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Interactions between the external mycelium of the mycorrhizal fungus Glomus intraradices and other soil microorganisms as affected by organic matter

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Abstract

The influence of organic matter on the interactions between external mycelium of the arbuscular mycorrhizal (AM) fungus *Glomus intraradices*, the bacterium *Burkholderia cepacia* and other soil microorganisms was studied in a root-free sand environment. Organic matter amendment, in terms of ground barley leaves, markedly increased the growth of the external mycelium of *G. intraradices* as estimated both with the fatty acid biomarker 16:1ω5 and hyphal length measurements. Mycelial proliferation of *G. intraradices* in sand with organic matter was unaffected by both inoculation with *B. cepacia* and a soil filtrate containing a mixed population of indigenous microorganisms. On the other hand, in the absence of organic matter, both inoculation with *B. cepacia* and the soil filtrate reduced the growth of *G. intraradices*, as estimated with measurements of 16:1ω5. In contrast, *B. cepacia* inoculation increased hyphal length density of *G. intraradices* in the absence of organic matter. Overall, the presence of external mycelium of *G. intraradices* increased the bacterial biomass and counteracted a suppressive effect of *B. cepacia* on the growth of saprotrophic fungi.

Keywords: Mycorrhizosphere; Hyphosphere; Organic matter; Microbial interactions; Burkholderia cepacia; Signature fatty acids

1. Introduction

AM fungi are associated with the roots of most herbaceous plants. The external hyphal network of AM fungi plays an important role in plant nutrition, as the inflow of mineral nutrients to the host plant from beyond the root zone, is facilitated by the spread of AM mycelium. In exchange of translocating mineral nutrients from the soil to the host plant, AM fungi receive carbon from their host plant (Smith and Read, 1997).

External mycelium of AM fungi has been suggested to preferably associate with organic matter (St. John et al., 1983). AM fungi have been shown to colonize decomposing leaves developing arbuscules and vesicles in dead leaf cells (Aristizábal et al., 2004) and AM fungi also colonize sphagnum leaves (Warner, 1984). This may be important for the recycling of mineral nutrients released during mineralization of dead organic matter (Aristizábal et al., 2004). Growth enhancement of AM fungal external mycelium has mainly been observed with complex organic matter applied both to the rhizosphere (Joner and Jakobsen, 1995) and the hyphosphere (Green et al., 1999), but also with more simple substrates like bovine serum albumin applied to the hyphosphere (Ravnskov et al., 1999). In contrast, cellulose amendment has been shown to reduce growth of the external mycelium (Ravnskov et al., 1999), and glycine amendment did not affect AM mycelial growth (Hodge, 2001). Knowledge on the mechanisms behind the interactions between AM fungi and organic matter is scarce, but Gavito and Olsson (2003) showed that carbon in AM mycelium proliferating in organic matter as expected originates from plant photosyntate, as indicated by incorporation of ^{13}C in AM signature fatty acids, which had been pulse labelled to the plant as $^{13}CO_2$. However, mycelial growth of AM fungi may be limited for other nutrients such as N, which can be obtained from decomposed organic matter as suggested by Ravnskov et al. (1999). Indeed, AM mycelial N (Ames et al., 1983; Hodge et al., 2001) and P (Joner and Jakobsen, 1995)

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uptake from various sources of organic matter has been described.

AM fungi interacts with various groups of soil bacteria (Paulitz and Linderman, 1991), and mycorrhiza can change communities of rhizosphere microorganisms (e.g. Andrade et al., 1997; Wamberg et al., 2003), but AM mycelia mediated changes in soil microbial communities has been less studied (Olsson et al., 1996; Mansfeld-Giese et al., 2002). The bacterium *Burkholderia cepacia* is commonly found in the mycorrhizosphere, but not from a corresponding rhizosphere of non-AM plants (Andrade et al., 1997). However, in another study *B. cepacia* was not specifically associated with AM fungi (Mansfeld-Giese et al., 2002).

Burkholderia cepacia has competitive saprotrophic abilities, and is also known as a plant growth promotor and as a possible biocontrol agent (Roberts et al., 1997; Larsen et al., 2003). Ravnskov et al. (2002) examined the influence of five different strains of *B. cepacia* on soil mycelial growth of the AM fungus *G. intraradices* and found all possible strain specific interactions. On the other hand, the presence of mycelium of *G. intaradices* in root-free soil decreased the biomass of three out of five strains of *B. cepacia* as measured using cyclic fatty acids as biomarkers for *B. cepacia* (Ravnskov et al., 2002).

