Lack of increased availability of root-derived C may explain the low N_2O emission from low N-urine patches

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Abstract

Urine deposition on grassland causes significant N_2O losses, which in some cases may result from increased denitrification stimulated by labile compounds released from scorched plant roots. Two 12-day experiments were conducted in ^{13}C-labelled grassland monoliths to investigate the link between N_2O production and carbon mineralization following application of low rates of urine-N. Measurements of N_2O and CO_2 emissions from the monoliths as well as $\delta^{13}C$ signal of evolved CO_2 were done on day -4, -1, 0, 1, 2, 4, 5, 6 and 7 after application of urine corresponding to 3.1 and 5.5 g N m^{-2} in the first and second experiment, respectively. The $\delta^{13}C$ signal was also determined for soil organic matter, dissolved organic C and CO_2 evolved by microbial respiration. In addition, denitrifying enzyme activity (DEA) and nitrifying enzyme activity (NEA) were measured on day -1, 2 and 7 after the first urine application event. Urine did not affect DEA, whereas NEA was enhanced 2 days after urine application. In the first experiment, urine had no significant effect on the N_2O flux, which was generally low (-8 to 14 μg N_2O-N m^{-2} h^{-1}). After the second application event, the N_2O emission increased significantly to 87 μg N_2O-N m^{-2} h^{-1} and the N_2O emission factor for the added urine-N was 0.18 %. However, the associated ^{13}C signal of soil respiration was unaffected by urine. Consequently, the increased N_2O emission from the simulated low N-urine patches was not caused by enhanced denitrification stimulated by labile compounds released from scorched plant roots.

Keywords

^{13}C, denitrification, grassland, nitrification, nitrous oxide, root scorching, soil respiration, urine

Introduction

Urine deposited by grazing livestock is a major source of the nitrous oxide (N_2O) production in European grasslands. At present, N_2O emissions from agricultural soils account for 5 % of the European release of anthropogenic derived greenhouse gases (EEA 2002), and the main processes involved in the production are nitrification and denitrification (Firestone and Davidson 1989). The mechanism responsible for the increased N_2O emission following urine deposition is complex and not well understood. Vertès et al. (1997) found that 90 % of the urine patches deposited by grazing heifers contained between 3 and 50 g N m^{-2}. Urea (NH_2CONH_2) is the predominant component of urine and typically accounts for over 70 % of the urine-N content (Oenema et al. 1997). In the soil, urea is rapidly hydrolysed to NH_4^+, OH^- and HCO_3^-, which makes urea-N available for the nitrifying bacteria. However, recent studies revealed that urea-derived N only constituted a minor part of the N_2O-N emitted during the days after urine application (Bol et al. 2004; Clough et al. 2004). The major part of N_2O-N originated from other sources, e.g. from soil N. Nonetheless, studies have shown that the N_2O emission increases almost linearly with the amount of urine-N deposited (Van Groeningen et al. 2005a, b). Thus, the amount of urine-N appears to have an indirect effect on the rate of N_2O emission.

As heterotrophic bacteria play a major role in denitrification, the process is strongly dependent on the supply of easily decomposable organic matter, particularly in urine patches where N availability is expected to be non-limiting. Root scorching due to $NH_{3(aq)}$ formed after urea hydrolysis may result in release of labile carbon compounds into the rhizosphere (Shand et al. 2002). Monaghan and Barraclough (1993) suggested that these labile compounds stimulate denitrification activity and

thereby are part of the reason for the urine-induced N_2O emission. However, the degree of scorching depends on the amount of NH_3 formed (Ritchey et al. 2003), which is influenced by the amount of urea-N applied, soil pH and the cation exchange capacity of the soil (Bolan et al. 2004). The low N_2O emission from low N-urine patches may, in part, be caused by the lack of root scorching and thereby low availability of labile carbon compounds for the denitrifying bacteria.

In the present ^{13}C-labelling study, we examined the link between N_2O emission and carbon mineralization following urine application to soil under ^{13}C depleted grassland vegetation (*i.e.* grassland monoliths provided with depleted atmospheric CO_2 during 2 or 8 weeks). The artificial urine applied simulated a urine patch with low N content (3.1 or 5.5 g N m^{-2}). We tested the hypothesis that the low N_2O emission from low N-urine patches is caused by the lack of root scorching, and thus, the lack of increased availability of root-derived C for the denitrifying bacteria. Because the plant material was ^{13}C depleted in the grassland monoliths studied, our hypothesis implies that the N_2O emission should be paralleled by a constant $\delta^{13}C$ signal of CO_2 evolved by soil respiration.

