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Preceding crop and weed management history affect denitrification and denitrifier community structure throughout the development of durum wheat

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ABSTRACT

Aside from negative impacts on crop yield, the role of weeds in agricultural systems, particularly with respect to below ground function, is not well understood. In this study, we utilised a mesocosm approach with soils collected from a long-term rotation study to compare legacy effects of previous flax (*Linum usitatissimum* L.) or canola (*Brassica napus* L.) and different levels of weediness established over a decade by different herbicide use patterns. Despite few significant differences in initial soil chemical properties, the impact of level of weediness superseded that of the preceding crop and altered the growth of durum wheat (*Triticum durum* L.) during early development and the temporal dynamics of denitrification and the *nirK* denitrifier communities. The impact of the presence of durum plants in the mesocosm experiment was relatively small, however, the presence of durum plants did modify the legacy effects of preceding crop and weediness on the denitrifier community. Differences in denitrifier community structure were due to differential presence/absence and differential abundance of multiple T-RFs. To our knowledge, this is the first study to investigate the effects of long-term weed management strategies on denitrification. Our results indicate that weediness contributes to priming effects and unexplained variation associated with these soil processes and that weed management history should be taken into consideration when determining soil function and soil microbial community dynamics in agricultural systems.

1. Introduction

Plant species and plant communities influence soil microbial communities and soil function. In natural systems, plant diversity is associated with soil microbial diversity (e.g., van der Heijden et al., 1998; Bardgett et al., 1999) and both are important for plant productivity (e.g., Tilman et al., 2006; Maron et al., 2011). Below-ground, plant functional diversity can be more important than species richness at enhancing the reliability of soil microbial respiration, collembola densities and the presence of decomposers (Milcu et al., 2010). Plants culture unique, species- and cultivar-specific microbial communities in the rhizosphere, the thin layer of soil surrounding the roots which is thought to be driven in part by the directed release of root exudates (Berg and Smalla, 2009).

Plant species diversity contributes to ecosystem functioning. In monoculture agriculture, weeds are usually the only source of plant diversity and scientists are recognizing the above- and below-ground value of weeds. In the UK, scientists are focusing on the contributions of weeds to above-ground diversity of birds (Storkey and Westbury, 2007) and are determining the above-ground functional characteristics of weeds (e.g., Hawes et al., 2005). The recently developed resource pool diversity hypothesis (RPDH) (Smith et al., 2010) focuses on the role of plant diversity on resource pools in the soil and predicts that increased plant diversity moderates plant competition. In light of this, there is a need to better understand the role of plants and plant communities at shaping soil microbial communities and soil function in agricultural systems.

Soil microbes mediate a number of critical ecosystem functions including nutrient transformations (e.g., nitrogen, carbon cycling) with many important implications such as greenhouse gas production (e.g., N_2O, methane, CO_2). Denitrification, the use of

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nitrogen oxides as alternative electron acceptors is an important indicator of soil function in agricultural soils as this process, if incomplete, transforms mineral nitrogen to the potent greenhouse gas nitrous oxide (Knowles, 1982). Denitrification is mediated by a large group of mainly free-living soil bacteria and is influenced by carbon, oxygen and nitrate in the soil (Giles et al., 2012). Nevertheless, there is mounting evidence that plant species and plant community composition (Bremer et al., 2009) modify the denitrifier community and can influence denitrification. Increased denitrification has been shown in the rhizosphere of barley (Klemedtsson et al., 1987) and the presence of active nitrite reductase has been documented in the rhizosphere of wheat (Metz et al., 2003). The rate of denitrification may be influenced by the presence or absence of plants and the concentration of nitrate also contributes to this relationship (Smith and Tiedje, 1979; Qian et al., 1997). In addition, the composition of the denitrifier community is also influenced by the presence or absence of plants (Cheneby et al., 2003; Marschner et al., 2004; Costa et al., 2006).

The temporal dynamics of denitrifier communities, however, are not well understood although they are known to change over time in abundance (e.g., Dandie et al., 2008) and composition (e.g., Bremer et al., 2009). The relative importance of the factors that influence denitrifier community structure is not the same as those affecting denitrification. For example, root carbon exudates can significantly increase denitrification, but may not always have a significant effect on the structure of the denitrifier community (e.g., Mounier et al., 2004). The effect of plant species on denitrifier community structure is less clear. Using DNA based techniques, Deiglmayr et al. (2004) did not observe a plant species effect on the denitrifier community between the rhizospheres of *Lolium perenne* L. and *Trifolium repens* L. However, species and species combination-specific effects on the denitrifier community structure have been reported in grassland species (Patra et al., 2006; Bremer et al., 2009) and increased functional diversity has been shown to buffer the temporal variability in denitrification (McGill et al., 2010).

