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*Plant and Soil* **0:** 1–11, 2004.
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# Short-term nitrous oxide emissions from pasture soil as influenced by urea level and soil nitrate

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Received . Accepted in revised form

*Key words:* denitrifying enzyme activity (DEA), dissolved organic carbon, GC-IRMS, nitrous oxide, osmotic potential, potential ammonium oxidation (PAO), urea

## Abstract

Nitrogen excreted by cattle during grazing is a significant source of atmospheric nitrous oxide ($N_2O$). The regulation of $N_2O$ emissions is not well understood, but may vary with urine composition and soil conditions. This laboratory study was undertaken to describe short-term effects on $N_2O$ emissions and soil conditions, including microbial dynamics, of urea amendment at two different rates (22 and 43 g N $m^{-2}$). The lower urea concentration was also combined with an elevated soil $NO_3^-$ concentration. Urea solutions labelled with 25 atom% $^{15}N$ were added to the surface of repacked pasture soil cores and incubated for 1, 3, 6 or 9 days under constant conditions (60% WFPS, 14 °C). Soil inorganic N ($NH_4^+$, $NO_2^-$ and $NO_3^-$), pH, electrical conductivity and dissolved organic C were quantified. Microbial dynamics were followed by measurements of $CO_2$ evolution, by analyses of membrane lipid (PLFA) composition, and by measurement of potential ammonium oxidation and denitrifying enzyme activity. The total recovery of $^{15}N$ averaged 84%. Conversion of urea-N to $NO_3^-$ was evident, but nitrification was delayed at the highest urea concentration and was accompanied by an accumulation of $NO_2^-$. Nitrous oxide emissions were also delayed at the highest urea amendment level, but accelerated towards the end of the study. The pH interacted with $NH_4^+$ to produce inhibitory concentrations of $NH_3$(aq) at the highest urea concentration, and there was evidence for transient negative effects of urea amendment on both nitrifying and denitrifying bacteria in this treatment. However, PLFA dynamics indicated that initial inhibitory effects were replaced by increased microbial activity and net growth. It is concluded that urea-N level has qualitative, as well as quantitative effects on soil N transformations in urine patches.

## Introduction

For Western Europe it is estimated that, on average, 8% of total N excreted by dairy cattle is deposited on pastures (IPCC, 1997). Nitrogen intake and excretion is influenced by factors such as lactation stage, pasture quality (clover percentage, N concentration) and feed composition. Excess N is mainly excreted as urea in the urine, i.e., the proportion of urea-N increases with total urinary N concentration (Petersen et al., 1998). In pasture soil, urea is completely hydrolyzed within 24–48 h, and subsequent transformations of $NH_4^+$ and $NO_3^-$ via nitrification and denitrification make urine patches a potentially important source of $N_2O$ (e.g., Clough et al., 1998; Monaghan and Barraclough, 1993; Yamulki et al., 1998).

The regulation of nitrification and denitrification in urine patches is not well understood, and $N_2O$ emissions may result from a combination of several factors, including elevated soil moisture, stresses caused by dissolved ammonia, $NH_3$(aq), and/or low osmotic potential, and elevated oxygen demand due to carbon leakage from scorched roots and possibly

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lysed microorganisms (Monaghan and Barraclough, 1992; Richards and Wolton, 1975; Stark and Firestone, 1995). If denitrification is restricted by $NO_3^-$ availability, then overlapping urine patches with $NO_3^-$ from a previous deposition could have elevated rates of $N_2O$ emission.

This laboratory study was conducted to investigate the short-term turnover of urea in pasture soil under typical summer grazing conditions. A wide range of variables were monitored for characterization of the physicochemical environment, N transformations and associated microbial dynamics in urine patches.

## Materials and methods

Soil for the laboratory study was sampled in late May from an 8-yr old grazed pasture near Research Centre Foulum in Denmark (55°52′ N, 9°34′ E); the area sampled had not been grazed since the previous autumn. The sandy loam soil (Typic Hapludult) contained 2.7% C and 0.18% N, the pH($H_2O$) was 6.3, and total CEC was 87 cmol $kg^{-1}$. Soil (0–20 cm depth) was sieved ($<$ 4 mm) to remove roots and stones. Gravimetric soil moisture was 15.2%, or 80% of field capacity (FC). The soil was stored for a week at 4 °C, and then at the incubation temperature (14°C) for 24 h before initiation of the experiment.

### *Experimental set-up*

In the experiment, solutions of urea labelled with 25 atom% $^{15}N$ were added to repacked soil cores at a rate of 4 L $m^{-2}$. The treatments were: (i) *CTL* (0 g N $L^{-1}$); (ii) *LU* (5 g urea-N $L^{-1}$); (iii) *HU* (10 g urea-N $L^{-1}$); (iv) *LUN* (5 g urea-N $L^{-1}$ + 50 $\mu$g $NO_3^-$-N $cm^{-3}$); and (v) $NO_3^-$ only (50 $\mu$g $NO_3^-$-N $cm^{-3}$). Treatment (v) was only used for respiration measurements and final soil analyses.

One day before the experiment was initiated, soil portions of 100 g (dry wt. equivalent) were weighed out, and soil moisture adjusted to 46% water-filled pore space (WFPS) by drop-wise addition of distilled water or a $KNO_3$ solution. Each sample was mixed, transferred to cylinders (internal diameter, 44 mm), and packed to a bulk density of 1.2 g $cm^{-3}$. Urea solutions or water was added dropwise to the appropriate cylinders, which were subsequently sealed at both ends with Parafilm that was perforated with a needle to facilitate gas exchange. All treatments were prepared in triplicate for each of four sampling times (1, 3, 6 and 9 d) and incubated at 14 °C. The final moisture content of all treatments was 60% WFPS, and the two urea amendment levels corresponded to 22 and 43 g N $m^{-2}$, respectively. The total of 51 samples, including three replicates with $NO_3^-$ only for the last sampling, were weighed at regular intervals during incubation; water loss was negligible ($\sim$ 0.2 mL).