Materials and methods

Grassland monoliths

The study was conducted in grassland monoliths placed in a ^{13}C-labelling facility at Institut National de la Recherche Agronomique (INRA), Clermont-Ferrand, France. The former management practise and the experimental facility were described in detail by Klumpp (2004). Briefly, in June 2002 the monoliths (50 cm × 50 cm × 40 cm deep) were taken from an intensively managed semi-natural grassland dominated

by perennial ryegrass (*Lolium perenne* L.), white clover (*Trifolium repens* L.) and Yorkshire fog grass (*Holcus lanatus* L.). The slightly acidic sandy soil contained 4.1 % C and 0.42 % N, and the pH_{H2O} was 6.6. Monoliths were placed in temperature controlled transparent enclosures kept under natural daylight. The enclosures were part of an open flow ^{13}C-labelling system, where ambient CO_2 was scrubbed and replaced by fossil fuel derived CO_2, which is depleted in ^{13}C. Starting 22 April 2003, plants were provided with CO_2 having a $\delta^{13}C$ signal of about -21.5 ‰. The external climate (PAR, temperature and humidity) and temperature of each enclosure was monitored continuously.

Urine treatment

To simulate grazing, the vegetation of six monoliths was cut to a height of 6 cm on 22 April and 9 June. Two weeks after the first cut (*viz*. on 7 May) and one week after the second cut (*viz*. on 16 June) urine was evenly applied on three of the monoliths using a watering can (Day 0). The three remaining monoliths were controls and received urine at the end of each experiment. The artificial urine was prepared using the recipe described by Doak (1952). The urine had a total N content of 0.7 g N l^{-1} and consisted of urea (1.12 g l^{-1}), hippuric acid (0.42 g l^{-1}), allantoin (0.18 g l^{-1}) and creatinine (0.09 g l^{-1}) and pH was adjusted to 7 with NaOH. Delta ^{13}C of each urine component was determined on an elemental analyser (EA1110, Carlo Erba, Milano, IT) coupled in continuous flow mode to an isotope ratio mass spectrometer (IRMS; FinniganMAT Delta plus, Bremen, DE). The amount of N applied via urine corresponded to 3.1 g N m^{-2} at the first application event and 5.5 g N m^{-2} at the second event. To keep soil moisture constant, the monoliths were irrigated every evening with a total amount of

119 108 and 128 mm water during the first and the second 12-day experiment,
120 respectively.
121
122 *Gas and soil sampling in monoliths*
123 About four weeks prior to urine application, small white chambers consisting of two
124 3-cm diameter PVC pipes (80 ml) and three 5-cm diameter PVC pipes (112 ml) were
125 installed in each monolith between the plants. Every pipe was connected to a three-
126 way sampling valve on the outside of the enclosure via a 75 cm silicon tube. The
127 sampling valve was fitted with a 5 ml syringe and a needle. Starting four days before
128 urine application (Day -4), below-ground production of N_2O and CO_2 as well as the
129 $\delta^{13}C$ of the evolved CO_2 were determined by use of the static chamber method.
130 Measurement of CO_2 emission was done between 11 am and 1 pm by briefly lifting
131 the enclosures to seal the 3-cm pipes with rubber stoppers for 40 minutes. One 2-ml
132 gas sample was collected via the external valves after 0, 20 and 40 minutes of
133 incubation. To measure N_2O emission, the 5-cm pipes were sealed by rubber stoppers
134 for 90 minutes between 1:30 and 4:30 pm. Two 2-ml gas samples were taken at the
135 beginning and at the end of the incubation period. A volume of N_2 equal to the sample
136 volume was added to the pipe before each gas sampling to maintain atmospheric
137 pressure. All samples were stored in 2-ml crimp-seal vials, which had been evacuated
138 before use. After penetration by a needle the vials were sealed with Terostat IX to
139 allow long-term storage. Determination of gas emissions from urine-treated and
140 control monoliths was done on day -4, -1, 0, 1, 2, 4, 5, 6 and 7 after urine application.
141 Furthermore, $\delta^{13}C$ of the evolved CO_2 was measured once before the labelling started.
142 Two days after urine application, two soil cores (0-10 cm depth, 2 cm diameter)
143 were collected in each monolith for chemical analyses and determination of microbial

