Population Dynamics of Wheat Root Pathogens Under Different Tillage Systems in Northeast Oregon

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Abstract

No-till or direct seeding can be described as seeding directly into the crop stubble from the previous season without use of tillage. A reduction in tillage can result in many benefits, including increased soil organic matter, increased water holding capacity, and reduced fuel costs. However, the effect of no-till and reduced tillage on crop root disease profiles is poorly understood. To study the effect of tillage on disease dynamics, soil samples were collected from commercial wheat fields representing a wide range of tillage strategies in fall 2016 and fall 2017. Because precipitation might affect soilborne diseases, wheat fields located across a diverse gradient of precipitation zones of the dryland Pacific Northwest were selected. *Fusarium* spp., *Pythium* spp., and *Rhizoctonia* spp. were quantified from soil samples using soil dilution plating and quantitative PCR (qPCR) assays. Results of dilution

plating showed that the colony counts of *Fusarium*, *Pythium*, and *Rhizoctonia* at the genus level were negatively associated with tillage. However, the same patterns were not observed when specific causal agents of *Fusarium*, *Pythium*, and *Rhizoctonia* that are known to be pathogenic on wheat were quantified with qPCR. Furthermore, precipitation affected the population density of some fungal pathogens (*F. culmorum*, *P. ultimum*, and *R. solani* AG 8). Within the scope of inference of this study, results of this study indicate that the benefits of adopting reduced tillage likely outweigh potential risk for increased root disease.

Keywords: field crops, cereals and grains, disease management, cultural and biological practices, pathogen diversity

Wheat (*Triticum aestivum* L.) is an important staple grain crop to feed a growing human population. In the inland Pacific Northwest (iPNW) of the United States, wheat is the predominant crop, accounting for approximately 17% of the national wheat production (USDA National Agriculture Statistics Service 2017). In the past 20 years, the iPNW has seen an increase of wheat producers adopting a no-till or reduced tillage strategy. No-till acreage for all wheat in Oregon increased from 1% in 1996 to 17% in 2004, and from 3 to 13% in Washington (Smiley et al. 2005b). No-till acreage continues to increase in the iPNW annually (Yorgey and Kruger 2017). Some dryland counties in Oregon, including Wasco and Gilliam, are currently estimated to be over 75% no-till (Sandy McNab, *personal communication*).

No-till or direct seeding can be described as seeding directly into the crop stubble from the previous season without use of tillage that disturbs soil. No-till strategies are highly diverse. Although no-till versus conventional tillage is often discussed as a qualitative scale, it is important to recognize the highly quantitative tillage scales and unique tillage strategies used by individual farms. Many farms may utilize a no-till drill for seeding and fertilizing, but they may also

utilize vertical tillage implements, chisel plowing, field cultivating, or tandem disking to accomplish weed control and/or residue management at critical stages in the production cycle. Management strategies ranging between strict no-tillage and inversion tillage are generally referred to as conservation tillage or mulch tillage. Wheat yields in arid and rainfed conditions, such as in the dryland iPNW (Hagerty et al. 2019), are significantly improved in no-till conditions (Pittellkow et al. 2015).

Soilborne diseases caused by fungal pathogens are important constraints on wheat yield and grain quality (Dada 2017; Kazan and Gardiner 2018; Martin and Loper 1999). A shift in cultural management from conventional tillage to no-till or reduced tillage may present challenges with management of the root rot complex (Cook 2001; Paulitz 2006; Paulitz et al. 2002). The root rot complex, comprising *Fusarium* spp., *Pythium* spp., and *Rhizoctonia* spp., is a major contributor to wheat yield loss in the iPNW. *Fusarium* spp. can cause crown rot or foot rot of wheat. *Fusarium pseudograminearum* and *F. culmorum* are the predominant *Fusarium* species in the iPNW (Poole et al. 2013; Smiley and Patterson 1996; Smiley et al. 2005a). Infected wheat shows symptoms of chocolate to reddish-brown discoloration on roots and lower stem internodes. Genetic resistance to *Fusarium* crown rot is poorly understood (Smiley et al. 2005a). *Pythium* spp. cause root rot stunting and damping-off of wheat. *Pythium* root rot is prevalent in cool, wet soils, typical in direct-seeded wheat fields, and is one of the most widely distributed yield-limiting diseases of wheat in North America (Cook et al. 1980; Higginbotham et al. 2004; Paulitz et al. 2002). Among *Pythium* species, *Pythium ultimum*, *P. irregulare*, *P. abopressorium*, and *P. rostratifingens* were the most important species to wheat. Genetic resistance to *Pythium* is not available (Bockus et al. 2010). *Rhizoctonia* spp. cause rotting of wheat roots, damping-off of seedlings, stunting, and bare patches. According to the hyphal incompatibility, sequence variations, and host specificity, *Rhizoctonia solani* is classified into anastomosis groups (AGs) (Anderson 1982; González et al. 2006). *R. solani* AG 8 was most pathogenic to wheat (Roberts and Sivasithamparam 1986). *Rhizoctonia* root rot and bare patch caused by *R. solani* AG 8 can be severe in direct-seeded wheat (Cook et al. 2002; Paulitz et al.

