Dairy Cow Urine Sodium Content and Soil Aggregate Size Influence the Amount of Nitrogen Lost from Soil

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Abstract
Cow urine deposition on pasture soils is a major source of N-related environmental impacts in the dairy farming systems. The urine-N can potentially be lost in reactive forms to the groundwater as nitrate (NO\textsubscript{3}) and to the atmosphere as nitrous oxide (N\textsubscript{2}O) and ammonia (NH\textsubscript{3}). These N-related environmental impacts are possibly related to the sodium (Na\textsuperscript{+}) concentrations in urine. We sampled a pasture soil and separated it into three aggregate sizes (0–3, 3–5, 5–7 mm). Then, cow urine with variable Na\textsuperscript{+} concentrations (4.3–6.1 g Na\textsuperscript{+}/l) was added to the soil cores. We treated the cores with simulated heavy rains and measured the amounts of inorganic-N leached from the soils. Increasing Na\textsuperscript{+} concentration in urine decreased the loss of NO\textsubscript{3} (–20%), after repeatedly applied simulated rain treatments (30 mm × 3) but increased the loss of ammonium (31%). Field level studies and studies focusing on the mechanisms behind the changes in nutrient losses are needed.

Key Words
sodium, urine, aggregate sizes, nitrogen

Introduction
In modern dairy farming systems, large amounts of nutrients are imported to the systems as feeds and fertilizer. If these imported nutrients are not exported from the systems as products (e.g. milk), nitrogen can potentially be lost to the groundwater and to the atmosphere. Nitrogen loss to the groundwater can induce environmental problems. Thus, the loss of NO\textsubscript{3} from grazed pasture systems has to be minimized. The major source of the NO\textsubscript{3} loss from dairy farming systems is cow urine (Di and Cameron 2002). Another potential negative impact on the environment derived from urine patches is the emission of nitrous oxide (N\textsubscript{2}O), a greenhouse gas with a long-term global warming potential of about 298 times that of carbon dioxide.

The composition of urine is another important factor controlling the N-related environmental impacts from urine-affected soils. Urine contains not only the large amount of N but also other cations such as potassium (K\textsuperscript{+}) and sodium (Na\textsuperscript{+}). A previous study showed that Na contents in cow urine markedly varied (0.03–0.43 %) and the urine-Na concentrations in cows depends on their diet (Martinez and Guiraud 1990). The amount of other nutrients excreted as urine is also controlled by the nutrients consumed by cows (Li et al. 1992). In the modern dairy farming industry, the supplementary feeding of K\textsuperscript{+} and Na\textsuperscript{+} is often performed particularly during summer to avoid the heat stress of dairy cows (Clayton et al. 1992). This supplementary feeding would result in the increase in K\textsuperscript{+} and Na\textsuperscript{+} excreted on soils as urine although the balance between Na\textsuperscript{+} input and output in dairy cows is overly influenced by temperature (heat stress), pregnancy and lactation, the function of kidney and gastrointestinal tract (Xiang et al. 2008). Previous studies reported that the presence of Na\textsuperscript{+} in soils negatively influences nitrification due to reduced microbial activities (McCormick and Wolf 1988, Quanzhong and Guanhua 2009). Thus, there is a possibility that varying urine-Na concentrations influence the magnitudes of the N-related environmental impacts derived from urine patches. The objective of this study was to examine the effect of Na\textsuperscript{+} concentrations in urine and soil aggregate size on NO\textsubscript{3} leaching under urine affected grazed pasture soils.

Methods
Soil characteristics
The soil used in the experiment was an Andosol collected (0–5 cm depth) from a dairy farm pasture (43°4.38’N, 141°20.8’E, 13 m above sea level) in Oct 2014. Soils were air-dried and sieved to three different aggregate sizes: 0–3 (small), 3–5 (medium) and 5–7 mm (large), which were packed into modified PET bottles. The PET bottles were cut into half and the upper parts were turned upside down to act as funnel...
shaped pots. The bottom parts were placed underneath of the upper parts, collecting leachates. Gravel (5 mm diameter, 160 g bottle⁻¹) were placed at the bottom of the funnel shaped pots and the sieved soil was placed on top of the gravel. The caps of the PET bottle pots were left closed throughout the experiment but five holes (2 mm diameter) were drilled on each cap to allow water to pass through. The sieved soils were then placed in each funnel-shaped pots. The soil surface area was 34 cm² and the soil depth was 2.5 cm (85 cm³).

