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

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**Highlights**

•Preceding crop influenced microbial communities in the wheat rhizosphere.

•Seasonal shifts in microbial communities were observed.

• *Mycosphaerella graminicola* was identified in the rhizosphere/root of wheat.

• *Eumonhystera* nematodes increased in oilseed rape grown for two years.

**Keywords**

Rhizosphere; nematodes; *Mycosphaerella graminicola;* microbial diversity; oilseed rape; wheat.

**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; Lei et al., 2006; Li et al., 2010; Li et al., 2009; Li et al., 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 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 other potential pathogens to crop rotation, 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 suppresses 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

76 factors which shape composition of other groups, including nematodes, where most
77 understanding comes from studies of known plant-pathogens in isolation (McLeod et al., 2001;
78 Warnke et al., 2008). Here we examined the influence of crop sequence on bacterial, fungal
79 and nematode communities at three contrasting plant developmental stages to determine shifts
80 in communities that could be related to crop rotation and ultimately yield decline.
81
82 **2. Materials and methods**
83 *2.1 Field plot experimental design and sampling strategy*
84 An established long-term field trial based in East Anglia, UK (52$^{o}$ 33' N and 1$^{o}$ 2' E),
85 investigating the effect of different frequencies of cropping of oilseed rape (cv. Winner) and
86 winter wheat (cv. Brompton) on oilseed rape yield, was used to provide samples for this project
87 via NIAB TAG and funded by AHDB Cereals & Oilseeds (Project RD-2003-2922). The soil
88 type was a sandy clay loam (Cambic Arenosol) with a pH of 6.6 and available P, K, Mg and
89 $SO_4^{2-}$of 32.4, 111, 28 and 30.6 mg kg$^{-1}$, respectively (IUSS, 2015). The entire trial area was
90 ploughed and pressed each season ahead of establishment. The experiment had a completely
91 randomised block design with four replicate plots of 24 x 6 m that had the following treatments;
92 oilseed rape grown after oilseed rape (Oo), oilseed rape grown after wheat (Ow), wheat grown
93 after wheat (Ww), wheat grown after oilseed rape (Wo). The Wo was preceded by three seasons
94 of wheat, while Ow was a seasonal wheat-oilseed rotation as shown in Table A1. While specific
95 drilling dates varied according to season, oilseed rape was typically drilled in early September,
96 first winter wheat in the second half of September and subsequent wheat in mid-October
97 (Stobart, 2009). Local commercial best practice was adhered to for pesticide and fertilizer
98 inputs (Stobart and Bingham, 2013). For oilseed rape this included autumn herbicide
99 (diflufenican) and insecticide (cypermethrin), and spring insecticides (lamda cyalothrin and
100 cyclohexadione), together with nitrogen and sulphur inputs of 200 kg ha-1 and 30 kg ha-1

101 respectively. For wheat this included autumn herbicide (diflufenican) and spring fungicides
102 (propiconazole, chlorothalnil and cyproconazole) and 100 kg N ha-1

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

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

117

118 *2.2 DNA extraction and community analysis*

119 DNA was extracted from 0.5 g of each bulk soil and rhizosphere sample using the
120 FastDNA® SPIN Kit for Soil (MP Biomedicals LLC, UK), according to the manufacturers'
121 instructions, with the exception that samples were homogenized in a Mini Beadbeater-8 cell
122 disrupter for 3 minutes (Biospec products, Inc., USA). DNA samples were amplified with PCR
123 primers universal to the small subunit rRNA gene of fungi, bacteria or nematodes. The PCR
124 reaction (50 µl) contained the Megamix-PCR Master Mix (Microzone Limited, UK), 10 ng
125 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, 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 less than 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.

151

152 *2.3 Cloning and sequencing*

153 Unlabelled primers for each taxa were used to amplify DNA from pooled rhizosphere
154 DNA from four replicate plots of oilseed rape grown after oilseed rape (Oo) or wheat grown
155 after wheat (Ww) in June from year four of the field trial (Table A1). PCR products were cloned
156 using the QIAGEN PCR cloning plus kit (Qiagen, Crawley, UK). Plasmid DNA from 96
157 colonies underwent Templiphi™ amplification (GE Healthcare Life Sciences, UK).
158 Sequencing was carried out using the vector targeted PCR primers M13 F and M13 R on an
159 automated sequencer (ABI PRISM1 3130xl Genetic Analyzer) using the BigDye® version 3.1
160 sequencing chemistry. Sequences were assembled and trimmed to the primer sites using the
161 DNAstar, Inc. software suite. *In silico* restriction cut sites were then determined. The sequences
162 were compared with the Genbank database using the BLASTN program (Altschul et al., 1990)
163 and the ribosomal database project (RDP) (Wang et al., 2007) for phylogenetic comparison.
164 The sequences obtained in this study are available in GenBank under accession numbers
165 JF432891−JF433024 (oilseed rape fungi and bacteria), MF344912-MF344951; MF348000-
166 MF348008 (oilseed rape nematodes), MF314107-MF314112 (wheat bacteria), MF344903-
167 MF344911 (wheat fungi).

168

169 *2.4 Identification of OTUs using the clone libraries*

170 *In silico* digests of the clone libraries were used to identify OTUs, which were
171 contributing towards the differences in community structure. To confirm the OTU size, each
172 DNA clone of interest was digested with the restriction enzyme used for TRFLP analysis to
173 confirm the sizes. Each OTU was further validated by determining the presence of the predicted
174 size using a second restriction enzyme (*Msp*I for fungi and bacteria and *Aci*I for nematodes).
175 Identification was only possible for OTUs of high abundance or those that were well-spaced.

176 The identification of OTUs using the continuous oilseed rape or wheat rhizosphere clone
177 libraries is shown in Table A2.
178
179 *2.5 Real-time PCR*
180 Primers ST-rRNA F and ST-rRNA R were used for real-time PCR (Guo et al., 2006). Total
181 rhizosphere or bulk soil DNA (1 ng) was quantified using the Qubit HS kit (Invitrogen) and
182 used in the real-time PCR reaction. Each reaction was set up in triplicate in a 384-well plate
183 with the following components: 2 x LightCycler® 480 SYBR Green I Master (Roche) (5µl),
184 1 mM forward primer, 1 mM reverse primer, 1 ng total sample DNA, 400 µg $ml^{-1}$ non-
185 acetylated BSA and water added to 10 µl. Real-time PCR was carried out using the
186 LightCycler® 480 system (Roche) with default cycling conditions (95 $^{o}$C for 5 min followed
187 by 45 cycles of denaturation at 95 $^{o}$C for 10 s, annealing at 60 $^{o}$C for 10 s and extension at 72
188 $^{o}$C for 10 s). An average of the triplicate results was taken. The quantities of DNA obtained
189 were converted to copy numbers of target DNA/µg total DNA.
190
191 *2.6 Statistical analysis*
192 Community profiles were expressed in relative abundance and analysed for
193 resemblance using analysis of similarity (ANOSIM) and non-metric multidimensional scaling
194 (non-metric MDS) (PRIMER, version 6, Primer-E) (Clarke, 1993). ANOSIM reports the level
195 of dissimilarity between sample groups (global R) and the associated level of significance (*P*).
196 R is scaled to be within the range +1 to -1. Positive R values indicate that samples are more
197 dissimilar between groups than within groups. R values close to zero occur if the high and low
198 similarities are perfectly mixed and bear no relationship to the group. Negative R values
199 indicate that dissimilarities within groups are greater than dissimilarities between groups
200 (Clarke, 1993). Significance values were obtained by permutation tests. As ANOSIM does not

correct for multiple comparisons, we used the global R and the associated level of significance (*P*) to interpret the results. Where the R value was very low this indicated the factor had only a small effect on the variables and was not considered important. The relative contribution (%) of each OTU to the similarity matrix structure was assessed using SIMPER (Similarity Percentages - species contributions) (Clarke, 1993). ANOVA was used to analyse OTU relative abundance across rotations and yield data.

## 3. Results

### *3.1 Yield data*

The yield data from the four replicate plots for each preceding crop is shown in Fig. 1. The yield recovered after the same preceding crop was significantly reduced for oilseed rape by 10% ($P$=0.04) and wheat by 11% ($P$=0.01).

### *3.2 Fungal communities*

#### *3.2.1 Crop type and sample type*

Non-metric MDS with ANOSIM analysis of the data showed significant differences between the fungal communities of the crop types and sample types, with less similarity within the rhizosphere communities than the bulk soil (Fig. 2, Table A3). Across all time points there was a significant difference between the rhizosphere community of oilseed rape and wheat ($P$ = 0.002, R = 0.877) (Fig. 3, Table A3). Using SIMPER analysis, the OTUs that contributed most towards these differences were 284 (*Olpidium brassicae*), and 299 (*Trichosporon* sp.), which both had a higher relative abundance in the oilseed rape rhizosphere, and 143 (*Mycosphaerella graminicola*), which had a higher relative abundance in the wheat rhizosphere (Table A4a). Within the bulk soil there were also significant differences between oilseed rape and wheat ($P$=0.002 R= 0.347) (Fig. 3, Table A3). Using SIMPER analysis, the OTU that

contributed most towards these differences was 124/125 (*Gibellulopsis nigrescens*), which had a higher relative abundance in the oilseed rape bulk soil (19.1 %) compared with the wheat bulk soil (12.6 %).

*3.2.2 Sampling time*

The oilseed rape and wheat fungal communities showed significant differences between seasons in the rhizosphere and bulk soil (Fig. 2, Table A3). Seasonal fluctuations were examined further using SIMPER analysis. Oilseed rape rhizosphere samples showed a mid-season (March) peak in relative abundance of OTU 284 (*Olpidium brassicae*) (Table A4b). The next OTU contributing to the seasonal differences was 299 (*Trichosporon* sp.) which followed the opposite seasonal pattern (Table. A2b). Within the rhizosphere of the wheat samples, there was a mid-season (March) peak in relative abundance of OTU 143 *(M. graminicola)* (Table. A2b). Levels of *M. graminicola* dropped substantially by June (Fig. A2b) which contributed to the distinct June community (Fig. 2b).

*3.2.3 Preceding crop*

The only treatment to show significant differences were between the November wheat rhizospheres, Ww (wheat grown after wheat) and Wo (wheat grown after oilseed rape) ($P$=0.029 R=0.969) (Fig. 3, Table A3). This was due predominantly to OTU 143 (*M. graminicola),* which had a much higher relative abundance in Wo. SIMPER analysis for preceding crop (November) is shown in Table. A2c. The relative abundance of OTU 143 (*M. graminicola)* across the rotations is shown in Fig. 4a. There was a significantly higher relative abundance of *M. graminicola* in Wo than Ww in November ($P$ = 0.03) (Fig.4a). Quantitative PCR analysis with *M. graminicola* specific primers supported these results (Fig. 4b). There was also a significantly higher relative abundance of unidentified OTUs 337 ($P$=0.002) and

327b ($P$=<0.001) in the Ww rotation. There were no significant differences in the oilseed rape fungal community between rotations although the relative abundance of OTU 284 (*Olpidium brassicae)* was 13.5 % higher in Oo compared with Ow (Table A4c).

### *3.3 Bacterial communities*

#### *3.3.1 Crop type and sample type*

Non-metric MDS with ANOSIM analysis of the data showed significant differences between the bacterial communities of the crop types and sample types, with again less similarity within the rhizosphere communities than the bulk soil (Fig. 5, Table A3). Overall there was a significant difference between the rhizosphere of oilseed rape and wheat ($P$ = 0.038, R = 0.073), although the low R value indicates that the differences are small. Using SIMPER analysis, the OTUs that contributed most towards these differences were 245 (*Pseudomonas* spp.) and 248 which had a higher relative abundance in the wheat rhizosphere, and 523 (Burkholderiales) and 723 which had a higher relative abundance in the oilseed rape rhizosphere (Table A5a). Within the bulk soil there were no significant differences between oilseed rape and wheat.

