Applied Soil Ecology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Rhizosphere microbial communities of canola and wheat at six paired field sites

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ARTICLE INFO

Keywords:
Canola
Crop rotation
Glucosinolates
Microbial community
PLFA
Wheat

ABSTRACT

Plant physical and chemical characteristics are known to alter rhizosphere microbial communities, but the effect of introducing canola (*Brassica napus* L.) into monoculture wheat (*Triticum aestivum* L.) rotations is not clear. Results from a field study in eastern Washington showed that winter canola (WC) influenced the bulk soil microbial community and differentiated it from the community associated with winter wheat (WW). Abundance of soil fungi, including mycorrhizae, was reduced with the introduction of WC. The objective of this research was to determine the differences and similarities in the rhizosphere microbial communities of WC and WW. Canola and wheat rhizosphere soil was collected from six dryland farms in Adams and Douglas Counties, WA. Each farm was a paired site with WC and WW grown in adjacent fields of the same soil type, landscape orientation, and crop history. Canola, or any non-cereal crop, had never been grown previously at the experimental sites. Rhizosphere microbial biomass and community composition, determined using phospholipid fatty acid analysis (PLFA), revealed differences associated with landscape position at the initial fall sampling when WC and WW were in the rosette and tiller development stages, respectively. However, data from spring samples, when WC and WW were in the early bolting and stem elongation growth stages, respectively, showed significant differences in microbial communities between WC and WW rhizosphere soils. Data suggest that initial (fall) microbial community composition were an artifact of previous histories of monocrop wheat production and varied with expected differences in landscape position. As the crops developed, microbial communities became more dissimilar and were differentiated by crop species. Our results show that WC can have significant effects on rhizosphere microbial biomass of specific microbial groups and community structure in wheat-based cropping systems, such as reductions in fungi and gram+ bacteria in some locations. Crop-related changes in the abundance of specific rhizosphere microbial groups may play a key role in the yield of subsequent crops, and overall soil health and nutrient turnover.

1. Introduction

Root exudates are specific according to plant species (Jones et al., 2004; Zhalnina et al., 2018), and potentially vary even down to genotype (Donn et al., 2014; Sasse et al., 2017), This specialization facilitates development of particular rhizosphere microbial communities (Berg and Smalla, 2009; Edwards et al., 2015). Rhizosphere microorganisms associate with plant roots and can be beneficial, harmful, or neutral for both the plant and the organism (Lynch, 1990). In agroecosystems, the interactions of rhizosphere microorganisms, both with the host plant and with other members of the rhizosphere community, are important due to the potential impact on plant growth, health and crop yield (Raaijmakers et al., 2009; Mahoney et al., 2017).

In many cases, the largest influences on the structure of the microbial community are the edaphic factors from which the soil was formed, including texture, mineral composition, and precipitation regime (Germida et al., 1998; Berg and Smalla, 2009; Buyer et al., 2010). However, many recent studies are finding that plant species, variety, and even physiological stage of growth can impact root exudates composition, and subsequently the composition and function of the

Abbreviations: AM fungi, arbuscular mycorrhizal fungi; BGA, β-glucosidase enzyme activity; CV, canonical variate; DEA, dehydrogenase enzyme activity; DFA, discriminant function analysis; Gram−, Gram negative bacteria; Gram+, Gram positive bacteria; GSLs, glucosinolates; ITCs, isothiocyanates; PLFA, phospholipid fatty acid analysis; PNW, Inland Pacific Northwest of the USA; SW, spring wheat; T-PLFA, total phospholipid fatty acids; WC, winter canola; WW, winter wheat; 2-PEITC, 2-phenylethylisothiocyanate

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https://doi.org/10.1016/j.apsoil.2018.06.012
Received 6 February 2018; Received in revised form 28 May 2018; Accepted 16 June 2018
0929-1393/ Published by Elsevier B.V.

rhizosphere microbial community (Peiffer et al., 2013; Donn et al., 2014; Edwards et al., 2015; Mahoney et al., 2017; Zhalnina et al., 2018). Consequently, microbial communities vary with previous management (Donn et al., 2014), different crops in a rotation sequence (Grayston et al., 1998; O'Donnell et al., 2001) and patterns are frequently found to be associated with plant species (Marschner et al., 2001; Ladygina and Hedlund, 2010). From the microbial pool of a given soil, plants select and restructure microbial communities that will continue to develop and increase in diversity as the plant matures (Mougel et al., 2006; Chaparro et al., 2014; Donn et al., 2014). Simultaneously, plants may experience either enhanced or compromised performance in their association with the rhizosphere microbial community (Berendsen et al., 2012).

Many studies have shown plant species significantly influence the rhizosphere microbial community through specific composition of plant-derived carbon (Lu et al., 2002; Farrar et al., 2003; Ladygina and Hedlund, 2010). Plant species differ in carbon resources they produce which affect the structure and function of the rhizosphere microbial community (Grayston et al., 1998; Berg and Smalla, 2009). In addition to carbon, phytochemicals released from the decomposition of residues and root exudates have vast potential to affect rhizosphere community composition (Dong et al., 2014) and associated nutrient cycling (Kirkegaard et al., 1999; Ryan et al., 2006; O'Sullivan et al., 2017). The root exudates of brassica crops, such as canola, contain glucosinolates (GSL), which upon cell rupture and decay of residue hydrolyze to produce isothiocyanates (ITC). The GSL, and their hydrolysis product ITC, of brassica crops are of interest because of their allelopathic properties and potential use as a biofumigant (Brown and Morra, 1997; Matthiessen and Kirkegaard, 2006; Rumberger and Marschner, 2003).

