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The effects of simultaneous root colonisation by three *Glomus* species on soil pore characteristics

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ABSTRACT

The spatial and temporal nature of the precise interactions between soil fungi and roots and their subsequent role in developing soil structure is still a subject where our understanding is limited. This research examines the relationship between three species of arbuscular mycorrhizal fungus (AMF) and soil structural characteristics. *Plantago lanceolata* was inoculated with one of: *Glomus geosporum*, *Glomus mosseae* or *Glomus intraradices*, and every combination of the fungal species. Infectivity was similar for each individual species, but *G. mosseae* and *G. intraradices* together resulted in the lowest per cent root length colonised. Despite the lower percentage colonisation, this combination induced the greatest mycorrhizal growth response. Aggregate stability and aggregate size distribution were unaffected by AMF but were increased by the presence of roots. Microbial biomass-C was also enhanced by roots. Pore size, pore size distribution and nearest neighbour distance were all reduced by *G. mosseae* and increased by *G. intraradices*. All AMF inocula containing *G. intraradices* resulted in greater distances between pores within the experimental soils. Porosity (%) was increased by *G. mosseae* suggesting that more, smaller pores with less distance between them enhanced overall porosity.

1. Introduction

Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with most terrestrial plant species (Clapp et al., 2002) and play an important role in plant nutrition (Smith et al., 2011), in protecting the plant from pathogens (Wehner et al., 2010) and from herbivorous insects (Gange et al., 2005). AMF in addition to roots and saprotrophic organisms also contribute to the formation of soil structure and stabilisation (Tisdall and Oades, 1982; Six et al., 2004; Rillig and Mummey, 2006). Roots stabilise soil aggregates through enmeshment and by the release of organic residues that bind soil particles and support many soil organisms, which in turn produce exudates and hyphae that assist in stabilisation (Morel et al., 1991; Jastrow et al., 1998; Hinsinger et al., 2009). These processes also influence the water sorptivity and repellency of soil aggregates (Czarnes et al., 2000; Traoré et al., 2000).

AMF are widely acknowledged to benefit soil formation and stabilisation, albeit to varying degrees. AMF develop extraradical hyphae which radiate away from the mycorrhizal roots into the surrounding soil. These hyphae secrete extracellular compounds such as glomalin, glomalin-related soil proteins and polysaccharides which bind soil particles with varying degrees of physical strength and hydrophobicity (Wright and Upadhyaya, 1996, 1998; Wright and Anderson, 2000; Rillig et al., 2002). In addition, hyphal products influence microbiota and food webs which may in turn change the soil structure (Filion et al., 1999; Marschner and Baumann, 2003). Fungal mycelia also exert direct effects on soil structure when hyphae enmesh soil particles (Tisdall and Oades, 1982; Miller and Jastrow, 1990). Hyphal morphology, including width, wall thickness and branching characteristics differs depending on species (Rillig and Mummey, 2006), causing variability in the tensile strength applied to soil.

Mycorrhizal fungal diversity influences plant productivity and community composition (Van der Heijden et al., 1998a; Hartnett and Wilson, 1999) and similarly, plant community diversity affects AMF species richness (Johnson et al., 2003); both factors influence soil structure. Rillig and Mummey (2006) argued that an understanding of soil and microbial processes at different scales is required to appreciate the complexities of the systems involved.

AMF affect individual plants by modifying root morphology (Van der Heijden et al., 1998b; Copetta et al., 2006) and altering nutrient dynamics (Oliveira et al., 2006). Extraradical mycelium (ERM) can have different forms of growth and different phosphate

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uptake efficiencies (Jakobsen et al., 1992; Helgason et al., 2002; Munkvold et al., 2004; Avio et al., 2006) that could modify plant growth and soil structure. Van der Heijden et al. (1998b) observed that as the number of AMF taxa within the plant root increased, the shoot and root biomass increased until a certain point after which biomass declined. Schreiner et al. (1997) reported that *Glomus mosseae* stabilised large aggregates (2–4 mm) whilst *Glomus etunicatum* and *Gigaspora rosea* did not. Smaller aggregates were not affected by AMF species.

There is therefore evidence that AMF play an important role in stabilising aggregates and in other aspects of maintaining and developing a good soil structure. Nevertheless, there is little information on the effects different species of AMF have on aggregate stability or on soil pore dynamics, or indeed whether different AMF species in disturbed conditions have complimentary functions in relation to soil structure.

The aim of this investigation was to determine the relationship between three AMF species, a host plant and the development of soil structure in terms of aggregates and resulting porosity. Soil macrocosms were established which contained sieved homogenous soil, assembled communities of indigenous microflora (originating from a soil–water 'slurry') and *Glomus geosporum, G. mosseae* and *Glomus intraradices* individually and in every combination. A split-pot design enabled quantification of the effects that roots, mycorrhizas and hyphae had on aggregate stability, aggregate size distribution, pore size and pore nearest neighbour distance. Drew et al. (2003) demonstrated that pore size affected morphology of *G. mosseae* and *G. intraradices* and concluded that extraradical hyphae adapt to the environment but exhibit different levels of adaptability. Here we determine not how the species were affected by pore size but instead, how the species modify pore size and porosity in a homogeneous soil.

