

Research Article

Sodium Contents in Dairy Cow Urine and Soil Aggregate Sizes Influence the Amount of Nitrogen Lost from Soil

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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 to the ground water as nitrate (NO_3^-) and to the atmosphere as nitrous oxide (N_2O). These N-related environmental impacts are possibly related to the sodium (Na^+) concentrations in urine. We sampled a pasture soil and separated it into three aggregate size groups (0–3, 3–5, and 5–7 mm). Then, cow urine with variable Na^+ concentrations ($4.3\text{--}6.1\text{ g Na}^+ \text{ L}^{-1}$) was added to the soil cores. We treated the cores with simulated heavy rains and measured the amounts of calcium (Ca^{2+}), Na^+ , potassium (K^+), and inorganic-N leached from the soils. N_2O emission rates were also determined throughout the experimental period. Increasing Na^+ concentration in urine decreased the loss of NO_3^- (–20%), after repeatedly applied simulated rain treatments ($30\text{ mm} \times 3$), whereas it increased the loss of ammonium (31%) and K^+ (19%). For the loss of Ca^{2+} and the emissions of N_2O , the effect of the Na^+ concentrations was unclear. Field level studies and studies focusing on the mechanisms behind the changes in nutrient losses are needed.

1. 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), the nutrients can potentially be lost to the ground water and to the atmosphere [1]. The nutrient loss to the ground water can induce environmental problems; for example, nitrate (NO_3^-) lost from dairy farm soils to the water system is known as a pollutant [2]. High NO_3^- in drinking water causes health problems such as blue baby syndrome [3]. The eutrophication of aqueous environment is also induced by high NO_3^- in water [4]. Thus, the loss of NO_3^- from grazed pasture systems has to be minimized [5]. The major source of the NO_3^- loss from dairy farming systems is cow urine [2]. Cow urine deposited on grazed pasture systems forms a patch in soils with high nitrogen (N) concentrations (urine patch) and the risk of NO_3^- pollutions is high under the urine patches [6]. Another potential negative impact on the environment derived from urine patches is the emission of nitrous oxide (N_2O), a greenhouse

gas with a long-term global warming potential of about 298 times that of carbon dioxide [7]. Under urine patches, microbial processes, namely, nitrification and denitrification, are responsible for N_2O emissions [8].

Previous studies reported that there are multiple factors influencing the magnitudes of NO_3^- leaching and N_2O emissions under urine patches (e.g., [9–12]). Within the factors, soil physical factors are particularly important. For example, Di and Cameron [2] reported that NO_3^- leaching losses are usually less from fine-textured soils when compared to coarse-textured soils due to slower drainage and greater potential for denitrification. Similarly, Scholefield et al. [13] showed that the drainage capacity of soils determined the amount of NO_3^- lost from the soils because of the preferential flow of water through large pore spaces. For N_2O emissions, the soil anaerobicity, influenced by the soil physical factors, is an important factor controlling their magnitudes. For example, Uchida et al. [14] showed that the highest peak of N_2O emissions, after an addition of urine to a pasture soil, occurred from the smallest and most compacted aggregates when aggregates of different sizes (range: 0–5.6 mm) were

compared. Contrastingly, Robinson et al. [15] reported that the varied aggregate sizes (1–5.6 mm) did not influence the cumulative N_2O emissions over 300 days following a urine application. Thus, further studies are needed to investigate the effect of soil physical factors, including aggregate sizes on NO_3^- leaching and N_2O emissions from soils with added urine. Also, many previous studies focusing on N_2O emissions did not consider the loss of NO_3^- as leachate. Therefore to understand the factors affecting the magnitudes of the N-related environmental impacts due to the addition of urine on soils, more studies focusing on both NO_3^- leaching and N_2O emissions, in relation to soil physical factors, are needed.

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^+) and sodium (Na^+) [16]. Previous study shows that Na contents in cow urine markedly varied (0.03–0.43%) and the urine-Na concentrations in cows depend on their diet [17]. The amount of other nutrients excreted as urine is also controlled by the nutrients consumed by cows [18]. Edwards et al. [19] reported that urine-N concentrations are generally low ($2\text{--}3\text{ g N L}^{-1}$) when low-N diet is fed to cows. In the modern dairy farming industry, the supplementary feeding of K^+ and Na^+ is often performed particularly during summer to avoid the heat stress of dairy cows [20]. This supplementary feeding would result in the increase in K^+ and Na^+ excreted on soils as urine although the balance between Na^+ input and output in dairy cows is overly influenced by temperature (heat stress), pregnancy, and lactation, the function of kidney and gastrointestinal tract [21]. Previous studies reported that an addition of potassium chloride (KCl) to soils had a negative effect on nitrification in soils [22]. Also, the presence of Na^+ in soils negatively influences nitrification and ammonia volatilization rates [23–26]. Thus, there is a possibility that varying urine-Na concentrations influence the magnitudes of the N-related environmental impacts derived from urine patches.

