Research on the mycorrhizal colonization mechanism induced by differentiated fertilization in the grassland ecosystem

(Summary of Phd Thesis)

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Introduction

In the last decades, research has focused on the conservation of biodiversity, which has suffered from pollution, the excessive use of mineral fertilizers and animal breeding, which are the most visible human activities in the agricultural field. Anthropogenic inputs together with climate change have led to changes in the climactic states of ecosystems.

Grasslands are one of the most widespread types of ecosystems in the world, covering a fifth of the Earth's surface. These ecosystems occur naturally on all continents, with an estimated area of 52.5 million km ^{2}, or 40.5% of the land surface, except for Greenland and Antarctica. This type of ecosystem is characterized by the predominance of grass species and other species and their main use is provided by animal feed. Unfavorable environmental conditions such as: limited amount of nutrients, low water regime and soil pH in the grassland ecosystem do not support the development of tree or tree species.

In addition to nutrient-poor environments, the high dominance of some species in plant communities can reduce the resources available to other biotic groups, promoting competition and therefore reducing plant diversity. In the grassland ecosystem, most species are perennial and can develop a nutrient deficiency in the root zone due to constant nutrient uptake. This intense activity, together with the oligotrophy present in grasslands, drastically influences the ecological niche conditions of plants. Thus, grassland species are forced to adopt different strategies to survive the pressure in these ecosystems. Achieving mutualism interaction with mycorrhizal fungi represents a viable biological mechanism for practical species to survive and exhibit luxuriant development in natural grassland ecosystems.

The symbiotic relationships between plant roots and fungi (defined as mycorrhizae) have been recognized since the early nineteenth century. Mycorrhizal associations are ubiquitous in all ecosystems, with a high number of cropped species, most of grassland species and even in forests. Mycorrhizal fungi are symbionts in the roots of most evolved plants, about 80% of plant species forming these types of associations. About 40,000-50,000 fungal species form mycorrhizal associations with almost 250,000 plant species. The symbiotic mechanism is given by the process through which the fungal hyphae adhere to the surface of plant roots and colonize them. Following this process, the plant receives a greater amount of water and nutrients, and at the opposite pole the fungus receives some of the metabolites assimilated by the vascular plant through the photosynthesis process. In addition to the main role, which is

still discussed in the literature, that of supplying nutrients to host plants, mycorrhizal fungi intervene in the circuit of elements, and in the decomposition of organic matter. Mycorrhizal plants are often more resistant to diseases such as the ones produced by microbial pathogens from soil, and the symbiont induce to the terrestrial plants an improved resistance to abiotic factors (water, temperature, heavy metals).

Mycorrhizal fungi present in grassland ecosystems can be included in four types, based on the criteria of morphological differentiation of root tissues and host plants: vesicular-arbuscular mycorrhizae, ectomycorrhizae, ericoid mycorrhizae and orchid mycorrhizae.

The study of mycorrhizal symbiosis is a subject that covers several important fields, such as: agriculture, horticulture, pedology and ecology. Due to the benefits to the entire ecosystem, more detailed studies are needed to fully understand the mechanisms of mycorrhizae because they can be a biological source for the rehabilitation and improvement of natural ecosystems. Most studies to date have focused mainly on agricultural crops, with mycorrhizal mechanisms in natural ecosystems being less studied.

Research objectives

The aim of the research was to study the influence of fertilization on the mycorrhizal colonization mechanism in the dominant species of a grassland ecosystem of high natural value in the Apuseni Mountains.

1. The level of native colonization of the dominant species in the grassland ecosystem.
2. The effects of long-term differentiated fertilization on mycorrhizal colonization parameters.
3. Development and complete assessment of fungal strategies shaped by different types of fertilization.
4. The elaboration and export of relevant mycorrhizal maps for each species and for each type of fertilization.

The results of this thesis were published in six articles: the first three in **ISI** indexed Journals: the first article was published in the *Plants* Journal (Basel) (**Q1. I. F. 4,658**), the second ISI article was published in the *Agronomy* Journal (**Q1, I.F. 3,949**), and the third ISI article was published in the *Agriculture* Journal (Q1, I.F: 3,408). And three other articles were published in **BDI** Journals (the first in the *Research Journal of Agricultural Science*, the second in the *Romanian Journal of Grassland and Forage Crops*, and the third in the *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca Agriculture*).

