Highlighting the Structures and Patterns of Fungal Colonization in the Species *Festuca rubra*

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RESEARCH ARTICLE

Abstract
Grasslands are the most dynamic ecosystems are characterized by high biodiversity both above-ground and underground. The biggest problem in these ecosystems is oligotrophy. The phenomenon affects the stability and resilience of these ecosystems, increasing the impact of biological processes. The symbiotic relationship between plants and mycorrhizas is a natural option with a great impact on maintaining the high natural value of ecosystems. This article aims rating colonization *Festuca rubra*, dominant in a mountain grassland ecosystem. Roots were cleared and stained with ink for the detection of mycorrhizas in roots. All root samples were analyzed with an optical microscope for the detection of mycorrhizal structures. The results obtained highlight a main mycorrhizal pattern as well as specific subcategories. Both types of arbuscules were identified: *Arum* and *Paris*. The results obtained highlight a main mycorrhizal pattern as well as specific subcategories. Vesicles are structures with a constant lack of consistency, which indicates their fluctuating nature. The mycorrhizal pattern of *Festuca rubra* as the dominant species in the pratol ecosystem may be the first step towards a more detailed understanding of the fungal strategy as well as the increased importance in the stability of the pratol ecosystem.

Keywords: arbuscules, grassland ecosystem, colonization strategy.

INTRODUCTION

In Europe, grasslands are a dominant land use, covering about 80 million ha, or 22% of the European Union's land area (Bugalho and Abreu, 2008). The grassland ecosystem has the highest diversity of plant species (Aguiar, 2005). The high biodiversity of plants is often reflected by a high diversity of animals and fungi (Pärtel et al., 2015). But the main problem in these ecosystems is the negative relationship between inputs versus output. This has led to the formation of a nutrient-poor soil over time, which is unfavorable for practical species that are mostly perennial. Perennial herbaceous species have small root systems that cannot cross the nutrient-deficient zone that has formed near the root (Pärtel and Helm, 2007). Thus, the practical species are more opportunistic in the formation of different partnerships with micro and macro soil organisms. Weigel et al. (2009) correlate different agricultural processes with the effect produced by the biological mechanisms in grasslands to increase and maintain production. However, this relationship is important because saprotrophic microorganisms in the decomposition chain can, given their important role in the decomposition of bedding and dead roots, control soil carbon storage (Persiani et al., 2008). Arbuscular mycorrhizae are the most important microbial symbiosis for most plants and, under conditions of P limitation, influence the development of the plant community, nutrient absorption, water relations and above-ground...
productivity (Jeffries et al., 2003). In natural ecosystems, plants get up to 80% of their nitrogen requirement and up to 90% of phosphorus from mycorrhizal fungi (Van Der Heijden et al., 2009).

Mycorrhizal fungi form different structures, both inside the roots and in the soil. Mycorrhizal fungi cannot grow without a host, obligatory biotrophy (Declerck et al., 2005). Spores of mycorrhizal fungi last in the soil for decades until they receive chemical signals from a possible plant (Denison and Kiers, 2011). Due to the very low ratio between plants and fungi, it is considered that their specificity in the choice of host is very low (Hartmann et al., 2008). Spores are asexual globose structures with several walls, and the organization of their walls is the main morphological attribute used to identify fungal mycorrhizae at the species level (Souza, 2015). These structures, in addition to the established role in fungal taxonomy, play an important role in the propagation and survival of mycorrhizal fungi. It has numerous nuclei, high amounts of lipids and carbon. The hyphae correspond to the

**RESULTS AND DISCUSSIONS**

The experimental area is part of a large experimental field established in 2001, in a high natural value grassland form the Apuseni Mountains (Corcoz et al, 2022). The roots were harvested after flowering, July 15, 2020. They were cleaned of large particles of soil and soaked in 10% NaOH solution for 24 hours. After this stage, they were rinsed and soaked in a coloring solution (5% Pelikan blue ink + 5% vinegar + tap water) for about 48 hours. After preparing the roots or cut into 1 cm pieces that were examined under a microscope. An image was made for each microscopic field, so the biological phenomenon was digitized. After obtaining the pictures, a grid of 10x10 was placed on each picture, and for each square a number was given from zero to six depending on the structure of the fungal component present in the square. After encoding the pictures, the table was introduced into the MycoPatt tool which automatically calculates all fungal parameters (frequency (%), intensity (%), colonization degree (%), arbuscules (%), vesicles (%), mycorrhizal/nonmycorrhizal area reports and produces the colonization maps that can be exported.
is characterized by extensive development of intracellular coiled hyphae which spread directly from cell to cell (Luginbuehl and Oldroyd, 2017).

