


RESEARCH ARTICLE

Mycorrhizal inoculation and application of cattle manure in field-grown maize in semiarid conditions

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Abstract

We evaluated the effects of cattle manure and inoculation with arbuscular mycorrhizal fungi (AMF) in maize plants growing in a semiarid area of Brazilian north-east in 2012 and 2013. Three isolates of AMF (*Acaulospora longula* URM-FMA 07 and URM-FMA 03, *Claroideoglossum etunicatum* UNIVASF 06A) were used, with or without the application of cattle manure, during two growing cycles. In the first year, significant effects of inoculation were detected for straw yield only when the manure was applied. In the second year, there was an interaction between fertilisation and inoculation for plant height and grain yield, with the highest values in the fertilised treatments. Inoculation with *A. longula* demonstrated that mycorrhizal inoculation in field-grown plants could be an alternative management for improving plant growth and grain yield, reducing the use of cattle manure. The AMF sporulation and mycorrhizal colonisation were improved after inoculation, and *A. longula* URM-FMA 07 increased sporulation by more than 15 times while inoculation with *C. etunicatum* increased sporulation by more than 3 times. The mycorrhizal inoculation is a management practice that can be useful for recovering or maintaining AMF infective propagules in soil, showing potential to be used in large-scale field conditions in Brazilian semiarid. Although mycorrhisation presents high agricultural relevance due to benefits promoted to the soil and plants, the knowledge about the factors influencing the interactions among microorganisms, soil and plants need to be broadened aiming to achieve successful crop management in semiarid regions.

Keywords: Agriculture; Crop production; Manure; Mycorrhizal fungi

Introduction

Agricultural expansion requires crop management practices for increasing plant productivity with low environmental impact. Microorganisms such as arbuscular mycorrhizal fungi (AMF) can be useful to achieve such objective as those mycobionts are naturally found in soil and improve the use of natural resources (Oyewole *et al.*, 2017). While transferring nutrients to plants, AMF receive photosynthates in a mutualistic association known as arbuscular mycorrhiza, which enhances plant growth (Oyewole *et al.*, 2017). An urgent concern about the maintenance of AMF communities in cultivated soils has been raised (Dai *et al.*, 2013), so that countries where the economy is strongly influenced by agricultural practices can no longer neglect managements that favour sustainable agriculture. Therefore, they should improve and expand the knowledge of how the use of microorganisms can promote environmental sustainability.

Field experiments involving inoculation with AMF of the major grain crops have been documented (Berta *et al.*, 2014) and studies with inoculation have been shown to increase the productivity of maize in Italy (Cozzolino *et al.*, 2013). There are few studies that address the effects of mycorrhizal inoculants on maize crops established in semiarid regions. In general, only the occurrence of AMF in maize plantations (Benedetti *et al.*, 2005) or the effects of manure application on crop yield are documented (Marin *et al.*, 2007; Mundus *et al.*, 2008). In addition, the evidence of the benefits provided by AMF is generally observed under greenhouse conditions and with sterilised soil.

Maize productivity in Northeast Brazil (2915 kg ha⁻¹) is much lower than in the rest of the country (6302 kg ha⁻¹) (Conab, 2018) due to water and nutrient limitations. In such semiarid region, mineral fertilisation is seldom used but manure is frequently applied to small plots of the predominant family-based agricultural systems. However, manure availability is not sufficient to supply the demand (Marin *et al.*, 2007) and increases in its use efficiency could have a positive impact on maize productivity. For example, AMF inoculation can improve nutrient uptake and thus increase the efficiency of fertilisers (Cozzolino *et al.*, 2013), while alleviating water-deficit symptoms (Oyewole *et al.*, 2017).