In sheep farming systems, feeding animals with high NaCl diet was reported as an option to reduce soil N_2O emissions [27]. It is difficult to estimate whether this is the case for more intensively managed dairy farming systems. The interactions among soil physical properties, heavy rainfall events, and Na^+ concentrations in urine on NO_3^- leaching and N_2O emissions are still unknown. The study by Liu and Zhou [27] focused on the influence of NaCl in animal feeds on urine volume and urine-N concentrations, but Na^+ concentrations in urine were not measured. Also, they did not measure NO_3^- leaching.

Thus, a study is needed to investigate the influence of increasing Na^+ concentrations in cow urine on NO_3^- leaching and N_2O emissions from urine patches, particularly under the dairy farming systems. Thus, the objective of this study was to examine the effect of Na^+ concentrations in urine and soil aggregate size on NO_3^- leaching and N_2O emissions under urine affected grazed pasture soils.

TABLE 1: Soil pH and nutrients (NO_3^- -N, NH_4^+ -N, exchangeable- K^+ , and exchangeable- Ca^{2+}) in soil ($mg\text{ kg}^{-1}$ soil) measured for each aggregate size. The soil:water ratio for the soil pH measurement was 2:5. The inorganic-N was measured using a colorimetric method. Exchangeable- K^+ and $-Ca^{2+}$ were measured using an atomic absorption method after the extraction with a NH_4COOH solution.

	Small	Medium	Large
pH	5.8 ± 0.1	5.8 ± 0.0	5.8 ± 0.0
NO_3^- -N $mg\text{ kg}^{-1}$	25.8 ± 0.8	23.6 ± 1.7	18.8 ± 1.4
NH_4^+ -N $mg\text{ kg}^{-1}$	15.2 ± 0.7	17.7 ± 0.6	13.8 ± 1.0
K $mg\text{ kg}^{-1}$	539.3 ± 9.6	552.3 ± 7.0	555 ± 17.1
Ca $mg\text{ kg}^{-1}$	6392 ± 90.5	6307 ± 133.4	6118 ± 275.3
CEC $meq\text{ kg}^{-1}$	421.3 ± 11.7	422.3 ± 10.5	416.7 ± 2.1
Organic matter %	6.5 ± 0.3	5.4 ± 0.1	5.2 ± 0.4
Soil texture			
Sand %	13.7 ± 3.2	14.8 ± 3.5	18.4 ± 4.5
Silt %	56.7 ± 1.4	56.5 ± 6.5	51.8 ± 4.5
Clay %	29.5 ± 4.3	28.7 ± 3.3	29.8 ± 3.7

2. Materials and Methods

2.1. Soil Characteristics. The soil used in the experiment was an Andosol collected (0–5 cm depth) from a dairy farm pasture ($43^\circ 4.38'N$, $141^\circ 20.8'E$, 13 m above sea level) at October 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. Gravels (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 gravels. 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 pot. The soil surface area was 34 cm^2 and the soil depth was 2.5 cm (85 cm^3). Each aggregate size (small, medium, and large) pot contained the equivalent of 79.2, 72.5, and 70.6 g dried soil, respectively; thus the density of the packed soils was 0.93, 0.85, and 0.83 g cm^{-3} for small, medium, and large aggregate size, respectively. Before the experiments were started, the characteristics of each aggregate size were measured by Tokachi Federation of Agricultural Cooperatives and were shown in Table 1. The smaller the aggregates were, the more NO_3^- -N and organic matter were presented in the soil.

2.2. Treatments and Leachate Collection. The day when the urine treatment was applied was noted as day zero (0). The pots were slowly wetted up from the bottom on day -7 and were left to drain for 7 days before urine treatments were applied. Soil moistures were expressed as water-filled pore space (WFPS) and this was achieved by weighing the whole apparatus (soil, gravel, and pet bottle) every 2–3 days.

TABLE 2: The components of cow urine were analyzed. Urine component was measured by atomic absorption.

	All values are in g N, Ca, K, or Na (L cow urine ⁻¹)			
	N	K	Na	Ca
Urine		5.02 + 0.09	4.28 + 0.02	0.004 + 0.00026
Urine_Na	2.26 + 0.17	6.02 + 0.30	5.34 + 0.11	0.003 + 0.00226
Urine_NaNa		5.75 + 0.12	6.09 + 0.03	0.009 + 0.00264

The particle density of the soil was assumed to be 2.65 g cm⁻³ to calculate the WFPS. 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 Na L⁻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 (Table 2). The nutrient characteristics of the urine used in the current experiment were shown in Table 2. The experimental design was completely randomized factorial (3 aggregate size × 4 urine treatments) with three replicates. The nutrient composition of the urine is described in Table 2. The pot experiments were performed in a temperature-controlled greenhouse and the average temperature during experiments was 25 degrees C.

To simulate heavy rains, inducing the nutrient leaching from soils, each pot received 30 mm of water on days 2, 8, and 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.

