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The legacy effect of cover crops on soil fungal populations in a cereal rotation

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ABSTRACT

The use of rotations and minimum tillage in agriculture can permit more sustainable production through increasing soil organic matter and nutrients, and breaking of pathogen lifecycles. Soil fungal populations make an important physical and chemical contribution to soil. For example, mycorrhizal species are important in plant nutrition but are often overlooked when considering management practices for efficient soil function. We undertook DNA metabarcoding (Ion Torrent) using novel PCR primers and high-throughput sequencing of the D1 region of the large ribosomal subunit of the rRNA locus, to assess the effect of different forages and cereal tillage methods on the soil fungal community. The study comprised five forage treatments, perennial ryegrass (*Lolium perenne*) with either low or high N, chicory (*Cichorium intybus*), red clover (*Trifolium pratense*) or white clover (*Trifolium repens*) grown over 3 harvest years (2010–2012). Cultivation of chicory, red clover or white clover led to significantly divergent soil fungal communities, with a notably lower diversity of fungal populations under clover, suggesting a link to soil N dynamics. Consistent with this, was a negative correlation of soil nitrate-N levels with populations of arbuscular mycorrhizal fungi (AMF) and other root-associated fungal groupings (dark septate endophytes, 'CHEG', Sebacinales and Ceratobasidiaceae). In contrast, abundance of Fungi belonging to the genera *Mortierella* and *Cryptococcus* were positively correlated with soil nitrate-N, with *Mortierella* also being negatively correlated with soil P. Spring wheat was sown on the same plots (April 2013) followed by winter barley (October 2013). Half of each plot was sown either after ploughing or by direct drilling. A legacy effect of the preceding forage crop on the fungal community was detected after both cereal crops, with plots previously cultivated with ryegrass being most divergent. No overall effect of establishment method on fungal communities was detected but AMF and CHEG fungi were more abundant on direct-drilled plots and pathogenic fungi were more abundant on ploughed plots after the sowing of winter barley.

1. Introduction

Given the need for the sustainable land use in agriculture, there is an urgent need to understand the complexities of plant-soil-microbe interactions, the role of microbes in plant nutrition and maintenance of soil function and how different agricultural management practices may alter the soil ecosystem and environment. The use of forage and cover crops in rotations and a reduction in tillage frequency, can play an important role in mixed farms by increasing the organic matter content of soils, thereby contributing to carbon sequestration and soil productivity (Six et al., 1998; West and Post, 2002). Such practices also improve the hydrological properties of soil, for instance water infiltration, moisture retention and erosion rates (Augé et al., 2001).

The benefit of leguminous forage crops, such as red (*Trifolium pratense*) and white clover (*Trifolium repens*) in providing nitrogen for following crops has long been appreciated (Ebelhar et al., 1984), but these and other forage crops can provide additional benefits within agricultural rotations. Forage crops can be an important element in the control of certain pathogens through breaking the lifecycle. For example, *Gaeumannomyces graminis* var. *tritici* causes root rot in wheat and other susceptible crops such as barley and rye but can be controlled via rotation with non-susceptible forages

Abbreviations: PRG0N, perennial ryegrass with low N; PRG200N, perennial ryegrass with high N; CY, chicory; RC, red clover; WC, white clover.

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such as clover and chicory (Cook, 2003). Chicory has also been shown to reduce the levels of helminth parasite infection of grazing sheep by interfering with the free-living stages of the life cycle of the parasites within the pasture environment and hence reducing their ability to infect grazing livestock (Marley et al., 2006).

Kingdom Fungi comprises a morphologically diverse group of organisms ranging from single celled yeast to macrofungi that can form networks in soil over many metres. In the context of arable agriculture, Fungi attract attention as major crop pathogens, for instance wheat rust (Aime, 2006) and ear blight but they also play a central role in nutrient cycling through the catabolism of dead organic matter and as mycorrhizal mutualists (Smith and Read, 2007). Historically the arbuscular mycorrhizal fungi (AMF) (Glomeromycota) have been viewed as the main mycorrhizal symbionts in arable and grassland soils (Smith and Read, 2007; Joergensen and Wichern, 2008). However, recent advances in plant-soil interactions have revealed that a wider range of Fungi than previously suspected may be involved in mutualistic interactions with higher plants in grasslands and thus play an important role in plant nutrition (Smith and Read, 2007; Heijden et al., 2015). Notable among these are the dark septate endophytes (DSE) a diverse group of filamentous ascomycetes, many belonging to the order Helotiales (Wilberforce et al., 2003; Mandyam and Jumpponen, 2014), as well as many members of order Sebacinales (Oberwinkler et al., 2013) and family Ceratobasidiaceae (Veldre et al., 2013). These groups comprise taxa with highly divergent mycorrhizal morphologies, which, depending on the host plant, can range from typical sheathing ectomycorrhizas to intracellular hyphal coils or undifferentiated intercellular hyphae. These groups also include a few taxa are known to cause plant disease and for the majority there is only sporadic evidence that the interactions are mutualistic (Jones and Smith, 2004). Thus the outcomes of these symbioses may vary with particular plant/fungus combinations and specific environmental conditions (the "mutualism-parasitism-continuum"; (Mandyam and Jumpponen, 2014)), with the interplay between direct and mycorrhizal pathways of nutrient uptake (Smith and Read, 2007). Therefore research to elucidate the role of these fungi in effective soil function is needed to assist in the development of our understanding of soil-plant interactions and, thereby reduce our reliance on inorganic inputs within agricultural systems.

