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Luciana Aparecida Giacomini, Carlos Aparecido de Siqueira Junior

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The influence of forest litter on the endomicorritic fungi community in a natural regeneration area in São Paulo State, Brazil[1]

Luciana Aparecida Giacomini[1,3], Carlos Aparecido de Siqueira Junior[2], Gustavo Padovani Ré[2] and André Cordeiro Alves dos Santos[1]

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Endomicorritic fungi in a natural regeneration

Luciana Aparecida Giacomini: https://orcid.org/0000-0002-7647-9092

Carlos Aparecido de Siqueira Junior: https://orcid.org/0000-0002-8106-334X

Gustavo Padovani Ré: https://orcid.org/0000-0003-3782-927X

André Cordeiro Alves dos Santos: https://orcid.org/0000-0001-6323-5200

[1]Parte da Dissertação de Mestrado do primeiro Autor

[1] Universidade Federal de São Carlos, Departamento de Biologia, Rodovia João Leme dos Santos (SP-264), Bairro do Itinga Km 110, 18052-780 Sorocaba, São Paulo, Brasil
[2] Universidade Cruzeiro do Sul, Departamento de Ciências Biológicas, Praça Regente Feijó 181, Centro, 13300-023 Itu, São Paulo, Brasil
[3] Corresponding author: lucianagiacominni@gmail.com

ABSTRACT – (The influence of forest litter on the endomicorritic fungi community in a natural regeneration area in São Paulo State, Brazil). Natural areas of high biological diversity are being degraded to meet man's need to convert them to various uses. The ecological restoration through litter transposition enables for the reestablishment of ecological processes and may contribute to the increase of microorganisms. It aimed to evaluate the influence of litter on the community of mycorrhizal fungi in an area of Cabreúva, São Paulo State, Brazil. Ten plots were delimited, five control and five treatment. Soil samples were collected during two distinct periods for physical, chemical and microbiological analysis. Results indicated that the edaphic conditions of the place are consistent with the presence of AMF; also, the transposed organic matter did not increase species richness and did not facilitate the colonization of plant species, whereas gravimetric humidity and grass may have influenced species richness.

Keywords: degraded soil, ecological restoration, glomeromycota, organic matter

RESUMO – (Influência da serapilheira florestal na comunidade de fungos endomicorrízicos em área de regeneração natural no interior do Estado de São Paulo, Brasil). Áreas naturais de elevada diversidade biológica estão sendo degradadas para suprir a necessidade do homem em convertê-las em usos diversos. A restauração ecológica por meio da transposição de serapilheira possibilita o reestabelecimento dos processos ecológicos e pode contribuir com o aumento de microrganismos. Objetivou-se avaliar a influência da serapilheira na comunidade de fungos micorrízicos em uma área de Cabreúva, SP. Dez parcelas foram delimitadas, sendo cinco controle e cinco com tratamento. Foram coletadas amostras de solo em dois períodos para análises físicas, químicas e microbiológicas. Os resultados indicaram que as condições edáficas do local condizem com a presença de FMA, a matéria orgânica transposta não promoveu aumento da riqueza de espécies e não facilitou a colonização de espécies vegetais, enquanto que a umidade gravimétrica e a gramínea podem ter influenciado na riqueza de espécies.

Palavras-chave: glomeromycota, matéria orgânica, restauração ecológica, solo degradado

Introduction

Tropical forests provide key ecosystem services to humans, conserve biodiversity and regulate the climate (Berenguer *et al.* 2018). The practice of burning, the complete removal of natural vegetation, the inadequate preparation of the soil for agricultural use and exploitation, without the replacement of organic matter or nutrients from the soil, are examples of actions that directly impact the soil (SBCS 2015). Deforestation can cause soil exposure, increased vulnerability to erosive processes (Flores *et al.* 2019) and loss of biodiversity (Berenguer *et al.* 2018), **rendering the soil impacted but still capable to regenerate naturally** (IBAMA 2011). In order to restore a disturbed area, ecological restoration is a form of human intervention used to trigger or accelerate natural succession (Secretaria do Meio Ambiente do Estado de São Paulo 2014). It is based on the reestablishment of ecological processes without relying on traditional forestry techniques; it seeks to emulate nature, using minimal inputs and restoring the health, integrity and sustainability of the ecosystem through practices implemented in partial areas, called nuclei (Bechara 2006).