### *Sampling*

Carbon dioxide and $N_2O$ evolution rates were determined after c. 0.2, 0.5, 1, 3, 6 and 9 d. Three replicates from each treatment were randomly selected and transferred to 1 L gas tight containers equipped with a septum for gas sampling. Carbon dioxide was analyzed at to and again after 60 min. At this time, 13 mL headspace gas was transferred to evacuated exetainers for isotope ratio mass spectrometry (IRMS) analysis of $^{14+15}N_2O$ and $^{14+15}N_2$. At the four last samplings, the replicates used for gas flux measurements were then destructively sampled for determination of pH, electrical conductivity (EC), dissolved organic carbon (DOC), inorganic and total N, and phospholipid fatty acid (PLFA) composition. Soil for total N determination was wetted with $NaH_2PO_4$ (0.5 $M$, pH 4.3) to prevent $NH_3$ volatilization during air-drying. On day 3, soil was also sub-sampled for determination of potential ammonium oxidation (PAO) and denitrifying enzyme activity (DEA). These assays were assumed to reflect the metabolic capacity for each process at the time of sampling.

### *Analytical techniques*

Urea solutions were prepared from a 99 atom% stock (Eurisotop, Saint Aubin, France) and unlabelled urea. Carbon dioxide was analyzed by a HP-P200 portable GC equipped with a thermal conductivity detector and a Poraplot Q column using He as a carrier gas. Nitrous oxide concentrations and $^{15}N$-$N_2O$ were determined using a continuous flow triple collector isotope ratio mass spectrometer linked to a GC and with automated cryogenic pre-concentration (ANCA-TGII system, IRMS, PDZ Europa). The sample initially passed through a water and $CO_2$ trap. Nitrous oxide was cryofocused before passing through a GC column (Poraplot Q, Chrompack) and through to the MS. Nitrogen was purged through a 5 Å Molecular Sieve GC column (Chrompack) and then through to the MS. GC flows were optimized so that nitrous oxide was detected by the MS prior to nitrogen. Lab standards were calibrated against atmospheric $N_2(\delta^{15}N = 0)$

for $\delta^{15}$N. The lab standard used was 50 ppm $N_2O$ in $N_2$ ($\delta^{15}N\text{-}N_2O = -0.9872$). Overall precision (machine error plus sample preparation error) for nitrogen isotopic composition was 0.37‰.

An automated combustion elemental analyzer interfaced with an IRMS (ANCA-SL system) was used to measure total nitrogen content as well as the nitrogen isotopic composition of soil samples (14 ± 0.1 mg). Samples were prepared as described in Schepers et al. (1989). Sharpsburg silty clay loam ($\delta^{15}N = 10.647$‰) was used as the soil working standard. Overall precision (machine error plus sample preparation error) for nitrogen isotopic composition was 0.3–1‰.

Ammonium and $NO_2^-$ were determined colorimetrically and $NO_3^-$ by ion chromatography (Keeney and Nelson, 1982). Isotopic composition of $NH_4^+$ and $NO_2^- + NO_3^-$ was determined by IRMS after microdiffusion (Sørensen and Jensen, 1991). However, the results for $^{15}N\text{-}NH_4^+$ were not reliable due to instrument overload and had to be estimated (see next section).

DOC was extracted in 0.5 *M* $K_2SO_4$ (Vance et al., 1987) and filtered extracts analyzed on an DC-180 Carbon Analyzer (Dohrmann, Xertex). pH and EC was measured in 1:1 soil:water mixtures (Smith and Doran, 1996). EC results were expressed as osmotic potentials using the expression:

$$\psi_o = -EC_e(\Theta_s/\Theta)0.036, \tag{1}$$

where $EC_e$ is the electrical conductivity of a saturated extract (dS $m^{-1}$), $\Theta_s$ and $\Theta$ are the volumetric water contents of the saturated extract and the fresh soil, respectively, and 0.036 is an empirical conversion factor (MPa $dS^{-1}$ m) (Rawlins and Campbell, 1986). In the present experiment, EC was strongly correlated with inorganic N ($r^2 = 0.62$, $P < 0.001$).

PAO was determined according to Belser and Mays (1980), and DEA as described by Tiedje et al. (1989). Phospholipid fatty acid analyses followed Petersen et al. (2002). A total of 34 fatty acids were consistently observed in the pasture soil, although in this context only total concentrations and proportions of selected fatty acids related to physiological status will be presented.

*Isotope calculations*

The fractions of soil N pools derived from urea, $N_{dfu}$, were calculated according to a standard equation for fertilizer uptake studies (Nason and Myrold, 1991):

$$N_{dfu} = [^{15}\text{N atom\%, fraction} - 0.366] / [^{15}\text{N atom\%, urea} - 0.366] \tag{2}$$

However, labelling of $NH_4^+$ had to be estimated (possible for days 3, 6 and 9 only) on the basis of net changes in total $NO_3^-$ and $^{15}NO_3^-$ concentrations during each time interval:

$$[^{15}NH_4^+]_{t2} = \left(\Delta_{t1,t2}[^{15}NO_3^-]\right) / \left(\Delta_{t1,t2}[^{14+15}NO_3^-]\right). \tag{3}$$

Equation 3 assumes that labelling of the $NO_3^-$ produced corresponded to the labelling of the substrate pool, and that there was no turnover of the $NO_3^-$ pool.

The $^{15}$N content of $N_2O$ emitted was calculated by subtracting the background in air (310 ppm $N_2O$ with 0.366 atom% $^{15}$N). Accumulated emissions of $N_2O$ were estimated assuming linear rate changes between samplings, and the fraction of $N_2O$ derived from urea at each sampling was calculated using Eq. 2.

