FEMS Microbiology Letters, 366, 2019, fnz189

https://doi.org/10.1093/femsle/fnz189
Advance Access Publication Date: 5 September 2019
Research Letter

RESEARCH LETTER – Environmental Microbiology

Preceding crop and tillage system affect winter survival of wheat and the fungal communities on young wheat roots and in soil

Hanna Friberg[1,*,†], Paula Persson[2], Dan Funck Jensen[1] and Göran Bergkvist[2]

[1]Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, P.O. Box 7026, 75007 Uppsala , Sweden and [2]Department of Crop Production Ecology, Swedish University of Agricultural Sciences, P.O. Box 7043, 75007 Uppsala , Sweden

*Corresponding author: Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, P.O. Box 7026, 75007 Uppsala, Sweden. Tel: +46 70 393 11 06; E-mail: hanna.friberg@slu.se

One sentence summary: Agricultural practices like tillage and the cropping sequence influence soil fungal communities and thereby crop health and performance.

Editor: Prof. Sergio Casella
†Hanna Friberg, http://orcid.org/0000-0003-3181-1597

ABSTRACT

Agricultural practices like tillage and cropping sequence have profound influence on soil-living and plant-associated fungi, and thereby on plant growth. In a field experiment, we studied the effects of preceding crop and tillage on fungal communities in the soil and on young winter wheat roots in relation to plant winter survival and grain yield. We hypothesized that plant performance and fungal communities (described by amplicon sequencing) differ depending on tillage system and preceding crop; that the effect of preceding crop differs depending on tillage system, and that differences in fungal communities are reflected in plant performance. In line with our hypotheses, effects of preceding crop on plant growth and fungal communities on plant roots and in soil were more pronounced under non-inversion tillage than under inversion tillage (ploughing). Fungal communities on plant roots in treatments with low winter survival were different from those with better survival. In soil, several fungal OTUs (operational taxonomic units) differed significantly between tillage systems. OTUs representing putative plant pathogens were either more abundant (*Parastagonospora sp.*_27) or less abundant (*Fusarium culmorum/graminearum*_5) after non-inversion tillage. Our findings highlight the influence of cultural practices on fungal communities and thereby on plant health and yield.

Keywords: cropping system; cropping sequence; crop rotation; break crop; tillage; legacy effects

INTRODUCTION

Adopting an appropriate cropping system is essential for a healthy crop. One critical factor for plant health is a well-planned cropping sequence, where disease control is achieved through the absence of a suitable host, resulting in a decline in the inoculum density of many plant pathogens (Angus *et al.* 2015). It is also possible that a specific cropping sequence can lead to an increase in the population of beneficial soil organisms that are able to suppress the development of plant diseases or stimulate plant growth by other mechanisms (Garbeva, van Veen and van Elsas 2004; Benitez, Taheri and Lehman 2016;

Received: 16 August 2018; **Accepted:** 2 September 2019

Lenc *et al.* 2016). Effects on fungi are of special interest because fungi are expected to be of major importance both in the case of problems with build-up of pathogenic populations and in the case of advantages from beneficial populations (Kirkegaard *et al.* 2008).

In cropping systems with non-inversion tillage (sometimes referred to as ploughless, reduced or minimum tillage), the importance of the cropping sequence, especially the preceding crop, can be expected to be higher than with inversion tillage (Bockus and Shroyer 1998). Soils under non-inversion tillage are only mixed superficially, in contrast to traditional mouldboard ploughing, where the soil is inverted down to a depth of 18–30 cm (Rasmussen 1999). In non-inversion tillage systems, crop residues are left on or close to the soil surface and have a slower rate of degradation, thereby supporting the inoculum of plant pathogens such as *Fusarium graminearum* for longer time than crop residues buried deeper in the soil (Pereyra, Dill-Macky and Sims 2004). Furthermore, crop residues on the soil surface are in closer contact with above-ground plant parts, which promotes pathogen dispersal within the crop. The problems with root diseases in crop rotations with low variation can also be expected to be higher under non-inversion tillage because the young roots are in contact with more crop residues from the preceding crop. Watt, Kirkegaard and Rebetzke (2005) found that without ploughing, young, new wheat roots followed old root channels, which resulted in over 50% of the length of new roots being in direct contact with root remnants from the preceding crop. Because of this, pathogenic fungi surviving on the dead roots from one crop can easily colonize the roots of the following crop.

