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# The fate of $^{15}\text{N}$ from dairy cow urine under pasture receiving different rates of N fertiliser

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**Abstract** —  $^{15}\text{N}$ -labelled urine was used to study the fate of N applied as urine in spring, summer and autumn on ryegrass receiving two different fertiliser rates (100 and 300 kg N·ha<sup>-1</sup>·yr<sup>-1</sup>) in Brittany (France). Ammonia volatilisation of labelled urine N was less than 4% and immobilised urine N represented 21–31% of the applied  $^{15}\text{N}$ . Recovery of labelled N in plants ranged from 30 to 65% of N input as urine, showing a decrease in autumn for the highest fertiliser nitrogen treatment. The plant recovery of urine N was relatively high in autumn (49%) when urine was deposited on ryegrass receiving a fertiliser rate of 100 kg N·ha<sup>-1</sup>·yr<sup>-1</sup>. Lower N fertiliser regimes can therefore lower the leaching potential of urine N in pastures.

urine / grassland / fertiliser rate / leaching /  $^{15}\text{N}$

**Résumé** – Devenir de l'azote des pissats de vache émis sur une prairie recevant différents niveaux de fertilisants azotés. De l'urine de vache laitière marquée au  $^{15}\text{N}$  a été utilisée pour étudier le devenir de l'azote urinaire apporté au printemps, en été et à l'automne sur un ray-grass anglais fertilisé à 2 doses d'azote (100 et 300 kg N·ha<sup>-1</sup>·an<sup>-1</sup>). La volatilisation d'ammoniac est toujours inférieure à 4 % de l'azote apporté et l'azote immobilisé dans le sol sous forme organique représente 21 à 31 % de l'apport. Le coefficient réel d'utilisation de l'azote par les plantes varie de 30 à 65 %, diminuant à l'automne pour le traitement 300. Le CRU de l'apport d'automne demeure cependant relativement élevé pour la prairie ne recevant que 100 kg N·ha<sup>-1</sup>·an<sup>-1</sup> (49 %). Cela peut conduire à un risque de lessivage de l'azote urinaire plus faible dans les régimes de fertilisation modérée.

pissat / prairie pâturée / fertilisation / lessivage /  $^{15}\text{N}$

## 1. INTRODUCTION

Increased concentration of nitrate N in groundwater is a major concern in Brittany (Western France). Leaching of nitrate N into ground and surface waters leads to eutrophication of watercourses and contamination of drinking water. Dairy farming is the most important farming system in Brittany, both on an area basis (40% of agricultural area is grazed grasslands [7]) and for animal N loading (51% of total animal N in Brittany is due to cattle farming [14]). Thus, the risk of pollution of groundwater via nitrate leaching under grazed pastures must be minimised. Various authors [30, 31] have reported rates of leaching between 160 and 300 kg N·ha<sup>-1</sup>·yr<sup>-1</sup> under grazed grasslands receiving 420 kg N·ha<sup>-1</sup>·yr<sup>-1</sup> in a mineral form. Simon et al. [33] found a relationship between the stocking rate

and the amount of N leached under pastures, but the large variability observed in leaching data was not fully explained.

Three factors can explain the large variability observed for leaching: (i) increased fertiliser application allows both higher stocking rates and N concentration in plants; consequently, higher amounts of N in urine and dung are returned to the pasture; (ii) management of fertiliser inputs and grazing, and (iii) differences in soil types and hydrological conditions. The first two points are characteristic of grazed systems where large quantities of N are spatially unevenly distributed, potentially leading to major N losses.

Urine, containing N mainly in urea form, is excreted as a concentrated solution over a small area (from 2 to 21.6 g N·L<sup>-1</sup>, mean 6 g N·L<sup>-1</sup> on 0.45 m<sup>2</sup> [4, 27]). This results in a N loading rate, under a urine patch, ranging from 30 to 120 g N·m<sup>-2</sup> [40,

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41], which is far beyond the immediate requirements of pasture plants. Thus, urine patches are thought to be a significant source of nitrate N leaching [3, 8, 38].