#### *3.3.2 Sampling time*

There were significant differences in the bacterial communities between seasons in the rhizosphere and bulk soil of oilseed rape and wheat (Fig. 5, Table A3). Within the samples, seasonal fluctuations were examined further using SIMPER analysis, which are shown in Table A5b. This showed that the OTU contributing the most towards the difference in oilseed rape rhizosphere communities over time was 245 (*Pseudomonas* spp.), which peaked mid-season (March). The next contributing OTU was 523 (Burkholderiales), which declined in relative abundance over time. Within the bulk soil of oilseed rape, OTU 245 (*Pseudomonas* spp.) also

276 contributed the most towards the communities over time, where it increased over the growing
277 season (Table A5b). The next contributing OTU was 722 (Acidobacteria gp6), which declined
278 in relative abundance over time. Within the wheat rhizosphere, OTU 523 (Burkholderiales)
279 contributed the most towards the differences in communities over time, where it peaked mid-
280 season (March) (Table A5b). The bacterial community of the wheat rhizosphere in June
281 showed much less similarity to the other sampling times (between March and June *P*=0.001
282 and R=0.860, Fig. 5). This was due mainly to a reduction in the relative abundance of OTU
283 523 (Burkholderiales) and an increase in OTUs 245 (*Pseudomonas* spp.), 248 and 135 (Table
284 A5b).
285
286 *3.3.3 Preceding crop*
287 The only samples to show significant differences were again between the November
288 wheat rhizospheres, Ww and Wo (*P*=0.029, R=0.969) (Fig.6, Table A3). Using SIMPER
289 analysis, the OTU that contributed most towards these differences was 245 (*Pseudomonas* spp.)
290 (Table. A3c). The relative abundance of OTU 245 (*Pseudomonas* spp.) was significantly higher
291 in the rhizosphere of Ww (*P*=<0.001) and is shown for the different preceding crops in Fig.
292 A1.
293
294 *3.4 Nematode communities*
295 *3.4.1 Crop type and sample type*
296 Non-metric MDS with ANOSIM analysis showed significant differences between the
297 nematode communities of the crop types and sample types. However, in contrast to bacteria
298 and fungi, there was less similarity within the bulk soil samples compared with the rhizosphere
299 (Fig. 7). Overall, there was a significant difference between the rhizosphere of oilseed rape and
300 wheat (*P*=0.001, R=0.520, Table A3). The differences between the rhizosphere of oilseed rape

and wheat were most pronounced during the March sampling time (Fig. 8). Using SIMPER analysis, the OTUs that contributed most towards the differences in crop rhizosphere were 304 (*Pratylenchus neglectus*) and 302 which had a higher relative abundance in the oilseed rape rhizosphere, and 413 (*Chiloplacus propinquus)*, 145 (Plectidae family) and 143 *(Bitylenchus dubius)* which had a higher relative abundance in the wheat rhizosphere (Table A6a).

*3.4.2 Sampling time*

There were significant differences in the nematode communities between seasons in the rhizosphere and bulk soil of oilseed rape and wheat (Fig. 7, Table A3). Seasonal fluctuations were examined further using SIMPER analysis. Within the oilseed rape rhizosphere there was a mid-season peak in relative abundance of OTUs 304 (*Pratylenchus neglectus*), 302 and 298, and a mid-season decrease in OTU 413 (*Chiloplacus propinquus)* (Table A6b). OTU 611 decreased throughout the growing season (Table A6b). Within the wheat rhizosphere the seasonal trends of OTUs were quite different. There was a mid-season peak in relative abundance of OTUs 413 (*Chiloplacus propinquus)*, 145 (Plectidae family) and 143 *(Bitylenchus dubius)*, whereas the relative abundance of OTUs 304 (*Pratylenchus neglectus*) and 302 increased during the growing season (Table A6b).

*3.4.3 Preceding crop*

There were significant differences between the oilseed rape rhizospheres grown after different crops (Oo and Ow) in November, ($P$=0.029 R=0.667) (Fig. 8, Table A3). The OTUs that contributed most towards these differences using SIMPER analysis were 611 and 610 (*Eumonhystera* spp.) which had a higher relative abundance in Oo, and 302, 298 and 145 (Plectidae family) which had a higher relative abundance in Ow, Table A6c. The relative abundance of OTUs 610 and 611 (*Eumonhystera* spp.) were significantly higher in the

November rotation Oo ($P$=<0.001) and is shown in Fig. A2. The relative abundance of OTU 302 was significantly higher in Ow ($P$=0.007). There were also significant differences between the March Ww and Wo rhizospheres ($P$=0.029 R=0.656) (Fig.9, Table A3). Using SIMPER analysis, the OTUs that contributed most towards these differences were 413 (*Chiloplacus propinquus)* and 145 (Plectidae family) which had a higher relative abundance in Ww and 304 (*Pratylenchus neglectus*), 302 and 143 *(Bitylenchus dubius)* which had a higher relative abundance in Wo, Table A6c. Out of these OTUs there was a significant difference in relative abundance between Ww and Wo in 413 (*Chiloplacus propinquus)* ($P$=0.024) and 302 ($P$=0.046).

## 4. Discussion

This study has demonstrated that preceding crop can influence the rhizosphere and bulk soil bacterial, fungal and nematode communities of oilseed rape and wheat. Within the fungal community there was less similarity within the rhizosphere samples than the bulk soil and there were clear crop specific differences. In particular the high abundance of *Olpidium brassicae* in the rhizosphere of oilseed rape which has previously been found where oilseed rape has been grown more than once (Bennett et al., 2014; Hilton et al., 2013; Tkacz et al., 2015). The relative abundance of *Olpidium brassicae* was 13.5 % higher in oilseed rape grown after oilseed rape, although this was not a significant increase. Within the wheat rhizosphere there were high levels of *Mycosphaerella graminicola*, the fungus which causes septoria tritici (leaf) blotch of wheat. This was unexpected as it is a foliar disease of wheat that infects via the stomata (Orton et al., 2011). *M. graminicola* overwinters as mycelium, on wheat crop debris, autumn sown crops and volunteers (AHDB, 2016). There are no reports of mycelium infecting via wheat roots or inhabiting roots or the rhizosphere, but the domination of the wheat rhizosphere (which

includes the root in this study) with *M. graminicola* and the large seasonal shifts in its abundance suggests that the rhizosphere or root may be involved in the life-cycle of this fungus.

Within the wheat rhizosphere, *M. graminicola* was much more prevalent in the rhizosphere of wheat grown after oilseed rape (Wo) than wheat grown after wheat (Ww). This is counter-intuitive, as it is a pathogen of wheat and not of oilseed rape. A possible explanation is that there is a natural enrichment of antagonistic organisms in the wheat rhizosphere following wheat, which suppress *M. graminicola*, which may not have developed when oilseed rape was the previous crop.

Within the bacterial community there was again less similarity within the rhizosphere community than the bulk soil and there were crop specific differences in the rhizosphere community but not in the bulk soil. Preceding crop had a large and significant effect in November between the rhizosphere of Ww and Wo. Interestingly, these are the same samples and sampling time that showed significant differences in the fungal community. The OTU mostly responsible for the differences were *Pseudomonas* spp. which had a significantly higher relative abundance in wheat grown after wheat. *Pseudomonas* species are known plant growth-promoting rhizobacteria (PGPR) and are biocontrol agents of several recognised root fungal pathogens including *Gaeumannomyces graminis* var. *tritici* (take-all of wheat), *Fusarium oxysporum* (wilt diseases); *Pythium* spp., *Rhizoctonia solani* (damping-off of seedlings) and *M. graminicola* (Flaishman et al., 1996; Levy et al., 1992; Raaijmakers et al., 2002). There was a negative correlation between the OTUs for *Pseudomonas* spp. and *M. graminicola* in the November samples ($r=-0.747$ $p=0.033$). It is possible that a higher relative abundance of *Pseudomonas* spp. in the rhizosphere of Ww could have suppressed fungi including *M. graminicola,* which had reduced levels in Ww compared with Wo, thus contributing to the differing fungal communities between Ww and Wo. This is analogous to the mechanism behind take-all decline (Kwak and Weller, 2013). There were also large increases in two unidentified

375 OTUs in Ww compared with Wo. Their identification could not be resolved, which is one of
376 the drawbacks of TRFLP. Next generation sequencing technologies will help resolve sequence
377 identification and provide depth not achievable with TRFLP analyses.

378 Nematodes are a key component of the soil food web, occupying a range of trophic
379 levels and forming links between plants, bacteria, fungi and other soil fauna. However,
380 responses of nematodes at a community level to preceding crops are poorly understood. Within
381 the nematode community there was less similarity in bulk soil communities than rhizosphere
382 communities. This was in contrast to fungi and bacteria. The reason for this may lie in the
383 sampling strategy. Nematodes are much less abundant in bulk soil numerically than fungi or
384 bacteria, but each nematode is likely to contain much more DNA. Therefore, a small sample
385 may not be representative of the whole community. This is why bulk soil sampling for
386 nematodes generally involves large soil samples and a subsequent extraction procedure before
387 DNA extraction to ensure a DNA sample which is representative of the community (Foucher
388 and Wilson, 2002). However, the sampling strategy for the rhizosphere samples, particularly
389 in November when the plants were small, involved using nearly all the root material for
390 sampling, and so we felt was an appropriate sampling strategy for the rhizosphere, particularly
391 as the nematodes would be concentrated in the rhizosphere compared with the bulk soil.
392 Compared with wheat, the oilseed rape rhizosphere had an increase in relative abundance of
393 *Pratylenchus neglectus* which is the root lesion nematode, a plant-pathogenic nematode with a
394 broad host range (Oldach et al., 2014). *Pratylenchus* spp. are migratory endoparasitic
395 nematodes that feed and migrate within root cortical tissue causing necrosis and reduced lateral
396 branching of roots upon infection (Vanstone et al., 1998). *P. neglectus* peaked in abundance at
397 different times in the growing season for wheat and oilseed rape. The wheat rhizosphere had a
398 higher relative abundance of another plant-pathogenic nematode, *Bitylenchus dubius* otherwise
399 known as stunt nematodes, which are root surface tissue feeders (Siddiqi, 2000). However,

neither of these plant pathogenic nematodes contributed significantly to differences in the community after different preceding crops so are unlikely to contribute to the yield decline observed. However, nematodes were the only taxa found to be influenced by preceding crop in the oilseed rape rhizosphere community. This was in the November samples and was due predominantly to an increase in relative abundance of *Eumonhystera* spp. and a decrease in an unidentified OTU (302). These OTUs are interesting potential contributors to oilseed rape yield decline which warrants further exploration.

Our results demonstrated that season had a strong effect on the community composition of the bulk soil of all three taxa. There was generally a stronger effect on the rhizosphere community which is likely due to the developmental stage of the plant which has been shown to influence community structure (Chaparro et al., 2014; Philippot et al., 2013). This may be due to the changes in root exudation patterns which have been found to be strongly affected by the plant developmental stage (Chaparro et al., 2013; Micallef et al., 2009). The largest community shifts occurred in the June samples, which could be due to the onset of senescence in the plants.

Overall the major drivers of community composition were crop type, soil type (rhizosphere or bulk soil) and sampling time. Preceding crop was found to have a strong effect on the composition within particular taxa at certain growth stages/seasonal times. This highlights the importance of investigating community composition throughout the growing season, as these changes and presence of potential plant pathogens could otherwise be missed. Further classification and isolation of organisms identified that differed with preceding crop, would be the next step in understanding the effect of preceding crop on rhizosphere community and yield decline. However, we have identified changes in potential pathogens and antagonists which could contribute to plant health in wheat-oilseed rape rotations, and highlight the need

for these rotations to be carefully managed to optimise the yield of these globally important crops.

**Acknowledgements**

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## Figures and tables

Figure 1. Yield data from plots of oilseed rape (O) and wheat (W), with different preceding crops, taken from the fifth year of the field trial. Grain yield is corrected for moisture. Error bars are ± standard errors of the mean. Ow= Oilseed rape grown after wheat; Oo = Oilseed rape grown after oilseed rape; Ww = Wheat grown after wheat; Wo = Wheat grown after oilseed rape.

Figure 2. Non-metric multi-dimensional scaling plots represent rhizosphere (solid) and bulk soil (open) fungal DNA profiles, obtained from oilseed rape (a) and wheat (b) at different sampling times.

