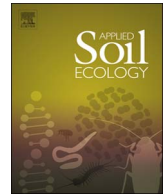




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## Soil microbial biomass, activity, and community composition as affected by dairy manure slurry applications in grassland production

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## ABSTRACT

The response of soil microorganisms to applications of whole dairy manure slurry or the liquid fraction of the manure is not fully understood. This study evaluated the effect of whole dairy manure slurry in two trials of different durations and with different cultivation histories, and the effect of the liquid fraction after solids removal on microbial biomass, enzyme activity, and community composition in the soil of tall fescue (*Festuca arundinacea* Schreb.) swards in the south coastal region of British Columbia, Canada. Two trials were carried out, and fertilizer was applied four times per year. The trial 1 plots were treated with whole dairy manure slurry annually at 0, 400, or 800 kg total N ha<sup>-1</sup> (control, M-low, or M-high, respectively), whereas the trial 2 plots were not treated with manure (control) or were treated with whole manure dairy slurry (WS) or a separate liquid fraction (LF) at 400 kg total N ha<sup>-1</sup>. Soil samples were collected at the 0-to-10-cm soil depth after each harvest in 2013 and 2014. Results from two trials show that soil microbial and bacterial biomasses and dehydrogenase and alkaline phosphomonoesterase activities increased with the application of whole dairy manure. Soil microbial community composition was not significantly influenced by whole dairy manure in trial 1. In contrast, in trial 2, LF and WS had a similar influence on soil microbial biomass and dehydrogenase activity, but LF had a greater impact on soil microbial community structure, mainly by reducing the fungal abundance. We conclude that the response of soil microorganisms changed with the types and application rates of dairy manure slurry in grassland production.

### 1. Introduction

In grassland ecosystems, soil microbial communities play a key role in recycling nutrients especially nitrogen (N) and phosphorus (P) via the decomposition of organic matter and the enhancement of plant nutrient availability (Bardgett et al., 1999). Organic fertilization, which is a common agricultural management practice in grassland soils, could affect the biomass, activity, and composition structure of soil microbial communities (Bittman et al., 2005; Deboz et al., 1999; Lalonde et al., 2000; Luo et al., 2015; Marschner et al., 2003; Parham et al., 2002; Peacock et al., 2001), through the modification of soil physical and chemical characteristics; this is especially true for the addition of readily available forms of carbon (C) (Edmeades, 2003).

Dairy manure slurry is an alternative to chemical fertilizers and an important source of nutrients for perennial grass swards, from both an economic and an environmental perspective (Bittman et al., 1999). Whole dairy manure slurry, a material containing 90% to 98% water

and small amounts of nutrient-rich solids, is the fertilizer normally applied to forage swards on dairy farms (Bittman et al., 2011). Previous studies reported that multiyear applications of whole dairy manure slurry, in comparison with mineral fertilizer application, increased soil microbial biomass and activity (Neufeld et al., 2017) and changed the ratio of bacterial biomass to fungal biomass as well as soil microbial community structure; however, high and low rates of application did not significantly affect these microbial variables (Bittman et al., 2005; Peacock et al., 2001). All those studies were conducted in swards around 5 yr after initiation of the manure application or after grassland renovation. Renovation, which include plowing and reseeding, is performed to maintain or increase the productivity of swards. Grassland renovation could result in changes of mineral N and nitrate leaching (Seidel et al., 2009). Little information is available on the response of soil microorganisms to the application of whole dairy manure slurry for different durations and different cultivation histories, especially following renovation practices.

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When whole manure application matches the N demand, other nutrients such as P are likely to be applied in excess, because the N:P ratio is lower in manure (5:1) than in crops (8:1) (Bittman et al., 2011). This practice can lead to an accumulation of P in soil and increase the leakage of P into the environment (Sharpley and Moyer, 2000). To mitigate P accumulation in soil, settling and separating of dairy manure slurry is recommended as a low-cost method for partially removing solids from the slurry and increasing the N:P ratio of the fertilizing material (Bittman, 2009). Bittman et al. (2011) found that at equal N rates, grass yield and N uptake were improved with the multiyear application of only the liquid fraction of manure rather than the whole manure. Removing solids from the manure slurry reduces the contents of organic C, organic N, and total P (Vetter et al., 1987; Whitehead, 2000). The response of microorganisms to the multiyear application of the separated liquid fraction of manure remains largely unknown. To our knowledge, only one previous study considered that question and reported that separating the solid fraction from the liquid fraction of manure did not reduce microbial community size and activity (Neufeld et al., 2017). More knowledge about the management of dairy manure slurry is required to understand its impact on soil biology.

The effects of management practices on soil quality are best evaluated using long-term experimental sites (Mitchell et al., 1991). For this study, we used two trials in perennial grassland soils with different durations of manure application and different cultivation histories under a humid maritime climate: trial 1 was established in 1993 and renovated in 2011–2012, whereas trial 2 was established in 2002 and received renovation in 2005–2006. The objective of this study was to determine the effects of dairy manure slurry type and application rate on microbial biomasses (C, N, and P), dehydrogenase and phosphomonoesterase activities, and microbial community structure, as determined by phospholipid fatty acid (PLFA) analysis, in grassland soils during the growing seasons of 2013 and 2014.

