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Arbuscular mycorrhizal fungal species differ in their capacity to overrule the soil's legacy from maize monocropping

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are promoted as biofertilizers for cleaner agricultural production. So far, most researchers have investigated the effects of AMF on plant growth under highly controlled conditions with sterilized soil. However, how the soil microbial community shapes AMF's impact on host plant performance is still poorly documented. To focus on the impact of belowground interactions (plant-AMF-soil microbes) alone, we compared sterilized *versus* non-sterilized soil, inoculating maize (*Zea mays* ssp. *mays*) seedlings with five commercial AMF inoculants (*Claroideoglomus claroideum*, *Funneliformis mosseae*, *Gigaspora* sp., *Rhizophagus irregularis* and *Scutellospora* sp.). Plants were pot-cultivated for nine weeks using soil which had been used for maize monocropping in the field. AMF inoculation was successful, despite an abundant native AMF community. As hypothesized: i) the soil microbial community interfered with AMF's benefits for maize growth; ii) these benefits depended on the AMF species, as *C. claroideum*, *F. mosseae* and *Gigaspora* sp. overruled the soil's legacy from maize monocropping. When plants were grown in sterilized soil, we found little to no effects of AMF inoculation on maize growth and nutrients acquisition. AMF's benefits to the host plants could not be explained by improved nutrition alone, since interaction with the remainder soil microbes also differed between inoculated AMF. Data show that the soil microbial community and AMF species should be taken into consideration when applying AMF inoculants in agriculture.

1. Introduction

Human population growth and changing consumption patterns affect food demand and quality, livestock and fibre production, energy use (fossil- and bio-fuel), and land use management (Rockström et al., 2009). As a result, food demand is forecast to double by 2050, while its environmental footprint must be reduced (for the EU, see Directive 2009/128/EC regarding the sustainable use of pesticides), creating an urgent need for cleaner agronomic practices capable of boosting crop yields while decreasing environmental impacts (Dias et al., 2015).

The ecological soil legacy (i.e. the carryover, or memory, of the system with regard to past events – Moorhead et al., 1999) from monocropping is responsible for significant crop yield losses *via* negative plant-soil feedbacks (from here referred to as feedbacks). These feedbacks occur because plant roots live in a highly populated and diverse environment, the soil, where they interact with animals and microbes that affect plant performance (e.g. germination, survival, growth, vegetative propagation and seed production – Bonanomi et al., 2005) and demography, as well as that of other plant species (Bever et al., 1997; Bever, 2003; van der Putten et al., 2013), and can be positive, neutral or negative (Bever et al., 1997; Bever, 2003). Since increases in nutrient availability and in plant density may shift plant-microbe interactions from mutualistic to neutral or parasitic (Anacker et al., 2014), negative feedbacks in agriculture have been well-known since ancient times (Dias et al., 2015), and avoided using appropriate crop rotations. Manipulating biotic interactions (e.g. plant-animal, plant-microbe, microbe-microbe) to provide the desired services and thus reduce or eliminate the need for external inputs is fundamental to a cleaner agricultural production. The challenge is to favour positive interactions, while reducing the negative ones (Shennan, 2008).

In line with this perspective, there is a steadily growing appreciation of the vital role of soil life in agricultural sustainability (Bender et al., 2016), including plant symbiotic associations. One important approach is to implement or revitalize eco-friendly technologies, such as

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biofertilizers (i.e. products containing soil microbes which promote plant growth – Herrmann and Lesueur, 2013). Among these products, those based on mycorrhizae (the widespread symbioses between fungi and plant roots – Smith and Read, 2008) are of special interest because mycorrhizae commonly overrule negative feedbacks on plant growth (Fitzsimons and Miller, 2010). Furthermore, almost all important crops (e.g. maize, wheat, soybean) form associations with arbuscular mycorrhizal fungi (AMF), which are therefore a permanent and natural component of agrosystems. Besides the well-known improvement in plant nutrition (e.g. Dias et al., 2015), other examples of AMF's role in agrosystems include pathogen suppression, pollination enhancement, herbivore protection and improved water relations (Verbruggen and Kiers, 2010). Despite their enormous potential, farmers have not yet explored the full potential of AMF (Berruti et al., 2016).

AMF generally form mutualisms with plants by trading soil resources and other benefits (e.g. protection from pathogens and stress factors) for photosynthates (Smith and Read, 2008). However, not all AMF partnerships are equally beneficial to plants; neutral and parasitic AMF symbioses also occur (Johnson et al., 2008). Furthermore, since AMF are obligate biotrophs (Smith and Read, 2008), AMF are often applied in experiments (pot and field trials) and agricultural production without considering the specificity of the AMF inoculants, compatibility with the target environment and competition with other soil organisms (Berruti et al., 2016). In fact, inoculant production is mostly driven by the ease of growing one isolate rather than its effects on plant performance (above a certain positive impact).