The purpose of the nucleation technique is to find various elements (microorganisms and seeds, among others) in forest areas, and then move them to the disturbed area in order to create small nuclear habitats that will produce environmental heterogeneity over time and space. These nuclei function as ecological triggers for the natural regeneration process, and allow for the arrival of living organisms that can establish ecological interactions (Reis *et al.* 2010, Reis *et al.* 2014). The literature describes several nucleating techniques used in the restoration process, such as soil transposition, direct seeding, hydrosowing, transposition of branches, artificial perches, and the planting of seedlings within high diversity islands (Reis *et al.* 2003).

The topmost layer of the forest soil, called serapillera or forest blanket, is a complex system formed by residues of plants and animals at various degrees of decomposition, as well as living microorganisms (De Souza *et al.* 2016). The use of biological methods such as nucleation in the restoration of areas is an economical and efficient way to promote restoration or even recovery of

these areas (Almeida 2016). The increase in serapillera favors the cycling of nutrients, the improvement of soil fertility (Primo *et al.* 2018), and can contribute to the inoculation of arbuscular mycorrhizal fungi (AMF) that interact with plants and increase the efficiency in nutrient absorption by the root system (Almeida 2016, House & Bever 2018). Mycorrhizae can be severely influenced by damage to vegetation and soil caused by natural processes or human intervention (Brundrett *et al.* 1985, House & Bever 2018). The complete removal of vegetation cover, the loss of the arable layer and **intense soil overturning** are agricultural practices harmful to mycorrhizae (De Souza *et al.* 2017). To understand the response of secondary forests to disturbances throughout the successional process, especially those that influence the dynamics of the serapillera, a better understanding of the relationships between shrub mycorrhizae and the change in vegetation cover is needed (Maia *et al.* 2015).

Due to the importance of serapillera in environmental restoration and in the establishment of endomycorrhizal fungi in the soil, the aim of this study was to assess the influence of serapillera transposition on the AMF community within a disturbed area.

Material and methods

Experimental Area - the study site is an private area located in the countryside of thexxx State of São Paulo, Brazil (UTM 7420062.55 S and 285802.26 E). According to the Köppen classification, the climate of the municipality of Cabreúva is Cwa, humid subtropical, with an average annual temperature of 19°C and average annual precipitation of 1,320 mm (CLIMATE-DATA 2019). The soil cover is formed by red-yellow clay, with a textural B horizon, high acidity, and has an increased clay ratio in deeper layers (Dos Santos *et al.* 2018). In the past, the area was utilized as pasture, for approximately two years it is **covered** by *Urochloa brizantha* (Hochst. Ex A. Rich.) R. D. Webster without management actions.

Description of The Experiment - twenty-five continuous 7 x 7 m plots were delimited with string and stakes. The grass was mowed with a brush cutter and removed from the site after ten permanent plots were selected by the Research Randomizer system (https://www.randomizer.org/): five treatment plots and five control plots. An integrated soil sample from a depth of 0-20 cm was collected according to the method laid out by De Arruda *et al.* (2014) for the determination of physical and chemical properties. Two composite samples (October 12th, 2017) from each of the ten plots were collected from a depth of 0-20 cm with the aid of an auger (De Arruda *et al.* 2014) for chemical (organic matter), physical (humidity) and microbiological analyses, totaling 20 samples.

In five of the ten plots, the treatment was implemented by transposing 1.2 liters per parcel of serapillera from the second organic sub-horizon of a fragment of Semideciduous Seasonal Forest at an intermediate stage of regeneration, located at a distance of seven kilometers from the experimental area. After nine months following treatment implementation (July 12th, 2018), a new integrated soil sample was collected for chemical analysis as well as two samples from each plot for chemical, physical and microbiological analyses.