571 Figure 3. Non-metric multi-dimensional scaling plots represent rhizosphere (solid) and bulk
572 soil (open) fungal DNA profiles for rape (black) and wheat (grey) obtained from different
573 rotations shown at three sampling times, November, March, and June.
574
575 Figure 4. (a) Relative abundance of OTU 143 (*Mycosphaerella graminicola*) in the rhizosphere
576 of different rotations of OSR (O) and wheat (W) (see Table A1 for rotation explanation). (b)
577 Absolute quantification using specific quantitative PCR primers to *Mycosphaerella*
578 *graminicola* in different rotations of OSR and wheat (see Table A1 for rotation explanation).
579 Error bars are standard errors of the mean for the four replicate plots.
580
581 Figure 5. Non-metric multi-dimensional scaling plots represent rhizosphere (solid) and bulk
582 soil (open) bacterial DNA profiles, obtained from oilseed rape (a) and wheat (b) at different
583 sampling times.
584
585 Figure 6. Non-metric multi-dimensional scaling plots represent rhizosphere (solid) and bulk
586 soil (open) bacterial DNA profiles for rape (black) and wheat (grey) obtained from different
587 rotations shown at three sampling times, November, March, and June.
588
589 Figure 7. Non-metric multi-dimensional scaling plots represent rhizosphere (solid) and bulk
590 soil (open) nematode DNA profiles, obtained from oilseed rape (a) and wheat (b) at different
591 sampling times.
592
593 Figure 8. Non-metric multi-dimensional scaling plots represent rhizosphere (solid) and bulk
594 soil (open) nematode DNA profiles for rape (black) and wheat (grey) obtained from different
595 rotations shown at three sampling times, November, March, and June.

Figure A1. Relative abundance of OTU 245 (*Pseudomonas* spp.) in the rhizosphere of different rotations of oilseed rape (O) and Wheat (W) in the November samples. Error bars are ± standard errors of the mean for the four replicate plots. Bars with different letters denote significant differences (ANOVA, $p < 0.05$).

Figure A2. Relative abundance of OTUs 610 and 611 (*Eumonhystera* spp.) in the rhizosphere of different rotations of oilseed rape (O) and Wheat (W) in the November samples. Error bars are ± standard errors of the mean for the four replicate plots. Bars with different letters denote significant differences (ANOVA, $p < 0.05$).

Table A1. Cropping history of rotations sampled. O = oilseed rape; W = wheat. Rhizosphere and bulk soil samples were collected in the 5$^{th}$ year of the trial (O = oilseed rape, W = wheat).

Table A2. Identification of OTUs using the oilseed rape (Oo) or Wheat (Ww) rhizosphere clone libraries. NCBI BLAST was used to assign fungi (a) and nematodes (b) and the Ribosomal Database Project (RDP) (at 80 % confidence) for bacteria (c). Peak sizes and equivalent restriction enzyme sites in the clones are shown. The accession number of the closest match of the consensus of the clones is shown for the fungal and nematode clones. * = An overlapping restriction site occurs resulting in a double peak.

Table A3. Results from Analysis of Similarities (ANOSIM) between communities (Bray-Curtis dissimilarity). The effects of treatments (crop, season/sampling time, preceding crop and soil type) on the microbial communities of the rhizosphere and bulk soil for fungi, bacteria and

nematodes. R values close to zero indicate most similarity. Values in bold highlight significant differences ($P$=<0.05).

Table A4. Similarity Percentage Analysis (SIMPER) analysis identifying the top five fungi which contribute (Contrib. %) towards dissimilarity (Av. dissim) in community compositions of (a) oilseed rape and wheat rhizosphere (b) seasons and (c) preceding crop.

Table A5. Similarity Percentage Analysis (SIMPER) analysis identifying the top five bacteria which contribute (Contrib. %) towards dissimilarity (Av. dissim) in community compositions of (a) oilseed rape and wheat rhizosphere (b) seasons and (c) preceding crop.

Table A6. Similarity Percentage Analysis (SIMPER) analysis identifying the top five nematodes which contribute (Contrib. %) towards dissimilarity (Av. dissim) in community compositions of (a) oilseed rape and wheat rhizosphere (b) seasons and (c) preceding crop.

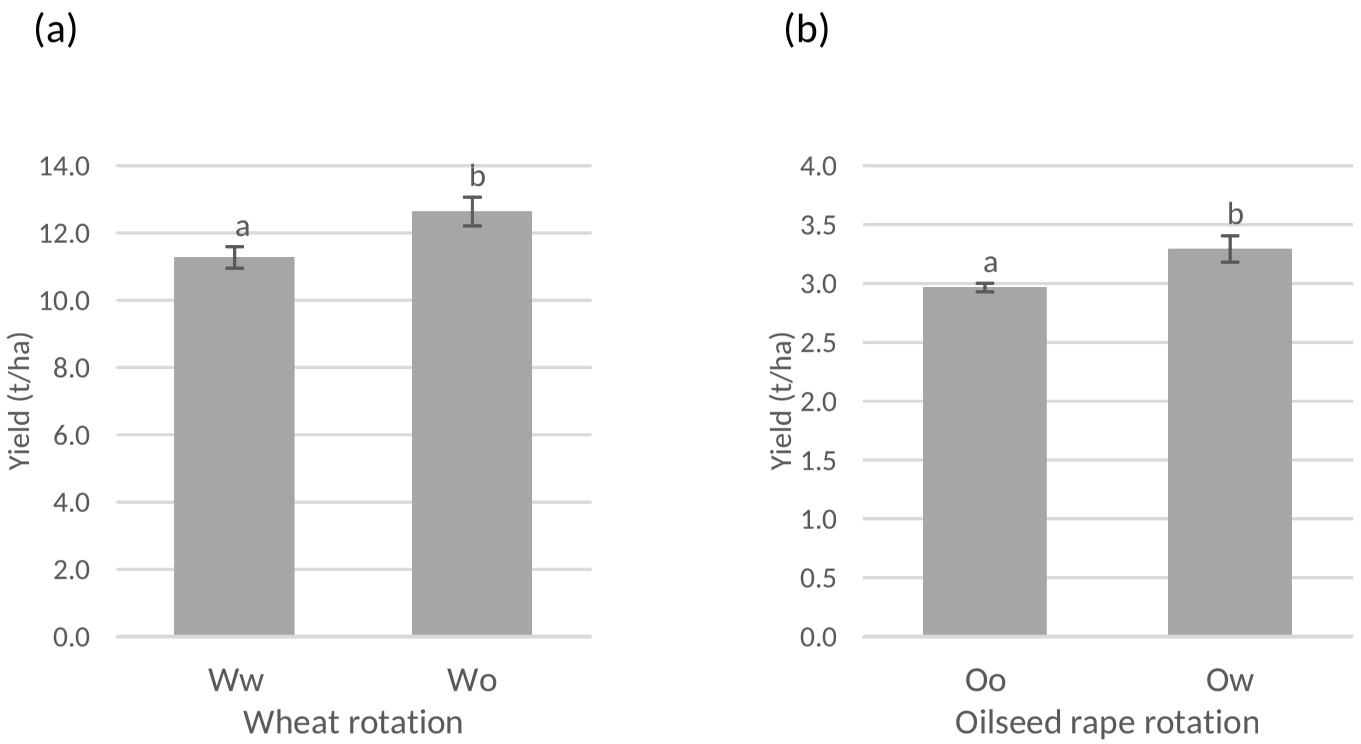

(a)
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Yield (t/ha)
a
b
Ww
Wo
Wheat rotation
(b)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Yield (t/ha)
a
b
Oo
Ow
Oilseed rape rotation

Fig. 2.

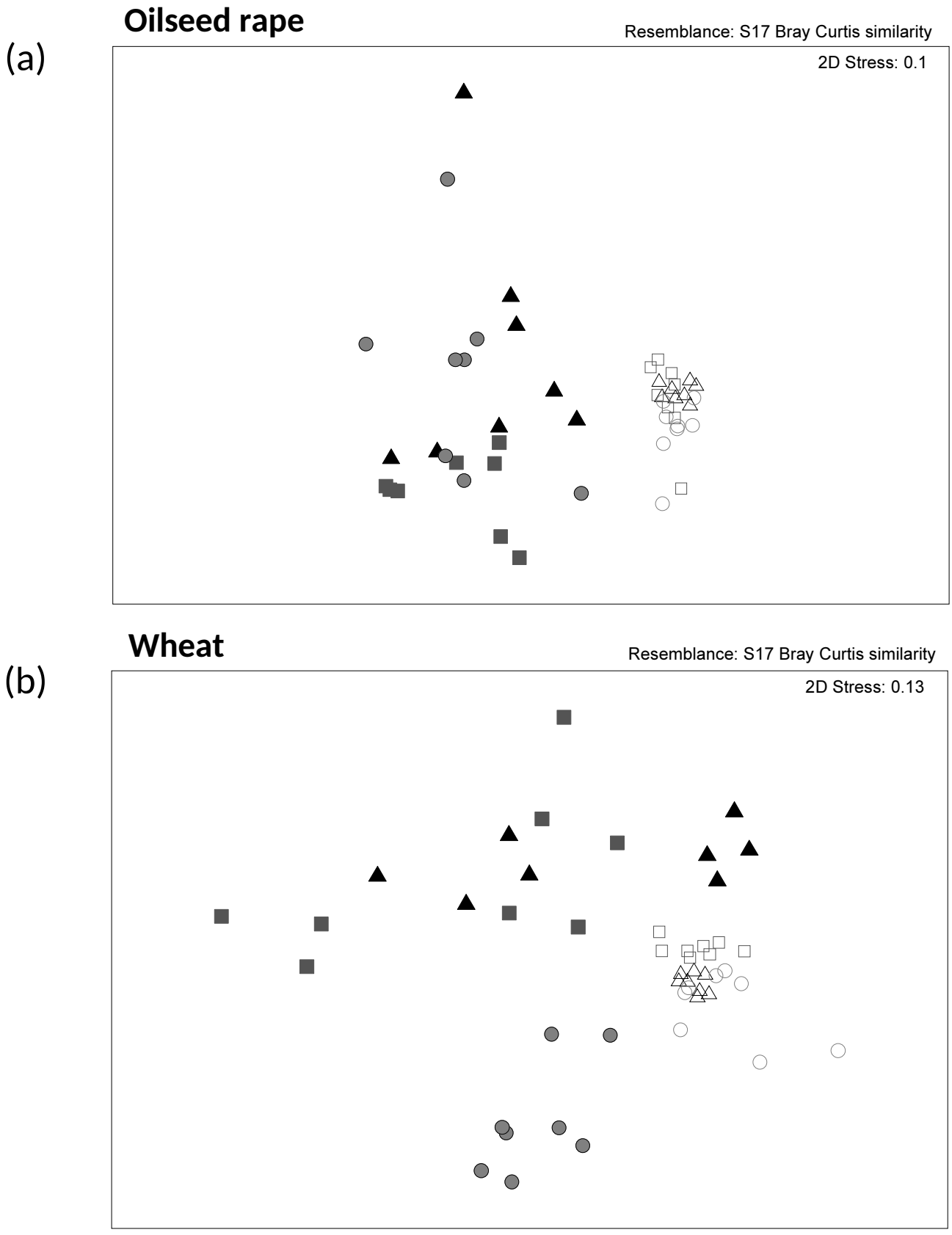

(a)
Oilseed rape
Resemblance: S17 Bray Curtis similarity
2D Stress: 0.1
(b)
Wheat
Resemblance: S17 Bray Curtis similarity
2D Stress: 0.13
Rhizosphere
November
March
June
Bulk soil
November
March
June

Fig. 3.

