Nitrogen leaching from ¹⁵N labelled cow urine and dung applied to grassland on a sandy soil

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Abstract

Nitrogen (N) leaching under grazed pastures can be very high directly under urine spots. The amount of N which is returned by one excretion of urine or dung can locally exceed 1000 kg ha⁻¹ a⁻¹ which is far more than the uptake by surrounding plants during one grazing period. We therefore quantified the contribution of N deriving from urine and dung to the total N leaching under urine and dung patches. Dung N and urine N was separately sampled from a cow feed with ¹⁵N labelled grassilage, and were amended on lysimeters in October 2000 and October 2001. Lysimeters (350 mm diameter and 800 mm length) were filled with sand, and an intact grass sod from a pasture, 4 lysimeter each were amended with the ¹⁵N labelled dung and urine; 4 lysimeters without an application of dung or urine served as control. During 11 months after dung and urine amendment the amount of leachate was monitored and leachate was analysed for nitrate, ammonium and total N. ¹⁵N in these fractions was measured. Dung and urine applications of 1052 and 1030 kg N ha⁻¹ in autumn increased N leaching. Leaching loss of nitrate and dissolved organic N deriving from dung was only 37 kg N ha⁻¹ in both years, whereas under urine patches 447 kg nitrate-N ha⁻¹, 108 kg N ha⁻¹ ammonia-N and 53 kg ha⁻¹ dissolved organic N leached on average of both experimental years. N not deriving from dung and urine exceeded the leached N under the control by about 36 and 136 kg ha⁻¹ on average of both years, suggesting the contribution of different priming processes.

Introduction

Grazing animals excrete 75–90% of the N taken up (Ball et al. 1979). Under practical farming conditions these amounts of locally excreted N exceed the plant uptake by far, resulting in significant N losses. In climates with a mild winter N leaching under pastures mainly depends on grazing intensity and on the N input via mineral fertilizer and slurry (Wachendorf et al. 2004). Under the prevailing climatic conditions, if pastures are grazed throughout the growing season, NO_3 leaching losses can amount up to 200 kg ha⁻¹ (Benke et al. 1992; Watson et al. 2000). Depending upon the grazing intensity and the duration of the growing season, 40–50% of the area is covered by dung and urine (Vinther 1998) resulting in a high variability of N-deposition. The overall amount of nitrogen which is returned by grazing animals is high. Per excretion locally 1100 kg N ha⁻¹ is returned with urine (Whitehead 1995) and 750–1300 kg N ha⁻¹ is returned with dung (Holmes

1968; O'Connor 1974). Although the amount of N which is excreted with urine and dung can be similar, the N-fluxes under urine and dung patches differ because of the higher amount of available N in urine. Urine consists to 60-90% of urea, which is hydrolyzed within a few days. N-losses under urine patches via nitrogen leaching are of great concern, whereas under dung patches N mineralization-immobilization processes dominate (Thompson and Fillery 1997). The total amount of N leached under urine patches varies between 18 and 58% of the applied N, depending on the soil texture (Clough et al. 1998), application time (Cuttle and Bourne 1993; Ledgard et al. 1988; Di and Cameron 2002; Stout 2003), the amount of urine (Thompson and Fillery 1997; Stout 2003), and the botanical composition of the pasture (Loiseau et al. 2001). An estimation of the contribution of dung to N-losses under pasture is difficult as the results from incubation experiments (Barrow 1961; Takahashi et al. 2004) are not translatable to field conditions. Nevertheless, Kyvsgaard et al. (2000) suggested the apparent digestibility of the feed as a robust predictor for faeces N mineralization. Within the discussion of the effect of N-losses on economy and ecology, dissolved organic nitrogen (DON) is becoming more and more important (Siemens and Kaupenjohann 2002), but is rarely investigated in grassland soils (Jones et al. 2004) and under urine patches (Shand et al. 2000).

Thus, the objective of this study was to determine the NH_4 -, NO_3 - and DON-leaching losses from dung and urine patches applied in autumn. Besides ¹⁵N labelling of urine and dung via feeding a cow with ¹⁵N labelled grass enabled a partitioning of leached N derived from the urine and dung and that derived from the soil, but the contribution of different priming processes could not be estimated.

