Mycobiota present in soils under two tillage systems in Buenos Aires province, Argentina

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Abstract

The present study aimed to describe and compare the fungal soil community under two systems of tillage: direct sowing and conventional tillage. This work was performed in the context of a twelve-year long-term trial, where crops and pasture rotation as well as tillage systems were tested. It was conducted in the experimental field of the National Atomic Energy Commission, located in the province of Buenos Aires. Soil samples from each plot were collected separately. The Simpson Index (1-D) for each plot was also calculated separately. For plate fungus isolation, the plate dilution technique was used. The results were expressed in CFU $g^{-1}$of soil. The fungal genera identified under both tillage systems were characterized by their degree of similarity through hierarchical cluster analysis. The total number of CFU g^{-1} of soil was higher in conventional tillage compared to direct sowing. The diversity index values were very similar for both tillage systems, with a slight difference in favor of direct sowing. The relatively high alfa diversity for both systems would indicate that the stability of the fungal community that presupposes the value obtained was not altered by conventional tillage.

Keywords *Fungi, Soil, Tillage, Community, Diversity.*

I. INTRODUCTION

The soil is an environment that presents a great variety of microhabitats for the microbial biodiversity, which includes virus, bacteria, fungi, seaweeds and protozoa [1]. Among these microorganisms, the bacteria are the most abundant and the fungi represent the mayor percent of the microbial biomass in the soil. In agricultural soils, the fungi are more numerous in the surface layers where there is a great diversity of genera. These genera also differ from those that are detected at greater depth [2].

Among the functions of fungi in the soil, their contribution in the degradation of organic matter and particle aggregation, stand out. Also certain species of *Alternaria, Cladosporium, Gliocladium, Humicola* and *Helminthosporium*, among others, produce similar substances to the humics substances of the soil. Therefore, they can be important in the maintenance of soil organic matter. The fungi are able to metabolize carbon compounds of very difficult degradation, like cellulose, hemicelluloses and lignin, as well as simple sugars, alcohols, amino acids and nucleic acids. Therefore, the fungi play important roles in the ecosystem processes, i.e. they decompose substances that in other way would accumulate like waste, they have cooperative-and parasitic-relationships with animals and plants, they participate in carbon cycling, and they mediate processes that involve plants nutrition and survival[3].

The fungi are very important in soils with crop residues, where their fast growing and intense degrading activity allow to keep the ecosystem in balance. They produce enzymes and metabolites that contribute to softening and transformation of the organic substances [4] [5].The fungi that are most frequently associated with plant roots include different species of *Aspergillus, Penicillium, Rhizopus* and *Trichoderma*. *Aspergillus* and *Penicillium* mobilize soil phosphorous and nitrogen and *Trichoderma* keeps moisture in the roots in drought conditions. The crops and the tillage systems affect the composition of the fungi community. The land use pattern influences the abundance and diversity of soil fungi [6].

The fungi that are developed in direct sowing are better adapted to interact with roots, in decomposition as a source of food and they can exist as root endophytes. While in conventional tillage, the fungi can use mature plants residues as pioneer colonizers and then produce large quantities of conidia [7]. Nevertheless, a mayor proportion of the fungi community is not affected by tillage and they can be specialized and generalists. Other authors found that one third of the identified fungi responded to the type of tillage. *Fusarium* as an example was more abundant in direct sowing and with corn as previous crop. While *Phoma* showed a significant association with conventional tillage and rapeseed previous to the crop [8].

The present paper was proposed with the objective to describe and compare the fungi community in the soil under two tillage systems: direct sowing and conventional tillage.

II. MATERIALS AND METHODS

Environment conditions

The study was conducted in the context of a crop and a pasture rotation test under different tillage systems, installed in 2005. Considered as a twelve-year long-term trial, in the experimental field of the National Atomic Energy Commission (CNEA), located in Ezeiza,34° 49´00"S, 58° 34´17" W, Province of Buenos Aires, in the sub-region of Undulating Pampa. The agricultural cycle present the following crop rotation: soybean as first crop (2005/06); wheat/ soybean double-cropping scheme (2006/07); corn (2007/08); soybean as first crop (2008/09); wheat (2009); corn (2010/11); soybean as first crop (2011/12); soybean as first crop (2012/13); corn (2013/14); wheat (2014) and pasture (2015).

