Research Journal of Agricultural Science, 47 (1), 2015

SYNERGISTIC EFFECT OF FERTILIZATION AND MANAGEMENT ON PLANT MYCORRHIZATION IN A MOUNTAIN GRASSLAND

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Abstract. *The balance of grassland ecosystems in Romania is constantly under strong pressure due to the socio-economic aspect that created the premise either of abandonment or intensification of these areas, leading to the increasing occurrence of nutritional perturbations and unidirectional allocation of resources to the dominant plants in the sward. Mycorrhizal fungi have the ability to adjust the transfer processes of nutrients between plants, maintaining the balance of the sward. This study aims to assess the reaction of mycorrhizas in the root system of Festuca rubra L. and Trifolium repens L. plants at fertilization and management. Mowing or mulching are traditional solutions to maintain the mycorrhizal nutrition area of dominant plants in the sward. The application of zinc sulphate or fungicide is a coherent alternative to preserve the stability of grassland ecosystems in the absence of these management types.*

Key words: *mycorrhizal colonization, fertilization, management, nutritional disturbances.*

INTRODUCTION

At global level fertilization intensification tends to produce increasingly stronger perturbations on grasslands, amplifying the effects of management applied in these ecosystems. In Romania, due to the socio-economic conditions, areas occupied by grasslands are subject to either drop or intensification. This context leads to strong nutritional disturbances and sward depreciation (ROTAR et al., 2014.). Due to the concomitant evolution with higher plants, mycorrhizal symbiotic fungi are a component capable of interconnection of the root with the soil particles, reacting quickly even to low levels of perturbation (VIDICAN et al., 2014). Mycorrhizas role in the ecosystems is the well-balanced allocation of nutrients resources between plants (LAMBERS et al., 2009), maintaining their overall balance. The analysis of mycorrhizal parameters is suitable for identifying effective methods for maintaining the stability of sward.

MATERIAL AND METHODS

A two-factor experimental field was installed in 2009 on a *Festuca rubra* L grassland type, located at an altitude of 1135 m, 46 ° 29 '27.5964 "latitude / 22 ° 48' 50.8284" longitude in the village Gârda de Sus, Apuseni Mountains, on a terra rossa soil. The first experimental factor consists of 4 types of management, grouped in traditional and alternative management. Traditional management was done by mowing (M1) and mulching (M2) at July 15 - which corresponds to the maximum of vegetation period in the area. As alternatives, were tested treatments with zinc sulphate (M3) and fungicide (M4), without any cut or mulching – which correspond to abandonment of the area. The second factor comprises six graduations of fertilization, applied annually at the beginning of the growing season for each type of management. Graduation starts from an unfertilized variant (N1), to 10 t ha-1 manure (N2 – total NPK – 30:29:25 kg ha-1), 10 t ha-1 manure + mineral NPK (N3 – total NPK – 80:54:50 kg ha-1), mineral NPK – 50:25:25 kg ha-1 (N4), Eurofertil mezocalc 120 kg ha-1+ N50 kg ha-1 (N5 – total NPK– 50:16:25 kg ha-1) and Eurofertil mezocalc 120 kg ha-1 (N6 – total NPK–

0:16:25 kg ha-1). Mycorrhizal parameters studied were frequency (freq) and intensity (int) of colonization in roots of red fescue *(Festuca rubra* L.) and white clover (*Trifolium repens* L.), dominant plants (<50%) in the sward (BRAUN– BLANQUET, 1932). Each experimental plot had 3 replications. To highlight the reaction of mycorrhizas to fertilization was preferred RDA ordination and Tukey HSD test to assess fluctuations between years, all the tests being carried out with R Statistics software (R Core Team, 2013).

RESULTS AND DISCUSSIONS

Traditional management, mowing and mulching, produce strong oscillations of mycorrhizal colonization frequency in roots of white clover (Table 1). Application of Eurofertil mezocalc (N5) along with mowing or fertilization with NPK (N4) under mulching conditions represents optimal variants for maintaining a stable frequency from one year to another. For unfertilized areas (N1), a treatment with zinc sulphate can maintain the frequency close to 100%, and a treatment with fungicide works well along with an organic fertilization (N2). This indicates that for the same conditions of fertilization, colonization frequency in *Trifolium repens* is less sensitive to the application of alternative treatments than to traditional management.