Materials and methods

Experimental site and management conditions

The experiment was conducted at the experimental farm 'Karkendamm' of the Faculty of Agricultural and Nutritional Sciences at the University of Kiel in northern Germany $(53^{\circ}55' \text{ N}, 9^{\circ}55' \text{ E}; 14 \text{ m a. s. l.})$. The climate is moderate oceanic, the average annual temperature (1961–1990) is 8.4 °C and the average annual rainfall is 824 mm. Daily precipitation was recorded at 2 m height, precipitation at ground level was collected weekly. Free drainage lysimeters (no suction was applied) with a diameter of 350 mm and a height of 800 mm were installed with four replicates in each treatment. On the experimental farm the gleyic podzols were deeply ploughed in 1980. Like that, the B-horizons are in a tilted position causing very variable flow conditions, whereas the frequently ploughed A-horizon was evenly mixed. Therefore only for the upper layer of the lysimeters undisturbed soil monoliths from the site were taken. Underneath, up to a depth of 600 mm the lysimeters were filled with medium sand which is the same soil texture of the A- and B-horizons of the site. The bottom of the lysimeters was filled with fine gravel and had a cylindrical form with an opening covered by a nylon mesh (2 mm) which was connected to a hose leading to the collection bottle. The undisturbed upper soil layer was sampled from a pasture which was not grazed for 1 year but maintained by frequent cutting and fertilized with 200 kg N ha⁻¹, 35 kg P ha⁻¹ and 133 kg K ha⁻¹. The monolith was taken by pressing a steel cylinder into the sward to a depth of 20 cm. Very few roots penetrated below this depth. The content of organic carbon and nitrogen of the upper horizon was 43 and 2.7 mg g^{-1} . The washed sand and gravel used in the layers beneath were free of organic carbon. The cation exchange capacity in the A-horizon was 7.4 cmol(+) kg⁻¹. The pH (H_2O) was 5.9, 7.3 and 7.8 in the A-horizon, the sand and the gravel, respectively. Field capacity of the lysimeter was estimated 96 mm which was lower than for the gleyic podzols at the site. The lysimeters were installed in a trench on the experimental farm, the soil surface of the lysimeter was at ground level leaving 20 mm of casing protruding at about ground level to avoid surface run-off from the the lysimeters. The remaining pasture on the lysimeter was cut to a stubble height of 50 mm and the herbage was removed. In the following year herbage in the lysimeter was harvested five times throughout the grazing season to simulate moderate to intensive grazing by clipping all herbage above 50 mm. N fertilization was conducted according to the common farming practice with 50 kg N ha⁻¹ applied as calcium

ammonium nitrate at the end of March and before each of the first three re-growths (Table 1).

Production and application of ^{15}N labelled dung and urine

A third cut perennial ryegrass (Lolium perenne) was fertilized with 80 kg N ha⁻¹. ¹⁵N was applied as KNO₃ and NH₄NO₃ (4.63 atom% excess). The grass was harvested as small bale silage with an ¹⁵N enrichment of 2.11 atom% excess. A culling cow was fed with ¹⁵N labelled grass silage for 17 days without any other supplements. Urine and dung was collected daily and frozen at -20 °C. The daily charges of urine and dung were bulked from day 10 to 17 and 7 to 17, respectively. The N concentration in the urine was 10.4 (SE 0.05) mg g fresh matter $(FM)^{-1}$ and in dung 4.4 (SE 0.03) mg g fresh matter $(FM)^{-1}$. The dry matter content in the dung was 252.4 (SE 6.2) mg g FM⁻¹. The urine and dung had an enrichment of 1.22 (SE 0.001) and 1.67 (SE 0.007) atom% excess, respectively. Urine, equivalent to 1030 kg N ha⁻¹ and dung, equivalent to 1052 kg N ha⁻¹, was applied directly to the centre of lysimeters to simulate dung and urine deposition by the animal.

Sampling and analysis of soil and leachate

The lysimeters were checked for leachate weekly or after rainfall events throughout the year. Leachate volume was recorded. Subsamples (250 ml) were filtered (cellulose-nitrate, $0.45 \,\mu$ m, Satorius) the same day and stored at 5 °C. After 11 months soil samples were taken in the upper soil layer (0–150 mm) with a soil probe (22 mm diameter) in 8

Table 1. Dates of application of urine and dung and mineral N-fertilizer and dates of cutting in two experimental years.

