

## Scientific Paper

# Arbuscular-mycorrhizal inoculation and reduction of organic and nitrogen fertilization in *Megathyrsus maximus* cv. Likoni

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## Abstract

The effect of the arbuscular-mycorrhizal inoculation on the reduction of organic and nitrogen fertilization in Guinea grass (*Megathyrsus maximus* cv. Likoni), cultivated on a Ferruginous Gley Nodular soil, located in the Cascajal locality, Villa Clara province, Cuba, was evaluated. Thirteen treatments were studied, formed by: absolute control, applications of 12,5 and 25 t of cattle manure ha<sup>-1</sup> combined with 0, 105 and 150 kg N ha<sup>-1</sup> year<sup>-1</sup>, with and without inoculation of the mycorrhizal fungus *Funneliformis mosseae*. The design was randomized blocks with four replications. The cattle manure increased pH and the contents of organic matter, assimilable P and exchangeable K of the soil; the highest effects were obtained with 25 t ha<sup>-1</sup>. In the absence of inoculation, the highest dry matter yields (16,57 t DM ha<sup>-1</sup>) and N (18,9 g kg<sup>-1</sup> DM), P (2,3 g kg<sup>-1</sup> DM) and K concentrations (17,9 g kg<sup>-1</sup> DM) in the biomass were reached with the addition of 25 t of manure ha<sup>-1</sup> and 105 kg N ha<sup>-1</sup>. These treatments showed the highest frequency and intensity of mycorrhizal colonization (57,7 and 58,2 %) and the highest number of spores (503 and 491 spores g<sup>-1</sup> of soil) of arbuscular-mycorrhizal fungi (AMF) in the rhizosphere. With the inoculation of *F. mosseae* at the moment of planting, the cattle manure doses and the nitrogen fertilizer can be reduced in 50 and 30 %, respectively, without affecting the nutrient content in the biomass or the yield, during two years of crop growth.

**Keywords:** chemical composition, plant nutrition, yield

## Introduction

Pastures and forages constitute the main source of feedstuffs for ruminants in the tropic; however, the low fertility of most soils dedicated to animal husbandry limits their yields and nutritional value and, consequently, the productivity of these crops (Ram and Trivedi, 2012).

Fertilization is one of the ways to reconstitute the nutrients that are extracted from the soil, and also an alternative to increase the forage offer per surface and time unit; nevertheless, with the decrease of soil fertility it becomes an increasingly complex activity, not only for its effect on the increase of agricultural production costs, but also because of the increase of contamination risks implied by the addition of increasingly higher amounts of chemical fertilizers (Merlin *et al.*, 2014).

Previously, the studies related to fertilization were based on the increase of animal production per surface unit and the establishment of economic limits for the use of fertilizers. At present, without neglecting the economic aspect, great importance is ascribed to environment conservation and the

access of the population to healthy food, and thus the need to design agricultural models that include strategies for this practice, aimed at guaranteeing adequate crop nutrition and, in turn, ensuring the protection of natural resources, has increased (Lara-Mantilla *et al.*, 2011).

Those strategies include the management of the arbuscular-mycorrhizal symbiosis, due to its potentialities to improve crop productivity and, in turn, to reduce the need for fertilizers, from the rise in the efficiency of nutrient absorption by the plants (Yang *et al.*, 2014). Such management can be achieved through the inoculation of arbuscular-mycorrhizal fungi (AMF) species, previously selected for their high efficiency to promote crop growth, especially when the resident communities of these microorganisms are not able to establish effective symbiosis with the host plants (Priyadharsini and Muthukumar, 2015).

Works conducted by Carneiro *et al.* (2011) and González *et al.* (2015) proved that the inoculation of efficient AMF species constitutes an effective way to decrease the fertilizer doses to be applied to

pastures, without reducing their yield or nutritional value. Yet, although the functional and ecological importance of AMFs in pasturelands and the benefits which can be obtained with their inoculation are acknowledged, the complexity of these agroecosystems suggests to continue evaluating their contribution to the increase of pasture productivity, the rise of nutrient use efficiency and the decrease of fertilizer doses.

Based on the above-explained facts, this study was conducted in order to evaluate the effect of arbuscular-mycorrhizal inoculation on the reduction of organic and nitrogen fertilization in Guinea grass (*Megathyrsus maximus* cv. Likoni).