Table 1

Inter-annual oscillations of mycorrhizal frequency due to management type

Year	Manag	*Trifolium repens*						*Festuca rubra*					
		N1	N2	N3	N4	N5	N6	N1	N2	N3	N4	N5	N6
2010	M1	78.9^{bcd}	92.2^{ab}	88.9^{ab}	88.9^{ab}	92.2^{ab}	88.9^{ab}	57.6^{de}	45.6^{e}	100.0^{a}	84.4^{bc}	71.1^{cd}	51.1^{e}
	M2	80.0^{abcd}	86.7^{abc}	90.0^{ab}	97.8^{a}	68.9^{cde}	88.9^{abc}	100.0^{a}	68.9^{f}	93.3^{abc}	48.9^{g}	73.3^{def}	64.4^{f}
	M3	100.0^{a}	97.8^{abc}	88.9^{abcd}	82.2^{bcd}	100.0^{a}	98.9^{ab}	72.2^{bc}	55.6^{cd}	94.4^{a}	85.6^{ab}	72.2^{bc}	55.6^{cd}
	M4	100.0^{a}	94.4^{ab}	91.1^{ab}	52.2^{d}	82.2^{ab}	85.6^{ab}	100.0^{a}	65.6^{b}	100.0^{a}	98.9^{a}	100.0^{a}	96.7^{a}
2011	M1	82.2^{abc}	72.2^{cde}	63.3^{de}	94.4^{ab}	96.7^{a}	61.1^{e}	90.0^{ab}	81.1^{bc}	94.4^{ab}	91.1^{ab}	83.3^{bc}	92.2^{ab}
	M2	98.9^{a}	51.1^{ef}	44.4^{f}	97.8^{a}	71.1^{bcde}	65.6^{de}	96.7^{ab}	72.2^{ef}	85.6^{bcd}	82.2^{cde}	86.7^{bc}	64.4^{f}
	M3	96.7^{abc}	88.9^{abcd}	81.1^{cd}	81.1^{cd}	72.2^{d}	51.1^{e}	92.2^{a}	34.4^{e}	100.0^{a}	41.1^{de}	84.4^{ab}	64.4^{c}
	M4	77.8^{bc}	93.3^{ab}	84.4^{ab}	75.6^{bc}	98.9^{a}	61.1^{cd}	67.8^{b}	44.4^{d}	65.6^{b}	51.1^{cd}	93.3^{a}	58.9^{bc}

Note: Margins sharing a letter in the group label are not significantly different at the 5% level.

For *Festuca rubra*, mowing or mulching creates a trend for an increase in frequency of colonization from one year to another (Table 1). In contrast, application of treatments acts toward a strong reduction of mycorrhizal frequency in roots of red fescue, from one year to another. Zinc sulphate maintain the frequency relatively stable only based on a fertilization with manure and mineral nitrogen (N3), a valid phenomenon for fungicide application only for a fertilization with Eurofertil mezocalc (N5). Traditional management determines a dependency phenomenon of plants for nutritional inputs. Simultaneously with the increase of nutrients is increased the above-ground biomass production (JOHNSON et al., 2008), which creates pools of nutrients. This type of accumulation reduces the expansion of roots in the soil while reducing their mycorrhization.

The spectrum of management influence on the frequency of colonization is well reflected RDA analysis (fig. 1). For *Festuca rubra* fertilization based on Eurofertil Mezocalc

(N6) or NPK (N4) provides slight increase of frequency in terms of mowing. In addition, the strong approach of NPK fertilization (N4) to the frequency registered in the variants treated with zinc sulfate indicate the synergistic influence of the two components on this parameter. Mulching is suitable for a manure and nitrogen type-based fertilizer (N2) or Eurofertil mezocalc (N6), both fertilization recipes similarly affecting the rate of colonization. Frequency locations outside the action of organo-mineral fertilization (N2) reveal a less effect of inputs compared with fungicide application.

Trifolium repens is more sensitive to the type of treatment applied than to fertilizer recipe. Among fertilization variants, the application of Eurofertil mezocalc supplemented with nitrogen (N5) highlights the strong effect of mowing, zinc sulphate and fungicide (fig. 1). The The location of zinc sulphate and fungicide frequencies outside the action area of fertilizer indicates the potential of this recipe for modifications towards positive or negative gradients. For mowing, frequency is located in the middle of the action of fertilizer, which indicates the conservative effect of these two techniques. A management through mulching increases the frequency dependency for the quantity of phosphorus applied.

