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Increasing the frequency of pulses in crop rotations reduces soil fungal diversity and increases the proportion of fungal pathotrophs in a semiarid agroecosystem

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ABSTRACT

In the Canadian Prairies, the production of pulse crops has increased considerably since the 1980's, including agronomically important crops such as field peas (*Pisum sativum* L.), lentils (*Lens culinaris* Medik.), and chickpeas (*Cicer arietinum* L.). As producers increase and intensify the use of pulse crops, more knowledge is needed to understand the impact these crops have on important soil biological resources. In this study, we used a high-throughput sequencing approach (454 amplicon sequencing) to determine if increasing the frequency of pulses in crop rotations affects the diversity and composition of soil and root-associated fungal communities and the proportion of functional guilds such as pathogens, saprophytes, and mutualists. This study was conducted in a semiarid region of the Canadian Prairies with nine different 4-year rotations including field pea, lentil, and chickpea grown once, twice, three times or not at all with wheat (*Triticum aestivum* L.). Soil fungal communities were assessed following the third year of the rotations and root-associated fungal communities were assessed during the fourth year when all of the rotations were seeded to wheat. Our results revealed that the inclusion of two or more pulses into 4-year crop rotations caused a significant shift in the composition of the soil fungal community, a decrease in fungal diversity, and an increase in the proportion of fungal pathotrophs compared to continuous wheat or rotations with only one pulse crop. Several important pathogens of pulse crops increased two to three-fold in pulse intensified rotations including *Fusarium avenaceum*, *F. redolens*, and *Alternaria alternata*, and crop-specific pathogens such as *Didymella pinodella* and *F. solani* increased in field pea intensified rotations. The build-up of fungal pathogens in the soil indicates that farmers in this region should avoid growing pulse crops in consecutive years or in close succession to avoid developing disease problems. This study also revealed that rotation sequence explained more of the variation in the fungal community compared to the previous crop and affected the relative abundance of several important fungal pathogens. This highlights the importance of crop selection in rotations and provides a tool that farmers can use to manage soil fungal communities to ensure the sustainability and productivity of agricultural systems.

1. Introduction

The inclusion of pulses in traditional cereal-based cropping systems of the Canadian Prairies has dramatically increased in recent years. In Saskatchewan alone, the amount of land seeded to pulse crops has gone from 89,000 ha in 1985–2,407,700 ha in 2015 (Statistics Canada, 2016). The adoption of pulse crops such as field pea, lentil, and chickpea that are well-adapted to the semiarid conditions of the Canadian Prairies has allowed producers to diversify and intensify their crop rotations by reducing the frequency of summer fallow (Gan et al., 2015). Diversifying crop rotations with pulses has been shown to help manage risks associated with changing weather and market patterns (Zentner et al., 2002; Miller and Holmes, 2005), increase the productivity of subsequent crops (Miller et al., 2003), and improve the environmental sustainability of agroecosystems in this region (Gan et al., 2011a,b). The primary factors responsible for the beneficial impact of pulses on subsequent crops or more generally the 'rotation

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effect' include reduced disease levels, increased soil fertility, and higher levels of residual water at lower soil depths (Kirkegaard et al., 2008; Knight, 2012; Wang et al., 2012). However, there are other factors that may play a role in the positive impact of pulses on subsequent crops such as changes in soil structure or the soil microbial community (Kirkegaard et al., 2008).

Soil microbes play an important role in various ecosystem processes that drive the productivity of agricultural systems (van der Heijden et al., 2008). Fungi account for a high proportion of the soil microbial biomass (Joergensen and Wichern, 2008; Högberg and Högberg, 2002) and directly impact crop productivity by playing key roles in nutrient acquisition (Willis et al., 2013; Bolan, 1991), development or suppression of disease (Fernandez, 2007; Sikes et al., 2009), nutrient cycling (Jones et al., 2009; Read and Perez-Moreno, 2003), and formation of soil aggregates (Rillig and Mummey, 2006). There is evidence that pulse crops and even cultivars can differentially alter the composition of soil fungal communities compared to cereal and oilseed crops (Vujanovic et al., 2012; Bainard et al., 2014; Bazghaleh et al., 2015). Other studies in the semiarid region of the Canadian Prairies have also revealed that changes in fungal community composition can have a positive or negative feedback on the productivity of durum wheat depending on the identity of the preceding pulse crop (Ellouze et al., 2013; Taheri et al., 2016). This effect was partly explained by the abundance of endophytic fungal antagonists and pathogens in durum wheat roots following the growth of different pulse crops and cultivars (Taheri et al., 2016). The drawback of these studies is they were limited to studying the culturable portion of the fungal community, which can bias the results. In addition, most studies have focused on the impact of the preceding pulse crop and it is unclear how other crops or multiple pulse crops in a rotation sequence affect the fungal community.