As a result, not much is known on how the abiotic context and soil microbial community shape biotic interactions and affect feedback magnitude and direction (Agrawal et al., 2007). AMF are a good model to study how contextual frameworks affect symbioses, because both the abiotic context and soil microbial community influence how AMF impact host plant performance (Hoeksema et al., 2010). Given the increasing evidence that non-mycorrhizal soil microbes significantly impact the formation and outcome of the mycorrhizal symbiosis (Garbaye, 1994; Frey-Klett and Garbaye, 2005; Bending, 2007; Frey-Klett et al., 2007; Mediavilla et al., 2016), we focused on how the soil microbial community alone shapes AMF's impact on host plant performance. We chose *Zea mays* L. subsp. *mays* as the host plant since it is: i) a fast-growing crop with great economic and nutritional importance worldwide (Ranum et al., 2014); ii) significantly affected by soil legacy effects from monocropping (e.g. in the early 1980 s, maize monocropping reduced production by 10–15% – http://corn.agronomy.wisc.edu/AA/A014.aspx); and iii) highly dependent on AMF (Aquino et al., 2015). We hypothesized that:

1. AMF's benefits to maize growth and nutrient acquisition are dependent on the soil microbial community;
2. AMF's benefits to maize growth and nutrient acquisition are dependent on the AMF species.

Negative feedbacks can, non-exclusively, be due to: release of allelopathic compounds by organic matter decomposition (Bonanomi et al., 2005; van de Voorde et al., 2012), nutrient depletion (Bonanomi et al., 2005) and changes in soil microbial communities (including accumulation of pathogens and parasites – Bever et al., 1997). Since we wanted to focus on the impact of belowground interactions (plant-AMF-soil microbes) alone, of the several feedback approaches (Brinkman et al., 2010; van der Putten et al., 2013), we chose to compare sterilized *versus* non-sterilized soil. Although decomposition of maize straw releases compounds that may enhance or reduce pathogenicity (Javaid, 2008) and affect the subsequent crop (Qi et al., 2015), as far as we know, maize is not auto-allelopathic. To exclude nutrient depletion, we used a very poor soil To overcome autoclaved-induced increases in nutrients availability (Berns et al., 2008), plants were supplemented weekly with readily available nutrients (Brinkman et al., 2010). Therefore, differences in plant growth between the sterilized and non-sterilized soil treatments would describe the soil legacy from maize monocropping, while differences between AMF species treatments would describe the feedback, i.e. interactions of each AMF with the soil microbes (Frey-Klett et al., 2007).

2. Materials and methods

2.1. Experimental design

Our experimental design consisted of two factors: AMF inoculation and soil sterilization. The design was fully factorial, resulting in 12 treatments with 6 replicates (pots) each (72 pots in total). To test whether the benefit to the host plant varied between AMF species, we assessed plant response to five AMF isolates with distinct characteristics: *Claroideoglomus claroideum*, *Funneliformis mosseae*, *Gigaspora* sp., *Rhizophagus irregularis* and *Scutellospora* sp. *C. claroideum*, *F. mosseae*, *Gigaspora* sp. and *R. irregularis* were purchased from Symbion, while *Scutellospora* sp. was donated by Fritz Oehl (Agroscope, Bern). To test the soil legacy from maize monocropping, and whether AMF's benefits to the host plant were dependent on the soil biotic community, we assessed plant response in the presence and absence of a pretrained soil microbial community (feedback). By using soil collected from a maize field in northern Portugal (Vagos, Aveiro – 40°33′N – 8°31′W), we ensured the pre-training of the soil under real agricultural conditions.

The soil, collected in April 2010, contained 0.4% organic matter, 2.2% humic substances, 0.1% total N, 182 ppm total P and 77 ppm K, and had pH (H_2O) 6.5. Mineral N was 37 ppm (Dias et al., 2014) while extractable P and K (Egner-Riehm method) were 8 and 40 ppm, respectively. Soil had a fine sandy loam texture (70% sand, 10% clay, 20% silt) determined by the gravimetric method. Given that mycorrhization is often negatively affected by high nutrient availability, soil was mixed with sterilized river sand in a 1:4 proportion to dilute soil nutrients. Both sand and soil (only for the sterilized soil treatments) were autoclaved at 121 °C; 1.1 atm for 60 min. Soil and sand were autoclaved three times on consecutive days, then left untouched for one week.

Maize (*Zea mays* ssp. *mays* L.) seeds from the cultivar Sincere (Syngenta) were washed under running tap water overnight to remove the antifungal coating, then sterilized by immersion (1/10 v/v seeds/solution) in ethanol 70% (v/v) for one minute; followed by immersion (1/10 v/v seeds/solution) in sodium hypochlorite 2.5% (v/v) for 10 min; and finally washed (1/10 v/v seeds/solution) in sterilized distilled water. Seeds were then germinated for five days in sterilized trays containing autoclaved perlite, and then transferred to the pots. Seedlings were planted in previously sterilized (with 70% alcohol) 3 L pots, with 20 cm diameter, containing the 1:4 soil:sand mixture.

