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Effects of using different host plants and long-term fertilization systems on population sizes of infective arbuscular mycorrhizal fungi

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Abstract

The effect of cultivation of mycorrhizal and non-mycorrhizal plants and mineral fertilization on the arbuscular mycorrhizal fungal (AMF) community structure of maize (*Zea mays* L.) plants was studied. Soil samples were collected from two field experiments treated for 5 years with three fertilization systems (Control – no fertilization; Mineral – NPK fertilization; and Organic – Farmyard manure fertilization). Soil samples containing soil and root fragments of rapeseed (*Brassica napus* L., non-mycorrhizal plant) and wheat (*Triticum aestivum* L., mycorrhizal plant) collected from the field plots were used as native microbial inoculum sources to maize plants. Maize plants were sown in pots containing these inoculum sources for four months under glasshouse conditions. Colonization of wheat roots by AMF, AMF community structure, AMF diversity (Shannon's index), AMF dominance (Simpson's index) and growth of maize were investigated. Sixteen AMF species were identified from rhizosphere soil samples as different species of genera *Acaulospora, Claroideoglomus, Dentiscutata, Funneliformis, Gigaspora, Quatunica, Racocetra*, and *Rhizoglomus*. Maize plants grown in manure-fertilized soils had a distinct AMF community structure from plants either fertilized with mineral NPK-fertilizer or non-fertilized. The results also showed that inoculum from non-mycorrhizal plants combined with mineral fertilization decreased AMF diversity (Shannon's index), AMF dominance (Simpson's index) and growth of maize. Our findings suggest that non-mycorrhizal plants, such as *B. napus*, can negatively affect the presence and the effects of soil inoculation on maize growth. Also, our results highlight the importance of considering the long-term effect of rapeseed cultivation system on the reduction of population sizes of infective AMF, and its effect on succeeding annual crops.

Keywords Arbuscular mycorrhizal fungi · Biodiversity · Long-term trial experiment · Organic cultivation systems · Conventional cultivation systems · Non-mycorrhizal plants

1 Introduction

Arbuscular mycorrhizal fungi (AMF) are soil fungi broadly known for their ability to establish symbiosis with terrestrial species of flowering plants by forming mycorrhizas into their roots (Smith and Read 2008). This symbiotic relationship has been shown to improve growth, nutrient and water supply to their hosts in several natural and agricultural ecosystems (Harikumar et al. 2015). It is widely known that non-mycorrhizal plants, intensive agricultural management practices and the continuous input of inorganic fertilizers result in the reduction of AMF diversity and functionality (Belay et al. 2015; Pakpour and Klironomos 2015). For instance, the family Brassicaceae has been reported as non-mycorrhizal by Eltrop and Marschner (1996), Olsson and Tyler (2004) and Valetti et al. (2016). These authors also highlight that rapeseed (*Brassica napus*) cultivation decreases AMF root colonization by 30% on succeeding cropping of *Glicine max* L. This non-mycorrhizal plant does not have the specific cleavage site with target genes (e.g., miR171b, *LOM1*, and miR171 family members) that occurs in mycotrophic species and is responsible for the activation and enhancement of root mycorrhization (Couzigou et al. 2017).

However, understanding the effects of long-term cultivation of non-mycorrhizal plants, such as rapeseed (*Brassica napus* L. var. H401) under inorganic NPK fertilization, on

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the functioning of local mycorrhizas is essential to explain how the continuous use of these practices negatively affects the population sizes of infective AMF that form symbioses with many annual crops (Zhong et al. 2010; Sharma et al. 2011; Carneiro et al. 2015; Pakpour and Klironomos 2015).

Inorganic fertilization is usually applied to increase crop productivity in conventional cultivation systems, especially in soils with a very low P availability, such as a Ferralsol, an old and severely weathered soil with high concentrations of oxides and hydroxides of aluminum and iron (Lambers et al. 2010). However, the long-term use of inorganic NPK-fertilizers may affect both the aboveground (Souza et al. 2015a) and underground ecosystems (Hassan et al. 2013). Over time, inorganic fertilization may result in a decline of soil organic matter, soil quality and AMF diversity and functionality (Geisseler and Scow 2014), whereas organic fertilization enhances soil fertility and biodiversity with less input of inorganic fertilizers, energy, herbicides and pesticides (Mäder et al. 2000; Mikanová et al. 2013). In organic systems, many practices improve ecological stability, biodiversity (Lavelle et al. 2006; Barrios-Masias et al. 2011), and reduce environmental degradation (Jackson et al. 2007).

We hypothesize that the population sizes of infective AMF are decreasing as a consequence of long-term cultivation of rapeseed and mineral fertilization on a Ferralsol. Some AMF species are more sensitive to non-mycorrhizal plant cultivation and fertilization than others (Hassan et al. 2013, Pakpour and Klironomos 2015). According to Oehl et al. (2011), some AMF species respond specifically to the intensity of land use, agricultural practices, and fertilization, which suggests that AMF genera, such as *Acaulospora, Claroideoglomus, Funneliformis,* and *Gigaspora*, are bioindicators of both cultivation systems and fertilization effects. Generally, AMF species from Order Gigasporales (e.g. *Gigaspora, Racocetra,* and *Scutellospora*) are bioindicators of acid soil (soil pH < 6.0) with low levels of nitrogen (<5 mg kg^{-1}) and available phosphorous (<20 mg kg^{-1}) (Ramos et al. 2008a, b, c; Bressan 2001, b; Siqueira et al. 1982, b), whereas AMF species from Order Diversisporales (e.g. *Acaulospora, Diversispora,* and *Quatunica*) and Glomerales (e.g. *Glomus, Funneliformis,* and *Rhizoglomus*) are bioindicators of soil with neutral pH (soil pH ranging between 6.5 and 7.2), presence of organic molecules and high soil fertility (Fracchia et al. 2001, b; Silva et al. 2005, b; Kiriacheck et al. 2009). It may be argued that the long-term utilization of inorganic NPK-fertilizer may create unfavorable conditions to sporulation, root colonization by AMF, and survival of more sensitive AMF species in cultivation areas of rapeseed and wheat in the Brazilian Northeast, thus decreasing soil quality and subsequent annual crop yield.

In fact, AMF are one of the soil microbial groups that are severely affected by changes in vegetation cover and physicochemical characteristics (Belay et al. 2015). However, there is limited information on how long-term cultivation of rapeseed and inorganic fertilization systems may affect local mycorrhizas. The main objectives of this study are (i) to evaluate the effect of long-term cultivation of rapeseed and fertilization on AMF community structure and AMF root colonization; (ii) to determine whether a local history of rapeseed cultivation and fertilization are associated with reduced mycorrhizal colonization and plant growth of succeeding maize; (iii) to relate the effect of rapeseed root extract from different fertilization systems on mycorrhizal fungal spore germination. To accomplish these, we combined field sampling of two root zone types, i.e., mycorrhizal and non-mycorrhizal plants, characterized the soil chemical characteristics (e.g., soil pH, soil organic carbon, total nitrogen and available P), mycorrhizal root colonization, and harvest yield; and performed an inoculum potential bioassay with maize under glasshouse conditions. Fungal inoculum was obtained from field samples and used by establishing a trap culture with maize, a standard host plant used in several mycorrhizal inoculum potential assays according to the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi, INVAM (http://invam.caf.wvu.edu/). This plant species produces thin fibrous roots that are preferred to optimize opportunity for mycorrhiza establishment. The bioassays allowed us to investigate the long-term effects of cultivation of mycorrhizal and non-mycorrhizal plants and mineral fertilization on the AMF community structure (e.g. number of AMF spores, diversity index, dominance index, and frequency of occurrence of AMF), maize root colonization, infectivity potential by AMF, maize dry biomass, and germination of *Acaulospora tuberculata* spores.