In earlier field studies performed in Sweden, where frost is common during winter, it was found that an important effect of the preceding crop on winter wheat is on the ability of crop plants to survive the winter and that differences among preceding crops in this regard are exacerbated by non-inversion tillage (Olofsson 1993). There is little knowledge on how the fungal community influence the effects of the preceding crop and tillage system on winter wheat performance. The aims of the present study were to determine effects of four different preceding crops to winter wheat under inversion and non-inversion tillage on the establishment of wheat, winter survival and yield, as well as to link preceding crop effect to fungal communities in the bulk soil in late autumn and on young wheat plants sampled in spring. We expected the links between fungal community and crop performance of wheat would be complex rather than explained by one or a few fungal pathogens. Therefore, broad community analysis using amplicon sequencing was chosen for analysis of the fungal communities. High-throughput sequencing techniques offer examination of fungal communities in plant and soil material, with a considerably higher resolution than older techniques (Lindahl *et al.* 2013).

Our hypotheses were: (i) Winter wheat survives better and produces higher yield when preceded by a non-cereal crop. The effect is greater under non-inversion tillage than after inversion tillage. (ii) Fungal communities in soil and on wheat roots are influenced by the preceding crop, i.e. cereal preceding crops result in community structures more similar to each other than to those after non-cereal preceding crops. These effects are more pronounced under non-inversion tillage. (iii) Fungal communities associated with wheat plant roots in early spring reflect plant winter survival.

MATERIALS AND METHODS

Experimental design and management

A field experiment was conducted at Kungsängen (59°50′N, 17°40′E), Uppsala, Sweden. The soil is clayey (48% clay, 30% silt, 22% sand) with an organic matter content of 3.2 g 100 g^{-1} air-dry soil. The experiment was set up using a strip-plot design (plot size 6 m x 12 m). Four preceding crops were compared: winter wheat (*Triticum aestivum* cv. Olivin), spring oat (*Avena sativa*, cv. Belinda), spring oilseed rape (*Brassica napus* ssp. *napus* cv. Petita) and spring pea (*Pisum sativum* cv. Brutus). All preceding crops were studied under inversion tillage and non-inversion tillage. Each preceding crop × tillage combination had 3 replicates. Winter wheat was sown on the whole experimental area on 19 September (year 1). The following spring (year 2), the wheat was removed from all plots that were to be grown with other preceding crops, by killing the wheat with glyphosate and harrowing. Oat, oilseed rape or pea was sown on 15 April (year 2). Winter wheat and oilseed rape were fertilised with 100 kg N ha^{-1} and oat with 70 kg N ha^{-1}. P and K was not applied. Pea was not fertilized. Weeds were controlled with herbicides appropriate for the different crops. All preceding crop treatments were harvested on 31 August (year 2). The two tillage treatments were applied after harvest of preceding crops. The soil was ploughed with a mouldboard plough to 23 cm depth on 6 September, constituting the inversion tillage treatment, or tilled with a rigid tine cultivator to 12 cm depth on 11 September, constituting the non-inversion tillage treatment. The soil was harrowed twice after the inversion treatment and once after the non-inversion treatment, on 19 September. Winter wheat (cv. Olivin) (main crop) was sown on 24 September (year 2). The crop was fertilised with 110 kg N ha^{-1} and 8 kg P ha^{-1}. K was not applied. No fungicides were applied. Weeds were controlled with herbicides (one treatment with Ariane S, Dow AgroSciences, Indianapolis, USA) at 2.4 L ha^{-1} on 29 May (year 3). The winter wheat was harvested on 24 August (year 3).

Sampling and DNA extraction

In late October (year 2), when the winter wheat main crop was just established, soil samples were collected from the upper 10 cm, through systematic sampling across each plot. One pooled sample of approximately 3 kg soil was obtained from each plot (biological replicate, $n = 3$). Each sample was mixed thoroughly and air-dried in room temperature (20°C) for a maximum of 4 hr to enable sieving at 2 mm. Thereafter, soil samples were stored at −20°C for molecular analyses. Soil dry matter content was determined by drying the soil at 105°C for 2 days. DNA was extracted by the FastDNA™ SPIN Kit for soil (MP Biomedicals, USA) in three technical replicates of 500 mg. DNA extracts were diluted to 4 ng DNA μL^{-1} and stored at −20°C.

Wheat plants were sampled in early May (year 3), when plants were at the tillering stage (Lancashire *et al.* 1991). Five plants were collected across each experimental plot and washed in water. DNA was extracted from roots by combining the 5 roots from one replicate, grinding them together in liquid nitrogen, and using aliquots for further DNA extraction using Qiagen DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). DNA extracts from the same replicate plot in the field experiments were pooled, diluted to 4 ng DNA μL^{-1} and stored at −20°C.

The number of winter wheat plants of the main crop was counted in three 0.25 m^2 sub-areas in each plot after establishment (October 31, year 2) and in one 0.25m^2 sub-area in early spring (April 18, year 3). Grain yield was determined from a 23 m^2 harvested area, in each plot using a plot combiner. The harvested products were weighed and dry matter content determined on subsamples.