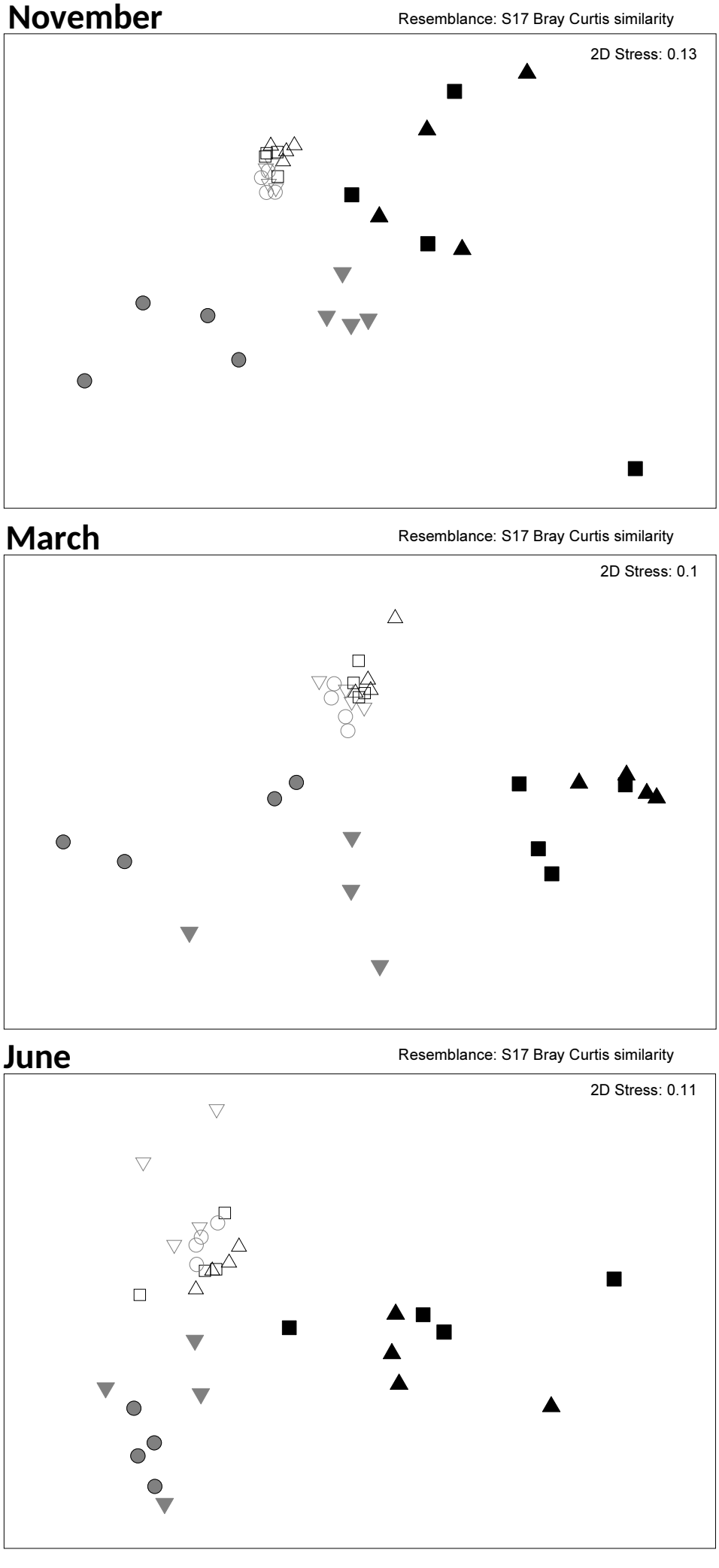

November
Resemblance: S17 Bray Curtis similarity
2D Stress: 0.13
March
Resemblance: S17 Bray Curtis similarity
2D Stress: 0.1
June
Resemblance: S17 Bray Curtis similarity
2D Stress: 0.11
Rhizosphere
Rape after rape (Oo)
Wheat after wheat (Ww)
Rape after wheat (Ow)
Wheat after rape (Wo)
Bulk soil
Rape after rape (Oo)
Wheat after wheat (Ww)
Rape after wheat (Ow)
Wheat after rape (Wo)

Fig. 4.

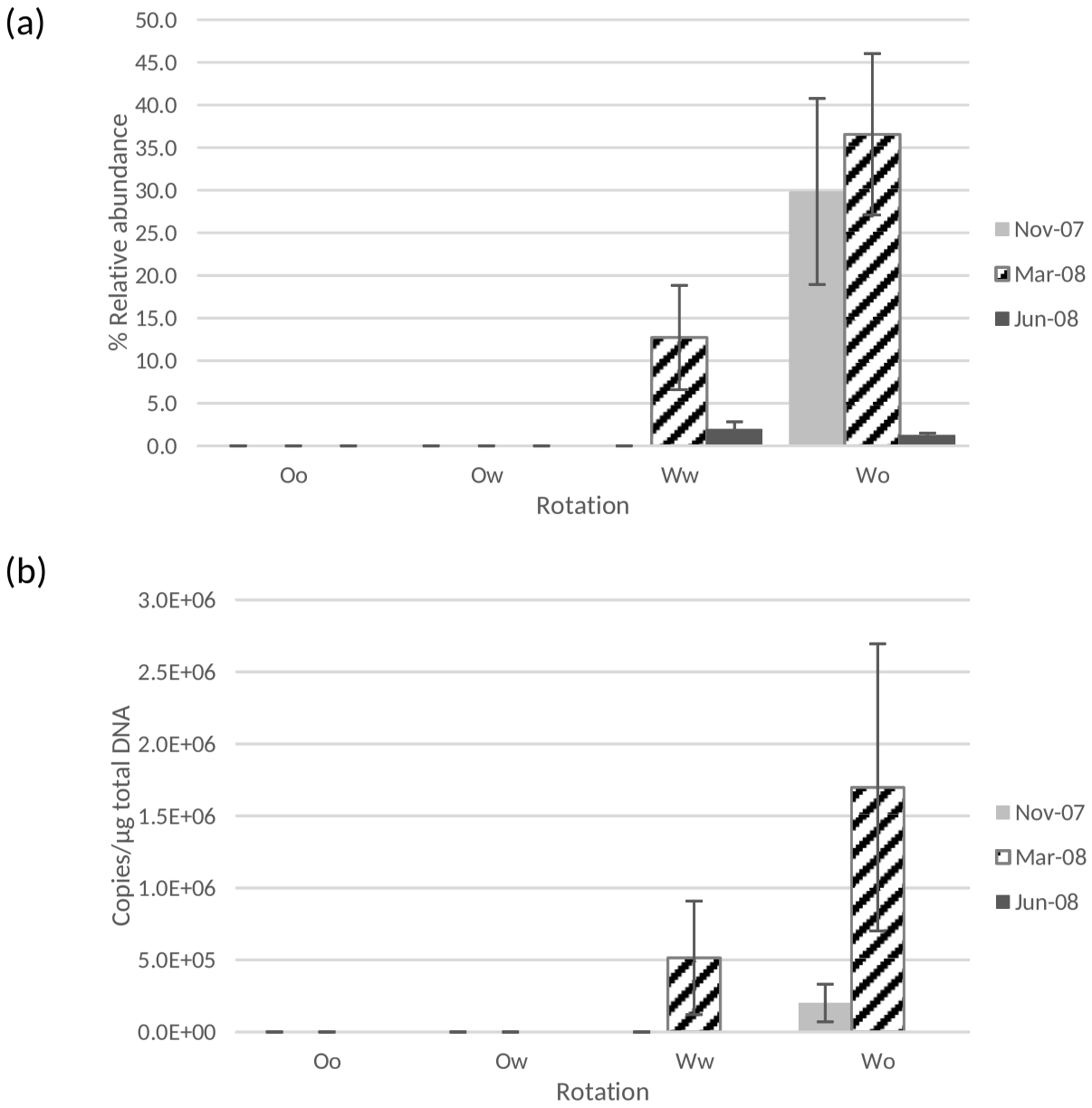
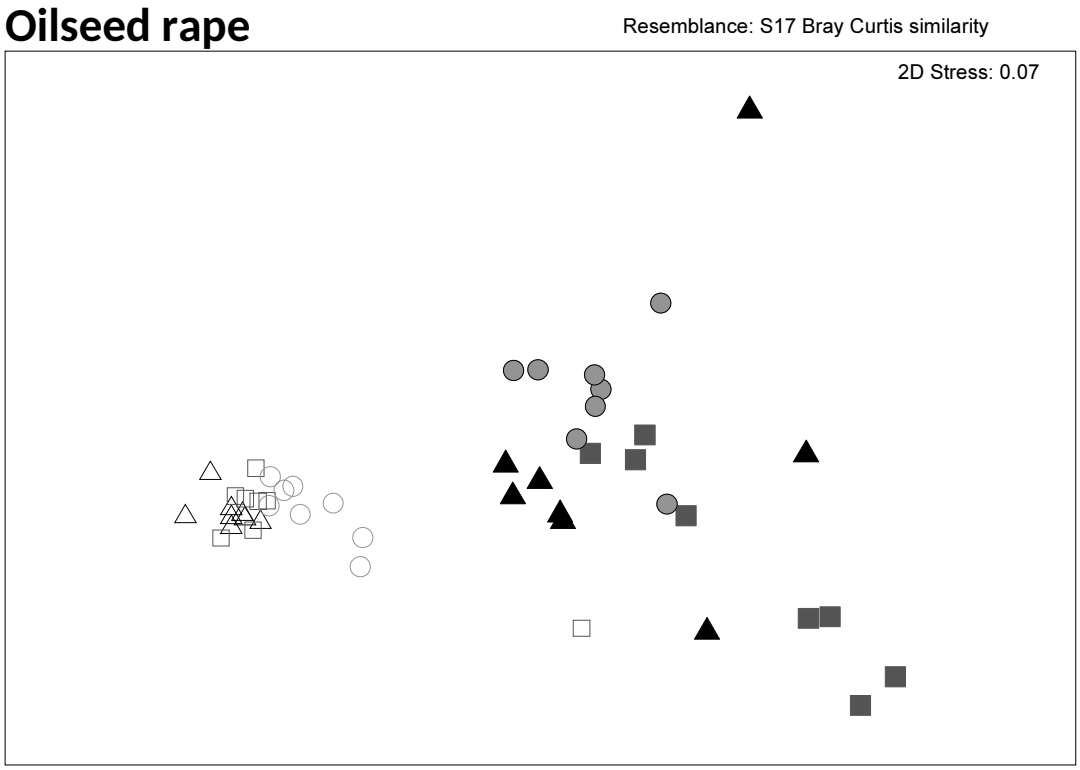
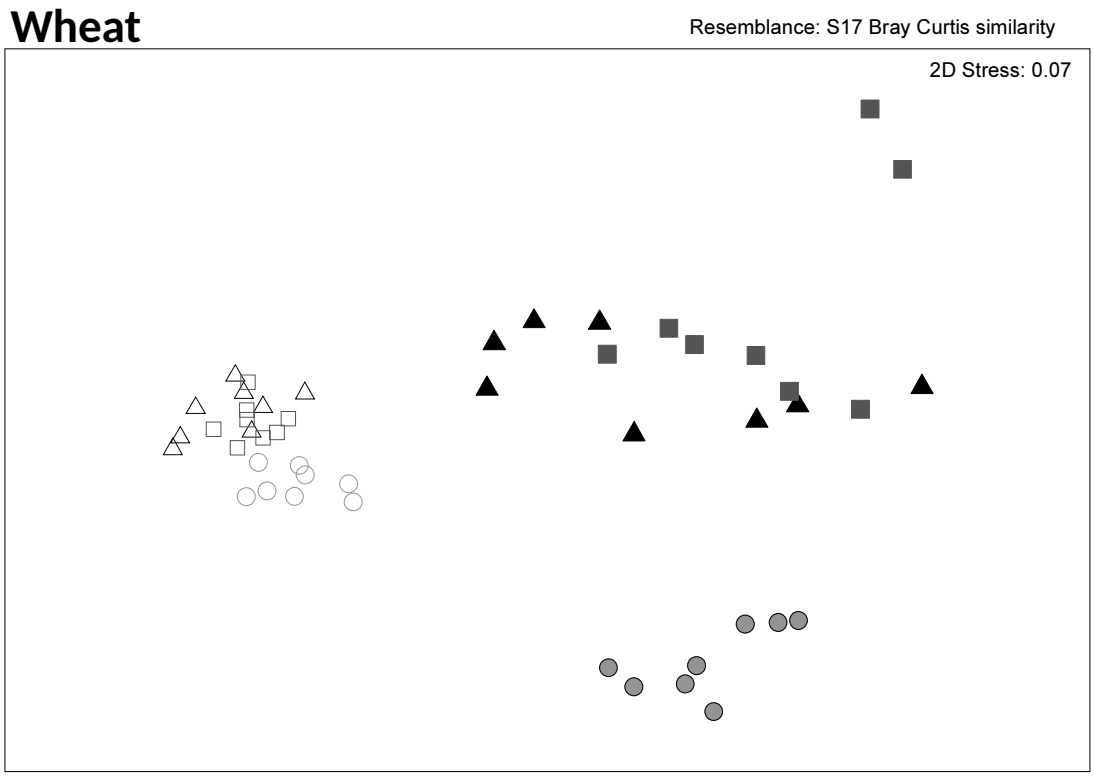

(a)
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
% Relative abundance
Oo
Ow
Ww
Wo
Rotation
Nov-07
Mar-08
Jun-08
(b)
3.0E+06
2.5E+06
2.0E+06
1.5E+06
1.0E+06
5.0E+05
0.0E+00
Copies/µg total DNA
Oo
Ow
Ww
Wo
Rotation
Nov-07
Mar-08
Jun-08

Fig. 5.

