Pedobiologia 55 (2012) 211–218

Contents lists available at SciVerse ScienceDirect

Pedobiologia - International Journal of Soil Biology

journal homepage: www.elsevier.de/pedobi

Effect of soil moisture and bovine urine on microbial stress

J.E. Bertram [a,*], K.H. Orwin [b], T.J. Clough [a], L.M. Condron [a], R.R. Sherlock [a], M. O'Callaghan [c]

[a] Faculty of Agriculture and Life Sciences, PO Box 84, Lincoln University, Lincoln 7647, New Zealand
[b] Soil and Ecosystem Ecology Laboratory, Lancaster Environment Centre, Lancaster University, LA1 4YQ, United Kingdom
[c] Biocontrol, Biosecurity and Bioprocessing Section, AgResearch, Lincoln Research Centre, Private Bag 4749, Christchurch, New Zealand

ARTICLE INFO

Article history:
Received 7 December 2010
Received in revised form 21 March 2012
Accepted 26 March 2012

Keywords:
Bovine urine
Microbial stress
Phospholipid fatty acid analysis
Soil moisture content
Urinary salt

ABSTRACT

While many studies have examined the cycling of urinary nutrients, few have focused on the effects ruminant urine might have on the soil microbial community. Urine application can cause microbial communities to become stressed, potentially changing community composition and microbial function with subsequent effects on nutrient dynamics. Identification of the factors that stress microbes may assist in explaining ruminant urine effects on nutrient cycling. In this laboratory study bovine urine, with either a high (15.0 g K^+ l^{-1}) or low (10.4 g K^+ l^{-1}) salt concentration, was added to repacked soil cores maintained at high or low soil moisture contents (70 or 35% water-filled pore space, respectively). Control cores did not receive urine. Microbial stress was measured using phospholipid fatty acid (PLFA) biomarker ratios. Urine addition increased stress as indicated by a decrease in the iso15:0/anteiso15:0 PLFA ratio from >1.35 to <0.95 in both wet and dry soils and by an increase in the 18:1ω9trans/18:1ω9cis PLFA ratio from 1.4 to 1.9 from day 8 onwards in wet soils. Higher stress was indicated by a lower Gram-positive/Gram-negative PLFA ratio in the urine treatments than in the control treatments on day 29 and this may have been a response to the reduction in substrate availability as the experiment progressed. The PLFA biomarkers showed that the salt treatments did not induce stress. Stress induced by urine addition and wet soil treatments was also indicated by principal component analyses and the metabolic quotient for CO_2, respectively. Thus microbial stress was induced by both urine addition and high soil moisture content, but not specifically by increasing the urinary salt concentration.

Introduction

Ruminant urine contains compounds that can alter the chemical and physical properties of the soil onto which it is deposited (Haynes and Williams 1992), and as a consequence urine deposition may affect soil microbes (Petersen et al. 2004a; Nunan et al. 2006). However, although many studies have investigated urine patch chemistry in pastoral systems, there have been few investigations into the effects of ruminant urine on soil microbiology. Stressed microbes may divert resources from reproduction to survival, leading to inhibition of microbial growth and function (Schimel et al. 2007). Changes in soil microbial function alter nutrient dynamics, including the production of greenhouse gases (GHGs), and can thus influence processes on a global scale (Williams et al. 2000; Petersen et al. 2004b).

Those studies that have included microbial measurements have found that the microbial biomass may decline (Lovell and Jarvis 1996), or remain unchanged (Williams et al. 2000) following urine application to soil, while the microbial community structure can be affected both in the short-term (several weeks) by urine application (Nunan et al. 2006) and in the long-term (several years) by grazing intensity (Patra et al. 2005). Both stimulation and stress of the microbial community have been indicated after urine application, by phospholipid fatty acid (PLFA) analysis and the metabolic quotient for CO_2 (qCO_2). These previously measured changes in microbial dynamics were linked to changes in soil N and C concentrations following urine deposition. However, other factors, such as urinary salt concentration and the existing soil moisture content, may interact with these changes in soil N and C to influence microbial dynamics. Salt and moisture both affect the osmotic potential of the soil solution, and osmotic stress can inhibit microbial growth and cell functions such as glycolysis and carbohydrate transport (Roth et al. 1985; Roseberg et al. 1986; Walter et al. 1987). Severe stress may result in microbial cells lysing or releasing organic compounds to counter the osmotic changes (Kieft et al. 1987; Halverson et al. 2000). Microbial stress could also be induced after urine deposition due to the rapid rise in pH and increased concentrations of NO_2^-, NO_3^-, or free NH_3 (Hunik et al. 1992, 1993; Villaverde et al. 1997; Baatout et al. 2007).