The climate is humid temperate, the average annual rainfall is 1050.4 mm (source: Ezeiza Meteorological Station series: 1959-2009). An annual ETP of 826 mm, without water deficit at any time of the year. The highest rainfall occurs during spring. The winter temperature is 10.7°C. The field trial is located in a landscape with normal relief and an average slope of 1%.

The field was carried out in a Vertical Argiaquoll (Soil Survey Staff, 2006), with the following profile sequence: Ap, A2, BA, Bt1, Bt2, BC. It has a moderate phosphorus and nitrogen content and is imperfectly drained. The reaction throughout the profile is neutral to slightly acidic and the organic matter content is approximately 4,31% (with a range between 2,99 to 5,78%). The soil shows features of hydromorphism (mottled Fe and Mn<9 from the BA horizon.

Soil Sampling

The soil samples were collected at three specific points on each plot to obtain a composite sample (250 g), at a depth of 10 cm. Plots harvest was done by separate according to the different treatments: direct sowing and conventional tillage. Once the soil samples were obtained they were air dried for 15 days running in Petri dishes at the FCA-UNLZ Soils Laboratory. Once dried, the samples were screened with a 2 µ mesh to remove small stones and larger plant material.

Fungi Isolation

For the isolation and identification of fungi, 10 g of each sample of sieved soil were taken. Each sample was processed using the plate soil dilution isolation technique, for which successive dilutions were obtained in sterile distilled water. A 10^{-3} dilution was plated in Petri dishes containing 2% potato dextrose agar, three repetitions were performed for each sample and incubated for 8 days at 22°C for subsequent colony count.

Colony Count and Identification

After the incubation, the colony forming units (CFUs) were counted and the results were expressed in CFUs per gram of soil (CFUs g^{-1}). For the taxonomic identification of the fungal genera, macroscopic observations of the colonies as well as microscopic observations of the fungal structures were made. Microscopic observations required preparations on slides and cover slips using physiological serum as a mounting solution. Specific taxonomic keys were consulted [9] [10] [11] and once the microorganisms were identified their relative frequency was computed.

Data analysis

The trial was designed according to an experimental design consisting of completely randomized blocks (DCA) with two treatments: Direct Sowing (SD) and Conventional Tillage (LC) with four repetitions. Each plot consisted of a 250 m^2 surface and was subjected to the same treatment since the trial was installed in 2005. In the plots under SD, no tillage work was carried out, they were only treated with 3 L ha^{-1} of glyphosate before sowing. The plots with LC consisted of a single pass of moldboard plow and two passes of a disc harrow.

The fungal genera identified in both sites were characterized by their degree of similarity through hierarchical cluster analysis, using the InfoStat program [12]. The attributes used in the characterization were the following: CFUs g^{-1} of soil and relative frequency for each genus separately using the following formula: N° CFUs g^{-1} genus / total of CFUs g^{-1} of soil.

Other attributes used were: phyto-pathological potential of the fungal specimens, resistance structures, expansive growth and main soil habitat; for these attributes, presence (1) and absence (0) was considered. The analysis also included the analysis of variance.

The Simpson Index (1-D) [13] for each site was also calculated separately, using the formula $D = \Sigma\, n\,(n-1) / N\,(N-1)$. Where n = total number of CFU of a particular genre and N = total number of CFU of fungal genera.

III. RESULTS AND DISCUSSION

Conventional tillage

Count and Identification of Fungal microorganisms

Within the plots under conventional tillage, eleven fungal genera were detected (Figure 1). Among which species of the genera *Aspergillus, Penicillium* and *Rhizoctonia* showed the highest values in CFU g^{-1} of soil, 5500, 4200 and 3500, respectively. While the lowest values corresponded to species of the genera *Pythium, Sclerotinia* and *Phytopthora* with 600 and 200 CFU $g^{-1}$of soil respectively. Among all the fungi identified, species of Ascomycota genera

predominated, followed by Pseudofungi genera species.

The prevalence of *Aspergillus* and *Penicillium* species coincides with the results shown in other studies [14]. As for the prevalence of Ascomycota observed, it has been associated with the functional role of key decomposers in agricultural soils [15]. As for the soil-borne pathogenic root fungi and oomycetes (*Pythium* and *Phytopthora*) identified, the high relative value of *Rhizoctonia* CFUs is highlighted compared to oomycete and *Fusarium* values. Since *Rhizoctonia* survives in the soil associated with crop debris [16], it is found mainly in the 10-15 cm of the upper soil layer. The large amount of CFU that was determined in the present study in soil samples under conventional tillage is surprising. This could be explained by the fact that the fungi of the *Rhizoctonia* complex are present in a wide variety of habitats [17]. Finally, the count of 16,400 CFU g^{-1} of soil (out of a total of 37,120 CFU) corresponding to Mycelia sterilia (mainly hyaline hyphae) is in the range of the values cited in the literature. It has been reported that the CFU of Mycelia sterilia may account for more than half of the total CFU isolated from soil [18].

