Applied Soil Ecology 107 (2016) 91–98

Contents lists available at ScienceDirect

Applied Soil Ecology

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

Enhanced transfer of biologically fixed N from faba bean to intercropped wheat through mycorrhizal symbiosis

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ARTICLE INFO

Article history:
Received 18 September 2015
Received in revised form 12 May 2016
Accepted 14 May 2016
Available online xxx

Keywords:
Arbuscular mycorrhizal fungi
Facilitation
^{15}N isotopic dilution
Intercropping systems
Low-input agricultural management
Nitrogen fixation

ABSTRACT

In Morocco, the use of seed legumes is limited because significant water deficits and the low availability of soil phosphorus (P) limit nitrogen fixation. However, little is known about the ability of faba bean-rhizobium symbiosis to fix nitrogen in P-deficient soils and to transfer fixed nitrogen (N) to intercropped wheat. Arbuscular mycorrhizal fungi (AM) and their extraradical hyphae networks play an important role in the facilitation process by promoting interconnectivity and the transfer of nutrients, such as N and P, between associated plants. The aim of this study was to analyse the impact of AM inoculation on N_2 fixation and the transfer of fixed N from faba bean to intercropped wheat. Germinated faba bean and wheat seeds were transferred into 1–l pots filled with a P-deficient soil that was collected from the Haouz valley near Marrakech (Morocco). Plants from the two species were grown in pots in either pure or mixed stands under greenhouse conditions, and each cropping system was subjected to three mycorrhizal inoculation treatments with a non-inoculated (AM0) and two concentrations of *Rhizophagus irregularis* inoculants containing 1000 (AM1) or 2000 (AM2) spores pot^{-1}. The ^{15}N isotope dilution method was used to determine the amount and proportion of atmospheric N fixed by faba bean (Ndfa%) and the fixed N that was transferred to wheat. Mycorrhizal inoculation had a significantly positive effect on the shoot dry weights and total shoot N in faba bean, but not in wheat. The cropping system had no significant effect on the plant growth and total shoot N in both faba bean and wheat. The Ndfa percentage was very high in all of the treatments, varying from 86 to 91%. The total N fixed by faba bean was 27% significantly higher in the AM2 treatment compared with the AM1 and AM0 treatments for both cropping systems combined. The estimated proportions of fixed N that were transferred from faba bean to wheat were far higher in AM1 (50%) and AM2 (32%) treatments than in AM0 (15%) treatment as well as for the total transferred fixed N. As corroborated by a parallel observation of root mycorrhizal colonization, these results suggest that the development of mycorrhizal networks stimulates the transfer of fixed N from faba bean to wheat, which could significantly contribute to the facilitation process under intercropping conditions.

1. Introduction

Key processes that are involved in maintaining the productivity and stability of natural ecosystems could be integrated into agricultural management systems to address our increasing agricultural problems. Natural processes have evolved over long periods, resulting in ecosystems that are highly productive, resistant to pests and able to retain nutrients (Ewel, 1999). Among

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http://dx.doi.org/10.1016/j.apsoil.2016.05.008
0929-1393/

the natural processes that are involved in the stability and productivity of non-disturbed ecosystems, facilitation between plants could be considered as a powerful tool to maximize growth and productivity (Callaway and Walker, 1997). Facilitation is an ecological process that occurs in communities around the world (Valiente-Banuet et al., 2006) when the presence of one plant enhances the growth, survival, and reproduction of a neighbour (Callaway, 2007). The maintenance of facilitation over time shows that the benefits of this association are not restricted to germination and seedling establishment but are extended to growth and long-term survival (Montesinos-Navarro et al., 2012). The primary mechanisms resulting from the facilitation process are (i) microclimate improvement beneath the canopies (Gomez-Aparicio et al., 2004), (ii) protection from herbivores (Baraza et al., 2006), and (iii) enhancement of physical, chemical and biological soil properties (Puerta-Pinero et al., 2006; Duponnois et al., 2011; Hafidi et al., 2013). Some studies have shown that mycorrhizal fungi could be a key ecological component for understanding facilitation in plant communities (Van der Heijden and Horton, 2009; Van der Putten, 2009). Mycorrhizal fungi can interconnect plant individuals from different species or genera in natural communities (Newman, 1988). These links between plants provide pathways for the transfers of major mineral nutrients such as nitrogen (N) and phosphorus (P) (Smith et al., 2001).

Facilitation is one of these natural processes that is integrated into agricultural and agroforestry practices such as intercropping, which is defined as the growing of two or more species simultaneously on the same area of land (Callaway and Walker, 1997). The potential for direct facilitative interactions could result from the transfer of symbiotically fixed N_2 from legumes to intercropped non-legumes (Jensen, 1996), the release of P from organic compounds (Dakora, 2003), or the dissolution of inorganic P due to the lowering of the pH by N_2-fixing legumes (Hinsinger, 2001). They can also act indirectly by promoting beneficial soil microbes (Wamberg et al., 2003) such as arbuscular mycorrhizal (AM) fungi (Francis and Read, 1994).