The studies aimed at the objectives proposed by the doctoral thesis were carried out in the experimental field in the village of Gheţari, Alba County, Apuseni Mountains, of the Department of Grasslands and Forage Crops, Faculty of Agriculture, USAMV Cluj. The following aspects were studied: the effect of organo-mineral fertilization on the emergence and development of plant-fungus symbiont associations, the assessment of the level of native colonization for each of the dominant species in the meadow ecosystem and the effects of differentiated fertilization on mycorrhizal colonization parameters. The roots were processed and evaluated in the Microbiology Laboratory of the Faculty of Agriculture, within the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, under the guidance of the coordinator Prof. Dr. Roxana Vidican.

The doctoral thesis entitled "**Research on the mycorrhizal colonization mechanism induced by differentiated fertilization in the practical ecosystem**" includes 25 figures, 8 tables and is structured in two main parts: the current study of knowledge containing three review articles (**chapter 1, 2 and 3**) and the original research containing the working hypothesis/objectives (**chapter 1 of the second part**) and the general methodologies (**chapter 2**), followed by the articles containing the own research (**chapters 3-5**), general conclusions and recommendations (**chapter 6-7**), respectively the originality and innovative contribution of the thesis (**chapter 8**).

The present thesis evaluates the mycorrhizal colonization mechanism induced by differential fertilization in the practical ecosystem for two dominant species.

Regarding the first part (**"State of the Art" - review articles**), data from the literature were studied using: Web of Science Core Collection and Google Scholar. Through this bilbiographical study, it was found that most fungal communities represent promoters of the good development of plants and the stability of ecosystem functions. In the grassland ecosystem, the analysis of root symbiosis is necessary for a large number of actions: evaluating the stability of the phytocenosis with understanding the level of mycoheterotrophy for each species; future expansion of invasive plants in relation to secondary and tertiary successions; integrative models of plant – microorganism – climate interactions and the potential for survival in new climatic conditions, the increased importance of mycorrhizal symbioses to support plant species negatively influenced by oligotrophy in practical ecosystems. The last review article presents the methodology used in the evaluation of the symbiont process.

As for the second part, it includes the experimental elements as follows: the sampling from the experimental field in the village of Gheţari, of the roots of the plants from the two analyzed species: *Festuca rubra* and *Agrostis capillaris*; the processing of the roots and their analysis was carried out in the Microbiology Laboratory. To evaluate the symbiont process in dominant species using MycoPatt. All data analysis was performed with R Studio software. All packages have been selected for specific tests, which allow a full exploration of the databases: "agricolae", "ape", "psych", "stats" and "vegan". The selected test allows surface exploration of the data to deep exploration: normality histograms, basic statistics, ANOVA and LSD comparative tests, scatterplots and regressions, cluster analysis and two types of ordinations – PCA vs. NMDS.

Chapter 3 presents the native colonization model of mycorrhizal fungi. Thus, the main aim of the study was to use the MycoPatt tool to assess the native mycorrhizal potential of the *Festuca rubra* species in a mountain meadow ecosystem exploited by long-term mowing.

Chapter 4 presents the applicability of the MycoPatt tool in evaluating the fungi-plant symbiont process. The aim was to evaluate the characteristics and stability of the mycorrhizal mechanism for the *Festuca rubra* species in response to the long-term application of differentiated treatments. Thus we demonstrated that the use of mycorrhizal maps allows a deep scan of the colonized roots, identifying the actual positioning of the fungal structures, together with their development potential and the evaluation of the colonization strategy.

Chapter 5 presents the applicability of the MycoPatt tool for framing the symbiont process in different strategies. The aim was to analyze in depth the mycorrhizal colonization in the roots of *A. capillaris*, shaped by the long-term application of the treatments.

Following the experiments, the following conclusions were drawn:

The native fungal colonization for the two dominant species in the grassland ecosystem was different, which demonstrates the major impact of the host in the symbiotic process. The species *Festuca rubra* had a native colonization with a frequency value of just fewer than 50%, with an intensity value of approximately 19%, and the structures specific to the fungal component have similar values. Arbuscules are present with 1.95% and vesicles with 1.80% within colonized roots.

The species *Agrostis capillaris* in the native mycorrhizal conditions had a value of the colonization frequency over 63%, with the colonization intensity at half of the frequency value, with the value of the arbuscules of 2.88% and the value of vesicles set to 1.53%.

The type of fertilizer as well as its doses drastically influences the fungal community and implicitly the whole plant-fungal symbiotic process. Organic fertilizers are promoters of the process, and at the opposite pole mineral fertilizers negatively influence the fungal colonization in the root cortex of the two dominant grass species. Organic fertilization stimulates the symbiotic mechanism for both species, up to 55% colonization frequency in *F. rubra* and 68.27% in *A. capillaris*, and the formation of a large arbuscules network, up to 3.15% for *F. rubra* and 2.92% for *A. capillaris,* within the total colonized area.

