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Proceeding Paper

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

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Abstract: Arbuscular mycorrhizal fungi (AMFs, Glomeromycota) are biotrophic mutualistic symbionts of 80% of terrestrial plants. AMFs increase their hosts' growth through their contribution to water and nutrient absorption from the 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 the 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 uses selected were native forest (Caldenales), *Eragrostis curvula* (Schrad.) Nees pasture, *Medicago sativa* L. cropfield and soybean (*Glycine max* (L.) Merill) cropfield. AMF spores were extracted from soil by the traditional method and were identified by their morphological features. Cluster analysis divided the land uses into two groups; Kruskal–Wallis tests showed significant differences in AMF abundance and richness between land uses; the 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 suggest that land use and vegetation richness have a strong influence on the AMF community. Agricultural activities would negatively influence the AMF species diversity but would not negatively affect the spore abundance.

Keywords: mycorrhiza; forest; crops

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1. Introduction

Arbuscular mycorrhizal fungi (AMFs) 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 their 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.

In Argentina, some semiarid forests are dominated by "Caldén" (*Prosopis caldenia* Burkart, Fabaceae), a xerophilous deciduous tree species endemic in 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 such as corn (*Zea mays* L.), soybean (*Glycine max* (L.) Merill) and sunflower (*Helianthus annuus* L.) fields [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 the lack of 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 uses 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 into three regions or districts. The Caldén region is dominated by forest of the Caldén tree (*P. caldenia*), an endemic specie 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). The average temperature ranges from 16 to 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).

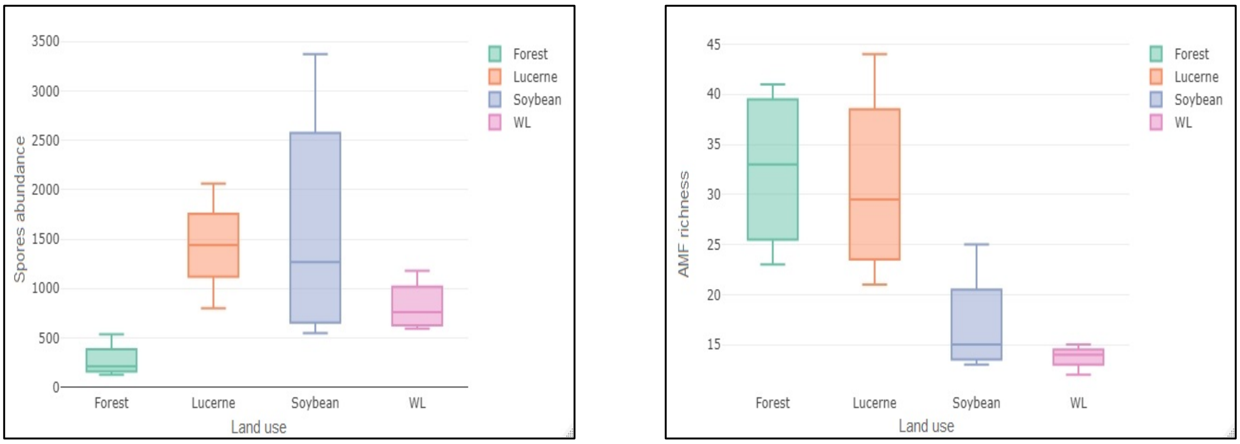
In the summer of 2017, 16 permanent plots of 90 m × 30 m were established and soil samples were collected. In each plot, 1 soil sample was collected. The sample was composed of 5 subsamples extracted at each vertex of the plot and the center of it, and the 5 subsamples were placed in a plastic bag, homogenized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

2.3. AMF Diversity

The collected samples were dried at room temperature for 48 h. A portion (100 g) of soil was processed for the analysis of the 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 those of the reference isolates described by INVAM (International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu; accessed on 26 September 2020), and the descriptions of the species compiled by Schenck and 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 were performed using an optical microscope at 40× and 100× magnification.



(**a**) (**b**)

Figure 1. Box plots of different land uses in Caldén district: (**a**) AMF spore abundance; (**b**) AMF species richness. Reference: WL, weeping lovegrass.

2.4. Soil Analysis: Physical and Chemical Properties

The soil pH and electrical conductivity were determined by the saturation paste method, the percentage of organic matter was determined using the Walkley–Black method, the percentage of total nitrogen content was determined by the micro-Kjeldahl method, and the phosphorus content was determined by the Bray and Kurtz method. The 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

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

3. Results

3.1. AMF Diversity

The abundance and richness showed significant differences when the studied land uses were compared (Table 1). The soybean plots showed the highest abundance, and the forest plots, the lowest abundance (Figure 1a). The richness was the highest in the woods and lucerne plots, and the richness was the lowest in the weeping lovegrass plots (Figure 1b).

Table 1. AMF richness and abundance in different land uses in the 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 means ± SEs. Different lowercase letters in each column indicate significant differences between the means.

3.2. Soil Analysis

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

Table 2. Soil chemical characteristics in different land uses in the 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 Matter (%)	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 means ± SEs. Different lowercase letters in each column indicate significant differences between the means.

3.3. Cluster Analysis

The cluster analysis grouped the studied plots into two large groups. A group was formed by only three forest plots, and another was formed by the rest of the plots, with the (1) forest plot and all the weeping lovegrass, lucerne and soybean plots (Figure 2).

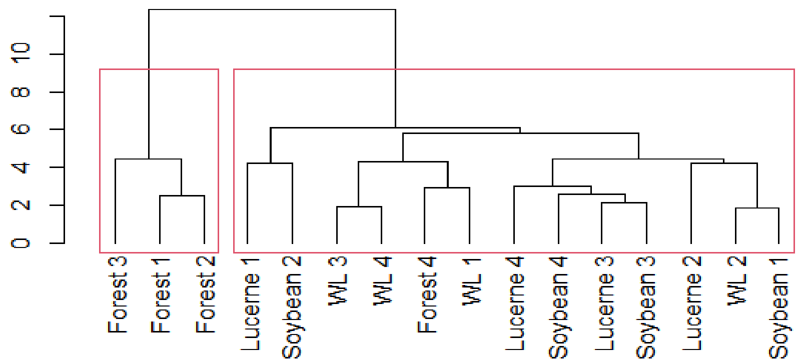


Figure 2. Cluster analysis showing two groups that include all the plots in the Caldén region.

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 early spore production [18] and appear to be dominant in disturbed environments [19]. Second, the phosphorus concentration is a variable that has a close relationship with the spore abundance [1], and in our study, the plots that showed the lowest abundance had the highest phosphorus concentrations. Additionally, a negative correlation between the spore abundance and the tree species richness was observed, 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 completing the life cycle and sporulation.

The richness of the AMFs also showed significant differences according to the land-use conditions. Thus, the highest number of species was 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 the 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 supports the highest diversity because it has high plant diversity. In fact, great plant diversity would increase the variety of hosts available 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 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 suggest 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 community composition.

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