2.3. Measurements of Leached Nutrients. 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). Additionally, calcium (Ca²⁺), K⁺, and Na⁺ ion concentrations in the leachate samples were measured by compact water quality meters (Horiba, Ltd., Japan). To improve the accuracy of the measurements, the calibration curves were made for the water quality meters using >4 standards within the concentration ranges for each ion type, instead of using a single concentration standard, provided by the supplier.

2.4. Remaining Inorganic N in the Soil. 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.

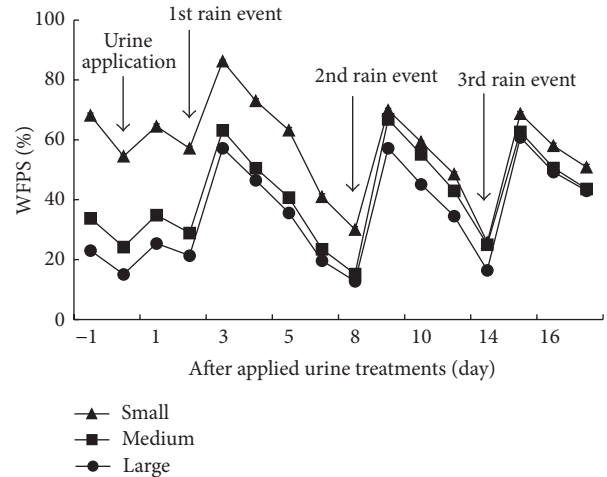


FIGURE 1: Changes in water-filled pore space (WFPS %) during the experiment. Triangles, squares, and circles are small, medium, and large aggregates, respectively. The errors were standard deviations ($n = 3$). The arrows express the timings for urine application and three simulated heavy rains.

2.5. Gas Measurements. Soil N₂O flux measurements were performed 16 times within the experimental period. Each measurement was performed between 1100 and 1400 hours. To measure N₂O, each pot was placed inside a glass bottle (2L). Then the bottles were capped for 30 min. Then the ambient air and the headspace gas inside the jar were sampled. The gas samples (30 mL) were taken using a gas-tight syringe via septa embedded in the lids of the jars and placed in evacuated vials (20 mL). The N₂O samples were measured by a gas chromatograph (GC-2014, Shimadzu Co., Japan) and analysed within 48 h of sampling.

2.6. 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 Tukey's method for multiple comparisons. The same statistical analysis was performed for the soil inorganic-N data. The cumulative N₂O flux data were log-transformed ($\log_{10}(\text{flux} + 1)$), so that data had a normal distribution, and the data were subjected to the two-way analysis of variance (ANOVA) with aggregate size and urine as factors.

3. Results

3.1. Changes in Soil Moisture during the Experiment. Throughout the experiment, soil moisture (WFPS) was small > medium > large aggregate size, when averaged across the urine treatments (Figure 1). Within the same aggregate size, the urine treatment did not influence the soil moisture contents throughout the experiment. The heavy rain treatments immediately increased WFPS and between the heavy rain treatments, WFPS sharply declined for all aggregate size treatments (Figure 1).

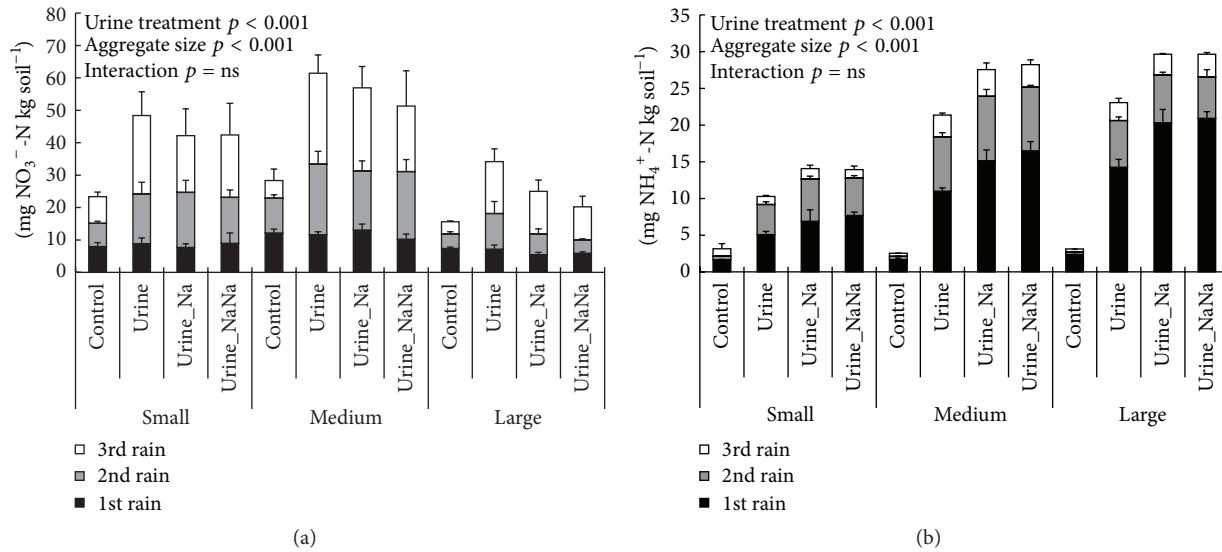


FIGURE 2: The amount of nutrient loss from soil cores after simulated rain events. Different coloured bars indicate the nutrient loss after each simulated rain treatment. The loss of (a) $\text{NO}_3^- \text{-N}$ and (b) $\text{NH}_4^+ \text{-N}$ was shown in separate figures. The error bars indicate standard deviations ($n = 3$).