After deposition, urea hydrolysis occurs within 24 h [10]. Nitrogen uptake by the plant and immobilisation in the soil are the primary mechanisms by which urine N is retained. Increased grass growth on urine patches normally lasts for 2–4 months and has been observed even following high rates of N fertiliser application [11, 25]. Up to 47% of the N deposited as urine can be taken up by aerial parts of plants, while up to 6% has been found in roots [2, 15, 16, 38]. The soil microbial biomass can immobilise 13 to 34% of the N added as urine in grassland soil [8, 36, 38].

Gaseous losses are variable. The proportion of the urine N volatilised as ammonia ranges from 1 to 52% [8, 28, 32, 39], depending on the weather following the deposition: dry, hot and windy conditions increase volatilisation, while a 10 mm irrigation just after application suppresses it (Black et al., 1987 in [8], [12]). Chemical and biological denitrification can also occur. Reported values vary from 0 to nearly 30% of deposited N. Inputs of urine N in summer lead to denitrification rates lower than 7%, and are often zero [5, 17, 22, 26, 42, 43]. During winter and spring, urine N deposition can lead to higher denitrification rates, especially in wet or water-logged soils [12, 26]. Fraser et al. [12] estimated that 28% of the urine N input is denitrified in winter on irrigated soils (irrigation immediately after application, then water input equivalent to twice the evapotranspiration over one year). After urine deposition in winter on four soil types, Clough et al. [8] measured  $N_2O$  emission levels that were lower than 2% of the urine N input, but by applying the  $N_2-N/N_2O-N$  ratio they measured in a parallel experiment, they concluded that 25% urine N had been denitrified, mainly in the  $N_2$  form. Nevertheless, this experiment can lead to an overestimation of the denitrification because  $N_2O$  fluxes in the second experiment (where  $N_2-N/N_2O-N$  ratio was measured) were much lower than in the first one (< 0.33% vs. 0.8 to 1.9% of the N applied).

To study the consequences of urine N patches on N losses in grazed pasture, we investigated the fate of urine N applied at three different times of the year on ryegrass pasture. Dairy-cow urine was spiked with  $^{15}N$ -labelled urea and its fate tracked in grassland micro-plots. Few experiments have studied the variability of the fate of urine N as a function of fertiliser N inputs; hence, we used pasture receiving either 100 or 300 kg  $N \cdot ha^{-1} \cdot yr^{-1}$  as inorganic N.

## 2. MATERIALS AND METHODS

### 2.1. Site

The study was carried out at the experimental farm of INRA near Rennes in Western France (48°7'3"N, 1°47'6"W), under temperate oceanic conditions. The average annual temperature and rainfall are 11.4 °C and 700 mm, respectively. The experimental field is nearly flat with a well-drained silt loam soil developed on clayey-stony alluvial deposits. It is classified as a redoxic loam neoluvisoil (13.8% clay, 72.3% silt and

13.9% sand with 2.1% organic matter and a total CEC of 6.6  $Cmol^+ \cdot kg^{-1}$  in the 0–30 cm layer).

### 2.2. Experimental approach

To monitor the nitrogen transformation and cycling under urine patches, 3 L of dairy-cow urine spiked with  $^{15}N$ -labelled urea were applied in spring (22/03/99 – day 1), summer (30/05/99 – day 69) and autumn (20/09/99 – day 182) in the centre of 3 series of micro-plots (3 m<sup>2</sup>, one series per application date). These micro-plots were randomly located on two ryegrass swards (*Lolium perenne*, Belfort variety) that had received in 1999 either 100 (RG100) or 300 (RG300) kg  $N \cdot ha^{-1} \cdot yr^{-1}$  as ammonium nitrate (33.5% N) on three dates (04/05/99: 25 or 100; 08/06: 25 or 100; 30/06: 50 or 100 kg  $N \cdot ha^{-1}$ , respectively, for RG100 and RG300). Ryegrass had been established in autumn 1997 and had received a homogeneous fertiliser rate in 1998 (100 kg  $N \cdot ha^{-1} \cdot yr^{-1}$ ). Just before each urine application, the sward was cut to 2 cm above soil level. For each application date, 3 replicate micro-plots received urine and 1 control micro-plot received 3 L of de-ionised water, to analyse separately the effects of water and nitrogen inputs.