Acaulospora longula Spain & N.C. Schenck, *Gigaspora albida* Schenck & Smith and *Claroideoglossum etunicatum* (W. N. Becker & Gerd.) C. Walker & A. Schüßler are the species of wide occurrence in Brazilian soils and isolates of these species have promoted the growth of sugarcane (Pereira *et al.*, 2016) and maize (Novais *et al.*, 2014) plants on sterilised substrates. However, few studies tested their effects on established plants in the field (Souza *et al.*, 2010). Considering that the performance of maize plants changes due to the applied AMF isolate (Novais *et al.*, 2014), it is important to investigate if introduced inocula establish a functional symbiosis and how the dynamics of fungus–plant relationship occurs under natural conditions. Such information is needed to ensure the success of inoculation in the field, especially in farming systems whose native mycorrhizal potential may be insufficient in quantity and quality (Koide and Mosse, 2004). Thus, the objective of this study was to evaluate how the application of mycorrhizal inoculum in soil interacts with organic fertilisation and affects soil microbiota activity and maize productivity.

Materials and Methods

Experimental design, inoculation treatments and plant material

Two experiments were conducted in São João municipality (8°52'32"S, 36°22'00"W, 716 m a.s.l.), Pernambuco, Brazil, in the growing seasons of 2012 and 2013. The area has a hot and humid climate, type As' according to the Köppen classification. Rainfall data of 2012 and 2013 are shown in Table 1. The experimental area was used for coffee production from 1960 to 2000, subsistence crops (beans and cassava) from 2001 to 2010, and it was left fallow in 2011. In 2012, the superficial soil layer (depth 0–20 cm) was sampled and analysed (P = 24 mg dm⁻³; Na = 0.04 cmol_c dm⁻³; K⁺ = 0.23 cmol_c dm⁻³; Ca⁺² = 1.0 g kg⁻¹; Mg⁺² = 0.7 cmol_c dm⁻³; H+Al = 14 g kg⁻¹).

The experiments were set up in a randomised block design in a 4 × 2 factorial arrangement with four inoculation treatments (*A. longula* URM-FMA 07; *A. longula* URM-FMA 03; *C. etunicatum* UNIVASF 06-A and a control, not inoculated) and two fertiliser treatments (with or without cattle manure), with four replications. Each block was divided into eight 4 × 4 m plots, each one with four planting lines 4 m long and 1 m apart. The two external lines and the 0.5-m terminal sections of the two central lines were considered as border areas. In each plot, eight plants were harvested for grain yield and nutritional analyses, with nutrient concentration being expressed in g kg⁻¹ of dry straw weight (stem + leaves + cob straw).

Cured cattle manure was spread each year on the soil surface at a rate equivalent to 15 Mg ha⁻¹ (24 kg plot⁻¹). Planting holes were opened every 0.5 m along the lines and three maize (*Zea mays* L. var. 'Pontinha') seeds were sown manually in each hole. In the inoculation treatments, soil

Table 1. Monthly and annual rainfall and air temperature during the experimental years in the São João municipality, Pernambuco state, Brazil

Months	2012				2013			
	Rainfall (mm)	Temperature (°C)			Rainfall (mm)	Temperature (°C)		
		Max	Min	Average		Max	Min	Average
Jan	15.2	31.0	17.7	24.3	12.5	31.8	19.5	25.6
Feb	14.0	30.9	18.0	24.4	0.00	32.0	20.1	26.1
Mar	19.0	31.2	17.8	24.5	3.6	32.1	20.2	26.1
Apr	18.0	30.2	16.6	23.4	70.0	30.8	19.6	25.2
May	53.0	28.7	15.9	22.3	53.1	28.1	18.7	23.4
Jun	57.5	27.3	15.9	21.6	111.5	27.3	18.2	22.8
Jul	73.0	26.0	15.1	20.5	137.9	25.8	17.5	21.7
Aug	70.7	26.2	14.8	20.5	64.4	25.4	17.3	21.3
Sep	15.0	27.9	16.1	22.0	15.6	27.7	17.6	22.7
Oct	11.0	31.5	18.5	25.0	44.0	30.0	17.4	23.9
Nov	0.0	31.5	19.3	25.4	27.0	30.5	18.2	24.3
Dec	0.0	31.5	18.9	25.2	0.00	31.8	18.6	25.2
Annual	346.4	–	–	–	539.6	–	–	–

Sources: Instituto Agrônomo de Pernambuco-IPA: rainfall; AgriTempo: air temperature.

inoculum containing approximately 300 (first experiment) or 400 (second experiment) spores of the corresponding AMF treatments were placed close to the seeds in each hole. Only one plant per hole was left when they reached stage V3 (three fully expanded leaves), removing the additional plants and leaving a total density at 20,000 plants ha⁻¹.

