DIVERSITY OF FUNGI IN THE *ALLIUM URSINUM* L COVERED SOIL FOREST

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ABSTRACT

In the soil,ecosystem there are differences in the diversity and spatial distribution of the fungal community. Forest soil samples were harvested in the spring season from the area of influence of plants of Allium ursinum L., in the western part of the country.

The study of fungal diversity was carried out on the "soil grain method" on the sifted and ungrounded soil samples. The composition of fungal species is diverse, but there are also repetions (rehearsals) where the number of species is limited. The species present in both forest soil samples is Circinella spp, followed by Penicillium spp and Aspergillus spp, the latter being isolated only from the sifted soil sample. The low-frequency species are: Torula herbarum (species isolated from both soil samples), Chaetomium spirale (highlighted only in sampled (sifted) soil), Fusarium spp, Helminthosporium spp and Mortierella monospora, the last species isolated from the unsifted soil sample.

INTRODUCTION

Allium ursinum L. is a bulbous plant from the family *Alliaceae*, very common in our forests, which accumulates in a relatively short time (3 months), a rich biomass, comparable in terms of the amount of nutrients with the mature beech leaves.

Between plants, soil and microorganisms, complex interactions are created, materialized by chemical, biochemical and biological reactions (Hinsinger and colab., 2003; Richter and colab., 2007; Lambers and colab., 2009), with a positive impact on both, plants and microorganisms.

According to some authors, the plants influence the microbial community from the soil through the root secretions in their area of influence.

Jaeger and colab. (1999), say that the structure of the microbial community is influenced by both the amount and the composition of the organic matter coming from the plants.

Soil microorganisms represent an important fraction of living biomass, with values of 10^3-10^4 kg / ha in the surface layer (Fierer and colab., 2007).

Between the microbial groups that dominate the soil, because of the celular biomass, there are fungi (Ali-Shtayeh and Jamous, 2000; Rane and Gandhe, 2006).

The experimental results obtained by Ling-Ling Shi et al (2014), have shown that plant diversity in forest areas, temperatures and latitudes influence the composition of soil fungi.

Fungi play an important role in soil degradation processes and soil nutrient cycles (Bailey et al., 2002; Bue´e et al., 2009).

Although fungi have an important role in soil processes, most studies refer to the diversity of bacteria (Roesch et al., 2007; Lauber et al., 2009; Borozan et al., 2013). In addition, the complexity of fungal communities in this ecosystem has not allowed complete investigation, and sequential approaches to assessing fungal diversity are limited and only a small part of the fungal species have been described worldwide (Hawksworth, 2001; McGuire and Treseder, 2010).

This argument comes in support of the studies in this paper, which are concerned with the diversity of fungi in the forest soil covered with *Allium ursinum* L, whose area of influence on soil microorganisms is insufficiently studied.

MATERIAL AND METHOD

The study area is located in the western part of Romania and is covered with deciduous forests, which contain insulated carpets of wild garlic. Soil samples were harvested from the root area of wild garlic (*Allium ursinum* L), in the spring season during the flowering of garlic.

For the isolation of filamentous microorganisms (fungi, actinomycete), an initial soil sample was used, from which vegetable residues was not removed (unsifted soil) and a soil sample from which plant residues was removed (sifted soil).

In addition, for the elimination of errors, given by the high production of spores, the degree of population of the soil with particles and the identification of filamentous fungi by the "soil granule method".

The principle of the method is after Parkinson D., Balasooriya I. and Winterhalder K (1968), with the improvements made by Neonila Petre (Patent for invention OSIM nr.77633 from 1981) and Ștefanic (1991), who has made contributions by finding a way to interpret the results (Borozan et al., 2005).

According to this method, the soil samples are processed and the soil granules are deposited on agarized medium. The soil granules, 5 of them, represent the repetitions within each sample or variant.