Laboratory Analysis - the organic matter was determined through the muffle furnace method (Goldin 1987), with the following modifications: 10 grams from each soil sample were weighed in porcelain crucibles and subsequently dried in an oven at 90 ºC for 24 hours. The samples were then weighed again and placed in a muffle furnace and incinerated at a temperature of 550 ºC for one hour. Finally, the crucibles containing the sample residues were weighed as to determine the weight of the organic matter therein.

To identify the glomerospores, 50 grams of each soil sample were weighed. The glomerospores were extracted by wet sieving (Gerdemann & Nicolson 1963) and density gradient centrifugation (Daniels & Skipper 1982), and fixed on microscopy slides with PVLG (lacto-glycerol polyvinyl alcohol) and Melzer resin (De Novais *et al.* 2017). **The spores were characterized morphologically (Schenck & Pérez 1990) and identified taxonomically (Morton 2018, Blaszkowski 2018, Goto & Jobim 2019).**

The determination of the total number of spores of the species found was conducted by microscopic observation of individual slides (De Paula 2016), and the ecological indices related to species richness – total sampled individuals, absolute frequency (Fa) and relative frequency (Fr) – were determined according to the following equations:

$$Fa = ui / ut * 100 \quad (1)$$

$$Fr = Fa_i / \sum_{i=1}^{n} Fai \quad (2)$$

where,

ui= number of plots where the ith species appears in the experimental unit per group (control or treatment)

ut: total number of plots per group

Fai: absolute frequency of a given species

$\sum_{i=1}^{n} Fai$: sum of the absolute frequencies of all species.

Statistical Analysis - the software R version 3.6.0 was used to compare data concerning species richness, gravimetric humidity and organic matter of dependent samples by means of the paired t-test with a significance level of $p < 0.05$.

Results and Discussion

Evaluation of The Natural Regeneration of Plant Species - when monitoring the experimental unit, it was observed that, from the treatment implementation period (October 12th, 2017) to the second collection of samples (July 12th, 2018), there was massive growth of *Urochloa brizantha* (Hochst. Ex A. Rich.) R. D. Webster on all plots, and there was no germination of seedlings of shrub/arboreal plant species and other weeds.

The genus *Urochloa* consists of exotic invasive species that proliferate and interact negatively with native species through interspecific resource competition, cover growth and chemical inhibition through the release of secondary compounds (Horowitz *et al.* 2007, Scabora 2011). In this

competition, the exotic species is favored and may inflict a depressant effect on the native plants, eliminating them from the site, which explains their absence on the plots.

Physico-chemical Analysis of Soil Integrated Samples - the soil particle size analysis performed on the first integrated sample characterized the soil as sandy clay with an increased ratio of clay in deeper layers, which categorizes it as "argissolo" (in the Brazilian Soil Classification System), according to Dos Santos *et al.* (2018).

The chemical properties of the two integrated samples (table 1) were interpreted in accordance with Instituto Agronômico de Campinas (Raij *et al.* 1997) and Embrapa (Sobral *et al.* 2015), considering the soil with a perennial culture regarding the presence of *Urochloa*.

The second sample was shown to contain less organic matter than the first, suggesting that the organic matter underwent decomposition, considering the increment (addition of serapillera) received by the treated plots. Phosphorus content decreased by 3 mg dm^{-3} between samples. It is possible to correlate this result with the presence of *U. brizantha* in the soil because, in addition to being a good host for FMA, this plant has a high photosynthetic rate and a high demand for phosphorus in the early stages of its development (Smith & Gianinazzi-Pearson 1988, Carrenho *et al.* 2010). Another way to understand the reduction of phosphorus content between samples is offered by Quesada *et al.* (2010): after analyzing soil samples from six South American countries, including Brazil, they pointed out that the total phosphorus content may decline due to a loss of dissolved organic and inorganic phosphorus, caused by weathering processes that produce leaching or a reduction of soil mass, in addition to the permanent occlusion of soil minerals.