PCR and sequencing

Fungal communities on roots and in soil samples were analysed using amplicon sequencing of the ITS2 region of the ribosome-encoding genes, with primers targeting mainly fungi within Basidiomycota and Ascomycota. PCR was conducted using fITS9 (Ihrmark *et al.* 2012) and ITS4 primers (White *et al.* 1990), extended with 8 bp sample identification tags as described in Ihrmark *et al.* (2012). The three technical replicates of DNA extracts from each soil sample were amplified using unique identification tags.

PCR amplification was conducted in a 2720 Thermal Cycler (Life Technologies, Carlsbad, CA, USA) in 25 μL reactions of 20 ng DNA, 300 nM tagged ITS4 and 1000 nM fITS9, 0.025 U/μL polymerase (DreamTaq Green, Thermo Scientific, Waltham, MA, USA) in PCR buffer. The number of PCR cycles (approx. 25 for root samples and 30 for soil samples) was adapted for each sample to avoid oversaturation and distortion of the PCR pool. To determine the number of cycles, test runs were conducted to find the number of cycles giving weak to moderately strong bands on agarose gel. PCR conditions were: 5 min at 94°C and cycles of [30 s at 94°C; 30 s at 55°C; 30 s at 72°C] and 7 min at 72°C.

PCR products were purified using the AMPure kit (Beckman Coulter, Brea, CA, USA) and concentrations of purified products were determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). PCR products from all soil and root samples were mixed in equal molar proportions into a general sample, and further purified using the GeneJet™ PCR-Purification Kit (Fermentas) before being freeze-dried. Addition of sequencing adaptors (by ligation) and 454-sequencing were performed by LGC Genomics GmbH (Berlin, Germany) on a GL FLX Titanium system (Roche, Basel, Switzerland). Demultiplexed raw sequence data were deposited in the Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra) under accession number SRP076984.

Sequence analysis

Sequences were analysed using the SCATA pipeline (https://scata.mykopat.slu.se). Sequences with an average quality score below 20 or with a score below 10 at any position were discarded, using the high quality region (HQR) extraction option. Clustering was based on 38 bp of the LSU, the entire ITS2 region (122–245 bp) and 50–55 bp of the 5.8S unit. Sequences were then compared for similarity, using BLAST as a search engine with minimum length of pair-wise alignments set to 90% of the longest sequence. Pairwise alignments were scored using a scoring function with one in penalty for mismatch, zero for gap opening and one for gap extension. Homopolymers were collapsed to 3 bp before clustering (Miller *et al.* 2008). Sequences were assembled into clusters by single-linkage clustering, using a maximum distance of 2%. Sequences that only occurred once in the entire dataset (global singletons) were excluded.

The most common sequence in each OTU was used for taxonomic assignment (Lindahl *et al.*, 2013) using rdp classifier (Wang *et al.* 2007) and UNITE Fungal ITS trainset 07/04/2014. Only assignments with a confidence threshold of 95% or more were kept. The 60 most common operational taxonomic units (OTUs) were also blasted against GenBank (http://www.ncbi.nlm.nih.gov/genbank). In cases where assignments were uncertain, OTUs were assigned at lower resolution. Taxonomic assignments of the most common OTUs are presented in Tables S1 and S2 (Supporting Information). OTUs given the name of a genus or higher taxon still represent a specific OTU, not all the OTUs belonging to the same taxon. OTUs of non-fungal origin (mainly wheat (3%), ciliates and bryophytes) were removed before further analyses. FUNGuild (Nguyen *et al.* 2016) was used to assign a putative trophic group (plant pathogen or saprotroph) to each OTU. The result was corrected to exclude plant pathogens on other host plants than wheat from the category 'plant pathogen'.

Statistical analyses

Statistical analyses were performed using the R-software (R Core Team 2016). In all cases, statistical analyses were made based on the three biological replicates in the field experiments ($n = 3$). When data was obtained from several technical replicates (e.g. DNA extractions from soil), the mean of these were used in the statistical analyses.

Plant data were analysed using the ANOVA procedure, with preceding crop, tillage strategy and their interaction as factors. Experimental block had no significant impact on the plant data, and was not included in the model. Pair-wise comparisons were performed using Tukey's HSD test. Plant data was checked for the assumptions of normality (Shapiro test) and homogeneity of variance (Bartlett's test).

Non-metric multidimensional scaling (NMDS) and the envfit function was used to describe fungal community structures in relation to treatments (vegan package; Oksanen *et al.* 2012). Effects of tillage and preceding crop on fungal community structures in soil and root samples were tested using GLM-based models. Using a negative binominal probability distribution, models including number of 454 reads, experimental block in the field experiment, tillage and preceding crop were fitted to each OTU, using the *manyglm* function in package 'mvabund' (Wang *et al.* 2012). Significances were assessed using the function anova.manyglm, which provides a multivariate test for the community composition and univariate tests for each OTU. Likelihood-ratio tests were used and *P*-values were adjusted for multiple testing using a step-down resampling procedure.