*Statistical analyses*

Treatment effects and temporal dynamics were analyzed by a linear mixed model, and using a Tukey multiple comparisons test to identify differences.

## Results

*Inorganic N dynamics*

Pools of $NH_4^+$ and $NO_3^-$ in the soil solution of treatments *CTL, LU, HU* and *LUN* are shown in Figure 1 (note different scale for *CTL*). The higher background of $NO_3^-$ in *LUN* was evident, as was the accumulation of $NO_3^-$ over time in all treatments with urea amendment.

Figure 2 shows $NO_2^-$ concentrations, which were initially negligible. In *LU* and *LUN* a transient, but non-significant accumulation of $NO_2^-$ was observed during the 9-d period ($P > 0.1$). The pattern in *HU* was very different, with a significant ($P < 0.0001$) accumulation of $NO_2^-$ between 3 and 9 days.

Based on the IRMS analyses, the average recovery of urea-N in the soil N was determined to be $84 \pm 1.1\%$ (mean ± standard error). The N balances for day 3, 6 and 9 are shown in Figure 3. Nitrate accumulation was delayed in *HU* compared to *LU* and *LUN*.

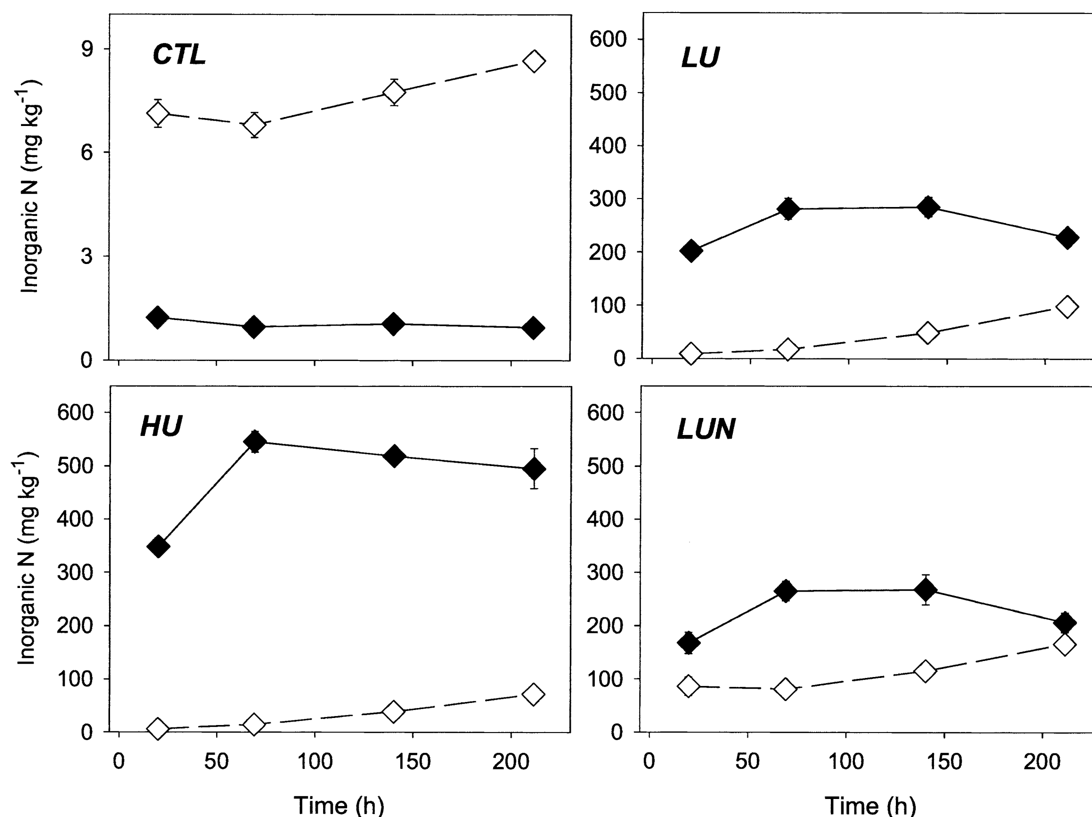


*Figure 1.* Concentrations of $NH_4^+$ (♦) and $NO_3^-$ (◊) in the treatments indicated. Error bars represent standard error ($n$ = 3).

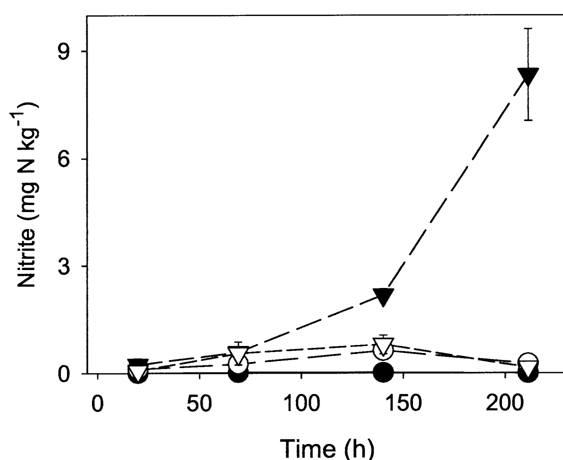


*Figure 2.* Concentrations of $NO_2^-$ in *CTL* (•), *LU* (○), *HU* (▼) and *LUN* (▽) during the 9-d experiment. Error bars represent standard error ($n$ = 3).

### *$N_2O$ emissions*

In Figure 4, $N_2O$ production rates are shown on an area basis. Emission rates from *LU* increased until day 6, while emission rates from *LUN* levelled off after day 3. Nitrous oxide emission, like nitrification, was delayed in *HU* during the first 6 days, but then increased dramatically.