Application of urine and dung	25.10.2000	24.10.2001		
 Fertilization Fertilization, 1. simulated grazing Fertilization, 2. simulated grazing Fertilization, 3. simulated grazing Simulated grazing Simulated grazing 	9.4.2001 4.5.2001 5.6.2001 3.7.2001 27.7.2001 3.9.2001	9.4.2002 6.5.2002 4.6.2002 2.7.2002 12.8.2002 8.10.2002		
6. Simulated grazing	15.10.2001	0.10.2002		

replicates and visible roots were removed. Replicates were combined to one bulk sample per lysimeter. After air drying, grinding and drying at 60 °C, samples were analysed with an isotope ratio mass spectrometer (see below). N content was corrected for water content (105 °C) and the amount of N was calculated considering the measured bulk density of the site of 1.27 (SE 0.12) (g cm³). The analysis of NO₃ and NH₄ was performed within a few days after sampling. Dissolved organic nitrogen (DON) was analysed within a week in refrigerated samples and exceptionally in frozen samples during the first year. Microdiffusion for ¹⁵N analyses was conducted on refrigerated or frozen samples. NH₄ in soil extracts was determined using a colorimetric method based on an inophenol-blue complex (Anonyma 1991), the detection limit was $0.03 \text{ mg } l^{-1}$. NO₃ was reduced to nitrite using zinc and determined by direct UV absorption at 210 nm (Keeney and Nelson 1982), detection limit was 0.5 mg l^{-1} . Total organic nitrogen (TON) was oxidized with K₂O₈S₂ (Cabrera and Beare 1993) and determined as NO₃ (see above). DON was calculated by difference of total dissolved nitrogen and NO₃ and NH₄.

Determination of ¹⁵N in fractions of the soil leachate was conducted by microdiffusion (Goerges and Dittert 1998). MgO was added to 0.2-20 ml of the soil leachate in a scintillation cap to liberate NH₄⁺. The NH₃ was trapped in an acid washed glass-fibre filter and a drop of KHSO₄, which was sealed between two membranes of teflon under the lid of the scintillation cap. The caps were end over shaken for 3 days. In a second step NO_3^- was reduced to NH_4^+ by the addition of 400 mg Devarda's alloy and the procedure was followed as for NH_4^+ . The two steps were combined in one if the NH₄ concentration was lower than 0.05 mg N l^{-1} . For ¹⁵N analysis of the total dissolved N 600 mg Devarda's alloy and 2-4 ml KCl was added to the $K_2O_8S_2$ oxidized soil leachate and 1-3 ml of 6.75 M NaOH was added to raise the pH above 13. The glass-fibre filter was dried and sealed in tin capsules. Total-N concentrations and relative ¹⁵N abundances were measured using an online system combining an elemental analyser (Carlo Erba NA 2500) for Dumas combustion and Finnigan Mat Delta plus gas isotope ratio mass spectrometer. Reproducibility of the analysis, checked with reference

material (RM 8548: IAEA-N2) from the international Atomic Energy Agency, was better than 0.2%.

Calculations

The amount of N derived from urine and dung in soil solution fractions was calculated using the equations:

1. Difference method: N derived from urine $(N_{dfu}) = T_u - T_c$; N derived from dung $(N_{dfd}) = T_d - T_c$. 2. ¹⁵N method: ¹⁵N from urine (¹⁵N_u) = $T_u * (A_s/A_u)$; ¹⁵N from dung (¹⁵N_d) = $T_d * (A_s/A_d)$.

where T_c , T_u and T_d is the total amount of N in each leachate N fraction in the control, urine and dung treated samples, A_s is the ¹⁵N atom% excess (¹⁵N atom% treatment-control) in the NO₃, NH₄, DON-fraction of the sample and A_u and A_d is the ¹⁵N atom% excess in the applied urine and dung, respectively.

The amount of leached N derived from soil was calculated using the equation:

Leached N derived from soil under urine patches $(N_{dfsu}) = N$ derived from urine $(N_{dfu}) - {}^{15}N$ from urine $({}^{15}N_u)$

Leached N derived from soil under dung patches $(N_{dfsd}) = N$ derived from dung $(N_{dfd}) - {}^{15}N$ from dung $({}^{15}N_d)$ Recovery of ${}^{15}N$ was calculated as the percent-

Recovery of ¹⁵N was calculated as the percentage of ¹⁵N in each fraction as well as the percentage of the amount of applied urine and dung-N. ¹⁵N analysis was conducted at least every second soil leachate sampling date, for calculation of the recovery rate after 11 months ¹⁵N atom% excess was interpolated. The figures presented only give measured data.