The hyphal forms arbuscular like branches sometimes grow from these coils, but there is very little (Dikson, 2004) Arum type arbuscules (Figure 2 c,d) are found mainly in cultivated species, in agricultural ecosystems, and the Paris type (Figure 2 a,b) is very common in species that grow in natural ecosystems. Due to the morphology of the arbuscules, these two types are mostly influenced by the host plant, by the malleability and the air-filled spaces in the root cells. The species Festuca rubra, from the mountain grassland in Romania, presents both types of arbuscules and intermediate ones. But mostly present it’s the Arum type, with a 3:1 ratio. Vesicles are thick-walled structures (Figure 2. e,f) of varying shapes from ovoid, irregularly lobed to box-like, depending on the species of fungus and where the vesicle is formed (Manuel Rodríguez-Morán et al., 2015). They contain abundant lipid and numerous nuclei, and it is likely that they are important storage organs, playing a significant role as propagules within root fragments (Smith and Read, 2008).Auxiliary cells is a fragile cells responsible for lipid storage, provide carbon for the formation of spores (Giovanetti et al., 2010). The vesicles are present in the root cells of the species Festuca rubra. These can indicate the presence of nutrients high in the soil, thus the fungal component depositing their surplus in these structures. Due to the interconnection of the species in the grassland, the high presence of the storage structures can also be explained by the transmission of nutrients to the neighboring species.

With the help of the MycoPatt tool, in addition to the multitude of mycorrhizal indices, the colonization maps can be exported. These maps visually transpose the symbiotic process, so that they can easily examine changes in the fungal component (Figure 3, Table 1.). Blue is represented by hyphae, red arbuscules, green vesicles, gray spores, purple entry points, and yellow auxiliary cells. For the first three maps, the predominance of nonmycorrhizal areas (white areas) indicates a resistance from Festuca rubra species in terms of acceptance of the development of the fungal component in the root cortex. The specific structures of the mycorrhizal fungi cover very small areas, being barely present. There are few hyphae present that fail to complete the entire symbiotic process. The following three maps show an intense development of the intraradicular mycelium, but without the simultaneous
development of the transfer or storage structures. These maps show the presence of intraradicular spores as well as adjoining cells but in limited proportions.

Map 6 shows a linear distribution of hyphae and very few uncolonized areas interspersed among these hyphae developments. The arbuscules present in maps 4 and 5 are grouped and individualized by the rest of the structures. The vesicles for these maps are grouped punctually and like the individualized transfer structures of other mycorrhizal fungus-specific formations. The red color massively present in maps 7 and 8 indicates an intense transfer of nutrients between mycorrhizal fungi and the *Festuca rubra* species. For both maps, most arbuscules are grouped around hyphae. Their development is linear, circular, or irregular. Map 8 shows a higher stability of the symbiotic process compared to map 7 which has more white areas. The last two maps are representative of the storage structure. The lack of transfer structures supports the idea, a hyphae can produce in most cases either arbuscules or vesicles. Map 10 shows the grouped storage structures in both linear and solitary forms.

The use of the MycoPatt tool in addition to exporting maps that allow the evaluation of the visual symbiotic process also offers a multitude of mycorrhizal indices that allow easy comparison of experimental variants. This type of analysis of the colonization process comes as an auxiliary study to those of genetics that can show in addition to the exact position of the fungal structures in the root cortex and the efficiency of the whole process by mapping strategies. It is important to note the direction of the process, which can start with a resistance strategy (the host has a high rigidity for fungal colonization) to a transfer strategy dominated by the presence of red color indicating a host plant’s flexibility in accepting arbuscules formation in root cortex cells. These structures indicate an increased efficiency of the symbiotic process, the arbuscules having a preponderant role in the transfer of nutrients. A strategy opposed to resistance is the proliferative one, where the host’s stiffness decreases, thus allowing the fungal component to explore more space in the cortex, but without allowing it to develop intracellular structures. And the

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**Figure 2. Details of arbuscules and vesicles development in roots of Festuca rubra:** a-b) Paris type arbuscules and coiled hyphae; c-d) Arum arbuscules and hyphae; e-f) Vesicles.

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The last strategy that can indicate a decline in the process is given by a storage strategy, mainly in this strategy the blisters will be present. The emergence of this strategy may indicate a sufficient source of nutrients available to plants. There is a need to allocate the patterns of colonization to these strategies because it is important to visualize the efficiency of the process, not just its presence.

**Figure 3.** Details of colonization strategy and expansion, with the development of mycorrhizal structures in roots of *Festuca rubra*.
CONCLUSIONS

The use of the MycoPatt tool in addition to exporting maps that allow the evaluation of the visual symbiotic process also offers a multitude of mycorrhizal indices that allow easy comparison of experimental variants. This type of analysis of the colonization process comes as an auxiliary study to those of genetics that can show in addition to the exact position of the fungal structures in the root cortex and the efficiency of the whole process by mapping strategies. There is a need to allocate the patterns of colonization to these strategies because it is important to visualize the efficiency of the process, not just its presence.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

REFERENCES