3.2. Loss of Inorganic-N from Soils. The aggregate size and the urine treatments had a significant effect ($p < 0.001$) on the total amount of $\text{NO}_3^- \text{-N}$ lost from 2.5 cm depth of soil. There was no interaction between the aggregate size and the urine treatments on the total amount of $\text{NO}_3^- \text{-N}$ leached (Figure 2(a)). According to Tukey's multiple comparison tests, when compared to the control soils, soils treated with cow urine (urine, urine_Na, and urine_NaNa) lost a significantly larger amount of $\text{NO}_3^- \text{-N}$ for all the aggregate sizes (Figure 2(a)). When averaged across the aggregate sizes and the urine treatments, the amount of $\text{NO}_3^- \text{-N}$ lost from soils was the largest after the 3rd simulated rain event, when compared to the 1st and the 2nd simulated rain events. The added Na^+ significantly decreased the amount of $\text{NO}_3^- \text{-N}$ loss by 41% only in the large aggregates when applied at the highest concentration (urine_NaNa) compared to the urine only treatment.

For the total amount of $\text{NH}_4^+ \text{-N}$ lost from the soils, similar to the loss of $\text{NO}_3^- \text{-N}$, the aggregate sizes and the urine treatments had a significant effect ($p < 0.001$) (Figure 2(b)). Soils with cow urine treatments lost a significantly larger amount of $\text{NH}_4^+ \text{-N}$ when compared to the control soils. The loss of $\text{NH}_4^+ \text{-N}$ occurred predominantly after the 1st simulated rain event, when compared to the 2nd and the 3rd simulated rain events. When comparing between the urine and the urine_Na treatments, the added Na^+ significantly increased the amount of $\text{NH}_4^+ \text{-N}$ loss by 30% but there was no significant difference between the urine_Na and the urine_NaNa treatments, when averaged across the aggregate sizes.

3.3. Inorganic-N Remaining in Soils after the Rain Events. For the $\text{NO}_3^- \text{-N}$ remaining in soils at the end of the experiment, the aggregate size and the urine treatments had a highly significant effect ($p < 0.001$) (Figure 3(a)). The cow urine treatments (urine, urine_Na, and urine_NaNa) significantly

increased the total amount of $\text{NO}_3^- \text{-N}$ 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 $\text{NO}_3^- \text{-N}$ concentrations when compared to the medium and large aggregates.

The amount of $\text{NH}_4^+ \text{-N}$ remaining in the soil, at the end of the experiment, was significantly ($p < 0.001$) increased with increasing aggregate sizes (Figure 3(b)). Cow urine treatments significantly ($p < 0.001$) increased the total amount of $\text{NH}_4^+ \text{-N}$ remaining in the soil when compared to the control. The interaction between the aggregate size and the urine treatments was also highly significant ($p < 0.001$). When compared among the urine treatments (urine, urine_Na, and urine_NaNa), the added Na^+ significantly increased the amount of $\text{NH}_4^+ \text{-N}$ in the medium and the large aggregates.

3.4. Nitrous Oxide Emissions. Nitrous oxide emissions peaked immediately following the first simulated rain treatment for soils that received cow urine (with and without Na), when averaged across the aggregate sizes (Figure 4(a)). The Na^+ concentrations of urine did not influence the time course of N_2O emissions and the cumulative N_2O emissions (Figure 5). When averaged across the urine treatments, the N_2O emissions were relatively higher for the small and the large aggregates, when compared to the medium aggregates (Figure 4(b)).

3.5. Leaching of Potassium, Sodium, and Calcium Ions from Soils. The aggregate sizes and the urine treatments had a significant effect ($p < 0.001$) on the amount of the total K^+ loss (Figure 6(a)). The addition of cow urine markedly increased the total amount of the K^+ loss when compared to the control soils. The K^+ loss was the largest after the 1st simulated rain event when compared to the 2nd and the 3rd