RESULTS

Plant data

Treatments with different preceding crops and tillage strategies influenced the ability of the winter wheat plants to survive the winter period. By the end of October (year 2), there were on average 369 plants m^{-2} and no differences were found between treatments (Table 1). By the April count (year 3), there was a significant effect of both preceding crop and tillage strategy on the number of plants, but no significant interaction between these two factors (Table 1). In the pairwise comparisons, there was a significant difference between the number of plants (in April year 3) after wheat or oats under non-inversion tillage compared to pea as preceding crop and inversion tillage (Fig. 1).

The mean grain yield of winter wheat was 7.0 tonnes dry weight ha^{-1}. Treatment effects on the yield were significant only for preceding crop (Table 1), where winter wheat as preceding crop resulted in 9% lower yield than oats as preceding

Table 1. Levels of significance (*P*-values) from statistical analyses for crop variables and fungal community structures (FCS) in soil and on wheat roots. Crop response variables are based on ANOVA and Tukey's test, FCS responses are based on multivariate testing (anova.manyglm). Significant effects of tested factors ($P < 0.05$) are in bold ($n = 3$). Block was not a significant factor for the crop variables and therefore excluded from the final models.

Response variable	Treatment factor Block	Preceding crop	Tillage	Tillage × Preceding crop
Plants in autumn	–	0.641	0.301	0.812
Plants in spring	–	**0.031**	**0.004**	0.276
Grain yield	–	**0.034**	0.457	0.081
Soil FCS	**0.001**	**0.001**	**0.001**	**0.001**
Root FCS	**0.004**	**0.032**	**0.003**	**0.002**

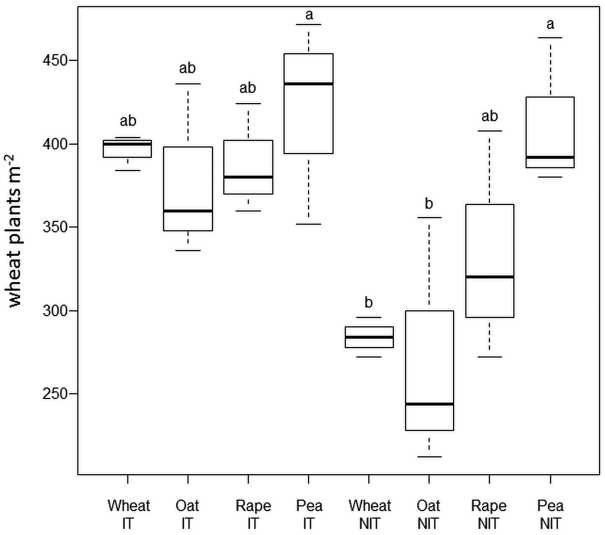

Figure 1. Number of wheat plants per m^2 in early spring, in treatments with the preceding crops winter wheat, oats, oilseed rape and peas under inversion tillage (IT) and non-inversion tillage (NIT). The horizontal line in the box plot shows the median value, the bottom and top of the box the 25th and 75th percentiles and the dashed lines the minimum and maximum values. Different letters above bars indicate significant differences between treatments ($P < 0.05$, Tukey's test; $n = 3$).

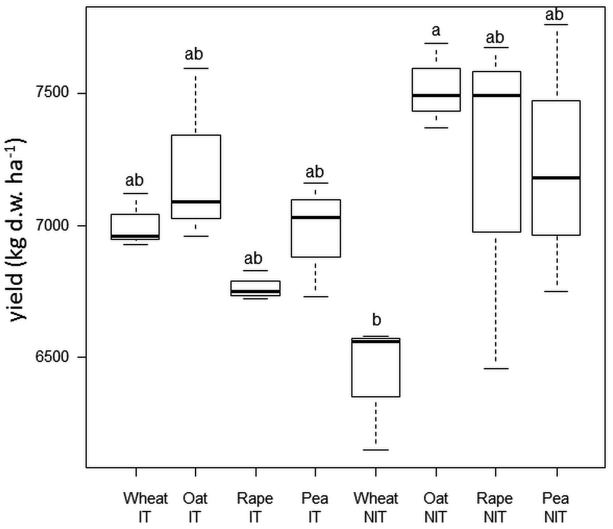

Figure 2. Wheat yield in treatments with the preceding crops winter wheat, oats, oilseed rape and peas under inversion tillage (IT) and non-inversion tillage (NIT). The horizontal line in the box plot shows the median value, the bottom and top of the box the 25th and 75th percentiles and the dashed lines the minimum and maximum values. Different letters above bars indicate significant differences between treatments ($P < 0.05$, Tukey's test; $n = 3$).

crop ($P = 0.02$). The yield after winter wheat was particularly low in the non-inversion tillage system, where it was significantly lower than after oats (Fig. 2).