The predominate GSL found in canola roots is gluconasturtiin (Sarwar et al., 1998) which hydrolyzes to form 2-phenylethylisothiocyanate (2-PEITC). Glucosinolates are continuously released to the rhizosphere as roots expand and surface cells senesce (McCully et al., 2008). Brassica root tissue content of GSL and ITC is greater compared to the above-ground tissues, and is in direct contact with soil microorganisms, particularly rhizosphere organisms (van Dam et al., 2009). Therefore, the release of ITC from brassica roots effectively suppresses some soil-borne pathogens (Haramoto and Gallandt, 2004), and influence soil and rhizosphere microbial communities (Rumberger and Marschner, 2003; van Dam et al., 2009). It follows that canola as a rotational crop has the potential to disrupt a soil microbial community adapted to non-GSL producing crops (Smith et al., 2004; Valetti et al., 2016).

In a 6-year on-farm rotation study conducted near Davenport, WA, Schillinger and Paulitz (2018) reported grain yield for spring wheat (SW) after WC was significantly reduced by an average 17% compared with SW after WW. Measurements of soil moisture, soil nutrients, foliar and root diseases, weeds, and root lesion nematodes were unable to explain the yield reduction. Schillinger and Paulitz (2018) found pronounced visual reduction in SW plant height and spike density after WC versus WW and speculated that the difference could be related to soil microbiology. The yield decline of wheat following canola documented by Schillinger and Paulitz (2018) does not agree with the most reports from around the world on the effects of canola and other break crops in wheat-based systems. Several studies in Australia and the Canadian and northern US Great Plains have reported positive benefits of canola on subsequent wheat yield (Angus et al., 2015; Kirkegaard et al., 2008; Kirkegaard and Ryan, 2014; Larney and Lindwall, 1994). Using PLFA and microbial enzymes, Hansen et al. (2018) determined microbial activity, biomass, and community composition of bulk soil associated with WC and WW in the Davenport, WA study mentioned above. Abundance of fungi, mycorrhizae, and total microbial biomass were significantly less in WC compared to WW. The reduction in fungi and mycorrhizae was also observed in SW following WC, indicating a residual effect. Based on these findings, we decided to continue field and laboratory research on the direct influence of canola roots on rhizosphere communities.

The objective of the study reported here was to investigate the short-term impact of canola as a rotational crop on the rhizosphere microbial communities of WC versus WW. We hypothesized that the microbial communities would be differentially influenced by exposure to root exudates of canola and demonstrate pronounced patterns of reduced microbial biomass and shifts in the rhizosphere community composition of canola compared to wheat.

2. Materials and methods

2.1. Site description and experimental design

A field experiment was conducted during the 2016 crop year at six on-farm sites in the low (< 300 mm annual) precipitation zone of east-central Washington. Three farms were located in Adams County and three farms in Douglas County. At each site, WC and WW were grown in adjacent fields of the same soil type and topography. On all fields, the only crop produced in the last 60 years was wheat. A monoculture WW-summer fallow rotation is dominant throughout the low-precipitation zone and was the rotation utilized at all sites in this study. Latitude and longitude coordinates, previous crop, and crop rotation for each field are listed in Table S1 of the Supplementary Data. The soil at the Adams County sites is a Ritzville silt loam (coarse-silty, mixed, superactive, mesic Calcidic Haploxeroll) with 0–5% slope and a depth of greater than two meters to restrictive layers (NRCS, 2018). The soil at the Douglas County sites is a Taunton loamy fine sand (coarse-loamy, mixed, superactive, mesic Xeric Haplodurids) with 0 to 10% slope and a depth of 0.5–1.0 m to restrictive layers (NRCS, 2018).

The experimental design was split plot with each of the six farms/sites used as replicates. Winter canola and WW were the whole-plot treatments at each farm. Two landscape positions of draw (D) and ridge (R) were the subplot treatments, resulting in four treatment combinations (WC-R, WC-D, WW-R and WW-D). A sample from each of the four treatments was a composite of rhizosphere soil from five plants within one meter of each other.

Rhizosphere soil is defined here as soil loosely adhering to roots after the plant is extracted from the ground. Samples were obtained by first moving aside surface debris from the base of the plant, then excavating the plant root mass with a spade (Sullivan et al., 2013). Excavation was to a depth of 45 cm with the bulk of the root mass not extending beyond 30 cm. Rhizosphere soil was obtained by removing the plant from the soil and shaking off the loosely adhering soil from the roots. This soil was collected and used for subsequent analysis. During collection of the sample, care was taken to avoid root damage and to exclude any root material from the sample.

Fall samples were collected in mid-November 2015 and spring samples collected in late March 2016. At the November sampling, WC was at growth stage 2 (rosette stage) (Sylvester-Bradley and Makepeace, 1984) and WW was at growth stages 2 to 3 (tillers forming) (Large, 1954). In late March, WC was at growth stages 2 to 3 (advanced rosette and early bolting) and WW was at growth stage 4 (stem elongation). Samples were immediately transported on ice in the dark to the USDA laboratory located in Pullman, WA. Subsamples of the composite rhizosphere soil were collected in sterile tubes and stored at −80 °C until analysis.

2.2. Rhizosphere soil chemical analyses

Soil pH and electrical conductivity (EC) were determined by preparing a slurry of 1:1 soil to distilled, deionized water (McLean, 1982). Centrifuge tubes containing the soil slurry were mixed end-over-end overnight at room temperature. The slurry was then centrifuged at 4000 rpm to separate the solid from the liquid. The pH of the soil solution was determined with an Orion Research 811 (Boston, MA) pH meter, and EC was measured using a digital conductivity meter (VWR