## Materials and Methods

The trial was conducted at the Pastures and Forages Research Station of Cascajal, located in the Santo Domingo municipality –Villa Clara province, Cuba–, on a petroferic Ferruginous Nodular Gleysol soil (Hernández *et al.*, 2015); its main chemical characteristics are shown in table 1. According to Paneque and Calaña (2001), it is a soil with low base exchange capacity; low contents of organic matter, assimilable P and exchangeable K; and high acidity.

Rainfall distribution during the period in which the trial was conducted (2012-2013) is shown in figure 1.

**Treatments and design.** Thirteen treatments were studied, combined as follows:

Treatment	Manure (t ha <sup>-1</sup> )	N (kg ha <sup>-1</sup> )	Inoculation
1 (control)	0	0	NI
2	12,5	0	NI
3	12,5	105	NI
4	12,5	150	NI
5	25	0	NI
6	25	105	NI
7	25	150	NI
8	12,5	0	I
9	12,5	105	I
10	12,5	150	I
11	25	0	I
12	25	105	I
13	25	150	I

The treatments were distributed in a randomized block design with four replicas. The plots constituted the experimental unit and had a surface of 25,2 m<sup>2</sup> and a calculation area of 16,8 m<sup>2</sup>.

The soil was prepared conventionally, with a sequence of plowing up (plow), harrowing, crossing (plow) and harrowing, at approximate intervals of 20-25 days between each one. The pasture was planted in March (2012), in rows separated by 70 cm and by drilling, with a dose of 10 kg of total seed ha<sup>-1</sup> (1 kg of pure germinable seed ha<sup>-1</sup>) and at a depth of 1,5 cm. The cattle manure, whose chemical composition is shown in table 2, was spread over the plot surface after the first harrowing and it was incorporated to the soil with the crossing. The manure was from the dairy farms of the station and had a deposition time in the manure heap of four months. The nitrogen fertilizer (urea) was applied in a fractioned form at the moment of planting and after each cutting, in doses of 0, 35 and 50 kg N ha<sup>-1</sup>, equivalent to 0, 105 and 150 kg N ha<sup>-1</sup> year<sup>-1</sup>. The trial was conducted without irrigation.

The strain INCAM-2 of the AMF species *Funneliformis mosseae* (Nicol. & Gerd.) Walker & Schüßler (Shüßler and Walker, 2010) was selected, from the collection of the National Institute of Agricultural Sciences (INCA, for its initials in Spanish) –Mayabeque, Cuba–, due to its high efficiency to increase pasture yield with the edaphic conditions under which this experiment was conducted (Ramírez *et al.*, 2006). For its application a solid inoculant was used, which was previously multiplied in a clayey substrate sterilized in autoclave at 120 °C one hour during three days, with the use of *Brachiaria decumbens* cv. Basilisk as host plant. It had 35 spores of the selected AMF species per gram of substrate, as well as abundant quantities of rootlet and hyphae fragments.

The inoculation was made through the seed covering method, for which they were submerged in a fluid paste, elaborated from the mixture of a quantity of solid inoculant equivalent to 10 % of their weight (1 kg) and 600 mL of water (Fernández *et al.*, 2001). After the seeds were covered and the inoculant was solidified, they were planted.

The pasture was cut three times in the first year and three times in the second year, which coincided with the rainy season, at a height of 10 cm from the soil surface: the first one 90 days after planting (June, 2012) and the other two at intervals of approximately 60 days (August and October, 2012) and in June, August and October, 2013.

In each cutting the green mass (GM) of the aerial part framed within the calculation area of the plots was weighed, and 200-g samples were taken to determine the dry matter (DM) percentage and

Table 1. Chemical characteristics of the soil (0-20 cm).

pH H <sub>2</sub> O	OM (%)	P <sub>2</sub> O <sub>5</sub> (mg 100 g <sup>-1</sup> )	Exchangeable cations (cmol <sub>c</sub> kg <sup>-1</sup> )				
			Ca	Mg	Na	K	BEC
4,7	2,52	1,5	3,62	1,12	0,05	0,10	4,89
(0,2)	(0,09)	(0,3)	(0,12)	(0,03)	(0,01)	(0,02)	(0,16)

Averages of ten samples taken at the beginning of the trial. Values between parentheses show the confidence interval of the means ( $\alpha = 0,05$ ).