The inocula of the three AMF isolates were provided by the Laboratory of Mycorrhiza, Department of Mycology, UFPE (Brazil). The inocula of *A. longula* URM-FMA 07 and *C. etunicatum* UNIVASF 06-A were multiplied in flowerbeds, on a substrate composed of sand (60%), clay (35%), sugarcane bagasse (2.5%) and *Leucaena* residue (2.5%), with *Sorghum bicolor* (L.) Moench as a host plant. The inoculum of *A. longula* URM-FMA 03 was produced in a greenhouse in pots with soil, using *S. bicolor*, *Zea mays* L. and *Brachiaria decumbens* Stapf as host plants.

The plants were grown for 120 days each year: from June to October in 2012 and from May to September in 2013. Four composite samples for each treatment (from five soil subsamples) (2 kg) were collected in the superficial layer (0–20 cm) at the time of planting and harvest during each crop year, totaling 32 samples per sampling time. The soil samples were used for microbiological analysis. Weed control was performed every fortnight with the aid of a hoe, and formicide was applied whenever necessary, following the indications of the manufacturer.

Growth parameters and NPK (Nitrogen-Phosphorus-Potassium) concentrations

Before harvesting, plant height and stem diameter were measured using a tape measure and a caliper, respectively, with the ears being counted in all plants of each plot. After harvest, the plants were separated into the straw (stalks and leaves), cob and grains and were weighed. Samples of these materials were dried in a forced air oven at 65 °C for 72 h, weighed, ground in a Wiley type mill, digested with sulfuric acid (H₂SO₄) and hydrogen peroxide (H₂O₂) and analysed. Harvest grain indices were calculated by the ratio between the total mass of dried grains and the total aboveground mass of plants. Contents of P were determined by colorimetry (Silva *et al.*, 1999), K by flame photometry (Silva *et al.*, 1999) and total N by distillation (Bremner and Mulvaney, 1982).

Mycorrhizal colonisation and glomerospores extraction

At harvest, fine roots from each plant were separated, washed with tap water, cleared and stained (Phillips and Hayman, 1970), and the degree of mycorrhizal colonisation was estimated by the gridline intersect method (Giovanetti and Mosse, 1980). The total number of glomerospores

in the soil was determined in soil samples (50 g) collected at planting and harvest time, totalling 64 soil samples per harvest. Glomerospores were extracted from the soil by wet sieving (Gerdemann and Nicolson, 1963) and sucrose centrifugation (Jenkins, 1964), and counted on a Petri plate, under a stereomicroscope (40×).

Microbial biomass carbon and soil respiration

Microbial biomass carbon (MB-C) was estimated at the beginning and end of each experiment using 10 g of soil samples that were fumigated or not with the chloroform free of ethanol, extracted with 25 mL potassium sulphate (0.5 M). Extracts were treated with 1 mL potassium dichromate (0.66 mM) in a medium with 5 mL concentrated sulfuric acid and 0.5 mL concentrated phosphoric acid and titrated with ammonium iron sulphate (0.033 N), using 1% dyphenylamine as indicator, according to De-Polli and Guerra (1997). The emission of CO₂ from the soil samples was calculated following the procedure as in Grisi (1978): a 100 g of soil was incubated in screw bottles with 10 mL KOH (0.5 N) for 14 days and CO₂ was quantified by titration with HCl 0.1 N, using phenolphthalein (0.1% in ethanol) and methyl orange (1.0%) as indicators of pH.

Statistical analysis

Data of soil respiration, microbial biomass carbon and glomerospores number were submitted to a three-way analysis of variance (ANOVA), considering the inoculation (control, inoculation with *A. longula* 07, *A. longula* 03 and *C. etunicatum*), fertilisation (with or without) and sampling time (before and after harvest) as factors. Mycorrhizal colonisation, straw, [K], [P], [N], height, stem diameter, number of ears and grain yield were submitted to a two-way ANOVA, considering the inoculation and fertilisation treatments as factors. These analyses were performed using the functions 'fat3.dbc' and 'fat2.dbc' for three- and two-way ANOVA included in the package 'ExpDes.pt' (Ferreira *et al.*, 2013) for the R environment (R Core Team, 2017), respectively.