The high potassium content indicates the presence of primary minerals and little weathering, which is common for soils in drier regions (Sobral *et al.* 2015). Amongst micronutrients, zinc in particular is absorbed by arbuscular mycorrhizae (De Novais *et al.* 2017), which may have accounted for the reduction in its content between samples. Manganese content was considered high (Sobral *et al.* 2015), but decreased in the dry season (July), which was also observed in a study conducted by Bezerra (2017). Aluminum content also decreased in the dry season, while still maintaining levels

that did not affect sporulation. Cardoso & Kuyper (2006) reported the increased uptake of these same elements by plant-associated AMF, leading to a decrease in their content in the soil and even to a reduction in soil toxicity for the plants. Finally, results concerning the integrated soil samples from the experimental unit are consistent with the presence of AMF throughout the research.

Species Diversity - including the two sampling periods, 27 species were recorded (table 2), distributed across eight genera: *Pacispora* (one species), *Diversispora* (one), *Funneliformis* (one), *Archaeospora* (two), *Scutellospora* (three), *Dentiscutata* (four), *Acaulospora* (five) and *Glomus* (10), belonging to six families (Acaulosporaceae, Archaeosporaceae, Dentiscutataceae, Diversisporaceae, Glomeraceae, Scutellosporaceae).

Glomus is a dominant genus in areas used for different purposes, including the Atlantic Forest (Pereira *et al.* 2014), agroforestry systems (Bezerra & De Mello 2015), the Amazon (Reyes *et al.* 2019) and other Brazilian ecosystems (Da Silva *et al.* 2014). In this study, we found this genus present in disturbed areas with soil pH between 4.5 and 4.6, as pointed out by Borba & De Amorim (2007) when they reported on *Glomus* dominance in two disturbed areas. Its high prevalence in both rainy and dry seasons indicates greater sporulation capacity and adaptability to soil conditions (Caproni *et al.* 2003).

The second genus in terms of highest richness and absolute frequency was *Acaulospora*, located in areas where the pH ranged between 4.4 and 5.0, as found by Da Silva *et al.* (2006). However, neither genera are restricted to the edaphic conditions of the experimental unit and the Brazilian climate. They are widely distributed, being found across seven continents: North America, South America, Africa, Europe, Asia, Antarctica and Oceania, and within the four climate zones: tropical, subtropical, temperate, and boreal/austral (Stürmer *et al.* 2018). *Glomus* and *Acaulospora* are the world's highest species-rich genera, with 54 and 52 species respectively, described in the Glomeromycota clade (Da Silva *et al.* 2014; Goto & Jobim 2019).

The species *Dentiscutata erythropus*, *Dentiscutata reticulata*, *Glomus aggregatum*, *Glomus heterosporum*, *Glomus macrocarpum*, *Funneliformis geosporum*, *Archaeospora trappei* are found on four or more continents and are considered to be cosmopolitan in distribution (Stürmer *et al.* 2018).

Considering all identified species (n = 27), 14 were found in all groups in both sampling periods. The species *Acaulospora alpina* and *Glomus aggregatum* did not appear in the first period, which suggests a propensity for sporulation in the dry season. Total species richness displayed a tendency to decrease by six (species 2, 11, 14, 20, 26 and 27) between sampling periods. This trend is probably explained by sporulation inactivity in the dry season, but the number of sample plots in this study was small, thus it is believed that a larger number of plots could strengthen the reliability of this assumption. Studies aiming to investigate AMF diversity in disturbed soil with varying gravimetric moisture and organic matter percentages throughout the year may provide guidance in understanding the species most susceptible to these variables.

Physicochemical and Microbiological Analysis of Individual Soil Samples - when comparing the percentages of gravimetric humidity, the control group showed no significant difference ($p = 0.11$) between the samples, whereas the treatment group exhibited a significant difference ($p = 0.01$) between the sampling periods; this difference may be indirectly related to the presence of litter. As the humidity of all plots decreased, species richness decreased in four of the five plots, so it can be assumed that the difference ($p = 0.06$) found between species over the two periods is significant (figure 1).