The study of soil fungi, historically focused culture-based or microscopic approaches, has hampered attempts to reliably identify and quantify the abundance of different taxa. However, recent developments in DNA sequencing technology and the expansion of large databases of sequence and taxonomic data, such as NCBI and curated database of fungal sequences such as RDP (Cole et al., 2014) and UNITE (Abarenkov et al., 2010), mean that sequencing and identification from environmental sample such as soil is now possible. As a result we are now beginning to understand the distribution and the changes in fungal communities from diverse habitats (Geml et al., 2014; Jumpponen and Jones, 2014; Tedersoo et al., 2014; Franke-Whittle et al., 2015).

Here we present an analysis of fungal communities under a crop rotation of forage crops and cereal crops using a novel primer combination that amplifies sequences from a diverse range of fungi from basal groups such as Chytridiomycota to the more advanced dikarya (Basidiomycota and Ascomycota). The aims of this paper are to determine the effect of five different forage crop regimes on soil fungal communities and then to monitor the effects of either direct drill or ploughing on establishment of cereal crops on these soil communities. It was hypothesised that the type of forage crop will lead to the development of divergent soil fungal communities and that direct drilling to establish follow-on cereal crops will cause less disruption to these communities than ploughing.

2. Materials and methods

2.1. Experimental design and establishment

2.1.1. Forages

The study comprised of 5 forage treatments, perennial ryegrass (*Lolium perenne*) with either low (PRG0N) or high N (PRG200N), chicory (*Cichorium intybus*)(CY), red clover (*Trifolium pratense*)(RC) or white clover (*Trifolium repens*)(WC) grown over 3 harvest years (2010–2012). The experimental plots were established at the Institute of Biological, Environmental and Rural Sciences (IBERS), Aberystwyth University (52.4331, -4.0261) on an area of stony, well-drained loam of the Rheidol series. Full details of the experimental site, plot establishment and crop management are described in (Crotty et al., 2015) and a summary schematic of plot layout and crop/sampling timelines is presented in Suppdata 1. In brief, after initial application of herbicide (4 l ha^{-1}) to the initial ryegrass ley in April 2009, the area was ploughed, dolomitic limestone (5 t ha^{-1}) and inorganic fertiliser (60 kg P_2O_5 and 60 kg K_2O ha^{-1}) was applied. The area was power-harrowed and rolled before the experiment was established as a randomised block design with four replicate plots (7.5 × 12 m), on the 29th June 2009. Five treatments (20 plots in total) were established, consisting of perennial ryegrass with either low N (80 kg N ha^{-1}, applied once in March 2011) or high N (200 kg N ha^{-1} yr^{-1}; as four monthly applications: April–July), chicory (200 kg N ha^{-1} yr^{-1}; as for high N ryegrass), white clover and red clover (no N fertiliser addition). Plots were seeded at a rate of 33 (ryegrass), 16 (red clover) and 6 (chicory, white clover) kg seed ha^{-1}. Plots were cut and herbage removed five times each season (monthly: May–September; from 2010 to 2012). All plots received P and K fertiliser (as required to maintain a soil P index of 2), and N fertiliser was applied to the relevant plots as ammonium nitrate.

2.1.2. Cereals

In February 2013, all plots were treated with herbicide (360 g l^{-1} glyphosate at 4 l ha^{-1}; Gallup 360) and each plot was split into sub-plots (3.75 × 12 m) and allocated at random to two cultivation treatments, ploughing (P) or direct drill (DD) giving a total of 40 plots. The P sub-plots were ploughed to a depth of 175 mm (20th March) and power-harrowed (4th April), whilst DD sub plots were undisturbed prior to sowing. Spring wheat (*Triticum aestivum*) was sown (253 kg seed ha^{-1}; 5th April) and fertiliser was applied with the seed (49 kg N, 9 kg P_2O_5, 28 kg K_2O and 16 kg SO_3 ha^{-1}). Prilled lime was top dressed at 370 kg ha^{-1} post-sowing to avoid any tillage effects on soil pH. A second fertiliser application (21st May) supplied 127 kg N, 22 kg P_2O_5, 72 kg K_2O and 42 kg SO_3 ha^{-1} to achieve sufficiency status according to RB209 recommendations (soil N index 1 to target the recommended spring wheat N application of 180 kg N ha^{-1}). Wheat was harvested on 29th August.

On 10 October 2013 all plots were treated with herbicide (4 l ha^{-1}) and the same sub-plot establishment treatments remained for barley. The P sub-plots were ploughed on the 14th October 2013 and power-harrowed on 15th October. Winter barley (*Hordeum vulgare*) was then sown (196 kg ha^{-1}) on the 15 October 2013. No fertiliser was applied during establishment. Fertiliser was applied on the 13 March 2014 (52 kg N, 52 kg P_2O_5 and 52 kg K_2O ha^{-1}) and prilled lime was top dressed at 370 kg ha^{-1}. On the 16th April 2014 a second fertiliser application supplied 80 kg N, 14 kg P_2O_5, 45 kg K_2O and 24 kg SO_3 ha^{-1} and the crop was harvested on 15th July.

2.1.3. Measurements

Soil samples were taken from each plot with a 25 mm auger to a depth of 15 cm (fourteen cores per plot) on 14th September 2012