Dairy Farm Effluent Effects on Urine Patch Nitrous Oxide and Carbon Dioxide Emissions

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ABSTRACT

Dairy farm effluent (DFE) comprises animal feces, urine, and wash-down water collected at the milking shed. This is collected daily during the milking season and sprayed onto grazed dairy pastures. Urine patches in grazed pastures make a significant contribution to anthropogenic N_2O emissions. The DFE could potentially mitigate N_2O emissions by influencing the N_2O to dinitrogen (N_2) ratio, since it contains water-soluble carbon (WSC). Alternatively, DFE may enhance N_2O emissions from urine patches. The application of DFE may also provide a substrate for the production of CO_2 in pasture soils. The effects of DFE on the CO_2 and N_2O emissions from urine patches are unknown. Thus a laboratory experiment was performed where repeated DFE applications were made to repacked soil cores. Dairy farm effluent was applied at 0, 7, or 14 d after urine deposition. The urine was applied once on Day 0. Urine contained ^{15}N-enriched urea. Measurements of N_2O, N_2, and carbon dioxide (CO_2) fluxes, soil pH, and soil inorganic N concentrations were made. After 43 d the DFE had not mitigated N_2O fluxes from urine patches. A small increase in the N_2O flux occurred from the urine-treated soils where DFE was applied 1 wk after urine deposition. The amount of WSC applied in the DFE proved to be insignificant compared with the amount of soil C released as CO_2 following urine application. The priming of soil C in urine patches has implications for the understanding of soil C processes in grazed pasture ecosystems and the budgeting of C within these ecosystems.

D UE TO ITS DUAL ROLE as a greenhouse gas (Duxbury et al., 1993) and as a precursor to ozone-depleting gases (Crutzen, 1981) there is interest in mitigating the emissions of nitrous oxide (N_2O). Agricultural soils are a major source of anthropogenic N_2O (Food and Agriculture Organization of the United Nations, 2001) and intensive grazing systems have relatively high emissions of N_2O compared with cropping systems. For N_2O mitigation strategies to be adopted they must be economical, easily applied to existing farming methods, and/or require minimal disruption to existing practices. In New Zealand, legislation prevents the direct discharge of DFE to surface waters and current farming practice consists of applying the DFE to pasture soils. Dairy farm effluent is collected at the milking shed. Animal effluent is deposited in the concrete yard area as animals wait to be milked and during milking. The yard is washed down after milking, with the wash-down water and effluent gravity-fed into tanks where pumps then empty the DFE from the tanks by spray irrigating nearby pasture. The predominant constituents of the DFE are urine and dung. Dairy farm effluent composition varies according to animal numbers, feed quality, and volume of wash-down water. Dairy farm effluent contains nitrogen (N) as urea, ammonium (NH_4^+), nitrate (NO_3^-), and organic N forms. The total N content of DFE varies but reported values range from 260 to 280 mg N L^{-1} (Barton and Schipper, 2001; Di and Cameron, 2004). The C content of DFE also varies with feed quality. Barton and Schipper (2001) found DFE from pasture-fed animals had a total C content of 0.2%, a dry matter content of 0.4%, and a pH of 7.9. Nitrogen application rates of DFE to pasture thus depend on its composition and the volume of irrigation. The timing of DFE application also varies with respect to the time of urine deposition and the resulting urine patches. Dairy farm effluent may be applied immediately after grazing or several days after grazing depending on the grazing rotation employed on the farm and the DFE application strategy. It is also feasible that a urine patch may receive repeat applications of DFE.

Nitrous oxide emissions can be promoted by DFE application to pastures. Barton and Schipper (2001) found DFE irrigation to be a source of N_2O due to increases in soil N, water content, and available C when DFE was applied to peat and mineral soils. However, the study of Barton and Schipper (2001) did not examine the potential relationship between N_2O and dinitrogen (N_2) fluxes or the possible effect of repeated applications of DFE. The deposition of bovine urine N onto pasture soils also stimulates N_2O emissions. The availability of water-soluble carbon (WSC) can influence denitrification rates and/or the ratio of N_2O to N_2 (Burford and Bremner, 1975; Firestone, 1982). Priming effects are short-term changes in the turnover of soil organic matter where large amounts of C, N, and other nutrients may be released or immobilized in a very short time (Kuzyakov et al., 2000). Urine addition to soils can result in increases in CO_2 fluxes, over and above the amounts of C applied, with the release of native soil C indicative of a priming effect (Clough et al., 2003). The interaction of DFE and urine patches, with respect to N_2O emissions, has not been reported on. It is possible that DFE application to urine patches (i) increases N_2O emissions from urine patches due to the addition of extra N substrate and nutrients or (ii) alters the ratio of N_2O to N_2 due to the addition of C and irrigation water creating conditions conducive to the further reduction of N_2O. The objectives of this study were to assess the effects of DFE on urine patch N_2O, N_2, and CO_2 emissions.

MATERIALS AND METHODS

Soil and Treatments

A Temuka silt loam soil [Fluvaquentic Endoaquept; Soil Survey Staff (1998)] was collected from a 2-m^2 area of a dairy

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Abbreviations: DFE, dairy farm effluent; WFPS, water-filled pore space; WSC, water-soluble carbon.

