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Bacterial communities of an agricultural soil amended with solid pig and dairy manures, and urea fertilizer

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ABSTRACT

Agricultural management practices impact the bacterial diversity of soil but it is unclear how bacterial communities respond to different nutrient sources. This study examined the impacts of manure and granular urea N additions on the diversity and composition of soil bacterial communities. Bacterial communities in an annual cropping system were examined in the short-term (within season) and medium-term (after three successive annual additions) following manure and urea N applications. Soil samples were collected from an experimental field site in fall 2007 prior to imposition of treatments, and post-planting, mid-season, and post-harvest in 2010 following three successive annual applications. Treatments included: solid pig manure (SPM), solid dairy manure (SDM), granular urea N-fertilizer, and unamended control. Pyrosequencing was used to characterize bacterial communities in soil and the manure added. *Psychrobacter* was the most abundant genus in both SPM and SDM, however it was not detected in soil. Solid pig manure treatments had greater diversity than urea and control treatments and diversity was greatest at post-harvest in fall than post-planting in spring. In 2010, the relative abundances of many bacterial taxa were affected by treatment and sample season but not their interaction. Where Actinobacteria, Firmicutes, Gemmatimonadetes and Bacteroidetes were significantly different for the urea and manure treatments, Proteobacteria declined in relative abundance over the growing season. Communities of manure and urea treated soils converged with progression of growing season. Redundancy analysis showed SO_4^{-2}, NO_3^- and NH_4^+ concentrations were significant, explaining 44% of the variation observed in bacterial communities across treatments and sample seasons. In conclusion, bacterial diversity increased with manure treatment and with progression of the growing season, the former being not as a result of introduction of taxa from the manure but likely from nutritional resources provided in the organic amendments.

1. Introduction

Within a single gram of soil, the bacterial community is immensely diverse containing roughly 10,000 different species (Curtis et al., 2002). These bacteria support biochemical cycling, improve plant productivity, degrade pollutants, and contribute to climate regulation (Griffiths and Phillipot, 2012; Zhang et al., 2006). Increased biodiversity can improve the stability of ecosystem function over time, leading to greater resistance and/or resilience to environmental perturbations (Campbell et al., 2011; Jiang and Pu, 2009; Konopka, 2009). Conversely, the loss of biological diversity can reduce the efficiency of ecosystem functions among all trophic levels, including bacteria (Balvanera et al., 2006; Cardinale et al., 2012, 2006).

Until recently, characterization of bacterial communities in soil has relied upon molecular fingerprinting methods such as terminal restriction fragment length polymorphism and denaturing gradient gel electrophoresis (Naether et al., 2012; Sun et al., 2004). Bacterial diversity is often underestimated using these methods due to identical banding patterns/terminal fragment lengths displayed by closely related bacteria. Additionally, bacterial taxa composition is often not examined and requires considerable efforts with downstream Sanger sequencing to identify dominant members of communities (Griffiths et al., 2011; Sun et al., 2004). The recent transition to high-throughput sequencing has allowed soil bacterial communities for land-use types and along ecological gradients to be examined in detail. These studies have provided

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enhanced descriptions of bacterial diversity and bacterial community composition (Acosta-Martinez et al., 2008, 2010; Lauber et al., 2009, 2013; Li et al., 2012; Nacke et al., 2011; Poisot et al., 2013).

Bacterial communities respond to changes in soil conditions such as: moisture, temperature, pH, soil type, and nutrient availability (Chaudhry et al., 2012; Girvan et al., 2003; Lauber et al., 2009; Nautiyal et al., 2010). Given that >99% of bacteria remain 'unculturable', the association of high throughput sequencing analysis of bacterial communities and soil conditions (metadata) may identify the response of individual bacterial taxa and communities to specific changes in soil conditions. The observed response can provide additional insight into the physiological capabilities and ecological roles of particular bacteria (Lauber et al., 2009; Malik et al., 2008).

Nitrogen fertilization of land with synthetic sources and animal manures is a common agricultural practice boosting world food production by 40% (Canfield et al., 2010; Fixen and West, 2002). The benefit of fertilization for crop production is extraordinary, however the short- (within season) and medium- (several years) and long-term (many years) impact of different nitrogen sources on soil bacterial communities has not been fully explored.

Bacterial communities respond to synthetic nitrogen source additions. Fierer et al. (2012) demonstrated that although bacterial diversity was unaffected, bacterial taxa composition (community structure) was altered by additions of NH_4NO_3. Particularly the relative abundance of Proteobacteria increased whereas that of Acidobacteria decreased with higher N inputs. Manure and synthetic N source additions increased and decreased, respectively, bacterial diversity of soil (Chaudhry et al., 2012; Sun et al., 2004). Those studies were based on a single sample season, which did not allow for within season or medium- to long-term impacts on bacterial communities to be apparent. Additionally, the impact of manure on soil bacterial communities has been characterized for solid cattle or poultry manure additions but not other manures (Chaudhry et al., 2012; Parham et al., 2003; Sun et al., 2004). We recently examined the impact of pig slurry additions on bacterial communities of grassed soil using terminal restriction fragment length polymorphism, Sanger sequencing and pyrosequencing (Hamm, 2014). There, slurry addition in spring increased Firmicutes and specifically *Clostridium* spp., however, by the end of the growing season the relative abundance of the slurry bacteria returned to levels of that prior to manure addition at spring.