Ammonia volatilisation was measured immediately after application. Total N and  $^{15}N$  present in foliage components were determined regularly between application and final measurements. All micro-plots were destructively sampled on 06/03/2000 (168, 280 and 350 days after urine application) to measure total N and  $^{15}N$  in roots and soil. The  $^{15}N$  budget was compiled for each micro-plot and the unaccounted-for  $^{15}N$  was assumed to be due to losses by leaching or denitrification, to measurement errors, or other losses such as entrapped gases in the soil pore space (Clough et al., [9]). As urine was applied in spring, summer or autumn on a well-drained and well-structured soil, without any compacted layer or hydromorphic features, we assumed that denitrification was low. As measurement errors were also assumed to be low, the unaccounted-for  $^{15}N$  was considered to be the potential leaching loss.

Lombaert [21] and Lantinga et al. [18] found that an area of up to 0.7–1.5 m<sup>2</sup> of soil and plants could be affected by urine deposition. Based on these results, plants and soil were sampled from a 2-m<sup>2</sup> circle centred on the urine patch. We checked that no  $^{15}N$  was present in herbage or soil outside the sampling area by measuring the decrease in  $^{15}N$  concentration in plants and soil from the centre of the patch to the rim of the sampling circle. In a few cases, it could be shown that a small amount of urine N was outside the sampling area. These data were corrected by attributing the  $^{15}N$  concentration measured at the rim of the sampling circle to the area with urine N initially unsampled. When the correction exceeded 5% of the total  $^{15}N$ , that is to say, when 5% of the urine was out of the sampled area, the data was suppressed.

### 2.3. Urine collection and application

Fresh cow urine was collected from Prim'Holstein animals fed with a low-N diet in order to obtain low N concentrations in the urine. Urine was kept at –18 °C and defrosted just before application at 4 °C. The urea N content of the urine was determined 1 day before application to ascertain the amount of

**Table I.** Total N concentrations ( $\text{g N}\cdot\text{L}^{-1}$ ) of urine, at.%  $^{15}\text{N}$  and total urine N quantity applied ( $\text{g}\cdot\text{patch}^{-1}$ ) for each treatment (mean and standard error of three micro-plot replicates).

		Urine application					
		Spring (22/03/1999)		Summer (31/05/1999)		Autumn (20/09/1999)	
		Mean	SE	Mean	SE	Mean	SE
RG100	Total N concentration	6.25	0.23	7.04	0.52	7.39	0.31
	At.% $^{15}\text{N}$	0.97	0.02	0.89	0.06	0.88	0.004
	Total urine N	18.76	0.68	21.13	1.57	22.16	0.94
RG300	Total N concentration	6.13	0.63	6.23	0.38	7.50	0.16
	At.% $^{15}\text{N}$	1.02	0.09	0.99	0.07	0.87	0.01
	Total urine N	18.40	1.89	18.69	1.13	22.51	0.48

N urea and  $^{15}\text{N}$  urea required to yield a concentration of about  $6\text{ g total N}\cdot\text{L}^{-1}$  and enrichments of approximately 1 at.%  $^{15}\text{N}$ . At the field site, 3 L of urine were applied through a PVC funnel on a tripod (1.2 m tall) placed centrally in each micro-plot. The N applied ranged from 18.3 to  $22.5\text{ g N}\cdot\text{patch}^{-1}$  (Tab. I). The N concentrations and the  $^{15}\text{N}$  contents of the urine applied are also given in Table I.

#### 2.4. Meteorological data

Temperatures were recorded hourly in the air (2 m) and at the soil surface (0.01 m above soil level). Rainfall, air relative humidity, global radiation and wind velocity were measured at 2 m above the soil surface. All micrometeorological data were recorded every minute and averaged over an hour interval with a data logger (21X or CR10X, Campbell Scientific, Shepshed, UK). Potential evapotranspiration data was recorded at an official meteorological station located 1 km from the field site. These data allowed us to run a volatilisation model [23, 24] used to integrate the instantaneous fluxes of measured ammonia volatilisation.

#### 2.5. Ammonia volatilisation

Two replicate micro-plots of RG300 per urine application date received an apparatus designed to measure  $\text{NH}_3$  volatilisation sporadically over the first 100 hours after each urine application.