Statistical analysis

Analysis of variance was performed using the procedure MIXED of SAS software (SAS Institute 1995) considering the full-randomized layout of the trial. Data presented are least square means produced in the analysis. Only common standard errors for least squares means are given in the tables and diagrams. For a conservative interpretation the highest value was chosen in case of different standard errors among treatments. The critical probability value generally was P < 0.05. The level of significance is described with ns: not significant, *: P < 0.05, **: P < 0.01, ***: P < 0.001.

Results

Rainfall, temperature and leaching

Soil temperature during winter was mild, and did not decline below 0 in the first experimental year (Table 2). Precipitation was higher in the second year than in the first year especially during winter. Moreover, precipitation in the second year was characterized by occasional heavy rainfall (>30 mm day⁻¹) (data not shown). The amount of leachate in 2000/2001 from the lysimeter was in accordance with the calculated average of 257 mm in 1997–2002 for undisturbed soil (Wachendorf et al. 2004) but leachate in 2001/2002 was higher than the calculated value because of the lower field capacity of the

Table 2. Soil temperature (at 50 mm depth) and rainfall in both experimental years (October–September) and during the winter period (October–March).

		2000/2001	2001/2002
Soil Temperature (°C)	Average experimental year	9.5	10.2
	Average winter period	4.7	5.2
	Max. winter period	11.3	13.3
	Min. winter period	0.4	-0.2
Rainfall (mm)	Sum experimental year	810	967
	Winter period	193	425

lysimeter and the intense rainfall events during that period (Figures 1a and 2a). Average leaching from the lysimeters differed significantly between treatments in the first year but not in the second year (Table 3). The drainage represented 31 and 48% of annual precipitation and 60–62% of the total collected leachate drained during winter in year one and two, respectively.



Figure 1. Rainfall and average leaching (a) from lysimeters treated with urine and dung and control lysimeters and the effect of urine and dung on leached ammonium (b), Nitrate (c) and dissolved organic nitrogen (d) after application in October 2000. Bars indicate the standard error of the mean.





Figure 2. Rainfall and average leaching (a) from lysimeters treated with urine and dung and control lysimeters and the effect of urine and dung on leached ammonium (b), nitrate (c) and dissolved organic nitrogen (d) after application in October 2001. Bars indicate the standard error of the mean.

N fractions in soil leachate

Nitrate leaching was the main contributor to the N leaching loss within all treatments but NH₄

leaching under urine patches also occurred (Table 3). The percentage of N leached as DON varied from 10 to 31% of the total N leaching loss. The N loads of the control, the urine and dung

Experimental year	1					2				
Treatment	Control	Urine	Dung	SE	Sig. ^a	Control	Urine	Dung	SE	Sig. ^a
Drainage volume (mm)										
(Oct.–March)	155.8	139.5	128.6	7.1	*	294.8	285.6	285.6	9.4	n.s.
(OctSep.)	273.5	239.0	214.0	13.9	*	496.1	415.5	467.6	23.5	n.s.
Leached N (kg N ha^{-1})										
NO ₃ -N	20.2	591.8	93.1	20.3	***	48.9	493.8	94.0	7.0	***
NH ₄ -N	0.1	88.0	0.1	5.9	***	0.1	137.8	0.1	10.8	***
DON	9.3	180.7	26.1	5.9	***	7.8	72.5	20.5	8.9	**
Sum N-leaching	29.6	860.5	119.5	30.8	***	56.7	704.3	114.7	17.9	***
Leached ${}^{15}N$ (kg N ha ⁻¹)										
¹⁵ N-NO ₃		494.8	30.7	23.0	***		398.6	28.1	8.1	***
¹⁵ N-NH ₄		80.1	n.d.	5.8			135.4	n.d.	13.0	
¹⁵ N-DON		79.6	7.1	16.7	***		25.9	9.3	9.5	**
Sum ¹⁵ N-leaching		654.4	37.5	28.4	***		559.9	37.4	18.9	***
Leached N derived from soil	(kg N ha^{-1})									
NO ₃	(0)	78.0	42.1	6.2	**		46.6	17.2	3.2	***
NH_4		7.7		2.4			2.4		0.3	
DON		91.6	9.7	10.3	**		38.9	3.5	3.9	***
Sum N-leaching		177.4	51.9	15.4	**		87.8	20.7	4.5	***
¹⁵ N-in soil layer (kg N ha ⁻¹)		139.9	846.3	13.9	**		131.3	741.1	61.2	***

Table 3. Mean and standard error (SE) of the drainage volume and the accumulated amount of nitrogen leached in lysimeters applied with 15 N labelled urine and dung and control lysimeters of the experimental years 2000/2001 and 2001/2002.