Seedlings were inoculated at the time of transfer to the pots. AMF inoculum, containing ~250 AMF spores, was added to each of the 12 (6 with sterilized soil +6 with non-sterilized soil) pots used per AMF treatment. Bacteria present in each AMF inoculant were extracted by suspending 10 g of each inoculant in 100 mL of sterile water. The bacterial suspensions from the five AMF inoculants were mixed to create a common bacterial pool. After filtration (45 µm pore to exclude AMF spores), 5 mL of this suspension were added to each pot (including control pots).

Plants were watered daily with 100 mL of tap water except on the days when they were supplied with nutrient solution. All plants were fertilised weekly with 100 mL of a 1/4 strength Hoagland's solution (1.5 mM KNO_3; 1 mM $Ca(NO_3)_2$; 0.5 mM $NH_4H_2PO_4$; 0.25 mM $MgSO_4$; 50 µM KCl; 25 µM H_3BO_3; 2 µM $MnSO_4$; 2 µM $ZnSO_4$; 0.5 µM $CuSO_4$; 0.5 µM $(NH_4)_6Mo_7O_{24}$; 20 µM FeNaEDTA), which represented the weekly addition of 5.6 mg N; 1.6 mg P; 6.0 mg K; 4.0 mg Ca; 0.6 mg Mg; 0.8 mg S; 27.5 µg B; 177.5 µg Cl; 3.2 µg Cu; 112 µg Fe; 11 µg Mn; 33.6 µg Mo; and 13.1 µg Zn. Plants were grown for nine weeks, between July and September 2012, in a greenhouse under a non-sterile environment, with natural light (~15 h day/9 h night), maximum photosynthetic

active radiation between 600 and 1000 µmol m^{-2} s^{-1}, and ambient temperature between 17 and 40 °C. Pots were randomized once a week.

2.2. Harvest and analysis

At harvest, maize plants were separated into roots and shoots, dried to constant mass at 60 °C, and weighed. From the six replicates per treatment, four maize shoots were randomly chosen and analysed for their concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), boron (B), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni) and zinc (Zn). The dried plant material was ground to powder using a ball mill (Retsch MM 2000). N concentrations in the plant material were determined using an elemental analyzer (EuroVector) by combustion – DCT (Rodrigues et al., 2009), while the concentrations of all the other nutrients was determined using Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES – Spectro Ciros CCD, Spectro, Germany), after acid digestion. The shoot nutrient contents (Figs. S1 and S2) were calculated by combining shoot biomass and the respective concentrations. The natural abundances of ^{13}C and ^{15}N in maize shoots (Fig. S3) were determined using mass spectrometry (IRMS, Micromass-GV Instruments, UK) and the expressions: $\delta^{13}C$ = (R sample/R standard − 1) × 1000, where R is the ratio $^{13}C/^{12}C$, in the sample and in the standard; and $\delta^{15}N$ = (R sample/R standard − 1) × 1000, where R is the ratio $^{15}N/^{14}N$, in the sample and in the standard. The carbon isotope standard used was PeeDee Belemnite (PDB), a Cretaceous marine fossil (*Belemnitella americana*). PDB has relatively more ^{13}C than most of the terrestrial biosphere, and therefore terrestrial plants and animals tend to have negative $\delta^{13}C$ values. The original PDB standard has now been exhausted and replaced by a substitute called Vienna-PDB (V-PDB). Positive or negative $\delta^{13}C$ values mean that the sample has more or less ^{13}C than the standard in parts per thousand (permil). The standard for nitrogen ($^{15}N/^{14}N$) was atmospheric nitrogen (air) (Sandberg et al., 2012).

Mycorrhizal colonization of roots was evaluated on segments of 1 cm length cut 1–2 cm above the root apices. These root segments were stained (Koske and Gemma, 1989), and mycorrhizal colonization was evaluated as presence *versus* absence on quadrilateral plaques in accordance with Giovannetti and Mosse (1980). Another root sample was used to characterize the microbial community on the surface of, and inside the roots (including endophytes), of plants in soil with a legacy from maize monocropping (those grown in the non-sterilized soil). Root tips from each of the six replicates per treatment were collected, bulked together in the same proportion, and immediately stored at −20 °C until analysis. DNA was extracted using the GeneMATRIX Plant & Fungi DNA Purification Kit (EURx, Poland). DNA amplification and molecular identification of microorganisms was carried out by sequencing the PCR amplified 16SrRNA gene sequence for prokaryotes (Case et al., 2007) and LO/LOR for fungi (Delgado, unpublished). The operational taxonomic units (OTUs) were identified to at least the phylum level.