Results

Grain yield, straw and NPK concentrations

Differences in rainfall between the growing seasons affected crop yield, with 2012 being atypically dry with very low rainfall throughout the year (Table 1). This caused poor crop development and there was no grain production (Tables 2 and 3). In such dry year, precipitation ceased at the most critical phenological stages, i.e., V8 and tassel emission. There was an average of 13 mm of rainfall in the last 60 days of the experiment (Table 1). At 120 days, plants were very dry and the few atrophied spikes were incorporated into the straw analysis (straw yield), because their harvest and evaluation were not feasible.

In 2012, plants supplied with manure were taller than plants in non-supplied plots (Table 2). Significant effects of inoculation were detected for straw yield, only when the manure was applied. Higher straw yields were observed in plants inoculated with *C. etunicatum* when compared to the ones inoculated with *A. longula* 07 (Table 3). K concentration was affected only by manure, and it was higher ($p < 0.05$) in fertilised (12 g kg⁻¹ dry weight of straw) than in unfertilised plots (8.7 g kg⁻¹ dry weight of straw). There was no effect of the inoculation and manure application on plant N (average of 7.6 g kg⁻¹ dry weight of straw) and P (average of 2.84 g kg⁻¹ dry weight of straw) concentrations.

In 2013, higher stem diameter was found in manure fertilised plots when compared to unfertilised plots and in plots inoculated with *A. longula* 07 when compared to ones not inoculated (Table 2). There was an interaction between fertilisation and inoculation for plant height and grain yield, with the highest values being observed in the fertilised treatments (except for *A. longula* 07). Differences among inoculation treatments were observed in the unfertilised plots, where the highest grain yield and plant height were found in plots inoculated with *A. longula* 07 (Tables 2 and 3).

Table 2. Plant height, stem diameter and number of ears of maize plants inoculated with AMF (*A. longula* 07, *A. longula* 03 and *C. etunicatum* 06-A) and supplied with cattle manure. Control plants were not inoculated and did not receive manure

Treatments	Height (m)		Stem diameter (cm)		Mean inoculation	No. of ears	
	Manure		Manure			Manure	
	With	Without	With	Without		With	Without
2012							
<i>A. longula</i> 07	0.79	0.70	20.1	20.8	–	0.0	0.0
<i>A. longula</i> 03	0.80	0.83	21.1	19.0	–	0.0	0.0
<i>C. etunicatum</i> 06-A	0.92	0.74	21.9	18.8	–	0.0	0.0
Not inoculated	0.86	0.65	23.7	16.2	–	0.0	0.0
Mean manure	0.84A	0.72B	21.9A	18.7B	–	–	–
CV (%)	16.65		14.47			–	
2013							
<i>A. longula</i> 07	2.20aA	2.05aA	27.1	22.2	24.6a	9.5	9.2
<i>A. longula</i> 03	2.29aA	1.56bB	26.9	18.7	22.8ab	10.5	8.0
<i>C. etunicatum</i> 06-A	2.17aA	1.76abB	27.1	19.9	23.5ab	10.5	8.2
Not inoculated	2.27aA	1.67bB	26.8	17.6	22.2b	10.7	7.0
Mean manure	–	–	26.9A	19.6B		10.3A	8.1B
CV (%)	9.80		7.68			17.60	

Means followed by the same letter do not differ by the Tukey test (5%).

CV, coefficient of variation; Lower case letter = comparison in the column; uppercase letter = comparison in the line, in each year.