Sustainable crop intensification, demanding an increase in crop productivity while simultaneously reducing environmental impacts of agriculture, requires a better understanding of the contribution of plants to below-ground processes. Contemporary agricultural systems are simple and rely almost exclusively on monoculture where a single crop species dominates large areas of the landscape. These systems require high levels of energy and technology to maintain productivity and their simplicity has been associated with a decline in soil organic matter (Williams et al., 2005) and a buildup of plant pathogens (Schnitzer and Klironomos, 2011). Modern herbicide programs are highly efficient and effective and have led to a general decrease in weed densities and concomitant increase in weed spatial aggregation over the past four decades (e.g., Leeson et al., 2005). In western Canada, for example, this has led to significant areas in fields that are devoid of plant diversity following the application of herbicides. The impact of this on soil microbial communities and function is not understood.

In 1999, a long-term field experiment was initiated at the University of Manitoba to evaluate the effects of in-crop herbicide omission. Part of the study consists of an annual crop rotation typical of the region at that time [flax (*Linum usitatissimum* L.) – oats (*Avena sativa* L.) – canola (*Brassica napus* L.) – wheat (*Triticum aestivum* L.)] managed using no-till methods (Schoofs et al., 2005). In each block, this rotation is repeated three times and subjected to different in-crop herbicide-use regimes including: (i) the control rotation (low seedbank density), where selective in-crop herbicides were applied to all annual crops, (ii) the same rotation where in-crop herbicides have not been applied to the oat crop (medium seedbank density) and (iii) the same rotation where in-crop herbicides have been omitted from each oat and flax crop (high seedbank density). These herbicide-use patterns have resulted in distinctly different above ground weed population densities (Schoofs et al., 2005). Over time, this shift is also reflected in weed seedbank populations where the lowest in-crop herbicide-use (2 in 4 years) has resulted in a more than 2-fold greater weed seedbank population density throughout all crops in rotation while the intermediate treatment (3 in-crop herbicides in 4 years) showed marked differences in seedbank densities following the flax crop when compared to the highest in-crop herbicide use intensities, but only an average increase of 30% over all crops in rotation (Gulden et al., 2011). Changes in weed seedbank community composition among the treatments were relatively minor. Weed seedbanks are a reflection of management practices (Cavers, 1995) and have been proposed to play a functional role in agro-ecosystems (Franke et al., 2009) although relatively little is known about this functional role. This field experiment forms a platform from which to address the longer-term impacts of subtle changes in weed management on crop performance, below-ground function and plant-microbe interactions.

The objectives of this study were to investigate the legacy effects of different intrinsic weed population densities and divergent preceding crops (flax and canola) on denitrification and associated denitrifier community dynamics utilising planted and unplanted mesocosms over time. The following questions were addressed: (i) What are, and how important are the legacy effects of preceding weed density and crop on denitrification in planted and unplanted mesocosms over time? and (ii) using *nirK* and *nirS* genes as markers what are, and how important are the legacy effects of preceding weed density and crop type on denitrifier community dynamics in planted and unplanted mesocosms over time? We expected higher weed population densities to contribute to increased denitrification and drive different denitrifier communities in soils with different preceding crops and weediness treatments. We also expected the durum plants in the planted mesocosms to moderate the legacy effects of weediness and preceding crop on the denitrifier communities over time compared to unplanted mesocosms.

2. Materials and methods

2.1. Soil collection and preparation

Soils for this experiment were collected from a long-term field experiment described elsewhere (Schoofs et al., 2005; Gulden et al., 2011). In brief, part of the experiment consists of an annual crop rotation (flax–oats–canola–wheat) arranged as an RCBD with three blocks. The experiment was fully-phased, where all crops were present each year and was managed using no-till. Before seeding, weeds were managed using glyphosate only in all plots. No pre-emergent, soil-residual herbicides were used. The specific herbicide programs are described in detail in Gulden et al. (2011). Briefly, all canola grown throughout the study was resistant to glufosinate and received one in-crop application of this herbicide in all three rotations. In flax, in-crop herbicides included the active ingredients bromoxynil, MCPA and sethoxydim and were applied to the low and medium seedbank density rotations only while flax in the high seedbank density rotation did not receive an in-crop herbicide application throughout this study. In wheat and oats, the crops not sampled for this experiment, the in-crop herbicides were clodinafop and thifensulfuron/tribenuron and the in-crop herbicides used in the oat crop in the low seedbank density rotation only was thifensulfuron/tribenuron. No additional fungicides and insecticides were applied or needed between 2005 and 2011. The resulting weed biomass (Schoofs et al., 2005) and weed seedbanks (Gulden et al., 2011) have been described in detail elsewhere.