Fungal community data

Sequencing of fungal communities in root and soil samples yielded in total 351 775 sequences. Of these, 59% passed the quality control. The sequence analysis and clustering resulted in a total of 1628 OTUs in root and soil samples combined, with 1531 OTUs in the soil samples and 438 in the root samples.

Fungal communities in the soil sampled in late autumn year 2 were significantly influenced by preceding crop, tillage and the interaction between them. There was also a significant block effect, particularly evident in the treatments under inversion tillage (Fig. 3, Table 1). Fungal community structures were similar after all preceding crops under inversion tillage, but were influenced by the preceding cropunder non-inversion tillage (Fig. 3). The preceding crops winter wheat and oats yielded similar structures of the fungal community under non-inversion tillage. The treatment with pea and non-inversion tillage gave the community structure most different from those found under inversion tillage. Oilseed rape treatment resulted in a community structure overlapping with both that from pea

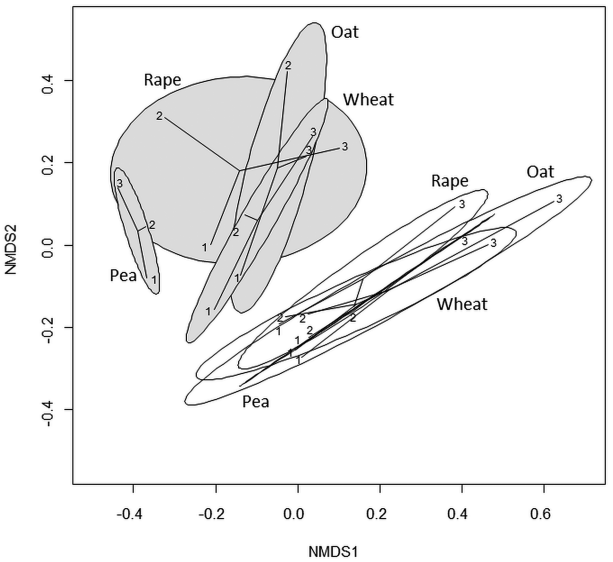

Figure 3. Fungal community structures in soil samples. NMDS plot of data from fungal ITS amplicons showing sample scores. Observations from identical treatments in different blocks (numbers 1–3) are connected to the barycentre of each treatment combination. The variation within treatments is indicated with 95% confidence circles (white for inversion tillage and grey for non-inversion tillage).

Table 2. Fungal OTUs in soil and root samples with $P < 0.2$ for at least one factor in statistical analyses (block, preceding crop, tillage or the interaction between preceding crop and tillage). Significant differences ($P < 0.05$) are in bold (anova.manyglm; $n = 3$).

Sample type	OTU	Block	Preceding crop	Tillage	Preceding crop × Tillage
Soil	Ascomycota_1	0.169	0.865	0.604	0.229
	Ascomycota_2	0.995	1.000	**0.001**	0.992
	Fusicolla merismoides_4	**0.037**	1.000	0.785	0.992
	Fusarium culmorum/graminearum_5	0.717	1.000	**0.025**	0.992
	Articulospora proliferata_6	0.999	0.503	**0.020**	0.999
	Pleosporales_7	0.999	0.076	**0.001**	0.129
	Sordariomycetes_10	0.998	0.622	0.183	0.274
	Mycosphaerella tassiana_13	0.998	1.000	**0.001**	0.992
	Alternaria metachromatica_16	0.995	0.93	**0.001**	0.323
	Solicoccozyma fuscescens_17	**0.001**	0.981	0.845	0.992
	Apiotrichum gracile_20	0.644	0.998	0.145	0.323
	Pseudaleuria_25	**0.006**	0.757	0.221	0.229
	Parastagonospora sp_27	0.999	**0.033**	**0.049**	0.992
	Apodus sp_28	**0.036**	1.000	0.367	0.226
	Mortierella sp_31	**0.017**	1.000	0.604	0.992
	Parastagonospora_32	0.999	0.096	0.121	0.999
	Ascomycota_49	0.998	0.440	**0.011**	0.999
	Helotiales_53	0.620	1.000	0.179	0.857
	Pleosporales_58	0.830	1.000	**0.030**	0.999
	Sordariomycetes_78	0.995	0.816	0.067	0.994
Root	*Articulospora proliferata_6*	1.000	1.000	0.288	**0.044**
	Ascomycota_26	0.925	0.588	0.131	0.904
	Ophiosphaerella sp_29	0.319	0.946	1.000	0.129
	Tremellomycetes_35	0.999	1.000	0.135	0.671
	Podospora sp_40	0.996	1.000	0.999	0.159

