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Temporal variation outweighs effects of biosolids applications in shaping arbuscular mycorrhizal fungi communities on plants grown in pasture and arable soils

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ABSTRACT

Landspreading of biosolids (treated sewage sludge) in agroecosystems is a common waste management practice worldwide. Evidence suggests biosolids may be detrimental to arbuscular mycorrhizal fungi (AMF); however, previous studies focused on arable systems and often unrealistically high biosolids application levels. We investigated the effects of biosolids on AMF communities in grassland and arable agroecosystems, in the context of the natural seasonal dynamics of AMF community composition and diversity. A pasture and arable system under commercial farming management were amended annually with two different types of biosolids, applied at levels meeting current European Union regulations, in a factorial, replicated field-scale plot experiment. AMF root colonisation and community composition were measured in *Lolium perenne* roots from the pasture and *Trifolium repens* roots growing in arable soil across the seasons of two years. AMF community compositions were assessed by terminal-restriction fragment length polymorphism analyses. Biosolids had no significant effect on AMF root colonisation or community composition in either agroecosystem. Soil chemical analyses indicated several changes in the top 0–5 cm layer of the pasture soil, including small increases in heavy metal concentrations in biosolids relative to control plots. Temporal AMF dynamics were detected in soils from both agroecosystem indicating that the effect of seasonality outweighed that of biosolids application.

1. Introduction

Arbuscular mycorrhizal fungi (AMF) of the phylum Glomeromycota (Schüßler et al., 2001) form symbiotic associations with the roots of most land plants, thereby mediating plant nutrient dynamics and AMF fungal carbon allocation (Smith and Read, 2008). Arbuscular mycorrhizal fungi have been shown to influence several ecosystem processes, including nutrient cycling, plant productivity and diversity, and soil aggregation (van der Heijden et al., 1998, 2006; Klironomos et al., 2000; Maherali and Klironomos, 2007; Leifheit et al., 2014).

The compositions of AMF communities associated with plant roots have been studied in various ecosystems around the world (Öpik et al., 2006, 2013). Different habitats are known to host different AMF communities (Helgason et al., 1998; Öpik et al., 2003, 2006) and AMF community compositions have been related to various soil physical and chemical characteristics (Lekberg et al., 2007; Hazard et al., 2013). Furthermore, communities of AMF are affected by agricultural management practices, with agriculture intensification reducing AMF richness (Oehl et al., 2003; Hijri et al., 2006; Gosling et al., 2010).

The practice of applying biosolids (treated municipal sewage sludge) as a soil amendment to agricultural lands has been used for decades (Fytili and Zabaniotou, 2008). Biosolids have been shown to improve soil physical conditions, supply nutrients, enhance microbial activity and benefit plant productivity (Garcia et al., 1994; Pascual et al., 1999; Sullivan et al., 2006a; Cogger et al., 2013). While many studies have investigated the effects of biosolids on soil bacterial and fungal communities (e.g. Sullivan et al., 2006b; Anderson

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et al., 2008; Ippolito et al., 2009; Mattana et al., 2014), few studies have specifically investigated such effects on AMF communities.

Available evidence suggests that biosolids could alter AMF community composition, despite the nature of impacts varying between studies. AMF colonisation of plant roots, spore densities in soils and species richness of spores or on roots has been found to increase, decrease or show no effect to biosolids application (Arnold and Kaputska, 1987; Weissenhorn et al., 1995; del Val et al., 1999a,b; Jacquot et al., 2000; Jacquot-Plumey et al., 2001; Barbarick et al., 2004; Toljander et al., 2008). Contradictory results between AMF biosolids studies may be attributed to several factors, including differences between biosolids, application levels, agricultural management, soil properties and study methodologies. Thus, further AMF biosolids studies are required to investigate effects on AMF community compositions and clarify the factors causing contradictory results.

Published AMF biosolids studies thus far have focused on arable systems, and often been conducted in experimental arable fields or on experimentally constructed soils, and often used unrealistically high biosolids application levels. There is currently a dearth of information on the impact of biosolids application on AMF communities in grassland systems, with only one grassland study by Barbarick et al. (2004) that investigated AMF root colonisation of blue grama (*Bouteloua gracilis*) six years after a single application of biosolids. Understanding the impact of biosolids in grassland systems represents a significant knowledge gap, as the AMF communities associated with perennial plants in grasslands are more diverse than those in arable fields (Oehl et al., 2003, 2010; Öpik et al., 2006). Also, studies are required to investigate effects of biosolids under commercial farming systems using realistic application rates and different sewage products. Further, studies are needed which take into consideration the seasonal dynamics of AMF (Dumbrell et al., 2011).

