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Arbuscular mycorrhizal fungal activity responses to winter cover crops in a sunflower and maize cropping system

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ABSTRACT

The symbiosis between plants and arbuscular mycorrhizal fungi (AMF) benefits the health, nutrition and abiotic stress tolerance of the host plant. The maintenance of potential AMF inoculum in the winter is important because it will affect the colonization process in the subsequent crop. The objective of this study was to evaluate the effect of winter cover crops (CC) on AMF parameters (root colonization, length of hyphae and number of AMF spores), other variables indirectly related to AMF (the easily extractable glomalin-related soil protein (EE-GRSP) and the enzymatic activity of β-glucosaminidase), along with water-stable aggregates (WSA) as a soil quality indicator. In addition, the effect of two sampling dates on the variables in maize and the relationships among all of the variables were studied. The samples were obtained from a field experiment established in 2006 located in Aranjuez (Central Spain) under a Mediterranean semiarid climate. The treatments were winter cover crops of barley (*Hordeum vulgare* L.) or vetch (*Vicia villosa* L.) and fallow as a control. The study covered two seasons in 2011–12 and 2012–13 with sunflower (*Helianthus annuus* L.) and maize (*Zea mays* L.) as the main crop, respectively, with both sown in the spring. The main crops were irrigated according to the crop demand. Compared with the bare fallow conditions, cover crops improved most of the variables, maintaining the benefits of CC on AMF under the semiarid conditions of the Mediterranean climate. Barley as a cover crop gave the best results, whereas the performance of vetch was poorer. In sunflower, barley increased by 80% the hyphae length and β-glucosaminidase activity and by 30% other variables compared with the fallow; whereas in maize, 60–70% increments were found in AMF spores and the hyphae length and 2-fold in the enzyme activity. The sampling date affected all of the variables analyzed in the maize crop, except for the EE-GRSP and the WSA. Positive relationships were found between the variables directly related to AMF, EE-GRSP content and β-glucosaminidase activity. This suggests that the variables indirectly related to AMF, mainly the EE-GRSP, could be used as indicators of AMF. Finally, the enhancement of soil aggregate stability by the CC via AMF promotion was corroborated.

1. Introduction

Winter cover crops (CC) prevent soil erosion (Bowman and Shirley, 2000), increase soil organic matter (Kuo et al., 1997) and reduce the leaching of nitrate and other nutrients (Gabriel et al., 2013), among other advantages. Another effect of CC is the enhancement of the inoculum level of the arbuscular mycorrhizal fungi (AMF) (Galvez et al., 1995), which may promote the health of the subsequent crop, the mineral nutrition, and the abiotic stress tolerance, as well as provide protection against pathogens and soil aggregation processes (Azcón-Aguilar and Barea, 1997; Jeffries et al., 2003; Lekberg and Koide, 2005; Van Der Heijden et al., 2006).

Replacing the winter bare fallow with CC improves the conditions for AMF development by providing a host plant and a supply of organic carbon to these obligate symbionts (Kabir and Koide, 2002). However, in semiarid climates, the drier conditions generated by CC with respect to fallow (Gabriel et al., 2012) might be a limitation for the maintenance of potential AMF inoculum, affecting the process of colonization in the subsequent crop. Thus, the climatic and environmental factors could be more important than winter cover cropping on AMF development (Higo et al., 2014). The effect of CC on AMF has been evaluated in various temperate areas in the USA (Galvez et al., 1995; Kabir and Koide, 2002; White and Weil, 2010) northwestern Europe (Sorensen et al., 2005) and Asia (Deguchi et al., 2007; Higo et al., 2014). Limited information about CC and AMF is available from Mediterranean areas and semiarid conditions. In Italy, Njeru et al. (2014) reported a beneficial effect of CC on the AMF colonization of the subsequent

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organic maize crop. Other studies in the Mediterranean areas of SE Spain reported the effects of management practices on AMF (Alguacil et al., 2014), but most of the research is focused on natural ecosystems (Barea et al., 2011; Ortiz et al., 2015). Additional investigations in cropping systems under Mediterranean conditions are required to clarify whether the positive effect of CC on AMF is maintained through their mild and relatively dry winter conditions.

Most studies of the influence of management practices on AMF have focused on root colonization as a measurement of fungal abundance. In addition, some propagules as number of spores or extra-radical hyphal measurements are often determined to quantify the inoculum potential for infection. Especially the extra-radical hyphal length determination would be a good option to measure the AMF abundance in the soil, due to the fact that not all AMF produce spores (Morton and Redecker, 2001) and that would result in a limitation to the methods of spore count. Compared with fallow conditions, CC increased the root colonization of the subsequent crop in a temperate climate, for example, in maize (Kabir and Koide, 2000, 2002; Lehman et al., 2012; White and Weil, 2010), leeks (Sorensen et al., 2005) and soybean (Higo et al., 2014). However, other crops, such as sunflower, that are typical in rotations in Mediterranean areas have received no attention. Differences in colonized root length are likely to be observed after main crop planting, and they may disappear over time (Gavito and Miller, 1998; Sorensen et al., 2005); for this reason, sampling should be conducted in the early crop stages. At that time, AMF infection may be more important because it can be decisive in the success of the seedling establishment phase (Boswell et al., 1998). Therefore, the influence of the sampling date should be considered along with the effect of the CC on AMF.

