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ADVANCED RESEARCH IN LIFE SCIENCES
7, 2023, 33 - 37

www.degruyter.com/view/j/arls

DOI: 10.2478/arls-2023-0004
Research Article

Differences in Mycorrhizal Status Induced by Long-Term Fertilization in Roots of Perennial Grassland Species

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Received December, 2022; Revised February, 2023; Accepted February, 2023

Abstract
Perennial species in a mountain grassland where there is high oligotrophy need support for good development. Soil nutrients, either in large amounts or in small quantities, are the driving force in the management of communities of microorganisms that compete and proliferate nutritionally. The symbiosis between vascular plants and fungi in the soil is extraordinary support for perennial species. The roots of the two dominant species in the meadow were harvested after the moment of flowering. They were prepared and stained according to standard methods for evaluating the colonization process. And the process was quantified with the MycoPatt tool. For both species, differentiated fertilization induces similar changes in fungal strategy. But at the level of the formation of the specific structures of the fungi as well as in their development in the cells of the host plants the differences are visible. Maps exported from the MycoPatt tool can be analysed based on strategies or punctually based on color distribution. One can analyse the distribution of each colour (hyphae-blue, arbuscules-red, vesicles-green) as well as the white areas that are represented by parts of the root where the plant does not allow the development of fungi. The use of the MycoPatt tool simplifies the understanding of the symbiotic process and in addition to the multitude of exported indices, it also provides colonization maps that easily show the changes induced by fertilization on the host plants. Thus, being able to predict the best management practice of the whole ecosystem, to sustainably develop grasslands.

Keywords: *Agrostis capillaris*, *Festuca rubra*, MycoPatt tool, fungal strategy.

Introduction

Natural/semi-natural grassland ecosystems have high biodiversity, both above ground and underground. These biomes coexist with each other, so different competitions will take place between them to access the available resources [1]. The chemical nature of organic matter is the result of interactions between flower composition, plant phenology, and soil fertility [2]. Soil microbial communities are involved in the key processes that govern the entire carbon and nitrogen cycle of the ecosystem, thus correlating plant diversity with ecosystem function. Soil nutrients, either in large amounts or in small quantities, are the driving force in the management of communities of microorganisms that compete and proliferate nutritionally. There is strong evidence that perennial grassland systems have higher phosphorus, higher biological activity, better water retention properties, and higher levels of organic carbon in the soil compared to annual systems [3]. Fertilization can disrupt and inhibit the growth of some species to the detriment of others [4]. Thus, through fertilization, the ecological niche is changed both above and below ground. Benizri and Amiaud [5] showed the negative influence of fertilization on floristic diversity in a grassland. Friedrich *et al*. [6] argue that nitrogen fertilization for early perennial species is the necessary impetus for above-ground development. Applying manure to mineral fertilizers is a common practice in the grassland ecosystem. Nitrogen balances in areas that have received manure are often higher than in those that have received only mineral

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fertilizer [7]. The problem arises when nitrogen is taken up from mineral fertilizers much faster, and compounds made of mineral fertilizers are much more difficult to solubilize. Nitrogen fertilization in the practical ecosystem has led to the promotion of saprophytic fungi [8]. A change in the ratio of symbiont and saprophytic fungi has major consequences for the whole ecosystem.

The composition of the mycorrhizal fungus community can drastically influence the dynamics of the plant community [9]. The abundance of nitrogen in the soil has a negative relationship with the fungal community in the soil [10]. The same is true for the phosphorus content, this element reduces the fungal activity by increasing the diameter of the root at the same time as the amount of phosphorus [11]. The sensitivity of fungal communities to different types of nitrogen fertilizers is influenced by their host species [12]. Semi-natural grasslands in temperate zones are dominated by slow-growing species, respectively *Festuca spp*. and *Agrostis capillaris*, which occupy the largest areas and prefer acidic, well-drained, and nitrogen-limited soils [13]. In the non-fertilized meadow, such as *Agrostis capillaris* and *Festuca rubra* from the Apuseni Mountains, the percentage of composition is 15% and 7%, respectively [14]. Soils that are limited in phosphorus are the soils that best support the development of *Agrostis capillaris* [15]. Mineral nitrogen fertilization is considered to increase the growth of grass species and also has a legume inhibiting effect [16]. In the same experimental field, other researchers Păcurar *et al*. [17] demonstrated the installation of the *Festuca rubra* species favorably on plots with organic fertilization, and the *Agrostis capillaris* species extended better to mineral fertilization.