* Corresponding author. Tel.: +64 3 321 8230; fax: +64 3 325 3607.
E-mail addresses: Janet.Bertram@lincoln.ac.nz, jbertramnz@yahoo.co.nz (J.E. Bertram).

0031-4056/$ – see front matter © 2012 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.pedobi.2012.03.004

Microbial community stress can be monitored using PLFA analysis and the qCO_2. Since PLFAs comprise a relatively constant proportion of the microbial biomass (Zelles et al. 1992; Frostegård and Bååth 1996) and degrade rapidly on cell death (Hill et al. 2000), they allow the living microbial biomass to be targeted. The use of signature fatty acids allows quantification and comparison of various microbial groups including fungi and bacteria, and more specifically Gram-positive and Gram-negative bacteria (Harwood and Russell 1984; Frostegård and Bååth 1996). Since their cell structure is believed to make Gram-negative bacteria more stress resistant than Gram-positive bacteria, an increase in the Gram-positive/Gram-negative (Gram+/Gram−) ratio has been used to indicate that a given microbial community is stressed (Kaur et al. 2005). Increased stress may also be indicated by an elevated cyclopropyl 17:0/16:1ω7 ratio (cy/ω7) as cell dormancy induces conversion of cis-monounsaturated FAs (e.g. 16:1ω7) to the more stable cyclopropyl FAs (Petersen et al. 2004a; Kaur et al. 2005). Stress is also indicated by an elevated trans/cis 18:1ω9 (t/c) ratio and a reduced iso15:0 /anteiso15:0 (i/a) ratio, both changes that reduce membrane permeability and protect cells in hyperosmotic conditions (Guckert et al. 1986; Heipieper et al. 1996; Loffeld and Keweloh 1996; Chihib et al. 2003; Kaur et al. 2005). An elevated metabolic quotient for CO_2 (qCO_2) also indicates microbial stress, since it shows that energy has been diverted from microbial growth to cell maintenance (Odum 1985; Wardle and Ghani 1995).

To test whether soil moisture content and urinary salt content affect microbial stress levels, we used samples from the study described in Orwin et al. (2010). That study found that altering the urinary salt and soil moisture content affected the community structure of N-cycling bacteria as assessed by denaturing gradient gel electrophoresis (DGGE), and reported increases in the pH, EC and inorganic N concentrations after urine addition that could potentially induce microbial stress. This provided an ideal context to test our hypothesis that microbial stress would increase following urine addition, with the higher urinary salt content enhancing the stress effect by increasing the soil osmotic potential and the higher soil moisture reducing the stress effect by decreasing the soil osmotic potential.

Materials and methods

Experimental design

The experiment was set up using a 2 × 3 factorial design, with soil moisture at two levels (wet and dry) and cow urine at three levels (no urine, low salt urine and high salt urine), resulting in six treatments (Table 1). A Templeton silt loam soil (Typic Immature Pallic; New Zealand Soil Classification (Hewitt 1998), pH 5.8, 3.4% total C, 0.3% total N, CEC 13 meq 100 g^{-1}, 18% clay) was collected (0–10 cm) near Lincoln, Canterbury, New Zealand (43°38.4S, 172°26.6E). This was sieved (<4 mm) and packed to 6 cm depth in 7 cm lengths of PVC pipe (5 cm ID) to a bulk density of 0.89 g cm^{-3}. The soil moisture content was adjusted, by air-drying or by adding DI water, to attain pre-treatment levels of 23 or 58% water-filled pore space (WFPS). Urine application took the soil moisture content to 35 and 70% WFPS for the dry and wet urine treatments, respectively, while the control soils remained at the pre-treatment levels (Table 1). The soil moisture content of the control soils was not amended to the same levels as the urine-treatments because alteration of the soil moisture is a fundamental effect of urine deposition onto soil. The cores were incubated at 20 °C throughout the experiment, and moisture levels were adjusted three times a week by pipetting DI water onto the soil surface to return the soil cores to their initial weight. There were a total of 180 soil cores which allowed destructive sampling of five replicates of each treatment on six sampling occasions.