144 respiration. Sampling holes were closed with cement filled PVC tubes to prevent
145 aeration of the soil and drainage of water. The two soil samples from each monolith
146 were pooled. Roots and stubbles were removed by tweezers during a period of one
147 hour per sample.
148
149 *Destructive harvest of monoliths*
150 To measure the $\delta^{13}C$ signal of unlabelled and ^{13}C-labelled plant material**,** four
151 monoliths were harvested on 5 May (unlabelled) and 10 June 2003 (^{13}C-labelled).
152 Root samples from the 0-10 cm soil layer were obtained by wet sieving of air-dried
153 soil slices (40 cm × 6 cm × 10 cm). Root samples and plant shoot samples were oven-
154 dried at 60 °C for 48 h, ground and analysed for $\delta^{13}C$ on the elemental analyser and
155 IRMS. On 5 May, samples of 40 g fresh 'root free' soil were obtained by sieving (2
156 mm) and removing roots by tweezers for 40 minutes per sample in order to measure
157 $\delta^{13}C$ of unlabelled soil C pools.
158
159 *Soil analyses*
160 Within 36 hours of soil sampling or destructive harvest, two 10 g portions of each
161 fresh 'root free' soil sample were extracted in 1 M KCl (1:5, w:vol), stirred on a
162 rotary shaker for one hour (only one portion on 5 May). The extracts were filtered
163 through Whatman 40 filters and kept at -20 °C until further analysis. Concentration of
164 ammonium and nitrate in the extracts were analysed colorimetrically on an
165 autoanalyzer (Bran+Luebbe, Norderstedt, DE). Dissolved organic carbon (DOC) in
166 the extracts was measured on a TOC/TN analyzer (Formacs, Skalar, Breda, NL). To
167 determine $\delta^{13}C$ of DOC, 10 ml of each extract was freeze-dried for 2 days, and the
168 solid residue was then analysed for $\delta^{13}C$ on the elemental analyser and IRMS.

To establish the $\delta^{13}C$ of CO_2 evolved by microbial respiration, 10 g portions of fresh 'root free' soil were incubated for 24 h at 25 °C in 250 ml screw capped serum bottles mounted with rubber stoppers. Empty bottles were included as controls. Gas samples for determination of CO_2 concentration and $\delta^{13}C$ of CO_2 were taken after 0 (ambient), 1, 3, 10 and 24 hours of incubation and stored in 2-ml vials. A volume of N_2 equal to the sample volume was added to the bottle before each gas sampling.

Soil pH was determined in a 10:25 (w:vol) suspension of fresh soil in distilled water (not soil from 5 May). The remaining of the 'root free' soil was air-dried and analysed for $\delta^{13}C$ on the elemental analyser and IRMS. In addition, total C and total N was measured on soil samples from 5 May.

Gas analyses

To measure N_2O concentrations, the vials were pressurized by adding 2 ml N_2 before analysis by gas chromatography (GC-14B, Shimadzu, Kyoto, JP). The samples for CO_2 determination were added 0.5 ml N_2 and the concentrations were established by gas chromatography (HP 6890, Agilent, Palo Alto, US). The $\delta^{13}C$ of CO_2 was determined following condensation in two successive cool traps (liquid N_2) and chromatographically separation of CO_2 on a trace gas preparation-concentration unit (PreCon FinniganMAT, Bremen, DE) coupled in continuous flow mode to the IRMS. Gas samples were analysed for CO_2, N_2O and $\delta^{13}C$ of CO_2 within 26, 36 and 61 days of sampling, respectively.

Denitrifying and nitrifying enzyme activities

To determine denitrifying and nitrifying enzyme activities, two soil cores (0-10 cm depth, 2 cm diameter) were collected in each monolith on day -1, 2 and 7 after the