The aim of this work was to assess the colonization dynamics in roots of *Festuca rubra* and *Agrostis capillaris* species. Fungal strategies and developed structures were analyzed in terms of presence/absence and input influence.

Material and Methods

The experimental field is in the Apuseni Mountains, a grassland with high natural value. It was established in 2001 and has 5 experimental variants, one variant, V1, is represented by the natural grassland (unfertilized), and the other 4 fertilizers were applied: V2- exclusively organic fertilization (10 t/ha manure), V3- mixed with organic and mineral fertilization (10 t/ha + 50N $25P_2O_5$ $25K_2O$), V4- exclusively mineral (100N $50P_2O_5$ $50K_2O$), and V5- mixed with a higher dose of mineral (10 t/ ha + 100N $50P_2O_5$ $50K_2O$). The roots of the two dominant species were harvested after the time of flowering, on July 15, 2020. All samples were prepared and analyzed based on MycoPatt tool, with the methodological changes performed by Corcoz *et al*. [18].

Results and discussion

The maps exported from the MycoPatt tool can be used to view the entire symbiont process from the roots of the host plants. One can easily notice the change generated by the long-term fertilization on root colonization in the two dominant species in the mountain grassland (Figure 1., Table 1.). The maps exported from the MycoPatt tool can be used to view the entire symbiont process from the roots of the host plants. One can easily notice the change generated by the long-term fertilization on root colonization in the two dominant species in the mountain grassland (Figure 1., Table 1.).

Table 1.

Mycorrhizal parameters related to colonization development the perennial species

Colonization maps	Frequency	Intensity	Arbuscules	Vesicles	Non-mycorrhizal areas	Colonization degree	Mycorrhizal/ non-mycorrhizal report
A.c.1	59.73	29.60	0.93	0.93	70.00	23.90	0.54
A.c.2	98.67	63.67	17.60	1.87	36.00	62.97	2.06
A.c.3	82.73	51.60	6.93	1.27	48.00	44.70	1.39
A.c.4	58.07	35.20	0.40	0.07	65.00	23.00	0.60
A.c.5	28.13	13.53	0.00	0.07	86.00	6.57	0.18
F.r.1	73.73	28.87	0.67	0.73	71.00	22.49	0.43
F.r.2	78.13	42.60	4.60	2.20	57.00	35.27	0.85
F.r.3	60.53	25.47	5.00	0.13	75.00	18.78	0.40
F.r.4	38.27	21.07	1.13	0.00	79.00	11.61	0.33
F.r.5	28.27	10.13	0.00	0.20	90.00	6.42	0.13

Note: A.c. – *Agrostis capillaris*; F.r. – *Festuca rubra*; Vales 1-5 represent the variants V1 to V5.