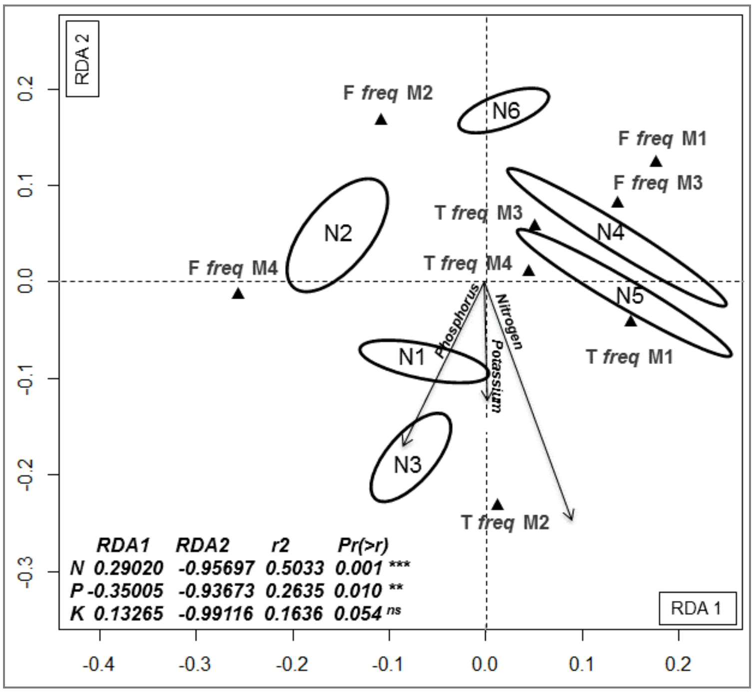

Fig. 1. RDA ordination of colonization frequency response to management and fertilization.
Legend: Plant species: F – *Festuca rubra* L., T – *Trifolium repens* L.; Management: M1 – mowing, M2 – mulching, M3 – zinc sulphate, M4 – fungicide; N 1-6 – fertilization level; Mycorrhization: *freq* – frequency

Fertilization and management act specifically on the two dominant plants in the sward, causing fluctuations in the intensity of colonization (Table 1). In the absence of fertilization, the species *Trifolium repens* proves a strong increase in the intensity produced by the application of zinc sulphate, while mulching produce a significant decrease of this parameter. The absence of fertilization allows the expression of full perturbative potential of management. For red fescue, under the same conditions, zinc sulfate and fungicide drastically reduce the intensity, while mowing and mulching acting to preserve the values of this parameter.

Manure (N2) and management produce strong oscillations in both species, global action beeing the decreasing of intensity of colonization from one year to another (Table 1). Supplementation of manure with nitrogen (N3) causes a slight increase from one year to another in *Trifolium repens* roots, only when is applied a fungicide treatment. For *Festuca rubra*, the application of manure produce a stability of the colonization intensity in both experimental years, regardless of the type of management. Unilateral NPK fertilization (N4) causes reduced oscillations to white clover, regardless of management, instead it produce strong inter-annual oscillations in *Festuca rubra*. Mulching and fungicide acts more powerful to reduce the intensity of red fescue root colonization, when applied over an Eurofertil mezocalc fertilization supplemented with nitrogen (N5), this fertilizer having a similar action if applied without nitrogen