Table 1. Timing of dairy farm effluent (DFE) treatment applications and total rates of inorganic N and water-soluble carbon (WSC) applied in the DFE treatments. The control and urine treatments did not receive DFE.

	Day						
Treatment†	0	7	14	21	28	Inorganic N	WSC
						kg ha^{-1}	
DFE	✓	✓	✓			1.5	49
UDFE0	✓	✓	✓			1.5	49
UDFE1		✓	✓	✓		4.0	29
UDFE2			✓	✓	✓	3.6	47

† Urine was applied on Day 0 in all of the DFE treatments. DFE applications, indicated by the symbol ✓, commenced on Days 0, 7, and 14 for treatments UDFE0, UDFE1, and UDFE2, respectively.

farm pasture, at a 0- to 10-cm depth (43°38.70′ S, 172°28.62′ E; 8 m above mean sea level). The soil bulk density in situ averaged (±standard error mean) 0.76 ± 0.06 Mg m^{-3}. The soil had a pH$_{(water)}$, loss on ignition, C, and N contents of 5.6, 16.0%, 7.3%, and 0.7%, respectively. Pasture species were principally perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). This pasture had a history of more than 50 yr of intensive dairying but had not previously received DFE.

The soil was air-dried, sieved (<0.4 cm), and then repacked into PVC cylinders (8.3-cm i.d. by 7.5 cm deep) to a bulk density of 0.77 Mg m^{-3}. These soil cores were then placed on tension tables as described in Clough et al. (2004), with suction applied using a hanging column of water (−5.0 cm from the base of the soil cores), and allowed to equilibrate at this moisture content for 2 wk (75% water-filled pore space, WFPS).

The first set of soil cores, subsequently referred to as "Group 1," consisted of four treatments: a control, DFE, urine, and a urine + DFE treatment where DFE applications commenced on the same day as urine application (UDFE0). The DFE applications were applied on three occasions, at 7-d intervals (Table 1). Treatments were replicated thrice with four destructive sampling times, giving 48 cores in total for the Group 1 soil cores. Group 1 soil cores were used to determine changes in soil inorganic N concentrations over time, as described below. Dairy farm effluent composition and application rates, along with the rate of urine N in the treatments, are also noted below. A second set of soil cores, subsequently referred to as "Group 2," was comprised of four replicates by six treatments, 24 cores in total. The six treatments were a control, DFE, urine, UDFE0, UDFE1, and UDFE2. The UDFE1 and UDFE2 treatments consisted of urine + DFE with DFE applications commencing 7 and 14 d after urine application, respectively. Again, for all treatments receiving DFE, the application of the DFE occurred on three occasions, with each application 7 d apart (Table 1). The Group 2 soil cores were used to measure soil N$_2$O and N$_2$ fluxes, soil pH, CO$_2$ fluxes, WSC, and inorganic N as described below.

Urine applications consisted of synthetic urine, formulated according to Fraser et al. (1994), and were applied at the conservative rate of 500 kg N ha^{-1}. In the Group 1 soil cores, the urine N applied was not enriched with ^{15}N (i.e., it was at a natural abundance level of ^{15}N). In the Group 2 soil cores, the urea N, in the urine applied, had a ^{15}N enrichment of 40 atom % excess relative to ambient air. This was to enable the determination of the ^{15}N enrichment in the N$_2$O and N$_2$ gas fluxes. The synthetic urine also contained C as: urea C (106 mg core^{-1}), glycine C (39 mg core^{-1}), and potassium bicarbonate (70 mg core^{-1}). The rate of DFE was determined on a volume basis, 0.025 L per soil core (equivalent to 0.09 pore volumes), to represent 5 mm of DFE irrigation. This provided the equivalent of 2 to 4 kg inorganic N ha^{-1} and 29 to 49 kg WSC ha^{-1} (Table 1). Dairy farm effluent was applied fresh from the Lincoln University dairy shed at each application date. The original intention was to keep the DFE composition identical throughout the experiment by collecting and freezing the DFE, followed by thawing before its application. However, a test showed that DFE inorganic N concentrations changed after freezing, with NH$_4^+$ and NO$_3^-$ concentrations increasing and decreasing, respectively. The effect of freezing DFE on its microbial populations was also unknown. Thus DFE was collected and applied fresh at each application date. The inorganic N concentrations, WSC, and the pH of the DFE applied are shown in Table 2. As might be expected, the DFE constituents and pH differ slightly due to factors noted above. The dry matter content of the DFE was <1%. The soil cores were maintained at 20°C for the duration of the experiment.

Measurements

Soil surface N$_2$O and N$_2$ fluxes were measured from the Group 2 soil cores on 10 occasions over the 43-d period of the experiment. To facilitate headspace sampling, a gas-tight screw-on PVC lid containing a rubber o-ring was fitted creating an 81-mL headspace. Gas samples were taken 3 h after lid closure via a septum in the lid. Samples were obtained using a gas-tight syringe (SGE, Austin, TX) fitted with a push button valve and were transferred to 12-mL vials (Exetainer tubes; Labco, High Wycombe, UK). The lids were then removed from the soil cores. Gas samples were analyzed for N$_2$O and N$_2$ on a continuous flow isotope ratio mass spectrometer (PDZ-Europa, Crewe, UK) using the methods of Stevens et al. (1993). The ^{15}N enrichment of the N$_2$ gas (atom % ^{15}N) and the percentage N$_2$ in the headspace were used to calculate an N$_2$–^{15}N flux. The flux of ^{15}N labeled N$_2$O was calculated from its concentration and the enrichment of the N$_2$O (atom % ^{15}N). The flux ratio of N$_2$O–^{15}N to (N$_2$O–^{15}N + N$_2$–^{15}N) was also determined.