2 Materials and methods

2.1 Field experiment

This study was conducted at the Agrarian Science Centre, Federal University of Paraiba, using two adjacent long-term field experiments at the "Chã-de-Jardim" Experimental Station (06°58′12" S, 35°42′15" W, altitude 619 m) comprising an old camp of *Brachiaria decumbens* Stapf., from which plants had been removed prior to the field experiments. The climate is Tropical wet and dry type (As′ accordingly Köppen classification), with average annual precipitation and temperature of 1500 mm and 21 °C, respectively. Data on the climatic conditions of the study area for each year of experimental work (2007–2011) has been described in more detail by Souza et al. (2015a).

The soil at the experimental field site was classified as Ferralsols (WRB 2006). Soil was collected from two experimental fields, the first one with a mycorrhizal plant (*Triticum aestivum* L.) and the second one with a non-mycorrhizal plant (*Brassica napus* L.), each treated for 5 years with: (1) no

fertilization (Control), (2) NPK fertilization according EMBRAPA's recommendation for *T. aestivum* cv. BRS-Guamirim and *B. napus* cv. H401, respectively (Mineral); and (3) organic fertilization according to regional familiar agriculture sustainable systems (Organic) [For more details on fertilizers, doses, and application mode, see Souza et al. (2015b, 2016)]. The soil was collected form two long-term field experiments, three fertilization treatments, and four blocks per year. In total, 24 soils samples containing root fragments were collected per year from a depth of 0–20 cm when rapeseed and wheat plants were in bud formation stage and heading growth stage, respectively. The soil samples from each treatment were split into three parts: one part was used for chemical soil characterization, the second one was used to extract plant roots for root colonization assessment, and the last one was used as inoculum for the maize inoculation bioassay.

2.2 Soil chemical analyses

After removal of roots and soil debris, samples were brought to the laboratory for chemical analyses. Soil pH, total organic carbon, total nitrogen and available phosphorous were assessed. Soil samples were air-dried and passed through a 2 mm sieve. Soil pH was determined in a suspension of soil and distilled water (Black 1965). Soil organic carbon was estimated according to the methodology described by Okalebo et al. (1993). Total soil nitrogen content was estimated using the Kjeldahl method (Black 1965). Available phosphorous (Olsen's P) was extracted using sodium bicarbonate for 30 min and the extract was analyzed by spectrophotometer at 882 nm (Olsen et al. 1954). Soil chemical characteristics are given in ESM_1. Detailed data on the chemical characteristics of the soils for each year (2007–2011) is provided in Souza et al. (2015a).

2.3 Mycorrhizal root colonization

Wheat roots of each studied condition were collected and stored in 50% ethanol until staining. Roots were cleared in 2% KOH for 1 h at 90 °C. Subsequently, they were left to acidify overnight in 1% HCl. Staining was done with blue ink (Parker ink) for 30 min at 60 °C, followed by destaining in lactoglycerol (Phillips and Hayman 1970). The amount of colonization was estimated using the grid-intersect method with examination of 100 intersects under a compound microscope at 200× magnification (Giovannetti and Mosse 1980). Root-intersects that contained vesicles, arbuscules and hyphae were scored as mycorrhizal. The decision to score hyphae as mycorrhizal was based on the cumulative presence of either vesicles, arbuscules, or spores, and the morphology of the mycelium. Roots with no cortex were excluded from the analysis.

2.4 Harvest yield

After 140 days, rapeseed and wheat were harvested. Rapeseed plants were harvested at 10 cm above the ground level, air dried and threshed through manual thresher, whereas wheat plants were harvested at 8–10 cm above the ground level and threshed through power-operated thresher. Thus, grain yield of these annual crops was recorded, and used to estimate the harvest yield in kg ha^{-1}.

2.5 Preparation of inoculum sources for the maize inoculum potential bioassay

To determine whether a local history of cultivation of mycorrhizal and non-mycorrhizal plants and mineral fertilization are associated with reduced mycorrhizal colonization and a lower growth rate of maize, soil samples containing roots and soil debris were brought to the laboratory. During each year (5 years) soil samples were collected for the maize inoculum experiment, and during each year 6 inocula were prepared: (1) soil inoculum from rapeseed field with no fertilization (Control-Rapeseed field soil); (2) mineral NPK fertilization (Mineral-Rapeseed field soils); (3) organic fertilization (Organic-Rapeseed field soil); (4) soil inoculum from wheat field treated for 5 years with no fertilization (Control-Wheat field soil); (5) mineral NPK fertilization (Mineral-Wheat field soil); and (6) organic fertilization (Organic-Wheat field soil).

2.6 Structure of AMF community

Spores from the inoculum for the maize inoculum potential bioassay were extracted by the wet sieving technique (Gerdemann and Nicolson 1963) followed by sucrose centrifugation (Jenkins 1964). For this, we used 100 g of inoculum obtained from each of the six treatments. Initially, the extracted spores were examined in water under a dissecting microscope and they were separated based on morphology. Subsequently, they were mounted in polyvinyl alcohol lactoglycerol (PVLG) with or without the addition of Melzer's reagent (Walker et al. 2007). Species identification was based on the descriptions provided by Schenck and Perez (1987), publications with descriptions of new families and genera (Oehl et al. 2008), and by consulting the INVAM database (http://invam.caf.wvu.edu). Here, we followed the classification proposed by Oehl et al. (2008), including recently described taxa (Goto et al. 2012; Sieverding et al. 2015). In addition to species identification, we also assessed spore abundance by counting the total number of spores, spore abundance of each AMF species by recording the number of spores of each AMF species recorded in the samples, and the species occurrence frequency (FO_i) of each AMF species. FO_i was calculated using the following equation: $FO_i = n_i/N$, where n_i is the number of times an AMF species was

observed and N is the total of AMF spores observed from each studied inoculum treatment. After AMF species identification, we calculated the following ecological indices: diversity index (H) proposed by Shannon and Weaver (1949), and dominance index (C) proposed by Simpson (1949).

2.7 Experimental setup of the maize inoculum potential bioassay

Maize inoculum potential bioassay was conducted in a glasshouse at the Agrarian Science Centre, Federal University of Paraiba (details provided above) using a 2 × 3 factorial scheme in a completely randomized design with 24 replicates, as follows: (1) two long-term annual crop field types: rapeseed field (Non-host) and wheat field (host), and (2) three long-term fertilized soil types: unfertilized soil (Control), mineral NPK- fertilized (Mineral), and farmyard manure fertilized (Organic). Maize seeds (*Zea mays* L. cv. AG 1051) were routinely sterilized (Vincent 1970), germinated in trays containing autoclaved sand (Twice at 121 °C for 20 min in two consecutive days). Seven days after emergence, seedlings (plant height varying 2–4 cm) were selected and individually transferred to plastics pots.

Maize plants were grown in plastic pots (4 kg), each with two plants, containing sterilized sand-field soil (3:1) under glasshouse conditions for 5 months. All pots were covered with aluminum wrap around the seedling to prevent dehydration and external contamination, and the plants were watered with sterilized water as necessary. Maize plants received, weekly, 50 mL plant^{-1} of a nutrient solution (Hoagland and Arnon 1939) containing (in mg L^{-1}): 202.0 KNO_3, 136.0 KH_2PO_4, 236.0 $Ca(NO_3)_2{\cdot}4H_2O$, 493.0 $MgSO_4{\cdot}7H_2O$, 80.0 NH_4NO_3, 1.81 $MnCl_2{\cdot}4H_2O$, 0.05 $CuSO_4{\cdot}5H_2O$, 0.22 $ZnSO_4{\cdot}7H_2O$, 2.86 H_3Bo_3, 0.12 $Na_2MoO_4{\cdot}2H_2O$, and 15.0 Na–Fe EDTA. The nutrient solution was previously sterilized by UV radiation to avoid external contamination. The average temperature in the glasshouse was 28 °C, ranging from 20 °C to 35 °C, an irradiance of up to 70% of full sun, relative humidity ranging from 65 to 75%, and photoperiod of 16:8 h L:D. Each treatment involved 24 replicates, each replicate representing an independent soil sample from a long-term field experiment. In total, we cultivated maize in 144 (6 inoculum sources × 24 replicates) pots during each year, i.e., 720 pots altogether.