Cover crops can affect other soil variables, such as glomalin, an insoluble and hydrophobic proteinaceous substance, which is a component of the hyphae and spore walls of AMF (Driver et al., 2005). Glomalin has a slow turnover rate (Steinberg and Rillig, 2003) and can accumulate in soils (Rillig et al., 2001b) where it enhances the stabilization of soil aggregates (Rillig and Mummey, 2006). While parameters traditionally used to measure AMF (root colonization or length of hyphae) are complex and time-consuming, some of the glomalin fractions, such as the glomalin-related soil proteins (GRSPs), are easily measured. Because studies found a positive correlation between GRSPs and parameters directly related to AMF (Bedini et al., 2007; Peng et al., 2013), GRSP might be used as a simple indicator of AMF. However, many studies found no relationship between the content of this protein and the AM fungal parameters (Lovelock et al., 2004; Lutgen et al., 2003; Rillig et al., 2001a; Wright et al., 1999; Wright and Upadhyaya, 1999), which may call into question its ability to indicate AMF abundance. Glomalin is also of interest due to its role in microaggregate and macroaggregate formation and stabilization (Rillig and Mummey, 2006), contributing to the explanation of the positive effect of AMF on soil structure (Bronick and Lal, 2005). Because the percentage of water-stable aggregates (WSA) is an important physical indicator used to evaluate soil structure, it was included in this study to test the positive effects of CC on aggregation through AMF enhancement, as well as to examine its relationship with glomalin. Among all of the biological soil properties, enzyme activities are the most sensitive indicator of soil quality changes related to land use, soil management and environmental stress (Ekenler and Tabatabai, 2003). The enzymatic activity of β-glucosaminidase is responsible for degrading chitin, a major structural compound in insects and fungal cell walls that is abundant in the soil and plays a key role in C and N cycling (Geisseler and Horwath, 2009). Some studies (Andersson et al., 2004; Miller et al., 1998; Reeslev et al., 2003) showed how the β-glucosaminidase activity was related to fungal biomass so that it could be used as a simple and sensitive indicator of soil fungal biomass.

The general objective of this study was to determine whether replacing the traditional winter fallow in Mediterranean areas with CC would enhance AMF activity. The specific objectives were as follows: (i) to evaluate the CC effect on several variables related to AMF under two different main crops, sunflower and maize, and (ii) to study the relationships among the variables directly and indirectly related to AMF. The directly related variables were the percentage of mycorrhizal colonization, the length of extra-radical mycelium and the number of AM fungal spores. The indirect variables were the soil content of the easily extractable fraction of the GRSP and β-glucosaminidase activity. Finally, the percentage of soil water-stable aggregates was selected as an indicator of the benefits of AMF to soil quality.

2. Materials and methods

2.1. Study site and experimental design

The study was conducted during two seasons (2011–12 and 2012–13) at La Chimenea Field Station (40°03′N, 03°31′W, 550 m a.s.l.) in the central Tajo River basin near Aranjuez (Madrid, Spain) in a trial established in 2006. The soil was classified as Typic Calcixerept (Staff, 2003) with the following top soil (0–20 cm) properties in 2012: pH_{water} (1:2.5), 8.4; organic matter, 1.8%; calcium carbonate, 15.7%; sand (2000–50 μm), silt (50–2 μm) and clay (<2 μm) content, 290, 420 and 290 g kg^{-1}, respectively, and loam textural class. The climate of this area is Mediterranean semiarid (Papadakis, 1966). The mean annual temperature is 14.2 °C, and the mean annual precipitation is 350 mm. Measurements of temperature, humidity, radiation, and wind were recorded throughout the experimental period in a CR23X micrologger from Campbell Scientific (Logan, Utah, USA).

Twelve plots (12 × 12 m^2) were randomly distributed in four replications of three treatments: barley (*Hordeum vulgare* L., cv. Vanesa) and vetch (*Vicia villosa* L., cv. Vereda) as CC during the fall-winter period, with fallow as the control. The cover crops were disseminated by hand over the stubble of the previous crop and covered with a shallow cultivator (~5 cm depth) that passed over all of the plots in October (18/10/2011 and 05/10/2012). All of the plots were treated with one application of glyphosate 2% (N-phosphonomethyl glycine) in March to terminate the CC. The main crop was sown in early spring over the chopped CC residue by direct sowing. The first year, sunflower (*Helianthus annuus* L., var. Sanbro) was sown (20/04/2012), and the second year, maize (*Zea mays* L., G-98 Pioneer) was sown (18/04/2013). The main crop was harvested in early autumn (sunflower on 12/09/2012 and maize on 07/10/2013). Water was uniformly applied using a sprinkler irrigation system (12 × 12 m, 9.5 mm h^{-1}) according to crop evapotranspiration (ETc) requirements calculated by the FAO method (Allen et al., 1998). Additional details about the soil and the experimental site can be found in the literature (Gabriel and Quemada, 2011). Throughout the experiment, all plots received the same amount of synthetic fertilizer. From 2006 to 2011, ammonium nitrate (210 kg N ha^{-1}) split into two applications when maize had 4 and 8 leaves, and before sowing the main crop 120 kg P ha^{-1} as triple superphosphate and 120 kg K ha^{-1} as potassium sulfate. Fertilization was suspended in 2012 and 2013. Cover crop residues remained in the field, whereas the maize and sunflower residues were removed from the experiment, leaving the same amount (≈1000 kg ha^{-1}) in all of the plots. A summary of the experimental history is shown in Table 1.

2.2. Field measurements, sampling and laboratory analysis

The soil water content was monitored periodically in this study using the Diviner® capacitance probe (Sentek Pty Ltd., Stepney,