The objective of the present experiment was to examine the influence of other soil microorganisms, on the growth enhancing effect of organic matter, in terms of ground barley leaves, on growth of the external mycelium of *G. intraradices*. Our main hypothesis was that bacterial inoculation of sterile organic matter would increase the proliferation of mycelium of *G. intraradices* in the organic matter.

2. Materials and methods

2.1. Experimental design

Compartmented pots with two root-free compartments were used as experimental units (Fig. 1). The experiment had a factorial design with 12 treatments with three main factors: (1) *G. intraradices* (with and without), (2) organic matter (with and without) and (3) microbial inoculations (water, soil filtrate, *B. cepacia*). Each treatment had four replicates. Treatments in the main units (called root compartment) consisted of non-AM plants or of plants colonized with *G. intraradices*. Two matching sub-units (called root-free compartments) were attached to the root compartment, one amended with organic matter and the other without. The root-free compartments contained one of three applications: (1) water, (2) soil filtrate and (3) *B. cepacia* (see Fig. 1).

2.2. Experimental set-up

Cucumber plants (*Cucumis sativus* L., cultivar Aminex) were used as host plants for *G. intraradices* (Schenck and Smith, BEG 87) and grown in a mixture of a sandy loam soil and quartz sand (1:3, w/w) with a low phosphorus content (8 mg P kg^{-1} soil) Olsen P, and a pH of 6.1. To eliminate indigenous mycorrhiza propagules, the soil was irradiated

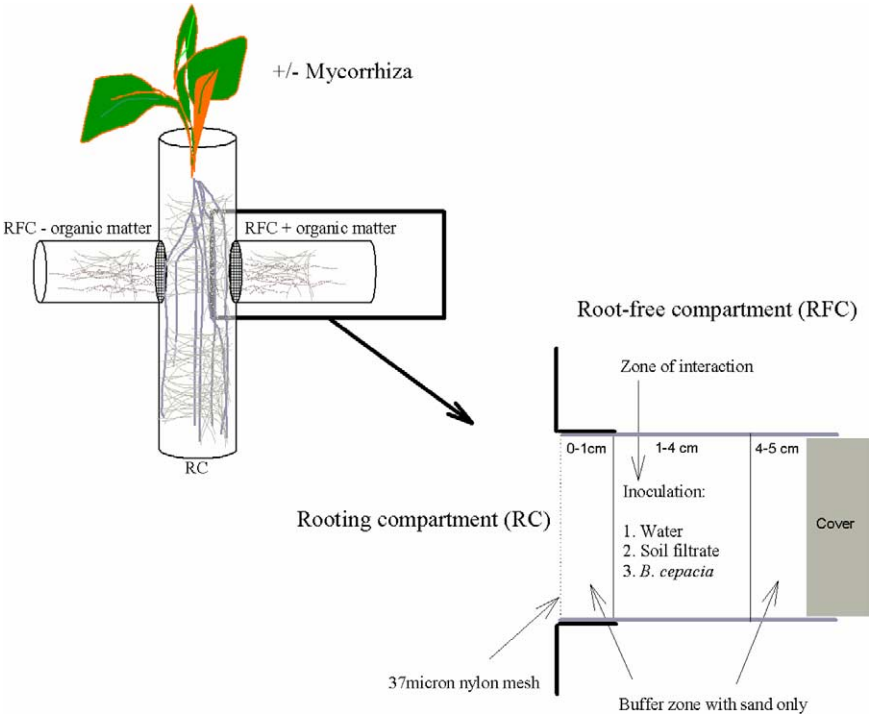

Fig. 1. Schematic drawing of the compartmented growth units with a central root compartment (RC) and two root-free compartments (RFCs). For details see Larsen and Jakobsen (1996). Each of the RFCs contained a zone of interaction where samples for hyphal length and fatty acid measurements were taken. The zone of interaction was surrounded by a 1-cm bufferzone in each ends with sand only, whereas the zone of interaction in one of the RFC of each unit were applied with organic matter and the other RFC of the same unit received no organic matter. Both RFCs of the same unit either received water, soil filtrate or *B. cepacia*.