**Treatments and leachate collection**

Four cow urine treatments were applied to each aggregate size group with three replicates, namely, control (water), urine (cow urine collected from the Hokkaido University dairy farm during an afternoon milking), urine Na (the urine with the addition of approximately 1 g NaL⁻¹ as NaCl), and urine NaNa (the urine with the addition of approximately 2 g Na L⁻¹ as NaCl). The amount of N added on soils as urine-N was 51 kg N ha⁻¹. The urine collected contained 4.28 g Na L⁻¹; thus the Na concentrations for urine Na and urine NaNa were 5.34 and 6.09 g Na L⁻¹. The pot experiments were performed in a temperature-controlled greenhouse and the average temperature during experiments was 25°C.

To simulate heavy rains, inducing the nutrient leaching from soils, each pot received 30 mm of water on day 2, 8, 14. Then, at 24 hours following each rain event, the leachate from each pot was collected from the bottom halves of the PET bottles placed under the funnel shaped pots (the upper halves of the PET bottles). The volumes of leachate (ml) were recorded and the chemical characteristics of the sampled leachate were measured as described in the next section.

**Measurements of leached N and remaining inorganic N in the soil**

The leachate was measured for NO₃⁻-N and NH₄⁺-N concentrations using a colorimetric method with a flow injection analyzer (AQLA-700, Aqualab Co., Ltd, Japan).

The NO₃⁻-N and NH₄⁺-N concentrations in soils, at the end of the experiment (17 days after the urine application) were measured as follows. The 5 g of each soil was placed in a polyethylene bottle and 25 ml of 10% KCl was added. After shaking for 30 minutes, the eluate was filtered through a filter paper (45 µm). The NO₃⁻-N and NH₄⁺-N of the filtrate were measured by the flow injection analyzer as described above.

**Statistics**

For the amounts of nutrients in leachate, the data were analyzed using a mixed model for repeated measurements (three simulated rain events) to investigate the effects of aggregate sizes and of urine treatments. The comparisons between treatments were performed using the Tukey’s method for multiple comparisons. The same statistical analysis was performed for the soil inorganic-N data.

**Results**

**Loss of inorganic-N from soils**

The added Na⁺ significantly decreased the amount of NO₃⁻-N loss by 41% only in the large aggregates when applied at the highest concentration (urine_NaNa) compared to the urine only treatment. The aggregate size and the urine treatments had a significant effect on the total amount of NO₃⁻-N lost from the soil. There was no interaction between the aggregate size and the urine treatments on the total amount of NO₃⁻-N leached (Fig. 1a). When compared to the control soils, soils treated with cow urine lost a significantly larger amount of NO₃⁻-N for all the aggregate sizes (Fig. 1a).

When comparing between the urine and the urine_Na treatments, the added Na⁺ significantly increased the amount of NH₄⁺-N loss by 30% but there was no significant difference between the urine_Na and the urine_NaNa treatments, when averaged across the aggregate sizes. For the total amount of NH₄⁺-N lost from the soils, similar to the loss of NO₃⁻-N, the aggregate sizes and the urine treatments had a significant effect (Fig. 1b). Soils with cow urine treatments lost a significantly larger amount of NH₄⁺-N when compared to the control soils. The loss of NH₄⁺-N occurred predominantly after the 1st simulated rain event, when compared to the 2nd and the 3rd simulated rain events.

**Inorganic-N remaining in soils after the rain events**

For the NO₃⁻-N remaining in soils at the end of the experiment, the aggregate size and the urine treatments had a highly significant effect (Fig. 2a). The cow urine treatments (urine_Urea, and urine_UreaNa) significantly increased the total amount of NO₃⁻-N remaining in the soil when compared to the control.
When compared among the soils with added urine, including the urine_Na and urine_NaNa treatments, the small aggregates showed approximately 1.41 and 1.67-fold higher soil NO$_3^-$-N concentrations when compared to the medium and large aggregates.