Table 3. Grain yield and straw (leaves + stem + cob) of maize plants inoculated with AMF (*A. longula* 07, *A. longula* 03 and *C. etunicatum* 06-A) and supplied with cattle manure. Control plants were not inoculated and did not receive manure

Treatments	Grain (kg ha ⁻¹)		Straw (kg ha ⁻¹)	
	Manure		Manure	
	With	Without	With	Without
2012				
<i>A. longula</i> 07	0	0	897bA	821aA
<i>A. longula</i> 03	0	0	1098abA	957aA
<i>C. etunicatum</i> 06-A	0	0	1402aA	754aB
Not inoculated	0	0	1326abA	581aB
CV (%)	–		27.50	
2013				
<i>A. longula</i> 07	2932aA	2305aA	4719	3425
<i>A. longula</i> 03	2862aA	1286bB	3866	1973
<i>C. etunicatum</i> 06A	3147aA	1592abB	5497	2267
No inoculation	2940aA	813bB	4821	1812
Not inoculated	–	–	4457A	2209B
CV (%)	24.3		27.1	

Means followed by the same letter do not differ by the Tukey test (5%).

CV, coefficient of variation; Lower case letter = comparison in the column; uppercase letter = comparison in the line.

The number of ears and straw yield were increased when supplying manure (Tables 2 and 3), as well as K concentration (17 vs. 12 g kg⁻¹ dry weight of straw with and without manure, respectively). There was no effect of the inoculation and manure application on N (average of 5.5 g kg⁻¹ dry weight of straw) and P (average of 1.46 g kg⁻¹ dry weight of straw) concentrations.

Mycorrhizal colonisation, glomerospores number and soil microbial activity

In both the years, mycorrhizal colonisation was higher in plants inoculated with AMF and when the soils were fertilised (Table 4). The inoculation also increased the number of soil glomerospores (Table 5). In 2012, glomerospores were 2.5 times greater in the unfertilised plots inoculated with

Table 4. Mycorrhizal colonisation of maize roots inoculated with AMF (*A. longula* 07, *A. longula* 03 and *C. etunicatum* 06-A) and supplied with cattle manure. Control plants were not inoculated and did not receive manure

Treatments	Mycorrhizal colonisation (%)		
	Manure		Mean inoculation
	With	Without	
2012			
<i>A. longula</i> 07	73.6	65.7	69.7a
<i>A. longula</i> 03	73.7	64.2	69.0a
<i>C. etunicatum</i> 06-A	68.1	59.6	63.9a
Not inoculated	53.7	46.6	50.2b
Mean manure	67.31A	59.0B	
CV (%)	10.96		
2013			
<i>A. longula</i> 07	83.25	68.25	75.7a
<i>A. longula</i> 03	80.50	72.25	76.4a
<i>C. etunicatum</i> 06-A	81.50	73.50	77.5a
Not inoculated	62.25	53.50	57.9b
Mean manure	76.9A	66.9B	
CV (%)	7.75		

Means followed by the same letter do not differ by the Tukey test (5%).
 CV, coefficient of variation; lower case letter = comparison in the column; uppercase letter = comparison in the line, in each year.

Table 5. Glomerospore numbers (in 50 g of soil) at planting and harvest times in maize rhizosphere inoculated with AMF (*A. longula* 07, *A. longula* 03 and *C. etunicatum* 06-A) and supplied with cattle manure. Control plots were not inoculated and did not receive manure

Treatments	Glomerospore number (50 g ⁻¹ soil)			
	Manure		Without	
	With	Without	Planting	Harvest
2012				
<i>A. longula</i> 07	58aA	80bA	65aA	151aA*
<i>A. longula</i> 03	90bA	356aA*	44aA	138aB*
<i>C. etunicatum</i> 06-A	67aA	150bA	88aA	104aA
Not inoculated	53aA	110bA	77aA	149aA
CV (%)	52.62			
2013				
<i>A. longula</i> 07	112aA	156bA	117aA	183bA
<i>A. longula</i> 03	104aA	196bB	52aA	807aA*
<i>C. etunicatum</i> 06-A	118aA	408aA*	121aA	249bB*
Not inoculated	140aA	258bA*	64aA	218bA*
CV (%)	34.68			

CV, coefficient of variation.
 Lower case letter, in the column, compares inoculation treatment; upper case letters compare the manure treatment (with or without) within each sampling time (before or harvest), and asterisks (*) compare the sampling time within each manure treatment (with or without). Means followed by the same letter did not differ by the Tukey test (5%).