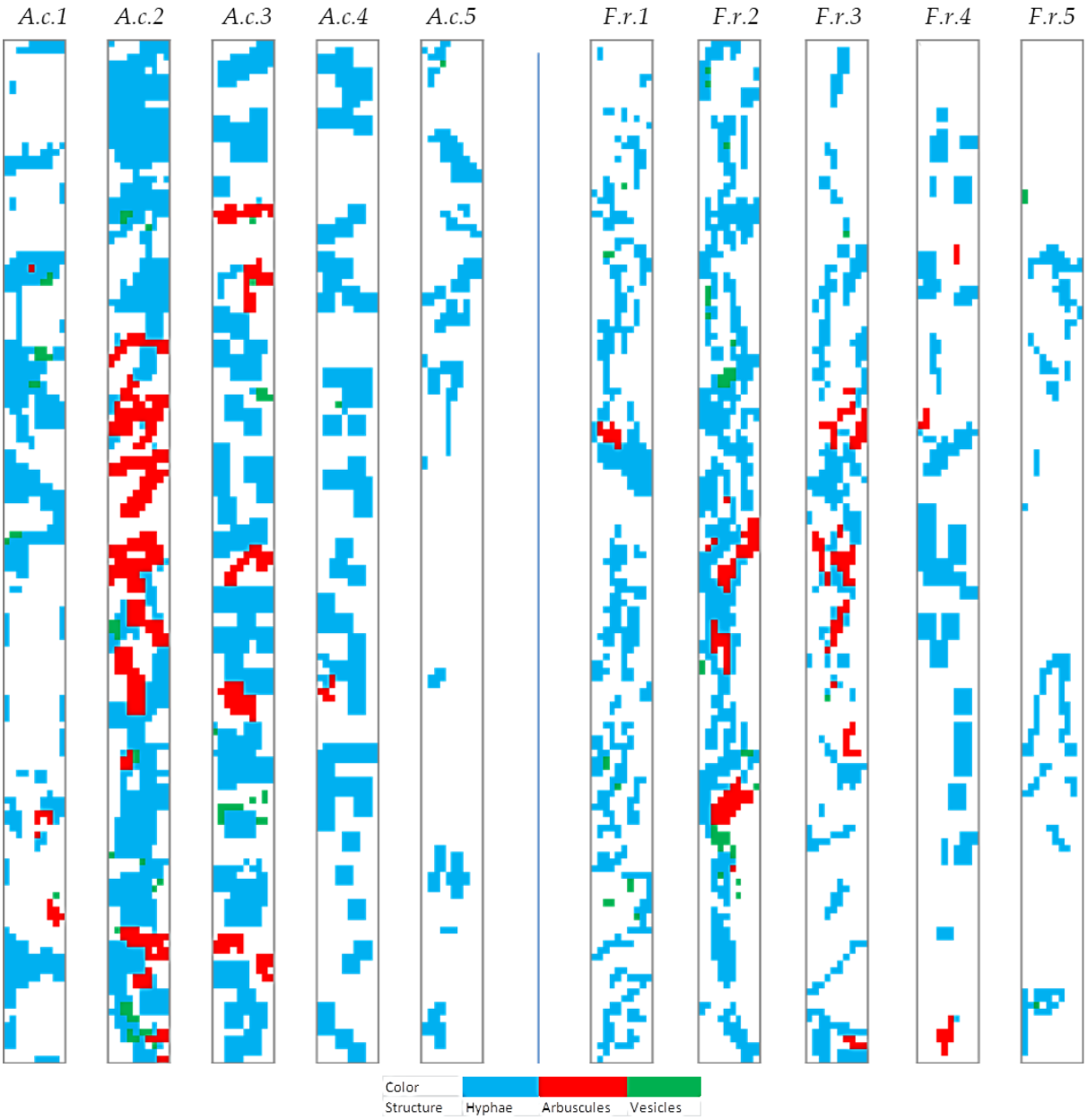


Figure 1. Maps of strategy fungal in the root of *A. capillaris* and *F. rubra*

The control variant shows the native mycorrhizal community, present in the roots of the species: *Festuca rubra* and *Agrostis capillaris*. In both variants, the maps project a proliferative strategy, in which the blue color dominates. In other words, the hyphae of the fungus are present, but in the native environment, both species do not allow the fungal component to developing its specific structures, the permissiveness of the host root being quite low. When applying organic fertilizers, the transposition of the proliferative strategy into a transfer one is observed. The amount of nutrients applied stimulates the symbiotic process, so you can see the red colour that represents the arbuscules. These are structures that play a role in the transfer of nutrients. The application of fertilizers with small amounts of ore maintains a balance, being the average of the other two maps, presented above. Under the pressure of this type of fertilization, fungi manage to colonize a high intake of cells of the root cortex, as well as form specific structures. For the *Festuca rubra* species, the vesicles are very rare, while for the *Agrostis capillaris*, these structures with a role in storage

are more present. Exclusive mineral fertilization intervenes in the decline of the symbiotic process. This type of fertilization is a proliferative strategy, in which the host species becomes more restrictive than the fungal component. This can be attributed to the nutrients in the fertilizer directly accessible to plant species, so there is no need for an intermediate (fungal partner). The combined fertilization between organic plus mineral, with a high dose, represents a barrier in the development of the symbiotic process. This type of fertilization describes a strategy in conditions of resistance, predominantly being the white areas.

Conclusions

For both species, differentiated fertilization induces similar fungal strategy changes. But at the level of formation of fungus-specific structures as well as in their development in the cells of host plants the differences are visible. Maps exported from the MycoPatt tool can be analyzed based on strategies or punctually based on color distribution. It is possible to analyze the distribution of each color (hyphae-blue, arbuscules-red, vesicles-green) as well as the white areas that are represented by parts of the root where the plant does not allow the development of fungi. In order to direct the symbiotic process towards a proliferative or transfer strategy, it is recommended to fertilize the oligotrophic meadows with organic fertilizers or a mixture of organic with mineral (low doses of mineral). By promoting transfer or proliferative strategies in terms of the fungal microbiome, the stability of the ecosystem is ensured.

Acknowledgment

This paper is part of a PhD study in the thematic area of *Mycorrhizal Status and Development of Colonization in Mountain Grassland Dominant Species*, conducted by the first author Larisa Corcoz, under the coordination of prof. dr. Roxana Vidican.

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