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2002; Smiley et al. 1996). However, *R. solani* AG 2-1 is pathogenic to dicotyledonous crop species belonging to Brassicaceae, such as canola, and is weakly pathogenic to barley and wheat roots (Tewoldemedhin et al. 2006). Mazzola et al. (1996) and Paulitz et al. (2002) reported that more than 50% of the *Rhizoctonia* spp. isolated from iPNW wheat fields was *R. oryzae*, which caused damping-off and reduced root numbers of wheat, and suggested it might play an important role in wheat root disease. In contrast, Smiley and Uddin (1993) suggested that AG 8 was more important because they found *R. oryzae* did not cause root rots or limit yields of winter wheat. To date, no resistant commercial wheat cultivars are available to growers.

Environmental factors, such as temperature, moisture, soil type, nutrition, and geographical region, affect soilborne pathogens and the diseases they cause. Among them, temperature and moisture are the most important factors and are essential to the growth, survival, and distribution of many pathogens. In a conservation tillage system, increased surface soil moisture and cooler soil surface temperatures, particularly in springtime, may contribute to increased levels of root disease (Bockus and Shroyer 1998; Cook 2001). Wong et al. (1984) demonstrated that there was a significant interaction between temperature and moisture for *F. avenaceum*, *P. irregulare*, and *R. solani* or their combinations. Fusarium crown rot severity was negatively correlated with precipitation and was positively correlated with temperature (Smiley and Yan 2009). Damping-off pathogens are also favored in wet and cool soils (Lamichhane et al. 2017). Furthermore, larger stubble size and intact wheat crowns in conservation tillage systems take longer to break down than smaller substrate pieces that have been broken down with tillage (Smiley et al. 2012). Larger straw pieces with longer time to decay may increase available refugia for pathogenic fungi to complete the lifecycle and infect the following crop.

Tillage has varying effects on root diseases caused by soilborne fungal pathogens, with either increases or decreases in observed diseases (Bockus and Shroyer 1998; Larkin 2015). The effect of no-till or reduced tillage on root diseases of winter wheat is not fully understood. Therefore, additional research is needed to reveal how tillage influences disease dynamics. We hypothesized that the population density of three yield-limiting pathogens (*Fusarium* spp., *Pythium* spp., and *Rhizoctonia* spp.) in soil collected from commercial winter wheat fields would correlate with tillage intensity and annual precipitation zone in the iPNW. The objectives of this study were (i) to examine the relationship between tillage and the population density of *Fusarium* spp., *Pythium* spp., and *Rhizoctonia* spp. using conventional dilution plating and quantitative PCR (qPCR) assays; (ii) to determine how these fungal pathogens are distributed across a range of precipitation zones in Umatilla County, OR; and (iii) to investigate if crop tillage and annual precipitation influence the soilborne pathogens using statistical modeling.

Materials and Methods

Field locations. All wheat farms included in this study represented a wide range of tillage practices in Umatilla County, OR. Total farm acreage ranged from approximately 2,000 acres to 6,000 acres. Surveyed farms practice a winter wheat–summer fallow rotation typical of the region except for one field, which was unexpectedly planted to canola in 2017. Although crop rotation is a key cultural practice to limit soilborne disease, producers within the rainfall zone studied do not have the annual precipitation to support nonwheat crops. Upon sampling each of the 30 fields, photographs of the fields were taken to objectively identify the tillage strategy utilized by the grower. The photographs were organized from no tillage to complete inversion tillage by utilizing key features of tillage strategy: soil clod size and aggregation, and mulch/residue layer on the soil surface. This strategy resulted in a 10-point scale with like fields grouped in the same category, with normal distribution along the 10-point scale. In addition, annual precipitation data were obtained for each sampled field from Climate Engine for 2016 and 2017, respectively (Huntington et al. 2017). Fields surveyed ranged from 21.6 to 55.13 cm of annual precipitation.

Soil sampling. Soil was collected within 3 weeks in fall 2016 and fall 2017 using the same method. Briefly, samples were taken at least 30 m into the field to avoid edge effects. Ten soil cores per field of 30 cm depth by 2 cm diameter were taken every 60 m along a 610-m transect utilizing a stainless-steel soil probe (JMC Soil Samplers, Newton, IA), and GPS coordinates were marked at each of the 10 sample locations along the transect. Cores were collected between rows for a bulk soil sample with minimal root matter (i.e., this was not a soil rhizosphere sample). For this study, only the 0 to 7.5-cm depth was utilized and passed through a 2-mm sieve in preparation for the pathogen quantification procedures. For 2016 samples, 10 cores were randomly divided into two groups of five and combined for dilution plating to result in two representative samples per field. For 2017 samples, the same fields were used, and five of the 10 cores were randomly selected and kept separate for qPCR analysis.