A. longula 07 compared to the condition before inoculation (Table 5). At harvest, plots inoculated with *A. longula* 03 had three times more glomerospores compared to the initial condition, regardless of manure supplying (Table 5). In 2013, the number of glomerospores was 15 times higher in the unfertilised plots inoculated with *A. longula* 03 at harvest, and two and three times higher in unfertilised and fertilised plots inoculated with *C. etunicatum*, respectively (Table 5). Increases in the number of glomerospores were also found between planting and harvesting, regardless of manure supplying (Table 5).

In 2012, there was no effect of manure or AMF inoculation on soil respiration and MB-C from soil samples, with an average of 4.8 µg C-CO₂ g⁻¹ soil and 63 µg C g⁻¹ soil, respectively. In 2013,

manure supply increased ($p < 0.05$) soil respiration (8.0 vs. 2.8 $\mu\text{g C-CO}_2 \text{ g}^{-1}$ soil in fertilised and non-fertilised treatments, respectively) with higher ($p < 0.05$) values being found at harvest as compared to planting (8.0 vs. 4.6 $\mu\text{g C-CO}_2 \text{ g}^{-1}$ soil). There was also no effect of fertilisation or AMF inoculation on MB-C, which averaged 204 $\mu\text{g C g}^{-1}$ soil.

Discussion

The positive effects of fertilisation with cattle manure observed in this study were also observed by Marin *et al.* (2007), who reported significant increases in cob and grain yields in response to manure, the main organic fertiliser used to improve the fertility of north-east Brazilian soils (Mundus *et al.*, 2008). In 2012, none of the AMF applied proved to be better than the AMF native populations in terms of increasing plant performance and grain yield under low water availability (Tables 2 and 3). However, the benefits of inoculation with *A. longula* 07 for crop yield in 2013 (Table 3) revealed that the practice of mycorrhizal inoculation may be an alternative management for improving plant growth and grain yield when plants do not face water stress, while saving cattle manure.

Among the three AMF isolates studied, only *A. longula* (URM 07) was effective in increasing growth (Table 2) and grain yield of maize when soil was not fertilised (Table 3), demonstrating the importance of knowing the best fungus–plant combination before large-scale application. Our data indicate that maize plants were benefited by the mycorrhizal symbiosis in an environment with low availability of nutrients, which is in agreement with Cozzolino *et al.* (2013). Differences in plant performance caused by isolates of the same species, as observed in the present study, have been observed in other studies (Guo *et al.*, 2014; Novais *et al.*, 2014). Although there is no specificity between the fungus and the host, preferences or selectivity between them have been reported (Guo *et al.*, 2014).

The highest AMF colonisation was found in plants inoculated with AMF and in fertilised plots (Table 4), which is a likely consequence of increased plant growth and root development resulting in more roots to be colonised. Berta *et al.* (2014) also observed high rates of mycorrhizal colonisation in maize plants inoculated with AMF, showing that the intensity of colonisation is higher when plants receive mycorrhizal inoculants even in roots naturally colonised by AMF (native and exotics). Another point to be highlighted is that mycorrhizal colonisation rates in 2013 could be favoured by the AMF inoculation carried out in 2012, demonstrating that consecutive inoculation can also benefit symbiosis formation, possibly due to the increase of infective propagules of AMF in the soil.


In general, the number of glomerospores in the soil increased with inoculation, mainly for *A. longula* (Table 5). The introduced fungi may have increased sporulation or induced some native mycorrhizal fungi to sporulate. *Acaulospora* species produce many spores, and they frequently occur in maize fields (Hu *et al.*, 2015). While evaluating the influence of maize and wheat on AMF community, Hu *et al.* (2015) observed great abundance of *Acaulospora* spores and attributed such dominance to the production of small spores in large quantity and in a short period of time. From an agricultural point of view, AMF isolates producing large amount of spores that germinate quickly are of interest to the application in the soil, considering that the glomerospores are excellent infective propagules promoters of mycorrhizal symbiosis. Inoculation usually favours both root colonisation and glomerospore numbers (Cozzolino *et al.*, 2013; Guo *et al.*, 2014), and it should be a practice in crop fields given the beneficial effects of the symbiosis.