The AMF species richness of the control group ($p = 0.91$) and the percentage of organic matter in both control ($p = 0.32$) and treatment groups ($p = 0.81$) did not show significant differences between sampling periods (figure 1). The wide variety of niches and opportunities in severely degraded areas also contributes to the greater richness of AMF species at the beginning of plant succession, according to research conducted in forest areas at various successional stages in the municipality of Alcântara, MA (Reyes *et al.* 2019). However, the reduction in AMF richness over time in the treatment group may be related to reduced fungal niche competition, higher population stability and a predominance

of k-strategist species, with occasionally low levels of sporulation and better performance in competitive survival, as mentioned by Pereira *et al.* (2014). The amount of litter transposed into the treatment plots was not sufficient to promote a significant difference in the total percentage of organic matter between samples.

The richness of AMF species in the treatment group during the dry season may have been influenced by gravimetric humidity and the presence of *Urochloa brizantha*. In order to make the restoration of the area possible, its complete removal is indispensable; manual or mechanized removal techniques, however, may produce changes in the physical and microbiological structure of the soil. The adoption of a chemical approach, in addition to contaminating the soil, may affect the life cycle of microorganisms, so it becomes necessary to develop a technique that has minimal impact on soil structure and composition.

Therefore, based on the above, in order to improve agricultural practices, as well as preserve soil health and quality and specially to reduce the use of phosphorus-based chemical inputs, the genera found in this research can be used in the municipality's agricultural crops.

Conclusion

The transposition of litter from the Semideciduous Seasonal Forest to the soil disturbed with *Urochloa brizantha* did not promote increased richness in arbuscular mycorrhizal fungus species, and did not facilitate the colonization of plant species that are useful in the ecological restoration process, thus suggesting that, before actions aimed at recovering an area are taken, the grass must be removed completely.

Conflicts of interest

The authors have no conflicts of interest to declare.

Author Contributions

Luciana Aparecida Giacomini: were involved in the study concept, design, implementation, data collection, data analysis, writing, critical revision and reviews of the manuscript versions.

Carlos Aparecido de Siqueira Junior and Gustavo Padovani Ré: were involved in data collection and data analysis.

André Cordeiro Alves dos Santos: were involved in design, data analysis, critical revision and reviews of the manuscript versions.

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Table 1. Chemical analysis of integrated soil samples in the two sampling periods: Oct 2017 (1) and Jul 2018 (2) at the experimental unit of the Sol site, Cabreúva, São Paulo State, Brazil. *Based on Technical Bulletin #100 - Raij *et al.* (1997). ** Based on Documents #206 - Sobral *et al.* (2015).

Attribute (method)	Units	Values		Note* **
		Sample 1	Sample 2	
Organic matter (oxidation)	g dm^{-3}	40	31	Clayey soil*
pH (active acidity) - KCl	-	4,5	4,6	Presence of exchangeable aluminium**
Al^{3+} (exc. acidity) - KCl	$mmol_c$ dm^{-3}	2	1	Low**
Phosphorus (resin)	mg dm^{-3}	16	13	Medium*
Calcium (resin)	$mmol_c$ dm^{-3}	22	30	High*
Magnesium (resin)	$mmol_c$ dm^{-3}	8	11	High*
Potassium (resin)	$mmol_c$ dm^{-3}	4,7	3,4	High*
Cation Exchange Capacity– CTC (SB+ (H+Al))	$mmol_c$ dm^{-3}	98,4	79,6	Presence of clay**
Iron (DTPA)	mg dm^{-3}	158	63	High*
Copper (DTPA)	mg dm^{-3}	1,4	1	High*
Zinc (DTPA)	mg dm^{-3}	4,5	3,1	High*
Boron (hot water)	mg dm^{-3}	0,26	1,05	Medium to high*
Manganese (DTPA)	mg dm^{-3}	13,0	11,6	High*

Table 2. Arbuscular Mycorrhizal Fungi; absolute frequency (Fa) and relative frequency (Fr) of the AMF species collected over the two sampling periods: Oct 2017 (1) and Jul 2018 (2), at the experimental Sol site in Cabreúva, São Paulo State, Brazil.