Soil dilution plating and baiting. In 2016, soil was analyzed by soil dilution plating and baiting procedures.

A modified clarified V8 agar selective medium was used to determine *Pythium* spp. populations in soil, which was amended with 10 mg/liter of natamycin, 20 mg/liter of rifampicin, 20 mg/liter of rose bengal, 250 mg/liter of ampicillin, and 120 mg/liter of penta-chloronitrobenzene. For each sample, 10 g of soil was added to 90 ml of 0.1% water agar and mixed thoroughly. Soil solution (0.5 ml) was then pipetted onto a plate of the selective medium and was spread evenly across the surface using a glass cell spreader. Then the plates were cultured at room temperature for 36 h. The total number of *Pythium* spp. colonies per plate was then counted. Each sample included four replicates.

A modified Komada's selective medium was used to determine *Fusarium* spp. populations in soil. Komada's selective medium was amended with 1 mg/liter of benomyl and 1 g/liter of lithium chloride (Komada 1975). Two milliliters of the soil solution from the *Pythium* dilution plating procedure was added to 18 ml of 0.1% water agar and mixed thoroughly. Soil solution (0.5 ml) was then pipetted onto a plate of modified Komada's selective medium and was spread evenly across the surface using a glass cell spreader. The plates were cultured under constant light at room temperature for 1 week, at which point the total number of *Fusarium* spp. colonies per plate was counted. Each sample included four replicates.

To measure *Rhizoctonia* spp. populations in soil, 10 g of sieved soil was added to 1.5 ml of double-distilled water (ddH₂O) in a 15-ml plastic disposable test tube and mixed thoroughly. The cap of the tube was loosely screwed on, and the samples were cultured in the dark at room temperature for 24 h. After incubation, four sterilized toothpicks were inserted to a depth of 5 cm into the soil. The cap of the tube was loosely screwed on, and the samples continued to incubate in the dark at room temperature for 48 h. Then the toothpicks were removed from the soil using sterile forceps and placed onto plates of *Rhizoctonia* selective medium (20 g of agar/liter of deionized water, supplemented with 100 µg/ml of chloramphenicol and 1 µg/ml of benomyl), and *Rhizoctonia* spp. colonies were counted for each toothpick, as described by Paulitz and Schroeder (2005).

qPCR analysis. In order to create the DNA standard curves for qPCR, we used the root fungal pathogen cultures from the collection of T. Paulitz (USDA-ARS, Pullman), which included nine fungal pathogen isolates: *Fusarium culmorum*, *F. pseudograminearum*, *Pythium ultimum*, *P. irregulare*, *P. abapressorium*, *P. rostratifingens*, *Rhizoctonia solani* AG 8, *R. solani* AG2 1, and *R. oryzae*. These fungi were grown in potato dextrose agar at room temperature. Mycelial mats were washed into centrifuge tubes with sterile ddH₂O and centrifuged at maximum speed. The mycelial pellets were then utilized for DNA extraction. Fungal DNA was extracted using the FastDNA Kit (Qbiogene, Carlsbad, CA) according to the manufacturer's instructions and a FastPrep bead beater (MP Biomedical, Santa Ana, CA) using the fungal program.

qPCR primers for *F. culmorum* and *F. pseudograminearum* were designed from the translation elongation factor 1-α gene (TEF1-α). The TEF1-α sequence of the other *Fusarium* spp. was retrieved from GenBank and aligned using ClustalW. Species-specific primers were

designed with a minimum formation of secondary structure and an amplicon size of 100 to 200 bp, as well as using BLAST analysis to avoid cross reactions with other *Fusarium* spp. and fungi (Table 1). qPCR primers for *P. rostratifingens* were designed from the variable regions of ITS1 and ITS2 using similar criteria as *Fusarium* spp. qPCR primers for *Pythium* spp. and *Rhizoctonia* spp. were previously designed by Schroeder et al. (2006, *personal communication*) and Okubara et al. (2005) (Table 1).