2.8 Infectivity potential by AMF, AMF root colonization and plant dry biomass

Five months after planting, maize plants were harvested. Roots were separated from shoots, and fresh roots were weighed immediately. A total of 2.0 g of each fresh root sample was used for determination of root colonization accordingly to McGonigle et al. (1990). To estimate shoot and root dry biomass, the remaining root material and shoots were oven-dried at 72 °C for 48 h. The mycorrhizal infectivity potential was measured using soil samples from the maize inoculum potential bioassay accordingly to Klironomos (1995) and Gai et al. (2015).

2.9 Germination of *Acaulospora tuberculata* spores

Roots were collected from the rapeseed and wheat fields at each fertilization system, and brought to the laboratory. Root extracts obtained by soaking the roots in distilled water for 48 h were prepared from each treatment. We tested the effect of six root extracts on the spore germination of a common AMF, *Acaulospora tuberculata* Janos & Trappe (Silva et al. 2014) using microcosms in the laboratory as described in Stinson et al. (2006). Each root extract treatment had 24 replicates. *A. tuberculata* spores from field samples were separated based on their morphotypes (Acaulosporoid for genus *Acaulospora*) and specific morphological characteristics. This AMF species was selected, because during the characterization of the AMF community, we observed that this AMF species had its frequency of occurrence significantly reduced by the cultivation of the non-host plant of AMF independently of the fertilization treatment.

2.10 Statistical analyses

Three-way ANOVA was used to compare data on root colonization by AMF, infectivity potential by AMF, plant dry biomass, and germination of *A. tuberculata* spores for soil samples from rapeseed and wheat fields grown under three fertilization systems in a long-term field experiment (2007–2011). Linear regression was employed to determine relationships between rapeseed and wheat yield and infectivity potential by AMF from field samples; and plant dry biomass and infectivity potential by AMF from the maize inoculum potential bioassay. Data sets not meeting assumptions for ANOVA were transformed as required (arcsin square root for percentage variables and logarithmic for other variables). Results are presented here in their original scale of measurement (mean and standard deviation) (Zar 1984). Multiple comparisons of means were performed by the Bonferroni test ($P < 0.05$) after performing three-way ANOVA. Three-way ANOVA, linear regression, and Bonferroni's multiple comparison tests were conducted using SAS 9.1.3 Portable.

3 Results

3.1 Field experiment

Overall, we did not detect any colonization of roots by AMF in the rapeseed cultivation under the three studied fertilization systems after five years of their utilization (Fig. 1). Significant

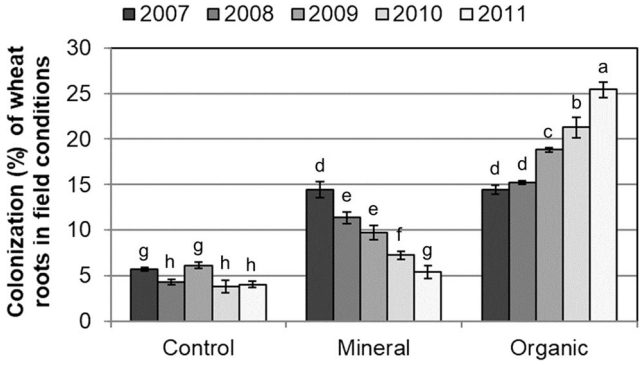

Fig. 1 Colonization of wheat roots by AMF under field conditions (%, mean ± SD, $n = 24$) and under three fertilization system treatments (Control, Mineral, and Organic) in a long-term experiment (2007–2011). Different letters indicate significant differences among years assessed by the Bonferroni test after performing three-way ANOVA ($P < 0.05$)

differences ($F_{8,91} = 21.33$, $p < 0.001$) between long-term fertilization among studied years were found for AMF root colonization in wheat field. This variable was significantly enhanced in the wheat field upon long-term utilization of organic fertilizer (e.g. farmyard manure), whereas the continuous use of mineral fertilization (e.g. NPK fertilizers) and non-fertilization treatments significantly decreased the colonization of roots by AMF in the wheat cultivation (Fig. 1). Root colonization was improved from 14.5% in the first year to 26.4% in the end of the experiment with continuous use of organic fertilizer in the wheat field. Conversely, colonization of wheat roots by AMF was reduced from 14.4 to 5.6% and 5.3 to 4.0% after five years under mineral fertilization and non-fertilization treatments, respectively (Fig. 1).

Rapeseed and wheat yields showed a significant correlation with infectivity potential by AMF on maize plants (Fig. 2a) during the five years of the experiment. Rapeseed yield had a stronger correlation ($r^2 = 0.78$) than wheat yield ($r^2 = 0.55$) with infectivity potential by AMF. Moreover, long-term rapeseed cultivation accounted for a smaller harvest yield in the mineral fertilization (e.g. NPK fertilizer) and non-fertilization treatment than in the organic fertilization (Fig. 2b). This is especially pronounced in treatments of the last three years of the long-term field experiment where colonization of wheat roots was reduced (Fig. 1). In both trials, harvest yield decreased with the continuous use of mineral fertilization and non-fertilization treatments (data not shown). Plant dry biomass shows a significant correlation with infectivity potential by AMF (Fig. 2b), using the data collected during 2007–2011.

3.2 Effect of local history of cultivation of mycorrhizal and non-mycorrhizal plants combined with mineral fertilization on AMF community structure

For all studied inoculum sources, the number of spores ($F_{8,91} = 13.75$; $P < 0.01$), diversity ($F_{8,91} = 24.59$; $P < 0.001$) and dominance ($F_{8,91} = 10.09$; $P < 0.01$) of AMF species were significantly different between rapeseed and wheat field soil samples (Table 1). The number of spores and diversity of AMF were significantly reduced upon long-term utilization of mineral fertilization (e.g. NPK fertilizers) and non-fertilization treatments, whereas the continuous use of organic fertilizer (e.g. farmyard manure) significantly increased the number of AMF spores and diversity of AMF in the rapeseed and wheat cultivation (Table 1). The dominance of AMF species was significantly higher in rapeseed field soil samples from the mineral and non-fertilization treatments compared with the other treatments (Table 1).

In total, we identified 16 different AMF species corresponding to 8 genera – *Acaulospora* (1), *Claroideoglomus* (2), *Dentiscutata* (2), *Funneliformis* (3), *Gigaspora* (3), *Quatunica* (1), *Racocetra* (3), and *Rhizoglomus* (1). The most abundant taxa in all studied treatments were species from the Order Glomerales. AMF species from the genus *Acaulospora*, *Claroideoglomus, Funneliformis*, and *Gigaspora* were mostly found in both studied situations, under host and non-mycorrhizal plants (Fig. 3).