Family/Species	Control 1	Treatment 1	Control 2	Treatment 2
	Frequencies (%): Fa (Fr)			
Acaulosporaceae J.B Morton & Benny				
Acaulospora alpina Oehl, Sykorova & Sieverd	0(0,00)	0(0,00)	100(8,06)	100(8,62)
Acaulospora capsicula Blaszk.	20(1,59)	100(5,62)	0(0,00)	0(0,00)
Acaulospora gedanensis Blaszk.	100(7,94)	100(5,62)	100(8,06)	100(8,62)
Acaulospora sp. 1	80(6,35)	100(5,62)	100(8,06)	100(8,62)
Acaulospora sp. 2	40(3,17)	80(4,49)	20(1,61)	20(1,72)
Archaeosporaceae J.B. Morton & D. Redecker				
Archaeospora sp. 1	0(0,00)	20(1,12)	20(1,61)	40(3,45)
Archaeospora trappei (R.N. Ames & Linderman) J.B.Morton & D.Redecker	80(6,35)	100(5,62)	80(6,45)	80(6,90)
Dentiscutataceae Sieverd., F.A. Souza & Oehl				
Dentiscutata erythropus (Koske & C. Walker) C. Walker & D. Redecke	20(1,59)	40(2,25)	40(3,23)	60(5,17)
Dentiscutata nigra (J.F. Readhead) Sieverd., F.A. de Souza & Oehl	100(7,94)	100(5,62)	100(8,06)	100(8,62)
Dentiscutata reticulata (Koske, D.D. Mill. & C. Walker) Sieverd., F.A. Souza & Oehl	100(7,94)	100(5,62)	60(4,84)	40(3,45)
Dentiscutata sp. 1	20(1,59)	60(3,37)	0(0,00)	0(0,00)
Diversisporaceae C. Walker & A. Schüssler emend. Oehl, G.A. Silva & Sieverd.				
Diversispora sp. 1	80(6,35)	80(4,49)	40(3,23)	60(5,17)
Pacispora sp. 1	0(0,00)	20(1,12)	20(1,61)	40(3,45)
Glomeraceae Piroz. & Dalpé emend. Oehl, G.A. Silva & Sieverd.				
Funneliformis geosporum (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler	0(0,00)	40(2,25)	0(0,00)	0(0,00)
Glomus aggregatum N.C. Schenck & G.S. Sm. emend. Koske	0(0,00)	0(0,00)	40(3,23)	20(1,72)
Glomus geosporum (T.H. Nicolson & Gerd.) C.Walker	80(6,35)	100(5,62)	40(3,23)	20(1,72)
Glomus glomerulatum Sieverd.	100(7,94)	100(5,62)	60(4,84)	60(5,17)
Glomus heterosporum G.S.Sm. & N.C. Schenck	0(0,00)	60(3,37)	40(3,23)	40(3,45)
Glomus macrocarpum Tul. & C. Tul.	100(7,94)	100(5,62)	80(6,45)	60(5,17)
Glomus rubiforme (Gerd. & Trappe) R.T. Almeida & N.C. Schenck	20(1,59)	40(2,25)	0(0,00)	0(0,00)
Glomus sp. 1	100(7,94)	100(5,62)	100(8,06)	100(8,62)
Glomus sp. 2	100(7,94)	100(5,62)	100(8,06)	60(5,17)
Glomus sp. 3	40(3,17)	40(2,25)	40(3,23)	0(0,00)
Glomus sp. 4	20(1,59)	60(3,37)	60(4,84)	20(1,72)
Scutellosporaceae Sieverd., F.A. Souza & Oehl				
Scutellospora sp. 1	40(3,17)	100(5,62)	0(0,00)	40(3,45)
Scutellospora sp. 2	0(0,00)	40(2,25)	0(0,00)	0(0,00)
Scutellospora sp. 3	20(1,59)	0(0,00)	0(0,00)	0(0,00)