Urine was collected from Friesian dairy cows 24 h before application and stored at 4 °C. The urine contained 4.3 g N l^{-1} and 10.4 g K^+ l^{-1}. To ensure a strong and obvious response to urine application, urea was added to the collected urine to take the total urine-N content to 15.0 g N l^{-1}, the upper range found in bovine urine (Bristow et al. 1992). For the high salt treatment KCl was added to the urine to give a final concentration of 15 g K^+ l^{-1}. This concentration of K^+ is high for cow urine (Williams 1988), and was selected to give clear differentiation between the high and low urinary salt treatments. Each soil core received 9.8 ml of urine (750 kg N ha^{-1}).

Sampling and analyses

On each sampling occasion, headspace gas sampling for CO_2 was carried out as described in Bertram et al. (2009). Following this the cores were destructively sampled. The top 2 cm of soil was used for all analyses, as initial experiments showed that this zone underwent the strongest effects of urine application. After thorough mixing, this fresh soil was sub-sampled for PLFA and phosphate (PO_4^{3-}-P) analyses. Analysis of PLFAs (carried out 3, 8, 15 and 29 days after urine addition) was based on the lipid extraction method of Bligh and Dyer (1959) with modifications by White et al. (1979) and Frostegård et al. (1991). The nomenclature used for FAs is as described by Ratledge and Wilkinson (1988) and Frostegård et al. (1993a,b). Gas chromatographic analyses were carried out on a Shimadzu GC-2010 Gas Chromatograph (Shimadzu, Kyoto, Japan) fitted with a Restek Rtx®-5MS column (30 m length × 0.25 mm ID × 0.25 μm). The sample (1 μl) was injected at high pressure (240 kPa held for 5 min) in a splitless injection with the inlet at 250 °C. The column programme started at 150 °C and increased at 1.5 °C min^{-1} to 200 °C, then at 4 °C min^{-1} to 240 °C. At this point the peaks of interest had eluted and the temperature was ramped up to 300 °C at 30 °C min^{-1} and held for 7 min to remove any remaining residue from the column. Column flow was 1 ml min^{-1} and detection was by flame ionisation detector at 310 °C.

The FAs in the samples were identified by comparison with the retention times of a bacterial acid methyl ester standard (CP Mix 47080-U; Supelco) using 'GCsolution' software (Shimadzu). Internal standards (13:0 and 19:0; Sigma–Aldrich) were used to identify the retention times of the peaks of interest. Each run included two blank samples that underwent the same preparation as the soil samples. The peak area data were corrected by subtracting the maximum blank value for that compound, and were then converted to nmol values by comparison with the quantitative 19:0 internal standard (Tunlid et al. 1989). These nmol values were converted to units of nmol g^{-1} dry soil and the microbial biomass (nmol g^{-1} dry soil) was calculated by adding the values for all the identified peaks. The fungal/bacterial ratio was calculated by dividing

Table 1
Summary of the experimental treatments. The experiment was a 2 × 3 factorial design with 2 levels of soil moisture and 3 urine treatments.

Moisture	Urine		
	No urine	Low salt urine (10.4 mg K^+ l^{-1})	High salt urine (15.0 mg K^+ l^{-1})
Dry (WFPS)	Dry control (DC) (23%)	Dry + low salt (DLS) (35%)	Dry + high salt (DHS) (35%)
Wet (WFPS)	Wet control (WC) (58%)	Wet + low salt (WLS) (70%)	Wet + high salt (WHS) (70%)