3.3 Maize inoculation bioassay

Soil inoculum from long-term rapeseed and wheat fields associated with mineral and non-fertilization treatments accounted for a smaller plant dry biomass than with organic fertilization. Maize plants showed reduced growth in soil inoculum from the rapeseed field, especially in treatments of the last three years of the long-term field experiment where infectivity potential by AMF was reduced (Fig. 4a and b). Rapeseed cultivation, fertilization treatments and years of

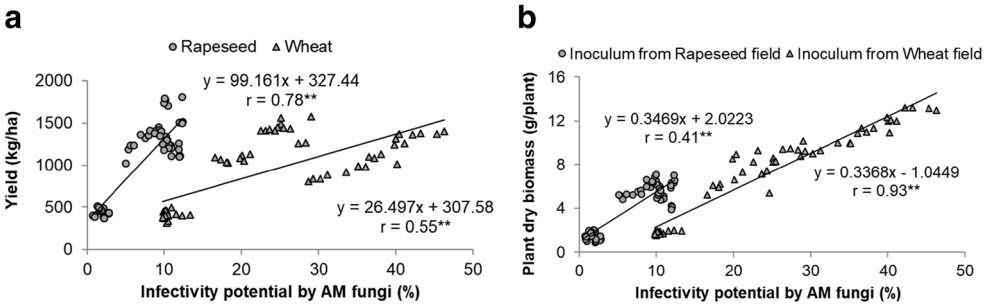

Fig. 2 Linear correlations and Pearson correlation coefficients between infectivity potential by AMF on maize plants (%) and (**a**) rapeseed (circle) and wheat (triangle) yields (kg ha^{-1}), and (**b**) plant dry biomass. Significant correlations ($P < 0.01$) are marked with two asterisks

Table 1 AMF community properties and ecological indexes for the AMF communities of rapeseed and wheat field from maize inoculation bioassay (mean ± SD, $n = 24$)

Field Soil /Year	Number of AMF spores (spore g^{-1} soil)			H'[a]			D[b]		
Rapeseed	Control	Mineral	Organic	Control	Mineral	Organic	Control	Mineral	Organic
2007	1.7 ± 0.12 a	1.4 ± 0.18 a	1.8 ± 0.12 a	2.71 ± 0.26 a	2.71 ± 0.18 a	2.72 ± 0.28 a	0.98 ± 0.01 a	0.99 ± 0.01 a	0.98 ± 0.01 a
2008	1.2 ± 0.12 b	0.8 ± 0.10 c	1.6 ± 0.15 a	2.64 ± 0.13 a	2.46 ± 0.31 a	2.72 ± 0.31 a	0.98 ± 0.01 a	0.96 ± 0.02 a	0.98 ± 0.02 a
2009	0.7 ± 0.21 c	0.4 ± 0.09 d	1.7 ± 0.15 a	2.35 ± 0.24 b	1.93 ± 0.19 c	2.70 ± 0.26 a	0.98 ± 0.02 a	0.94 ± 0.02 b	0.99 ± 0.01 a
2010	0.3 ± 0.10 d	0.3 ± 0.08 d	1.7 ± 0.12 a	1.81 ± 0.23 c	1.65 ± 0.15 d	2.70 ± 0.24 a	0.96 ± 0.02 a	0.94 ± 0.03 b	0.99 ± 0.01 a
2011	0.1 ± 0.05 e	0.2 ± 0.09 d	1.7 ± 0.13 a	0.99 ± 0.19 e	1.61 ± 0.21 d	2.71 ± 0.33 a	0.89 ± 0.04 c	0.93 ± 0.04 b	0.98 ± 0.02 a
Wheat									
2007	3.2 ± 0.16 d	3.3 ± 0.33 cd	3.1 ± 0.28 d	2.6 ± 0.17 a	2.5 ± 0.25 a	2.6 ± 0.23 a	0.92 ± 0.03 a	0.91 ± 0.03 a	0.92 ± 0.01a
2008	3.1 ± 0.25 d	2.9 ± 0.26 d	3.5 ± 0.26 d	2.7 ± 0.21 a	2.0 ± 0.19 b	2.7 ± 0.10 a	0.92 ± 0.04 a	0.85 ± 0.04 b	0.93 ± 0.01 a
2009	2.9 ± 0.18 d	2.3 ± 0.41 e	4.2 ± 0.38 b	2.4 ± 0.28 a	1.7 ± 0.13 c	2.7 ± 0.19 a	0.90 ± 0.02 a	0.82 ± 0.02 b	0.93 ± 0.01 a
2010	3.2 ± 0.19 d	2.0 ± 0.29 f	4.4 ± 0.19 b	2.4 ± 0.21 b	1.7 ± 0.15 b	2.7 ± 0.20 a	0.90 ± 0.05 a	0.81 ± 0.05 b	0.93 ± 0.01 a
2011	2.3 ± 0.26 e	1.9 ± 0.39 f	5.0 ± 0.14 a	2.4 ± 0.19 b	1.7 ± 0.11 b	2.6 ± 0.10 b	0.90 ± 0.05 a	0.80 ± 0.02 b	0.92 ± 0.02 a
Rapeseed versus Wheat[c]	15.69**	17.33**	11.63**	13.45**	16.89**	12.65**	7.16*	12.13*	11.98**

[a] H', Diversity index; [b] D, Dominance index; [c] Independent sample *t* test comparing Rapeseed × Wheat soil groups; ** $P \leq 0.01$; Different letters indicate significant differences assessed by the Bonferroni test after performing three-way ANOVA ($P < 0.05$)

their utilization also influenced infectivity potential by AMF, plant dry biomass and germination of *A. tuberculata* spores. We recorded the best infectivity potential in soil from the wheat field and the worst in soil from the rapeseed field. We also observed a positive effect of organic fertilization system on infectivity potential by AMF in soil from the rapeseed and wheat fields, whereas mineral fertilization had a negative effect on this variable (Fig. 4a). The host-plant (maize) was significantly less productive when grown in soil collected from the rapeseed field, as well as in soil with the continuous use of mineral fertilizers. Conversely, we observed that the continuous use of organic fertilization increased plant dry biomass in both cultivation systems (rapeseed and wheat) (Fig. 4b), but plant dry biomass was larger in soil inoculum from the wheat field than in soil inoculum from the rapeseed field. We also found significantly reduced *A. tuberculata* spore germination rates when they were exposed to extracts of rapeseed roots from the long-term experiment under three fertilization systems compared to when they were exposed to extracts of wheat roots in the same conditions (Fig. 4c).

4 Discussion

Our results provided evidence for changes in population size of AMF caused by long-term cultivation of rapeseed and mineral fertilization. In fact, rapeseed cultivation may influence AMF community by phytochemical inhibition, as described by Lambers et al. (2015). These results support our hypothesis that rapeseed combined with mineral fertilizers may negatively impact AMF functionality by changing population size of AMF in a long-term field experiment on a Ferralsol. It is not usual to report colonization of rapeseed roots by AMF in field conditions (Souza et al. 2005; Lambers and Teste 2013), but results herein showed that rapeseed cultivation under mineral fertilization can have a more negative impact on local mycorrhizas than it does under organic fertilization. Pakpour and Klironomos (2015) reported that plant species from the family Brassicaceae, such as *Brassica nigra* L., do not shown any root colonization by AMF fungi. They also reported negative effects of this plant on AMF diversity and on the growth of native plant species from several study sites. Our results on maize dry biomass indicated that this component experienced positive effects after organic fertilization, both in soils from rapeseed and wheat fields. This finding suggests that this treatment include essential nutrients in order to maintain sustained high production. In a previous study, we found that mineral fertilization can maintain higher levels of soil N than organic fertilization over a longer period of time (Souza et al. 2015a). These results are in agreement with previous works by Gao et al. (2015) and Zhang et al. (2008), whose concluded that long-term mineral fertilization causes acidification and degradation of soil structure.