In relation to the calculation of the fungal biodiversity, it was obtained a Simpson biodiversity index (1-D) equal to 0.73545, which indicates the presence of an alfa diversity relatively high in the soil samples analyzed.

According to the grouping analysis performed (cutting distance 0.32), the formation of three groups was observed (Figure 2). The first group included species of *Phytopthora* and *Pythium* included in the class Oomycete. This class includes saprophytic species and plants parasites that are among the most devastating crop pathogens. However, in our study the presence of these Oomycete was not very abundant. The second group includes species of genera that -except *Trichoderma*- behave like saprophytes or parasites. For the species of *Fusarium* and *Sclerotinia* there are data about the relationship between the type of tillage and the prevalence of diseases in crops. In different studies, lower incidence levels of this fungal diseases were observed in conventional tillage [19] [20] compared to the values observed in direct sowing. The third group included species of two genera of Eurotiomycetes (*Penicillium*and *Aspergillus*) that are among the most abundant in soil and hay, and are also the main decomposers in agricultural soils [21] [22].In other studies, no relationship was found between the level of abundance the Eurotiomycetes and the tillage system [7].

Direct Sowing

Count and Identification of Fungal microorganisms

Within the plots under direct sowing, ten fungal genera were detected (Figure 3). Among these genera, species of the genera *Penicillium*, *Trichoderma* and *Aspergillus* showed the highest values in CFU g^{-1} of soil, 8600, 2000 and 1800, respectively. While the lowest values corresponded to species of the genera *Cercospora*, *Fusarium* and *Cladosporium* (400, 300 y 200 CFU g^{-1} of soil, respectively). The majority of the species identified belong to different genera of Ascomycota. The highest value corresponded to *Trichoderma* and the lowest to fungi with phyto-pathogenic potential, which matches with the indicated for direct sowing in Argentina soybean crops [23]. The results observed could be due to the natural control of pathogenic fungi of the soil performed by *Trichoderma*. Nevertheless, in other studies about tillage systems and qualitative composition of microorganisms transmitted by the ground, authors did not detect changes on the fungal community [24].The number of CFU corresponding to Mycelia sterilia is within the expected values for the soil habitat [18].

The Simpson diversity index (1-D) was 0.79. This indicates the presence of an alfa diversity relatively high in the soil samples analyzed.

The grouping analysis performed, allowed to determine, at a distance of 0.50, three groups (Figure 4). The first was integrated by species of the genera *Penicillium*, *Trichoderma* and *Aspergillus*, which in addition to sharing high values of CFU g^{-1} of soil, are important decomposers of organic material. In the case of *Trichoderma*, they are also potential biological control agents [22]. The second group included species of genera *Cladosporium*and *Cercospora*, which include phyto-pathogenic species. *Cladosporium* a common fungus in the soil that colonizes aerial parts of the plants, produces a large number of spores and can also be present in air samples. In a long-term study about the composition of the fungal community in relation to the tillage system, it was found that *Mycosphaerella tassiana* (perfect form of *Cladosporium herbarum*) was one of the most abundant fungi in conventional tillage [7]. On the contrary, in our study *Cladosporium* species were the most abundant in direct sowing.

Finally the third group included species of the genera *Phytophthora*, *Rhizoctonia*, *Fusarium* and Mycelia sterilia.The first three fungi mentioned are phyto-pathogens of crops and pastures planted during the test. Regarding the relationship with the tillage system, different studies show opposite results for *Fusarium*. Indicating high levels of incidence of *Fusarium* in direct sowing [21] [22] and absence of relationship [7]. In contrast, for *Rhizoctonia* the results achieved in our study match whit those that indicate that direct sowing has a suppressive effect of this fungus [7], probably mediated by the suppressive effect of other soil fungi [26]. The opposite happened with *Phytophthora.* Different studies show that the increasing use of direct sowing increases the problem of this fungus in Ohio soybean cultivation [27]. In

Argentina, the increase of the virulence complexity of *Phytophthora* is probably associated with continuous soybean production with the Rps genes, and the wide adoption of direct sowing [28].