The  $\text{NH}_3$  trapping system used was described by Ball et al. [1]. It consisted of a square steel volatilisation chamber (50 cm sides) equipped with an electrical air mixing fan and sealed onto a base (5 cm in height) inserted at approximately 5 cm depth in the soil in the centre of the plot. Ambient air was drawn through the chamber via polyethylene tubing from an inlet placed on a 2-m mast. Three holes drilled opposite to each other in the chamber wall formed the air inlet (1 hole) and outlet (2 holes). The air was drawn through a vacuum pump and a total of about  $22\text{ L}\cdot\text{min}^{-1}$  passed through the chamber. Out of the total pumped volume,  $2.1\text{ L}\cdot\text{min}^{-1}$  passed through an acid trap. The flux was measured by a commercial gas flow meter placed in the line. The acid traps are made up of glass bubblers fitted to glass tubes containing 50 ml of 0.2N  $\text{H}_2\text{SO}_4$ . Ambient air  $\text{NH}_3$  was also determined by monitoring a line directly from the air inlet mast. The chamber was moved after one hour of measurement to avoid perturbations on the sward.

Ammonia was systematically analysed twice by colorimetry [37] and the background values of  $\text{NH}_3$  at 2 m height were subtracted from the chamber values for each period. Data from analytical replicates were averaged.

#### 2.6. Plant N uptake

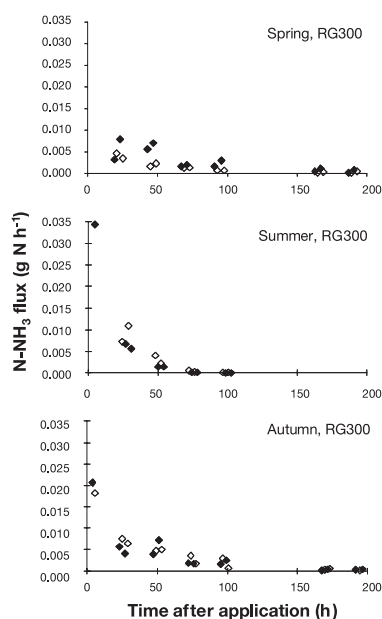
Between the spring application of urine (22/03/99) and the end of the experiment (06/03/00), seven cuts were made at 2 cm height using a micro-mower on 14th April, 27th May, 28th June, 26th July, 14th September, 18th October and 23rd November. Plant samples were bagged, weighed, dried at  $60^\circ\text{C}$  and then re-weighed at each sampling date.

The final harvest was carried out on day 350 (06/03/00) and involved collecting roots and cutting plant shoots to ground level. Roots were separated as much as possible from the upper 12.5 cm layer of the soil, and then washed, dried at  $60^\circ\text{C}$  and weighed. This layer contained most of the root mass, as only a few of the roots penetrated below 12.5 cm depth. All the above-ground components and roots from each micro-plot were finely ground and sealed in tin capsules for analysis. Total N in shoots and roots was analysed by the Kjeldahl method and  $^{15}\text{N}$  contents were determined by mass spectrometry [29]. All  $^{15}\text{N}$  contents were corrected to the normal value for atmospheric  $\text{N}_2$  of 0.3663 at.%  $^{15}\text{N}$ .

In this paper, we present the total amount of  $^{15}\text{N}$  taken up by ryegrass (partitioned between aerial parts and roots) or remaining in the soil, as well as the total accumulation of aerial dry matter by the plant between the date of urine application and the end of the experiment in March 2000, and the dry matter weight of the roots at the end of the experiment.

#### 2.7. Soil sampling and statistical analysis

After the final harvest and removal of roots, 26 soil cores were taken from each of the  $2\text{-m}^2$  micro-plots using a 3.5 cm diameter cylindrical gauge and separated into 4 layers (0–20 cm, 20–30 cm, 30–50 cm and 50–100 cm). The samples were bulked to form a composite sample by layer and immediately placed in a cool room. Each sample of soil was mixed thoroughly and sub-samples were taken for mineral and organic N,  $^{15}\text{N}$  and water content determinations. A 1M KCl solution was used to extract mineral N by shaking samples of soil (200 ml KCl for 100 g of soil). Mineral N content (ammonium and nitrate) in the filtrates was analysed by colorimetry [37].



**Figure 1.** Measured ammonia fluxes ( $\text{g N}\cdot\text{h}^{-1}$ ) after each urine application on 300 N ryegrass (open and closed symbols for 2 micro-plot replicates per application).

