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Pyrosequencing reveals profiles of soil bacterial communities after 12 years of conservation management on irrigated crop rotations

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ABSTRACT

Potato and sugar beet, which are widely grown in southern Alberta, may degrade soil quality because they return little C to the soil, and their harvesting methods cause soil disturbance that increases erosion risk. To reverse these effects, a 12-yr study was established to evaluate soil conservation (CONS) management systems for rotations that included potato, sugar beet, dry bean and wheat. These systems, comprising addition of feedlot manure compost, reduced tillage, diverse crop rotations and use of cover crops, were applied to 3- to 5-yr crop rotations. They were compared with conventional (CONV) management systems that did not have any of the CONS practices. In the final year of the study, pyrosequencing was used to determine differences in soil bacterial community profiles between the two systems (CONS vs. CONV) in wheat rhizosphere and bulk soil. Thirteen phyla were observed, and the most abundant were Proteobacteria (39.6%), Actinobacteria (19.1%) and Acidobacteria (14.9%). Soil bacterial α-diversity increased under CONS relative to CONV management. However, whereas the relative abundances of Bacteroidetes and Firmicutes were greater under CONS than CONV management, the reverse was observed for Acidobacteria and Gemmatimonadetes. Proteobacteria were also more abundant under CONS than CONV management, but only in bulk soil. The community structures of the bacterial communities were in agreement with the differences in relative abundances. These differences were consistent with the ecological classification of soil bacteria as copiotrophic or oligotrophic. Therefore, CONS management systems altered the soil bacterial community profiles and increased the productivity of these soils.

1. Introduction

Growing root crops like potato (*Solanum tuberosum* L.) and sugar beet (*Beta vulgaris* L.) usually results in soil degradation, yet their acreage and that of dry bean (*Phaseolus vulgaris* L.) in southern Alberta has increased 2- to 3-fold recently (Alberta Agriculture and Forestry, 2015). Soil degradation occurs due to reductions in soil organic C because these crops produce less biomass and therefore return less C to the soil than the cereal or forage crops that they replace in irrigated crop rotations (Li et al., 2015). In addition, potato is usually grown on raised beds which require extra tillage passes, and the harvesting methods of both potato and sugar beet necessitate greater soil disturbance, making the soil susceptible to wind and water erosion (Chow et al., 1990; Carter and Sanderson, 2001). Annual soil losses by water erosion from continuous potato plots on 8 and 11% slopes have been estimated at 17 and 24 Mg ha^{-1} yr^{-1}, respectively, in New Brunswick, Canada (Chow et al., 1990). Eroded soils lose some of their organic C and nutrients, and soil tillage further reduces organic C by accelerating its decomposition (Lupwayi et al., 2004).

A field study was conducted from 2000 to 2011 to determine if soil conservation practices could be applied to southern Alberta irrigated cropping systems to address the soil degradation issues. These practices included addition of feedlot manure compost, reduced tillage, use of cover crops, and solid-seeded narrow-row dry bean, applied as a package. Yields of dry bean (Larney et al., 2015), potato (Larney et al., 2016b) and sugar beet (Larney et al., 2016a) increased under conservation management relative to conventional management. The increased crop yields were probably a result of the soil building nature of the soil conservation practices as indicated by increases in soil organic C and total N under CONS management during the study (Larney et al., 2017). The stability of soil aggregates also improved significantly under CONS management relative to CONV management (Li et al., 2015).

An examination of the soil microbial responses to these soil conservation practices is also important. Soil microbial properties are

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sensitive indicators of soil quality because they respond quickly to changes in soil management (Doran et al., 1996; Bruggen and Semenov, 2000; Stone et al., 2016), and in addition, the soil microbial community mediates many key biological processes for sustainable agriculture. These processes include biological nitrogen fixation (Jensen and Hauggaard-Nielsen, 2003; Gaby and Buckley, 2011), biomass decomposition and nutrient cycling (Schneider et al., 2012; Lupwayi and Soon, 2015), formation and maintenance of soil aggregates (Six et al., 2004; Blaud et al., 2012), biological disease and pest control (Janvier et al., 2007; Mendes et al., 2011), detoxification of agro-chemicals (Shelton and Doherty, 1997; Itoh, 2014) and regulation of climate through C and N cycles (Baldock et al., 2012; Gregorich et al., 2015). Using phospholipid fatty acid biomarkers in the final two years of the current field study, Lupwayi et al. (2017) reported greater soil microbial biomass (including that of fungi, Gram-negative bacteria, Gram-positive bacteria and actinomycetes) under CONS management than under CONV management. Soil β-glucosidase activity was also greater under CONS management than under CONV management. The objective of this study was to expand on those results by using next-generation sequencing to examine how CONS management affected soil bacterial diversity, composition and community structure by the final year of the 12-yr study.

2. Materials and methods

2.1. Study location, experimental treatments and management

A 12-yr (2000–2011) irrigated field study was conducted at Vauxhall, Alberta (50° 03′ N, 112° 09′ W, elev. 781 m). The soil was a Brown Chernozem (Soil Classification Working Group, 1998) (Haplic Kastanozem in World Reference Base) which had 520 g kg^{-1} sand, 340 g kg^{-1} silt, 140 g kg^{-1} clay, and 12.9 g organic C kg^{-1} soil and pH 6.9 (0–15 cm depth) at the start of the experiment. It is a typical soil type of southern Alberta. The 30 year (1971–2000) mean annual precipitation in the research area was 303 mm with a mean annual air temperature of 5.7 °C.

