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Proceeding Paper

Effect of Land-Use Change over Arbuscular Mycorrhizal Fungi Diversity in an Argentinean Endemic Native Forest †

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Abstract: Arbuscular mycorrhizal fungi (AMF, Glomeromycota) are biotrophic mutualistic symbionts of 80% of the terrestrial plants. AMF increase their hosts' growth through its contribution to the water and nutrients absorption from soil to the plant roots. The different AMF taxa vary in their edaphic and nutritional preferences, the host species ranges and the seasonal changes in sporulation features. The increase in world human population and the global demand for natural resources have acted as an important driving force for agricultural changes in Argentina in the last 150 years. Particularly, the *Prosopis caldenia* Burkart forests (or "Caldenales") have suffered an important reduction in the last 10 years. Here, we studied AMF abundance and diversity in four land uses and their relationship with soil and vegetation characteristics. The land use selected were Native Forest (Caldenales), *Eragrostis curvula* (Schrad.) Nees pasture, *Medicago sativa* L. cropfield and soybean (*Glicine max* (L.) Merill) cropfield. AMF spores were extracted from soil by the traditional method and were identified by morphological features. Cluster analysis divided the land uses into two groups, Kruskal-Wallis show significant differences in AMF abundance and richness between land uses, AMF abundance and tree richness were negatively correlated, showing less abundance of AMF spores in the plots with the highest richness of tree species. Our results suggested that land use and vegetation richness have a strong influence on the AMF community. Agricultural activities would negatively influence AMF species diversity but would not affect negatively spores abundance.

Keywords: Mycorrhiza; forest; crops

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1. Introduction

Arbuscular mycorrhizal fungi (AMF) are biotrophic mutualistic symbionts associated with most terrestrial plants and their effect on the growth of plant species is well known due to their contribution to nutrient absorption [1]. This symbiosis is established through the colonization of the roots of its hosts and through the mycelial network that also prevents erosion and interconnects the plants, redistributing resources [2,3].

AMF spores are fundamental components of soil microorganism communities and are propagules that remain dormant in the absence of hosts [1]. Spore formation may represent a crucial life-history strategy of AM fungi for surviving in periodically disturbed habitats such as cultivated agro-ecosystems [4]. Land uses influence the diversity of these fungi and their sporulation.

Semiarid forests dominated by "Caldén" (*Prosopis caldenia* Burkart, Fabaceae), a xerophilous deciduous tree species endemic from Argentina that thrives at the dry edge of the Pampas. Caldén's woodlands originally covered 169,333 km^2 in central Argentina but these forests have been severely affected by deforestation for more than one century [5,6].

In the Caldén forest, the current deforestation rate of 0.82% per year is mainly due to the conversion of woodlands into grazing pastures such us lucerne (*Medicago sativa* L.) and weeping lovegrass (*Eragrostis curvula* (Schrad.) Nees) cropfields, and croplands as corn (*Zea mays* L.), soybean (*Glicine max* (L.) Merill) and sunflower (*Helianthus annuus* L.) [7]. Only 18% of the original Caldén's woodland is still in place, covering approximately 8438 km^2 [6,7].

Native forests, croplands, and the microorganisms associated with them are very important because the microbial communities of soils have an important influence on primary production and the health of natural and agricultural ecosystems. Considering non-existing data describing AMF diversity and its relation with land use in the Caldén woodland region, we undertook a study of AMF abundance and diversity in the most common land uses in the region and their relationship with soil and vegetation characteristics. The land use selected were Caldén Forest, *Eragrostis curvula* pasture, *Medicago sativa* cropfield and soybean cropfield

2. Materials and Methods

2.1. Study Area

Caldén forests are semiarid woodlands covering about 170,000 km^2 of central Argentina. This xerophytic open forest is a transitional ecosystem between the Pampas grasslands and the dry Monte shrublands. This phytogeographical area is denominated Espinal and subdivided in three regions or districts. The Caldén region is dominated by forest of Caldén tree (*P. caldenia*) an endemic species in Argentina. These woodlands thrive on the edge of the driest area of the Argentinean Pampas, at 34–36° S and 64–66° W [8,9]. Across its natural distribution area, the total annual precipitation varies from 450 to 620 mm, and it is concentrated in the spring and summer months (78%, from October to March). Temperature ranges from the annual isotherms of 16–18 °C. The area is a well-drained plain with moderate slopes produced by wind and fluvial processes [7].

2.2. Experimental Design

In the district of Caldén, four land uses were analyzed: Caldén forest (Forest), weeping lovegrass (WL), perennial Lucerne monoculture (Lucerne), and soybean monoculture (Soybean) with four replicates each. A total of 16 sites were selected for sample collection (Figure 1).

