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Preceding crop and seasonal effects influence fungal, bacterial and nematode diversity in wheat and oilseed rape rhizosphere and soil

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ABSTRACT

Crop rotation can have major influences on yield, which may be the result of changes in the composition of the rhizosphere microbiome. In particular there is evidence that yields of both oilseed rape and wheat can be influenced by the frequency in which they are grown in rotation with each other. In the current study we investigated the effect of preceding crops (either wheat or oilseed rape) on wheat and oilseed rape yield, with associated changes in the rhizosphere and bulk soil communities of fungi, bacteria and nematodes using terminal restriction fragment length polymorphism (TRFLP) of rRNA genes. Yield of wheat and oilseed rape were reduced by 11 and 10% respectively when grown two years consecutively. Rhizosphere populations were significantly different to bulk soil populations for all groups of organisms. Seasonal shifts in the communities were observed in the rhizosphere for all groups. Communities of fungi, bacteria and nematodes were all significantly influenced by the preceding crop in the wheat rhizosphere, while just the nematode population was affected by preceding crop in the oilseed rape rhizosphere. In particular when two consecutive crops of oilseed rape were grown, relative abundance of members of nematodes within the genus *Eumonhystera* increased markedly. The fungal foliar pathogen *Mycosphaerella graminicola*, the teleomorph of *Zymoseptoria tritici* which causes septoria leaf blotch in wheat, was identified in the rhizosphere of wheat and was significantly more abundant in wheat grown after oilseed rape. We conclude that overall, preceding crop had less impact on community composition than season or crop type, but that specific changes in communities at particular plant growth stages may have substantive impacts on crop growth.

1. Introduction

A wheat and oilseed rape crop rotation is a popular rotation due to the high demand for oilseed rape as cooking oil, animal feed and as a source of biofuel. Wheat yields benefit from 'break crops' such as oilseed rape or other non-host crops to break the life-cycle of crop-specific pathogens. However, if oilseed rape is grown too frequently in the rotation it can result in a subsequent yield decline of oilseed rape, which can be up to 25% (Berry et al., 2014; Berry and Spink, 2006; Hilton et al., 2013; Sieling and Christen, 1997).

Many crops are susceptible to yield decline, in which crops grown in short rotation have reduced yields relative to crops grown in longer rotation, or for the first time. The causes of yield decline are complex and a range of factors have been implicated, including alteration of soil physico-chemical properties by land management practices and biotic factors, particularly changes in the composition of soil or rhizosphere microbial communities, including increased prevalence of plant pathogens (Bennett et al., 2012).

A wide range of biotic and abiotic factors can influence the composition and function of rhizosphere microbial communities. Rhizodeposition by plant roots results in increased microbial growth in the rhizosphere compared with the bulk soil, a phenomenon often referred to as the 'rhizosphere effect' (Hunter et al., 2014; Philippot et al., 2013; Vanstone et al., 1998). However, the quality and quantity of rhizodeposits can also vary markedly between plant species and developmental stages, thereby affecting rhizosphere community composition (Chaparro et al., 2014; Houlden et al., 2008; Turner et al., 2013).

When crops are grown continuously or in short rotation there is typically a change in rhizosphere community composition and often a decline in microbial diversity (Alvey et al., 2003; Larkin, 2003; Li et al., 2010, 2009, 2016; Lupwayi et al., 1998; Venter et al., 2016). In the case of oilseed rape, yield decline is known to be associated with changes in rhizosphere microbial communities. This includes increased abundance of a number of fungi, two of which were subsequently shown to act as