The abundance of fungal pathogens from the 2017 samples was analyzed with qPCR assays. Five cores were randomly chosen, and DNA was extracted from individual cores and treated as individual observations. Soil (0.5 g) was extracted using the MoBio PowerSoil kit (MoBio/Qiagen, Carlsbad, CA) according to the manufacturer's instructions and a FastPrep bead beater (MP Biomedical) using the soil program. DNA concentrations were measured using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA).

qPCR was performed in a total volume of 10 µl using KAPA SYBR FAST qPCR Master Mix kit I (Roche Applied Science, Indianapolis, IN) on a Roche LightCycler (Roche Applied Science). Each 10-µl PCR reaction consisted of 5 µl of KAPA SYBR FAST qPCR Master Mix, 2 pmol of each primer, 4.6 µl of fungal DNA or 2 µl of soil DNA (concentration was diluted to 2 ng/µl), and ddH₂O to 10 µl. A standard curve was generated for each pair of primers, using nine fungal pathogen DNAs as a template, respectively. The DNA concentrations for each fungal pathogen were series diluted with 10-fold reductions (DNA concentrations from 4 to 8 ng/µl to 0.4 to 0.8 pg/µl). The PCR program used was 95°C for 3 min, followed by 45 cycles of 95°C for 10 s, 60°C for 20 s, and 72°C for 1 s. To evaluate amplification specificity, melt curve analysis was generated followed by each PCR run. A melt curve profile was obtained by heating the mixture to 95°C for 5 s, cooling to 65°C for 1 min, and then incremental increases of 5 to 10°C up to 97°C with continuous measurement of fluorescence. All samples were amplified in triplicate. Each DNA concentration of fungal pathogens was calculated based on standard curves and expressed as picogram per nanogram of soil DNA.

Statistical analysis. The 2016 soil dilution plating and baiting resulted in a count of colonies for each of three fungal and fungal-like pathogens per sample, with two samples per field. A generalized linear mixed-effects model with the Poisson link function to account for the count response was fit for each fungal pathogen using the “glmer” function in the “lme4” package in R (Bates et al. 2014). Field was treated as a random effect. Fixed effects considered were precipitation, tillage, seeded or fallow, and all interactions between effects. Model selection was conducted independently for each pathogen response variable. Fixed effects to include in the final model were selected with likelihood ratio tests using backward selection.

The 2017 qPCR analysis resulted in an abundance measurement expressed as a concentration for each of nine fungal pathogens per biological replicate, with five biological replicates per field. A linear mixed-effects model was fit for each fungal pathogen using the “lmer” function in the “lme4” package in R (Bates et al. 2014). Field was treated as a random effect. Fixed effects considered were precipitation and tillage, as well as the interaction between tillage and precipitation. Model selection was conducted independently for each pathogen response variable. Fixed effects to include in the final model were selected with likelihood ratio tests using backward selection.

Results

Quantification of fungal pathogens in 2016 field samples using dilution plating assay. *Fusarium*, *Pythium*, and *Rhizoctonia* in the soil samples from 30 fields across four rainfall zones of Umatilla County, OR (Fig. 1) were detected and quantified using the dilution plating assay. Of the three most prevalent soil fungal and fungal-like pathogens, *Fusarium* spp. colony counts averaged across four technical replicates in all samples ranged from 0 to 6,850 CFU/g of soil. Reduced *Fusarium* spp. colony count occurred in fields with increased tillage in 2016 ($P = 0.03$) (Fig. 2A; Table 2). There was no effect of the precipitation in 2016 on *Fusarium* spp. colony counts ($P = 0.895$) (Fig. 2B; Table 2), but it improved the Poisson generalized linear mixed model (GLMM) (Table 2).

Pythium spp. colony counts averaged across four technical replicates in all samples ranged from 0 to 35 CFU/g of soil. Similarly,

Fig. 1. Map of surveyed region of Umatilla County, OR. Each of 30 points represent a surveyed field. Shade of the point refers to precipitation zone of the surveyed field.

Table 1. Species-specific primers of fungal pathogens for qPCR assays

Species	Primer	Sequence (5'→3')	Product size (bp)
<i>Pythium ultimum</i>	ULT1F	GACACTGGAACGGGAGTCAGC	414
	ULT4R	AAAGGACTCGACAGATTCTCGATC	
<i>P. irregulare</i> group IV	IIV7F	GTATCGTCTTGGCGGAGTGG	370
	ABA1R	TGCATAAACGAATATACCAACCGC	
<i>P. abappressorium</i>	ABA1bF	GTTGTTGTGCGTCTGCGGATTG	397
	ABA1R	TGCATAAACGAATATACCAACCGC	
<i>P. rostratifingens</i>	PROSF2	GCGCAGATAGAGAGACTGATTTGG	100
	PROSR2	ACTACCAACACACACACACACG	
<i>Fusarium culmorum</i>	FCKY648945F	AGTACCACTGCATCCCAACC	120
	FCKY648945R	CTTCTCGATGGTTTCGCTTGT	
<i>F. pseudograminearum</i>	FPKY927890F	GTGTCAATCAGTCACTAACAACC	194
	FPKY927890R	GAGGACAATAGTGACACATACC	
<i>Rhizoctonia oryzae</i> genotype III	RoGr3F	CTGTTGAAACCGGTTTACTATG	286
	RoGr3R	CTTCCAAGTCCAAATACAACAATC	
<i>R. solani</i> AG 8	Rs8F	GGGGGAATTTATTCAATTTATTGGAC	327
	Rs8R	GGTGTGAAGCTGCAAAAAG	
<i>R. solani</i> AG 2-1	AG 2-1F	GTTGTAGCTGGCCCATTCATTG	123
	AG 2-1R	CCTATTGCCTTTGTATTCCAAAAGC	