(a)




(b)




**Rhizosphere**

▲ November

■ March

● June

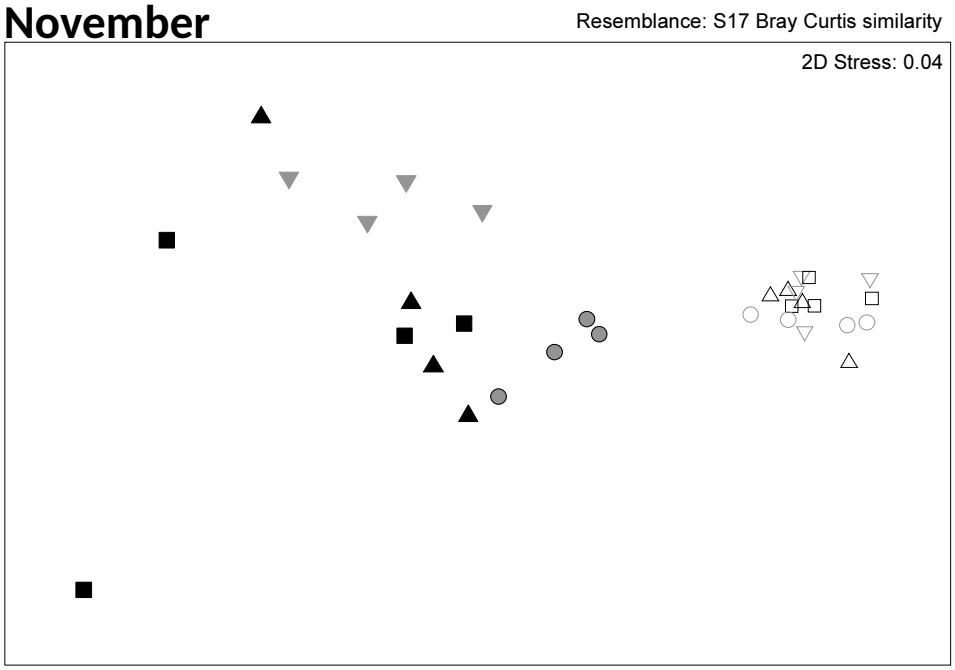
**Bulk soil**

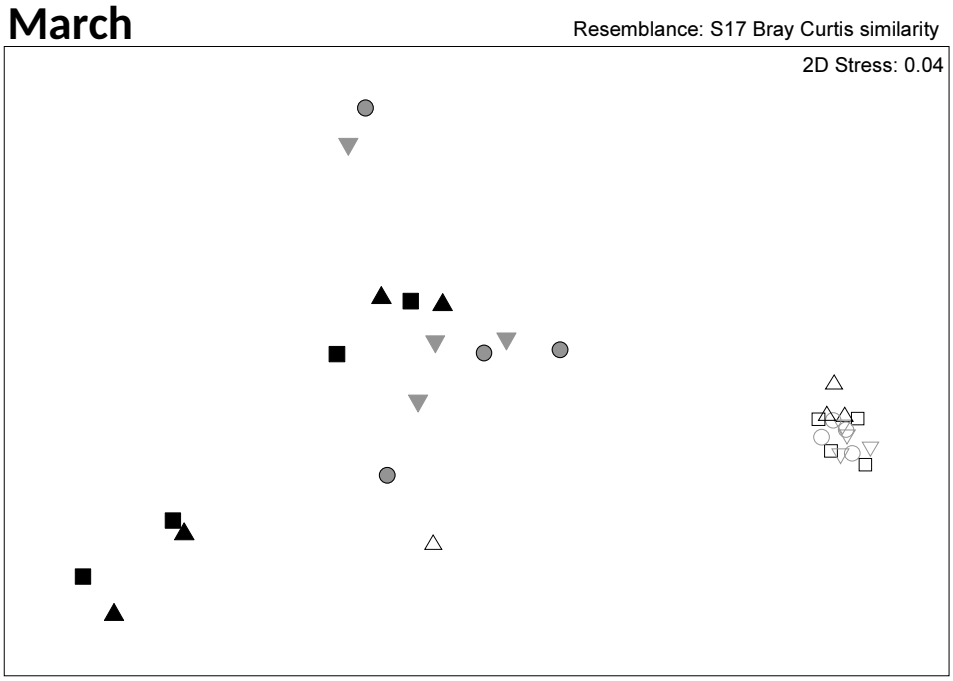
△ November

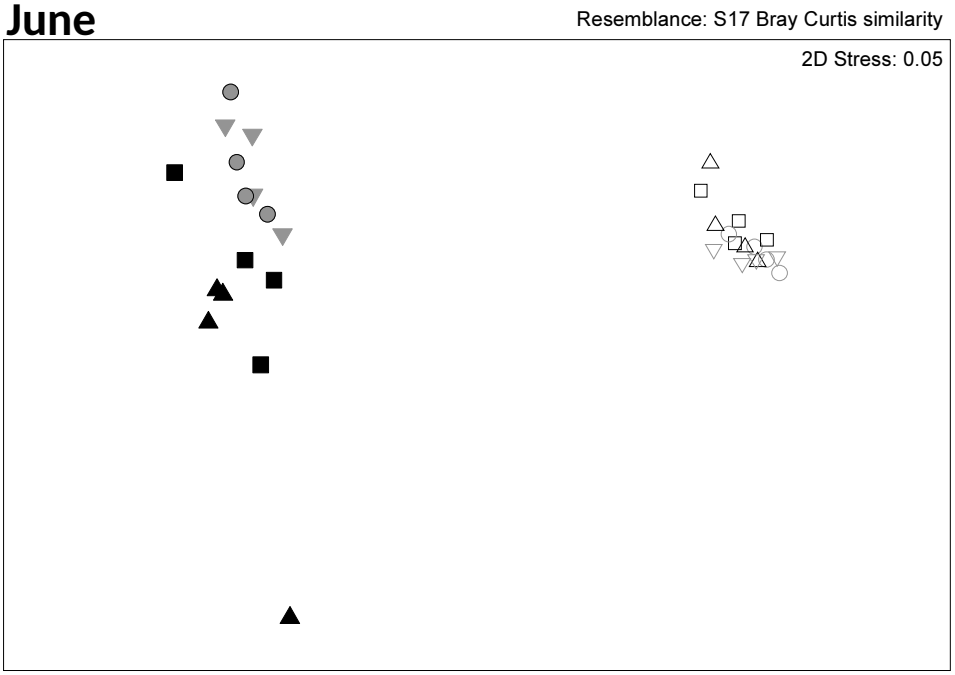
□ March

○ June

Fig. 6.




**Rhizosphere**

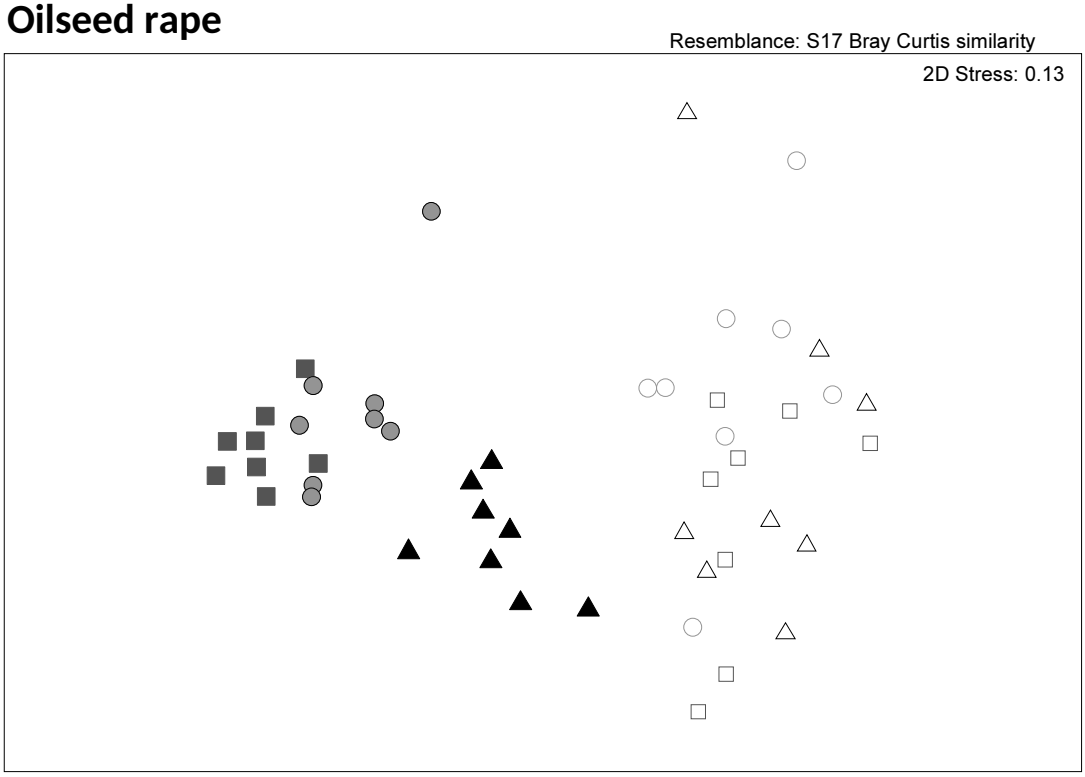
- ▲ Rape after rape (Oo)
- ▼ Wheat after wheat (Ww)
- ■ Rape after wheat (Ow)
- ● Wheat after rape (Wo)

**Bulk soil**

- △ Rape after rape (Oo)
- ▽ Wheat after wheat (Ww)
- □ Rape after wheat (Ow)
- ○ Wheat after rape (Wo)