The storage capacity, expressed by the formation of vesicles is highly visible in organic treated variants, with 2.13% in *F. rubra* and 2.32% in *A. capillaris*, sustained by a colonization degree set to 17.72% in *F. rubra* and 31.0% in *A. capillaris*.

Mineral-organic fertilized variants reduce the expansion of mycorrhizal symbionts in roots of both plants, with a minimum frequency of 35.63% recorded for *F. rubra* and 30.25% for *A. capillaris*.

Plants fertilized with mineral or organo-mineral fertilizers block the development of both arbuscules and vesicles, with a reduction of these structures presence to less than 1%, which restrict the overall transfer and storage mechanism between the symbionts.

Species *F. rubra* is more performant in the use of low-mineral organic treatments, with a colonization frequency with 9% higher than native profile, compared to *A. capillaris*, which have this parameter at a value of 16% less than the native profile.

In addition, the amount of nutrients accessible to the plants after fertilization is the driving force in directing the symbiotic process towards certain strategies. Differentiated fertilization induces a specific colonization strategy orientation in roots of grasses. The native colonization strategy is balanced, oriented mainly toward a proliferative expansion, followed by the development of both transfer and storage strategies.

The application of organic fertilizers change the colonization toward two clear strategies, in different areas of the root – one for storage, based on a large share of vesicles, and one for transfer due to the large presence of arbuscules.

Mineral fertilizers induce a resistance condition strategy, with a decrease in the colonization potential and the restriction of both arbuscules and vesicles formation.

Mycorrhizal maps are an important visual-based technique for the projection of colonization patterns and strategies, along with the clear positioning of mycorrhizal structures. The use of mycorrhizal maps is an important step-forward in the understanding of the symbiotic mechanism, along with a deep analysis of Mycorrhized root functionality which is inconsistent along the entire root.

Recommendations

Organic fertilization is recommended for the increase in the native colonization potential of *F. rubra* and *A. capillaris* species.

Mineral organic (10t/ha+50N25$P_2O_2$25$K_2$0) fertilization is recommended only for the improvement of *F. rubra* colonization, which is a species that use this mixed resource as a stimulus for the proliferation of fungal components.

Mineral and mineral-organic fertilizers should be avoided in the HNV grasslands, due to their perturbation potential on colonization mechanism up to the severe restriction of the vesicles and arbuscules development.

MycoPatt system and methodology is recommended for the analysis of mycorrhizal mechanism and the objective quantification of colonization parameters, along with the export of large databases of parameters and the assemblage of mycorrhizal maps.

The selection of mycorrhizal should be done based on recorded median, if the number of data entries is over 900 in the database, with the additional extraction of 25% higher and lower mycorrhizal maps.

If the database contains fewer than 900 entries, a cluster analysis is recommended for the selection of most relevant mycorrhizal map.

For both cases, an important step in the colonization analysis is the extraction of another two maps, for the root segments that show the maximum of arbuscules and vesicles.

The use of colonization strategies in the analysis of colonization mechanism is important to define the specific orientation of each root segment toward one clear strategy: restricted conditions, proliferative, transfer or storage.

Originality and personal contributions

1.This is the first study worldwide that highlights the mycorrhizal patterns for *F. rubra* and *A. capillaris* species, both the native pattern and the patterns influenced by differentiated fertilization. Overall, it is a study based on the assemblage and analysis of more than 20000 microscopic images, which is the largest database on mycorrhizal mechanisms and processes.
2. Can be considered a comprehensive study on the mycorrhizal mechanism for two dominant species in the grassland ecosystem.

3. The results presented in this thesis are useful for the scientific community in multiple fields of study: general and applied microbiology, biodiversity in grassland species and applied agronomy.
4. Elaboration of a specific methodology for objective quantification of the colonization process in grassland species.
5. The proposal of an agronomic expression of the mycorrhizal fungi influence in supporting grassland species coverage and survival potential.
6. Proposal of mycorrhizal strategies and elaboration of a new methodology for improved classification of mycorrhizal mechanism and colonization processes.
7. Establishment of separation and limit threshold of parameters for the detection of colonization strategies.
8. The elaboration and the clear establishment of a mycorrhizal map selection methodology, based on the lower and upper limits of certain parameters, as a new direction for enhancement of explaining a very complex biological symbiotic process.
9. Elaboration of a clear set of rules for mycorrhizal maps interpretation assembled in a new proposed Multi-point Analysis procedure.
10. Establishment of a complex data analysis system for the extraction of relevant maps from the Mycopatt databases based on Average values, Median values and/or Cluster analysis.
11. The proposal of a new synthetic indicator - the Arbucules/Vesicle ratio.

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