Throughout the experiment subsets of the Group 1 soil cores were destructively sampled for soil inorganic N concentrations at 1-wk intervals. Group 2 soil cores were destructively sampled on Day 43. A subsample of soil was extracted (10 g dry soil to 100 mL of 2 *M* KCl), shaken for 1 h, filtered, and analyzed for inorganic N using a flow-injection analyzer

Table 2. Mean composition of dairy farm effluent (DFE) at each application. Twenty-five milliliters of DFE was applied per core at each application.

Application time	pH	NH$_4^+$–N	NO$_3^-$–N	NO$_2^-$–N	Water-soluble carbon (WSC)		
					Total	Inorganic	Organic
d					μg mL^{-1}		
1 (fresh)	8.1 (0)†	8 (0)	2 (0)	0.1 (0)	466 (4)	45 (1)	421 (3)
7	7.8 (0)	15 (0)	1 (0)	0.2 (0)	444 (5)	30 (1)	414 (5)
14	8.7 (0)	6 (0)	0	0	153 (8)	52 (1)	101 (8)
21	8.1 (0)	64 (1)	0	0	454 (16)	118 (1)	336 (16)
28	8.0 (0)	7 (0)	0	0	400 (8)	38 (2)	362 (8)

† Numbers in parentheses are standard deviations of the means, *n* = 5.

(Model FS3000; Tecator and Alpkem, Saskatoon, SK, Canada). Inorganic N concentrations in the Group 2 soil cores were determined in an identical manner after 43 d. At this time the WSC content of the soil in the second set of soil cores was also determined according to the method used by Burford and Bremner (1975). Total, organic, and inorganic WSC were measured on a TOC-5000A carbon analyzer (Shimadzu, Kyoto, Japan). Gravimetric soil water contents were determined at the same time as the inorganic N determinations. Inorganic N data were then used to determine the net-nitrification rates by calculating the change in soil inorganic N concentrations divided by the number of days between sampling.

Nondestructive soil pH measurements were taken at the surface of the Group 2 soil cores throughout the experiment using a calibrated flat-surface pH electrode (Broadley-James, Irvine, CA). The CO_2 flux from the Group 2 soil cores was measured over time using an infrared gas analyzer (EGM-1; PP Systems, Hitchin, UK). This analyzer was placed over the soil core so that it formed a seal with the moist silica flour on the tension table. Each CO_2 flux measurement was 2 min in duration.

Statistical analyses were performed using Minitab 14 (Minitab, 2003). Analysis of variance was performed to determine statistical differences among treatments on individual days, following checks to ensure the assumptions underlying the analysis of variance were not violated. Error bars in figures are least significant differences between means at the 5% level of significance. Linear regression and correlation were performed to determine the relationship between CO_2 flux and soil pH.

RESULTS

Nitrous Oxide and Dinitrogen Gas Production

The application of DFE alone did not significantly increase the production rate of N_2O above that of the control (Fig. 1a). However, N_2O production increased in all the urine-based treatments following treatment application. The maximum N_2O production rate occurred on Day 2 at 26 ng g soil^{-1} d^{-1} in the UDFE0 treatment (Fig. 1a). Mean production rates of N_2O after urine application were rarely significantly higher than those of the control treatment, due to the data's large variability. When the urine-based treatments were compared with one another, N_2O production rates from the UDFE0 treatment were significantly higher than the urine-only treatment on Day 2, and those from the UDFE1 treatment were higher than the urine-only treatment on Days 1, 7, and 9 ($p < 0.01$). When integrated over the entire study period the amount of N_2O produced in the UDFE1 treatment (391 ± 176 [SD] ng N_2O-N g soil^{-1}) was significantly higher ($p < 0.05$) than in the control (54 ± 96 [SD] ng N_2O-N g soil^{-1}) but not the urine-only treatment (185 ± 154 [SD] ng N_2O-N g soil^{-1}). The atom % ^{15}N enrichments of the N_2O produced were less than the level of ^{15}N enrichment in the urine urea N applied, ≤29 atom % ^{15}N (Table 3), with no statistically significant differences among treatments ($p \geq 0.08$).