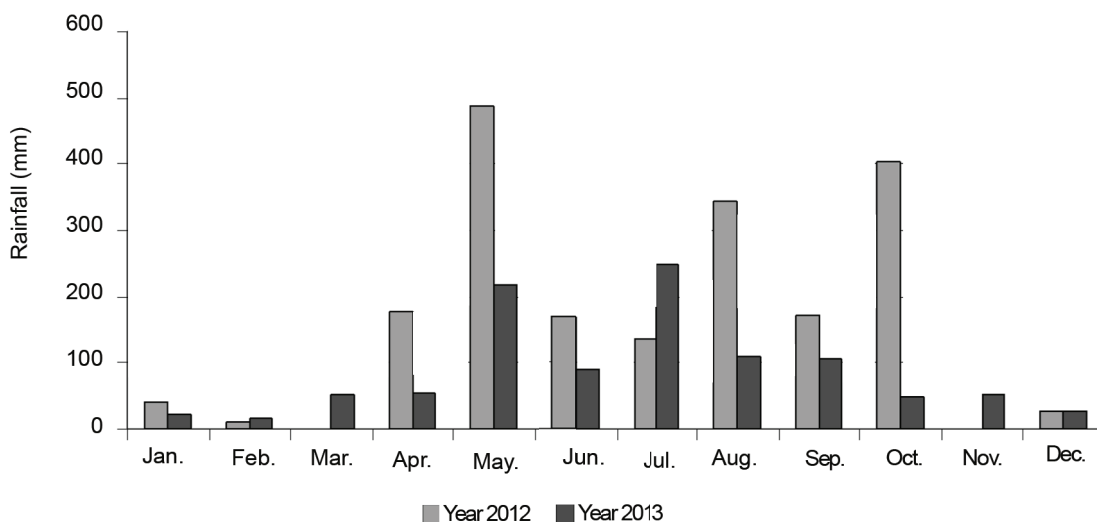


Figure 1. Rainfall distribution during the experimental period.

Table 2. Chemical characteristics of the cattle manure (dry basis).

OM (%)	N (%)	C:N ratio	P (%)	K (%)	Ca (%)	Mg (%)	pH	Humidity (%)
62,3	2,05	17,6	0,55	1,43	2,72	0,64	7,1	52,5
(0,8)	(0,03)	(2,1)	(0,01)	(0,02)	(0,05)	(0,01)	(0,1)	(0,6)

Averages of ten samples taken at the moment of manure application. Values between parentheses show the confidence interval of the means ( $\alpha = 0,05$ ).

the N, P and K concentrations. The DM yield was estimated from the GM yield and the DM percentage.

In the cuttings of June and August, 2012, and June and October, 2013, when there was higher moisture in the soil, 10 subsamples of the roots were taken at a depth of 0-20 cm, through the use of a metallic cylinder of 5 cm diameter and 20 cm of height. The sampling spots were equidistantly distributed and separated at 10 cm from the rows. The subsamples were homogenized to form a compound sample per plot, from which 1 g of rootlets was extracted for their staining and clarification (Rodríguez *et al.*, 2015); the mycorrhizal colonization frequency (Giovanetti

and Mosse, 1980) and visual density (Trouvelot *et al.*, 1986) were evaluated. Likewise, the number of spores in the rhizosphere was determined (Herrera *et al.*, 1995).

In the last cutting of each year five soil subsamples were taken at the depth of 0-20 cm, to form a composed sample per plot and perform the chemical analyses through the following techniques:

- pH H<sub>2</sub>O: potentiometric titration. Soil-water ratio: 1:2,5
- Organic matter: Walkley and Black
- Assimilable P<sub>2</sub>O<sub>5</sub>: Oniani
- Exchangeable cations: extraction with NH<sub>4</sub>Ac 1 mol L<sup>-1</sup> at pH 7 and determination by complexometric

titration (Ca and Mg) and flame photometry (Na and K).

The chemical analyses of soil, organic fertilizer and aerial biomass of the pasture were made according to the Manual de técnicas analíticas (Handbook of analytical techniques) of INCA (Panque *et al.*, 2010).