Pythium spp. colony number significantly reduced with increased tillage in 2016 ($P = 0.0009$) (Fig. 3A; Table 2). The precipitation in 2016 slightly affected *Fusarium* spp. colony number ($P = 0.057$) (Fig. 3B; Table 2). Seeded versus fallow did not have an impact on *Pythium* spp. colonies ($P = 0.32$) but improved the Poisson GLMM (Table 2).

Rhizoctonia spp. colony counts averaged across four technical replicates in all samples ranged from 0 to 6.9 CFU/g of soil. Interestingly, both tillage and precipitation in 2016 significantly affected *Rhizoctonia* colony counts. *Rhizoctonia* colony counts were reduced significantly with increased tillage ($P = 0.002$) (Fig. 4A; Table 2) but increased with increased precipitation ($P = 0.01$) (Fig. 4B; Table 2).

Fig. 2. Scatter plots of dilution plating quantification of *Fusarium* spp. colony forming units (CFU) per gram of soil collected from commercial winter wheat fields in Umatilla County, OR, under different tillage and precipitation regimes. **A**, Tillage scale: 0 = no tillage, and 10 = conventional inversion tillage. **B**, Different precipitation. Each point represents one technical replicate.

Table 2. Summary of best fit generalized linear mixed model for colony counts of fungal pathogens of commercial winter wheat fields under different environments in 2016^a

Fungal pathogens	Fixed effects	Coefficient	SE	P value
<i>Fusarium</i> spp.	Intercept	1.960	0.956	0.040*
	Precipitation	0.0004	0.003	0.895
	Tillage	-0.246	0.114	0.030*
<i>Pythium</i> spp.	Intercept	-2.154	1.678	0.199
	Precipitation	0.010	0.005	0.057
	Tillage	-0.691	0.208	0.0009***
	Seeded versus fallow	-0.950	0.948	0.316
<i>Rhizoctonia</i> spp.	Intercept	0.033	0.672	0.961
	Precipitation	0.006	0.002	0.010*
	Tillage	-0.248	0.082	0.002**

^a SE = standard error. Significance levels: *** = 0; ** = 0.001; and * = 0.01.

Quantification of fungal pathogens to species level in 2017 field samples using qPCR assay. qPCR was used to quantify fungal pathogens in the 2017 soil from 30 fields in the same locations. Specific primers were designed to identify and quantify fungal pathogens to species level. *F. culmorum* was detected in 99% of 150 soil samples at DNA concentrations ranging from 0.055 ± 0.033 to 16.804 ± 1.905 pg of DNA/ng of soil DNA. DNA quantity of *F. culmorum* in the soil was slightly affected by tillage ($P = 0.051$) but reduced dramatically with increased precipitation ($P = 0.0007$) (Fig. 5; Table 3). For *F. pseudograminearum*, the qPCR assay showed that 83% of 150 samples had detectable DNA at concentrations ranging from 0.030 ± 0.000 to 43.261 ± 5.487 pg of DNA/ng of soil DNA. However, all fixed effects tested, including tillage and precipitation, did not have an impact on DNA quantity of *F. pseudograminearum* (Fig. 6; Table 3).

The four most prevalent pathogenic *Pythium* spp., including *P. ultimum*, *P. irregulare*, *P. abappressorium*, and *P. rostratifingens*, were analyzed. More than 96% of 150 samples had detectable amounts of all four *Pythium* spp., and DNA concentrations for *P. ultimum*, *P. irregulare*, *P. abappressorium*, and *P. rostratifingens* ranged from 0.007 ± 0.000 to 5.574 ± 0.280 pg of DNA/ng of soil DNA, from 0.035 ± 0.003 to 1.422 ± 0.068 pg of DNA/ng of soil DNA, from 0.0004 ± 0.000 to 7.104 ± 0.678 pg of DNA/ng of soil DNA, and from 0.012 ± 0.001 to 0.095 ± 0.000 pg of DNA/ng of soil DNA, respectively. Interestingly, DNA quantities of three *Pythium* spp. (*P. ultimum*, *P. irregulare*, and *P. abappressorium*) in the soil were significantly reduced with increased tillage, and their P values were 0.026, 0.004, and 0.004, respectively (Figs. 7A, 8A, and 9A; Table 3). Moreover, DNA quantity of *P. ultimum* in the soil was dramatically reduced with increased precipitation ($P = 0.024$). A similar pattern of precipitation impact on *P. irregulare* and *P.*