The sources of $N_2O$ on each sampling day are shown in Table 1. The amendment of $NO_3^-$ alone (treatment *N*) did not stimulate $N_2O$ production in the soil. The treatments *LU*, *HU* and *LUN* stimulated the emission of soil-derived $N_2O$ similarly. With respect to $N_2O$ derived from urea the picture was more complex, reflecting that emissions from *LU* were higher than from *LUN* throughout the experiment, whereas in *HU* the emissions of $N_2O$ derived from urea were initially depressed, but greatly increased between day 6 and 9 (data not shown).

### *Soil solution composition*

Soil pH (Figure 5A) was immediately raised by urea amendment, though with different effects of the three treatments ($P < 0.0001$). In all treatments with urea, pH declined continuously during the experiment ($P < 0.05$). EC levels in *LU*, *HU* and *LUN* initially corres-

*Table 1.* Accumulated emissions of $N_2O$ derived from urea and soil, as well as accumulated rates of $CO_2$ evolution, after 9 days. The treatments were: *CTL*, water amendment; *LU*, 22 g urea-N $m^{-2}$; *HU*, 43 g urea-N $m^{-2}$; *LUN*, 22 g urea-N $m^{-2}$ + 50 $\mu g$ $NO_3^-$N $cm^{-3}$; N, 50 $\mu g$ $NO_3^-$N $cm^{-3}$. The $CO_2$ data were corrected for urea-derived $CO_2$ by assuming that urea was completely hydrolyzed and urea-C released to the atmosphere. Letters indicate significant ($P < 0.05$) differences within each column ($n = 3$)

| | $N_2O$ from urea | $N_2O$ from soil | $CO_2$, corrected |
|---|---|---|---|
| | mg N | | mg C |
| *CTL* | 0.3 c | 15.9 c | 8.2 c |
| *LU* | 27.6 a | 26.2 a | 19.0 b |
| *HU* | 22.8 ab | 22.0 a | 36.4 a |
| *LUN* | 15.2 b | 24.7 ab | 6.8 c |
| *N* | 0.4 c | 17.2 bc | 8.4 c |

ponded to osmotic potentials of −0.05 to −0.12 MPa after 1 d, decreasing to −0.14 to −0.19 MPa after 9 d (Figure 5B).

Relative to *CTL*, concentrations of DOC (Figure 6A) were elevated in urea amended soil after 1 and 3 days, and in *HU* and *LUN* throughout the experiment ($P < 0.05$). In *HU*, DOC decreased between day 1 and day 3, and then increased again to the original level ($P < 0.01$). *LU* showed a small, but significant ($P < 0.05$) decrease in DOC during the experiment.

*Soil respiration*

Soil $CO_2$ evolution from urea and soil respiration are shown in Figure 6B; the production was corrected for dissolved $CO_2$ and carbonates (Lindsay, 1979). Effects of all treatments had ceased by the end of the 9-d period, despite the differences in DOC availability. $CO_2$ evolution from *LUN* was always lower than from *LU*. In Table 1, the accumulated release of $CO_2$ from each treatment is shown.

*Microbial dynamics*

Potential ammonium oxidation (PAO) and denitrifying enzyme activity (DEA) were quantified after 3 d (Figure 7A). The buffered PAO assay (pH 7.4) was stimulated in *LU*, *HU* and *LUN* relative to *CTL* ($P < 0.05$). The DEA assay indicated a reduction in the potential for denitrification in both *LU*, *HU* and *LUN* ($P < 0.05$). The decrease appeared to be stronger in the *HU* treatment, but differences between *LU*, *HU* and *LUN* were not significant ($P = 0.10$–$0.15$). The DEA assay is not buffered, and pH of the slurries were 6.3 (*CTL*), 6.9 (*LU* and *LUN*) and 7.2 (*HU*), whereas the pH of the undisturbed soil was 6.4; all pair-wise differences were significant at $P < 0.01$, as determined by Tukey's multiple comparisons test.

Figure 7B presents concentrations of membrane lipid fatty acids (PLFA). Initial levels of PLFA in *HU* and *LUN* were elevated relative to *CTL* ($P < 0.01$). The levels of PLFA in *LU* and *LUN* remained constant throughout the experiment. In the *HU* treatment, the apparent decrease between 1 and 3 days was not significant ($P > 0.1$). Between day 3 and day 9, PLFA concentrations in *HU* increased by 25% ($P = 0.02$).

Figure 8 shows ratios of selected PLFA's which have been linked with the physiological status of microorganisms. These included the ratios between the cyclopropane fatty acids cy17:0 and cy19:0 and their metabolic precursors, palmitoleic acid (16:1$\omega$7c) and cis-vaccenic acid (18:1$\omega$7c), as well as the trans-cis ratio of 16:1$\omega$7. Both cyclopropane/precursur ratios remained constant in *CTL* and *LUN* during the experiment, whereas the cy17:0/16:1$\omega$7c ratio of *LU* and *HU* ($P < 0.01$)) and the cy19:0/18:1$\omega$7 ratio of *HU* ($P = 0.0003$) decreased. Ratios of 16:1$\omega$7t/c decreased significantly ($P < 0.05$) in the treatments *LU* and *HU* during the experiment. All significant changes occurred mainly between day 3 and day 9.