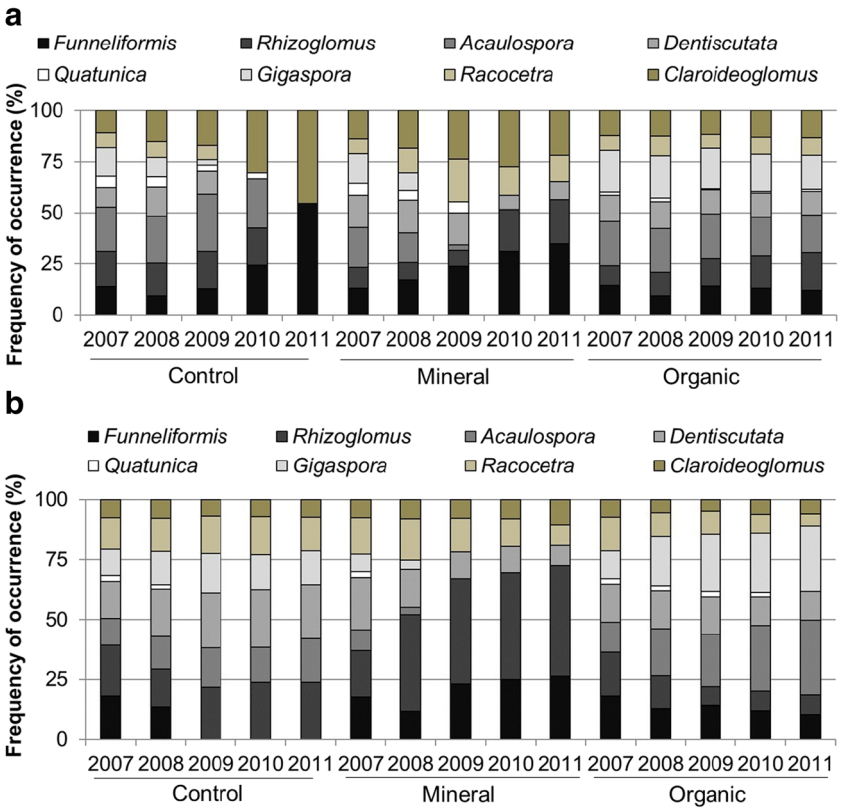

Fig. 3 Effects of different fertilization systems (control, mineral, organic), cultivation of a mycorrhizal and a non-mycorrhizal plants on AMF frequency of occurrence in rapeseed field experiments (Fig. 3a) and wheat field experiments (Fig. 3b) for each studied year (2007–2011)

In addition, the potential for maize to form mycorrhizas was significantly higher in soil inoculum from the wheat than from the rapeseed field. According to Lankau et al. (2011) and Warwick (2011), plant species from the family Brassicaceae produce antifungal compounds with negative phytochemical effects on infectivity potential by AMF in soil inoculum, which might have accounted for the result obtained here. Among the fertilization treatments, we also found cumulative negative effects of non-fertilization and mineral fertilization on this variable. The supply of mineral nutrients has a strong influence on AMF functionality (Eltrop and Marschner 1996; Ramos et al. 2009). The AMF infectivity potential is considerable reduced by high levels of nitrogen and phosphorous (Smith and Read 2008, Hodge and Storer 2014). Bressan (2001, b) and Siqueira et al. (1982, b) reported that the metabolites synthesis during the asymbiotic phase of AMF from genus *Claroideoglomus* and *Gigaspora* was negatively affected in substrate with high level of N, and Siqueira et al. (1985) reported that high level of P significantly reduced energy supply and root colonization during the asymbiotic, pre-symbiotic and symbiotic of AMF from genus *Claroideoglomus*, *Funneliformis*, *Gigaspora*, and *Glomus*. On the other hand, the positive effects of organic fertilization on infectivity potential could be attributed to improvement on mycelium growth, protein synthesis and sporulation during the pre-symbiotic and symbiotic phases of AMF from genera *Claroideoglomus*, *Gigaspora*, *Glomus*, and *Scutellospora* as described by Siqueira and Hubbell (1986), Vilariño and Sainz (1997), Fracchia et al. (2001, b) and Silva et al. (2005, b).

Inoculation with soil inoculum from the rapeseed field decreased plant performance measured as plant dry biomass. Conversely, plant dry biomass was enhanced by inoculation with soil inoculum from the wheat field combined with organic fertilization treatment. Soil inoculum from fields where Brassicaceae species grow has previously been reported to decrease plant growth, and plant dry biomass in bioassay conditions (Pakpour and Klironomos 2015). Mycorrhizal infection often leads to increased rates of photosynthesis, P uptake, N uptake, C demand (Smith and Read 2008, Hodge and Storer 2014), and these changes might be involved in increase plant growth, as described by Eltrop and Marschner (1996).

Finally, the cultivation of rapeseed might have reduced the germination of *A. tuberculata* spores along the five years independently of the fertilization treatment. The negative effects of this treatment suggest that rapeseed plants might produce an antifungal compound which affects the germination of AMF spores, as described by Pakpour and Klironomos (2015). Conversely, the results for the continuous use of organic fertilization in the samples from wheat field suggest that this treatment improves the germination of the spores from this AMF species. Many works had reported that the germination of AMF spores depend on favorable biotic and abiotic conditions. These include appropriate moisture levels, soil pH, mineral nutrient levels, organic matter, and soil microorganisms (Bécard et al.