2.3. Calculations and statistics

Feedback was calculated according to Kardol et al. (2007) as follows:

$$\text{Feedback} = \frac{(\text{Parameter non sterilized} - \text{Average parameter sterilized})}{\text{Average parameter sterilized}}$$

The effects of soil sterilization on plant biomass, on nutrient contents and on shoot isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) were tested separately using a two-way ANOVA, with soil and AMF treatments as fixed factors. The differences between sterilized and non-sterilized soil (i.e. soil legacy from maize monocropping) were analysed separately for each AMF treatment using Student's *t*-test ($p < .05$). The effects of the AMF treatments on feedbacks on plant biomass and on nutrient contents were tested separately using a one-way ANOVA, with AMF treatment as fixed factor. LSD (least significant difference) post hoc multiple comparisons tested for differences ($p < .05$) in feedbacks on plant biomass and on nutrient contents between AMF treatments. To relate feedbacks on plant biomass and on nutrient content, we used a principle component analysis (PCA), whereby we pooled all samples for which we had analysed shoot composition across the six AMF treatments (n = 24). Finally, we used a PCA to identify the microbial groups that most contributed to distinguish the microbial communities inhabiting maize roots of plants grown in the non-sterilized soil. For this analysis, the number of sequences per phylum of one composite sample per each of the six AMF treatments were pooled (n = 6). Preliminary analyses were performed to ensure there was no violation of the assumptions regarding the tests' application (e.g. Levene's test to check for homogeneity of variances; Kaiser-Meyer-Olkin Measure of Sampling Adequacy and Bartlett's test of sphericity). SPSS software, version 23.0, was used for all tests.

3. Results

Mycorrhizal colonization was confirmed in all plants from all treatments except those not inoculated with AMF grown in sterilized soil. Furthermore, molecular analysis of the root segments confirmed the presence of the inoculated AMF (data not shown). However, for the plants grown in the non-sterilized soil, it was not possible to separate the mycorrhization by native AMF from that by the inoculant.

The soil's legacy from maize monocropping was evident in control plants and those inoculated with *Rhizoglomus irregularis* or *Scutellospora* sp., which grew less (accumulated less root, shoot and total biomass) and contained fewer nutrients in non-sterilized than in sterilized soil (Figs. 1, S1 and S2). In contrast, inoculation with *Claroideoglomus claroideum*, *Funneliformis mosseae* and *Gigaspora* sp. enabled plants growing in non-sterilized soil to accumulate as much root, shoot and total biomass as those growing in sterilized soil, thus overruling the soil's legacy from maize monocropping. Since shoot biomass was highly correlated with total biomass (r = 0.98; $p = .000$), the impacts of AMF and soil sterilization on plant nutrients were assessed on shoots. Again, in general inoculation with *R. irregularis* or *Scutellospora* sp. did not overrule the soil's legacy from maize monocropping (i.e., negative impact of non-sterilized soil) on nutrients, while inoculation with *C. claroideum*, *F. mosseae* and *Gigaspora* sp. enabled maize plants growing in non-sterilized soil to accumulate as much nutrients as those growing in sterilized soil. Furthermore, negative feedbacks on biomass and shoot nutrient contents were evident in control plants and those inoculated with *R. irregularis* or *Scutellospora* sp., while inoculation with *C. claroideum*, *F. mosseae* and *Gigaspora* sp. resulted in neutral, or at least less negative feedbacks (Table 1 and Fig. 1).

Since Axis 1 of the PCA explained 62% of the variation, the feedback on biomass, on most macronutrients and even on some micronutrients (e.g. B) appeared as a particularly significant explanatory gradient influencing the response variables (Fig. 2). PCA of the combined feedbacks on biomass and on nutrients made clear the existence of a gradient ranging from: i) negative feedbacks when plants were not inoculated (control) within the same range as those inoculated with *Scutellospora* sp.; and ii) neutral or less negative feedback when plants were inoculated with *C. claroideum*, *F. mosseae* and *Gigaspora* sp. Inoculation with *R. irregularis* was neutral to slightly negative.

Besides AMF, roots' microbial community growing in the non-sterilized soil included many other eukaryotes and prokaryotes (data not shown). PCA of the number of sequences of eukaryotes and prokaryotes detected in these roots showed that the first two components explained 76% of the variation (Fig. 3). PC1, which explained 35% of the variation, was associated with a higher number of bacterial phyla sequences (inoculation with *C. claroideum*, *R. irregularis* and *Scutellospora* sp.), and in the opposite direction, with the number of Glomeromycota sequences (Control and inoculation with *Gigaspora* sp. and *F. mosseae*). In

Table 1
Impact of AMF inoculation on feedback on shoot macro- and micronutrients contents. Significant effects are shown within each row: *5% level; **1% level; and ***0% level. Different letters show significance at the 5% level. Values are the mean ± 1SE (n = 4).

