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Effects of cattle-lagoon slurry on a soil microbial community can be observed until depths of 50 m

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ABSTRACT

The large amount of effluent generated by concentrated animal feeding operations (CAFOs) has raised concerns about contamination of groundwater and pollution of streams by compounds that penetrate the vadose zone. However, the possibility that a microbial community in the vadose zone under cattle-slurry lagoons (CSLs) may also be affected has not been considered. In the present study, we investigated the influence of long-term (30 years) accumulation of cattle slurry on the vertical distribution of a soil microbial community (microbial biomass [MB], CO_2 evolution, substrate utilization ability), until a 50-m depth, compared to a control site. Total soluble nitrogen (TSN) was found to be elevated fourfold, and MB was found to be threefold higher under the CSL compared to a control site. In general, the increase in MB is associated with higher soil moisture and higher nitrogen content. Substrate utilization ability was found to be significantly higher in a CSL in comparison to the control site. At the CSL site, a higher utilization of aromatic carboxylic acids typical of cattle slurry was obtained in the deeper soil layers (7–30 m), indicating a degree of microbial adaptation even at these depths. The soil layers under the CSL were more dynamic as the microbial functional diversity was significantly different between the layers, while no such difference was seen at the reference site. Our results, therefore, suggest that the infiltration of cattle slurry can affect the microbial community throughout the vadose zone. We also suggest that activity of the microbial community, as characterized by its substrate utilization ability, can be a bioindicator for anthropogenic activities and environmental changes even at depths below the rhizosphere (30 cm).

1. Introduction

Organic manure is an inevitable waste product of animals and is produced in enormous amounts in concentrated animal feeding operations (CAFOs) such as dairy, swine, and poultry farms (Lee et al., 2007). In sustainable agricultural management, animal waste is spread on agricultural fields as an organic fertilizer, improving nutrient availability via decomposition processes (Jedidi et al., 2004; Shore and Pruden, 2009). This decomposition and recycling of organic matter is largely due to soil microorganisms that are capable of converting the organic components into nutrients available to plants (Steinberger and Shore, 2009). The soil microbial community can also be used as bioindicators for detecting environmental and human impacts on soil quality (Rodríguez-Añón et al., 2007) such as CO_2 enrichment, using PLFA and DNA fingerprint (DGGE) techniques at rhizosphere level (0–10 cm) (Ebersberger et al., 2004) or by substrate utilization of a soil microbial community.

Rillig et al. (1997) found that microbial communities associated with *Gutierrezia sarothrae* roots had different substrate utilization in response to CO_2 enrichment, which indicated rhizodeposition. Moreover, the microbial community is a tool for measuring environmental cattle- and sheep-grazing effects (Kohler et al., 2005), such as trampling, herbage removal, and dunging. In particular, cattle activity in successive years may modify the potential metabolic activity of certain microbial guilds (Kohler et al., 2005), thus inducing changes in microbial-community structure. Similarly, Williams et al. (2000) found that the addition of synthetic sheep urine to upland grassland resulted in a dramatic and short-term change in soil microbial-community structure and activity.

The objective of the present study was to explore the effect of a cattle-slurry lagoon of dairy-farm origin on the vertical distribution of a soil microbial community. In the site studied, untreated slurry accumulated in the lagoon for thirty years and the slurry infiltrated to deeper soil layers, causing changes in soil physical and chemical parameters that have already been described (Arnon et al., 2008).

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Table 1
Soil characteristics along the vertical profile beneath a cattle-slurry lagoon (CSL) and control sites.

Soil type/location	Clay (vertisols)	Sandy loam	Calcic sand	Brown–red sandy soils (podsoils)	Calcic sand with pebbles
CSL	0–6 m	6–8 m	8–50 m	–	–
Control	0–10 m	10–15 m	15–25 m	25–40 m	40–50 m

Table 2
Soil layers of the vertical profile beneath a cattle-slurry lagoon (CSL) and control sites.

Soil layer	CSL (m)	Control (m)
I	0.15, 0.5, 0.85	0.15, 0.5
II	1, 2, 4, 5	1, 2, 4
III	6, 7, 8, 9, 12, 15	7, 10, 15
IV	19, 25, 30	20, 25, 30
V	35, 40, 45	40, 45
VI	50	50