## Discussion

This incubation experiment aimed to describe the relationship between urea turnover and $N_2O$ emissions

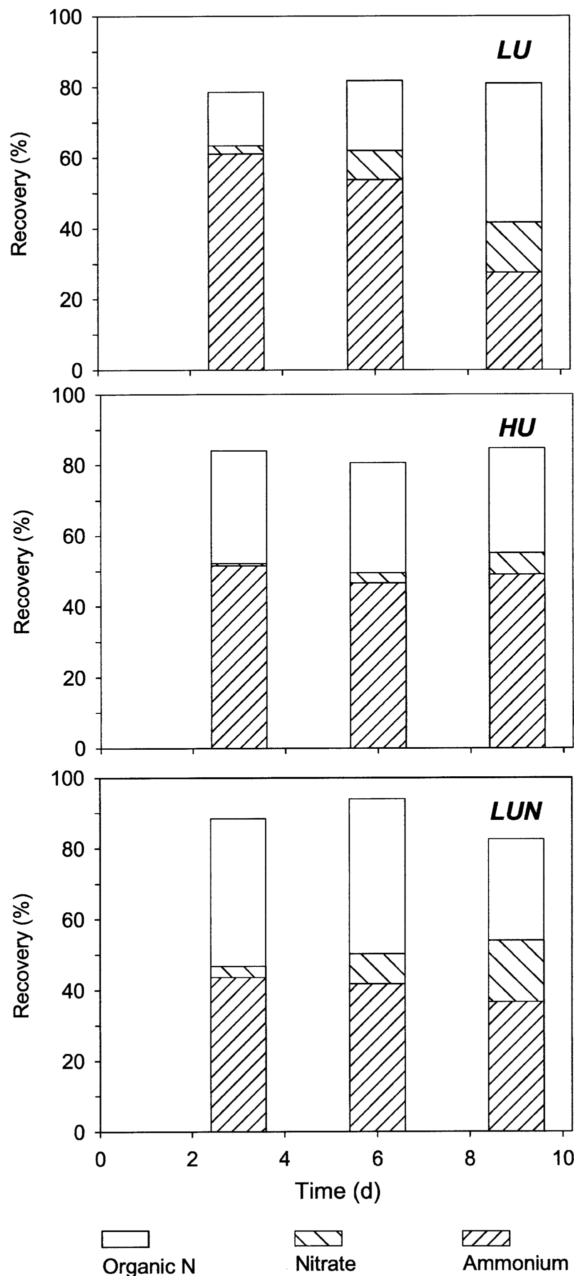


*Figure 3.* Total recovery of $^{15}$N added in urea as soil N, as $NH_4^+$ and as $NO_3^-$ ($N_2O$ emissions were insignificant).

in a pasture soil, and the possible interaction with $NO_3^-$ availability. The moisture content during incubation was kept at 60% WFPS where denitrification was not expected to occur unless stimulated by urea transformations. For nitrification, this moisture level was probably near optimal (Doran et al., 1988). Urea alone was added rather than artificial cattle urine, since

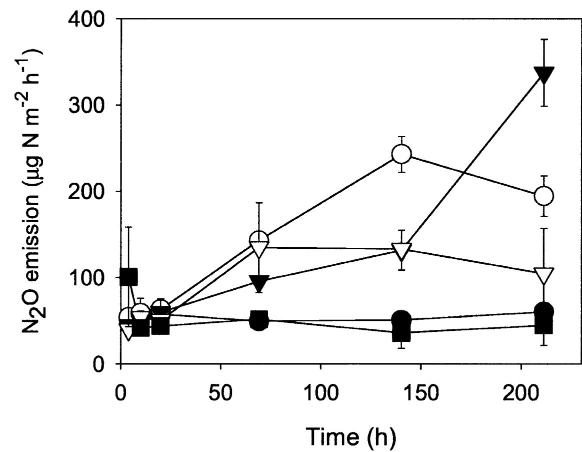


*Figure 4.* Nitrous oxide emission rates ($\mu$g m$^{-2}$ h$^{-1}$) in the *CTL* (•), *LU* (○), *HU* (▼), *LUN* (▽) and *N* ($NO_3^-$ only) (■). Bars represent standard errors ($n$ = 3).

we wanted to avoid the interference from turnover of organic constituents in the urine (Bristow et al., 1992). The absence of hippuric acid probably delayed urea hydrolysis in the soil (Whitehead et al., 1989), thereby dampening the initial increase in pH (Sherlock and Goh, 1984; Somda et al., 1997), as well as the osmotic down-shock. Thus, it is likely that any stresses imposed on soil organisms would be as great or greater in a pasture after deposition of cattle urine.

Concentrations of urea-N applied to the soil surface, 5 and 10 g N L$^{-1}$, were selected on the basis of previous analyses of urine from cattle in this grazing system (Petersen et al., 1998). The input to the soil corresponded to 22 and 43 g N m$^{-2}$, which is within the range of 20 to 80 g N m$^{-2}$ quoted by Oenema et al. (1997) as typical for urine patches.

The use of $^{15}$N-labelled urea made it possible to follow the turnover of the N introduced to the soil. For day 1, reliable data on $^{15}NH_4^+$ and $^{15}NO_3^-$ could not be obtained, but for subsequent sampling days the recovery of urea-N in mineral N and $N_2O$ was mostly between 40 and 65%, and total $^{15}$N recovery averaged 84%. The missing urea-N was presumably lost to the atmosphere as $NH_3$, or as $N_2$ which could not be detected against the background in atmospheric air. For comparison, gaseous losses of 19–32% from $^{15}$N-labelled urine were indicated in a 406-d field lysimeter experiment with four soil types (Clough et al., 1998).