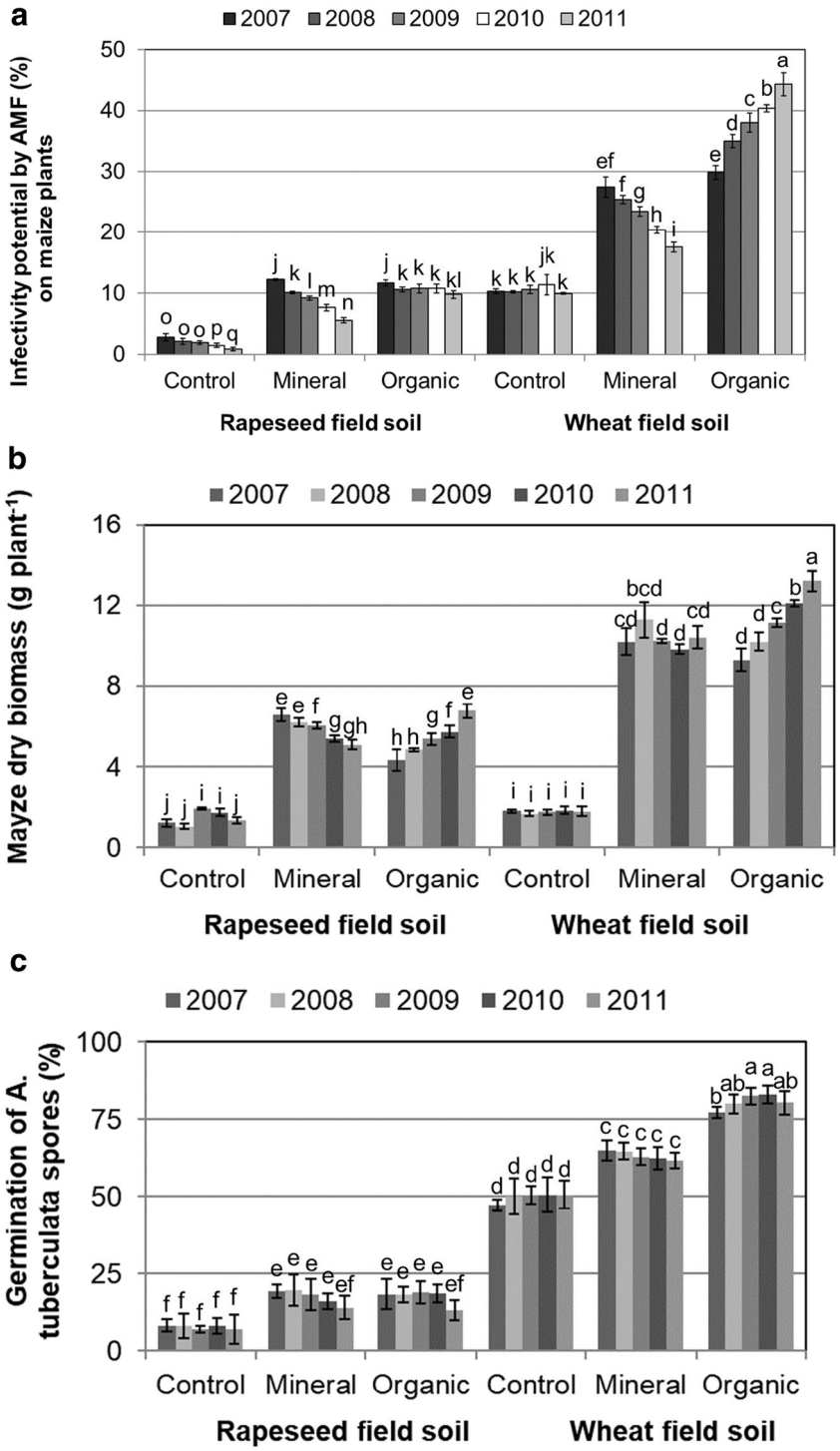

Fig. 4 Infectivity potential by AMF on maize plants (%) (Fig. 4a); maize dry biomass (g plant^{-1}) (Fig. 4b); and germination of *A. tuberculata* spores (%) (Fig. 4c) in soil inoculum from maize inoculation bioassay under six treatments, soils from rapeseed and wheat field and fertilization treatments (Control, Mineral, and Organic) in a long-term field experiment (2007–2011) (means ± SD); Bars of each parameter labeled by different letters indicate significant differences assessed by the Bonferroni test after performing three-way ANOVA ($P < 0.05$)

2004, Dalpé et al. 2005, Bais et al. 2006, Besserer et al. 2006, and Verdin et al. 2006).

Although our experiment was not designed to directly test whether fertilization systems affect soil fertility and subsequently AMF community through changes in soil pH and nutrient availability (e.g. available P), the negative effects of mineral fertilization on local mycorrhizas that we observed may be related to alterations in soil pH and P availability after the continuous use of this treatment. In fact, Souza et al. (2015a) reported negative effects of the continuous use of mineral fertilization on root colonization, and frequency of occurrence of *Acaulospora* species by changes in favorable soil conditions, such as soil pH, available phosphorous and soil organic carbon in a long-term field experiment with wheat plants. Previous studies also have shown that AMF community was affected by tilling, temperature, soil pH, soil

moisture, presence of inorganic and organic compounds, and root exudates (Córdova et al. 2017; Gao et al. 2011; Brady and Weil 2008; Oliveira et al. 2015).

AMF species from Order Diversisporales (e.g. genera *Acaulospora*, *Gigaspora* and *Scutellospora*) are very common in acid soil with low P availability (Ramos et al. 2008a, b, c). Volante et al. (2005) reported that *Gigaspora* species are bioindicators to ethylbenzene presence. According to Plenchette et al. (2005), high soil P availability and the conventional fertilization are the worst conditions for AMF from Order Diversisporales. Some AMF species respond specifically to the intensity of land use, cultural practices, and type of fertilization, which suggests that AMF genera, such as *Acaulospora*, *Claroideoglomus*, *Gigaspora*, and *Funneliformis* are a strong bioindicator of the continuous fertilization effects (Oehl et al. 2011). Accordingly, Summuna et al. (2017), *Glomus* species are indicators for high phosphatase activity on soil with alkaline pH (7.0), whereas *Acaulospora* species presence indicate low phosphatase activity on soil with acid pH (<6.0). To our knowledge, there are no studies available reporting (1) the effects of continuous use of mineral and organic fertilizers on genera *Acaulospora, Claroideoglomus, Funneliformis*, and *Gigaspora* diversity; and (2) the effects of rapeseed root extract on germination of *Claroideoglomus, Funneliformis*, and *Gigaspora* spores. Thus, further investigation addressing these issues is desirable.

In conclusion, the long-term rapeseed cultivation combined with mineral fertilization negatively affected local mycorrhizas in the rapeseed and wheat fields on a Ferralsols during five years of their utilization. Conversely, the positive effects of organic fertilization were evident mainly with inoculum from wheat soil. So, our findings suggest that inputs of organic matter promoted by organic fertilization system have positive effects on root colonization by AMF, infectivity potential by AMF, plant dry biomass and germination of *Acaulospora tuberculata* spores. The results of our study highlight the importance of considering the long-term effect of rapeseed cultivation system on the population sizes of infective AMF, and the beneficial effects of organic cultivation of a host plant, such as wheat, on the infectivity potential of the succeeding plant.

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References

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Ann Rev Plant Biol 57(233–266):314

Barrios-Masias FH, Cantwell MI, Jackson LE (2011) Cultivar mixtures of processing tomato in an organic agroecosystems. Org Agric 1(17–30):316

Bécard G, Kosuta S, Tamasloukht M, Sájalon-Delmas N, Roux C (2004) Partner communication in the arbuscular mycorrhizal interaction. Can J Bot 82(1186–1197):318

Belay Z, Vestberg M, Assefa F (2015) Diversity and abundance of arbuscular mycorrhizal fungi across different land use types in a humid low land area of Ethiopia. Trop Subtrop Agroecosyst 18(47–69):321

Besserer A, Puech-Pagès V, Kiefer P, Gomez-Roldan V, Jauneau A, Roy S, Portais J-C, Roux C, Bécard G, Sejalon-Delmas N (2006) Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. PLoS Biol 4:1239–1247

Black CA (1965) Methods of soil analysis, part 2. In: Black CA (ed) Agronomy monograph no 9. American Society of Agronomy, Madison 771–1572

Brady CN, Weil RR (2008) The nature and properties of soil. Pearson Prentice Hall, Upper Saddle River

Bressan W (2001) Interactive effect of phosphorus and nitrogen on in vitro spore germination of *Glomus etunicatum* Becker & Gerdemann, root growth and mycorrhizal colonization. Braz J Microbiol 32:276–280

Carneiro MAC, Ferreira DA, Souza ED, Paulino HB, Saggin Junior OJ, Siqueira JO (2015) Arbuscular mycorrhizal fungi in soil aggregates from fields of "murundus" converted to agriculture. Pesq Agrop Brasileira 50(4):313–321. https://doi.org/10.1590/S0100-204X2015000400007

Córdova AA, Cruz MCR, Cuevas LVH, Alarcón A, Narcía AT, Cruz RG (2017) Responses of arbuscular mycorrhizal fungi and grass *Leersia haxandra* Swartz exposed to soil with crude oil. Water Air Soil Pollut 228(65):1–12. https://doi.org/10.1007/s11270-017-3247-2

Couzigou JM, Lauressergues D, André O, Gutjahr C, Guillotin B, Bécard G, Combier JP (2017) Positive gene regulation by a natural protective miRNA enables arbuscular mycorrhizal symbiosis. Cell Host Microbe 21(1):106–112. https://doi.org/10.1016/j.chom.2016.12.001