In the summer of 2017, 16 permanent plots of 90 m × 30 m were established and soil samples were collected. In each plot, 1 soil sample was collected. The sample was composed of 5 subsamples extracted at each vertex of the plot and the center of it, the 5 subsamples were placed in a plastic bag, homogenized, and transported to the laboratory for further analysis.

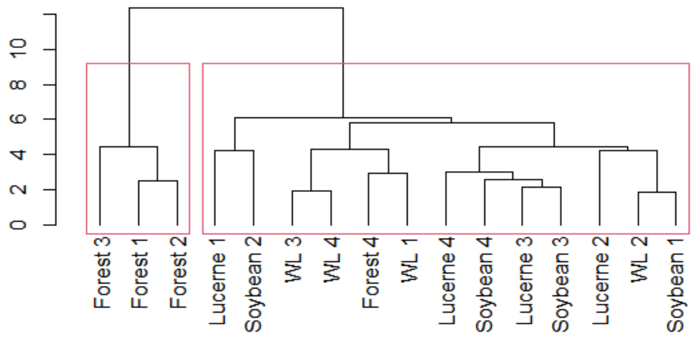


Figure 1. Cluster analysis showing two groups that include all plots in Caldén region.

2.3. AMF Diversity

The collected samples were dried at room temperature for 48 hours. A portion (100 g) of soil was processed for the analysis of AMF diversity following the methods of wet sieving and decantation [10], and sucrose gradient centrifugation [11]. AMF spores and sporocarps were isolated and mounted in a 70% *v*/*v* glycerin:water solution and in a mixture of this solution with Melzer's reagent.

The spore characteristics were compared with the reference isolates described by INVAM (International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu), the descriptions of the species compiled by Schenck & Perez (1990)[12] and Błaszkowski (2012) [13] were used; the systematic location followed the proposal by Tedersoo et al. (2018) [14]. Taxonomic identification and quantification was performed in an optical microscope at 40× and 100× magnification.

2.4. Soil Analysis: Physical and Chemical Properties

Soil pH and electrical conductivity were determinate by the saturation paste method, percentage of organic matter using the Walkley-Black method, percentage of total nitrogen content by micro Kjeldahl method, phosphorus content by the Bray & Kurtz method. Soil texture, which represents the granulometric distribution of its constituents (the proportion between small particles: clay, silt and sand), was calculated using the Robinson's pipette method [15,16].

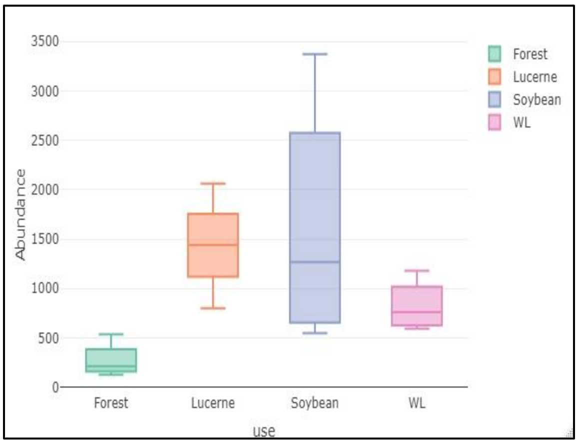
2.5. Statistical Analysis

Physical and chemical soil properties, abundance and richness each land use, were compared using a non-parametric Kruskal-Wallis test was performed followed by a Dunn's multiple comparison test, a $p < 0.05$ was considered significant for all tests. The correlation between the variables richness and abundance with the physical-chemical variables of the soil were analyzed with the Spearman correlation coefficient. The abundance of each AMF species in each plot was used to perform a cluster analyses. All statistical analyzes were carried out in R software [17].

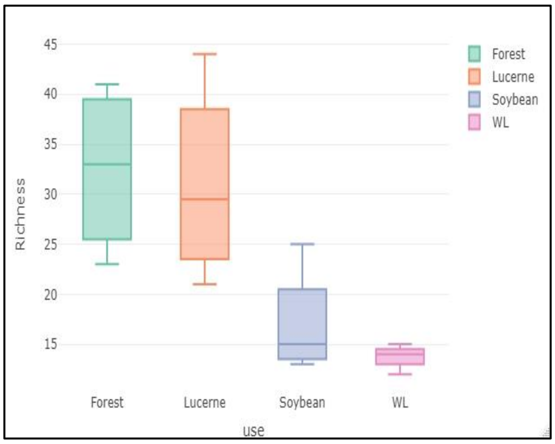
3. Results

3.1. AMF Diversity

Abundance and richness showed significant differences when the studied land uses were compared. Soybean plots showed the highest abundance and forest plots the lowest abundance. Richness was highest in the woods and lucerne plots, richness was lowest in the weeping lovegrass plots (Table 1).



(**a**)



(**b**)

Figure 2. Box plot of different land uses in Caldenal (**a**) AMF spore abundance; (**b**) AMF species richness. Reference: WL, weeping lovegrass.