Incubation was made at 28^0C, medium temperature, for 7 days. After the incubation period, the filamentous fungi were identified and at the same time the actinomycetes differentiated through microscopic observations on each soil granule.

Statistical analysis

The fungal communities were characterized in terms of diversity by calculating richness, Shannon index, and ACE (abundance-based coverage estimation) using PAST 3.06 program

RESULTS AND DISCUSSIONS

The results illustrated in Figure 1 show that the probability of the filamentous fungi species present in the two soil samples being repeated is 0.8885.

From the 2 soil samples, 10 species of filamentous fungi were isolated, to which was added unconverted mycelium in both samples, but present in a higher proportion in soil sample with vegetal residues (unsifted). Also, the actinomycetes were isolated in a large proportion, especially from the soil sample with vegetal residues (unsifted).

Total number of fungi isolated from the 2 soil samples during the spring season was of 25×10^3 cfu / sol.

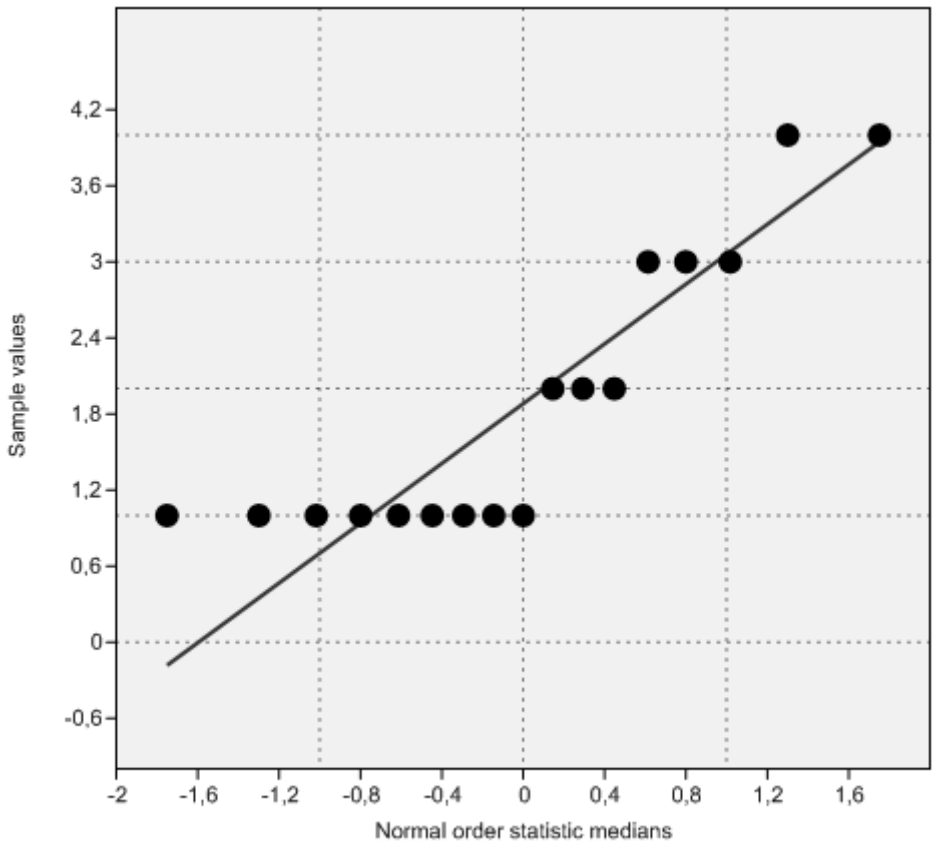

Figure 1. Description of fungus number based on abundance model

From studies conducted by Petr Baldrian and colaboratorii (2012), done on the forest soil, it appears that the fungus is numerically reduced, compared to the bacterial community, but instead there are some species of fungi that dominate numerically the bacteria.