In Saskatchewan, pulses are typically included in crop rotations every three to five years to minimize disease risk (Knight, 2012). However, due to the environmental and economic benefits of including pulses in crop rotations, the goal of this study was to evaluate the impact of increasing the frequency of pulse crops in rotations on the soil and root-associated fungal community. More specifically our objectives were to (1) determine if increasing the frequency of pulses in crop rotations affects the diversity and composition of the fungal community and the relative abundance of functional guilds (i.e., pathogens, saprophytes, and mutualists), and (2) determine whether the full rotation sequence explains more of the variation in the fungal community than the previous crop. The study was conducted in southwestern Saskatchewan, an important growing region for pulse and cereal crops. Field pea (*Pisum sativum*), lentil (*Lens culinaris* Medik.), and chickpea (*Cicer arietinum* L.) were included once, twice, three times or not at all (i.e., continuous wheat [*Triticum aestivum* L.]) in four year crop rotations with wheat. All of the rotations were seeded to wheat in the fourth year to evaluate the impact of increasing the frequency of pulses on the soil and root-associated fungal community using high-throughput sequencing technology (454 amplicon sequencing).

2. Materials and methods

2.1. Site descriptions and experimental design

The field experiment was conducted at the Swift Current Research and Development Centre (SCRDC) of Agriculture and Agri-Food Canada, located near Swift Current, Saskatchewan. The four year crop rotation experiment was conducted from 2010 to 2013 (site 1), and repeated from 2011 to 2014 (site 2) at a different location (site year) at the South Farm of the SCRDC (latitude: 50° 17′N; longitude: 107° 41′W, elevation: 825 m). The soil is an Orthic Brown Chernozem of the Swinton soil association that has a silt loam texture (see Table S1 for summary of chemical properties). The experimental design was a randomized complete block design with nine crop rotations (Table 1) and four replicates. The crop varieties included AC Lillian hard red spring wheat, CDC Meadow field pea, CDC Frontier kabuli chickpea, and CDC Maxim CL red lentil. Plots were 4 m wide and 12 m long, consisting of 16 rows at 25 cm spacing. In year three of the rotations, the crops were seeded at a rate of 83 (wheat), 214 (chickpea), 186 (pea), and 56 (lentil) kg ha^{-1} in site 1, and 91 (wheat), 188 (chickpea), 206 (pea), and 56 (lentil) kg ha^{-1} in site 2. In year four of the rotations, all plots were seeded to wheat at a rate of 100 kg ha^{-1} in site 1 and 97 kg ha^{-1} in site 2. Plots were treated with different fertilizers, herbicides, and fungicides pre- and post-emergence for optimal growth and control of weeds and pathogens (see Niu et al., 2017 for a detailed description of the plot management).

2.2. Sampling

Soil sampling took place in the fall following the harvest of the third year of the rotation at each site to examine the effects of the first three years of the rotations on the chemical properties and fungal communities. Rhizosphere soil samples were collected by digging up 3–4 plants at three randomly selected locations along the second or third row in each plot to a depth of approximately 30 cm. Rhizosphere soil was collected by brushing soil attached to roots into Ziploc bags and stored at −80 °C prior to DNA extraction. Root samples were collected at the mid bloom stage in the fourth year of the rotations to assess the effect of the rotations on the root colonizing fungal community associated with wheat plants. Root samples collected from three randomly selected plants in each plot were thoroughly washed and stored at −80 °C prior to DNA extraction.

2.3. Molecular analysis

Genomic DNA was extracted from 1 g of rhizosphere soil using the Ultra Clean Soil DNA Isolation Kit (MoBio) following the manufacturer's recommended protocol. To extract genomic DNA from root samples, 100 mg of fresh roots were placed in 2 ml screw-top microcentrifuge tubes with a 5 mm ceramic bead, frozen in liquid N_2, pulverized to a powder in a bead mill for 30 s, and then genomic DNA was extracted using a DNeasy Plant Mini Kit (Qiagen) following the manufacturer's recommended protocol.

The ITS1F/ITS2 primer set (Gardes and Bruns, 1993; White et al., 1990) was used to amplify the ITS1 region of the fungal community. The forward primer (ITS1F) included the A adaptor and a 10-bp MID (1 of 20 different Roche MIDs), and reverse primer (ITS2) included the B adaptor. Each 20 μl PCR mixture contained 16 μl of Platinum Supermix (Invitrogen), 1.6 μl of distilled water, 0.2 μl of each 20 μM primer, and 2 μl diluted (1:10) DNA. Thermocycler conditions consisted of an initial denaturing step at 95 °C for 3 min, 30 cycles of 45 s at 94 °C, 45 s at 55 °C and 75 s at

Table 1
Sequence of crops grown in the nine different 4-year rotations.

Rotation	Pre-test	Year 1	Year 2	Year 3	Year 4
WWWW	Wheat	Wheat	Wheat	Wheat	Wheat
PWWW	Wheat	Pea	Wheat	Wheat	Wheat
CWWW	Wheat	Chickpea	Wheat	Wheat	Wheat
PWPW	Wheat	Pea	Wheat	Pea	Wheat
PPPW	Wheat	Pea	Pea	Pea	Wheat
LWLW	Wheat	Lentil	Wheat	Lentil	Wheat
LLLW	Wheat	Lentil	Lentil	Lentil	Wheat
CWCW	Wheat	Chickpea	Wheat	Chickpea	Wheat
CCCW	Wheat	Chickpea	Chickpea	Chickpea	Wheat