194 first urine application. The two soil samples from each monolith were pooled and the
195 soil was sieved (2 mm).
196 Denitrifying enzyme activity (DEA) was measured on the fresh soil samples
197 over a short period according to Smith and Tiedje (1979) (for details, see Patra et al.
198 2005). Briefly, 10 g (equivalent oven-dried) soil was placed into 150 ml flasks, and
199 KNO_3 (200 µg NO_3-N g^{-1} dry soil), glucose (0.5 mg C g^{-1} dry soil) and glutamic acid
200 (0.5 mg C g^{-1} dry soil) was added. The atmosphere of each flask was evacuated and
201 replaced by a 90:10 He-C_2H_2 mixture. During incubation at 26 °C, gas samples (200
202 µl) were taken after 4 and 6 hours and immediately analysed for N_2O by
203 chromatography (Varian, STAR 3400 CX, Walnut Creek, US).
204 Nitrifying enzyme activity (NEA) was measured according to Lensi et al.
205 (1986). For each fresh soil sample, two sub samples (equivalent to 10 g oven-
206 dried) were placed in 150 ml flasks. One sub sample was used to estimate the
207 initial soil NO_3^- content. This sub sample was supplied with 6 ml of a suspension
208 containing a denitrifying organism (*Pseudomonas fluorescens*, O.D. 580 nm = 2)
209 in a solution of glucose and glutamic acid (final soil C concentration for each: 0.5
210 mg C g^{-1} dry soil). The atmosphere in the flask was replaced by a He-C_2H_2 mixture
211 (90-10) and N_2O accumulation was measured until soil NO_3^- was converted fully
212 to N_2O. The other sub sample was used to determine potential NO_3^- accumulation.
213 In this case, 4 ml of a $(NH_4)_2SO_4$ solution was added (final concentration 200 µg
214 N g^{-1} dry soil). Water was added to achieve 70 % water holding capacity. After
215 aerobic incubation (7 h at 26 °C), which allows nitrate to accumulate, the soil
216 samples were enriched with *Pseudomonas fluorescens* and incubated as described
217 above. Nitrous oxide was analysed on a Varian STAR 3400 gas chromatograph.
218

219 *Calculations and statistics*

220 Nitrifying enzyme activity was computed by subtracting the nitrate initially present in
221 the soil from that present after aerobic incubation. All results on $^{13}C/^{12}C$ ratios are
222 reported using the $\delta^{13}C$ notation, *i.e.*:

223 $$\delta^{13}C\ (‰) = 1000 \times (R_{sample} / R_{standard} - 1),$$

224 where $R = {}^{13}C/{}^{12}C$. Internal standards were used to check and correct for changes in
225 N_2O, CO_2 and $\delta^{13}C$ of CO_2 between time of sampling and analysis. The emission of
226 N_2O and CO_2 were calculated using linear regression and the $\delta^{13}C$ of the evolved CO_2
227 was established by Keeling plots (Keeling 1958). To simplify, $\delta^{13}C$ of CO_2 from soil
228 respiration and microbial respiration are referred to as $\delta^{13}C$ of soil respiration and
229 microbial respiration, respectively.

230 In general, the mean of the results obtained in each monolith was used, which
231 gives 3 replicates. Some data are reported as the overall mean ± standard error.
232 Analysis of variance (ANOVA), analysis of covariance (ANCOVA) and Tukey's
233 multiple comparison tests ($\alpha = 0.05$) were performed using SAS General Linear
234 Model procedure (SAS Institute 1997). Furthermore, ANCOVAs were performed
235 with SAS Mixed Model procedure on the repeated measurements of N_2O, CO_2 and
236 $\delta^{13}C$ of CO_2 using means of the measurements on day -4 and -1 before urine
237 application as covariate ($\delta^{13}C$ of CO_2 after the first application, covariate not
238 included). The ANCOVAs for CO_2 emission after the first application and N_2O
239 emission after the second application were performed on log transformed data.
240

241 **Results**

242

243 *Irrigation and temperature*

244 The distributions of the 108 and 128 mm water given in the first and the second
245 experiment, respectively, appear from Figure 1. Air temperature in the enclosures
246 during gas measurement ranged between 15 and 27 ºC in the first experiment (data not
247 shown). In the second experiment, the temperature was on average 12 ºC higher ($P < 0.0001$) and varied between 26 and 40 ºC. Air temperature did not differ between time
249 of CO_2 and N_2O measurements ($P = 0.14$).

250

251 *N_2O emission*

252 Homogeneity of variance was not obtained despite transformation when testing the
253 emission of N_2O after the first urine application. Thus, no statistical analysis was
254 performed on the N_2O data from the first experiment. Application of urine appeared to
255 have no significant effect on the N_2O flux from the grassland monoliths in the first
256 experiment (3.1 g N m^{-2}; Fig. 2 A). Overall the flux of N_2O was very low during the
257 first experiment, varying between -8 and 14 μg N_2O-N m^{-2} h^{-1}.