Table 1. AMF Richness and abundance in different land uses in Caldén region.

Variable	Forest	Weeping Lovegrass	Lucerne	Soybean
Richness	32.5 ± 4.21 ^{a}	13.65 ± 0.63 ^{b}	31 ± 4.98 ^{ac}	17 ± 2.74 ^{bc}
Abundance	271.5 ± 90.77 ^{a}	822 ± 131.23 ^{ab}	1435.25 ± 257.43 ^{b}	1613.25 ± 644.23 ^{b}

Values are the mean ± SE. Different lowercase letters in each column indicate significant differences between the means.

3.2. Soil Analysis

The content of carbon, phosphorus and organic matter, and electrical conductivity showed significant differences when comparing the different land uses (Table 2).

Table 2. Soil chemical characteristic in different land uses in Caldén region.

Variable	Forest	Weeping Lovegrass	Lucerne	Soybean
Carbon (%)	0.026 ± 0.004 ^{a}	0.026 ± 0.003 ^{a}	0.015 ± 0.001 ^{b}	0.017 ± 0.002 ^{ab}
Phosphorous (g/kg)	33.05 ± 0.77 ^{a}	25.05 ± 1.27 ^{ab}	23.33 ± 0.54 ^{b}	24.33 ± 1.52 ^{b}
Nitrogenous (mg/g)	0.066 ± 0,0020 ^{a}	0.064 ± 0,0031 ^{a}	0,068 ± 0,0024 ^{a}	0.071 ± 0,0034 ^{a}
Electrical Conductivity (dS/ms)	0.31 ± 0.02 ^{a}	0.29 ± 0.02 ^{a}	0.26 ± 0.01 ^{a}	0.29 ± 0.01 ^{a}
Organic Mater (%)	1.40 ± 0.20 ^{a}	1.05 ± 0.08 ^{a}	0.90 ± 0.05 ^{ab}	0.80 ± 0.03 ^{b}
pH	6.42 ± 0.17 ^{a}	6.18 ± 0.08 ^{a}	5.95 ± 0.11 ^{a}	5.91 ± 0.06 ^{a}

Values are the mean ± SE. Different lowercase letters in each column indicate significant differences between the means.

3.2. Cluster Analysis

The cluster analysis grouped the studied plots into two large groups. A group formed only by 3 forest plots and another formed by the rest of the plots (1 forest plot, and all weeping lovegrass, lucerne and soybean plots (Figure 1).

4. Discussion and Conclusions

The diversity of arbuscular mycorrhizal fungi, measured by spore abundance and species richness, was affected by land use in the Caldén region, Espinal phytogeographic province, Argentina.

The highest abundance of AMF spores was found in the soybean plots, and the lowest abundance in the forest plots. There are different explanations for this result. First, the land use change and the consequent replacement of natural vegetation and soil disturbance could favor the proliferation, establishment and dominance of species with ruderal life histories, which allocate a significant amount of energy in the early spore production [18] and appears to be dominant in disturbed environments [19]. Second, the phosphorus concentration is a variable that has a close relationship with the spores abundance [1], and in our study, the plots that showed the lowest abundance had the highest phosphorus concentration. Also, a negative correlation was observed between the spore abundance and the tree species richness, which can be explained by the fact that AMF species generally live in the fine roots of plants [20], which trees do not have being able to also have more competition when colonizing the root and fulfilling the life cycle and sporulation.

Richness of AMF also showed significant differences between the land use conditions. Thus, the highest number of species were found in the forest and lucerne plots; on the other hand, the lowest richness was observed in the weeping lovegrass and soybean plots. These differences can be explained by the disturbance in the soil caused by deforestation and by the constant and sustained physical disturbance that the weeping lovegrass plots have due to their constantly grazed condition. In the case of soybean plots, the lower richness can be explained by the type of cultivation that is carried out, because after soybean harvest these plots are not sown with other plant species and the soils remain bare until the next soybean culture, so the AMF species do not have hosts and should hold out until the next planting season. The forest support highest diversity because have high plant diversity. In fact, great plant diversity would increase the variety of host availability to the biotrophic and symbiotic AMF and their different mycorrhizal traits [21,22].

The cluster analysis of the studied plots resulted in two groups. One of them is made up of only three forest plots and the other is made up of the rest of the crop species (weeping lovegrass, lucerne, and soybean plots and one of forest. This plot grouping might suggest that most of the forest plots are similar to each other in their different components and have a similar ecosystem functioning in relation to the AMF spore diversity. On the other hand, the different crops have significant degrees of similarity, which would show that deforestation and land use change have produced similar effects on the AMF communities that inhabit them.

In general, our results suggests that the change in land use, mainly through agricultural activity and deforestation, affects the AMF communities that inhabit the soil of the Caldenal district, which undoubtedly has a negative effect on its diversity of AMF spores in terms of richness, abundance and communities composition.

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