and those from winter wheat and oats (Fig. 3). Communities in soil were dominated by OTUs belonging to the phylum Ascomycota. The proportion of OTUs within Ascomycota was higher after wheat (97%) than after oilseed rape (89%; 0.02. Several fungal OTUs were significantly influenced by block, preceding crop or tillage. Among them were some assigned to species or groups that contain plant pathogens on wheat. *Fusarium culmorum/graminearum_5*, was more common in treatments with inversion tillage. *Parastagonospora_32* was significantly influenced by both preceding crop and tillage, with higher abundances in treatments with non-inversion tillage (Table 2; Fig. 1, Supporting Information). Several OTUs assigned as putative plant pathogens (Table S1, Supporting Information) were part of the OTUs with higher abundances in non-inversion tillage treatments (Fig. 1, Supporting Information).

Fungal communities on wheat roots were significantly influenced by block, preceding crop, tillage system and treatment combinations (Table 1). In the NMDS of fungal communities on roots, there was an overlap between treatments with oats and wheat within each tillage system, and a separation between the tillage systems (Fig. 4.). In treatments with oilseed rape and pea, there was an overlap for the two crops and the two tillage systems. The within-treatment variation was higher in treatments under non-inversion tillage, especially when oat or winter wheat was the preceding crop (Fig. 4). OTUs belonging to Ascomycota represented on average 96% of the fungal community in roots. Tillage or preceding crop had no significant influence on the community structure at phylum level. One fungal OTU was significantly influenced by the interaction between tillage and preceding crop: *Articulospora proliferata_6* had higher abundance after cereal preceding crops and non-inversion tillage (Table 2; Fig. 2, Supporting Information). OTUs assigned as putative plant pathogens on wheat (Table S2, Supporting Information) were in several cases among the OTUs with higher abundances in

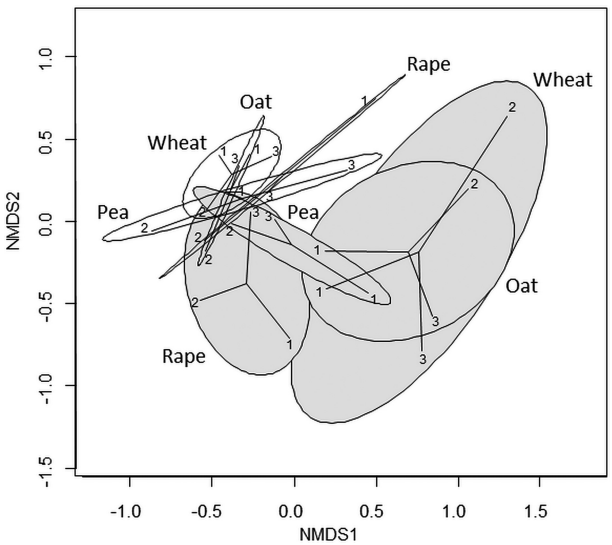

Figure 4. Fungal community structures in root samples. NMDS plot of data from fungal ITS amplicons showing sample scores. Observations from identical treatments in different blocks (numbers 1–3) are connected to the barycentre of each treatment combination. The variation within treatments is indicated with 95% confidence circles (white for inversion tillage and grey for non-inversion tillage).

treatments with cereal preceding crops and non-inversion tillage (Fig. 2, Supporting Information).

DISCUSSION

Our data show effects of preceding crop and tillage system on winter wheat performance as well as on fungal communities on roots and in soil. In relation to our hypotheses, we found

that: (i) Winter survival of wheat was lower after oats, and grain yield was lower after wheat. However, it was only under non-inversion tillage that effects of preceding crop could be established in the specific treatment combinations. (ii) Fungal community structure in the topsoil was influenced by the preceding crop in treatments with non-inversion tillage, but not in treatments with inversion tillage. Fungal community structure on wheat roots was influenced by the combination of preceding crop and tillage. (iii) The combination of a cereal preceding crop and non-inversion tillage resulted in fungal community structures on roots that were different from those in other treatments, and in lower winter survival of the wheat plants. OTUs representing putative plant pathogens were of some importance for the differences in community structures in soil and root communities. However, only in a few cases the abundances differed significantly between treatments. In soil, OTUs representing putative plant pathogens either increased or decreased in non-inversion tillage treatments.

