Applied Soil Ecology 125 (2018) 177–183

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

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

Teresa Dias[a,*], Patrícia Correia[a,b], Luís Carvalho[a,b], Juliana Melo[a], Amarilis de Varennes[c], Cristina Cruz[a]

[a] Centre for Ecology, Evolution and Environmental Changes (cE3c), Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[b] SoilVitae Lda, Rua Espiríto Santo, 18, 6060-071 Idanha-A-Nova, Portugal
[c] Linking Landscape, Environment, Agriculture and Food (LEAF), Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal

ARTICLE INFO

Keywords:
AMF
Feedback
Maize
Nutrients
Soil microbial community

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

* Corresponding author at: Faculdade de Ciências da Universidade de Lisboa, Edifício C2, Piso 5, sala 2.05.37, Campo Grande, 1749-016 Lisboa, Portugal.
E-mail address: mtdias@fc.ul.pt (T. Dias).

https://doi.org/10.1016/j.apsoil.2017.12.025
Received 29 June 2017; Received in revised form 26 December 2017; Accepted 31 December 2017

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