Organic N contents were determined by total N analysis on the residue (Kjeldahl method). Mineral and organic  $^{15}\text{N}$  contents were determined by mass spectrometry [29].

Each elementary sample was analysed systematically two times for mineral N determination, then the results were averaged. Only one analysis was performed for organic N and  $^{15}\text{N}$ . Volatilisation was measured on two field micro-plots on 300N ryegrass. All labelled urine N, aerial parts and root growth data came from three replicate micro-plots, leading to samples analysed separately or mixed (100N ryegrass for all data; 300N ryegrass for roots and soil determinations from summer urine application).

For data with field replicates, analyses of variance and comparisons of means (Newman – Keuls test at the 5% level of confidence) were performed using the urine application date as factor.

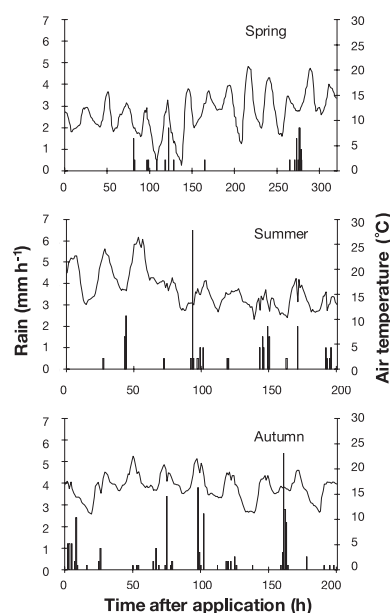
## 2.8. Urine N recovery in soil or plants

Labelled urine N recovered (in soil or in plants) was calculated by

$$\text{Labelled N (\% of input)} = 100 \left( \frac{N(E - E_0)}{N_u - E_u} \right)$$

where:

- $N$  is the quantity of nitrogen in soil (organic or  $\text{NO}_3^-$  or  $\text{NH}_4^+$ ) or in plants (aerial parts and roots) for the considered patch ( $2 \text{ m}^2$ ) on 1 m depth.
- $E$  and  $E_0$  are the  $^{15}\text{N}$  enrichments measured for the soil or the plants, respectively, with and without urine applied; on average,  $E_0$  is equal to 0.0025% at.%  $^{15}\text{N}$ .
- $N_u$  is the quantity of urine N applied to the patch.
- $E_u$  is the  $^{15}\text{N}$  enrichment of applied urine.



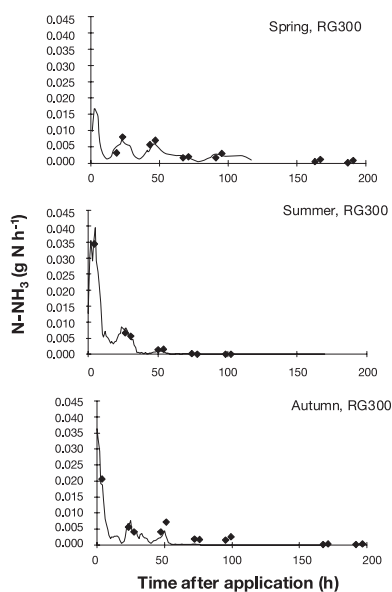
**Figure 2.** Rain and air temperature following each urine application.

Enrichments are all calculated by  $E = A - A_a$  where:  $A$  and  $A_a$  are the  $^{15}\text{N}$  abundance, respectively, for the sample and for the air with  $A = 100 \frac{^{15}\text{N}}{^{14}\text{N} - ^{15}\text{N}}$  and  $A_a = 0.3663 \text{ at.}\% ^{15}\text{N}$ .