Studies on the abundance of bacteria in samples of raw soil and unblended forest soil samples showed that there were no differences between the values cfu/g sol, after an incubation period of the bacterial isolate for 48 hours (Borozan and colab., 2013).

Dominant fungal species are distributed in both studied samples, but there are cases where certain species are found only in one of the soil samples (sifted and not sifted soil), (fig. 2).

After Petr and al. (2012), fungi are spatially restricted, and are present only on certain sites.

Regarding diversity, our studies indicate a fungal dominance of 0.07813. The smallest dominance is 0.08203 and the upper one is 0.1445.

The variety of fungal species is diverse, but we also have rehearsals in which species are restricted (fig. 5). Diversity Index Shannon-H is 2.68.

The species that is present in both samples of forest soil is *Circinella spp* ($5x10^3$ cfu/sol), followed in descending order of *Penicillium spp* ($4x10^3$ cfu/sol) and *Aspergillus spp* ($3x10^3$ cfu/sol), the last species being isolated only from the sifted sample (fig. 3,4). Both actinomycetes and uncontrolled mycelium were present in both soil samples.

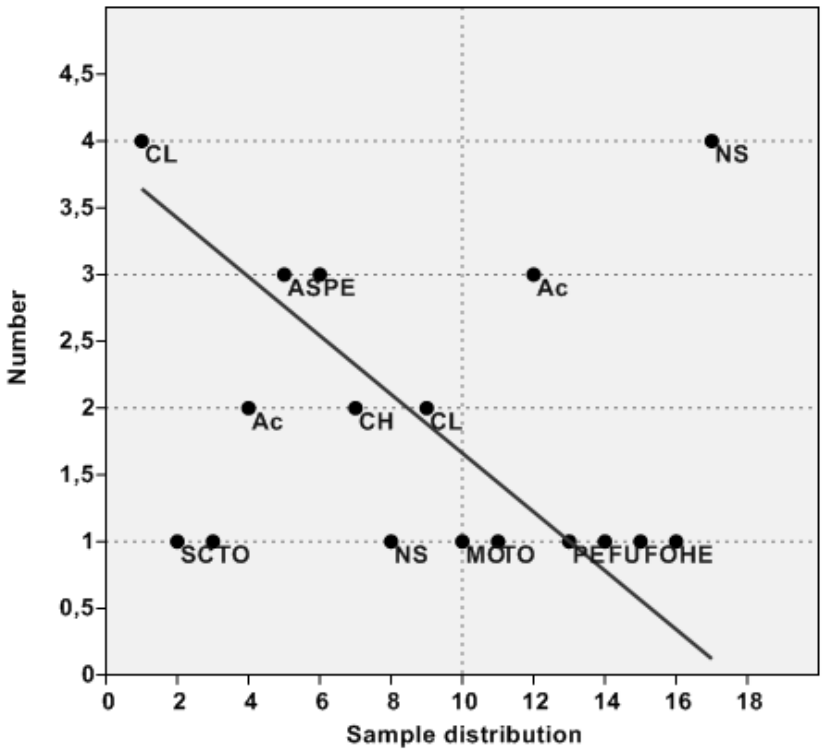

Figure 2 Direction of the development of fungi species from soil samples

Less frequencies are the following fungal species: *Torula herbarum* (species isolated from both soil samples), *Chaetomium spirale* (highlighted only in the sifted soil sample), *Fusarium spp, Helminthosporium spp* și *Mortierella monospora*, species present only in the unblended soil sample (fig. 4,5).

The studies made by *Anderson and Domsch (*1975), on the soil harvested from the deciduous forest, have shown that the largest share had imperfect fungi, follow in descending order of phycomycetes, ascomycetes and basidiomycetes. Among the types of isolated fungi (by the authors above), from the analysed soil samples were: *Penicillium*, *Absidia, Fusarium, Mucor, Trichoderma*, *Humicola, Gongronella, Candida, Cunninghamella* and *Paecilomyces*.