Following urine application, the production rate of ^{15}N-labeled N_2 increased from Day 1 to peak on Day 2 (343 ng g soil^{-1} d^{-1}) with no significant differences among ^{15}N-labeled treatments at this time (Fig. 1b).
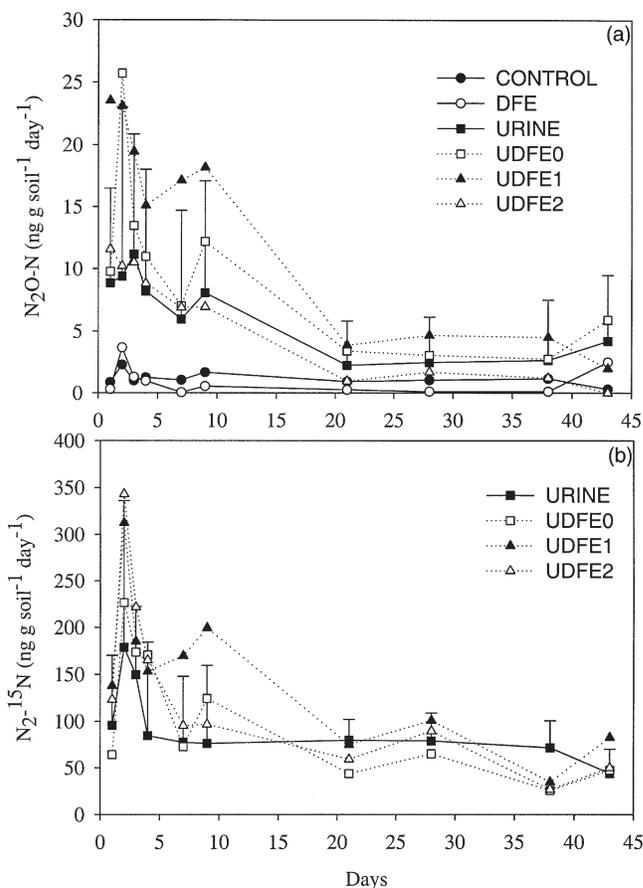

Fig. 1. Fluxes of (a) N_2O-N and (b) N_2–^{15}N over time. Data points are treatment means ($n = 4$). Error bars associated with the urine treatment are +LSD at $p < 0.05$ for treatment comparisons. DFE, dairy farm effluent; UDFE, urine + dairy farm effluent.

However, the production rate of N_2 did vary with treatments on Days 7 and 9, when the rate of N_2 production in the UDFE1 treatment was higher than that in the urine-only treatment ($p < 0.04$). When integrated over the entire study period the amount of N_2 produced in the UDFE1 treatment (4789 ± 1166 [SD] ng N_2–N g soil^{-1}) was again significantly higher ($p < 0.05$) than in the urine-only treatment (3132 ± 397 [SD] ng N_2–N g soil^{-1}). The enrichment of the N_2 gas in the headspace reached a maximum of 0.385 atom % ^{15}N on Day 2 in the UDFE2 treatment (Table 3).

The ratio of N_2O–^{15}N to (N_2O–^{15}N + N_2–^{15}N) did not differ significantly among the urine-based treatments ($p \geq 0.07$) on any given sampling occasion. However, the overall mean ratio did vary significantly with sampling time ($p < 0.01$). It was at a maximum between Days 3 to 6 [0.21 (SD = 0.07) to 0.32 (SD = 0.09)] and <0.15 outside this period with a minimum of value of 0.06 (SD = 0.02).

Inorganic Nitrogen Concentrations and Nitrification Rates

The soil NH_4^+–N concentrations increased immediately ($p < 0.01$) following urine and UDFE0 treatment applications, peaking on Day 7 at 345 µg NH_4^+–N g soil^{-1}

Table 3. Atom % ^{15}N enrichment of N_2O and N_2 fluxes from urine-based treatments.

Treatment†	Days since urine application									
	1	2	3	4	7	9	21	28	38	43
	atom % ^{15}N									
	N_2O									
Urine	4.7	7.2	15.9	21.1	22.0	25.2	18.9	18.9	16.9	20.1
UDFE0	3.6	3.6	15.9	20.9	24.0	24.5	12.8	12.8	19.3	11.8
UDFE1	2.5	4.5	16.4	19.6	22.0	22.5	19.5	19.5	16.0	18.2
UDFE2	5.7	13.2	24.5	27.0	29.0	27.4	19.7	19.7	15.4	20.5
LSD	2.6	7.9	6.7	8.3	14.3	14.4	10.2	7.9	6.0	13.0
	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	N_2									
Urine	0.372	0.376	0.375	0.371	0.371	0.371	0.371	0.371	0.371	0.369
UDFE0	0.370	0.379	0.376	0.376	0.371	0.374	0.369	0.370	0.368	0.369
UDFE1	0.374	0.383	0.377	0.375	0.376	0.378	0.371	0.372	0.369	0.371
UDFE2	0.373	0.385	0.379	0.376	0.372	0.372	0.370	0.372	0.368	0.370
LSD $\times 10^{-3}$	4	8	4	5	4	4	1	2	1	1
	NS	NS	NS	NS	*	*	**	NS	*	*

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
† DFE, dairy farm effluent; UDFE, urine + dairy farm effluent applied on Days 0, 7, and 14 for treatments UDFE0, UDFE1, and UDFE2, respectively.

in the urine-only treatment (Fig. 2a). Concentrations of soil NO_2^-–N were elevated in the urine and UDFE0 treatments after 7 d, reaching 0.68 µg NO_2^-–N g soil^{-1} at this time, but the high variability of the data meant these concentrations were not different from the control and DFE treatments (Fig. 2b). From Day 14 onward soil NO_2^-–N concentrations remained at <0.14 µg NO_2^-–N g soil^{-1} but with higher concentrations in the urine treatment than the control on Days 14 and 28 ($p < 0.05$). Soil NO_3^-–N concentrations increased above those in the control treatment following the addition of urine in both the urine and UDFE0 treatments ($p < 0.001$) peaking at 597 µg NO_3^-–N g soil^{-1} (Fig. 2c). The DFE-amended soils and controls did not differ with respect to soil NO_3^-–N concentrations (Fig. 2c).