258 In contrast, urine application equivalent to 5.5 g N m^{-2} in the second experiment
259 had a significant effect on the N_2O emission ($P = 0.047$), which increased to 87 ± 57
260 μg N_2O-N m^{-2} h^{-1} (Fig. 2 B). The emission remained elevated for at least 8 days, but
261 declined gradually with time ($P = 0.049$).

262

263 *Respiration and $\delta^{13}C$ of respiration*

264 Urine application had a significant effect on the amount of CO_2 emitted from the
265 grassland monoliths in the two experiments ($P \leq 0.040$; Fig. 3). A peak in the CO_2

emission took place on the day of application, which was probably mainly caused by the hydrolysis of urea, resulting in formation of HCO_3^-.

Mean $\delta^{13}C$ of soil respiration determined in the grassland monoliths during the study was -28.5 ‰. No decline in $\delta^{13}C$ of soil respiration was observed following urine application (Fig. 4) and urine had no significant effect on the ^{13}C signal ($P \geq 0.16$). The CO_2 peak on the day of urine application that partly derived from hydrolysis of urea ($\delta^{13}C$ -34 ‰) did only affect the $\delta^{13}C$ of soil respiration in the first experiment, where $\delta^{13}C$ of CO_2 from the urine treatment dropped significantly below that of the control on the day of application (Fig. 4 A). The ^{13}C signal of soil respiration increased following days with high irrigation, *viz.* day -1 and 5 in the first experiment (Fig. 1, 4 A). On day 5 after the first application event, the CO_2 emission increased as well (Fig. 3 A).

The rate of microbial respiration measured on 'root free' soil samples in the laboratory did not change over the course of the study or between treatments ($P \geq 0.79$) and the mean rate was 3.4 ± 0.2 μg CO_2-C g^{-1} dry soil h^{-1} (data not shown). Delta ^{13}C of microbial respiration established on the 'root free' soil samples was stable during the study ($P = 0.38$; Fig. 5) and the urine treatment had no significant effect on the ^{13}C signal ($P = 0.72$).

Delta ^{13}C of other C pools

Delta $\delta^{13}C$ of plant shoot and root measured just before the second experiment revealed that the vegetation had been significantly labelled ($P < 0.0001$; Fig. 5). However, the shoot material was more depleted than the roots. Delta ^{13}C of DOC tended to decrease over the course of the study ($P = 0.078$; Fig. 5). Furthermore, $\delta^{13}C$ of soil organic matter (SOM) declined significantly during the period from the start of

291 labelling to the first experiment (P = 0.05). The urine treatment had no effect on $\delta^{13}C$
292 of DOC and SOM (P ≥ 0.22). In general, $\delta^{13}C$ of DOC differed from $\delta^{13}C$ of soil
293 respiration measured at the start of labelling and on day 2 after urine application (P =
294 0.05), whereas $\delta^{13}C$ of SOM and microbial respiration was rather similar (P > 0.05).
295
296 *Denitrifying and nitrifying enzyme activities*
297 Measurements of DEA (Fig. 6 A) and NEA (Fig. 6 B) revealed that the monoliths
298 used for the urine treatment and the control in the first experiment differed
299 significantly before urine was applied (P ≤ 0.020). This difference was accounted for
300 in the statistical analyses by including the measurements before urine application as
301 covariate. Urine had no effect on DEA when measured on day 2 and 7 after
302 application (P = 0.88). In contrast, NEA appeared to increase following urine
303 application. However, due to the number of replicates (two or three), the effect of
304 urine on NEA was not statistically significant (P = 0.17).
305
306 *Inorganic N, DOC and soil pH*
307 The content of soil inorganic N in the 0-10 cm soil layer measured on day 2 after
308 urine application did not differ between the two experiments (P = 0.76) and was
309 significantly higher in the urine treatment (1.46 g N m^{-2}) than in the control (0.49 g N
310 m^{-2}; P = 0.042) (data not shown). The increased level of inorganic N in the urine
311 treatment was almost exclusively caused by a rise in the NH_4^+ content. The NO_3^-
312 content was below the detection limit in the first experiment and had a mean value of
313 0.05 g N m^{-2} (0-10 cm soil layer) in the second experiment.
314 The content of DOC in the upper 0-10 cm of the soil was similar in the urine
315 treatment and the control (17.8 g C m^{-2}; P = 0.88). Despite urea hydrolysis, no pH