Dalpé Y, Souza FA, Declerck S (2005) The life cycle of *Glomus* species in monoxenic culture. In: Declerck S, Strullu D-G, Fortin JA (eds) In vitro culture of mycorrhizas. Springer, Heidelberg, pp 49–71. https://doi.org/10.1007/3-540-27331-X_4

Eltrop L, Marschner H (1996) Growth and mineral nutrition of non-mycorrhizal and mycorrhizal Norway spruce (*Picea doies*) seedlings grow in semi-hydroponic sand culture. II. Carbon partitioning in plants supplied with ammonium or nitrate. New Phytol 133:474–486

Fracchia S, Menendez A, Godeas A, Ocampo JA (2001) A method to obtain monosporic cultures of arbuscular mycorrhizal fungi. Soil Biol Biochem 33(9):1283–1285. https://doi.org/10.1016/S0038-0717(01)00014-1

Gai J, Gao W, Liu L, Chen Q, Feng G, Zhang J, Christie P, Li X (2015) Infectivity and community composition of arbuscular mycorrhizal fungi from different soil depths in intensely managed agricultural ecosystems. J Soils Sediments 15(5):1200–1211. https://doi.org/10.1007/s11368-015-1060-3

Gao Y, Li Q, Lingn W, Zhu X (2011) Arbuscular mycorrhizal phytoremediation of soils contaminated with phenanthrene and pyrene. J Hazard Mater 185(2-3):703–709. https://doi.org/10.1016/j.jhazmat.2010.09.076

Gao W, Yang J, Ren S, Hailong L (2015) The trend of soil organic carbon, total nitrogen, and wheat and maize productivity under different long-term fertilizations in the upland fluvoaquic soil of North China. Nutr Cycl Agroecosyst 103(1):61–73. https://doi.org/10.1007/s10705-015-9720-7

Geisseler D, Scow KM (2014) Long-term effects of mineral fertilizers on soil microorganisms – a review. Soil Biol Biochem 75:54–63. https://doi.org/10.1016/j.soilbio.2014.03.023

Gerdemann JW, Nicolson TH (1963) Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Trans Br Mycol Soc 1:43–66

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol 84(3):489–500. https://doi.org/10.1111/j.1469-8137.1980.tb04556.x

Goto BT, Silva GA, de Assis DMA, Silva DKA, Souza RG, Ferreira ACA, Jobim K, Mello CMA, Vieira HEE, Maia LC, Oehl F (2012) I*ntraornatosporaceae* (*Gigasporales*), a new family with two new genera and two new species. Mycotaxon 119(1):117–132

Harikumar VS, Błaszkowski J, Medhanie H, Kanagaraj MK, Deepak Samuel V (2015) Arbuscular mycorrhizal fungi colonizing the plant communities in Eritrea, Northeast Africa. Appl Ecol Environ Res 13(1):193–203. https://doi.org/10.15666/aeer/1301_193203

Hassan SED, Liu A, Bittman S, Forge TA, Hunt DE, Hijri M, St-Arnaud M (2013) Impact of 12-year field treatments with organic and inorganic fertilizers on crop productivity and mycorrhizal community structure. Biol Fertil Soils 49(8):1109–1121

Hoagland DR, Arnon DI (1939) The water culture method for growing plant without soil. Calif Agric Exp Stn Circ 347:1–32

Hodge A, Storer K (2014) Arbuscular mycorrhizal and nitrogen: implications for individual plants through to ecosystems. Plant Soil 386: 1–19

IUSS WORKING GROUP WRB (2006) World reference base for soil. World soil resources reports. n. 103. FAO, Rome, p 128p

Jackson LE, Pascual U, Hodgkin T (2007) Utilizing and conserving agrobiodiversity in agricultural landscapes. Agric Ecosyst Environ 121(3):196–210. https://doi.org/10.1016/j.agee.2006.12.017

Jenkins WR (1964) A rapid centrifugal flotation technique for separating nematodes from soil. Plant Dis Rep 48:692

Kiriacheck SG, Azevedo LBC, Peres LEP, Lambais MR (2009) Regulação do desenvolvimento de micorrizas arbusculares. Rev Bras Ciênc Solo 33(1):1–16. https://doi.org/10.1590/S0100-06832009000100001

Klironomos JN (1995) Arbuscular mycorrhizal of *Acer saccharum* in different soil types. Can J Bot 73(11):1824–1830. https://doi.org/10.1139/b95-193

Lambers H, Teste FF (2013) Interactions between arbuscular mycorrhizal and non-mycorrhizal plants: do non-mycorrhizal species at both extremes of nutrient-availability play the same game? Plant Cell Environ 36:1911–2070

Lambers H, Brundrett MC, Raven JA, Hopper SD (2010) Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. Plant Soil 334(1-2):11–31. https://doi.org/10.1007/s11104-010-0444-9

Lambers H, Martinoia E, Renton M (2015) Plant adaptations to severely phosphorus-impoverished soils. Curr Opin Plant Biol 25:23–31. https://doi.org/10.1016/j.pbi.2015.04.002

Lankau RA, Wheeler E, Bennett AE, Strauss SY (2011) Plant-soil feedbacks contribute to an intransitive competitive network that promotes both genetic and species diversity. J Ecol 99(1):176–185. https://doi.org/10.1111/j.1365-2745.2010.01736.x

Lavelle P, Decaëns T, Aubert M, Barot S, Blouin M, Bureau F, Margerie P, Mora P, Rossi JP (2006) Soil invertebrates and ecosystem services. Eur J Soil Biol 42:3–15

Mäder P, Edenhofer S, Boller T, Wiemken A, Niggli U (2000) Arbuscular mycorrhizae in a long-term field trial comparing low-input (organic, biological) and high-input (conventional) farming systems in a crop rotation. Biol Fertil Soils 31:150–156

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol 115(3): 495–501. https://doi.org/10.1111/j.1469-8137.1990.tb00476.x

Mikanová O, Šimon T, Kopecký J, Ságová-Marečková M (2013) Soil biological characteristics and microbial community structure in a field experiment. Open. Life Sci 10:249–259

Oehl F, Souza FA, Sieverding E (2008) Revision of Scutellospora and description of five new genera and three new families in the arbuscular mycorrhiza-forming Glomeromycetes. Mycotaxon 106: 311–360

Oehl F, Jansa J, Ineichen K, Mäder P, van der Heijden M (2011) Arbuscular mycorrhizal fungi as bio-indicators in Swiss agricultural soils. Rech Agron Suisse 2:304–311

Okalebo JR, Gathua KW, Woomer PL (1993) Laboratory methods of plant and soil analysis: A working manual. Soil Sci East Africa Tech Publ 1:22–29

Oliveira RS, Galvão HC, de Campos MCR, Eller CR, Pearse SJ, Lambers H (2015) Mineral nutrition of campos rupestres plant species on contrasting nutrient-impoverished soil types. New Phytol 205(3): 1183–1194. https://doi.org/10.1111/nph.13175

Olsen SR, Cole CV, Watanable FS, Dean LA (1954) Estimation of available phosphorous in soils by extraction with sodium bicarbonate. US Department of Agriculture, Washington, DC (Circular 939)

Olsson PA, Tyler G (2004) Occurrence of non-mycorrhizal plant species in south Swedish rocky habitats is related to exchangeable soil phosphate. J Ecol 92:806–815

Pakpour S, Klironomos J (2015) The invasive plant, *Brassica nigra*, degrades local mycorrhizas across a wide geographical landscape. Roy Soc Open Sci 2(9):150300. https://doi.org/10.1098/rsos.150300

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–160

Plenchette C, Clermont-Dauphin C, Meynard JM, Fortin JA (2005) Managing arbuscular mycorrhizal fungi in cropping systems. Can J Plant Sci 85(1):31–40. https://doi.org/10.4141/P03-159

