the composition of spider families is also influenced by the possibility of trophic niches present in the ecosystems, which leads to the assumption that places where the energy flow is high have the greatest diversity of detritivore soil organisms (Braschler and Baur, 2016), which serve as prey to spiders. This fact may explain why the environmental variables of litter quality and C flow, allied to the composition of the spider fauna are sufficient to indicate differences between NF and RF, thus defining the Hahniidae, Lycosidae, Anapidae, Linyphiidae, Salticidae, Scytodidae, Corinnidae, Dipluridae, Nemesiidae and Palpimanidae families (Table 2) as sensitive indicators for different Araucaria forests.

Conclusions

The abundance of families of soil spiders is higher in native Araucaria forests only in the summer; in winter, there was no difference between the forests studied. Hahniidae, Anapidae, Linyphiidae, predominant in NF, were the ones that most contributed to the separation between native and reforested forests, showing the possibility of their application as indicators, even when the planted forests are in an advanced stage of regeneration. Lycosidae, Salticidae, Scytodidae, Corinnidae, Dipluridae, Nemesiidae and Palpimanidae, in general more numerous in RF, also contribute significantly to the separation of the forests studied and can serve as quality indicators. The soil properties related to the energy flow (C-org, C-CO₂, qCO₂), in addition to C-Lt, were determinant for the presence of different families of soil spiders in native and reforested Araucaria forests. In addition, physical soil properties such as total porosity, density and soil moisture also contribute to the differential distributions of the families of the soil spiders.

The great advantage of the identification of spiders at the family level, as opposed to species level is that it can be adopted when a rapid assessment on biodiversity is required, in areas where the spider fauna is poorly known taxonomically. On the other hand, identification at the species level is important information which sheds light on native species. Developing such knowledge will make it possible to infer management practices in the recovery of degraded forests, in agricultural environments or at the edges of forests to improve the efforts of preservation of both spiders and ecosystems.

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