## 2. Materials and methods

### 2.1. Experimental design

Two experiments were carried out in two fields, located about 500 m apart, at the Agassiz Research and Development Centre of Agriculture and Agri-Food Canada located in south coastal British Columbia, Canada (49°10'N, 121°15'W). This is a warm temperate and humid climatic zone, with an annual average air temperature of 10.2 °C and an annual average precipitation of 1770 mm during 1980–2010 (Environment and Climate Change Canada, 2017). The soils at this location are derived from medium-textured (silt loam to sandy loam), stone-free Fraser River deposits with moderately good drainage and contain about 6% organic matter (Luttmerding, 1981). The soils (Eutric Eluviated Brunisols) are classified as belonging to the Monroe series, which corresponds to the Typic Dystraxepts in the USDA soil classification system. A stand of tall fescue (*Festuca arundinacea* Schreb. var. *Festorina*) was established in 1993 for trial 1 and in 2002 for trial 2.

In trial 1, the 120 × 125-m experimental area was divided into 40 plots, each measuring 3 × 125 m, as described in Bittman et al. (2007). The three treatments in three complete blocks considered in trial 1 were an unfertilized control, and two whole dairy manure slurry treatments with different rates, one applied annually at a target rate of 400 kg total N ha<sup>-1</sup> (M-low) and the other applied at a target rate of 800 kg total N ha<sup>-1</sup> (M-high) in four equal doses. The manure treatments were applied in early April and again after each hay cut except the final cut in the fall. The stand was renovated by moldboard plowing to 25 cm and sowing back to grass in 2003 and maize (*Zea mays* L.) in 2011. The stand was reseeded with tall fescue in April 2012. No nutrients were applied and no crop biomass measurements were taken in the grass seeding years (2003 and 2012).

In trial 2, three treatments randomized in 3 × 45-m plots arranged in three complete blocks were selected: an unfertilized control, whole

dairy manure slurry (WS), and the separated liquid fraction (LF), with both manure treatments applied at a target annual rate of 400 kg total N ha<sup>-1</sup>, which is considered to be the best management practice at the time of publication. The whole manure application resulted in the addition of 200 kg ammonium-N ha<sup>-1</sup> and the separated liquid fraction application resulted in the addition of about 300 kg ammonium-N ha<sup>-1</sup>; the remainder of N was in organic N form. The tall fescue swards were renovated in 2006 using conventional tillage (moldboard plowing to 25 to 30 cm, offset disking to 15 to 20 cm, and S-tine cultivation to 15 cm). Maize was grown without fertilizer in the renovation year (2005), and no nutrients were applied in the grass seeding year (2006).

Both trials were treated intermittently with 2,4-D (Nufarm Agriculture Inc., Calgary, AB, Canada) at 1.0 L ha<sup>-1</sup> tank-mixed with Banvel II (480 g diglycolamine salt L<sup>-1</sup>) (BASF Canada, Toronto, ON, Canada) at 2.2 L ha<sup>-1</sup> to control broadleaf weeds and white clover. The manure for both trials was obtained from local high-producing commercial dairy farms on which cows (*Bos taurus*) were confined year-round in naturally ventilated, free-stall barns with sawdust bedding. The cows' diets consisted of about 70% locally grown grass and maize silage and 30% imported concentrate, largely made of cereal grain and canola meal. The whole slurry was obtained from manure storage tanks soon after agitation, whereas the liquid fraction of the slurry was collected from a secondary lagoon that received the overflow from a settling lagoon. The whole dairy manure slurry, which was used in both trials, averaged 5.9% dry matter, 16:1C:N, pH 7.0, and, on a wet basis, 4.1% C, 1.3 g ammonium-N kg<sup>-1</sup>, 2.5 g total N kg<sup>-1</sup>, and 0.49 g total P kg<sup>-1</sup>, for an N:P ratio of 5.1. The decanted liquid slurry averaged 1.6% dry matter, 13:1C:N, pH 7.6, and, on a wet basis, 1.89% C, 0.95 g ammonium-N kg<sup>-1</sup>, 1.45 g total N kg<sup>-1</sup>, and 0.15 g total P kg<sup>-1</sup>, for an N:P ratio of 9.7. (Bittman et al., 2005, 2011). The amount of total N in the manure was determined by the Kjeldahl method, and ammonium was determined by steam distillation followed by titration (McGill and Figueiredo, 1993). Total P was determined by inductively coupled atomic plasma-emission spectrophotometry after nitric acid digestion supplemented by peroxide treatment (US Environmental Protection Agency, 1996). The dry matter content of the manure was determined after oven drying at 60 °C for 24 h, and pH was measured in stirred manure samples using a pH meter (Model 810; Fisher Scientific, Ottawa, ON, Canada).

The plots were harvested four times during the growing season of 2013 and 2014: in May, July, September, and early November. Herbage yield was determined by harvesting a 1.5 × 7.5-m strip between the tanker tracks, in order to minimize the effect of compaction. The grass was cut to a height of 5 to 7 cm with a sickle bar mower. Immediately after harvesting, herbage from the entire sampling area in the plot was weighed fresh and then subsampled (~600 g) for dry matter determination.

### 2.2. Climatic data

On site, a data logger (HOBO Micro Station; Onset Computer Corp., Bourne, MA, USA) was installed inside the experimental plots to measure the soil temperature at a depth of 5-cm. Additional environmental data (daily precipitation and average air temperature) were obtained from a nearby meteorological station (2 km from the fields).

### 2.3. Soil sampling and chemical analysis

Soil samples (each a composite of five cores, 2.0 cm in diameter) were collected at the 0-to-10-cm depth on 27 May, 10 July, 9 (trial 1) or 16 (trial 2) September, and 9 November in 2013 and on 20 May, 1 July, 4 September, and 1 November in 2014, less than a week after harvest and before manure was reapplied. The soil samples were stored at 4 °C and shipped within 24 h by air to the Quebec Research and Development Centre of Agriculture and Agri-Food Canada (Quebec City), where most of the analyses were performed. Soil microbial

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