For the statistical processing of the data of dry mass, colonization, visual density and spores, as well as of the nutrient content of the aerial mass of the pasture, double-classification variance analysis was carried out and Duncan's (1955) test was used at  $p < 0,05$ . The confidence interval of the means was estimated on the variables corresponding to the soil analyses and the manure, for a significance of  $\alpha = 0,05$  (Payton *et al.*, 2000). The variables fulfilled the variance normality and homogeneity assumptions, for which in all the cases the original data were analyzed (Vásquez, 2011). The program SPSS Statistics 21 (IBM, 2012) was used.

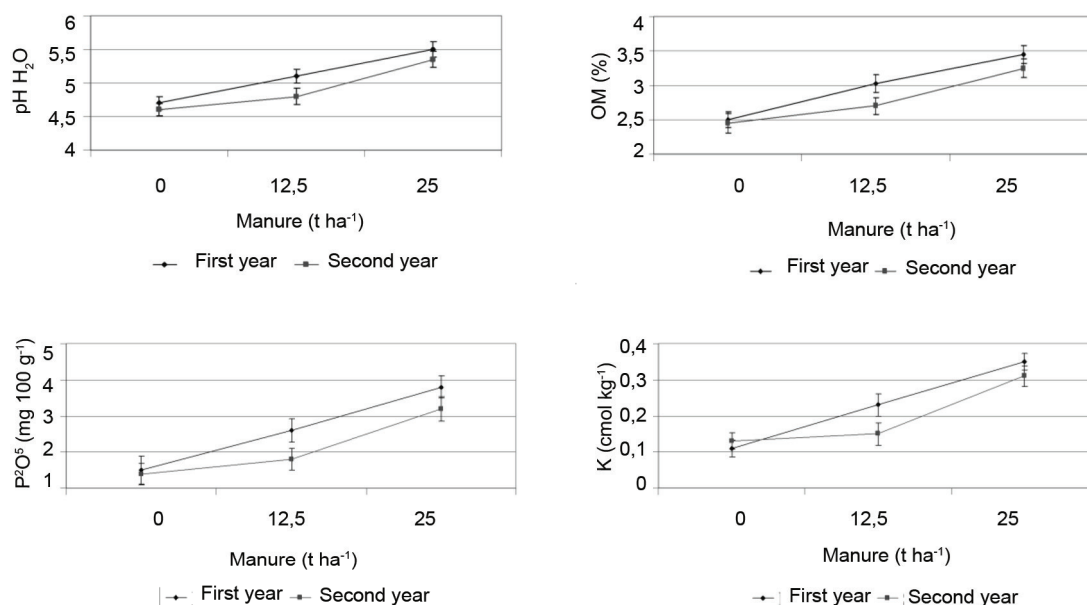
## Results and Discussion

The application of cattle manure contributed to the increase of pH and the contents of organic matter (OM), assimilable P and exchangeable K of the soil, and the highest values were reached with 25

t ha<sup>-1</sup> (fig. 2). Its effect on the soil remained during the two years of evaluation by adding the highest dose, because with 12,5 t ha<sup>-1</sup> significant increases in these variables were obtained only during the first year.

The effect of manure on the chemical characteristics of the soil was in correspondence with its OM and nutrient contribution. According to its chemical composition (table 2), per each ton of manure 296 kg of OM; 2,6 kg of P and 6,8 kg of K were incorporated to the soil, which contributed to increase the values of these elements, especially with the highest dose. Likewise, the pH increase seems to be a consequence of the Ca contribution, because with each ton of manure 13 kg of this nutrient were incorporated to the soil.

In this sense, Crespo *et al.* (2010) and Ghanbari *et al.* (2014) stated that the contents of organic substances and mineral elements of cattle manure confer excellent qualities to it as ameliorator of the chemical, physical and biological properties of the soil and as source of nutrients for the plants. In addition, its use as organic fertilizer generates other environmental services which are not less important, by contributing to the decrease of the environmental contamination of animal husbandry



The vertical bars represent the confidence intervals of the means; the absence of overlapping of the confidence intervals indicates significant difference ( $\alpha = 0,05$ ), according to Payton *et al.* (2000).

Figure 2. Effect of cattle manure on the chemical characteristics of the soil (0-20 cm).

ecosystems and to the mitigation of climate change, through the sequestration of important quantities of C in the soil (Crespo, 2011).