The net rates of change in soil inorganic N concentrations were significantly higher ($p < 0.01$) for the urine and UDFE0 treatments than in the nonurine treatments, up until Day 14. After this time there was no difference among treatments in the net rates of change in the soil NH_4^+–N and NO_3^-–N concentrations. Rates of change in soil NH_4^+–N concentrations were negative by Days 7 to 14, peaking at −31 µg NH_4^+–N g soil^{-1} d^{-1}. The maximum net rate of nitrification (i.e., the increase in the soil NO_3^-–N concentrations), 43 µg NO_3^-–N g soil^{-1} d^{-1}, also occurred between 7 and 14 d in the UDFE0 treatment. The net rate of change in soil NO_3^-–N concentrations had become negative by Day 28.

Soil Surface pH and Soil Moisture

Despite the high pH of the DFE applied, there was no significant difference in the soil surface pH of the control and DFE treatments (Fig. 3a). Where urine was a treatment constituent, the soil surface pH increased rapidly to be >8.0 following urine application. This was significantly higher than the control and DFE treatments from Day 2 to 10. From Day 11 to 18, there was no significant difference in the soil surface pH among treatments. After this time the soil surface pH in the urine treatments declined to be less than the DFE and control treatments with the UDFE0, UDFE1, and UDFE2 treatments hav-

ing soil surface pH values that lay between the values of the urine and control treatments (Fig. 3a). Soil moisture contents at Days 7, 14, 21, 28, and 43, were 76, 78, 78, 76, and 74% WFPS respectively, all with a standard error of the mean of 2%.