Comparison between conventional tillage and direct sowing

The total number of CFU g^{-1} of soil was higher in the samples coming from plots under conventional tillage (37.120) in comparison with plots under direct sowing (24.200). However, this difference was not statistically significant ($p < 0.5$). The richness in genera was very similar for samples from both treatments (Figures 1 and 2). The greater number of CFU in conventional tillage could be due to the loss of soils aggregates, what exposes the microsites to oxygen and increases fungal activity [7]. In contrast, conservation systems, such as direct sowing, contribute to increase the formation and stability of soil macro-aggregates [29].

The values of the diversity index were very similar for both tillage systems, with a slight difference in favor of direct sowing (0.79 vs 0.73). The high alfa diversity index found in both systems would indicate that the stability of the fungal community was not altered by the tillage system. These situation was evident in the richness, one of the characteristics of the fungal community, studied (Figures 1 and 3). These results differ from those shown in other studies of shorter duration, where it was discovered that the diversity and richness of fungi decreases with reduced tillage in comparison with conventional tillage [30]. They also differ from other studies where mayor diversity and richness is found under no tillage [7]. From the results achieved in the present investigation, it can be hypothesized that a higher diversity associated with the direct sowing compared to conventional tillage in the short-term, could tend to equalize in the long term based on temporal succession.

The relative frequencies in the different groups of fungi present showed coincidence in the predominance of species of *Penicillium* and Mycelia sterilia in both tillage systems. These fungi are specialized generalists and this could be the reason why they are not affected by the tillage system. Moreover, differences between tillage systems were found in the predominance of *Aspergillus* (conventional tillage) and *Pythium* (direct sowing) (Figures 1 and 3). *Aspergillus* can colonize crop remains as pioneer colonizer.

The relative frequency of species of the *Trichoderma* bio-controller fungus was similar in both tillage systems. While, when comparing the relative frequencies of species with phyto-pathological potential, the genera *Fusarium* and *Rhizoctonia* showed the higher values in conventional tillage, and *Pythium* and *Cladosporium*in direct sowing. According to other studies, the tillage system does not significantly affect the frequency of *Fusarium* isolation [31]. One similar effect has been reported for *Rhizoctonia*, in long term studies [32]. It is widely sustained that the zero tillage favors the development of phyto-pathogenic fungi, due to the conservation of crop residues on the soil surface [19]. However, our results do not show a trend about the species with phyto-pathological potential in relation to the tillage system. Therefore, for further understanding of the phyto-pathological dynamics, new tests should be performed. This consideration is also valid for the observed presence, with a relatively low frequency, of species of *Pilobolus* and *Sclerotinia* only in conventional tillage, and *Cercospora* only in direct sowing. The phyto-pathogenic fungi mentioned coexist with populations of the biological control agent *Trichoderma* in both tillage systems studied.

IV. CONCLUSIONS

The results achieved allow us to state the following conclusions:

The total number of CFU g^{-1} of soil was higher in samples coming from plots with direct sowing. This result could be due to soil aggregates breakage under tillage which exposes microsites to oxygen and increases the fungal activity.

The richness of the fungal community was very similar in samples of both tillage systems.

The values of the diversity index were very similar for both tillage systems, with a slight difference in favor of direct sowing. The relatively high alfa diversity for both systems would indicate that the stability of the fungal community that presupposes the value obtained was not altered by conventional tillage.

The relative frequencies show the predominance of species of *Penicillium* and Mycelia sterilia in both tillage systems. *Aspergillus* predominated in conventional tillage and *Pythium* in direct sowing.

The relative frequencies of species of the bio-controller fungus *Trichoderma* were similar in both tillage systems.

The relative frequencies of species of genera with phyto-pathological potential, *Fusarium* and *Rhizoctonia*, were higher in conventional tillage, and *Pythium* and Cladosporium, in direct sowing.

Our results do not sustain a trend in relation to species of genera with phyto-pathologic potential and tillage systems. For further understanding of the pathological dynamics new tests are required.

Finally, from the results achieved in the present long-term experiment, it may be considered as mode of hypothesis that the highest biodiversity values associated with direct sowing in comparison with conventional tillage, tend to equalize based on temporal succession.