The values of mycorrhizal colonization, visual density or colonization intensity and number of spores in the rhizosphere are shown in table 3. The inoculation of *F. mosseae* produced a significant increase of these indicators with regards to the non-inoculated treatments, which showed the level of fungal occupation of resident AMFs; however, during the first and second years, the highest values were obtained with the application of 12,5 t of manure ha<sup>-1</sup> combined with 105 or 150 kg N ha<sup>-1</sup> year<sup>-1</sup>. The effect of inoculation was extended until the second year, but only in those treatments in which the fungal variables reached the highest values during the first year.

In the presence of *F. mosseae*, the application of 25 t of manure ha<sup>-1</sup> with or without addition of N produced a depressive effect on the fungal variables, which was manifested not only in the lower value of root occupation in these treatments with regards to that of 12,5 t ha<sup>-1</sup>, but also in the permanence of the effect of inoculation, which was observed only during the first year.

These results indicate that, in the conditions under which the trial was conducted, there was

higher effectiveness of *F. mosseae* with regards to resident AMFs to colonize the pasture roots; as well as the need that, along with its inoculation, certain quantities of nutrients are added, from organic or mineral sources, to achieve higher root occupation and prolong its effect in time.

According to Grman and Robinson (2013) and Castillo *et al.* (2014), the fungal structures are closely related to the nutrient availability in the soil, because the mycorrhizal symbiosis is controlled by the carbon supply of the host. Thus, fungal structures can be increased with an adequate nutrient supply; or, on the contrary, they can be reduced when fertilizer quantities that exceed the crop requirements are applied, because the delivery of soil resources to the host plant through AMFs loses importance.

Such reasons could explain the higher colonization frequency and intensity, as well as the higher number of spores which were reached with the inoculation of *F. mosseae* combined with the application of 12,5 t of manure ha<sup>-1</sup> plus the nitrogen fertilization, and the depressive effect the highest dose of organic manure exerted on the fungal variables.

The fact that an effective inoculation of the pasture was achieved with the application of a

Table 3. Effect of the treatments on mycorrhizal structures

Manure (t ha <sup>-1</sup> )	N (kg ha <sup>-1</sup> year <sup>-1</sup> )	Inoc.	First year			Second year		
			Colonization (%)	Visual density (%)	Spores 50 g <sup>-1</sup>	Colonization (%)	Visual density (%)	Spores 50 g <sup>-1</sup>
0	0	NI	22,3 <sup>d</sup>	1,29 <sup>d</sup>	178 <sup>d</sup>	14,7 <sup>c</sup>	1,07 <sup>c</sup>	105 <sup>c</sup>
12,5	0	NI	21,8 <sup>d</sup>	1,31 <sup>d</sup>	180 <sup>d</sup>	15,2 <sup>c</sup>	1,11 <sup>c</sup>	112 <sup>c</sup>
12,5	105	NI	22,7 <sup>d</sup>	1,33 <sup>d</sup>	169 <sup>d</sup>	14,9 <sup>c</sup>	1,09 <sup>c</sup>	108 <sup>c</sup>
12,5	150	NI	21,5 <sup>d</sup>	1,27 <sup>d</sup>	183 <sup>d</sup>	15,0 <sup>c</sup>	0,96 <sup>c</sup>	119 <sup>c</sup>
25	0	NI	22,7 <sup>d</sup>	1,29 <sup>d</sup>	179 <sup>d</sup>	14,3 <sup>c</sup>	1,05 <sup>c</sup>	105 <sup>c</sup>
25	105	NI	21,4 <sup>d</sup>	1,32 <sup>d</sup>	188 <sup>d</sup>	15,9 <sup>c</sup>	0,97 <sup>c</sup>	103 <sup>c</sup>
25	150	NI	21,6 <sup>d</sup>	1,26 <sup>d</sup>	171 <sup>d</sup>	14,6 <sup>c</sup>	1,10 <sup>c</sup>	112 <sup>c</sup>
12,5	0	I	52,4 <sup>b</sup>	2,13 <sup>b</sup>	379 <sup>b</sup>	39,1 <sup>b</sup>	1,39 <sup>b</sup>	321 <sup>b</sup>
12,5	105	I	57,7 <sup>a</sup>	2,83 <sup>a</sup>	503 <sup>a</sup>	50,3 <sup>a</sup>	1,83 <sup>a</sup>	458 <sup>a</sup>
12,5	150	I	58,2 <sup>a</sup>	2,79 <sup>a</sup>	491 <sup>a</sup>	48,9 <sup>a</sup>	1,79 <sup>a</sup>	433 <sup>a</sup>
25	0	I	36,5 <sup>c</sup>	1,53 <sup>c</sup>	291 <sup>c</sup>	15,0 <sup>c</sup>	1,11 <sup>c</sup>	109 <sup>c</sup>
25	105	I	35,2 <sup>c</sup>	1,49 <sup>c</sup>	282 <sup>c</sup>	14,8 <sup>c</sup>	1,07 <sup>c</sup>	118 <sup>c</sup>
25	150	I	36,7 <sup>c</sup>	1,55 <sup>c</sup>	279 <sup>c</sup>	15,7 <sup>c</sup>	0,98 <sup>c</sup>	115 <sup>c</sup>
SE ±			2,0**	0,15**	38**	1,8**	0,13**	29**