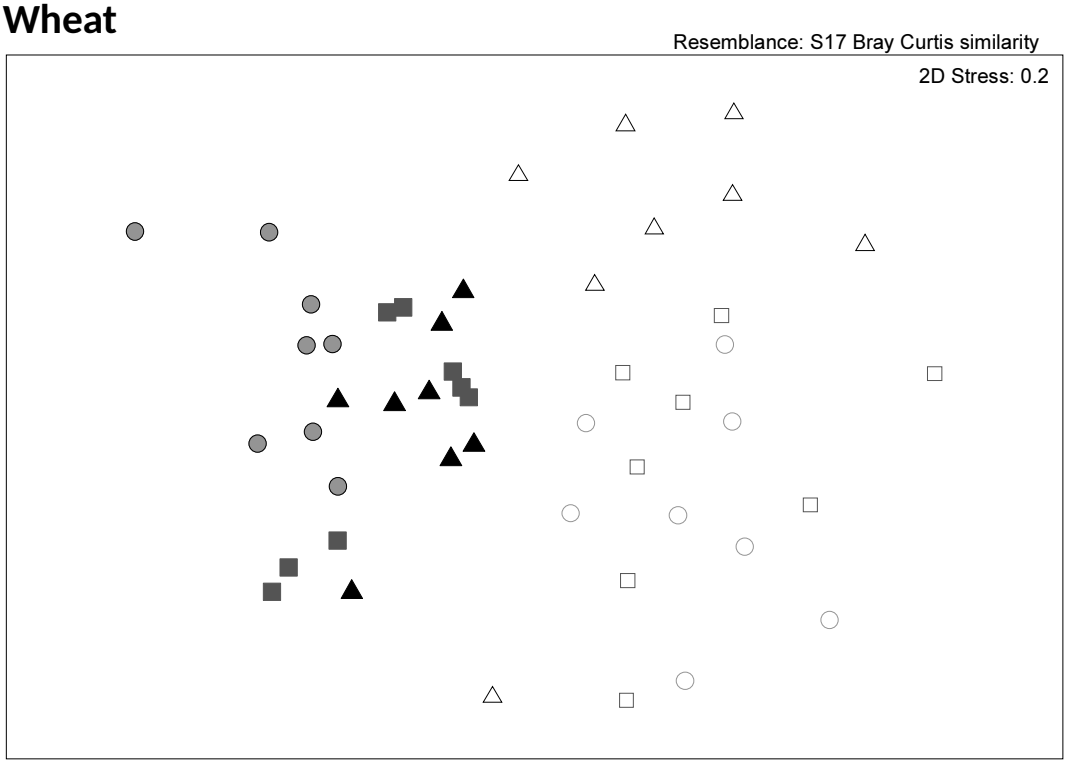
(a)

**Oilseed rape**




(b)

**Wheat**




**Rhizosphere**

▲ November

■ March

● June

**Bulk soil**

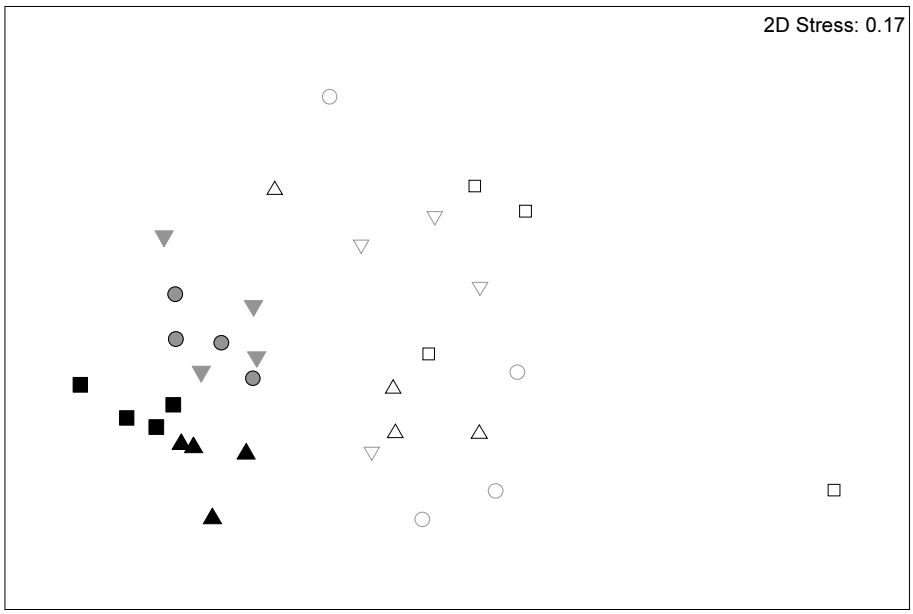
△ November

□ March

○ June

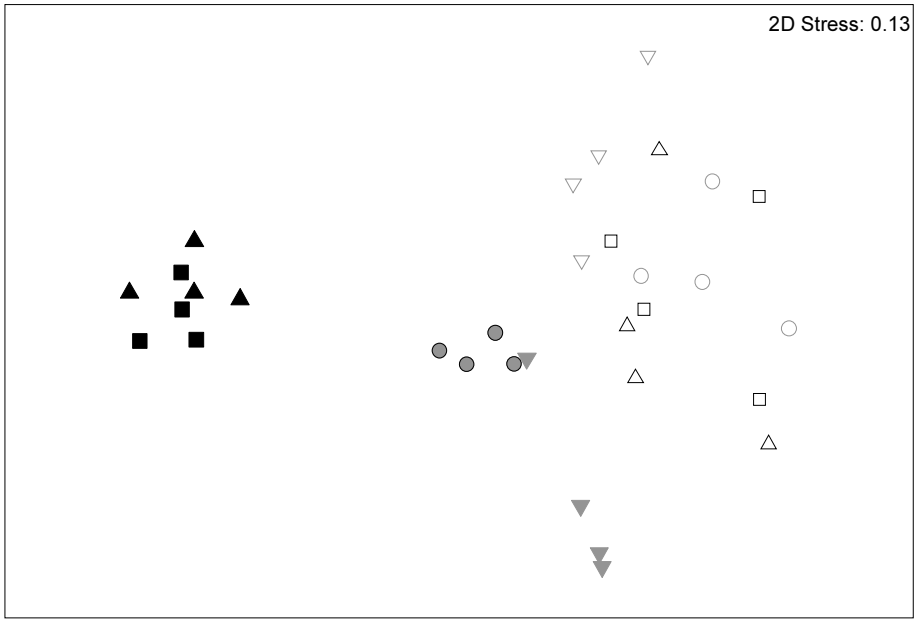
## November

Resemblance: S17 Bray Curtis similarity



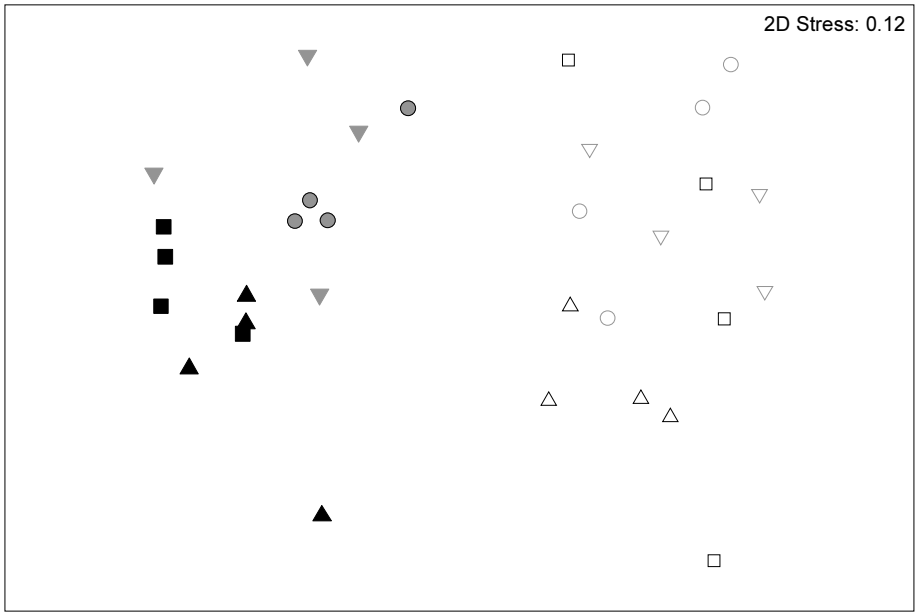

## March

Resemblance: S17 Bray Curtis similarity




## June

Resemblance: S17 Bray Curtis similarity

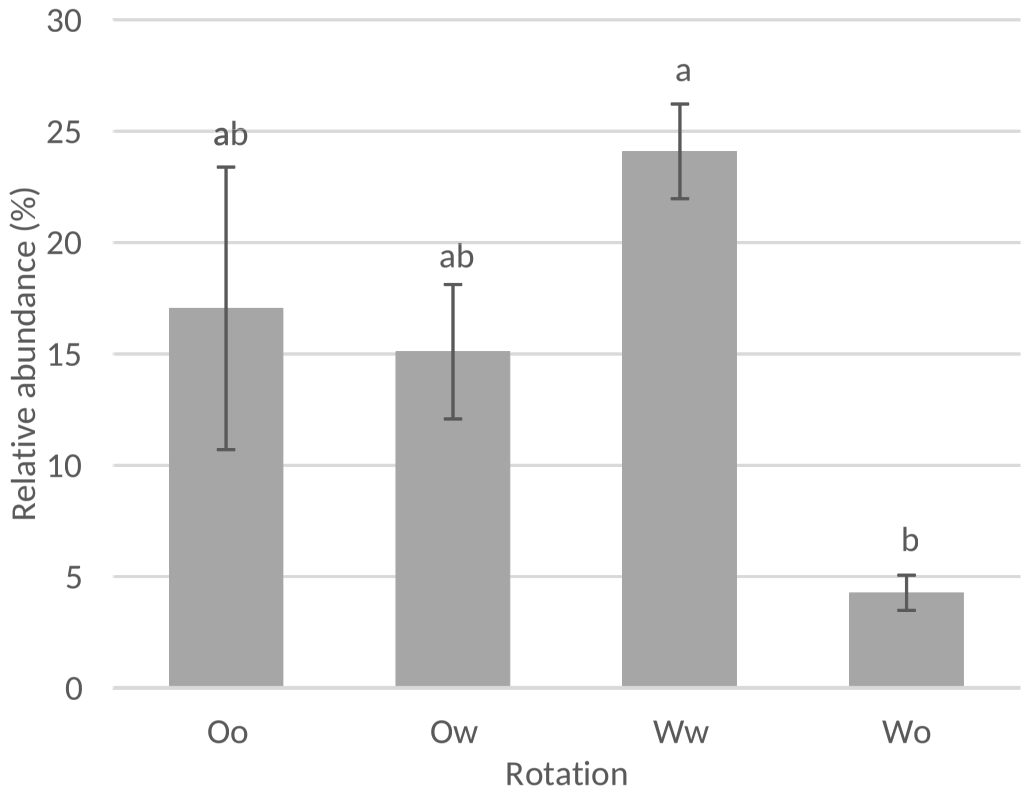



### Rhizosphere

- ▲ Rape after rape (Oo)
- ▼ Wheat after wheat (Ww)
- ■ Rape after wheat (Ow)
- ● Wheat after rape (Wo)

### Bulk soil

- △ Rape after rape (Oo)
- ▽ Wheat after wheat (Ww)
- □ Rape after wheat (Ow)
- ○ Wheat after rape (Wo)


30
25
20
15
10
5
0
Relative abundance (%)
ab
ab
a
b
Oo
Ow
Ww
Wo
Rotation

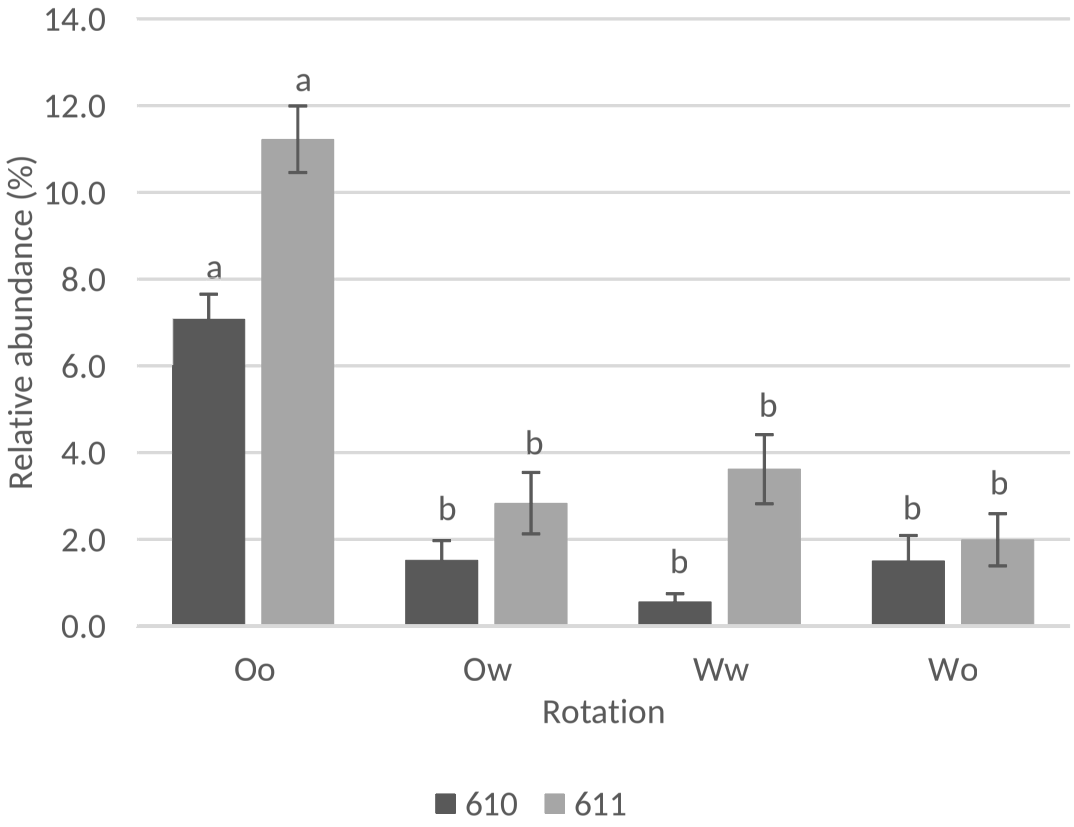

14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Relative abundance (%)
a
a
b
b
b
b
b
b
Oo
Ow
Ww
Wo
Rotation
610
611