Soil Carbon Dioxide Production Rates and Water-Soluble Carbon

Before treatment applications, the soil CO_2 production rates averaged 150 mg CO_2 kg soil^{-1} d^{-1} and did not differ significantly among treatments. When urine was a treatment constituent the CO_2 production rate increased ($p < 0.01$) immediately after urine application, with higher CO_2 production rates than in either the control or DFE treatments until Day 5 (Fig. 3b). The maximum CO_2 production rate was 1046 mg CO_2 kg soil^{-1} d^{-1} on Day 2 in the urine-only treatment compared with the control (Fig. 3b). No significant or consistent treatment differences in CO_2 production occurred after DFE application on Day 7. Following DFE applications on Day 14, the production rates of CO_2 were higher from the control and the DFE treatment than any of the urine-based treatments ($p < 0.05$). After Day 23, CO_2 production rates were still higher from both the control and DFE treatments, while the rate in the urine-only treatment was the lowest ($p < 0.05$). The CO_2 production rates for the DFE and urine treatments were of an interim in value compared with the other treatments (Fig. 3b). When integrated over 10 d the urine-treated soils (range 3638–3764 mg CO_2 kg soil^{-1}) produced more CO_2 ($p < 0.01$) than the control or DFE-treated soil (range 1602–1765 mg CO_2 kg soil^{-1}). However, when integrated over 39 d the difference among treatments became insignificant with the control soil producing 4401 ± 712 mg CO_2 kg soil^{-1} and the highest-yielding urine-treated soil 5042 ± 1215 mg CO_2 kg soil^{-1}; errors are standard deviations. Correlation of soil pH versus CO_2 production rate for Days 1 to 43 and Days 10 to 43 were 0.78 and 0.48 respectively ($p < 0.001$). The linear regressions of soil pH (x) versus CO_2 production rate (y) for Days 1 to 43 ($y = -0.18 + 0.06x$)
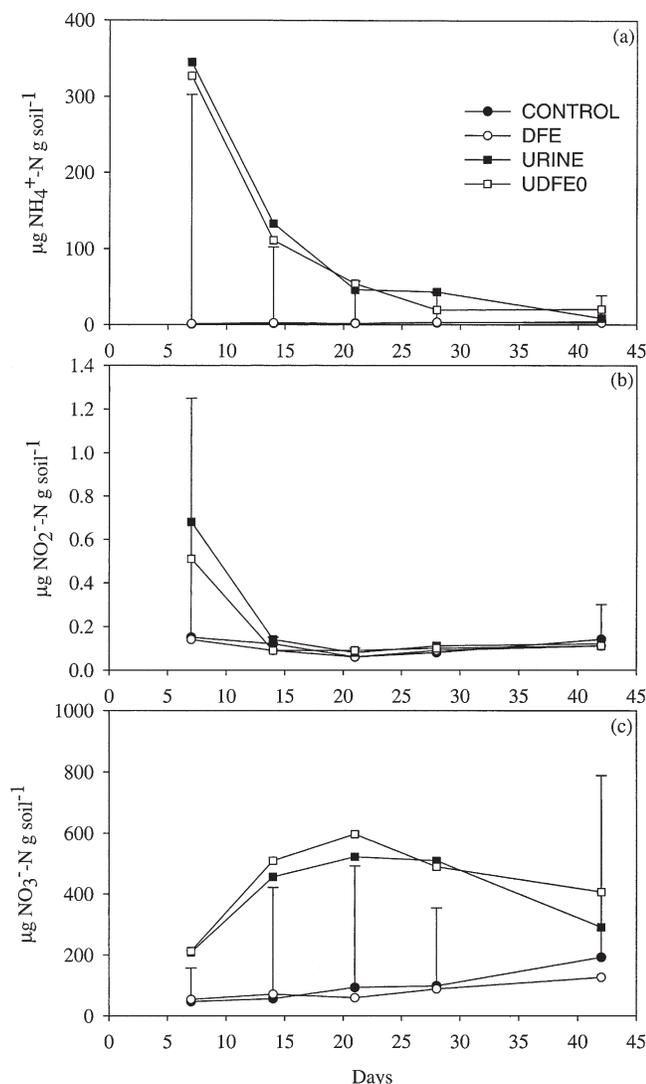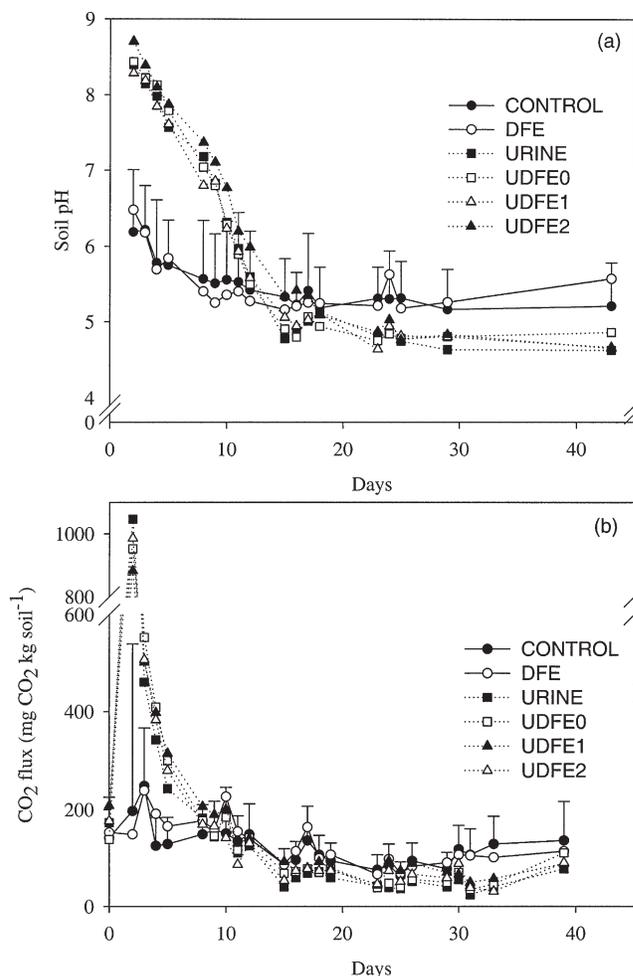
Fig. 2. Changes in soil inorganic N concentrations over time. Data points are treatment means ($n = 3$). Error bars associated with the control treatment are +LSD at $p < 0.05$ for treatment comparisons. Error bars are smaller than symbols between Days 10 to 30. DFE, dairy farm effluent; UDFE, urine + dairy farm effluent.

Fig. 3. Changes in (a) soil surface pH and (b) CO₂ flux over time. Data points are treatment means ($n = 4$). Error bars associated with the control treatment are +LSD at $p < 0.05$ for treatment comparisons. DFE, dairy farm effluent; UDFE, urine + dairy farm effluent.

and Days 10 to 43 ($y = -0.8 + 0.18x$) were also significant ($p < 0.001$) with r^2 values accounting for 60.3 and 22.5% of the variance, respectively.

The application of DFE alone did not significantly change the CO₂ production rate compared with the control treatment. However, there was a significant effect of urine application (Table 4) on CO₂ production rate. In the urine-based treatments, 61 to 68% of the total CO₂ flux for the 39-d measurement period occurred in the first 10 d. For the control and DFE treatments the corresponding range was 34 to 37% (Table 4). Assuming all the C substrates added in the urine evolved as CO₂ (i.e., 2526 mg CO₂ kg⁻¹ soil), the treatment effect excess attributable to increased soil microbial activity was 1112 mg CO₂ kg soil⁻¹. Dividing the excess CO₂ produced by the CO₂ produced in the control treatment produced a synthetic urine priming effect of 0.69.

After 43 d, WSC was dominated by organic C. Inor-

ganic WSC was below detection limits. Water-soluble organic carbon (WSOC) in the DFEU0 and DFEU1 treatments averaged 0.18 μg WSOC g soil⁻¹. This concentration was higher ($p < 0.01$) than that of the control, DFE, or urine-based treatments (average 0.07 μg WSOC g soil⁻¹). Assuming an average WSOC content of 330 μg mL⁻¹ in the DFE applied, then three 25-mL

Table 4. Cumulative CO₂ flux.