Feedback on:	Control	*C. claroideum*	*F. mosseae*	*Gigaspora* sp.	*R. irregularis*	*Scutellospora* sp.
Macro**	−0.4 ± 0.0^{bc}	0.0 ± 0.1^{a}	−0.2 ± 0.0^{ab}	−0.1 ± 0.0^{a}	−0.2 ± 0.1^{ab}	−0.5 ± 0.1^{c}
N**	−0.3 ± 0.0^{c}	0.1 ± 0.1^{a}	−0.2 ± 0.0^{c}	0.1 ± 0.1^{ab}	−0.2 ± 0.1^{bc}	−0.4 ± 0.1^{c}
P**	−0.5 ± 0.0^{ab}	−0.1 ± 0.1^{a}	−0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	−0.2 ± 0.1^{ab}	−0.6 ± 0.1^{b}
K**	−0.3 ± 0.0^{ab}	0.0 ± 0.1^{a}	−0.1 ± 0.0^{a}	−0.2 ± 0.1^{a}	−0.2 ± 0.1^{ab}	−0.5 ± 0.1^{b}
Ca**	−0.6 ± 0.0^{bc}	−0.3 ± 0.1^{a}	−0.4 ± 0.0^{ab}	−0.2 ± 0.1^{a}	−0.3 ± 0.1^{a}	−0.7 ± 0.1^{c}
Mg**	−0.5 ± 0.0^{cd}	−0.2 ± 0.1^{ab}	−0.3 ± 0.0^{bc}	0.0 ± 0.1^{a}	−0.3 ± 0.1^{bc}	−0.6 ± 0.1^{d}
S**	−0.4 ± 0.0^{bc}	−0.2 ± 0.0^{ab}	−0.3 ± 0.0^{abc}	−0.1 ± 0.1^{a}	−0.2 ± 0.0^{ab}	−0.6 ± 0.1^{c}
Micro	−0.3 ± 0.1	1.1 ± 1.1	0.5 ± 0.3	−0.5 ± 0.3	−0.8 ± 0.0	−0.3 ± 0.3
B**	−0.6 ± 0.0^{bc}	−0.2 ± 0.0^{a}	−0.3 ± 0.0^{a}	−0.1 ± 0.1^{a}	−0.4 ± 0.1^{ab}	−0.7 ± 0.1^{c}
Cu	−0.8 ± 0.1	−0.7 ± 0.1	−0.6 ± 0.1	−0.7 ± 0.2	−0.5 ± 0.3	−0.8 ± 0.1
Fe	0.0 ± 0.1	2.3 ± 2.0	1.2 ± 0.5	−0.5 ± 0.3	−0.8 ± 0.0	−0.2 ± 0.4
Mn**	−0.8 ± 0.0^{c}	−0.6 ± 0.0^{bc}	−0.5 ± 0.0^{ab}	−0.3 ± 0.1^{a}	−0.7 ± 0.0^{bc}	−0.8 ± 0.1^{c}
Mo	−0.3 ± 0.1	−0.2 ± 0.2	0.4 ± 0.2	0.3 ± 0.4	0.2 ± 0.2	−0.4 ± 0.2
Ni	1.0 ± 1.0	5.6 ± 2.9	18.7 ± 12.0	1.4 ± 1.2	0.9 ± 1.3	0.7 ± 0.6
Zn*	−0.6 ± 0.0^{ab}	−0.4 ± 0.1^{ab}	−0.4 ± 0.1^{ab}	−0.4 ± 0.1^{ab}	−0.2 ± 0.2^{a}	−0.7 ± 0.1^{b}

contrast, PC2, which explained 21% of the variation, roughly grouped the treatments according to the effects of the soils' legacy from maize monocropping on biomass and on nutrients: negative feedback (Control and inoculation with *R. irregularis* and *Scutellospora* sp.) was associated with higher number of Nematoda sequences, while neutral/less negative feedback (inoculated with *C. claroideum*, *F. mosseae* and *Gigaspora* sp.) was associated with a higher number of Ascomycota sequences. Since PC1 and PC2 contributed in similar ways to explain the variation, it is difficult to identify which microbial group(s) would most explain the gradient, influencing roots microbial communities. Furthermore, it shows that each AMF inoculant resulted in distinct socialization strategies at the root level.

4. Discussion

Our study allowed simultaneous examination of plant responses to both whole-soil communities and mycorrhizal isolates and showed that: i) the soil microbial community controls AMF's benefits to maize growth; ii) there is a gradient of AMF's effects ranging from negative to neutral feedbacks, depending on the AMF isolate; and iii) *C. claroideum*, *F. mosseae* and *Gigaspora* sp. overrule the soils' legacy from maize monocropping.