Table 2

Inter-annual oscillations of mycorrhizal intensity due to management type

Year	Manag	*Trifolium repens*						*Festuca rubra*					
		N1	N2	N3	N4	N5	N6	N1	N2	N3	N4	N5	N6
2010	M1	6.5^{d}	12.8^{ab}	14.7^{c}	6.3^{d}	12.0^{c}	6.4^{cd}	2.8^{d}	18.8^{cd}	8.1^{bc}	11.6^{ab}	17.3^{b}	8.1^{cd}
	M2	25.3^{b}	7.9^{bc}	40.9^{a}	8.5^{cd}	20.5^{a}	6.7^{cd}	3.8^{d}	23.1^{bc}	9.6^{b}	13.6^{a}	23.3^{a}	11.7^{b}
	M3	5.6^{d}	6.4^{cd}	12.1^{cd}	16.6^{a}	5.5^{d}	14.2^{b}	20.2^{b}	14.2^{d}	5.2^{c}	11.2^{ab}	5.5^{c}	17.1^{a}
	M4	19.9^{c}	13.9^{a}	8.1^{d}	9.9^{cd}	7.5^{d}	27.6^{a}	26.9^{a}	31.3^{a}	15.1^{a}	9.2^{b}	6.1^{c}	6.8^{de}
2011	M1	7.7^{d}	6.6^{cd}	10.5^{cd}	8.8^{cd}	11.3^{c}	2.4^{de}	2.5^{d}	6.5^{e}	6.7^{bc}	9.1^{b}	16.2^{b}	11.9^{b}
	M2	15.3^{c}	5.3^{cd}	34.7^{b}	7.1^{cd}	16.8^{b}	4.1^{de}	3.4^{d}	17.6^{cd}	8.4^{bc}	0.6^{c}	0.9^{d}	9.1^{c}
	M3	31.0^{a}	1.5^{d}	2.2^{e}	13.7^{ab}	4.8^{d}	10.1^{bc}	1.8^{d}	1.2^{e}	5.0^{c}	10.2^{b}	8.1^{c}	5.0^{e}
	M4	15.1^{c}	10.2^{abc}	10.6^{cd}	10.7^{bc}	7.6^{d}	1.0^{e}	10.9^{c}	27.2^{ab}	15.1^{a}	0.9^{c}	0.8^{d}	6.5^{de}
Note: Margins sharing a letter in the group label are not significantly different at the 5% level.													

Type of management and fertilization has a more aggressive synergistic effect on the intensity of colonization, causing a strong separation of variants (fig. 2.). The intensity indicates the acceptance of fungal advance in the root cortex, plants requiring strong hyphal networks only when nutritional resources are not directly available. For white clover, positioning of intensity on nitrogen and potassium gradient indicates a greater need for symbiosis if biomass is mowed or converted into mulch.

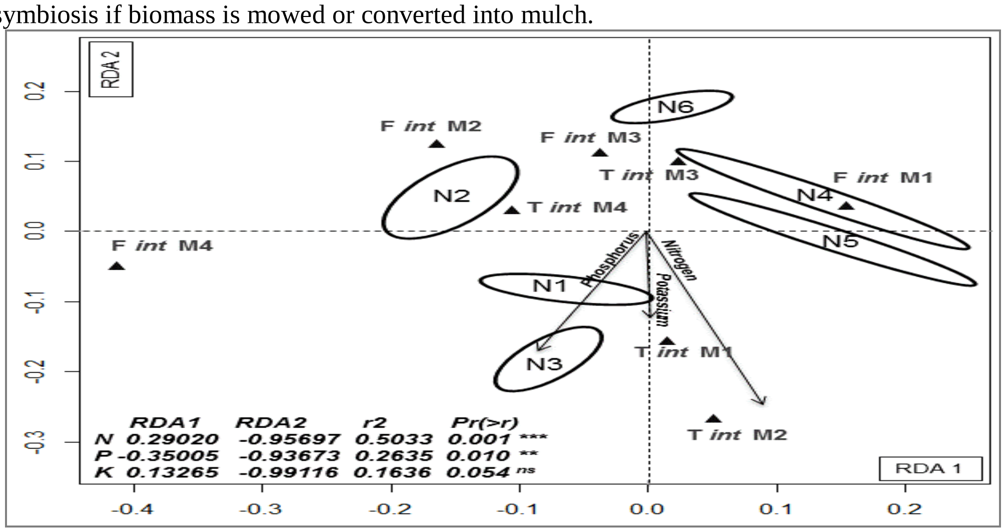

Fig. 2. RDA ordination of colonization intensity response to management and fertilization.
Legend: Plant species: F – *Festuca rubra* L., T – *Trifolium repens* L.; Management: M1 – mowing, M2 – mulching, M3 – zinc sulphate, M4 – fungicide; N 1-6 – fertilization level; Mycorrhization: *int* – intensity

In contrast, a treatment with zinc sulphate requires mineral fertilizer, and a fungicide treatment works with organic fertilizer, both variants raising the intensity of colonization (fig. 2.). For red fescue, mowing conditions demands the application of high amounts of mineral fertilizers to increase the intensity of colonization. Treatments with zinc sulphate positively influence the intensity of colonization, along with fertilizers with a slow rate of decomposition (N2, N6). Similarly, mulching requires an organic fertilization over 10 t ha^{-1} manure to enhance the intensity.

CONCLUSIONS

Optimizing fertilization and management applied on grasslands leads to a constant level of mycorrhization that maintain the nutritional balance of these ecosystems. For this purpose, treatments with zinc sulphate or fungicide may represent an alternative to mowing or mulching.

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