Table. 1

| | Year of trial | | | | |
|---|---|---|---|---|---|
| **Rotation** | **1** | **2** | **3** | **4** | **5** |
| Oilseed rape after oilseed rape (Oo) | O | O | O | O | O |
| Wheat after wheat (Ww) | O | W | W | W | W |
| Oilseed rape after wheat (Ow) | O | W | O | W | O |
| Wheat after oilseed rape (Wo) | W | W | W | O | W |

| A | % of Oo and Ww clone libraries | Accession numbers | *Hha*I peak (bp) | *Hha*I site (bp) | *Msp*I peak (bp) | *Msp*I site (bp) | Top NCBI Blast hit / Accession no. | ID/ range (bp) |
|---|---|---|---|---|---|---|---|---|
| | 11.9 Oo; 4.4 Ww | JF432964; JF432970; JF432976; MF344903; MF344907; MF344908; MF344909 | 124/125* | 125/127* | 130 | 132 | *Gibellulopsis nigrescens* / AM922222 | 99 %/ 505 |
| | 9.5 Oo; 0 Ww | JF432979; JF432982; JF432988; JF432998; JF433001; JF433008 | 284 | 286 | 290 | 291 | *Olpidium brassicae* / AB205209 | 99-100 %/ 600 |
| | 7.1 Oo; 1.1 Ww | JF432972; JF432989; JF433000; JF433002; JF433021; MF344906 | 123 | 124 | 135 | 141 | *Plectosphaerella cucumerina* / L36640 | 98-100 %/ 573 |
| | 7.1 Oo; 0 Ww | JF432973; JF433004 | 325 | 330 | 480 | 490 | *Trichothecium* sp. / EU754905 | 99 %/ 546 |
| | 2.4 Oo; 0 Ww | JF432995 | 98 | 99 | 83 | 84 | *Pyrenochaeta* sp. / AM921726 | 100 %/ 506 |
| | 2.4 Oo; 0 Ww | JF432975; JF432987 | 341 | 344 | - | - | *Tetracladium furcatum* / EU883432 | 100%/ 592 |
| | 2.4 Oo; 2.2 Ww | JF432993; JF433006; MF344910; MF344911 | 299 | 301 | 312 | 314 | *Trichosporon* sp. / FJ439589 | 100 %/ 528 |
| | 0 Oo; 2.2 Ww | MF344904; MF344905 | 143 | 146 | 81 | 83 | *Mycosphaerella graminicola* / AF181692 | 100%/545 |

| B | % of Oo clone library | Accession numbers | *Hae*I peak (bp) | *Hae*I site (bp) | *Aci*I peak (bp) | *Aci*I site (bp) | Top NCBI Blast hit / Accession no. | ID/ range (bp) |
|---|---|---|---|---|---|---|---|---|
| | 26.7 Oo | MF348000-MF348008; MF344926; MF344929; MF344931; MF344932; MF344934; MF344935; MF344938; MF344941; MF344943; MF344944; MF344945; MF344950 | 304 | 307 | 56 | 62 | *Pratylenchus neglectus* JQ303332 | 99%/887 |
| | 17.8 Oo | MF344915; MF344918; MF344922; MF344927; MF344930; MF344933; MF344936; MF344937; MF344940 | 413 | 416 | 136 | 141 | *Chiloplacus propinquus* KY119877 | 99%/887 |
| | 20.0 Oo | MF344951; MF344913; MF344914; MF344917; MF344920; MF344921; MF344923; MF344924; MF344925; MF344946; MF344947; MF344949 | 145 | 149 | 399 | 402 | Plectidae (*Ceratoplectus cf. armatus*) FJ474096 | 99%/889 |
| | 5.5 Oo | MF344912; MF344916; MF344919; MF344928; MF344942 | 143 | 148 | 97 | 102 | *Bitylenchus dubius* AY284601 | 99%/880 |
| | 2.1 Oo | MF344939; MF344948 | 610/611 | 615 | 477/479 | 483 | *Eumonhystera filiformis* KJ636238 | 98%/883 |

| C | % of Oo and Ww clone libraries | Accession numbers | *Hha*I peak (bp) | *Hha*I site (bp) | *Msp*I peak (bp) | *Msp*I site (bp) | RDP classification |
|---|---|---|---|---|---|---|---|
| | 4.4 Oo; 3.3 Ww | JF432908; JF432913; JF432920; JF432922; MF314107; MF314111 | 245 | 250 | 486 | 491 | *Pseudomonas* spp. |
| | 3.3 Oo; 5.5 Ww | JF432898; JF432926; JF432947; MF314108; MF314109; MF314112 | 523 | 528 | 487 | 492 | Burkholderiales |
| | 2.2 Oo; 0 Ww | JF432903; JF432931 | 338 | 344 | 300 | 306 | Acidobacteria Gp 6 |
| | 1.4 Oo; 2.2 Ww | JF432934; MF314110 | 721 | 727 | 300 | 306 | Acidobacteria Gp 6 |

| Community | Soil type | Sampling time | Treatments compared | P | R |
|---|---|---|---|---|---|
| Fungi | All | All | OSR rhizosphere and OSR bulk soil | **0.001** | **0.770** |
| | | | Wheat rhizosphere and wheat bulk soil | **0.001** | **0.462** |
| | Rhizosphere | All | OSR and wheat | **0.002** | **0.877** |
| | | | Season (OSR) | **0.003** | **0.245** |
| | | | Season (Wheat) | **0.001** | **0.541** |
| | | November 2007 | O(o) and O(w) | 1.000 | -0.229 |
| | | | W(w) and W(o) | **0.029** | **0.969** |
| | | March 2008 | O(o) and O(w) | 0.257 | 0.083 |
| | | | W(w) and W(o) | 0.143 | 0.292 |
| | | June 2008 | O(o) and O(w) | 0.629 | -0.063 |
| | | | W(w) and W(o) | 0.257 | 0.083 |
| | Bulk soil | All | OSR and wheat | **0.002** | **0.347** |
| | | | Season (OSR) | **0.001** | **0.470** |
| | | | Season (Wheat) | **0.001** | **0.393** |
| | | November 2007 | O(o) and O(w) | 0.057 | 0.448 |
| | | | W(w) and W(o) | 0.086 | 0.469 |
| | | March 2008 | O(o) and O(w) | 0.229 | 0.073 |
| | | | W(w) and W(o) | 0.886 | -0.094 |
| | | June 2008 | O(o) and O(w) | 0.343 | 0.063 |
| | | | W(w) and W(o) | 0.143 | 0.146 |
| Bacteria | All | All | OSR rhizosphere and OSR bulk soil | **0.001** | **0.812** |
| | | | Wheat rhizosphere and wheat bulk soil | **0.001** | **0.871** |
| | Rhizosphere | All | OSR and wheat | **0.038** | **0.073** |
| | | | Season (OSR) | **0.001** | **0.383** |
| | | | Season (Wheat) | **0.001** | **0.667** |
| | | November 2007 | O(o) and O(w) | 0.829 | -0.073 |
| | | | W(w) and W(o) | **0.029** | **0.969** |
| | | March 2008 | O(o) and O(w) | 0.629 | -0.104 |
| | | | W(w) and W(o) | 0.714 | -0.125 |
| | | June 2008 | O(o) and O(w) | 0.686 | -0.052 |
| | | | W(w) and W(o) | 0.686 | -0.115 |
| | Bulk soil | All | OSR and wheat | 0.959 | -0.051 |
| | | | Season (OSR) | **0.001** | **0.464** |
| | | | Season (Wheat) | **0.001** | **0.476** |
| | | November 2007 | O(o) and O(w) | 0.771 | -0.063 |
| | | | W(w) and W(o) | 0.829 | -0.167 |
| | | March 2008 | O(o) and O(w) | 0.286 | 0.052 |
| | | | W(w) and W(o) | 0.457 | 0.000 |
| | | June 2008 | O(o) and O(w) | 0.943 | -0.250 |
| | | | W(w) and W(o) | 0.857 | -0.188 |
| Nematodes | All | All | OSR rhizosphere and OSR bulk soil | **0.001** | **0.829** |
| | | | Wheat rhizosphere and wheat bulk soil | **0.001** | **0.606** |
| | Rhizosphere | All | OSR and wheat | **0.001** | **0.520** |
| | | | Season (OSR) | **0.001** | **0.792** |
| | | | Season (Wheat) | **0.001** | **0.611** |
| | | November 2007 | O(o) and O(w) | **0.029** | **0.667** |
| | | | W(w) and W(o) | 0.571 | -0.042 |
| | | March 2008 | O(o) and O(w) | 0.257 | 0.135 |
| | | | W(w) and W(o) | **0.029** | **0.656** |
| | | June 2008 | O(o) and O(w) | 0.286 | 0.094 |
| | | | W(w) and W(o) | 0.886 | -0.188 |
| | Bulk soil | All | OSR and wheat | **0.034** | **0.050** |
| | | | Season (OSR) | **0.001** | **0.317** |
| | | | Season (Wheat) | **0.001** | **0.557** |
| | | November 2007 | O(o) and O(w) | 0.086 | 0.292 |
| | | | W(w) and W(o) | 0.229 | 0.146 |
| | | March 2008 | O(o) and O(w) | 0.657 | -0.073 |
| | | | W(w) and W(o) | 0.200 | 0.104 |
| | | June 2008 | O(o) and O(w) | 0.086 | 0.135 |
| | | | W(w) and W(o) | 0.571 | -0.010 |

(a) Crop

| **Rhizosphere** | | | | **Average relative abundance** | |
|---|---|---|---|---|---|
| ID | Taxon | Av. dissim | Contrib. % | Oilseed rape | Wheat |
| *Olpidium brassicae* | 284 | 14.8 | 20.8 | 29.7 | 0.2 |
| *Trichosporon* sp. | 299 | 6.3 | 8.8 | 13.0 | 1.0 |
| *Mycosphaerella graminicola* | 143 | 2.3 | 3.2 | 0.3 | 15.7 |
| Unidentified | 337 | 2.2 | 3.1 | 3.2 | 5.4 |
| *Tetracladium* sp. | 341 | 1.9 | 2.7 | 3.2 | 3.8 |