Treatment†	Days 0 to 10	Days 0 to 39	Days 0 to 10 as % of Days 0 to 39
	mg CO₂ kg soil⁻¹		%
Control	1602	4740	34
DFE	1765	4822	37
Urine	3638	5375	68
UDFE0	3721	5796	64
UDFE1	3764	6133	61
UDFE2	3681	5650	65
LSD	723	1201	5
	**	NS	**

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
† DFE, dairy farm effluent; UDFE, urine + dairy farm effluent applied on Days 0, 7, and 14 for treatments UDFE0, UDFE1, and UDFE2, respectively.

DFE applications amounted to 80 μg WSOC g soil^{-1}, well in excess of the WSOC recovered.

DISCUSSION

Nitrogenous Gas Production

Apart from the UDFE0 and UDFE1 treatments the addition of DFE to the urine patch did not affect the N_2O production rate. Likewise the daily N_2 production rate was only affected in the UDFE1 treatment, while the ratio of N_2O–^{15}N to (N_2O–^{15}N + N_2–^{15}N) was not affected. The magnitude and the duration of N_2O production from the urine-treated soils were consistent with previous work, where comparable soils have received the same rate of urine N and had similar pH (Clough et al., 2001, 2004). It was hypothesized that the addition of WSC, in the DFE applications, would enhance heterotrophic microbial processes such as denitrification, since WSC has been shown to be well correlated with denitrification (Burford and Bremner, 1975). The addition of extra C was also expected to influence the ratio of the N_2O to N_2 produced (Firestone, 1982).

The increases in N_2O production in the UDFE0 and UDFE1 treatments on Days 2 and 7, respectively, were short lived and relatively small in comparison with the total N_2O flux at these times. When integrated over the entire study period the UDFE1 treatment produced more N_2O than the control and had there been increased replication it is possible that a treatment effect may have been seen between the UDFE1 and urine-only treatment. Likewise the UDFE1 treatment produced more N_2 than the urine-only treatment. Since the fresh DFE composition was very similar across all application times it is likely that this increase in denitrification in the UDFE1 treatment was due to conditions in the urine-affected soil 1 wk post-urine application. These conditions could have included the relative concentrations of inorganic N in the soil. The gateway through which N_2O must be formed is NO_2^-–N and it was at Day 7 that soil NO_2^-–N concentrations were at their highest. Master et al. (2003) showed the significance of soil NO_2^-–N concentrations while measuring N_2O production from effluent-treated soils. The small increase in N_2 production in the UDFE1 treatment was most likely a result of the increased N_2O production and its further reduction to N_2. The lack of significant differences in the ratio of N_2O–^{15}N to (N_2O–^{15}N + N_2–^{15}N) may have been due to added C sources being unavailable to the denitrifiers, the C released as a result of urine addition nullifying any potential affect of the added C, or inorganic N levels determining the ratio of N_2O–^{15}N to (N_2O–^{15}N + N_2–^{15}N) (Firestone et al., 1979).

In general the overall lack of any enhanced N_2O production or consumption following DFE application to the urine-treated soils may be a consequence of the added DFE substrates (C and N) being of an insignificant amount when compared with those either supplied in the urine or those substrates released from the soil due to urine addition as discussed below.

Alternatively the general lack of any enhanced N_2O production or consumption in the DFE plus urine treatments could have been due to the microbial population being influenced by soil chemical factors within the urine patch. It has recently been noted that different denitrifying species in the soil can be promoted or inhibited by the type of treatment applied to the soil. For example Wolsing and Prieme (2004) found that soils treated with mineral fertilizer or cattle manure produced different communities of denitrifying bacteria. Denitrifier community structure can influence N_2O fluxes (Cavigelli and Robertson, 2000; Munch, 1989). In our study, soil pH changes over time in the urine-based treatments may have influenced denitrifier function or community composition.

Previous work, at similar soil temperatures to this study, found N_2O fluxes increased when a mineral soil was treated with DFE (Barton and Schipper, 2001). However, the N_2O fluxes were only higher in the DFE-treated soils for 3 h in the autumn and 48 h in the spring (Barton and Schipper, 2001). Due to our moderate sampling regime any significant N_2O production would not have been detected in the 3 h following DFE application. The lack of significant DFE-induced N_2O production in our study, when compared with Barton and Schipper's (2001) results, may also be a consequence of the higher inorganic N concentrations in their DFE application, the presence of pasture and thus root mucilage increasing denitrifier activity (Mounier et al., 2004), the differences in soil depth, soil moisture effects, and soil drainage. It would be worthwhile repeating aspects of our study in conjunction with soil wetting and drying cycles and in the presence of pasture species to determine if, over the long term, DFE applications make any difference to urine-induced N_2O production. Dairy farm effluent, under the conditions of this study, was not a suitable management tool for mitigating N_2O emissions.

The atom % ^{15}N enrichment of the N_2O produced also indicates that N other than the ^{15}N-labeled urea N was contributing to the N_2O flux. That is, the atom % ^{15}N enrichment of the N_2O produced was less than the level of ^{15}N enrichment applied, particularly on Days 1 and 2. The hydrolysis of urea raises the soil pH and solubilizes soil organic matter that can, in turn, lead to significant deamination (Sen and Chalk, 1993). Such an unlabeled source of N could have provided further N substrate for the N_2O production mechanisms. Alternatively other natural abundance N, such as the glycine N applied in the urine, could have contributed to the substrates utilized by the N_2O producing mechanisms and contributed to a reduced N_2O-^{15}N enrichment.