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pathogens in glasshouse studies (Hilton et al., 2013), and may therefore be in part responsible for yield decline in this crop. However, the effect of crop rotation on other potential pathogens, particularly nematodes, which can result in significant crop losses in oilseed rape, is unknown. In the case of wheat, the soil-borne fungus *Gaeumannomyces graminis* var. *tritici* (Ggt), causing take-all in wheat and other cereals, is regarded as the most important disease on wheat in short rotations (Cook, 2003; Sieling and Christen, 2015). Effective controls require either crop rotation, or wheat monoculture which will eventually induce take-all decline, which involves build-up of populations of 2,4-diacetylphloroglucinol (2,4-DAPG)-producing fluorescent *Pseudomonas* spp. which suppress the take-all pathogen (Loper et al., 2012; Raaijmakers and Weller, 1998; Weller et al., 2007). However, crop rotation is favoured as it generally results in much higher yields than monoculture (Cook, 2003). Oilseed rape has been shown to be a favourable preceding crop to wheat, resulting in higher wheat yields when compared to wheat grown after wheat (Kirkegaard et al., 2008; Sieling and Christen, 2015; Sieling et al., 2007). Wheat grown after oilseed rape has been shown to increase yield by 13% and reduce take-all Ggt severity at maturity to a level with no yield penalties (Sieling and Christen, 2015). Therefore, the trends globally have been to shorten rotations in wheat-based cropping systems, which has been associated with reduced yields of oilseed rape used as a break crop.

It is clear that the sequence within a crop rotation is critical in order to maximise yield of the primary crop as well as the break crop. To be able to understand the belowground influences of microbes, in particular pathogens within wheat-oilseed rape rotations, we characterised the rhizosphere and bulk soil communities of oilseed rape and wheat when grown after different preceding crops (oilseed rape or wheat). Typically, studies of rhizosphere microbiota have focussed on bacterial and fungal communities, and much less is known of the factors which shape composition of other groups, including nematodes, where most understanding comes from studies of known plant-pathogens in isolation (McLeod et al., 2001; Warnke et al., 2008). Here we examined the influence of crop sequence on bacterial, fungal and nematode communities at three contrasting plant developmental stages to determine shifts in communities that could be related to crop rotation and ultimately yield decline.

2. Materials and methods

2.1. Field plot experimental design and sampling strategy

An established long-term field trial based in East Anglia, UK (52° 33′ N and 1° 2′ E), investigating the effect of different frequencies of cropping of oilseed rape (cv. Winner) and winter wheat (cv. Brompton) on oilseed rape yield, funded by AHDB Cereals & Oilseeds (Project RD-2003-2922) and managed by NIAB TAG, was used to provide samples for this project. The soil type was a sandy clay loam (Cambic Arenosol) with a pH of 6.6 and available P, K, Mg and SO_4^{2-} of 32.4, 111, 28 and 30.6 mg kg^{-1}, respectively (IUSS, 2015). The entire trial area was ploughed and pressed each season ahead of establishment. The experiment had a completely randomised block design with four replicate plots of 24 × 6 m that had the following treatments; oilseed rape grown after oilseed rape (Oo), oilseed rape grown after wheat (Ow), wheat grown after wheat (Ww), wheat grown after oilseed rape (Wo). The Wo was preceded by three seasons of wheat, while Ow was a seasonal wheat-oilseed rotation as shown in Table A1. While specific drilling dates varied according to season, oilseed rape was typically drilled in early September, first winter wheat in the second half of September and subsequent wheat in mid-October (Stobart, 2009). Local commercial best practice was adhered to for pesticide and fertilizer inputs (Stobart and Bingham, 2013). For oilseed rape this included autumn herbicide (diflufenican) and insecticide (cypermethrin), and spring insecticides (lamda cyalothrin and cyclohexadione), together with nitrogen and sulphur inputs of 200 kg ha^{-1} and 30 kg ha^{-1} respectively. For wheat this included autumn herbicide (diflufenican) and spring fungicides (propiconazole, chlorothalnil and cyproconazole) and 100 kg N ha^{-1}.

The field trial was in its fifth year when samples were collected in November 2007 (seedling stage), March 2008 (stem extension) and June 2008 (pre-harvest). Each plot was divided into three equal sub-plots longitudinally. The central sub-plot was used for yield data and the outer two sub-plots were used for destructive sampling.