^a *: P < 0.05, **: P < 0.01, ***: P < 0.001.

applied treatments differed significantly. In the first year NH₄ leaching losses occurred in a period of 120 days after urine application and it was related to precipitation and drainage volume (Figure 1a and b), whereas in the second year 94% of the NH₄ under urine patches was already leached after 50 days (Figure 2b). Under the control and dung patches NH₄ load remained low and the maximum amount of NH4 leached did not exceed $0.06 \text{ kg N} \text{ ha}^{-1}$ in both years. NO₃ leaching losses were not related to drainage volume (Figure 1a and b; Figure 2a and b). The majority of nitrate under urine patches was leached in a 60-day period, beginning later in the first than in the second year. Heavy rainfall after fertilization in July 2002 resulted in an increase of nitrate leaching in all treatments (Figure 2c). DON leaching under urine spots peaked in the first year when drainage volume exceeded 16 mm week $^{-1}$ (Figure 1a and d), much lower DON loads were detected in the second year (Figure 2d). With the exception of the first sampling after urine application in year one, DON accounted for 20% of total leached N within 150 days after dung and urine application and increased to 60% in all treatments and both years towards the end of the experiments.

N recovery

The percentage of leached NH₄ derived from urine was high from the first sampling after urine application and remained above 90% in both years (Figures 3a and 4a). The recovery of ¹⁵N in the NO3 fraction under urine patches showed different patterns in both experimental years but the average over the year was 68 and 70% in the first and second year, respectively. Under dung patches the average percentage of dung-N in NO₃ was similar with 25 and 32% in the first and second year, respectively. The DON fraction in the leachate of the urine treatment comprised by 50 and 31% of urine-N in the mean of the first 100 days after urine application in year one and two, respectively (Figures 3b and 4b). After 125 days this percentage decreased below 10% with the exception of one sampling date in year one. Under dung patches the maximum percentage of excretal-N in the DON fraction was 52 and 71% after 194 and 89 days in the first and second experimental year, respectively. The total leached labelled N of the urine and the dung treatments differed significantly in both experimental years (Table 3). Total recovery averaged over both years was 607 and



Figure 3. Percentage of labelled nitrogen in ammonium and nitrate (a) and dissolved organic nitrogen (b) leached from lysimeters amended with in ¹⁵N labelled urine and dung in October 2000. Bars indicate the standard error of the mean. level of significance ns: not significant, *: P < 0.05, **: P < 0.01, ***: P < 0.001.

37 kg N ha⁻¹, which is 59 and 4% of the applied ¹⁵N for the urine and the dung treatment, respectively. Average recovery of urine- and dung-N calculated by the difference method, was 72 and 7%, respectively, which is higher than the N recovery calculated by the ¹⁵N method. The difference between both methods which is due to the increase of soil N in the leachate of the urine and dung applied treatments compared to the control, was higher for the urine than for the dung treatment and was higher in the first than in the second year (Table 3).

Discussion

N leaching

Differences in experimental design make it difficult to directly compare observed N leaching losses in this study with results from other studies. Moreover, rarely all N fractions in soil leachate were analysed. The high N losses observed in this investigation are due to the sandy soil with high drainage volumes, the late application date allowing only a very small uptake of excretal-N by plants (Trott 2003; Wachendorf unpublished) and the small area on which the dung and urine were applied, creating a 'hot spot'. However, NO₃ leaching losses of up to 420 kg N ha⁻¹ after synthetic urine application in fall in lysimeters filled with silt loam were observed by Stout (2003), irrespective of whether 442, 885 or 1327 kg N ha⁻¹ were applied. Although the urea content in soil and in urine was not measured, hydrolysis of urea and ammonification was rapid as 102% of the urine-N was extracted as NH₄ in the upper soil layer 10 days after urine application (Lampe 2004). Up to 1090 kg NH₄-N ha⁻¹ below the centre of urine affected areas, was found by Ball and Ryden (1984). In the present experiment the NH_4 leaching loss of 113 kg N ha⁻¹ under urine patches (average of both years) points to the importance of this process in grazed pastures. NH₄



Figure 4. Percentage of labelled nitrogen in ammonium and nitrate (a) and dissolved organic nitrogen (b) leached from lysimeters amended with in ¹⁵N labelled urine and dung in October 2001. Bars indicate the standard error of the mean.