Inoc: inoculation, NI: not inoculated. Averages with different letters in the same column significantly differ at  $p < 0,05$  (Duncan, 1955).

low quantity of inoculant (1 kg ha<sup>-1</sup>), which also contributed a low quantity of spores per area, is interesting. Nevertheless, the presence of abundant fragments of rootlets and hyphae from the host plant, along with the inoculation method used, could have guaranteed that, besides the spores, other viable mycorrhizal propagules also remained in close contact with the Guinea grass seeds, facilitating the colonization of its roots since the moment of germination. In addition, the low population of resident AMFs, which is inferred from the low values of colonization frequency and intensity and the number of spores in the rhizosphere of the plants that were not inoculated, could also have facilitated the action of the inoculant.

Cattle manure, alone or combined with the nitrogen fertilizer and the inoculation of *F. mosseae*, increased the N, P and K values in the biomass of the aerial part of the pasture; the values for each evaluation period are shown in table 4.

The effect of fertilization and mycorrhizal inoculation on macronutrient concentration in the biomass was manifested during the first and second year; in both years the N values with the application of 12,5 t of manure ha<sup>-1</sup>, combined with 105 or 150 kg N ha<sup>-1</sup> year<sup>-1</sup> and inoculated with *F. mosseae*, did not differ from the ones reached with the addition of 25 t ha<sup>-1</sup> plus 150 kg N ha<sup>-1</sup> year<sup>-1</sup> without inoculation.

Likewise, the dose of 12,5 t ha<sup>-1</sup> plus the inoculation of *F. mosseae* produced P and K values similar to the ones achieved with the addition of 25 t ha<sup>-1</sup> in absence of inoculation. The N, P and K concentrations during the second year were increased in 13, 23 and 16 %, respectively, with regards to the first one.

If it is taken into consideration that with the inoculation of *F. mosseae* plus the addition of 12,5 t of manure ha<sup>-1</sup> and 105 or 150 kg N ha<sup>-1</sup> year<sup>-1</sup> the highest values of the fungal variables were obtained, it is deduced that the introduction of an efficient AMF strain, accompanied by fertilizer doses adapted to the needs of the inoculated pasture, contributed to improve the chemical composition of the biomass and to reach similar values to the ones that are obtained only with the addition of higher fertilizer doses in the absence of inoculation. This was possible from an effective mycorrhizae functioning, which was shown in higher colonization of the pasture roots by the introduced strain and, consequently, in the improvement of the utilization of the fertilizer and soil nutrients, which coincides with the report by Castillo *et al.* (2013).

In the non-inoculated pasture, the highest DM yield was obtained with 25 t of manure ha<sup>-1</sup> plus 150 N ha<sup>-1</sup> year<sup>-1</sup>; nevertheless, in the presence of *F. mosseae* with the addition of 12,5 t of manure

Table 4. Nutrient content in the aerial biomass of the pasture (g kg<sup>-1</sup> DM).