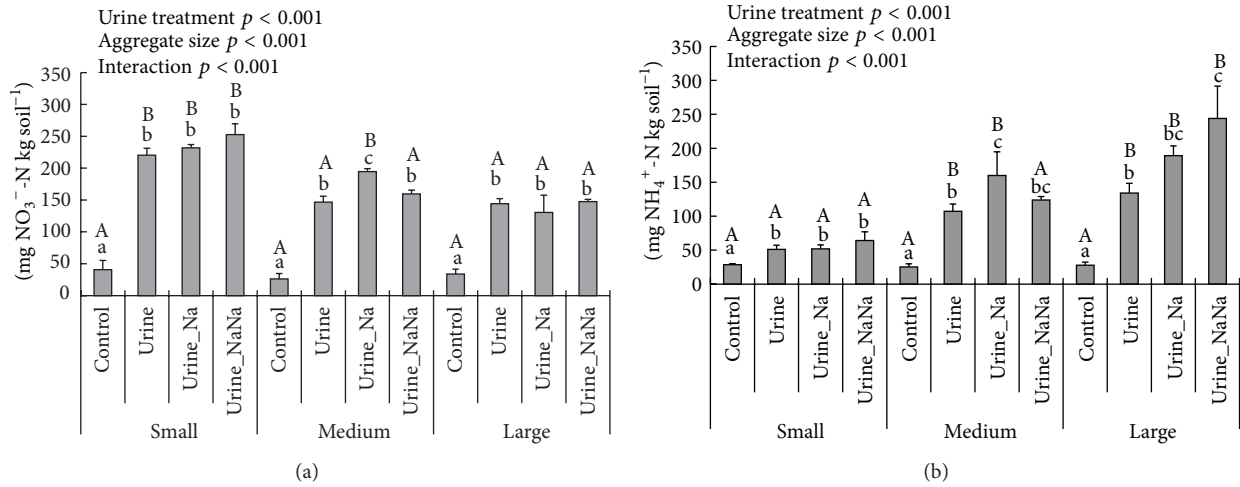


FIGURE 3: The amount of inorganic-N remaining in soils after the three simulated heavy rain events. The amounts of (a) NO_3^- -N and (b) NH_4^+ -N were shown in separate figures. The error bars indicate standard deviations ($n = 3$). The small letters on each bar indicate significant difference within each aggregate size. The capital letters indicate significant difference within each treatment to show the effect of aggregate size on each urine treatment.

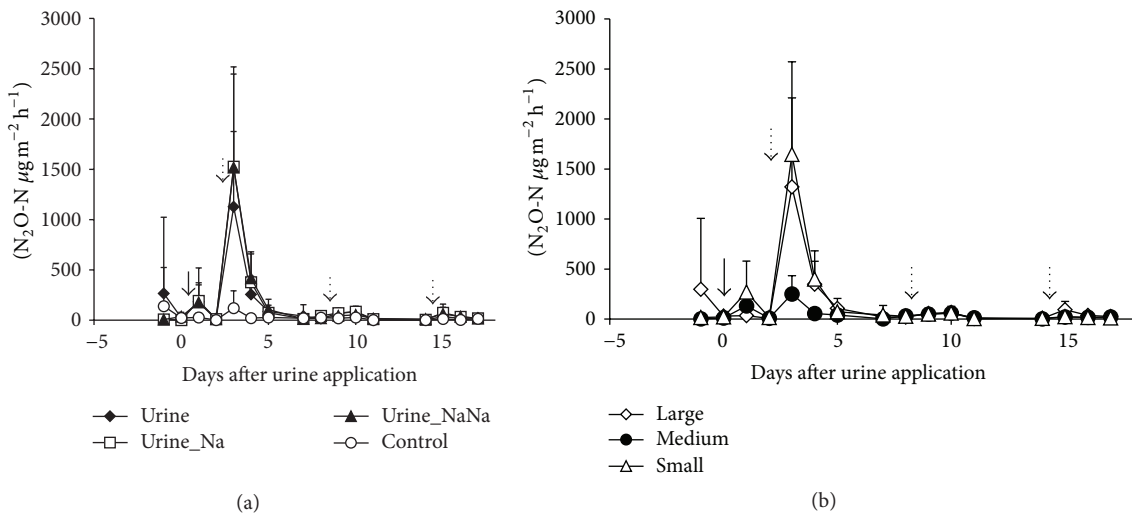


FIGURE 4: The effects of the application of (a) urine with different Na^+ concentrations and (b) different aggregate sizes on N_2O emissions. The timing of urine application is shown as a solid-line arrow. Dotted line arrows indicate the timing of the rain treatment. The error bars are standard deviations ($n = 9$ for (a) and $n = 12$ for (b)).

simulated rain events. The addition of Na^+ in urine increased the loss of K^+ compared to the urine treatment, but only from the small aggregate soil treatments.

For the total loss of Na^+ , the aggregate sizes and the urine treatments had a highly significant effect ($p < 0.001$) (Figure 6(b)). The amount of Na^+ leached was in order of urine_NaNa > urine_Na > urine > control, when averaged across the aggregate sizes. The amount of the leached Na^+ was the largest after the 1st simulated rain event when compared to the 2nd and the 3rd simulated rain events, when averaged across the urine treatments.

For the total Ca^{2+} loss, the aggregate sizes and the urine treatments had a highly significant effect ($p < 0.001$) but there was no interaction between them (Figure 6(c)). The addition of cow urine to soils increased the loss of Ca^{2+} but

the effect of the Na^+ contents in the urine was absent. The lost Ca^{2+} was the largest after the 1st simulated rain event when compared to the 2nd and the 3rd simulated rain events.