leaching is attributed to the low cation exchange capacity of the sandy soil, the high salt content in urine, as well as the delay of nitrification. The delay of nitrification under urine spots is due to the high pH, NH₃ and NO₂ concentration and osmotic strength of urine, and it may require more than a week before appreciable amounts of NO₃ appear under urine patches (Monaghan and Barraclough 1992). On average of both years 16, 20 and 22% of the total N lost in drainage water was in organic form under urine and dung patches and the control, respectively. These data, together with findings by Korsaeth et al. (2003), who measured 18-29% of DON in drainage water from lysimeters under perennial ley, emphasize the relevance of DON regarding N losses under grazed pasture in sandy soils. In contrast to other investigations (Williams et al. 1999; Shand et al. 2000) a significant effect of urine-application on DON load was found. This may be attributed to the application of natural urine in this study, which contains appreciable amounts of organic compounds like creatin und creatinin (1-10%) and allantoin (2-12%) of the total N in urine)(Whitehead 1995). Furthermore, high concentrations of salt under urine patches probably favoured the leaching of DON.

Recovery rate of applied N

The recovery of applied urine $-^{15}$ N in leachate was 64 and 53% in year one and two, respectively. This is relatively high compared to results from a lysimeter study from New Zealand with different soil textures, in which recoveries ranged from 13 to 32% in a sandy loam and a silt loam, respectively (Clough et al. 1998). The higher recovery in leached N is due to different climatic conditions, with higher precipitation in northern Germany and the sandy soil texture resulting in higher drainage volumes. The average recovery of 48% in the NO₃ fraction (difference method) is in the same range as results from a field experiment, where 38 and 60% of the urine-N applied in October and November, respectively, were recovered during the following leaching period (Cuttle and Bourne 1993). Following the application of ¹⁵N labelled urine 5% of the applied urine-N was detected in the DON

fraction, whereas no ¹⁵N was detected in the DON fraction sampled under soil applied with synthetic urine (Shand et al. 2000). These findings highlight the fact that it is important to use urine in which all N components were labelled to investigate effects of urine on DON.

Average recovery of applied ¹⁵N in leachate under dung patches was 4%, which is much lower than what may be expected from mineralization rates of 30% of dung-N after dung was incorporated in soil under ley pastures observed by Thompson and Fillery (1997) and observations by Sørensen et al. (1994a) in lysimeter experiments. This may be due to low mineralization rates in the patch itself or a high immobilization of dung-N in the top soil compared to incorporated dung, shown by recovery rates of 80 and 70% in the soil under dung patches after experimental year one and two, respectively (Table 3). Due to high leaching loss under urine patches recovery rates of ¹⁵N in soil under urine were much lower. About 13% of the applied ¹⁵N in urine remained in the upper soil layer after both experimental years, which is in accordance with results from Whitehead and Bristow (1990).

Thus, dung patches substantially increased the soil N pool and dung-N represented up to 15% of the total soil N, probably affecting mineralization processes in subsequent years.

An important sink for the excreted N by grazing animals is the plant biomass with recovery rates of 3 and 10% of urine-N and dung-N during the following year, respectively (Wachendorf 2004). Gaseous losses of N₂O 6 months after dung and urine amendment during the second year amounts to 0.05 and 0.33% of urine-N and dung-N, respectively (Lampe 2004). Losses by volatilization of NH₃ from the soil surface may be low, as 12 days after urine application 100% of urine-N was extracted as NH₄ in the upper soil layer (Lampe 2004).

Priming effect

Total N leaching calculated by the difference method was higher than the total leached 15 N. On average of both years the surplus amount of nitrate leached under the patches exceeded the amount of 15 N nitrate by 62 and 30 kg ha⁻¹ after urine and dung amendment, respectively. DON