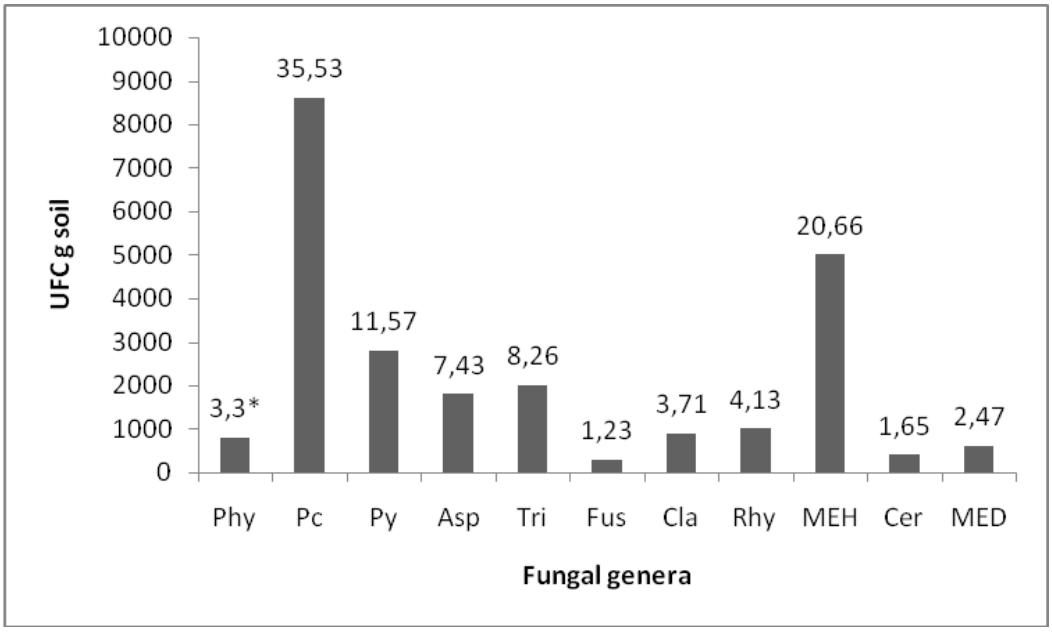

Fig. 1:. Colony forming units by g-1 of soil of species fungal genera and relative frequency (*) in plots where conventional tillage was used. Pc: *Penicillium*, Fus: *Fusarium*, Asp: *Aspergillus*, Pil: *Pilobolus*, Cla: *Cladosporium*, Tri: *Trichoderma*, Py: *Pythium*, Rhi: *Rhizoctonia*, Scle: *Sclerotinia*, Phy: *Phytophthora*, ME: Sterile mycelium

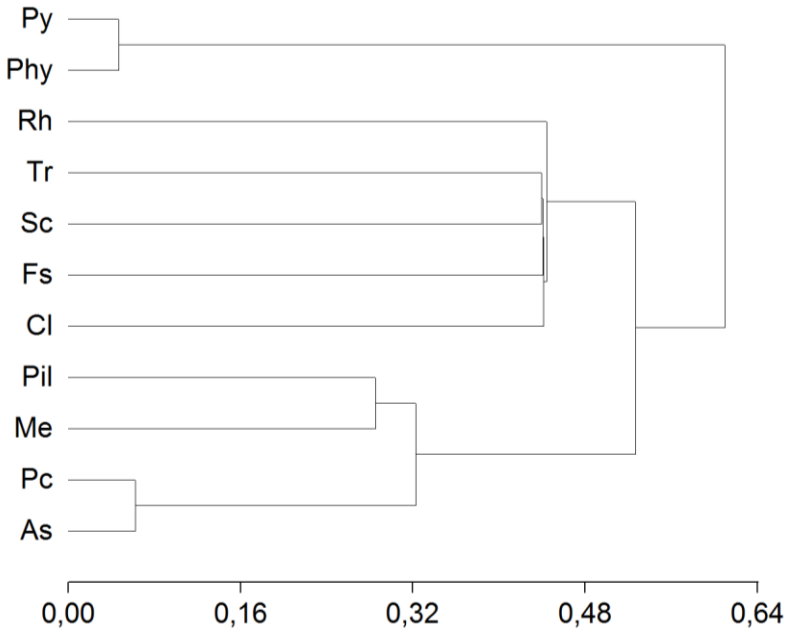

Fig. 2: Clustering analysis of fungal genus species detected in plots where conventional tillage was used Single linkage. Gower distance, cofenetics correlation = 0.926