Better performance of the maize crop in 2013 (year of higher rainfall) may have favoured AMF sporulation in the soil, contributing to greater recovery of glomerospores in this year in relation to the previous year (2012). As obligate symbionts, the AMF depend on physiologically active plants to complete their life cycle, which ends with the formation of new spores, especially in the extraradicular system. Considering the importance of mycorrhizal colonisation to form new AMF

propagules and maintain the fungus in the environment, the understanding of the mechanisms that regulate this symbiosis is extremely important for the proper management of the soil aiming its sustainability.

In the present study, MB-C and soil basal respiration (C-CO₂) were not influenced by mycorrhizal inoculation; however, in other studies positive responses of these attributes in relation to AMF inoculation were observed (Almehyeb *et al.*, 2013). Similarly, changes in MB-C due to organic fertilisation were not observed in any of the two years (2012 and 2013). The absence of microbial biomass responses to manure application was also reported by Cunha *et al.* (2011), indicating that soil microbial activity does not always reflect management practices and can be a consequence of the complex nature of environment and organism interactions.

In conclusion, manure application had significant effects on maize grain and straw yields, while mycorrhizal inoculation affected plants when manure was not supplied (Table 3). The inoculation with *A. longula* 07 increased plant growth and grain yield when plants did not face water shortage, presenting the potential to be used in large-scale field conditions. The mycorrhizal attributes (sporulation and mycorrhizal colonisation) were improved after inoculation with AMF, mainly when plants were inoculated with *A. longula*. The inoculation was found to be an alternative management practice for maintaining glomerospores in the soil, which could be useful for recovering or maintenance of AMF infective propagules in Brazilian semiarid soil. In spite of the research already done, the knowledge about the factors that influence the interactions among microorganisms, soil and plants need to be broadened aiming to achieve successful soil management for semi-arid regions.

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References

- Almehyeb M., Ruppel S., Paulsen H.M., Vassilev N. and Eichler-Löbermann B. (2013). Single and combined applications of arbuscular mycorrhizal fungi and *Enterobacter radicincitans* affect nutrient uptake of faba bean and soil biological characteristics. *Applied Agricultural and Forestry Research* 3, 229–234.
- Benedetti T., Antonioli Z.I., Giracca E.M.N. and Steffen R.B. (2005). Diversidade de fungos micorrízicos arbusculares na cultura do milho após uso de espécies de plantas de cobertura de solo. *Revista de Ciências Agroveterinárias* 4, 44–51.
- Berta G., Copetta A., Gamalero E., Boma E., Cesaro P., Scarafoni A. and D'Agostino G. (2014). Maize development and grain quality are differentially affected by mycorrhizal fungi and a growth-promoting pseudomonad in the field. *Mycorrhiza* 24, 161–170.
- Bremner J.M. and Mulvaney C.S. (1982). Nitrogen-total. In *Methods of Soil Analysis, Part. 2. Chemical and Microbiological Properties*, 595–624 (Agronomy Monograph, vol. 9) (Eds A.L. Page, R.H. Miller and D.R. Keeney). Madison: ASA-SSSA.
- Conab: Companhia Nacional de Abastecimento. *Acompanhamento da safra brasileira de grãos* (2018). V.11 Safra 2017/18 - Décimo primeiro levantamento/Agosto ISSN: 2318-6852, p. 148.
- Cozzolino V., Di Meo V. and Piccolo A. (2013). Impact of arbuscular mycorrhizal fungi applications on maize production and soil phosphorus availability. *Journal of Geochemical Exploration* 129, 40–44.
- Cunha E.Q., Stone L.F., Ferreira E.P.B., Didonet A.D., Moreira J.A.A. and Leandro W.M. (2011). Sistemas de preparo do solo e culturas de cobertura na produção orgânica de feijão e milho. ii - atributos biológicos do solo. *Revista Brasileira de Ciência do Solo* 35, 603–611.
- Dai M., Bainard L.D., Hamel C., Gan Y. and Lynch D. (2013). Impact of land use on arbuscular mycorrhizal fungal communities in rural. *Applied and Environmental Microbiology* 79, 6719–6729.
- De-Polli H. and Guerra J.G.M. (1997). *Determinação do carbono da biomassa microbiana do solo: método da fumigação-extração (Série Documentos 37)*. Seropédica: Embrapa-CNPAB.