316 increase was observed in the urine treated soil when measured on day 2 after
317 application (P = 0.23; data not shown).
318
319 **Discussion**
320
321 *Increased N_2O emission after urine application*
322 According to the peaks in CO_2 emission (Fig. 3), urea hydrolysis was completed
323 within 24 hours, which is in line with results obtained in other studies (Petersen et al.
324 1998; Bol et al. 2004). In the first experiment in May, urine application corresponding
325 to 3.1 g N m^{-2} did not lead to a significant increase of the N_2O emission (Fig. 2 A). In
326 contrast, the N_2O emission increased significantly following urine application
327 equivalent to 5.5 g N m^{-2} in the second experiment in June (Fig. 2 B).
328 A possible reason for the larger N_2O emission in the second experiment
329 compared to the first could be the temperature, which was about 12 ºC higher in the
330 second experiment (mean 34 ºC). Christensen (1983) found that the Q_{10} value for N_2O
331 production in soil was 2-3. The emission on the day of urine application was 6-fold
332 higher in the second experiment than it was in the first, which suggests that the
333 difference in N_2O production between the two experiments was too big to be
334 accounted for by a temperature effect only. The different responses at the two
335 application events may be explained partly by the larger amount of N added in the
336 second experiment (5.5 vs. 3.1 g N m^{-2}). Furthermore, nitrifying bacteria may
337 compete with plants for NH_4^+ (Verhagen et al. 1995; Kaye and Hart 1997). Compared
338 to plant growth in May, the growth rate was reduced during the second experiment in
339 June. Thus, probably the plants left more inorganic N for the nitrifying and

denitrifying bacteria in the second experiment, which enabled increased N_2O production.

Maximum N_2O emission measured was 87 ± 57 μg N_2O-N m^{-2} h^{-1}. In comparison, Williamson and Jarvis (1997) measured emission of 600 μg N_2O-N m^{-2} h^{-1} after application of similar amounts of urine-N (6 g N m^{-2}) to a grassland on poorly drained silty clay loam in November. However, the sandy soil and moderate soil moisture in the present study offered less favourable conditions for N_2O production, which largely explains the lower emission. More generally, relative N_2O emission at peak emission date (*i.e.* N_2O emission expressed per unit of applied urine-N) computed from published data (Allen et al. 1996; Clough et al. 1996; Yamulki et al. 1998; Bol et al. 2004; Van Groenigen et al. 2005b) varied between 2 and 123 μg N_2O-N h^{-1} g^{-1} urine-N. The median of these observations (n = 15) is 17 μg N_2O-N h^{-1} g^{-1} urine-N, which is close to the relative N_2O emission of 16 μg N_2O-N h^{-1} g^{-1} urine-N observed in our study.

Assuming an N loss of 20 % due to NH_3 volatilization and nitric oxide (NO) emission (IPCC 1997), the N_2O emission factor for the added urine-N measured over the 8 days was 0.18 ± 0.08 %. In the study by Williamson and Jarvis (1997), where a similar amount of urine-N was applied, the N_2O emission factor measured over 37 days was 5 % (De Klein et al. 2001). According to the guidelines issued by the Intergovernmental Panel on Climate Change (IPCC 1997), the N_2O emission from urine deposited by grazing livestock should be calculated as 2 % of the N remaining after NH_3 volatilization and NO emission, which are assumed to account for 20 % of the total N content. However, a review of 10 field studies showed that median N_2O emission factor of real urine was 0.9 % (Van Groenigen et al. 2005a), and the present study supports a reduction of the IPCC default emission factor as well.