## 3. RESULTS AND DISCUSSION

### 3.1. Urine N volatilisation

In all the experiments, nearly all measured volatilisation losses occurred within the first two days following application of the urine (Fig. 1) which is consistent with reported results [20]. We explain the variations of volatilisation between application dates by differences in rainfall and temperature (Fig. 2). To test this hypothesis, we used a dynamic volatilisation model known as STAL [23, 24]. This model takes into account the effects of air temperature, wind velocity and rainfall to simulate hourly  $\text{NH}_3$  fluxes volatilised from liquid wastes. It has been validated by aerodynamic measurements [13] and nitrogen mass budgets [23]. Rainfall acts mainly by accelerating the N infiltration into the soil, preventing volatilisation. Temperature increases the rates of the biotransformations of N which compete with the volatilisation (N uptake by plants and nitrification of ammonium). Figure 3 shows the measured volatilisation fluxes; their simulation by the STAL model, calibrated to these measured results, allowed us to estimate the total quantity of volatilised N by integration over time. For the spring application, the relatively low temperature ( $<10^\circ\text{C}$  during the first 24 h after application) explained the low measured fluxes. The summer application with high temperature and without rain at the moment of application resulted in a higher rate of volatilisation. For the autumn application, the rain



**Figure 3.** Ammonia fluxes ( $\text{g N} \cdot \text{h}^{-1}$ ) measured ( $\blacklozenge$ ) and simulated by STAL (—) after spring, summer or autumn applications of urine on 300N ryegrass.

immediately after the urine application explained the low level of volatilisation.

Total volatilised N for each application is given in Table II as a percentage of N applied as urine, corrected for the  $\text{NH}_3$  in ambient air. Over the three applications, it ranged from 1.0 to 2.6% of the applied N. These results are lower than most of the results reported in the literature. For example, Thompson and Fillery [36] found that 10–30% of the urine N was volatilised as ammonia when cattle urine was applied to the surface of grass swards in winter and spring, respectively. However, our results agree with Clough et al. [8], who irrigated the plots immediately after urine application to prevent ammonia volatilisation. We have a similar case for the autumn application, when rain fell immediately after application (Fig. 2), but not for the summer application.

By using STAL to integrate data over time instead of statistical fitting, we estimated the cumulated quantity of volatilised  $\text{NH}_3$  better, because we included the lowering effects of night temperature and low potential evapotranspiration.

These results suggest that the volatilisation of urine N does not represent an important flux under the temperate climate of

**Table II.** Volatilised urine N after each application estimated with the STAL model (% of N applied) (mean and standard error of two micro-plot replicates). Values with different letters are statistically different at the 5% level of significance (Newman-Keuls test).

Urine application					
Spring		Summer		Autumn	
Mean	SE	Mean	SE	Mean	SE
1.30 <sup>a</sup>	0.49	2.60 <sup>b</sup>	0.51	1.62 <sup>a</sup>	0.04

Brittany, where high temperature is exceptional even on sunny days in summer. Moreover, the high probability of rain soon after urine application in spring and autumn, causing faster infiltration of urine N into the soil, will minimise the volatilisation losses.

### 3.2. Plant nitrogen uptake

From the spring application, the  $^{15}\text{N}$  recovery in aerial parts of the plants accounted for up to 62% of the applied  $^{15}\text{N}$  (Tab. III). The total N present in root material has been rarely reported. In our study, up to 12.8% of the applied urine N was stored in roots for the autumn application (Tab. IV).

The  $^{15}\text{N}$  recovery in aerial parts of the grass was lower when the urine was applied in autumn (21.4 and 35.9% for RG300 and RG100, respectively). The shorter period between the autumn application and sampling (168 days vs. 280 and 350 in summer and spring) and the lower crop growth rate during autumn and winter led to a lower dry matter accumulation, which resulted in a lower  $^{15}\text{N}$  recovery, as shown in Figure 4.

Figure 5 shows a negative relationship between aerial growth and urine N recovery in the roots: the lower the aerial growth, the higher the urine N recovery in the roots. The shorter time to metabolise and transfer N towards aerial parts can partly explain this result, but the relationship between root density and N recovery observed in the roots (Fig. 6) can also corroborate and help to explain this result. We found that the root density was maximal for RG100 at the last urine application.

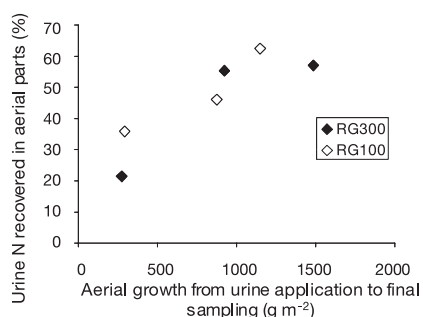
This is consistent with the finding that under low conditions of mineral nutrition, root growth is favoured relative to aerial growth [6, 19, 34]. Figure 7 shows that the ratio of root density/accumulated dry matter during the experiment is rather constant for all urine applications on RG300 and spring and summer applications on RG100, but is double for the autumn

**Table III.** Labelled urine N in above-ground parts of the sward (% of N applied) (mean and standard error of three micro-plot replicates). Values with different letters are statistically different at the 5% level of significance.