Fungal communities in the soil samples were clearly separated by the tillage treatments. The impact of tillage on microbial communities has been demonstrated in previous studies, especially the long-term effects of no-tillage systems (Sharma-Poudyal *et al.* 2017). Our study is based on a short-term experiment, where the tillage treatment was applied only once. It is of special interest that we see clear effects of tillage treatments on both plant performance and fungal communities, already the year after the treatment. There are several possible explanations to the effects of tillage on fungi. One important factor is that fungi are growing saprotrophically on the crop material, and that this material is present in larger amounts under non-inversion, as we sampled upper 10 cm of the soil. With inversion tillage, this material is mainly found at ploughing depth (Rasmussen 1999). Fungi favoured by less intense tillage could be root endophytes or species adapted to utilize intact decaying roots, while populations of opportunistic fungi can be expected to recover faster after disturbances such as tillage, and thus be favoured in more intense tillage systems (Detheridge *et al.* 2016; Sharma-Poudyal *et al.* 2017).

Among the OTUs assigned as putative plant pathogens, several were among the ones explaining differences between treatments seen in the NMDSes. It is, however, difficult to state categorically that these OTUs represent pathogens, since it is common for a single genus, and sometimes species, to contain both pathogens and non-pathogens (Stergiopoulos and Gordon 2014). In the soil samples, *Parastagonospora sp._27* was more abundant under non-inversion tillage. In contrast, *Fusarium culmorum/graminearum_5* was more abundant under inversion tillage. Although non-inversion tillage has an important influence on soil fungal communities, the effects on plant pathogens and plant disease is not always easy to predict, and the effects will depend on the crop sequence used (Schroeder and Paulitz 2006). While there is a clear risk for build-up of pathogen populations in non-inversion tillage systems when the crop rotation is dominated by one or a few crops, the risk is much less in varied crop rotations. When the same or a related crop is grown repeatedly, various soil-borne and residue-borne pathogens are favoured (Smith, Kirkegaard and Howe 2004; Kirkegaard *et al.* 2008). This effect is expected to be pronounced when residues are left at the soil surface, where the rate of decomposition is lower and the contact with young plants is greater. It could be assumed that OTUs representing potential plant pathogens would be more abundant on roots in treatments with low winter survival (i.e. a cereal preceding crop and non-inversion tillage). Such patterns were seen in the NMDS (Fig. S2, Supporting Information, *e.g. Parastagonospora_ 27* and *Parastagonospora_32*), but none of them differed significantly among treatments in the statistical analysis (Table 2). One of the most commonly found OTUs on roots, *Microdochium nivale_22*, (syn. *Fusarium nivale, Monographella nivalis*) is causal agent of snow mold, a disease that can cause severe winter survival problems of wheat. This OTU was, not influenced by preceding crop or tillage in our experiment. *Articulospora proliferata_6* was more abundant in wheat roots after a combination of cereal preceding crops and non-inversion tillage (Table 2; Fig. 2, Supporting Information). This species has been described as an aquatic hyphomycete, but also as an endophyte of plants (Baerlocker *et al.* 2010; Sieber and Grünig 2013). With the limited information about the biology of this species we have today, it is therefore not possible to conclude whether its presence has an influence on the winter survival of wheat plants.

Many of the most abundant fungal OTUs in our datasets were assigned to species of which there is limited information. A *Titea* species, assigned as *Titea maxilliformis* (syn. *Tetracladium maxilliformae*), was the most commonly found species on wheat roots (Table S2, Supporting Information) and the third most common in soil (Table S1, Supporting Information). Grudzinska-Sterno *et al.* (2016) and Klaubauf *et al.* (2010) studied fungi on wheat plants from Sweden and soil fungi in Austria, respectively, and also found species of this genus to be common. Klaubauf *et al.* (2010) suggested that these species are involved in plant debris degradation. In our soil samples, the most common OTU was classified as an unknown species within Dothideomycetes. However, neither *T. maxilliformis* nor the Dothideomycetes were significantly affected by preceding crop or tillage treatments.

Our findings show that the tillage system has a significant effect on fungal communities already the first year with non-inversion tillage. We also show that the effects of tillage on fungal communities and on crop performance should not be considered in isolation, but in relation to the crop rotation used.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSLE* online.

ACKNOWLEDGEMENTS

We thank Björn Lindahl, Mikael Brandström-Durling, Ida Karlsson, Katarina Ihrmark and Maria Jonsson for helpful discussions.

FUNDING

This work was supported by the Swedish Farmer's Foundation for Agricultural Research (SLF; Grant number H0633195), Centre for Biological Control and Swedish University of Agricultural Sciences (SLU).

Conflicts of Interests. None declared.

REFERENCES

Angus JF, Kirkegaard JA, Hunt JR *et al.* Break crops and rotations for wheat. *Crop and Pasture Sci* 2015;**66**:523–52.

Baerlocher F, Charette N, Letourneau A *et al.* Sequencing DNA extracted from single conidia of aquatic hyphomycetes. *Fung Ecol* 2010;**3**:115–21.

Benitez M-S, Taheri WI, Lehman RM. Selection of fungi by candidate cover crops. *Appl Soil Ecol* 2016;**103**:72–82.