365

366 *Assessment of the possible link between N_2O production and availability of root-*
367 *derived C in urine patches*

368 Urine deposition by grazing livestock is known to trigger significant N_2O production,
369 but the mechanisms involved are very complex and not well understood. It has been
370 suggested that labile compounds released from scorched plant roots stimulate
371 denitrification activity, and thus is part of the reason for the increased N_2O emission
372 following urine deposition (Monaghan and Barraclough 1993). We propose that the
373 small increase in N_2O emission from low N-urine patches partly is caused by the lack
374 of root scorching and associated release of labile carbon compounds. The aim of the
375 present study was to test this hypothesis by assessing the source of CO_2 emitted
376 following application of a low rate of urine-N. If the increased N_2O emission were a
377 result of higher denitrifying activity due to a supply of labile compounds released
378 from scorched plant roots (more depleted than other soil C pools in the monoliths
379 studied), then $\delta^{13}C$ of soil respiration would be expected to decline after urine
380 application. In line with the hypothesis, the increased N_2O emission in the second
381 experiment was not related to increased mineralization of plant-derived C, *viz*. $\delta^{13}C$ of
382 soil respiration was unaffected by the urine application (Fig. 4 B). The plant material
383 may have been inadequately ^{13}C-labelled in order to trace plant-derived C in other C
384 pools. However, the result may indicate that no significant root scorching occurred
385 following urea hydrolysis. Lack of urine-effect on other soil C measures (*i.e.* $\delta^{13}C$ of
386 microbial respiration, soil content and $\delta^{13}C$ of DOC) supported that root scorching
387 was probably negligible. The urine compounds remaining after urea hydrolysis
388 (hippuric acid, creatinine and allantoin; $\delta^{13}C$ -26 ‰) did not affect the results on $\delta^{13}C$

389 of DOC because of their low amount (< 3 g C m^{-2} vs. 17.8 g DOC-C m^{-2} in the 0-10
390 cm soil layer).

391 More generally, the urine-induced rise in N_2O emission was not linked to an
392 increase of soil respiration. The same result appears from a study by Bol et al. (2004),
393 where urine was applied corresponding to 23 or 40 g urea-N m^{-2}. In contrast, Lovell
394 and Jarvis (1996) found that soil respiration increased significantly following
395 application of urine equivalent to about 20 g N m^{-2}.

396 Our results show that application of 5.5 g urine-N m^{-2} gave rise to a $NH_{3(aq)}$
397 concentration in the soil solution that did not cause significant scorching of the roots
398 and, thus, that root scorching could not be responsible for the urine-induced N_2O
399 emission from the simulated low N-urine patch. In contrast, a related study
400 demonstrated that application of a high rate of urine-N (50.9 g N m^{-2}) significantly
401 increased the mineralization of plant-derived C, possibly as a result of root damage
402 due to scorching (P. Ambus, personal communication).

403

404 *Alternative processes explaining the urine-induced N_2O emission*

405 The concentration of N in livestock urine may vary between 1 and 20 g N l^{-1} (Oenema
406 et al. 1997), thus the concentration used in the present study (0.7 g N l^{-1}) was in the
407 lower end of this range. A nitrogen concentration of urine above 16 g N l^{-1} leads to
408 microbial stress due to $NH_{3(aq)}$ and low osmotic potential, and thereby to inhibition of
409 nitrification (Monaghan and Barraclough 1992; Bol et al. 2004). The low urine-N
410 concentration in the present study means that nitrification most likely occurred, and
411 the process might play a major role in the increase in N_2O emission. This view is
412 supported by the apparent increase of NEA in the urine treated soil during the first
413 experiment (Fig. 6) and the presence of soil NO_3^- on day 2 after the second

414 application event. Hence, the elevated N_2O emission immediately following urine
415 application was probably caused by a rapid nitrification-denitrification turnover of
416 urea-derived N. This mechanism is different from that following application of higher
417 rates of urine-N, where nitrification is typically inhibited for a couple of days
418 (Monaghan and Barraclough 1992; Bol et al. 2004).

419 In conclusion, the increased N_2O emission following urine application at rates
420 up to 5.5 g N m^{-2} was not caused by enhanced denitrification stimulated by an
421 increased availability of labile plant compounds. Furthermore, strong competition for
422 inorganic N between plants and microorganisms combined with low urine-N rates
423 limited the N_2O loss from this semi-natural grassland.

424

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435

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Figure 1. Distribution of irrigation during the first and second experiment.