4. Discussion

4.1. Loss of Inorganic-N from Soils. The current study indicated that cow urine added to soils significantly increased the loss of NO_3^- -N from soils after heavy rain events (Figure 2(a)). This was indicated by many previous studies; for example, Silva et al. [28] reported NO_3^- -N concentrations in drainage water under a grazed pasture treated with cow urine peaked at $120 \text{ mg NO}_3^- \text{ N L}^{-1}$. This was equivalent to $124 \text{ kg N ha}^{-1} \text{ y}^{-1}$. The loss of NO_3^- -N from soils observed in the current experiment was relatively smaller when compared

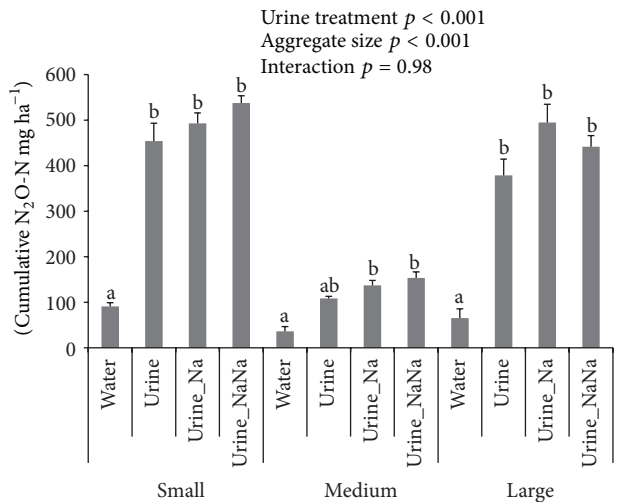


FIGURE 5: The effects of the urine application with different Na⁺ concentrations and aggregate sizes on cumulative N₂O emissions. The error bars are standard deviations ($n = 3$).

to Silva et al. [28]. In the current experiment, the concentration of leached NO₃⁻-N peaked at 16 mg NO₃⁻-N L⁻¹ and the total loss of NO₃⁻-N after the three heavy rain treatments was equivalent to 11 kg N ha⁻¹ when averaged across the urine treated soils. The amount of N added on soils as urine-N was much smaller in the current study (51 kg N ha⁻¹) when compared to the previous study by Silva et al. [28] (1000 kg N ha⁻¹ y⁻¹).

The amount of NO₃⁻-N lost from the urine treated soils was relatively higher when the urine was applied on the small (0–3 mm) and the medium aggregates (3–5 mm), when compared to the large aggregates (5–7 mm) (Figure 2(a)). Contrastingly, Di and Cameron [2] reported that NO₃⁻-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. Our data suggested that the small aggregates contained less sand but more silt when compared to the larger aggregates (Table 1), and we visually observed that soil was relatively more poorly drained in the small aggregates. In the current experiment, the significant proportion of N was lost as NH₄⁺-N, and the amount of NH₄⁺-N loss was positively correlated to the increasing aggregate size (Figure 2(b)). Thus, a reason for the smaller amount of NO₃⁻-N loss from the large aggregate size was likely to be because urine-N was lost as NH₄⁺-N before it was nitrified to NO₃⁻-N.

The amount of NO₃⁻-N remaining in the soils after the three simulated rain treatments was higher in the small aggregates compared to the medium and the large aggregates (Figure 3(a)). Contrastingly, the amount of NH₄⁺-N remaining in soils after the urine treatment was relatively higher in the large aggregates when compared to the small aggregates (Figure 3(b)). Thus, NO₃⁻-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 NH₄⁺-N in longer term because the NH₄⁺-N may be nitrified in a longer term and be leached from the soil.

The loss of NH₄⁺-N from soils as a leachate has been reported as negligible at the field level [29, 30] because the soil particles fix NH₄⁺-N [31]. The current experiment was conducted as a small scale laboratory experiment and the soil depth was very shallow (2.5 cm). This was why we observed the significant loss of NH₄⁺-N from soils. However, we still believe that our finding is valuable because the activity of soil microbes is markedly different in a few centimeters scales of soil depth, according to a study that observed the response of denitrifying microbes to flooding events at 0–1 cm and 1–3 cm [32]. 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 amounts of microbes and roots [33, 34]. However, soil disturbance might affect microbial activity because of physical process of sieving and repacking of the soil.

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 NO₃⁻-N, whereas they increased the loss of NH₄⁺-N (Figures 2(a) and 2(b)). 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 [26]. Quanzhong and Guanhu [26] compared soils with Na⁺ concentrations in the range 0–16 mg Na⁺ L⁻¹. In the current experiment, Na⁺ concentration in soils after the application of urine was assumed to be 454–724 mg kg⁻¹ soil; thus the impact of Na⁺ in the current experiment would be stronger although the amount of Na⁺ in urine was within the realistic level [17]. We also have to consider that high-Na diet can potentially increase the water uptake of dairy cows, resulting in the dilution of urinary-N, although the amount of N excreted as urine per a unit of time (e.g., per day) may be uninfluenced by the amount of Na⁺ uptake [35].