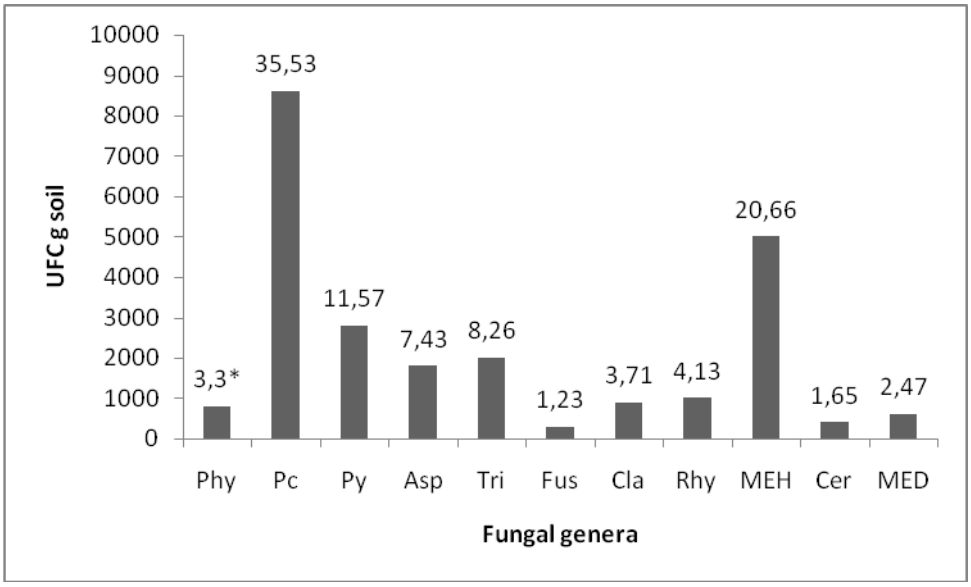

Fig. 3: Colony forming units by g-1 of soil of species fungal genera and relative frequency (*) in plots where direct sowing was used. Phy: *Phytophthora*, Pc: *Penicillium*, Py: *Pythium*, Asp: *Aspergillus*, Tri: *Trichoderma*, Fus: *Fusarium*, Cla: *Cladosporium*, Rhy: *Rhizoctonia*, MEH: Hyaline sterile mycelium, Cer: *Cercospora*, MED: Dematiaceo sterile mycelium.

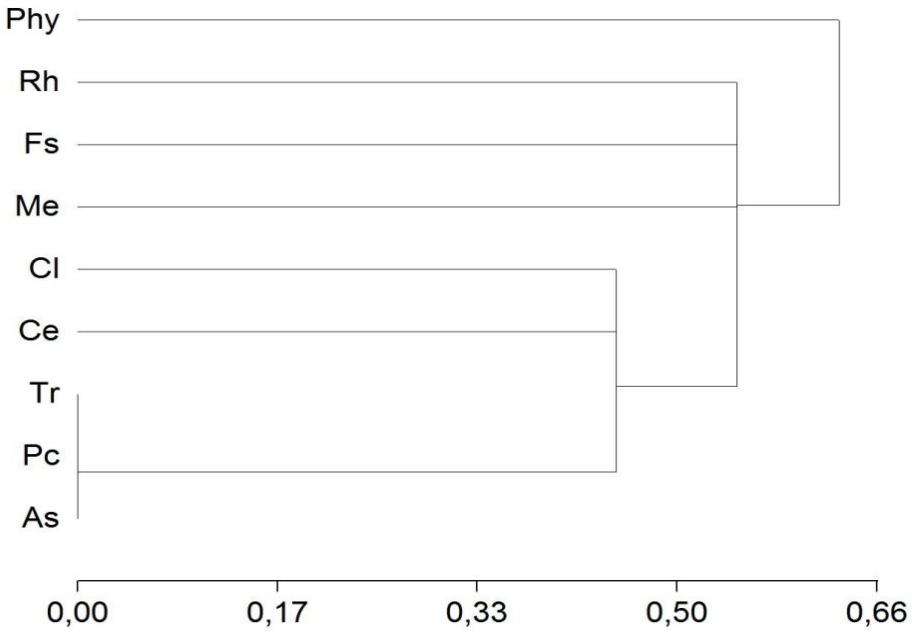

Fig. 4: Clustering analysis of fungal genus species detected in plots where direct sowing was used. Single linkage. Gower distance, cofenetics correlation = 0.890

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