Nitrous oxide emission rates ranged from ca. 50 $\mu$g $N_2O$-N m$^{-2}$ h$^{-1}$ in the *CTL* treatment to a maximum of 350 $\mu$g $N_2O$-N m$^{-2}$ h$^{-1}$ in *HU* by day 9 (Figure 4). This range was similar to, or lower, than initial

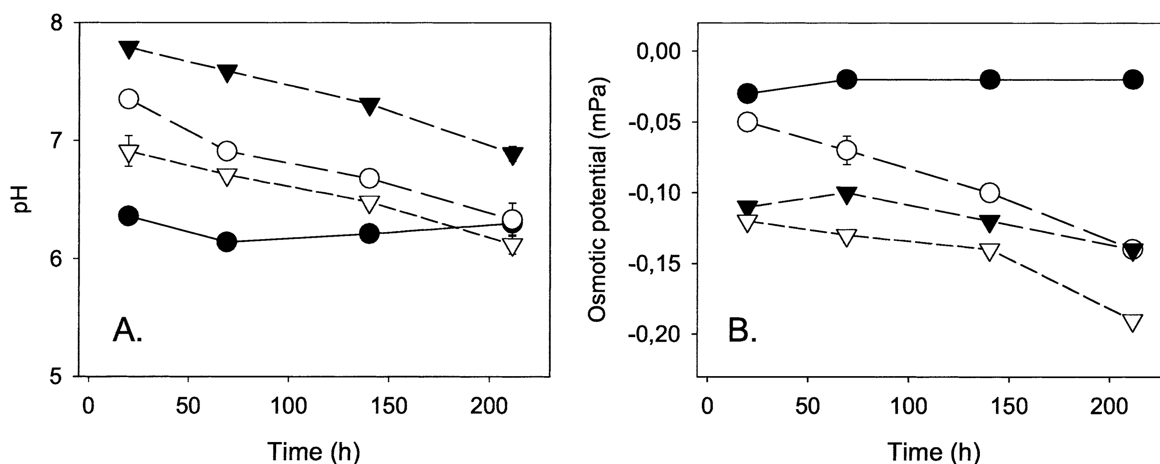


*Figure 5.* pH (A) and osmotic potentials (B) in *CTL* (•), *LU* (○), *HU* (▼) and *LUN* (▽) during the 9-d experiment. Error bars represent standard error ($n = 3$).

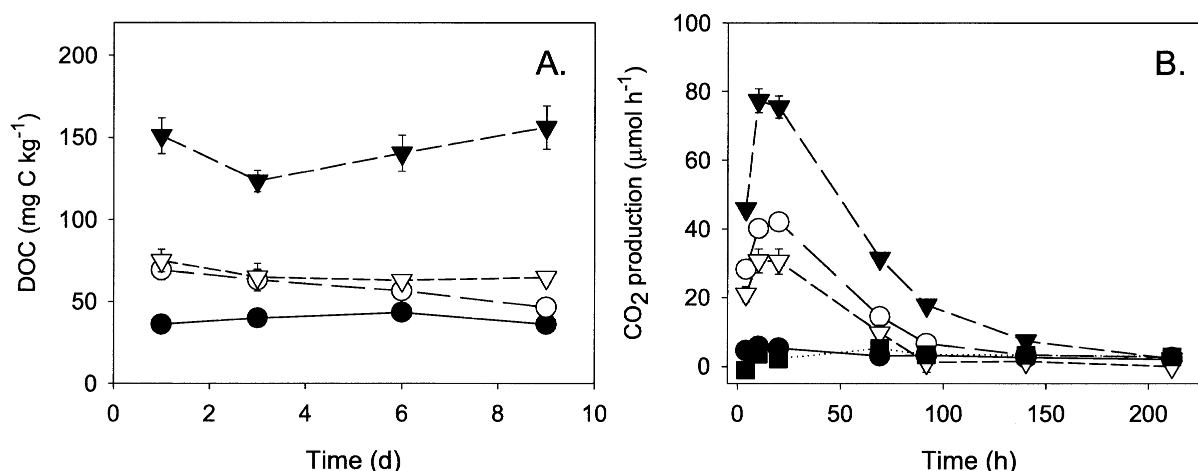


*Figure 6.* Dissolved organic C (A) and $CO_2$ evolution rates (B) in *CTL* (•), *LU* (○), *HU* (▼) and *LUN* (▽) during the 9-d experiment. Error bars represent standard error ($n = 3$).

emissions from urine-affected pasture soil observed in other studies (Anger et al., 2003; De Klein et al., 1999; Koops et al., 1997; Lovell and Jarvis, 1996; Yamulki et al., 1998). Emission rates in *HU* apparently increased beyond the 9-d period of this experiment, in accordance with other studies of $N_2O$ emission from urine patches where a maximum has been recorded after 2–4 weeks (Allen et al., 1996; Lovell and Jarvis, 1996; Monaghan and Barraclough, 1993). In the present study, accumulated $N_2O$ emissions during the 9 days represented 0.05–0.1% of the N inputs in urea. For comparison, $N_2O$ emissions equivalent to 0.2–0.3% of urinary urea-N were recorded during 7 weeks after deposition of 25.5 or 50.9 g urea-N $m^{-2}$ to monoliths from the pasture where soil for the present experiment was collected (Ambus, 2004).

### *Effects on nitrification*

Selected soil characteristics were monitored in order to throw light on the potential importance of nitrification and denitrification for the $N_2O$ emissions observed. Treatment effects on either pH or osmotic potential were not likely to inhibit $NH_4^+$ oxidation at the ranges observed (Low et al., 1997; Stark and Firestone, 1995), but the combination of pH and total ammoniacal nitrogen (TAN) in *HU* resulted in $NH_3$(aq) levels in the soil solution of up to 45 mg $L^{-1}$, as determined by the following modification of the Henderson-Hasselbach equation:

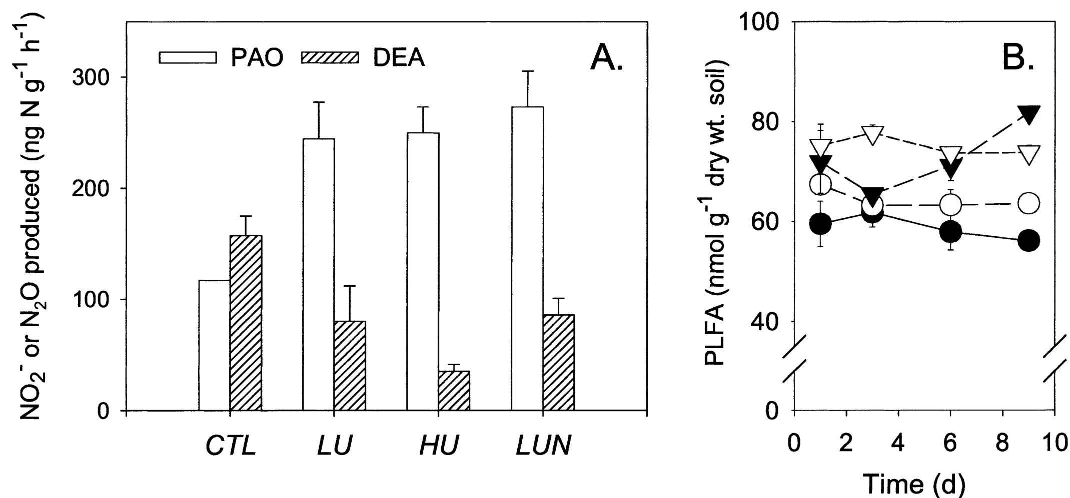


*Figure 7.* Potential ammonium oxidation (PAO) and denitrifying enzyme activity (DEA) by day 3 (A) and PLFA concentrations (B). Error bars represent standard error ($n = 3$). Key to symbols: *CTL* (•), *LU* (○), *HU* (▼), *LUN* (▽).

$$pH = 9.25 + \log([NH_3]/[TAN \div NH_3]) \,, \quad (4)$$

where 9.25 is the pKa of the $NH_4^+$-$NH_3$ equilibrium. According to the relationship described by Monaghan and Barraclough (1992), this level of $NH_3$(aq) could have given a > 50% reduction of nitrification rates. In the present experiment, net accumulation of $NO_3^-$ in *LU*, *HU* and *LUN* after 9 days were 90, 63 and 116 mg N $kg^{-1}$, confirming that nitrification was delayed at the higher urea level.

Nitrite oxidation is more readily inhibited than $NH_4^+$ oxidation (Harada and Kai, 1968), and this may have caused the $NO_2^-$ accumulation observed in *HU* (Figure 2). In this treatment, the $NO_2^-$ concentration followed a time course similar to $N_2O$ emissions (cf. Figures 2 and 3). Monaghan and Barraclough (1992) also observed $NO_2^-$ accumulation at high urine-N concentrations, while Stevens et al. (1998) found a direct relationship between $NO_2^-$ accumulation and $N_2O$ emissions at pH 8, but not at pH 5.6–6.5. Nitrous oxide can be produced by nitrifiers via two different pathways (Wrage et al., 2001). It is either derived from hydroxylamine ($NH_2OH$) as a byproduct of $NH_4^+$ oxidation, or it is produced via so-called nitrifier denitrification, in which case $NO_2^-$ is the substrate for a process leading to $N_2O$ and $N_2$ formation. The correlation of $N_2O$ emissions and $NO_2^-$ accumulation in *HU* was consistent with nitrifier denitrification as a source of $N_2O$ emissions. While a direct link between the two pools could not be established in this study because $^{15}NO_2^-$ was not determined, a strong correlation between $^{15}N$ labelling of $NO_2^-$ and $N_2O$ pools was recently reported for a pasture soil (Müller et al., 2004).

### *Effects on denitrification*

Denitrification is primarily regulated by (lack of) oxygen, carbon and $NO_3^-$ availability. Denitrification was not limited by $NO_3^-$ availability, as indicated by similar $N_2O$ emissions from *LU* and *LUN*, and by the absence of $N_2O$ in the N treatment ($^{15}N_2$ was not detected). Carbon availability could have varied between treatments, since the measurements of DOC indicated that a pool of soil organic matter was dissolved, in accordance with previous observations (e.g., Lovell and Jarvis, 1996). However, the degradability of this DOC appeared to be low, since elevated concentrations were maintained, especially in the *HU* treatment (Figure 6A), whereas $CO_2$ evolution rates declined to the background level by day 9 (Figure 6B). Also, Kalbitz et al. (2003) studied DOC in grassland soil from a fen area and concluded that only 5–9% of the dissolved organic matter was labile. We propose that DOC derived from soil organic matter did not significantly stimulate microbial activity in the present experiment, and that $CO_2$ emissions mainly reflected the effect of urea-N on soil microbial turnover.

Emissions of $N_2O$ via denitrification are often associated with transient conditions such as oxic-anoxic gradients or wetting of a dry soil (Højberg et al., 1994; Rudaz et al., 1991). Sustained $N_2O$ production via denitrification is mostly associated with low pH values and/or excess $NO_3^-$ (Stevens and Laughlin, 1998). In