Soil Carbon and Priming

Measured fluxes of CO_2 from synthetic urine and urea granules (Lockyer, 1984; Lovell and Jarvis, 1996; Tenuta and Beauchamp, 2000) have been accredited to the chemical reactions involved in urea hydrolysis and the ensuing hydrolysis of the carbonate ions to form CO_2 (Sherlock and Goh, 1983). Allowing for such chemical reactions, there was still a release of CO_2 from the soil equivalent to 303 mg C kg soil^{-1} over 10 d as a result of urine

application. This priming effect has been noted before in urine-treated soils (Clough et al., 2003) and the concept of priming has been reviewed by Kuzyakov et al. (2000). Insoluble organic forms of C, such as feces, were also applied in the DFE and some of this insoluble C may also have been mineralized and become available to microorganisms. It should also be noted that not all of the applied WSC in the DFE would have been readily available to the soil microorganisms (Lundquist et al., 1999). Overall the amount of C applied in the DFE was insignificant in comparison with the C released during soil priming. The priming effect of urine on CO$_2$ flux lasted 5 d and was possibly driven by the solubilization of soil organic matter, due to the high soil pH at this time. Another factor enhancing the priming of soil C could have been the high inorganic N concentrations in the soil resulting from urine application. Previous work has shown both positive and sporadic relationships between the amount of available mineral N and the amount of C mineralized (Chantigny et al., 1999; Liljeroth et al., 1990; Merckx et al., 1987). If priming of soil C increases the release of CO$_2$ for 5 d every grazing, and grazing occurs approximately every 3 wk in intensively managed pastures, then priming is a very significant feature of microbial activity in the soil urine patch. Carbon is also returned to pastures due to photoassimilation of C and has been reported to equal 1320 kg C ha^{-1} yr^{-1} under a temperate high-fertility dairy pasture (Saggar and Hedley, 2001). Thus the priming effect observed here does not necessarily represent a net loss of C from the urine patch. In fact other studies in grazed grasslands have found grasslands to be both a source and a sink for CO$_2$ (Leahy et al., 2004; Xu and Baldocchi, 2004). Further work needs to establish the direction of the net flux and the relative magnitudes of the mechanisms responsible (i.e., chemically induced CO$_2$ fluxes from urine patches and the release of CO$_2$ via soil respiration).

The soil surface pH values in the urine-treated soils declined to be less than that of the control and the DFE-treated soils, after 23 d. Over the same period the CO$_2$ production also declined at a greater rate in the urine-treated soils, when compared with the control and DFE treatments. Assuming all chemical production of CO$_2$ has ceased it is reasonable to assume that this decrease in CO$_2$ production indicates a decrease in soil microbial activity; possibly a result of the urine treatment effect on soil pH. At the same time as CO$_2$ production was decreasing, nitrogenous gas production was also decreasing. This could not be due to a lack of N substrate since soil NO$_3^-$–N concentrations were high and soil WFPS conditions were suitable for denitrification (Dobbie and Smith, 2001). This suggests C was becoming limiting at this time or the soil environment was not conducive to high rates of denitrification due to the lowering soil pH.

The close correlation between soil pH and CO$_2$ production over the first 10 d is readily explained by chemical processes. Increases in soil pH following urine application are a result of urea hydrolysis, while the decrease in soil pH that then follows is a result of hydrogen ions being produced as a consequence of the ammonia

volatilization and nitrification processes (Haynes and Sherlock, 1986).

Repeating aspects of this study in the presence of pasture species, in situ, would also overcome any possible artifacts of the laboratory methodology used here. Air drying of soil has been shown to release soil organic C (Merckx et al., 2001). It is theoretically possible that C was released during air-drying of the soils, partially masking the potential effect of DFE carbon on the soil denitrifiers; although equilibration of the soil cores on the water tension tables should have nullified this. Sieving of the soil could potentially also have disrupted microorganism community environments and populations. It has been shown that soil aggregate size can affect the production of N$_2$O from applied effluent (Master et al., 2003). Likewise any effects pasture species had on soil bulk density and soil aeration could be considered with in situ studies.

CONCLUSIONS

In summary the repeated application of DFE to urine patches, commencing at 0, 7, or 14 d after urine deposition, did not mitigate the N$_2$O production rates from the simulated urine patch, or change the ratio of N$_2$O to N$_2$ gas fluxes. The application of DFE to urine patches 7 d after urine deposition increased the total amount of N$_2$O produced over the 43-d measurement period when compared with the control soil but not when compared with a urine-only treatment. The application of DFE 7 d after urine deposition also resulted in small increases in N$_2$ gas production. The amount of WSC applied in the DFE was insignificant compared with the C released from the soil in the urine patch. This was evident from the measured cumulative CO$_2$ flux, which was greater than the C inputs in the urine and DFE combined and reasons for this are suggested. Dairy farm effluent, under the conditions of this study, was not a suitable management tool for mitigating N$_2$O emissions. However, a field-based study in the presence of pasture plants should be done to verify this. The priming of soil C in urine patches has implications for the understanding of soil C processes in grazed pasture ecosystems and the budgeting of C within these ecosystems.

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