Manure (t ha <sup>-1</sup> )	N (kg ha <sup>-1</sup> )	Inoc.	First year			Second year		
			N	P	K	N	P	K
0	0	NI	10,2 <sup>f</sup>	1,11 <sup>c</sup>	10,6 <sup>c</sup>	11,1 <sup>f</sup>	1,63 <sup>c</sup>	12,5 <sup>c</sup>
12,5	0	NI	11,5 <sup>c</sup>	1,58 <sup>b</sup>	12,8 <sup>b</sup>	13,3 <sup>c</sup>	1,96 <sup>b</sup>	14,6 <sup>b</sup>
12,5	105	NI	12,7 <sup>d</sup>	1,63 <sup>b</sup>	13,1 <sup>b</sup>	14,6 <sup>d</sup>	2,07 <sup>b</sup>	14,9 <sup>b</sup>
12,5	150	NI	14,0 <sup>c</sup>	1,57 <sup>b</sup>	12,9 <sup>b</sup>	15,9 <sup>c</sup>	1,95 <sup>b</sup>	14,8 <sup>b</sup>
25	0	NI	13,9 <sup>c</sup>	1,91 <sup>a</sup>	15,3 <sup>a</sup>	16,1 <sup>c</sup>	2,38 <sup>a</sup>	17,8 <sup>a</sup>
25	105	NI	15,4 <sup>b</sup>	2,03 <sup>a</sup>	15,6 <sup>a</sup>	17,4 <sup>b</sup>	2,41 <sup>a</sup>	18,1 <sup>a</sup>
25	150	NI	16,8 <sup>a</sup>	1,89 <sup>a</sup>	15,5 <sup>a</sup>	18,7 <sup>a</sup>	2,36 <sup>a</sup>	17,7 <sup>a</sup>
12,5	0	I	13,9 <sup>c</sup>	1,93 <sup>a</sup>	15,6 <sup>a</sup>	15,6 <sup>c</sup>	2,40 <sup>a</sup>	17,9 <sup>a</sup>
12,5	105	I	16,9 <sup>a</sup>	2,04 <sup>a</sup>	15,2 <sup>a</sup>	19,1 <sup>a</sup>	2,39 <sup>a</sup>	18,3 <sup>a</sup>
12,5	150	I	16,7 <sup>a</sup>	1,96 <sup>a</sup>	15,7 <sup>a</sup>	18,8 <sup>a</sup>	2,42 <sup>a</sup>	17,7 <sup>a</sup>
25	0	I	14,1 <sup>c</sup>	2,05 <sup>a</sup>	15,4 <sup>a</sup>	15,9 <sup>c</sup>	2,37 <sup>a</sup>	18,2 <sup>a</sup>
25	105	I	15,5 <sup>b</sup>	1,96 <sup>a</sup>	15,8 <sup>a</sup>	17,2 <sup>b</sup>	2,41 <sup>a</sup>	18,5 <sup>a</sup>
25	150	I	17,1 <sup>a</sup>	2,02 <sup>a</sup>	15,3 <sup>a</sup>	18,9 <sup>a</sup>	2,37 <sup>a</sup>	17,9 <sup>a</sup>
SE ±			0,3**	0,08**	0,4**	0,3**	0,09**	0,4**

Inoc: inoculation, NI: not inoculated. Averages with different letters in the same column significantly differ at  $p < 0,05$  (Duncan, 1955). \*\* $p < 0,01$ .



ha<sup>-1</sup> plus 105 or 150 kg N ha<sup>-1</sup> year<sup>-1</sup> similar results have been achieved, which confirmed the effect of the introduced strain on the reduction of the doses of both fertilizers (table 5). This also turned out to be evident when comparing the yield reached with 12,5 and 25 t of manure ha<sup>-1</sup>, with and without the inoculation of *F. mosseae*, respectively, and without complementary addition of N, whose values were similar.

The application of 25 t of manure ha<sup>-1</sup>, accompanied or not by nitrogen fertilizer and combined with *F. mosseae*, did not increase the yield with regards to these same treatments in the absence of inoculation, which suggests a decrease of the effectiveness of the introduced strain due to the addition of an organic fertilizer dose which, evidently, turned out to be high for the inoculated pasture. The lowest values of the fungal variables of this manure dose in the presence of *F. mosseae*, with regards to the other inoculated treatments, confirm such statement.

The influence of manure and mycorrhizal inoculation on the yield was observed in the first as well as the second year.