leached under urine patches not accounted for ¹⁵N and soil derived N under the control was on average of both years 65 kg ha^{-1} . The surplus N leaching can be explained by a real priming effect due to an accelerated SOM turnover, which may have been induced by an increased activity or amount of microbial biomass in soils amended with dung shown by Hatch et al. (2000). On the other hand excreted N is subjected to immobilization (Sørensen and Jensen 1995), due to the concurrent supply of dissolved carbon (Monaghan und Barraclough 1992). Causes leading to an apparent priming effect like pool substitution (i.e. uptake of dung and urine N instead of soil derived N by microbial biomass) can not be ruled out in this experiment, as immobilization of N was observed in the first year from day 7 to 33 with a NO_3 concentration being lower under urine patches than the control (data not shown). Other processes leading to apparent priming effects like isotopic exchange of ${}^{15}NH_4$ with unlabelled NH_4 or cation exchange of unlabelled NH4 with K from urine are assumed to be minor, because the enrichment of the NH₄ fraction remained high throughout the experiment. Non-uniformly labelling of the dung and urine may induce both positive as well as negative apparent priming effects. Sørensen et al. (1994b) showed that with manure in which the easily decomposable pool of organic manure is lower enriched by ¹⁵N than the total manure, the recovery of N in plants was underestimated (Sørensen et al. 1994a). Though the ¹⁵N enrichment of water soluble manure was higher than the enrichment of the total manure N, a part of the water soluble-N was very slowly decomposable (Sørensen et al. 1994b), leading to an overestimation of the actual transport of labelled N in the subsoil (Sørensen et al. 1994a). A further reason for real priming effects under urine patches is possibly the release of N through lysis and dying of microbial biomass through osmotic stress by high salt concentrations. But this effect is probably superimposed on an increased solubility of soil organic matter under alkaline conditions (Fillery and McInnes 1992).

Conclusions

The results of this research indicate that the application of ¹⁵N labelled urine and dung in au-

tumn directly contribute to 78 and 32% of the total amount of the total-N leached in the sandy soil, respectively. High ¹⁵N leaching loss under urine patches was connected with a high N loss deriving from other sources like microbial biomass and soil organic matter. Solubilization of organic matter by urea and high salt concentration are important processes causing surplus N leaching. Under dung patches accumulation of dung-N leads to an increase of soil organic N of 15%, significantly affecting mineralization processes in subsequent years.

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References

- Anonyma 1991. VDLUFA Methodenbuch Band 1. Die Untersuchung von Böden. 4. Auflage, Darmstadt, Germany.
- Ball P.R., Keeney D.R., Theobald P.W. and Nes P. 1979. Nitrogen balance in urine affected areas of New Zealand pastures. Agron. J. 71: 309–314.
- Ball P.R. and Ryden J.C. 1984. Nitrogen relationships in intensively managed temperate grasslands. Plant Soil 76: 23– 33.
- Barrow N.J. 1961. Mineralization of nitrogen and sulphur from sheep faeces. Aust. J. Agric. Res. 12: 644–650.
- Benke M., Kornher A. and Taube F. 1992. Nitrate leaching from cut and grazed swards influenced by nitrogen fertilization. In: Proceedings of the 14th General Meeting of the European Grassland Federation. Lahti, Finland, pp. 184– 188.
- Cabrera M.L. and Beare M.H. 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Sci. Soc. Am. J. 57: 1007–1012.
- Clough T.J., Ledgard S.F., Sprosen M.S. and Kear M.J. 1998. Fate of ¹⁵N labelled urine on four soil types. Plant Soil 199: 195–203.
- Cuttle S.P. and Bourne P.C. 1993. Uptake and leaching of nitrogen from artificial urine applied on grassland on dif-

ferent dates during the growing season. Plant Soil 150: 77-86.