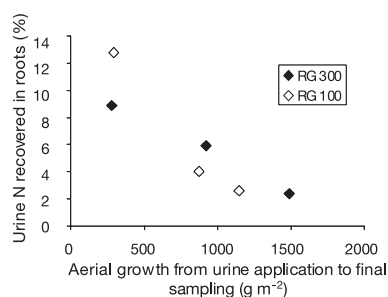
Days between application and harvest	Urine application					
	Spring		Summer		Autumn	
	350		280		168	
	Mean	SE	Mean	SE	Mean	SE
RG100	62.4 <sup>a</sup>	18.6	46.1 <sup>a</sup>	6.9	35.9 <sup>b</sup>	3.7
RG300	57.0 <sup>a</sup>	6.5	55.3 <sup>a</sup>	11.2	21.4 <sup>c</sup>	4.0

**Table IV.** Labelled urine N recovered in roots (% of N applied). Each value with SE is the mean of three micro-plot replicates, while the others come from composite samples.

Days between application and harvest	Urine application					
	Spring		Summer		Autumn	
	Mean	SE	Mean	SE	Mean	SE
RG100	2.6	-	4.0	-	12.8	-
RG300	2.4	0.7	5.9	-	8.9	3.3



**Figure 4.** Proportion of applied urine N in aerial parts of plants in relation to the aerial growth between urine application and final sampling.

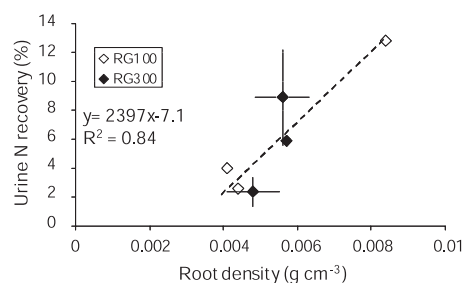


**Figure 5.** Proportion of applied urine N in roots in relation to the aerial growth between urine application and final sampling.

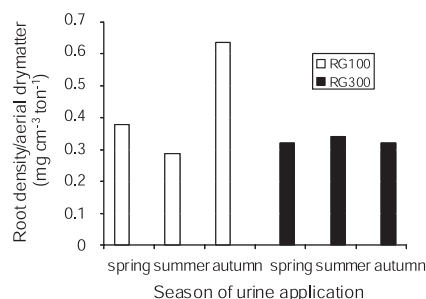
application on RG100. Thus, urine N recovery in the whole plant decreases for autumn application but the effect is less in the pasture with lower nitrogen availability (RG100) due to the higher root/shoot ratio, and consequently higher N recovery in the roots.

### 3.3. Nitrogen in soil

The amounts of labelled <sup>15</sup>N recovered in the soil at the end of the experiment are given in Table V. At the end of the experiment, i.e. from 168 to 350 days after application, 23–30.5% of the applied urine N was recovered in soil organic matter and very low soil mineral N concentrations were observed at the end of the experiment. Some of the <sup>15</sup>N recovered in the organic N fraction may have been previously absorbed by the pasture plants and returned to the soil following death of tissues, in particular through turnover of roots.



**Figure 6.** Urine N recovery in roots in relation to the root density (g dry matter·cm<sup>-3</sup>) with error bars (SE from 3 micro-plot replicates for RG300 spring and autumn urine applications).



**Figure 7.** Ratio of root density on aerial dry matter for each urine application (mg·cm<sup>-3</sup> of soil for 1000 kg of dry matter).

These recovery rates are consistent with other reported values. Clough et al. [8] found that 21–24% of applied <sup>15</sup>N was in soil organic form 400 days after application. Thompson and Fillery [36] observed that 25–34% had been immobilised one year after application.