Bockus WW, Shroyer JP. The impact of reduced tillage on soil-borne plant pathogens. *Annu Rev Phytopathol* 1998;**36**:485–500.

Detheridge AP, Brand G, Fychan R *et al*. The legacy effect of cover crops on soil fungal populations in a cereal rotation. *Agric Ecosyst Environ* 2016;**228**:49–61.

Garbeva P, van Veen JA, van Elsas JD. Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. *Annu Rev Phytopathol* 2004;**42**:243–70.

Grudzinska-Sterno M, Yuen J, Stenlid J *et al*. Fungal communities in organically grown winter wheat affected by plant organ and development stage. *Eur J Plant Pathol* 2016;**146**:401–17.

Ihrmark K, Bödeker ITM, Cruz-Martinez K *et al*. New primers to amplify the fungal ITS2 region - evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiol Ecol* 2012;**82**:666–77.

Kirkegaard J, Christen O, Krupinsky J *et al*. Break crop benefits in temperate wheat production. *Field Crops Res* 2008;**107**:185–95.

Klaubauf S, Inselsbacher E, Zechmeister-Boltenstern S *et al*. Molecular diversity of fungal communities in agricultural soils from Lower Austria. *Fungal Divers* 2010;**44**:65–75.

Lancashire PD, Bleiholder H, Langelûddecke P *et al*. An uniformdecimal code for growth stages of crops and weeds. *Ann Appl Biol* 1991;**119**:561–601.

Lenc L, Kwasna H, Jeske M *et al*. Effects of farming system on root-zone fungal populations in wheat. *J of Plant Path* 2016;**98**:471–82.

Lindahl BD, Nilsson RH, Tedersoo L *et al*. Fungal community analysis by high-throughput sequencing of amplified markers - a user's guide. *New Phytol* 2013;**199**:288–99.

Miller JR, Delcher AL, Koren S *et al*. Aggressive assembly of pyrosequencing reads with mates. *Bioinformatics*, 2008;**24**:2818–24.

Nguyen NH, Song Z, Bates ST *et al*. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fung Ecol* 2016;**20**:241–8.

Olofsson S. Influence of preceding crop and crop residue on stand and yield of winter wheat *(Triticum æstivum L.)* in different tillage systems, including zero tillage, *Crop Production Science*, Issue 18, Swedish University of Agricultural Sciences, Uppsala, Sweden. 1993.

Oksanen J, Blanchet FG, Kindt R *et al*. *vegan: Community Ecology Package*. 2012. R package version 2.0-5. http://CRAN.R-project.org/package = vegan.

Pereyra SA, Dill-Macky R, Sims AL. Survival and inoculum production of *Gibberella zeae* in wheat residue. *Plant Disease* 2004;**88**:724–30.

R Core Team, *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing: Vienna, Austria. 2016. ISBN 3-900051-07-0, URL https://www.R-project.org/

Rasmussen KJ. Impact of ploughless soil tillage on yield and soil quality: a Scandinavian review. *Soil & Tillage Res* 1999;**53**:3–14.

Schroeder KL, Paulitz TC. Root diseases of wheat and barley during the transition from conventional tillage to direct seeding. *Plant Disease* 2006;**90**:1247–53.

Sharma-Poudyal D, Schlatter D, Yin C *et al*. Long-term no-till: a major driver of fungal communities in dryland wheat cropping systems. *PLoS one* 2017;**12**:e0184611.

Sieber TN, Grünig CR. Fungal root endophytes. In: Eshel A, Beeckman T (eds). *Plant Roots: The Hidden Half*. CRC Press, Boca Raton, 2013. Chapter 38.

Smith BJ, Kirkegaard JA, Howe GN. Impacts of Brassica break-crops on soil biology and yield of following wheat crops. *Aust J Agric Res* 2004;**55**:1–11.

Stergiopoulos I, Gordon TR. Cryptic fungal infections: the hidden agenda of plant pathogens. *Front Plant Sci* 2014;**5**:506.

Wang Q, Garrity GM, Tiedje JM *et al*. Naïve Bayesian classifier for rapid assignment of rRNA Sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 2007;**73**:5261–7.

Wang Y, Neuman U, Wright S *et al*. mvabund: an R package for model-based analysis of multivariate abundance data. *Methods Ecol Evol* 2012;**3**:471–4.

Watt M, Kirkegaard JA, Rebetzke GJ. A wheat genotype developed for rapid leaf growth copes well with the physical and biological constraints of unploughed soil. *Func Plant Biol* 2005;**32**:695–706.

White TJ, Bruns T, Lee S *et al*. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, In: Innis MA, Gelfland DH, Sninsky JJ, White TJ , (eds). *PCR Protocols: A Guide to Methods and Applications*. Academic Press: San Diego, CA, 1990, pp. 315–22.