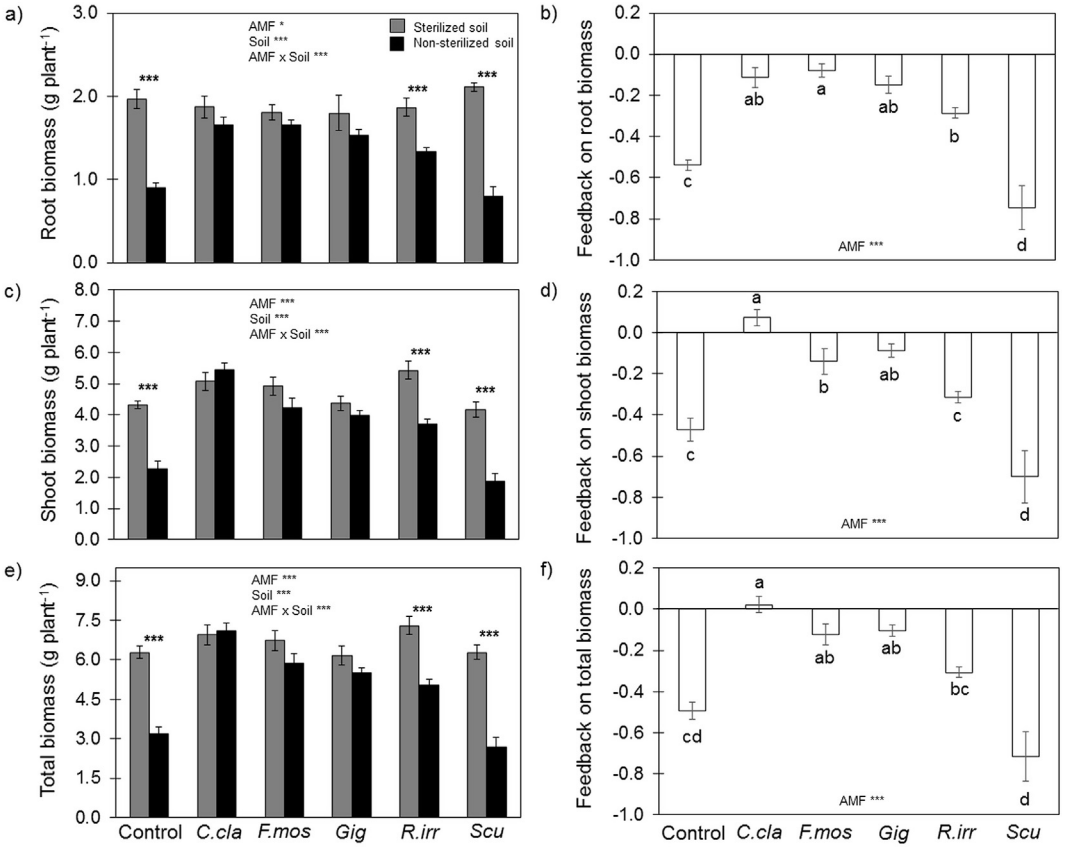

Fig. 1. Impact of AMF inoculation and soil sterilization on plant root (a), shoot (c) and total biomass (e) and their respective feedbacks (b, d and f). Significant effects are shown: *5% level; **1% level; and ***0% level. Different letters show significance at the 5% level. Symbols are the mean ± 1SE (n = 6).

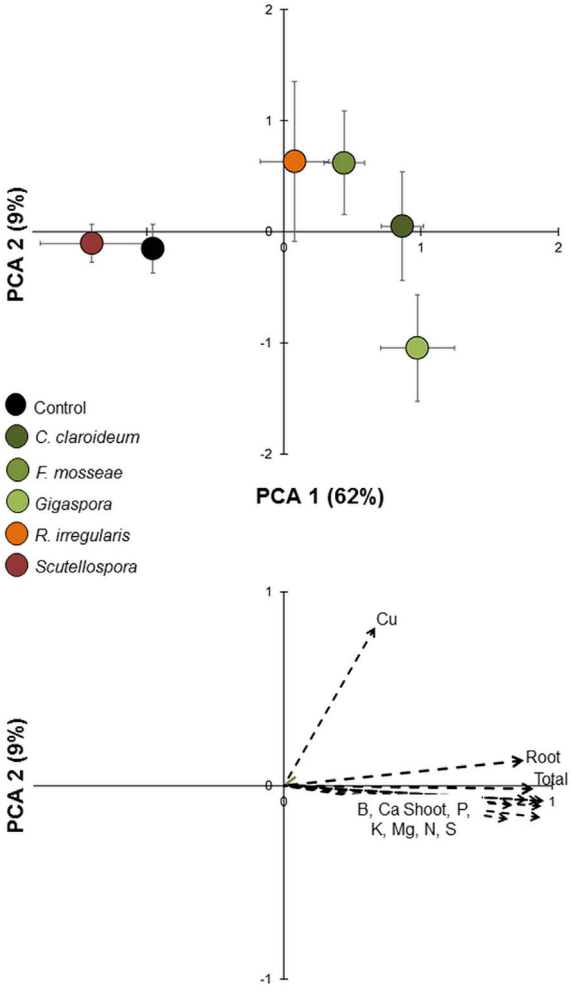

Fig. 2. Principal component analysis (PCA) of feedbacks on plant biomass and shoot nutrient contents (a). Symbols are the mean ± 1SE (n = 4 plants); PC1 explains 61% of the variance while PC2 explains 9%. b) Loading plot for the most important feedbacks (loading > 0.8) which are presented by vectors.