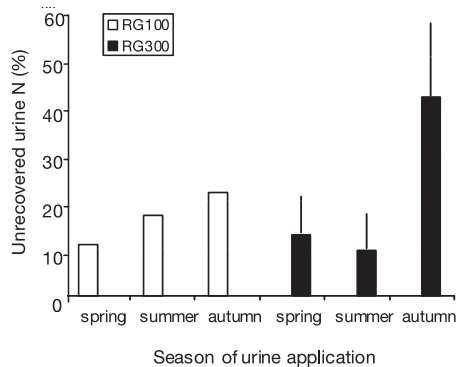
The absence of <sup>15</sup>N-mineral nitrogen in soils suggests that the ammonium and nitrate produced from the urine was all absorbed by plants, leached, or transformed into other forms.

### 3.4. Unaccounted-for <sup>15</sup>N

We assumed that losses of applied <sup>15</sup>N due to denitrification and due to measurement errors were low, so the <sup>15</sup>N that is unaccounted for provides an upper estimation of the potential amount of nitrate leached. Figure 8 presents the estimated N losses attributed to spring, summer and autumn applications

**Table V.** Labelled urine N in soil at the end of the experiment (% of N applied). Each value with SE is the mean of three micro-plot replicates, while the others come from mixed samples.

Days between application and harvest	Urine application						
	Spring		Summer		Autumn		
	350		280		168		
	Mean	SE	Mean	SE	Mean	SE	
RG100	N-NH <sub>4</sub> <sup>+</sup> (%)	0.0	-	0.0	-	0.1	-
	N-NO <sub>3</sub> <sup>-</sup> (%)	0.0	-	0.0	-	0.0	-
	N-organic (%)	23.0	-	30.5	-	28.0	-
	N total (%)	23.0	-	30.5	-	28.1	-
RG300	N-NH <sub>4</sub> <sup>+</sup> (%)	0.0	0.0	0.2	-	0.1	0.1
	N-NO <sub>3</sub> <sup>-</sup> (%)	0.1	0.0	0.1	-	1.7	1.7
	N-organic (%)	26.5	2.4	26.5	-	24.7	2.5
	N total (%)	26.6	2.5	26.8	-	26.5	4.8

**Figure 8.** Proportion of unrecovered urine N (assumed to be leached) (with top of histogram bar corresponding to mean value and error bars to SE from 3 micro-plot replicates).

at the end of the experiment. These losses range from 9 to 42% of applied N.

Under RG100, less than 22% of the N applied was lost. Under these conditions, immobilisation, plant uptake and root storage are important sinks for urine N.

Since a substantial proportion of the applied urine N was absorbed after spring and summer applications on RG300, the proportion of urine N available for leaching was generally small (9–13%). Late urine application with the 300 kg N·ha<sup>-1</sup>·yr<sup>-1</sup> treatment leads to an estimate of potential loss of 41.6% of the applied urine N, compared with 21.6% for urine N applied at the same periods with the 100 kg N·ha<sup>-1</sup>·yr<sup>-1</sup> treatment.

Under widely variable conditions, recoveries of applied <sup>15</sup>N-labelled urine N in the leachate from monolith lysimeters have been reported by authors to range from 8 to 50% of the <sup>15</sup>N input [12, 42]. The wide range reflects the variety of environmental and agronomic conditions encountered in experiments, particularly the fertiliser and water inputs. In our study, the date of application of urine interacts with the level of N fertiliser to determine the potential urine N leaching estimate. The mechanisms of N recovery by plants can explain a significant part of this variability.

#### 4. CONCLUSION

Livestock production systems can have negative impacts on water quality if not properly managed. The management of grazing and N fertiliser applications are the main practices to be considered by farmers. Many authors have suggested that animals should be removed from the pasture at the time of the year when NO<sub>3</sub> N derived from urine would be most subject to leaching [35]. Under moderately fertilised ryegrass (100 kg N·ha<sup>-1</sup>·yr<sup>-1</sup>), we show that the risk of urine N leaching is limited even if urine is applied late in the season. On the other hand, a higher level of N fertiliser appears to decrease the urine N uptake in the above-ground parts of the plant – and moreover in the roots – compared with when using low levels of N fertiliser, thus leading to higher risks of leaching when the urine N is applied at the end of the growth season. By explaining the interaction between the date of urine application and the fertiliser rate, the mechanism of autumn urine N recovery in roots appears to be highly relevant for understanding the overall N uptake in grassland as well as the fate of applied N.

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