Furthermore, increasing urine-Na concentrations might have influenced other N processes not measured in the current experiment (e.g., NH₃ volatilization and N immobilization). When the leached inorganic-N and soil inorganic-N were added, there was a trend that, with increasing urine-Na concentrations, the added N values were higher (Supplementary Fig. S1, in Supplementary Material available online at <http://dx.doi.org/10.1155/2015/275985>). Witter and Kirchmann [36] reported that NH₃ volatilization during the decomposition of poultry manure was significantly reduced through the additions of Ca and magnesium salts. Similarly, Westerman and Tucker [37] reported that the immobilization of N in soils was significantly decreased by high concentrations of Na, copper, and calcium chloride salts. We could not find previous studies specifically focused on the Na⁺ concentrations in cow urine on NH₃ volatilization and N immobilization; thus, further studies are required in this area.

4.2. N₂O Emissions. The addition of urine markedly increased the N₂O emissions from soils immediately after the 1st simulated heavy rain event (Figure 4, day 3). However, the sharp increases in N₂O emissions were not observed after

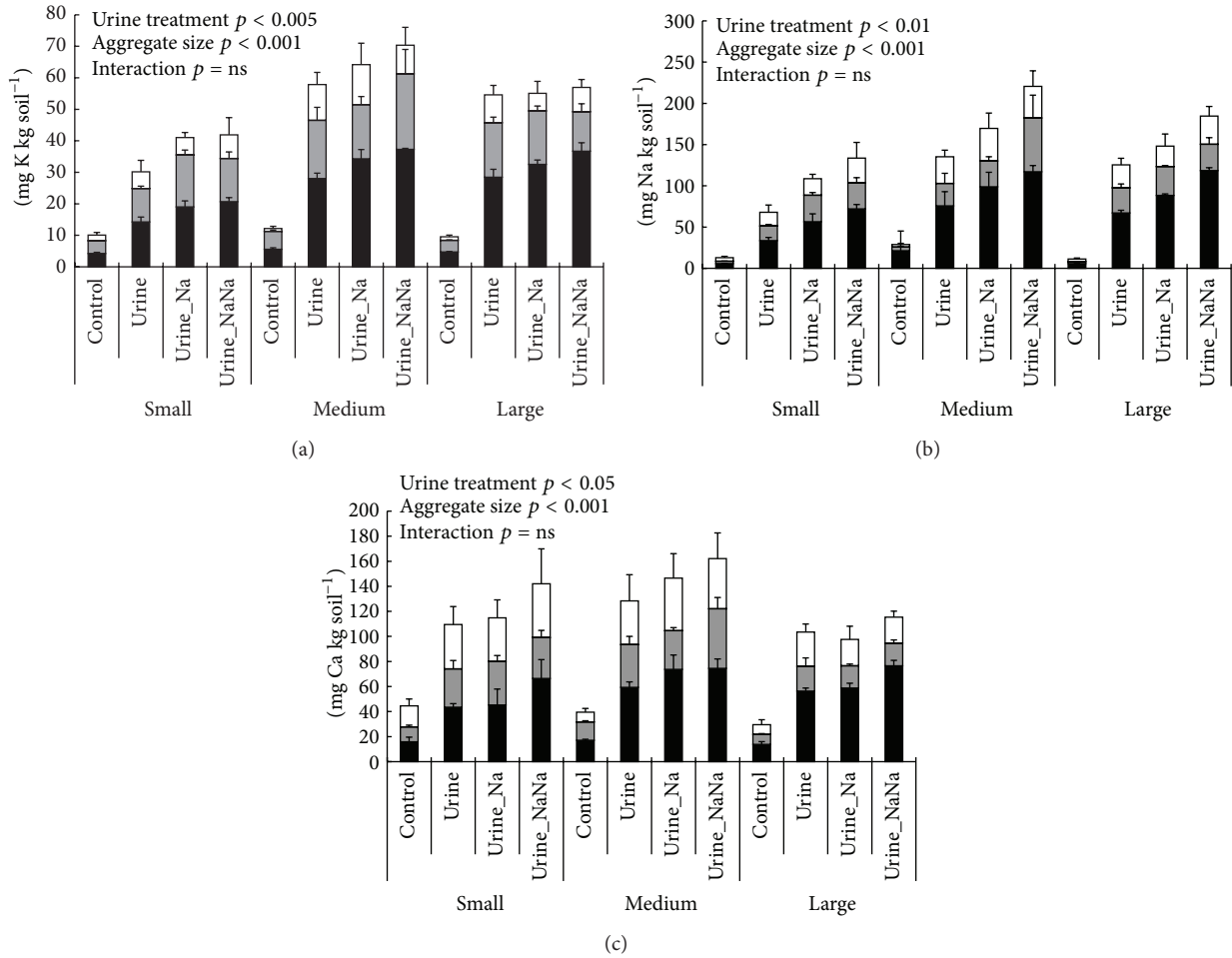


FIGURE 6: The amount of nutrient loss from soil cores after simulated rain events. Different coloured bars indicate the nutrient loss after each simulated rain treatment. The loss of (a) K⁺, (b) Na⁺, and (c) Ca²⁺ was shown in separate figures. The error bars indicate standard deviations ($n = 3$).