The purpose of this study was to compare the bacterial richness, diversity and taxa relative abundances (community structure) of soil following three successive annual additions of solid pig manure (SPM), solid dairy manure (SDM) and granular urea N fertilizer. An annual cropping system was examined to identify the effect of the N sources in the short-term, within growing season, and medium-term, after the successive annual additions. To gain insight into the response of bacterial communities to the N sources, the relationship of taxa to soil nutrient concentrations and properties was also examined. To determine if bacteria in the manure sources remained in soil, bacterial communities of the manures were also characterized by pyrosequencing and compared to those of manure amended and manure non-amended soil. Effects on richness and diversity were examined using univariate statistical approaches. Effects on relative abundances of taxa and also their association with soil properties were examined by univariate and multivariate statistical approaches.

2. Materials and methods

2.1. Experimental site and treatments

Soil was collected from the site of the Long-term Manure and Crop Management Field Laboratory established by the National Centre for Livestock and the Environment, at the University of Manitoba's Glenelea Research Station, 18 km south of Winnipeg, Manitoba (49°38′15″N, 97°9′25″W). The soil at the site is mapped as the Red River association that is an imperfectly drained Dystric Vertisol soils in the FAO and Humic Vertisol in the Canadian soil classification system. Soil characteristics (0–15 cm) were clay texture (700 clay g kg^{-1}, 260 silt g kg^{-1}, 40 sand g kg^{-1}), pH 5:1 H_2O 7.0, electrical conductivity 0.32 dS m^{-1}, organic C 21.0 g kg^{-1}, and bulk density 1.0 Mg m^{-3}.

Prior to initiating treatments of N sources, the site was seeded in 2007 to Legacy six-row malting barley (*Hordeum vulgare* L.) without addition of fertilizers or animal manures. The experimental design was a complete randomized block with four replicate plots (20 m × 20 m each) per treatment. The treatments examined in the current study were: control (no addition), granular urea N fertilizer, SPM, and SDM additions. Manures were obtained from the Glenlea Research Station and applied fall of 2007, 2008 and 2009. The average characteristics of the manures added over the three falls was for SPM, dry matter 304 (17.4 standard deviation), total N 9.99 (5.79), 2 M KCl extractable NH_4^+-N 2.16 (2.81), organic N 9.37 (5.89) and total P 5.41 (2.34) g kg^{-1} as applied material and for SDM, dry matter 223 (9.61), total N 7.03 (0.52), 2 M KCl extractable NH_4^+-N 1.32 (0.68), organic N 5.72 (0.75) and total P 2.12 (0.32) g kg^{-1}. Manure was surface applied and incorporated to 15 cm using a heavy cultivator implement. Granular urea (46-0-0), an inorganic N source, was broadcasted onto soil the surface just prior to seeding and incorporated into soil from disturbance of the hoe-openers of the seeder. Plant available nitrogen applied as manure was calculated assuming 25% of the organic N and 85% of manure extractable NH_4^+-N was available within one year of application (Prairie Provinces' Committee on Livestock Development and Manure Management, 2006). Manure and urea fertilizer were applied to provide 140 kg available-N ha^{-1} that included fall soil residual NO_3^- to 60 cm. The rate of SPM applied was 54, 22 and 58 Mg ha^{-1} in fall 2007, 2008 and 2009, respectively, and for SDM, 56, 40 and 45 Mg ha^{-1} in fall 2007, 2008 and 2009, respectively. The plots were planted to the barley variety again in 2008, to Red River 1826 Roundup Ready high-erucic-acid rapeseed (*Brassica napus* L.) in 2009, and Glenn spring feed wheat (*Triticum aestivum* L.) in 2010.

2.2. Sampling

Samples were collected 0–15 cm on four occasions: fall 2007 (prior to addition of amendments), spring 2010 (post-planting), summer 2010 (mid-season) and fall 2010 (post-harvest). These were chosen to identify short-term (2010 within year) and medium-term (after three years of additions fall 2007 to fall 2010) changes in bacterial communities. On each occasion, three soil samples were randomly collected from each plot using a spade and were composited (totaling about 6 kg) for a plot into a new polyethylene bag. Between plots, the spade was washed in fresh tap water with a brush. For each new plot, a soil sample was taken with the cleaned spade and the sample discarded. In fall 2009, a composite sample of each manure type was obtained during application. The manures were chopped so bedding straw pieces were less than 1 cm in length. The bag of each composite soil sample was manipulated by hand to mix the soil. A 100 g subsample of each composite soil and manure sample was stored at −80 °C prior to DNA extraction and nutrient analysis.

2.3. Pyrosequencing

Total microbial genomic DNA was extracted in duplicates of 0.25 g soil from each plot soil sample using the ZR Soil Microbe DNA MiniPrep Kit™ (Zymo Research Corporation, Irvine, CA) and in duplicates of 0.15 g manure for four subsamples of each manure