Fig. 3. Scatter plots of dilution plating quantification of *Pythium* spp. colony forming units (CFU) per gram of soil collected from commercial winter wheat fields in Umatilla County, OR, under different tillage and precipitation regimes. **A**, Tillage scale: 0 = no tillage, and 10 = conventional inversion tillage. **B**, Different precipitation. Each point represents one technical replicate.

abappressorium was not observed (Fig. 8B and 9B; Table 3). However, no significant association was found between the fixed effects tested and DNA quantity of *P. rostratiformis* (Supplementary Fig. 1; Table 3).

Three pathogenic *Rhizoctonia* spp., including *R. solani* AG 8, *R. solani* AG 2-1, and *R. oryzae*, were quantified in the 2017 soil samples with qPCR assay. Only precipitation had an impact on *R. solani* AG 8, and DNA quantity of AG 8 in the soil was negatively associated with precipitation ($P = 0.03$) (Fig. 10A; Table 3). The other fixed effects tested did not have an impact on DNA quantity of three *Rhizoctonia* spp. (Fig. 10B; Supplementary Figs. 2A and B, 3A and B; Table 3).

Discussion

Identification and quantitation of fungal pathogens in commercial wheat field soil is essential for disease control and to implement disease management strategies. In this study, prevalent soilborne fungal pathogens in soil collected from commercial winter wheat fields differing by tillage intensity and annual precipitation zone in the dryland Pacific Northwest were detected and quantified using dilution plating or qPCR assays in a continuous 2 years. Our study revealed higher colony counts of *Fusarium*, *Pythium*, and *Rhizoctonia* at the genus level with reduced tillage. However, the same patterns were not observed when specific causal agents of *Fusarium*, *Pythium*, and *Rhizoctonia* that are known to be pathogenic on wheat were quantified with qPCR. There were three pathogenic *Pythium* species (*P. ultimum*, *P. irregulare*, and *P. abappressorium*) that followed a similar trend as the dilution plating results and were negatively associated with tillage. Precipitation was found to affect some fungal pathogen abundance, such as *F. culmorum*, *P. ultimum*, and *R. solani* AG 8. Within the scope of inference of this study, our findings suggest that

the increase of root disease should not necessarily prevent producers from adopting a no-till strategy.

Soilborne fungal pathogens infect wheat and usually cause very similar symptoms, such as root rot, crown rot, stunting, seedling damping-off, and wilt. It is common that multiple species complexes exist in natural environments (Cook 2010; Moya-Elizondo 2013), which creates a challenge for disease diagnosis and detection. The combination of a conventional method and a molecular-based method, such as dilution plating and qPCR, can provide quantitative data of soilborne pathogens in farm soil, although the threshold of copy numbers and DNA concentrations of the different pathogens in wheat fields to cause disease is unknown. In this study, we employed a dilution plating assay for 2016 soil samples and a qPCR assay for 2017 samples. Two consecutive years of results did not show the same pattern except for three *Pythium* spp. The discrepancy might also be explained by the fact that dilution plating might detect the culturable species including pathogenic and nonpathogenic species but overlook those at low population density or slowly growing species. In contrast, the qPCR assay is very sensitive and capable of detecting specific species, nonculturables, and low densities of fungal pathogens (Okubara et al. 2008; Schroeder et al. 2006; Woodhall et al. 2013). Another reason for the discrepancy could be that the selective media used in dilution plating (Mihail and Singleton 1992) are designed to reduce other fungi contamination but do not eliminate them completely. In addition, fungi may exist in soil with different development stages; for example, *Rhizoctonia* produces dormant sclerotia that are in too low a population to be detectable. Moreover, the population dynamics of these fungal pathogens is very complex; for example, more than 10 *Pythium* species and dozens of *Fusarium* species cause root rot in wheat and barley (Chamswang and Cook 1985; Dweba et al. 2017; Higinbotham et al. 2004; Schroeder

Fig. 4. Scatter plots of soil baiting quantification of *Rhizoctonia* spp. colony forming units (CFU) per gram of soil collected from commercial winter wheat fields in Umatilla County, OR, under different tillage and precipitation regimes. **A**, Tillage scale: 0 = no tillage, and 10 = conventional inversion tillage. **B**, Different precipitation. Each point represents one technical replicate.