(b) Season

| **Oilseed rape rhizosphere** | | | | **Average relative abundance** | | |
|---|---|---|---|---|---|---|
| ID | Taxon | Av. dissim | Contrib. % | November | March | June |
| *Olpidium brassicae* | 284 | 11.6 | 22.4 | 25.6 | 38.7 | 24.7 |
| *Trichosporon* sp. | 299 | 9.8 | 18.9 | 17.8 | 1.5 | 19.7 |
| Unidentified | 271 | 1.6 | 3.1 | 4.2 | 0.4 | 1.3 |
| *Tetracladium* sp. | 341 | 1.6 | 3.1 | 1.2 | 4.8 | 3.8 |
| Unidentified | 130 | 1.6 | 3.1 | 0.7 | 5.1 | 0.5 |

| **Oilseed rape bulk soil** | | | | **Average relative abundance** | | |
|---|---|---|---|---|---|---|
| | Taxon | Av. dissim | Contrib. % | November | March | June |
| Unidentified | 327a | 2.0 | 5.8 | 1.0 | 6.9 | 1.6 |
| *Gibellulopsis nigrescens* | 125 | 1.5 | 4.5 | 12.3 | 9.0 | 11.4 |
| Unidentified | 335 | 1.4 | 4.1 | 0.4 | 0.8 | 4.5 |
| Unidentified | 383 | 1.3 | 3.9 | 0.9 | 3.7 | 2.3 |
| *Gibellulopsis nigrescens* | 124 | 1.1 | 3.3 | 7.8 | 7.3 | 9.5 |

| **Wheat rhizosphere** | | | | **Average relative abundance** | | |
|---|---|---|---|---|---|---|
| ID | Taxon | Av. dissim | Contrib. % | November | March | June |
| *Mycosphaerella graminicola* | 143 | 11.4 | 18.0 | 15.9 | 29.2 | 2.1 |
| Unidentified | 168 | 3.3 | 5.1 | 0.1 | 1.8 | 9.6 |
| Unidentified | 337 | 3.2 | 5.0 | 10.6 | 2.6 | 2.9 |
| *Tetracladium* sp. | 341 | 2.4 | 3.8 | 3.1 | 6.3 | 2.0 |
| Unidentified | 334 | 2.2 | 3.5 | 0.1 | 0.3 | 6.7 |

| **Wheat bulk soil** | | | | **Average relative abundance** | | |
|---|---|---|---|---|---|---|
| ID | Taxon | Av. dissim | Contrib. % | November | March | June |
| Unidentified | 286 | 3.2 | 9.4 | 6.0 | 5.4 | 10.7 |
| Unidentified | 327a | 2.4 | 7.0 | 1.5 | 8.5 | 2.2 |
| Unidentified | 418 | 2.1 | 6.0 | 0.5 | 0.2 | 6.1 |
| *Gibellulopsis nigrescens* | 125 | 1.2 | 3.4 | 7.5 | 6.8 | 7.3 |

c) Preceding crop

| **Oilseed rape rhizosphere** | | | | **Average relative abundance** | |
|---|---|---|---|---|---|
| ID | Taxon | Av. dissim | Contrib. % | Ow | Oo |
| *Olpidium brassicae* | 284 | 11.9 | 23.8 | 22.9 | 36.4 |
| *Trichosporon* sp. | 299 | 9.5 | 18.9 | 18.0 | 8.0 |
| Unidentified | 271 | 1.5 | 3.0 | 2.3 | 1.6 |
| *Tetracladium* sp. | 341 | 1.5 | 3.0 | 3.8 | 2.6 |
| Unidentified | 130 | 1.4 | 2.8 | 2.7 | 1.5 |

| **Wheat rhizosphere (November)** | | | | **Average relative abundance** | |
|---|---|---|---|---|---|
| ID | Taxon | Av. dissim | Contrib. % | Wo | Ww |
| *Mycosphaerella graminicola* | 143 | 13.0 | 20.3 | 26.1 | 0.1 |
| Unidentified | 337 | 7.6 | 11.8 | 3.0 | 18.2 |
| Unidentified | 327b | 4.2 | 6.6 | 1.5 | 9.9 |
| Unidentified | 326 | 3.2 | 4.9 | 1.7 | 7.3 |
| Unidentified | 271 | 3.0 | 4.7 | 6.3 | 1.2 |

(a) Crop

| **Rhizosphere** | | | | **Average relative abundance** | |
|---|---|---|---|---|---|
| ID | Taxon | Av. dissim | Contrib. % | Oilseed rape | Wheat |
| *Pseudomonas* spp. | 245 | 6.1 | 21.3 | 19.3 | 15.0 |
| Burkholderiales | 523 | 3.2 | 11.0 | 14.7 | 15.7 |
| Unidentified | 248 | 1.9 | 6.5 | 7.8 | 6.9 |
| Unidentified | 135 | 1.8 | 6.4 | 9.0 | 9.2 |
| Unidentified | 723 | 1.4 | 4.8 | 5.4 | 3.3 |

(b) Season

| **Oilseed rape rhizosphere** | | | | **Average relative abundance** | | |
|---|---|---|---|---|---|---|
| ID | Taxon | Av. dissim | Contrib. % | November | March | June |
| *Pseudomonas* spp. | 245 | 8.1 | 25.6 | 16.1 | 30.9 | 11.3 |
| Burkholderiales | 523 | 2.6 | 8.3 | 15.8 | 14.7 | 12.8 |
| Unidentified | 248 | 2.3 | 7.2 | 3.9 | 10.3 | 9.2 |
| Unidentified | 135 | 2.2 | 6.9 | 7.4 | 7.4 | 12.2 |
| Unidentified | 723 | 1.8 | 5.6 | 6.3 | 2.7 | 7.2 |

| **Oilseed rape bulk soil** | | | | **Average relative abundance** | | |
|---|---|---|---|---|---|---|
| ID | Taxon | Av. dissim | Contrib. % | November | March | June |
| *Pseudomonas* spp. | 245 | 3.1 | 20.0 | 2.1 | 5.5 | 8.5 |
| Acidobacteria Gp 6 | 722 | 1.0 | 6.5 | 8.4 | 7.0 | 6.0 |
| Acidobacteria Gp 6 | 339 | 0.8 | 4.8 | 9.9 | 9.7 | 9.1 |
| Unidentified | 132 | 0.7 | 4.8 | 5.7 | 5.7 | 4.3 |
| Unidentified | 135 | 0.6 | 4.1 | 12.2 | 11.7 | 11.9 |

| **Wheat rhizosphere** | | | | **Average relative abundance** | | |
|---|---|---|---|---|---|---|
| ID | Taxon | Av. dissim | Contrib. % | November | March | June |
| Burkholderiales | 523 | 4.8 | 17.1 | 16.7 | 22.1 | 8.3 |
| *Pseudomonas* spp. | 245 | 4.7 | 16.8 | 14.2 | 14.0 | 16.7 |
| Unidentified | 248 | 2.4 | 8.5 | 4.0 | 5.7 | 11.1 |
| Unidentified | 135 | 2.1 | 7.5 | 8.3 | 6.8 | 12.6 |
| Unidentified | 131b | 1.4 | 4.9 | 1.0 | 4.8 | 1.3 |

| **Wheat bulk soil** | | | | **Average relative abundance** | | |
|---|---|---|---|---|---|---|
| ID | Taxon | Av. dissim | Contrib. % | November | March | June |
| *Pseudomonas* spp. | 245 | 1.0 | 8.0 | 1.8 | 1.7 | 4.1 |
| Acidobacteria Gp 6 | 722 | 0.8 | 6.9 | 8.6 | 8.2 | 6.4 |
| Unidentified | 135 | 0.7 | 5.8 | 12.6 | 12.0 | 13.4 |
| Unidentified | 723 | 0.6 | 4.7 | 4.0 | 3.3 | 2.4 |
| Unidentified | 132 | 0.5 | 4.6 | 5.9 | 5.8 | 4.6 |

c) Preceding crop

| **Oilseed rape rhizosphere** | | | | **Average relative abundance** | |
|---|---|---|---|---|---|
| | Taxon | Av. dissim | Contrib. % | Ow | Oo |
| *Pseudomonas* spp. | 245 | 7.4 | 25.7 | 19.6 | 19.3 |
| Burkholderiales | 523 | 2.6 | 9.0 | 15.4 | 13.4 |
| Unidentified | 135 | 2.0 | 6.7 | 8.4 | 9.6 |
| Unidentified | 248 | 1.8 | 6.2 | 7.4 | 8.2 |
| Unidentified | 525 | 1.6 | 5.5 | 4.3 | 2.7 |

| **Wheat rhizosphere (November)** | | | | **Average relative abundance** | |
|---|---|---|---|---|---|
| ID | Taxon | Av. dissim | Contrib. % | Wo | Ww |
| *Pseudomonas* spp. | 245 | 9.9 | 39.4 | 4.3 | 24.1 |
| Burkholderiales | 523 | 2.3 | 9.0 | 18.5 | 14.9 |
| Unidentified | 135 | 1.7 | 6.7 | 10.0 | 6.6 |
| Acidobacteria Gp 6 | 339 | 0.9 | 3.6 | 5.5 | 3.8 |
| Unidentified | 723 | 0.9 | 3.6 | 4.6 | 2.8 |

(a) Crop

| **Rhizosphere** | | | | **Average relative abundance** | |
|---|---|---|---|---|---|
| ID | Taxon | Av. dissim | Contrib. % | Oilseed rape | Wheat |
| *Pratylenchus neglectus* | 304 | 8.9 | 17.9 | 25.4 | 8.4 |
| *Chiloplacus propinquus* | 413 | 5.5 | 11.1 | 8.9 | 18.6 |
| Plectidae | 145 | 4.2 | 8.4 | 3.6 | 11.4 |
| Unidentified | 302 | 2.9 | 5.9 | 12.1 | 9.7 |
| *Bitylenchus dubius* | 143 | 2.2 | 4.3 | 1.7 | 5.7 |

(b) Season

| **Oilseed rape rhizosphere** | | | | **Average relative abundance** | | |
|---|---|---|---|---|---|---|
| ID | Taxon | Av. dissim | Contrib. % | November | March | June |
| *Pratylenchus neglectus* | 304 | 7.7 | 17.7 | 15.0 | 36.4 | 24.9 |
| Unidentified | 302 | 3.6 | 8.3 | 7.4 | 17.1 | 11.7 |
| *Chiloplacus propinquus* | 413 | 3.4 | 7.8 | 13.9 | 4.7 | 8.0 |
| Unidentified | 298 | 2.2 | 5.0 | 3.4 | 7.0 | 3.2 |
| *Eumonhystera* sp. | 611 | 2.0 | 4.7 | 6.0 | 0.6 | 0.0 |

| **Wheat rhizosphere** | | | | **Average relative abundance** | | |
|---|---|---|---|---|---|---|
| ID | Taxon | Av. dissim | Contrib. % | November | March | June |
| Chiloplacus propinquus | 413 | 4.7 | 11.8 | 17.3 | 21.5 | 17.1 |
| Plectidae | 145 | 3.1 | 7.7 | 10.1 | 13.8 | 10.3 |
| *Pratylenchus neglectus* | 304 | 2.7 | 6.8 | 6.8 | 6.0 | 12.3 |
| *Bitylenchus dubius* | 143 | 2.6 | 6.4 | 6.3 | 8.5 | 2.3 |
| Unidentified | 302 | 2.4 | 6.1 | 8.6 | 9.6 | 10.9 |

(c) Preceding crop

| **Oilseed rape rhizosphere (November)** | | | | **Average relative abundance** | |
|---|---|---|---|---|---|
| ID | Taxon | Av. dissim | Contrib. % | Ow | Oo |
| *Eumonhystera* sp. | 611 | 4.2 | 14.1 | 2.8 | 11.2 |
| Unidentified | 302 | 3.0 | 10.0 | 11.7 | 5.7 |
| *Eumonhystera* sp. | 610 | 2.8 | 9.3 | 1.5 | 7.1 |
| Unidentified | 298 | 2.3 | 7.5 | 5.7 | 2.1 |
| Plectidae | 145 | 2.2 | 7.4 | 10.1 | 6.1 |

| **Wheat rhizosphere (March)** | | | | **Average relative abundance** | |
|---|---|---|---|---|---|
| ID | Taxon | Av. dissim | Contrib. % | Wo | Ww |
| *Chiloplacus propinquus* | 413 | 9.8 | 25.8 | 16.1 | 35.6 |
| Plectidae | 145 | 4.9 | 12.9 | 12.0 | 21.2 |
| Unidentified | 302 | 4.6 | 12.1 | 16.0 | 7.3 |
| *Bitylenchus dubius* | 143 | 3.6 | 9.5 | 12.8 | 7.9 |
| *Pratylenchus neglectus* | 304 | 3.0 | 7.9 | 10.3 | 4.5 |