Summerell B.A. and al. (1993), isolated from soil 13 species from *Fusarium* as: *F. oxysporum, F. solani F. moniliforme, F. proliferatum, F. beomiforme, F. chlamydosporum, F. compactum, F. equiseti, F. longipes, F. nygamai, F. scirpi, F. semitectum* and *F. subglutinan,* from wooded areas and unspoiled forests. The authors of this study observed that fungal diversity was not influenced by the isolation techniques used, but it was affected by precipitation. The most abundant fungal species, isolated from most of soils, were *F. oxysporum* and *F. solani.*

In studies conducted by Ling-Ling Shi and al. (2014), it is shown that in forest ecosystems, soil fungi communities are influenced by vegetation, but there is no correlation between fungal diversity and plant diversity, on the contrary where the diversity of plants was reduced, the fungal composition was more varied.

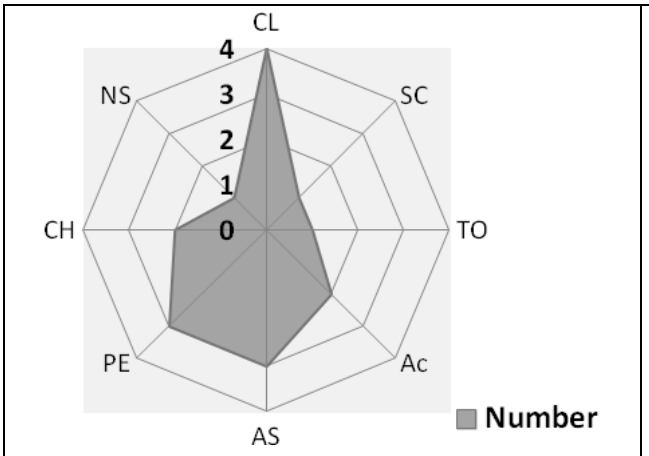

Figure 4 Diversity of fungi in the sifted soil sample

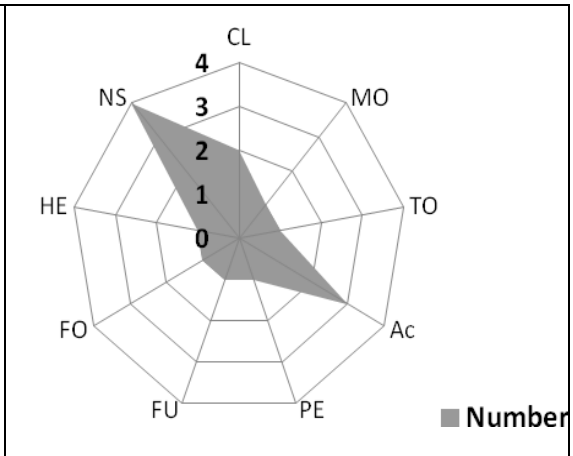

Figure 5 Diversity of fungi in the not sifted soil sample

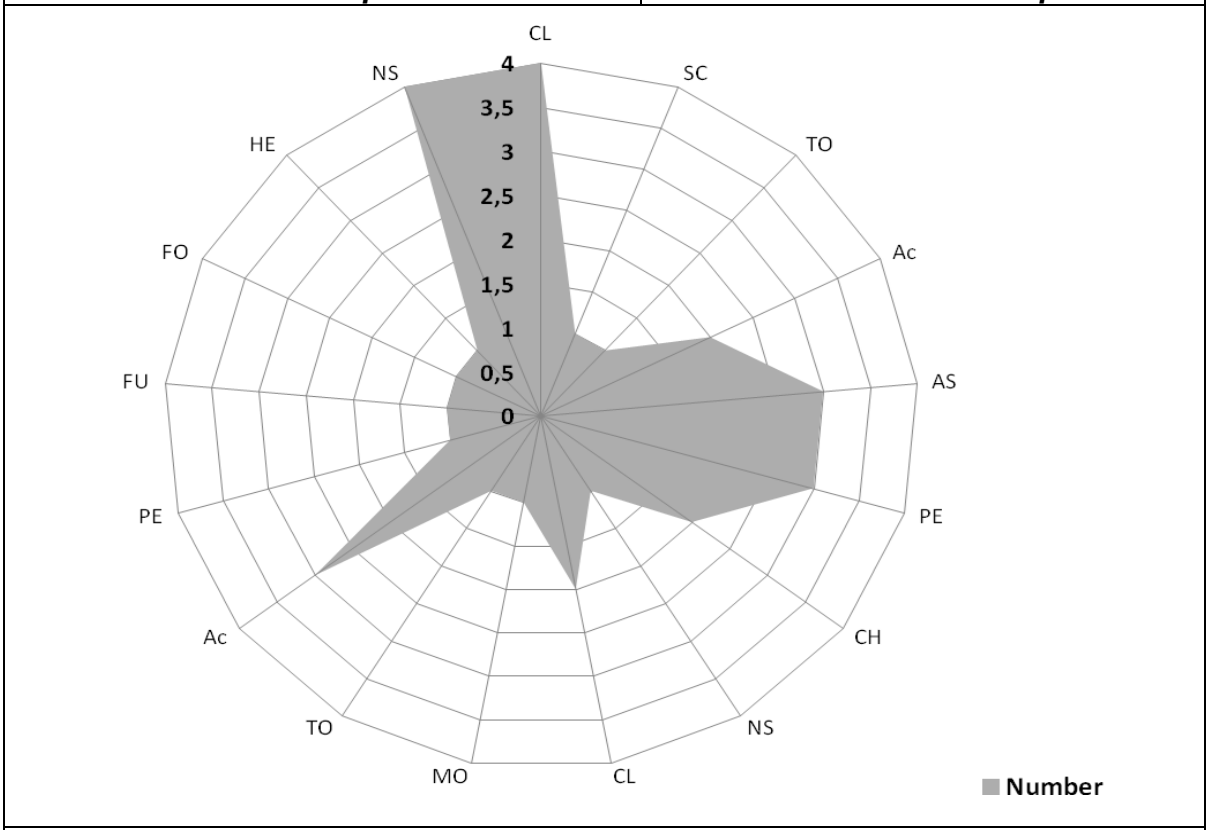

Figure 6 The diversity of filamentous fungi in the two soil samples

Legend: *CL-Circinella spp.; SC- Scopulariopsis spp;* TO- *Torula herbarum;Ac-* Actinomycetes; AS-*Aspergillus spp; PE-Penicillium spp; CH-Chaetomium spp spirale;* NS-Non-sporulated micelia; *MO-Mortierella monospora; FU-Fusarium spp; FO-Fusarium oxisporum; HE-Helminthosporium spp.*

CONCLUSIONS

Our data suggest changes to the composition of the fungal community, which may be due to the plant that covers the soil, side influences of woody plants (beech), the method of analysis and the method of soil processing in the laboratory.

In our studies, these influences were evident at the level of repetitions and sample taken as a whole. Some rehearsals have been characterized by a great fungal diversity, and in others the variation palette was restricted.

From the analyzed forest soil samples, ten species of fungi were isolated. The dominant species present in both soil samples was *Circinella spp*, followed by *Penicillium spp, Aspergillus spp* and *Chaetomium spirale*.

A high percentage of mycelium, almost quantitatively comparable to the species *Circinella spp,* was unsorted. Also, actinomycetes had a fairly high percentage in the analyzed samples. Among common species of both samples were *Penicillium spp* and *Torula herbarum.*

The composition of the fungi was also modified by the appearance of new species in both samples. In the sifted soil sample were found species such as: *Aspergillus spp, Chaetomium spirale, Scopulariopsis spp and* in the unblended soil sample there were species of *Fusarium* and *Helminthosporium.*

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