Fig. 5. Scatter plots of qPCR quantification of *Fusarium culmorum* pg of DNA/ng of soil DNA collected from commercial winter wheat fields in Umatilla County, OR, under different tillage and precipitation regimes. **A**, Tillage scale: 0 = no tillage, and 10 = conventional inversion tillage. **B**, Different precipitation. Each point represents one technical replicate.

et al. 2006). Out of this complex, only nine predominant species of *Fusarium*, *Pythium*, and *Rhizoctonia* that are pathogenic to wheat were analyzed with qPCR.

Fusarium can produce notoriously variable symptoms in the field (Smiley and Yan 2009) and has a stubble-borne portion of the life-cycle (Bockus et al. 2010). Tillage is often considered as a good practice to reduce the risk of *Fusarium* disease development in cereals (Hofgaard et al. 2016; Munger et al. 2014; Steinkellner and Langer

2004). However, it is sometimes difficult to find a direct link between tillage and *Fusarium* disease development (Lori et al. 2009; Maiorano et al. 2008). In our study, elevated levels of all *Fusarium* in our 2016 dilution plating effort were observed, whereas qPCR on 2017 samples indicated specific causal agents in our region, including *F. culmorum* and *F. pseudograminearum*, are not highly influenced by tillage. Statistical modeling efforts in this study show that *F. culmorum* is influenced by tillage, but we cannot conclude the

Table 3. Summary of linear mixed models for DNA quantity of fungal pathogens of commercial winter wheat fields under different environments in 2017^a

Fungal pathogens		Fixed effects	Coefficient	SE	P value
<i>Fusarium</i> spp.	<i>F. culmorum</i>	Intercept	14.702	2.707	9.59e-06***
		Precipitation	−0.0260	0.007	0.0007***
		Tillage	0.693	0.338	0.051
<i>Pythium</i> spp.	<i>F. pseudograminearum</i>	Intercept	2.231	0.431	1.45e-05***
		Precipitation	1.752	0.307	4.52e-06***
		Tillage	−0.090	0.038	0.026*
	<i>P. ultimum</i>	Intercept	0.808	0.105	2.2e-08***
		Tillage	−0.059	0.019	0.004**
	<i>P. irregulare</i>	Intercept	1.905	0.329	3.19e-06***
		Tillage	−0.186	0.060	0.004**
	<i>P. abapressorium</i>	Intercept	0.048	0.002	<2e-16***
		Precipitation	4.287	1.451	0.0036**
	<i>P. rostratifingens</i>	Intercept	−0.006	0.003	0.038*
<i>Rhizoctonia</i> spp.	<i>R. solani</i> AG 8	Intercept	0.129	0.032	0.0003***
		Precipitation	0.396	0.274	0.159
	<i>R. solani</i> AG 2-1	Intercept			
	<i>R. oryzae</i>	Intercept			

^a SE = standard error. Significance levels: *** = 0; ** = 0.001; and * = 0.01.

Fig. 6. Scatter plots of qPCR quantification of *Fusarium pseudograminearum* pg of DNA/ng of soil DNA collected from commercial winter wheat fields in Umatilla County, OR, under different tillage and precipitation regimes. **A**, Tillage scale: 0 = no tillage, and 10 = conventional inversion tillage. **B**, Different precipitation. Each point represents one technical replicate.

Fig. 7. Scatter plots of qPCR quantification of *Pythium ultimum* pg of DNA/ng of soil DNA collected from commercial winter wheat fields in Umatilla County, OR, under different tillage and precipitation regimes. **A**, Tillage scale: 0 = no tillage, and 10 = conventional inversion tillage. **B**, Different precipitation. Each point represents one technical replicate.

directionality of the response. *F. pseudograminearum* was not influenced by tillage or any of the fixed effects measured in this study. Additionally, highly replicated studies are needed to overcome the inherent in-field variability associated with the causal agents of Fusarium crown rot (Smiley and Yan 2009) to understand drivers of variability and make practical conclusions for wheat producers. Studies have shown that Fusarium crown rot incidence was significantly higher in systems with high stubble retention (Wildermuth et al. 1997). However, the opposite has also been shown; several studies have found less root rot disease under no-tillage compared with conventional tillage (De Boer and Kollmorgen 1988; Salas and Stack 1988; Tinline and Spurr 1991). Other studies show a neutral effect of tillage on soilborne plant pathogens (Conner et al. 1987; Lawn and Sayre 1989; Windels and Wiersma 1989).

Of the soilborne pathogens we assessed, *Pythium* was most highly influenced by tillage in this study. This result could be due, in part, to tillage physically disrupting aspects the *Pythium* life cycle, including germination of the oospore, formation of the zoosporangium, and/or vesiculation of the sporangium. However, the most likely driver of higher *Pythium* spp. in reduced tillage systems is the cooler and wetter soil environment in reduced tillage systems that function to promote *Pythium* infection. *Pythium* infection of winter wheat has been shown to be greatest in cool, wet, high-residue seed beds, which essentially define reduced or no-tillage conditions (Bockus and Shroyer 1998; Cook 2001; Cook et al. 1980; Ingram and Cook 1990). Limited studies support our result of increased *Pythium* in no-tillage systems of winter wheat (Cook et al. 1980; Pankhurst et al. 1995).