2. Materials and methods

2.1. Study site

A 30-year-old dairy farm located in the south-east section of the Israeli aquifer, with a semi-arid climate and 300 mm multi-annual precipitation, was used in the present study (Shahar, 2009). The dairy farm included 60 dairy cows and ~30 calves. The output of effluent solid matter per day was 7 kg/cow, resulting in 70 kg of effluent/day per cow, of which 10% was solid matter. The facility used was a 150 m^2 single-stage earthen unlined waste lagoon with an average depth of 0.5 m, which is common manure-management practice in the area. Excess wastewater overflowed directly into a dry creek, without any particular maintenance procedures such as drainage or removal of solids. A continuous slurry flow into a nearby creek over a period of 30 years created a slurry lagoon. Boreholes were mechanically drilled to a depth of 50 m in order to collect vertical sediment samples. One borehole was drilled under the cattle slurry lagoon (CSL) in summer, while a second borehole used as a control was drilled ~1 km east of the dairy farm in a typical, open agricultural field (Arnon et al., 2008). The boreholes were entirely cased with PVC pipes and perforated from ~1 m above the groundwater surface to the bottom of the borehole, which was ~7 m below groundwater level (groundwater levels were 47 and 42 m below ground surface for CSL and control, respectively).

In order to explore the vertical distribution of the soil microbial community, we used a dry-drilling method with a bucket auger. The bucket was cleaned between samples with pressurized water and a propane flame to prevent cross-contamination. Soil samples were taken from the soil surface to a 50-m-depth, with denser sampling at the top of the profile (20 samples under CSL and 14 samples under the control site; $n = 3$). Each soil sample was placed in an individual plastic bag that was placed in a cooler to prevent heating, and transported within 3 h to the lab, where they were kept at 4 °C till chemical and biological analyses were performed. The soil sediment properties at the study site were determined by Arnon et al. (2008). Along the vertical gradient, three types of sediment were found: clay mineral was found at the upper layer, followed by a transition layer of sandy-loam, and to a sand-calcareous layer. This is typical of the brown–red sandy soil of the Israeli coastal plain (Holocene-Upper Pleistocene), with a 50–60% clay fraction toward the deeper layers (Gal et al., 1974; Fitzpatrick, 1996) (Table 1). The soil pH was 8.17 ± 0.13 and 8.23 ± 0.08 for the CSL and control sites, respectively. Soil samples collected from each plot were divided, according to Ravikovitch (1981), into six compatible soil layers based on similarities in chemical, physical, and biological components (Table 2).

2.1.1. Soil moisture

SM was expressed as a percentage determined gravimetrically by drying 5 g soil samples for 72 h at 105 °C.

2.1.2. Organic matter

OM was expressed as a percentage determined by oxidation with 1 N potassium dichromate in acidic medium, according to Rowell (1994).

2.1.3. Total soluble nitrogen

TSN was measured by the extraction of 10 g soil samples in 25 ml 0.01 M $CaCl_2$, and determined with a Skalar Autoanalyzer (Houba et al., 1987; S.F.A.S., 1995).

2.2. Soil microbial community

The soil microbial community was determined by the MicroResp™ method (Campbell et al., 2003), with which we assessed the community level physiological profile (CLPP), microbial biomass (MB), and CO_2 evolution. This method is based on determining the potential utilization of 15 different carbon sources that are typical of the utilization of carbohydrates (Carb), carboxylic acids (CA), amino acids (AA), and aromatic carboxylic acids (ACA) by the soil microbial community (Campbell et al., 2003; Berg and Steinberger, 2008; Saul-Tcherkas and Steinberger, 2009). In order to determine microbial biomass (MB), glucose solution was added to soil samples, while no substrates were added to samples in order to determine basal CO_2 evolution (Anderson and Domsch, 1978; Carpenter-Boggs et al., 2000; Berg and Steinberger, 2010).

The dye plates were read twice in a spectrophotometer at 590 nm: just before they were placed on the deep plates containing the soil samples (Time 0) and after discerning colorimetric changes in the indicator plate (Time 1). After Time 0, the plates were incubated in the dark at 27 °C. The results per well were calculated in comparison to the 16th well that contained the same soil sample.

Microbial functional diversity was determined using the Shannon–Weaver index

$$(H') : H' - \sum P_i(\ln P_i),$$

where P_i is the ratio of the activity of a particular substrate and the sum of activities of all substrates (Zak et al., 1994).

2.3. Statistical analysis

All the data obtained in the present study were subjected to statistical analysis of variance (ANOVA) using the SAS model (Duncan's multiple range test and Pearson correlation coefficient [SAS Institute, Inc.], one-way ANOVA, and T test) for evaluating differences between separate means. Differences at the $p < 0.05$ level were considered significant ($n = 3$) (Kandeler et al., 1999).

3. Results

3.1. Soil moisture (SM)

The vertical distributions of soil moisture (SM) percentage expressed as mean values of samples collected along the 50 m from the cattle-slurry lagoon (CSL) (12.2%) and the control site (12.9%),