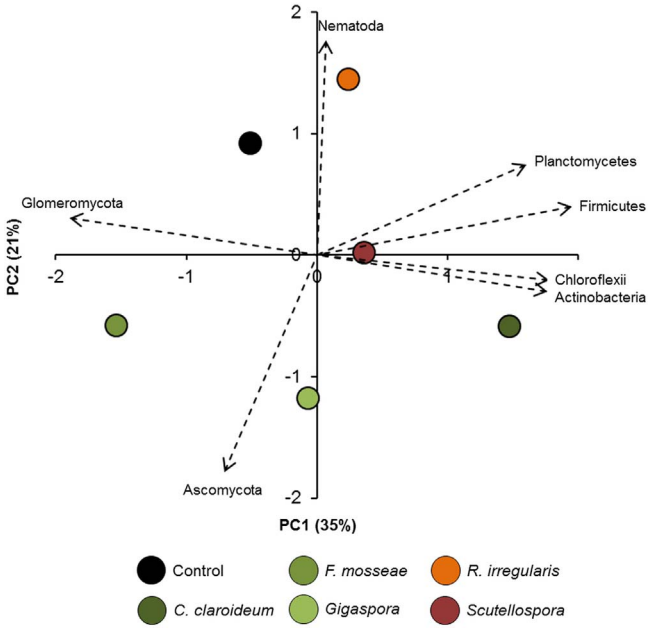

Fig. 3. Principal component analysis (PCA) of the root microorganisms (# sequences per phylum) in the different AMF inoculation in the non-sterilized soil. Symbols represent one bulk sample per treatment; PC1 explains 35% of the variance in the roots microbial community data, PC2 explains 21%. The microbial phyla most responsible for the variations in root microbial community composition (loadings > 0.8) are presented by vectors.

4.1. AMF benefits depended on the soil microbial community

As expected, there was a soil legacy from maize monocropping that resulted in negative feedbacks on plant biomass (Fig. 1) and nutrient contents (Table 1 and Figs. S1 and S2). Interference from nutrient depletion (Bonanomi et al., 2005) and increased nutrient availability due to autoclaving (Berns et al., 2008) were excluded from our study by using a very poor soil and supplying plants with readily available nutrients. Furthermore, the effects of distinct bacteria associated with each AMF inoculant were ruled out by creating a common pool of bacteria equally distributed among pots (including the controls). Therefore, at time zero, the only difference between the treatments was the presence (or absence in the controls) of a specific AMF isolate, so all the observed differences must be related with the activity of the inoculated AMF: i) directly on nutrient uptake; and/or ii) indirectly through distinct interactions with the rhizospheric microbes.

Sterilized and non-sterilized soil differ in soil microbial community (e.g. bacteria, fungi) (Kardol et al., 2007) and fauna (e.g. nematodes) (Voorde et al., 2012), including pathogens and parasites (Bever et al., 1997), which interacted with the inoculated AMF in different ways. As a result, plants grown in the sterilized soil grew more than those in the non-sterilized soil, and they also contained more macronutrients (Fig. 2), the 'building blocks' of plant biomass. Surprisingly, and in contrast to most studies, we found little to no effects of AMF inoculation on maize growth (Fig. 1) and extraction of nutrients (Table 1 and Figs. S1 and S2) when the organisms pretrained by maize monocropping were eliminated by soil sterilization. However, some studies also report a lack of AMF benefits for plants grown in very poor sterilized soils (e.g. Ceulemans et al., 2017), probably reflecting severe plant nutrient limitation, together with a lack of 'alternative' nutrient sources to be scavenged by AMF. Non-exclusively, this lack of AMF benefits highlights that mycorrhizal effects can range from fully mutualistic to parasitic interactions, depending on a complex interplay of both partners (Reynolds et al., 2006; Janouskova et al., 2013) under the conditions tested.