The amount of NH$_4^+$-N remaining in the soil, at the end of the experiment, was significantly increased with increasing aggregate sizes (Fig. 2b). Cow urine treatments significantly increased the total amount of NH$_4^+$-N remaining in the soil when compared to the control. The interaction between the aggregate size and the urine treatments was also highly significant. When compared among the urine treatments (urine, urine_Na, and urine_NaNa), the added Na$^+$ significantly increased the amount of NH$_4^+$-N in the medium and the large aggregates.

**Figure 1.** The amount of nutrient loss from soil cores after simulated rain events. Different coloured bars indicate the nutrient loss after each simulated rain treatment. The loss of (a) NO$_3^-$-N and (b) NH$_4^+$-N were shown in separate figures. The error bars indicate standard deviations ($n=3$). The small letters on each bar indicate significant difference within each aggregate size.

**Figure 2.** The amount of inorganic-N remaining in soils after the three simulated heavy rain events. The amounts of (a) NO$_3^-$-N, (b) NH$_4^+$-N were shown in separate figures. The error bars indicate standard deviations ($n=3$). The small letters on each bar indicate significant difference within each aggregate size. The capital letters indicate significant difference within each treatment to show the effect of aggregate size on each urine treatment.

**Discussion**

**Loss of inorganic-N from soils**

The Na$^+$ concentrations in urine clearly influenced the movement of inorganic-N in soils following the simulated heavy rain events. Overall, the increased Na$^+$ contents in urine decreased the loss of NO$_3^-$-N, whereas increased the loss of NH$_4^+$-N (Fig. 1a and 1b). This finding suggests that nitrification rates were decreased due to the increased Na$^+$ concentrations in urine. The decreased nitrification rates due to the increased Na$^+$ concentrations in soil solutions were previously reported (McCormick and Wolf 1980). We...
also have to consider that high-Na diet can potentially increase the water uptake of dairy cows, resulted in the dilution of urinary-N.

The amount of NO$_3^-$-N lost from the urine treated soils was relatively higher when the urine was applied on the small and the medium aggregates, when compared to the large aggregates (Fig. 1a). Contrastingly, Di and Cameron (2002) reported that NO$_3^-$-N leaching losses are usually less from fine-textured soils when compared to coarse-textured soils due to the slower drainage and the greater potential for denitrification in the fine-textured soils. In the current experiment, the significant proportion of N was lost as NH$_4^+$-N, and the amount of NH$_4^+$-N loss was positively correlated to the increasing aggregate size (Fig. 1b). Thus, a reason for the smaller amount of NO$_3^-$-N loss from the large aggregate size was likely to be because urine-N was lost as NH$_4^+$-N before it was nitrified to NO$_3^-$-N.

The amount of NO$_3^-$-N remaining in the soils after the rain treatments was higher in the small aggregates compared to the medium and the large aggregates (Fig. 2). Thus, NO$_3^-$-N loss potential in the small aggregates might be higher than the larger aggregates. However, in the large aggregates, questions remain in terms of the fate of the NH$_4^+$-N in longer term because the NH$_4^+$-N may be nitrified in a longer term and be leached from the soil. We still believe that our finding is valuable because the activity of soil microbes are markedly different in a few centimeters scales of soil depth, according to a study observed the response of denitrifying microbes to flooding events at 0–1 cm and 1–3 cm (Uchida et al. 2014). Thus, in the current experiment, we simulated the nutrient movement from the soil surface zone, where soil microbes and roots are the most active, to the next zone (>2.5 cm) where there are relatively smaller amount of microbes and roots.

**Conclusion**

The result suggested that nitrification rates may have slowed down due to the increased urine-Na concentrations and this can be a reason for reduced NO$_3^-$-N leaching from large aggregate soil (5–7 mm). Contrastingly, the loss of NH$_4^+$-N from soils was increased with increasing urine-Na concentrations regardless of the aggregate size groups (i.e. 0–3, 3–5, and 5–7 mm). The current experiment was performed as a small scale soil core experiment (soil depth = 2.5 cm), without the plant presence but simulated the potential impacts of high-Na feeds, commonly used in the dairy industries, on nutrient dynamics in pasture soils, via high-Na cow urine addition to the soils. We note that N below the soil depth of 2.5 cm can still be available for plants thus in this study, we aimed to identify the potential changes in N dynamics under pastoral soils when urine-Na concentration increased. Further studies are needed to implicate our findings to larger scales with the presence of plants.

**References**