The integral analysis of the results allows to state that the effect of cattle manure on the yield was related to its contribution of OM and nutrients, which was also proven when evaluating its influence on the chemical characteristics of the soil and the

nutritional status of the plants; yet, its contribution in the improvement of the soil physical and biological properties and in the increase of pasture productivity cannot be discarded either, taking into consideration the role played by OM (Zhang *et al.*, 2014).

The fact that with *F. mosseae* plus the application of 12,5 t of manure ha<sup>-1</sup> and 105 kg N ha<sup>-1</sup> year<sup>-1</sup> similar yields were obtained as the ones reached with 25 t of manure ha<sup>-1</sup> and 150 kg N ha<sup>-1</sup> year<sup>-1</sup> in the non-inoculated pasture confirms the thesis that when an efficient AMF strain is included, the quantity of nutrients to be applied to reach certain yield is usually lower than the one needed to achieve that same result in the absence of mycorrhizal inoculation, aspect which has also been proven in other crops (Rivera *et al.*, 2007; Tanwar *et al.*, 2013; Martín *et al.*, 2014).

If it is taken into consideration that the OM, P and K contents in the soil were lower with the application of 12,5 than with 25 t of manure ha<sup>-1</sup> and that in the second year they did not show significant differences between the doses of 0 and 12,5 t of manure ha<sup>-1</sup>, even when the effect of the introduced strain was extended until that period, it is deduced that the contribution of *F. mosseae* to the reduction of organic and nitrogen fertilization was closely linked to the improvement of the utilization of fertilizer and soil nutrients, aspects reported by

Table 5. DM yield (t ha<sup>-1</sup>) of the pasture.

Manure (t ha <sup>-1</sup> )	N (kg ha <sup>-1</sup> year <sup>-1</sup> )	AMF	First year	Second year
0	0	NI	7,11 <sup>f</sup>	3,03 <sup>f</sup>
12,5	0	NI	9,15 <sup>c</sup>	4,44 <sup>c</sup>
12,5	100	NI	10,22 <sup>de</sup>	5,11 <sup>de</sup>
12,5	150	NI	11,18 <sup>d</sup>	6,01 <sup>d</sup>
25	0	NI	12,85 <sup>c</sup>	7,79 <sup>c</sup>
25	100	NI	14,76 <sup>b</sup>	9,41 <sup>b</sup>
25	150	NI	16,42 <sup>a</sup>	11,13 <sup>a</sup>
12,5	0	I	12,92 <sup>c</sup>	7,77 <sup>c</sup>
12,5	100	I	16,81 <sup>a</sup>	10,93 <sup>a</sup>
12,5	150	I	17,13 <sup>a</sup>	11,11 <sup>a</sup>
25	0	I	12,89 <sup>c</sup>	7,95 <sup>c</sup>
25	100	I	15,02 <sup>b</sup>	9,19 <sup>b</sup>
25	150	I	16,57 <sup>a</sup>	10,89 <sup>a</sup>
SE ±			0,49**	0,39**

Inoc: inoculation, NI: not inoculated. Averages with different letters in the same column significantly differ at  $p < 0,05$  (Duncan, 1955). \*\* $p < 0,01$ .

Beltrano *et al.* (2013) and Ngwene *et al.* (2013). The possible contribution of the addition of OM to the improvement of the functioning of the introduced strain cannot be discarded either, which was reported by Hodge (2014).

The lower rainfall that occurred during the rainy season of the second year could have influenced the DM yield, which decreased 40 % as average with regards to the same period of the previous year. Between May and October of the second year an accumulated value of 829 mm of rainfall was reached, which represented only 48 % of the accumulated rainfall during similar period of the first year.

Thus, the values of the fungal variables and of the N, P and K concentrations in the biomass during the first year with regards to the second could have also been affected by the unequal growth of the crop during both periods. In the first case, the higher pasture growth could have increased the nutrient demand and, in fact, the formation of more mycorrhizal structures to have access to such resources (Bennett *et al.*, 2013; Bainard *et al.*, 2014); in the second case, the higher growth could have originated a decrease of the nutrient concentration in the biomass, due to a dilution effect (Shakhane *et al.*, 2013; Zangaro *et al.*, 2013).

The results allow to conclude that, through the inoculation with *F. mosseae* at the moment of planting, the applications of cattle manure and nitrogen fertilization can be reduced in 50 and 30 %, respectively, without affecting the nutrient content in the biomass or its yield, during two years of crop growth.

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