4.2. AMF benefits depended on the isolate

Despite the abundant native AMF community revealed by the mycorrhization of control plants, AMF inoculation was successful, as demonstrated by the greater biomass (Fig. 1) and nutrient extraction (Figs. S1 and S2) of the AMF-inoculated plants. These results are in agreement with other studies on AMF inoculation (Vosatka, 1995; Kohl et al., 2016). However, the gradient of inoculated AMF's benefits to their host plants (van der Heijden et al., 1998; Hart and Reader, 2002) could not be explained by improved nutrition alone, since interaction with the remaining soil organisms (i.e., priming effect of mycorrhiza) also differed between inoculated AMF (Fig. 3). Due to distinct socialization strategies between inoculated AMF and the remainder soil microbes and fauna (Fig. 3), inoculation with *R. irregularis* and especially with *Scutellospora* sp. did not overrule the soil's legacy from maize monocropping (and thus the negative feedbacks), while inoculation with *C. claroideum*, *F. mosseae* and *Gigaspora* sp. did (Table 1 and Figs. 1, S1 and S2).

Maize plants inoculated with *Scutellospora* sp. experienced a feedback on root and shoot biomass that was even more negative than that under control conditions (Fig. 1), suggesting mycorrhizal colonization. Since mycorrhized plants experience an initial growth depression in comparison to non-mycorrhized plants (Hart and Reader, 2002), and *Scutellospora*'s growth and mycorrhization is slow (Oehl, personal communication), it is possible that *Scutellospora*'s benefits would

require a longer period of growth to become apparent. This may be a disadvantage for the use of this AMF species in crops with short life cycles.

When grown in the sterilized soil, plants inoculated with *R. irregularis* presented the biggest shoots (Fig. 1). However, since plants grew less in the non-sterilized than in the sterilized soil, and roots accumulated the most nematodes (Fig. 3), inoculation with *R. irregularis* did not overrule the soil's legacy from maize monocropping (Fig. 1). In arable fields, nematode population densities in the upper soil layer can reach $10^7\,m^{-2}$, the equivalent of 2.0 kg C and 0.25 kg N ha^{-1}. Bacterivores often dominate this fauna, particularly Rhabditid and Cephalobid species (Bouwman et al., 1996), which were the most abundant nematodes in *R. irregularis* roots (data not shown). This suggests that nematodes, and possibly other parasites and pathogens, decreased *R. irregularis*' efficiency in extracting nutrients.

In contrast, the roots of plants inoculated with *C. claroideum*, *F. mosseae* and *Gigaspora* sp. accumulated the least nematodes (Fig. 3), in agreement with other studies (e.g. Sasanelli et al., 2009; Affokpon et al., 2011). Even though we cannot infer which mechanism(s) caused pathogen protection (changes in root architecture, activation of plant defence mechanisms, competition for infection sites and improved nutrient status – Wehner et al., 2011), the soil's legacy from maize monocropping was overcome, resulting in neutral or less negative feedbacks (Table 1 and Fig. 1). AMF's role in improving the growth and nutrition of the plant host is widely documented and recognized (Dias et al., 2015) for P (Kothari et al., 1991; van der Heijden et al., 2006, 2008), N (Cruz et al., 2007; Correa et al., 2014, 2015) and micronutrients (Kothari et al., 1991; Liu et al., 2000; Balakrishnan and Subramanian, 2012). AMF improve plant nutrition by scavenging 'alternative' nutrient sources that would not otherwise be accessible to plant roots (Smith and Read, 2008) and/or by acting as a 'pipeline' of plant-derived C to other soil microorganisms, trading the carbon for nutrients and transferring the nutrients to the plant (Nuccio et al., 2013). Our data does not support the hypothesis that AMF were scavenging 'alternative' nutrient sources, since shoot $\delta^{15}N$, an integrative indicator of the N source (Ariz et al., 2015), did not change (Fig. S3). Instead, our data suggest that *C. claroideum*, *F. mosseae* and *Gigaspora* sp. extended the root system and thereby took up more nutrients (Smith and Read, 1997), and enhanced their host's competition against free-living soil microbes (Schimel and Bennett, 2004).

5. Conclusions

In contrast to previous observations that AMF are not beneficial to agriculture, our data show that AMF inoculation of field soils can enhance the growth of maize irrespective of the pre-established microbial community, being able to compete successfully with native AMF (Kohl et al., 2016). We confirmed the clear biological consequences of belowground socialization of AMF with the other soil microbial communities on plant growth. Furthermore, this effect was AMF species-dependent under a more-structured and stable soil microbial community (i.e., non-sterilized soil), but not under a recently assembled soil microbial community (i.e., sterilized soil), where AMF had little to no effect.

Rhizophagus intraradices, *R. irregularis* and *F. mosseae* are very generalist symbionts that can colonize a wide variety of host plants, survive long-term storage, are geographically distributed all over the world (Opik et al., 2010), and can be easily and massively propagated, making them suitable premium inoculum components (Berruti et al., 2016). However, our data shows that other AMF (*C. claroideum* and *Gigaspora* sp.) may be equally or even more beneficial, and should be further assessed for their application in agriculture with the aim of developing cleaner productive systems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2017.12.025.

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