Bulk soil and rhizosphere samples were collected from the sub-plots of each of the four replicates of the four selected rotation treatments. For each replicate, three plants were excavated from the two sub-plots at approximately 6, 12 and 18 m along the length of the plot (six plants in total per replicate) and pooled. Bulk soil samples were collected at the same intervals, using a 30 cm auger (six samples pooled per replicate). Plants and bulk soil samples were taken back to the laboratory for processing. Roots were shaken free of loose soil and fine roots were cut into approximately 5 mm sections. Fine roots plus closely adhering soil were designated as the rhizosphere and sub-samples (0.5 g) of rhizosphere material were frozen for molecular analyses. Bulk soil samples were sieved using a 3 mm sieve and sub-samples (0.5 g) were also frozen for molecular analyses.

2.2. DNA extraction and community analysis

DNA was extracted from 0.5 g of each bulk soil and rhizosphere sample using the FastDNA® SPIN Kit for Soil (MP Biomedicals LLC, UK), according to the manufacturers' instructions, with the exception that samples were homogenized in a Mini Beadbeater-8 cell disrupter for 3 min (Biospec products, Inc., USA). DNA samples were amplified with PCR primers universal to the small subunit rRNA gene of fungi, bacteria or nematodes. The PCR reaction (50 µl) contained the Megamix-PCR Master Mix (Microzone Limited, UK), 10 ng DNA and taxon-specific forward and reverse primers. For fungi 25 pmol of PET labelled ITS1f (5′-CTT GGT CAT TTA GAG GAA GTA A-3′) (Gardes and Bruns, 1993) and unlabelled ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) (White et al., 1990) were used. For bacteria 5 pmol of VIC labelled 1087r (5′-CTC GTT GCG GGA CTT ACC CC 3′) (Hauben et al., 1997) and unlabelled 63f (5′-AGG CCT AAC ACA TGC AAG TC-3′) (Marchesi et al., 1998) were used. For nematodes 20 pmol of VIC labelled Nem_18S_F (5′-CGC GAA TRG CTC ATT ACA ACA GC-3′) and unlabelled Nem_18S_R (5′-GGG CGG TAT CTG ATC GCC-3′) were used (Floyd et al., 2005). Thermocycling consisted of an initial denaturation at 95 °C for 3 min followed by 30 cycles (bacteria and fungi) or 40 cycles (nematodes) of 95 °C for 30 s, 55 °C for 60 s, 72 °C for 60 s. The final extension was at 72 °C for 10 min. The PCR products were purified using a Qiagen PCR purification kit. Purified DNA (approximately 250 ng) was digested with *Hha*I (bacteria and fungi) or *Hae*III (nematodes) for 4 h at 37 °C and the reaction terminated by a further incubation at 95 °C for 15 min. These restriction enzymes were selected due to the production of evenly spaced peaks for downstream analysis. Aliquots (1 µl) of digested PCR products were mixed with 10 µl of HIDI formamide (Applied Biosystems™, Warrington, UK) and 0.15 µl of internal size standard LIZ 1200 (Applied Biosystems™, Warrington, UK) and then denatured for 5 min at 95 °C. Terminal restriction fragment length polymorphism (TRFLP) analysis was carried out on an automated sequencer, ABI PRISM1 3130xl Genetic Analyzer on a 36 cm capillary array (Applied Biosystems™, Warrington, UK). Terminal restriction fragments generated by the sequencer were analysed using GeneMarker 1.60 (SoftGenetics LLC®, USA). To avoid detection of primers and undigested PCR products, peaks < 50 bp or more than 500 bp (fungi), 900 bp (bacteria) or 800 bp (nematodes) were excluded from further analysis, this was based on the amplicon size. The relative abundance of OTUs was determined by calculating the percentage height of each peak in relation to the total peak height of all peaks within one sample. There were 110, 56 and 99 OTUs over 0.1% relative abundance for fungi, bacteria and nematodes, respectively.