Ramos AC, Façanha AR, Feijó JA (2008a) Ion dynamics during the polarized growth of arbuscular mycorrhizal fungi: from presymbiosis to symbiosis. In: Varma A, Hock B (eds) Mycorrhiza: biology, genetics, novel endophytes and biotechnology. Springer, Heidelberg, pp 241–261. https://doi.org/10.1007/978-3-540-78826-3_12

Ramos AC, Façanha AR, Feijó JA (2008b) Proton (H^+) flux signature for the presymbiotic development of the arbuscular mycorrhizal fungi. New Phytol 178(1):177–188. https://doi.org/10.1111/j.1469-8137.2007.02344.x

Ramos AC, Façanha AR, Lima PT, Feijó JA (2008c) pH signature for the responses of arbuscular mycorrhizal fungi to external stimuli. Plant Signal Behav 3:1–3

Ramos AC, Martins MA, Okorokova-Façanha AL, Olivares FL, Okorokov LA, Sepúlveda N, Feijó JA, Façanha AR (2009) Arbuscular mycorrhizal fungi induce differential activation of the plasma membrane and vacuolar H^+ pumps in maize roots. Mycorrhiza 19(2):69–80. https://doi.org/10.1007/s00572-008-0204-3

Schenck NC, Perez Y (1987) Manual for the identification of VA mycorrhizal fungi, Second edn. International Culture Collection of VA Mycorrhizal Fungi (INVAM), University of Florida, Gainesville

Shannon CE, Weaver W (1949) The mathematical theory of communication. University of Illinois Press, Urbana

Sharma MP, Reddy UG, Adholeya A (2011) Response of arbuscular mycorrhizal fungi on wheat (*Triticum aestivum* L.) grown conventionally and on beds in a sandy loam soil. Indian J Microbiol 51(3): 384–389. https://doi.org/10.1007/s12088-011-0134-1

Sieverding E, da Silva GA, Berndt R, Oehl F (2015) Rhizoglomus, a new genus of the Glomeraceae. Mycotaxon 129(2):373–386

Silva FSB, Yano-Melo AM, Brandão JA, Maia LC (2005) Sporulation of arbuscular mycorrhizal fungi using Tris-HCl buffer in addition to nutrient solutions. Braz J Microbiol 36:327–332

Silva IRS, Mello CMA, Ferreira Neto RA, Silva DKA, Melo AL, Oehl F, Maia LO (2014) Diversity of arbuscular mycorrhizal fungi along an environmental gradient in the Brazilian semiarid. Appl Soil Ecol 84: 166–175. https://doi.org/10.1016/j.apsoil.2014.07.008

Simpson EH (1949) Measurement of Diversity. Nature 163(4148):688–688

Siqueira JO, Hubbell DH (1986) Effect of organic substrates on germination and germ tube growth of vesicular-arbuscular mycorrhizal fungus spores in vitro. Pesq Agrop Brasileira 21:523–527

Siqueira JO, Hubbell DH, Schenck NC (1982) Spore germination and germ tube growth of a vesicular-arbuscular mycorrhizal fungus "in vitro". Mycologia 74(6):952–959. https://doi.org/10.2307/3792725

Siqueira JO, Sylvia D, Gibson J, Hubbel D (1985) Spores, germination, and germ tubes of vesicular-arbuscular mycorrhizal fungi. Can J Microbiol 31(11):965–997. https://doi.org/10.1139/m85-183

Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic, San Diego

Souza FA, Declerck S, Smith E, Kowalchuk GA (2005) Morphological, ontogenetic and molecular characterization of *Scutellospora reticulate* (Glomeromycota). Mycol Res 109(6):697–706. https://doi.org/10.1017/S0953756205002546

Souza TAF, Rodrigues AF, Marques LF (2015a) Long-term effects of alternative and conventional fertilization. I: effects on arbuscular mycorrhizal fungi community composition. Russ Agric Sci 41: 454–461

Souza TAF, Rodrígues AF, Marques LF (2015b) Long-term effects of alternative and conventional fertilization on macroarthropod community composition: a field study with wheat (Triticum aestivum L.) cultivated on a Ferralsol. Org Agric 6:323–330. https://doi.org/10.1007/s13165-015-0138-y416

Souza TAF, Rodrígues AF, Marques LF (2016) The trend of soil chemical properties, and rapeseed productivity under diferent long-term fertilizations and stubble management in a Ferralsols of northeastern Brazil. Org Agric. https://doi.org/10.1007/s13165-106-0164-4

Stinson KA, Campbell SA, Powell JR, Wolfe BE, Callaway RM, Theden GC, Hallett SG, Prati D, Klironomos JN (2006) Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. PLoS Biol 4(5):727–731. https://doi.org/10.1371/journal.pbio.0040140

Summuna B, Sheikh PA, Mushtaq T (2017) Evaluation of arbuscular mycorrhizal fungi of orchard soils for growth improvement in maize under protected conditions. Int J Curr Microbiol App Sci 6(3):1303–1313. https://doi.org/10.20546/ijcmas.2017.603.151

Valetti L, Iriarte L, Fabra A (2016) Effect of previous cropping of rapeseed (*Brassica napus* L.) on soybean (*Glycine max*) root mycorrhization, nodulation, and plant growth. Eur J Soil Biol 76: 103–106. https://doi.org/10.1016/j.ejsobi.2016.08.005

Verdin A, Sahraoui AL-H, Fontaine J, Grandmoungin-Ferjani A, Durand R (2006) Effects of anthracene on development of an arbuscular mycorrhizal fungus and contribution of the symbiotic association to pollutant dissipation. Mycorrhiza 16(6):397–405. https://doi.org/10.1007/s00572-006-0055-8

Vilariño A, Sainz MJ (1997) Treatment of *Glomus mosseae* propagules with sucrose increases spore germination and inoculum potential. Soil Biol Biochem 29(9-10):1571–1573. https://doi.org/10.1016/S0038-0717(97)00058-8

Vincent JM (1970) A manual for the practical study of root-nodule bacteria. Blackwell Scientific, Oxford IBP handbook no15

Volante A, Lingua G, Cesaro P, Cresta A, Puppo M, Ariati L, Berta G (2005) Influence of three species of arbuscular mycorrhizal fungi on the persistence of aromatic hydrocarbons in contaminated substrates. Mycorrhiza 16(1):43–50. https://doi.org/10.1007/s00572-005-0012-y

Walker C, Vestberg M, Demircik F, Stockinger H, Saito M, Sawaki H, Nishmura I, Schüßler A (2007) Molecular phylogeny and new taxa in the Archaeosporales (Glomeromycota): Ambispora fennica gen. sp. nov., Ambisporaceae fam. nov., and emendation of Archaeospora and Archaeosporaceae. Mycol Res 111(2):137–153

Warwick SI (2011) Brassicaceae in agriculture. In: Schmidt R, Bancrof I (eds) Genetics and genomics of the Brassicaceae. Springer, New York, pp 33–50. https://doi.org/10.1007/978-1-4419-7118-0_2

Zar JH (1984) Biostatistical analysis. Prentice Hall, Englewood Cliffs

Zhang HM, Wang BR, Xu MG (2008) Effects of inorganic fertilizer inputs on grain yields and soil properties in a long-term wheat-corn cropping system in South China. Commun Soil Sci Plant Anal 39(11-12): 1583–1599. https://doi.org/10.1080/00103620802071721

Zhong W, Gu T, Wang W, Zhang B, Lin X, Huang Q, Shen W (2010) The effects of mineral fertilizer and organic manure on soil microbial community and diversity. Plant Soil 326(1-2):511–522. https://doi.org/10.1007/s11104-009-9988-y