- Di H.J. and Cameron K.C. 2002. Nitrate leaching and pasture production from different nitrogen sources on shallow stoney soil under flood irrigated pasture. Aust. J. Soil Res. 40: 317– 334.
- Fillery I.R. and McInnes K.J. 1992. Components of the fertilizer nitrogen balance for wheat production on duplex soils. Aust. J. Experiment. Agric. 32: 887–899.
- Goerges T. and Dittert K. 1998. Improved diffusion technique for ¹⁵N:¹⁴N analysis of ammonium and nitrate from aqueous samples by stable isotope spectrometry. Comm. Soil Sci. Plant Anal. 29: 361–368.
- Hatch D.J., Lovell R.D., Antil R.S., Jarvis S.C. and Owen P.M. 2000. Nitrogen mineralization and microbial activity in permanent pastures amended with nitrogen fertilizer or dung. Biol. Fertil. Soils. 30: 288–293.
- Holmes W. 1968. The use of nitrogen in the management of pasture for cattle. Herbage Abstracts 38: 265–277.
- Jones D.L., Shannon D., Murphy D.V. and Farrar J. 2004. Role of dissolved organic nitrogen in soil N cycling in grassland soils. Soil Biol. Biochem. 36: 749–756.
- Keeney D.R. and Nelson D.W. 1982. Nitrogen inorganic forms. In: Page A.L. (ed., Methods of Soil Analysis, Part 2. Am. Soc. Agronomy Madison, Wisconsin, USA, pp. 643–698.
- Korsaeth A., Bakken L. and Riley H. 2003. Nitrogen dynamics of grass as affected by N input regimes, soil texture and climate: lysimeter measurements and simulations. Nutr. Cycl. Agroecosyst. 66: 181–199.
- Kyvsgaard P., Sørensen P., Møller E. and Magid J. 2000. Nitrogen mineralization from sheep faeces can be predicted from the apparent digestibility of the feed. Nutr. Cycl. Agroecosyst. 57: 207–214.
- Lampe C. 2004. Effect of nitrogen fertiliser and animal experiments on N₂O emissions from permanent grassland using ¹⁵N-labelling. Ph.D. thesis, University of Kiel, Germany
- Ledgard S.F., Steele K.W. and Feyter C. 1988. The influence of time of application on the fate of ¹⁵N-labelled urea applied to diary pasture. New Zeal. J. Agric. Res. 31: 87–91.
- Loiseau P., Carrère P., Lafarge M., Delpy R. and Dublanchet J. 2001. Effect of soil-N and urine-N on nitrate leaching under pure grass, pure clover and mixed grass/clover swards. Eur. J. Agron. 14: 113–121.
- Monaghan R.M. and Barraclough D. 1992. Some chemical and physical factors affecting the rate and dynamics of nitrification in urine-affected soil. Plant Soil 143: 11–18.
- O'Connor K.F. 1974. Nitrogen in agrobiosystems and is environmental significance. New Zeal. Agric. Sci. 8: 137–148.
- SAS Institute 1995. User Guide. Release 6.11. SAS Institute, Cary, NC, USA.
- Shand C.A., Williams B.L., Smith S. and Young M.E. 2000. Temporal changes in C, P and N concentrations in soil solution following application of synthetic sheep urine to soil under grass. Plant Soil 222: 1–13.
- Siemens J. and Kaupenjohann M. 2002. Contribution of dissolved organic nitrogen to N leaching from four German agricultural soils. J. Plant Nutr. Soil Sci. 165: 675–681.
- Sørensen P., Jensen E.S. and Nielsen N.E. 1994a. Labelling of animal manure with ¹⁵N. Plant Soil 162: 31–37.
- Sørensen P., Jensen E.S. and Nielsen N.E. 1994b. The fate of ¹⁵N-labelled organic nitrogen in sheep manure applied to

soils of different texture under field conditions. Plant Soil 162: 39–47.

- Sørensen P. and Jensen E.S. 1995. Mineralization and immobilization and plant uptake of nitrogen. Plant Soil 173: 283–291.
- Stout W.L. 2003. Effect of urine volume on nitrate leaching in the northeast USA. Nutr. cycl. Agroecosyst. 67: 197–203.
- Takahashi S., Ueno H. and Yamamuro S. 2004. Comparison of nitrogen mineralization from 15N-labeled organic amendments under flooded and upland conditions. Plant Soil 259: 307–314.
- Thompson R.B. and Fillery I.R.P. 1997. Transformation in soil and turnover to wheat of nitrogen from components of grazed pasture in the south of Western Australia. Aust. J. Agric. Res. 48: 1033–1047.
- Trott H.L. 2003.Medium-term effects of defoliation system and N intensity on performance and N balances of permanent grassland. Ph.D. thesis, University of Kiel, Germany.

- Vinther F.P. 1998. Biological nitrogen fixation in grass-clover affected by animal excreta. Plant Soil 203: 207–215.
- Wachendorf M., Büchter M., Trott H. and Taube F. 2004. Performance and environmental effects of forage production on sandy soils. II. Impact of defoliation system and nitrogen input on nitrate leaching losses. Grass Forage Sci. 59: 56–68.
- Watson C.J., Jordan C., Lennox S.D., Smith R.V. and Steen R.W.J. 2000. Inorganic nitrogen in drainage water from grazed grassland in Northern Ireland. J. Environ. Qual. 29: 225–232.
- Williams B.L., Shand C.A., Sellers S. and Young M.E. 1999. Impact of synthetic sheep urine on N and P in two pastures in the Scottish uplands. Plant Soil. 214: 93–103.
- Whitehead D.C. 1995. Grassland Nitrogen. CAB international, Wallingford.
- Whitehead D.C. and Bristow A.W. 1990. Transformations of nitrogen following the application of ¹⁵N-labelled cattle urine to an established grass sward. J. Appl. Ecol. 27: 667–678.

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