Rhizoctonia is arguably the most studied pathogen in regard to tillage. Life cycle features of mycelial growth in soil can be disrupted by tillage and may have the capacity to reduce disease. Tillage may also

change microbial activity in the soil to suppress *Rhizoctonia* (Paulitz 2006; Yin et al. 2017). We found *R. solani* AG 8 in the 2017 soil was negatively associated with precipitation, which supports the observation in dryland wheat production zones of Washington State (Okubara et al. 2014). However, we did not see trends of increased *Rhizoctonia* in reduced tillage environments, because tillage did not improve the model fit for *R. solani* AG 8, *R. solani* AG 2-1, or *R. oryzae*. Our result is in disagreement with several studies that have shown soil cultivation reduces *Rhizoctonia*. The literature on tillage effects with *R. solani* is abundant, especially from Australia, where researchers have documented that disease severity is significantly less in cultivated compared with direct-seeded treatments (MacNish 1985; Pumphrey et al. 1987; Roget 1995; Rovira 1986). *R. oryzae* may not be impacted by tillage as much as *R. solani*, because it infects from microsclerotia that are likely resistant to tillage disturbance (Anderson 1982; Okubara et al. 2014). In addition, cooler, wetter environmental conditions in no-till systems may favor *R. solani*. Greenhouse-based studies have shown that *R. solani* AG 8 causes more severe root rot at low temperatures than at high temperatures, but *R. oryzae* was less influenced by temperature (Smiley and Uddin 1993).

Reduced tillage was invented in the 1960s but did not gain adoption until the 1990s. Since its invention, no-till acreage has steadily increased worldwide; however, uncertainty related to pathogen shifts in no-till management has slowed the adoption of no-till farming globally. A likely cause of the conflicting evidence in the literature regarding pathogen dynamics in no-till systems is the duration of time the field has been managed without tillage. Abiotic benefits of conservation tillage, including increased organic matter, reduced erosion, and increased water infiltration, can take time to be realized by the farmer. Similarly, it may take

Fig. 8. Scatter plots of qPCR quantification of *Pythium irregulare* pg of DNA/ng of soil DNA collected from commercial winter wheat fields in Umatilla County, OR, under different tillage and precipitation regimes. **A**, Tillage scale: 0 = no tillage, and 10 = conventional inversion tillage. **B**, Different precipitation. Each point represents one technical replicate.

Fig. 9. Scatter plots of qPCR quantification of *Pythium abapressorium* pg of DNA/ng of soil DNA collected from commercial winter wheat fields in Umatilla County, OR, under different tillage and precipitation regimes. **A**, Tillage scale: 0 = no tillage, and 10 = conventional inversion tillage. **B**, Different precipitation. Each point represents one technical replicate.

many cropping cycles for changes in microbial communities, including pathogens, to be realized. Reciprocal effects between abiotic and biotic factors may further prolong the changes that occur after a transition to no-till.

The results of this study have a scope of inference limited to commercial winter wheat fields of Umatilla County in a regionally typical wheat-fallow rotation. Our results suggest that potential for increased soilborne disease should not be a major limiting factor for the adoption of no-till technology in the dryland Pacific Northwest. We hypothesize the benefits of adopting no-till technology outweigh the potential risk of increased soilborne disease. And negative pathogen-related effects of a transition from conventional tillage to no-tillage, including increased substrate for residue-borne and soilborne pathogens, are realized immediately. However, positive pathogenic-related effects, including the buildup of competitive and suppressive beneficial soilborne bacteria and fungi, may take many cropping cycles to take effect.

Microbiota management with farming practices could be a key next step to understanding the resilience of a total farming operation. Next-generation technology and a microbiome approach will allow us to utilize beneficial soil microbial communities to support sustainable agriculture and protect farm profitability. Research should support the no-till management strategy so that growers are equipped with data-driven decision-making tools for crop production. This project serves as a first step as we work to provide growers with a set of innovative disease management tools and decision-making strategies that can accommodate no-till and conventional production strategies. Transitioning from conventional tillage to no-tillage requires a large investment of equipment. Farmers need data to support this investment, so they may feel confident in making significant changes to cultural management.

Fig. 10. Scatter plots of qPCR quantification of *Rhizoctonia solani* AG 8 pg of DNA/ng of soil DNA collected from commercial winter wheat fields in Umatilla County, OR, under different tillage and precipitation regimes. **A**, Tillage scale: 0 = no tillage, and 10 = conventional inversion tillage. **B**, Different precipitation. Each point represents one technical replicate.

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