2. Materials and methods

2.1. Soil preparation

A sandy loam soil (Dunnington Heath series) was collected from the University of Nottingham farm site at Sutton Bonington, Leicestershire (SK 512 267). Soil was taken from a depth of 5–20 cm, air dried and sieved to <2 mm before γ-irradiating at 25 kGy (Isotron Ltd, Daventry, UK) to primarily remove indigenous arbuscular mycorrhizal fungi. The soil had the following physical characteristics: Sand 66%, silt 18%, clay 16%, organic matter 3.7% and pH 7.35.

Macrocosms (19.7 cm length × 10.8 cm internal diameter) were constructed by splitting pipes lengthways and placing 20 μm mesh (Cadisch Precision Meshes Ltd, London, UK) vertically through the centre of each column. This maintained roots within one half of the pot, but allowed AMF hyphae to penetrate the mesh and enter the root-free half of the pot. Mesh (400 μm) was also fixed to the base of each macrocosm.

Soil was packed into both sides of each macrocosm to a bulk density of 1 g cm^{-3}. A layer of AMF inoculum was placed into one side of the column (4.5 cm from the top) where seeds were later sown. Inoculum was added as a layer rather than mixed homogeneously throughout so that firstly, it would not interfere with soil structural properties and would be easily detected when columns were scanned and secondly, because roots would need to penetrate the layer thereby maximising the chance of encountering the inoculum. A layer of soil covered the inoculum and acted as a seed-bed. The AMF species used in this investigation originated from the same site, although not from the field where experimental soil was collected. Inoculum was originally sourced *via* PlantWorks Ltd (Sittingbourne, Kent, UK) and each species subsequently maintained in culture in sterilised Dunnington Heath field soil from the University of Nottingham farm, with *Plantago lanceolata* as the host. Inoculum used in this investigation consisted of three different AMF species: *G. geosporum*, *G. mosseae* and *G. intraradices*, applied as a mixture of substrate containing mycorrhizal roots, spores and extraradical hyphae. AMF species were inoculated individually and in every combination. Non-mycorrhizal treatments were given sterilised inoculum. The inoculum was split appropriately according to treatment. The non-mycorrhizal control received 12 g of sterilised inoculum; the one species inoculum consisted of 6 g of the appropriate species and 6 g of sterilised inoculum; the two-species mix consisted of 3 g of each species and 6 g of sterilised inoculum; the three-species mix consisted of 2 g of each individual AMF inoculum and 6 g of sterilised inoculum.

Following addition of AMF inoculum, indigenous microorganisms were introduced to each macrocosm by saturating with a soil slurry solution made from field fresh soil (Dunnington Heath sandy loam) and inoculum 'washings' by diluting in sterile, 1/4 strength Ringer's solution in a ratio of 10:1 and sieving. This ensured addition of a common microflora from the original field soil. Once the cores were saturated, they were left to drain for 2 days to reach field capacity and weighed. At the start of the experiment, three *P. lanceolata* seeds were sown into the top of each macrocosm in the half containing the AMF inoculum. Once seedlings had reached the one true leaf stage of growth, they were thinned to leave one seedling per macrocosm. Macrocosms were maintained in a glasshouse at 20–25 °C with a 16 h day-length supplemented by lights and maintained at field capacity (determined by weight) by watering with sterile deionised water. There were 8 replicates per treatment; six replicates were destructively harvested after 7 weeks and two were kept intact for X-ray CT imaging. The duration of the experiment was relatively short because the plants grew quickly so were harvested before pots became root bound.

2.2. Plant and mycorrhizal measurements

After 7 weeks shoot and root biomass was determined on oven-dried material (80 °C for 2–3 days to constant mass). Prior to drying, sub-samples of roots were weighed and then maintained in 70% ethanol for later determination of AMF colonisation which was carried out according to Brundrett et al. (1984). Roots were cleared in 10% KOH solution, rinsed in water and stained for 1 h at 90 °C in a 0.1% Chlorazol Black E lactoglycerol solution containing equal volumes of 80% lactic acid, glycerol and deionised water. After staining, the roots were transferred into glycerol for storage and destaining. Colonisation was quantified using the method of McGonigle et al. (1990) at ×200 magnification to give percentage root length colonised. Mycorrhizal growth response (MGR) was calculated from the following equation (Nadian et al., 1997) where M is mycorrhizal and NM is non-mycorrhizal:

$$\mathrm{MGR} = \frac{\text{Root dwt of M plants} - \text{Root dwt of NM plants}}{\text{Root dwt NM plants}}$$

Presence of extraradical AMF hyphae was determined each side of the central mesh after extraction by the membrane filter technique of Jakobsen et al. (1992) and staining with Trypan Blue. Presence of AMF hyphae in each field of view (×100 magnification) was recorded and distinguished from hyphae of non-mycorrhizal fungi by visual appearance (Piotrowski et al., 2004).

Microbial biomass-C was determined using the fumigation–extraction method described by Vance et al. (1987) and quantified using a correction factor of 0.45 (Wu et al., 1990).