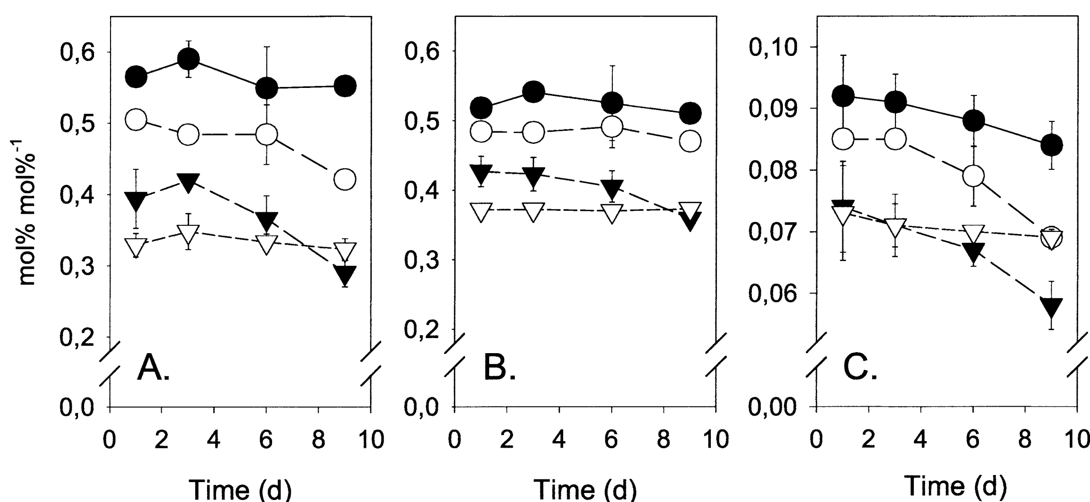


*Figure 8.* Ratios of cyclopropane fatty acids cy17:0/16;1ω7c (A), cy19:0/18:1ω7 (B) and the trans-cis ratio of 16:1ω7 (C) in treatments *CTL* (•), *LU* (○), *HU* (▼) and *LUN* (▽). Error bars represent standard error ($n$ = 3).

the present experiment, the pH in urea-amended soil was higher than in unamended soil, and there was no effect of increasing $NO_3^-$ availability. Therefore, it appears unlikely that significant amounts of $N_2O$ were produced via denitrification.

The possibility that $N_2O$ emissions were partly due to chemodenitrification (references in Nelson, 1982; Venterea and Rolston, 2000) cannot be ruled out. The process is mainly expected to occur under acidic conditions, and in the present study pH in urea-amended soil was generally above 7. However, more acidic conditions could have occurred in connection with nitrifying micro-sites.

*Microbial dynamics*

DEA was depressed in urea-amended soil by day 3. Simek et al. (2002) recently showed that DEA is sensitive towards pH of the soil slurry and often has an optimum near the natural pH of the soil. The pH in the slurries of *LU*, *HU* and *LUN* were 6.9–7.2, i.e., higher than the pH in *CTL* of 6.3, and a pH effect may thus have contributed to the reductions in DEA observed in urea-amended soil. If such a pH effect was important during the DEA assay, then denitrification activity must have been inhibited in the soil which also had elevated pH upon urea amendment, especially in *HU* (Figure 5A) . If, in contrast, a pH effect was not important for the DEA results, then the treatment effects must be interpreted as a decline in the potential for denitrification.

Urea amendment gave comparable stimulations of PAO in *LU*, *HU* and *LUN*, but this assay was buffered. An adaptation to ambient soil pH, similar to that observed for DEA, has been described for short-term nitrification activity in pasture soils from various sites in New Zealand (Bramley and White, 1990). This implies that the pH changes in urea-amended soil could well have affected nitrification activity during incubation in this experiment, as indeed suggested by the dealy in $NO_3^-$ accumulation in *HU* in comparison with *LU* and *LUN*.

The concentration of PLFA in soil is an index of microbial biomass that is strongly correlated with biomass C (Bailey et al., 2002). Compared to *CTL*, *HU* and *LUN* had elevated concentrations of PLFA even at the first sampling. Microbial dynamics could have been confounded by a shift in lipid extractability or partitioning during extraction at the higher ionic strength in *HU* and *LUN* (Frostegård et al., 1991; Nielsen and Petersen, 2000), but subsequent changes in PLFA did not correlate with changes in soil solution properties and were probably dominated by microbial dynamics.

In *HU*, PLFA appeared to decline between day 1 and day 3 but then increased dramatically, especially due to bacterial growth (data not shown). A possible interpretation of this pattern is that initial growth inhibition was transient and replaced by net growth. Active growth between day 3 and day 9 in *HU* was also indicated by the decline in fatty acid stress indicators (Figure 8). Cyclopropane fatty acids are pro-

duced in particular by Gram negative bacteria and appear when the organisms enter a stationary phase (Grogan and Cronan, 1997); hence, reduced proportions of these compounds suggest active growth. Elevated *trans/cis* ratios of membrane lipid fatty acids is another response to environmental stresses observed with some Gram negative bacteria, including *Pseudomonas* (Heipieper et al., 2003), and so a decline in 16:1$\omega$7t/c may be taken as an indication of stress relief. The observed trends thus imply that any inhibitory effects of urea deposition were replaced by vigorous growth after a few days.

## Conclusions

The microbial response to deposition of urea corresponding to 22–43 g N $m^{-2}$ was complex. There was evidence for inhibition of both nitrification and denitrification at the highest urea level, but also an average stimulation of potential ammonium oxidation activity and, after a few days, significant microbial growth. Inhibition and stimulation effects could have been spatially separated, and future work should describe the vertical stratification in more detail. The highest $N_2O$ emission rates coincided with $NO_2^-$ accumulation, and nitrifier denitrification is likely to be the main source of $N_2O$ in this laboratory study. It should be stressed that the well-defined experimental conditions of this study effectively minimized background $N_2O$ emissions, which are often associated with fluctuating climatic conditions, in order to focus on the direct effects of urea. Also, the exclusion of urine components other than urea reduced C availability and probably urea turnover rates. Hence, the extent of losses, as well as the balance between nitrification and denitrification in this model system may differ from the field situation. On the other hand, the simplicity of the experimental setup enabled a relatively detailed interpretation of N dynamics and microbial community changes leading to $N_2O$ emissions from urea in pasture soil. We conclude that urea concentration in urine deposited on pastures is likely to influence microbial dynamics and soil N transformations not only quantitatively, but also qualitatively.

## Acknowledgement

This study was supported by the EU Framework Programme 5 project MIDAIR (EVK2 CT-2000-00096), and by the Danish Research Centre for Organic Farming. The technical assistance of M. Astridou is greatly appreciated.

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