The entire plot area was planted to barley (*Hordeum vulgare* L.) in 1999 and treatments were established in the spring of 2000. There were six rotation treatments: continuous (monoculture) wheat (*Triticum aestivum* L.), two 3-yr rotations, two 4-yr rotations, and one 5-yr rotation (but with two wheat phases that were each sampled for this study) (Table 1). These rotations were managed utilizing CONV or CONS management practices (described below). All crop phases of each rotation were grown each year to account for varying environmental conditions over years. The treatments were arranged in a randomized complete block design (RCBD) with four replicates. The individual plot sizes were 10.1 by 18.3 m with 2.1 m buffer zones between plots.

For the CONS rotations (Table 1), a package of the following four practices was applied (for details, see Larney et al., 2015, 2016a,b):-

1. Direct seeding and/or reduced tillage where possible in the rotation.
2. Fall-seeded cover crops: oat (*Avena sativa* L.) and fall rye (*Secale cereale* L.) with entry points detailed in Table 1. However, due to sub-optimal fall establishment of oat, it was replaced with fall rye from fall 2003 onward. Oat cannot be used as a cover crop in the study area because it gets killed by frost before it produces enough dry matter to cover the soil.
3. Composted cattle manure: straw-bedded beef cattle feedlot manure compost (182, 15.4, and 5.4 g kg^{-1} dry wt. of total C, N, and P, respectively) was fall-applied to supply some of the N and P required (as determined by soil testing). The rest of the required N was supplied by inorganic fertilizer. A rate of 42 Mg ha^{-1} (fresh wt) was applied after dry bean and before potato in the 4- and 5- CONS rotations (Table 1). The shorter 3-CONS rotation received a lower rate (28 Mg ha^{-1}, fresh wt) after wheat and before potato. This lower rate was also applied at a second entry point in the 5-yr CONS rotation − after sugar beet and before wheat (Table 1).
4. Solid-seeded, direct-cut, narrow-row (19–23 cm) dry bean. There was no inter-row cultivation, and the crop was harvested by direct-cutting (no soil disturbance) with a plot combine.

Conventional management did not use any of the above practices, and hence the 3- and 4-yr CONV rotations (a) had more intensive tillage, (b) no cover crops, (c) no compost amendments (all nutrients were supplied by inorganic fertilizers), and (d) dry bean was grown in wide rows (60 cm), with inter-row cultivation for in-season weed control, and undercutting at harvest (causing soil disturbance and unanchored residue).

Tillage intensity was reduced as much as possible under CONS compared with CONV management. Crops were fertilized according to soil test recommendations, with soil testing done every three years. The crops were irrigated, using a wheel-move system, to maintain soil water (to 100 cm depth) at no lower than 50% field capacity, which was considered to be sufficient to eliminate moisture stress as a factor. Pre-seeding, in-crop and post-harvest herbicides were used as required for weed control. Each fall, mature crops were harvested for yield and quality assessment.

2.2. Microbiological soil sampling

In the final year (2011) of the 12-yr study, soil samples were collected in the wheat phase of 1- to 5-yr rotations. The samples were taken at the flag leaf growth stage of wheat on July 28. Plants were excavated from four random 0.5-m lengths of row. Loose soil was removed from the roots by shaking the plants vigorously by hand, and the remaining soil that had strongly adhered to the roots was carefully brushed off and recovered as rhizosphere soil. Bulk soil (0–7.5 cm depth) was sampled from the middle of two adjacent crop rows at four locations per plot. The four bulk or rhizosphere soil samples from each plot were combined, respectively, passed through a 2-mm sieve, stored

Table 1
Description of treatments indicating management differences from 2000 to 2011.

Rotation[a]	Crop sequence[b]	No. of crop phases	Tillage	Nutrient source	Cover crop	Bean row spacing[d]
1-CONT	<u>W</u>	1	Reduced	Fertilizer	None	No bean
3-CONV	P–B–<u>W</u>	3	Conventional	Fertilizer	None	Wide
3-CONS	P–B–<u>W</u>	3	Reduced	Fertilizer/Compost after W	After P & B	Narrow
4-CONV	P–<u>W</u>–SB–B	4	Conventional	Fertilizer	None	Wide
4-CONS	P–<u>W</u>–SB–B	4	Reduced	Fertilizer/Compost after B	After P	Narrow
5-CONS1[c]	P–<u>W</u>–SB–W–B	5	Reduced	Fertilizer/Compost after SB & B	After P	Narrow
5-CONS2[c]	P–W–SB–<u>W</u>–B	5	Reduced	Fertilizer/Compost after SB & B	After P	Narrow

[a] Integers refer to the length (years) of each rotation; CONT = Continuous wheat; CONV = conventional management; CONS = conservation management.
[b] B = dry bean; P = potato; SB = sugar beet; W = wheat. The <u>underlined</u> wheat phase of each rotation was sampled for soil microbiological analysis.
[c] In 5-CONS1 rotation, the first (<u>underlined</u>) wheat phase was sampled; in 5-CONS2 rotation, the second (<u>underlined</u>) wheat phase was sampled.
[d] Wide row spacing = 60 cm; narrow row spacing = 19 cm (2000–2003 and 2011) and 23 cm (2014-2010).