Non-N_2-fixing species have often been found to have better growth and yields when intercropped with N_2-fixing legume species (Fujita et al., 1992; Ledgard and Steele, 1992). This trend is primarily caused by the transfer of symbiotically fixed N_2 from legumes to non-legumes, which can be (i) an indirect transfer, through the decomposition of litter, roots and nodules (Johansen and Jensen, 1996); or (ii) through mycorrhizal uptake and translocation (Smith and Read, 1997); or (iii) a direct transfer through the common mycorrhizal networks (CMNs), which enable linkages to form between the root systems of both mixes of species (He et al., 2009) as well as (iv) through the rhizodeposition and subsequent uptake of released root exudates (Høgh-Jensen and Schjoerring, 2001; Paynel et al., 2008; Mahieu et al., 2014).

In many regions devoted to cereal crops in Morocco, the use of seed legumes is limited because of significant water deficits and the low availability of soil P, which strongly affect biological N_2 fixation. However, very few data are available with regards to the ability of faba bean-rhizobium symbiosis to fix N_2 in P-deficient soils as well as to transfer a proportion of the fixed N to wheat in intercropping or crop rotations. In addition to the direct and positive effects of AM fungi on nutrient uptake and plant growth, the extraradical hyphae networks are likely to play a major role in the facilitation process by promoting interconnectivity and the transfer of nutrients such as N and P between associated plants.

The aim of this study was to analyse the impact of AM inoculation on N_2 fixation and to study the transfer of fixed N from faba bean to intercropped wheat using the ^{15}N isotopic dilution method. We hypothesized that the AM inoculation and the abundance of AM propagules that mimic the mycorrhizal soil infectivity will be important biological factors that control the amount of fixed N that is transferred from faba bean to wheat.

2. Materials and methods

2.1. Soil properties

Soil was collected from a 0–15 cm depth horizon of cultivated fields in the agricultural zone of the Haouz valley, which is located approximately 30 km east of Marrakech (Morocco). The soil was a silty clay loam of the isohumic type (Lithosols) (FAO-UNESCO, 1974). Before its use, the soil was crushed and passed through a 2-mm sieve. Its chemical characteristics were as follows: pH H_2O = 7.2, organic carbon (%) 1.53, Total N (%) 0.08, C/N 20.4, Total P (mg kg^{-1}) 500 and available Olsen P (mg kg^{-1}) 25.2. The soil was disinfected (120 °C, 40 min) and mixed with sterilized river sand at a 1:4 (v:v) ratio to improve soil drainage and to avoid compaction by repeated watering during the experiment, and the mixture was autoclaved (120 °C, 40 min).

2.2. Experimental design and plant labelling

This experiment was set up in a completely randomized block design with four replications (*i.e.*, 4 pots) with the following treatments: three cropping system treatments that consisted of two monocultures (legume or cereal) and a mixture of both plants; each cropping system treatment was subjected to three mycorrhizal inoculation treatments including a non-uninoculated control and two different concentrations of mycorrhizal inoculant.

Seeds from *Vicia faba* L. cv. Aguadulce and *Triticum turgidum* L. subsp. *durum* were surface-sterilized with 1% NaOCl for 15 min and rinsed with demineralized water. After immersion in distilled water overnight, they were pre-germinated in vermiculite beds for 4 days and then transferred individually into pots. The 1–l pots were filled with a mixture of Haouz soil and sterilized river sand. Plants from both species were grown in pots for 6 weeks either alone or mixed under greenhouse conditions (with a day length of approximately 10 h and a mean temperature of 22 °C). Each cropping system was subjected to three different mycorrhizal inoculation treatments, namely a non-inoculated control (AM0) and two concentrations of *Rhizophagus irregularis* inoculants consisting in 1000 (AM1) and 2000 (AM2) spores pot^{-1}. The plant densities were one faba bean and ten wheat plants pot^{-1} in either pure or mixed cropping (one central faba bean surrounded by ten wheat plants). The plants were watered with deionized water throughout the duration of the experiment. The faba bean plants were nodulated in all of the treatments, although no rhizobial inoculation was performed. The primary explanation for this bacterial contamination was that the irrigation tap water may have contained indigenous *Rhizobium leguminosarum* strains. The AM inoculum was previously propagated on maize (*Zea mays* L.) that grew for 12 weeks in 1-l pots filled with calcined clay (with an average particle size of 5 mm) and Oil-Dri USspecial Ty/IIIR (Oil-Dri Company, Chicago, USA). The cultural substrate was then collected and AM fungal spores were extracted by wet sieving and decanting followed by sucrose centrifugation (Gerdemann and Nicolson, 1963). After centrifugation, the supernatant was poured through a 50-μm sieve and washed with tap water. Spores were counted under a stereomicroscope. The spores were surface-sterilized with a solution of chloramine T (0.2 g l^{-1}) and streptomycin (0.2 g l^{-1}) to exclude the mycorrhizosphere microflora. The spores were then suspended in sterile distilled water and inoculated to the plants at a rate of 5 ml of suspension per pot.

The ^{15}N isotope dilution method was used to assess the amount of nitrogen that was fixed by faba bean in each treatment and the amount of fixed N that was transferred to wheat. Isotopic labelling