Here, we investigated the short-term effects of biosolids on AMF in a grassland and arable field under typical commercial farming management in which biosolids were applied at levels meeting current European Union regulations (DoELG, 1998). Two contrasting biosolids types, differing in dry matter, nutrient and heavy metal concentrations, were compared. The specific aims of the experiment were (1) to compare the effects of two contrasting biosolids on AMF communities colonising *Lolium perenne* from a grassland and *Trifolium repens* grown in arable soils and (2) to determine the impacts of biosolids, relative to natural seasonal fluctuations, on AMF community dynamics.

2. Materials and methods

2.1. Description of field sites and experimental design

The pasture field was located near Tinahely, County Wicklow, Ireland (52°49′30″ N, 6°26′12″ W) on a sandy loam soil (10.4% clay content at 0–20 cm depth; pH 5.2) with a plant community consisting almost entirely of *Lolium perenne*. The site was grazed by sheep and cattle. The arable field was located near Aughrim, County Wicklow, Ireland (52°52′34″ N, 6°16′20″ W) on a sandy loam soil (8.9% clay content at 0–20 cm depth; pH 6.2) and has been used for the production of spring barley since the early 1980s following conventional farming practices. Coinciding with the months sampled in this study, seasonal climate conditions for the sites are provided in Table 1.

At each field site, 15 plots (each 20 m × 15 m), arranged in five blocks, were established in a complete randomised block design. Plots were separated by 7 m, and blocks by 10 m, in order to create a buffer zone between treatments. Plots were subjected to one of three treatments: Biocake, Biofert or control (no biosolids). The biosolids were supplied by the Ringsend wastewater treatment plant in Dublin, Ireland, where sewage sludge is treated by a thermal drying process resulting in 'Class A' pasteurised biosolids with 26% (Biocake) and 95% (Biofert) dry matter (DM) and differing in nutrient and heavy metal concentrations (Table 2). Using commercial machinery (large scale tractor-drawn applicator), biosolids were spread onto plots in March 2007 and 2008, and prior to ploughing and sowing of spring barley in the arable site. Biosolids were applied at the maximum level of 5 Mg DM ha^{-1} following European Union regulations (DoELG, 1998). Prior to this study, the field sites had never been treated with biosolids.

During the course of this experiment, farming practices continued as normal. At the arable field site mineral fertilizer was applied: 118 kg ha^{-1} of N (calcium–ammonium–nitrate); fungicides were applied: 0.25 l ha^{-1} of Bumper (Propiconazole), 0.5 l ha^{-1} of Amistar (Azoxystrobin) and 0.75 l ha^{-1} of Bravo (Chlorothalonil); herbicides were applied: 30 g ha^{-1} of Metsulfuron-methyl and 2.3 l ha^{-1} of CMPP [((chloro(methyl)phenoxy)) propionic acid]; and insecticide was applied: 165 ml ha^{-1} of Esfenvalerate.

2.2. Collection of Lolium perenne from the pasture site

Whole plant samples of *Lolium perenne* were randomly collected from each plot in March 2007, June 2007, October 2007, January 2008, March 2008 and October 2008. For this, five soil monoliths of 15 cm × 15 cm area by 30 cm deep were excavated from each plot using a spade, stored at 4 °C and processed within two weeks from collection. Soil was thoroughly washed from the plant roots with tap water. For each plot, one individual *L. perenne* plant was randomly selected from each of the five soil monolith samples and roots were bulked, rinsed with deionised water, blotted dry, homogenised and equally split into two subsamples. One sub-sample was stored in 70% ethanol at room temperature for AMF root colonisation analyses, and the other flash-frozen with liquid N and stored at −80 °C for molecular procedures.

2.3. Arable soil bioassay with Trifolium repens

An arable soil bioassay approach was used to bait for AMF as crop plants were not always present for sampling throughout the year. From the arable site, soil samples were collected from each of the plots in February, July and October 2007, and in January and October 2008. A total of five soil samples were collected within each plot using a standard, 20 cm depth × 5 cm diameter Edelman soil auger (Eijkelkamp Agrisearch Equipment BV, Giesbeek, NL). Soil samples were taken at 2 m intervals along a transect centred in the middle of the plot. For each plot, soil samples were bulked, homogenised and stored at 4 °C until used.

A subsample of this soil was used for a bioassay with *Trifolium repens* L. (Fabaceae) to bait for AMF. Pots (8 cm × 8 cm × 8 cm) were filled with a 1:1 mix of bulked soil and autoclaved sand (three replicate pots were prepared per plot per sampling date). Fifteen negative control pots contained autoclaved field soil and sand (1:1 mix). Seeds of *T. repens* were surface-sterilised (2.5% sodium hypochlorite for 15 min), rinsed three times in sterile water, and 10 seeds were sown into each pot. Pots were arranged randomly in a growth chamber and the seedlings were grown under environmentally-controlled conditions [8 h dark/16 h light (120 μmol photons m^{-2} s^{-1}) cycle, and a constant temperature of 20 °C] and plants were routinely watered as necessary. After three months, all plants were removed from the soil and roots were thoroughly washed free of soil using tap water. Roots grown in soils