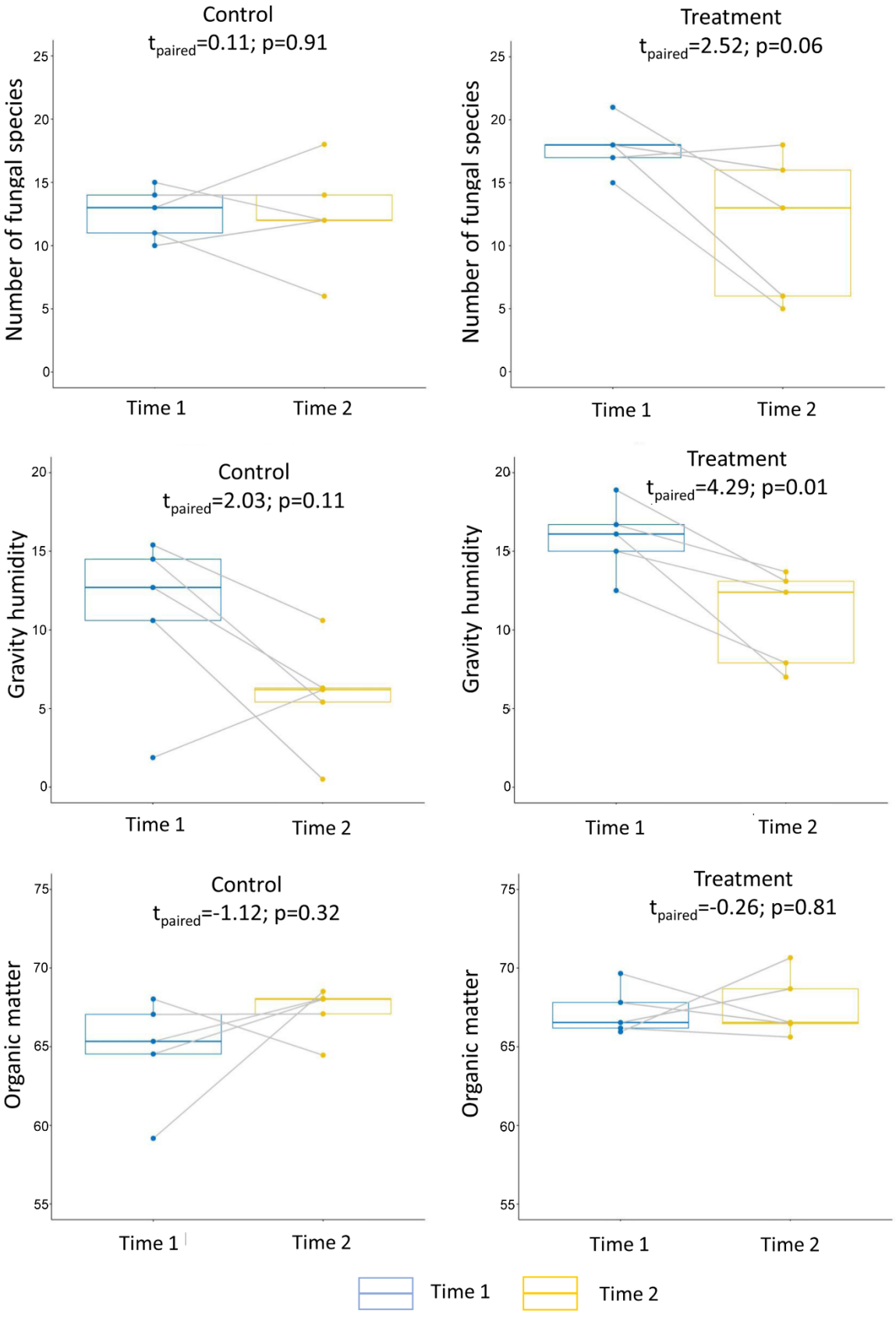

Figure 1. Paired t-test of AMF species richness, percentages of gravimetric moisture and organic matter of individual soil samples in the two sampling periods: october 2017 (1) and july 2018 (2) at the experimental Sol site in Cabreúva, São Paulo State, Brazil.

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