the 2nd and the 3rd simulated rain event, when averaged across the urine treated soils. The availability of NO₃⁻-N and NH₄⁺-N was still significantly higher in the urine-treated soils, even after the 3rd rain event (Figure 3); thus other factors such as the carbon availability might have limited the N₂O emissions after the 2nd and the 3rd rain events. The magnitudes of N₂O emissions following a heavy rain event are reported to be influenced by the amount of soluble carbon in soils [38].

The cumulative N₂O emissions were relatively higher for the small and the large aggregates when compared to the medium aggregates (Figure 5). In the small aggregates, the soil moisture was relatively higher throughout the experiment, when compared to the other aggregates (Figure 1); thus N₂O producing microbes which favour high soil moisture (i.e., denitrifiers) might be responsible for the N₂O emissions in the current experiment in the small aggregates. Uchida et al. [14] reported relatively higher N₂O emissions from soils with smaller aggregate sizes when four aggregate size categories (0-1, 1-2, 2-4, and 4-5.6 mm) were compared. The current study covered relatively larger aggregate sizes (up to 7 mm) when compared to the study by Uchida et al.

[14] (up to 5.6 mm). Therefore, the reason for the high N₂O emissions in the large aggregates in the current study might be due to the anaerobic sites in the middle of the large aggregates, stimulating the denitrification processes. The anaerobic sites within aggregates are more likely to occur in large aggregates and the anaerobic centre was found within an aggregate when the radius of the aggregate was >4 mm [39, 40].

The effect of varying Na⁺ concentrations in urine on N₂O emissions was not clear. It has been reported that the excess Na⁺ in soils damages soil structures [41] and the damage to soil structures increases N₂O emissions due to the decrease in the air permeability [42]. However, this was not observed in the current experiment. It is difficult to fathom reasons for this because the availability of inorganic N has been influenced by the urine-Na to an extent. We believe that this was simply because of the large variability in N₂O emissions in natural soils [43]. Additionally, cow urine can potentially damage soil structures due to its high pH solubilizing soil organic carbon [44]; thus the effect of changes in urine-Na concentrations on soil structures might be overshadowed by the soil structural damage caused by high pH of urine.

4.3. Loss of Other Cations from Soils. The addition of urine to soils significantly increased the amount of K^+ , Ca^{2+} , and Na^+ losses from soils, when compared to the control treatment (Figures 6(a), 6(b), and 6(c)). We note that, in our experiment, plants were not present. With plants, the amount of cations lost from soils would be reduced due to the plant uptake. For example, a previous study, performed using a lysimeter system with ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) pasture, showed that only 1.8% of the applied urine-K (the applied $K^+ = 55 \text{ g m}^{-2}$) was lost from the lysimeter (soil depth = 1200 mm) as a leachate [45]. Thus, further studies are needed to examine how the effect of aggregate size on cation leachate, which we observed in the current study, is influenced due to the presence of plants and to greater soil depth.

The current experiment suggested that more than half of the added urine-Na remained in soils even after the three simulated heavy rain treatments. High Na^+ concentrations in soils are problematic due to their negative effect on soil structures [41] and on the plant growth [46]. Further research is needed to investigate whether the use of a high-Na diet in the dairy industry results in the accumulation of Na^+ in soils and future studies should link the high-Na diet and its effect on the plant growth in relation to soil nutrient dynamics. Our results also showed that most of the Ca^{2+} lost from the soils originated from soil particles or soil solution, rather than from urine-Ca (Table 2). The removal of Ca^{2+} from cation exchange sites was likely to occur due to the addition of the large amount of K^+ and H^+ in urine [47]. Thus, the added urine-K might have been replaced with Ca^{2+} in cation exchange sites. Calcium in soils plays an important role in improving the aggregate stability [48] and the plant growth [49]; thus the loss of Ca^{2+} from soils should be minimized.

5. 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 increasing urine-Na concentrations also increased the loss of K^+ from soils while they had no influence on the amount of Ca^{2+} lost from soils. The relationship between N_2O emissions and Na^+ concentrations in urine was unclear. 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.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

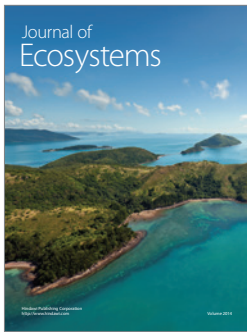
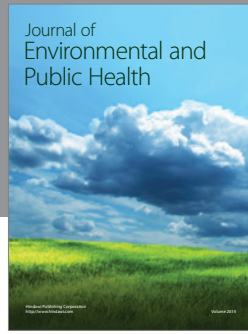
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