- Ferreira E.B., Cavalcanti P.P. and Nogueira D.A. (2013). ExpDes.pt: Experimental Designs package (Portuguese). R package version 1.1.2.
- Gerdemann J.W. and Nicolson T.H. (1963). Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society* **46**, 235–244.
- Giovanetti M. and Mosse B. (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist* **84**, 489–500.
- Grisi B.M. (1978). Método químico de medição da respiração edáfica: alguns aspectos técnicos. *Ciência e Cultura* **30**, 82–88.
- Guo W., Zhao R., Fu R., Bi N., Wang L., Zhao W., Guo J. and Zhang J. (2014). Contribution of arbuscular mycorrhizal fungi to the development of maize (*Zea mays* L.) grown in three types of coal mine spoils. *Environmental Science Pollution* **21**, 3592–3603.
- Hu J., Yang A., Wang J., Zhu A., Dai J., Wong M.H. and Lin X. (2015). Arbuscular mycorrhizal fungal species composition, propagule density, and soil alkaline phosphatase activity in response to continuous and alternate no-tillage in Northern China. *Catena* **133**, 215–220.
- Jenkins W.R. (1964). A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Disease Report* **48**, 692
- Koide R.T. and Mosse B. (2004). A history of research on arbuscular mycorrhiza. *Mycorrhiza* **14**, 145–163.
- Marin A.M.P., Menezes R.S.C. and Salcedo I.H. (2007). Produtividade de milho solteiro ou em aléias de gliricídia adubado com duas fontes orgânicas. *Pesquisa Agropecuária Brasileira* **42**, 669–677.
- Mundus S., Menezes R.S.C., Neergaard A. and Garrido M.S. (2008). Maize growth and soil nitrogen availability after fertilization with cattle manure and/or gliricidia in semi-arid NE Brazil. *Nutrient Cycling in Agroecosystems* **82**, 61–73.
- Novais C.B., Borges W.L., Jesus E.C., Saggin Júnior O.J. and Siqueira J.O. (2014). Inter- and intraspecific functional variability of tropical arbuscular mycorrhizal fungi isolates colonizing corn plants. *Applied Soil Ecology* **76**, 78–86.
- Oyewole B.O., Olawuyi O.J., Odebode A.C. and Abiala M.A. (2017). Influence of Arbuscular mycorrhiza fungi (AMF) on drought tolerance and charcoal rot disease of cowpea. *Biotechnology Reports* **14**, 8–15.
- Pereira C.C.M.S., Pedrosa E.M.R., Rolim M.M., Cavalcante U.M.T. and Pereira Filho J.V. (2016). Estresse hídrica e seus efeitos no desenvolvimento inicial e atividade bioquímica em cana-de-açúcar com a dupla inoculação de Meloidogyne incognita e fungos micorrízicos arbusculares. *Revista Brasileira de Agricultura Irrigada* **10**, 726–738.
- Phillips J.M. and Hayman D. (1970). Improved procedures for clearing roots and staining parasitic and vesicular arbuscular fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**, 158–161.
- Silva F.C., Eira P.A., Van Raij B., Silva C.A., Abreu C.A., Gianello C., Pérez D.V., Quaggio J.A., Tedesco M.J., Abreu M.F. and Barreto W.O. (1999). Análises químicas para a avaliação da fertilidade do solo. In *Manual de Análises Químicas de Solos, Plantas e Fertilizantes*, 75–169 (Ed F.C. Silva). Brasília: EMBRAPA.
- Souza R.G., Goto B.T., Silva D.K.A., Barbosa F.S.B., Sampaio E.V.S.B. and Maia L.C. (2010). The role of arbuscular mycorrhizal fungi and cattle manure in the establishment of *Tocoyena selloana* Schum. in mined dune areas. *European Journal of Soil Biology* **46**, 237–242.

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