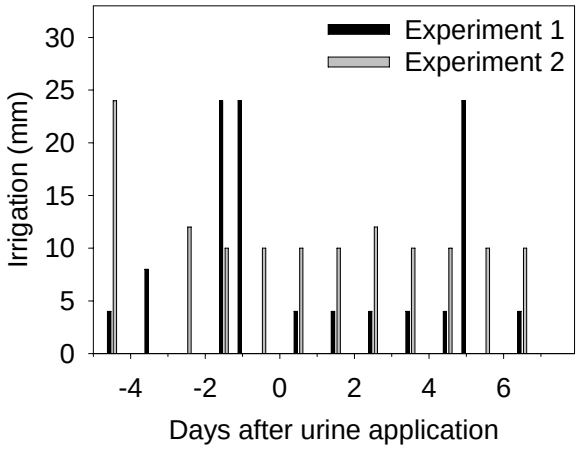
Figure 2. Emission of N_2O from the urine treatment and the control during (A) the first experiment and (B) the second experiment; n = 3; means ± SE.

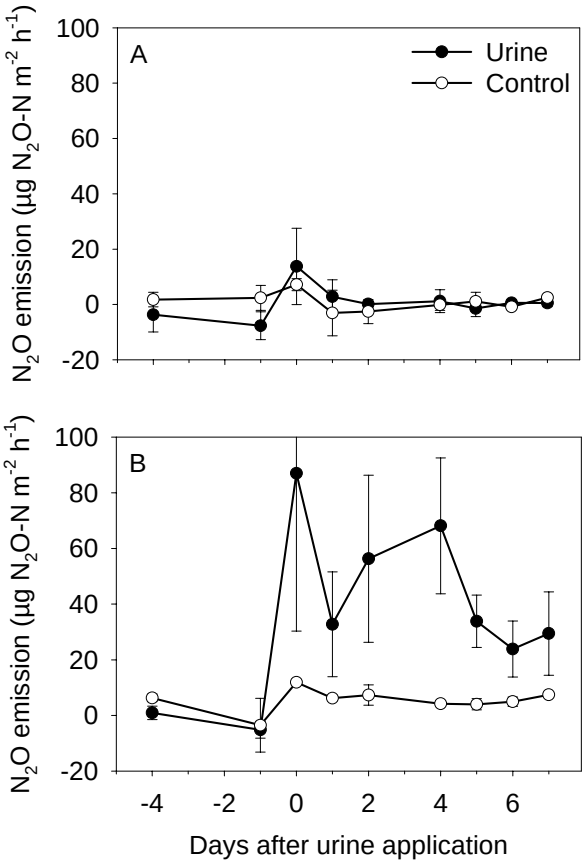
Figure 3. Emission of CO_2 from the urine treatment and the control during (A) the first experiment and (B) the second experiment; n = 3; means; the bars indicate the Minimum Significant Difference.

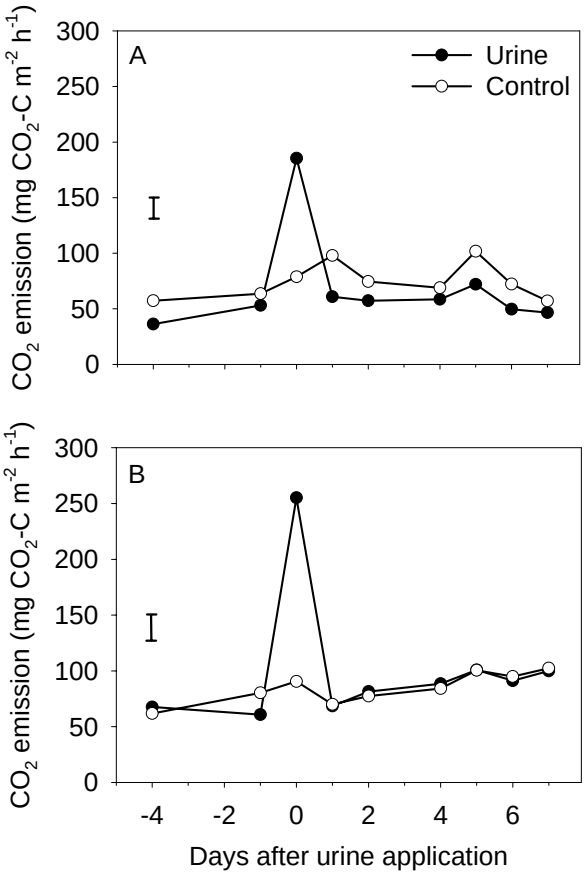
Figure 4. Delta ^{13}C of CO_2 evolved by soil respiration in the urine treatment and the control during (A) the first experiment and (B) the second experiment; n = 3; means ± SE. [a] Urine, n = 1; control, n = 2. [b] One outlying sub measurement was not included.

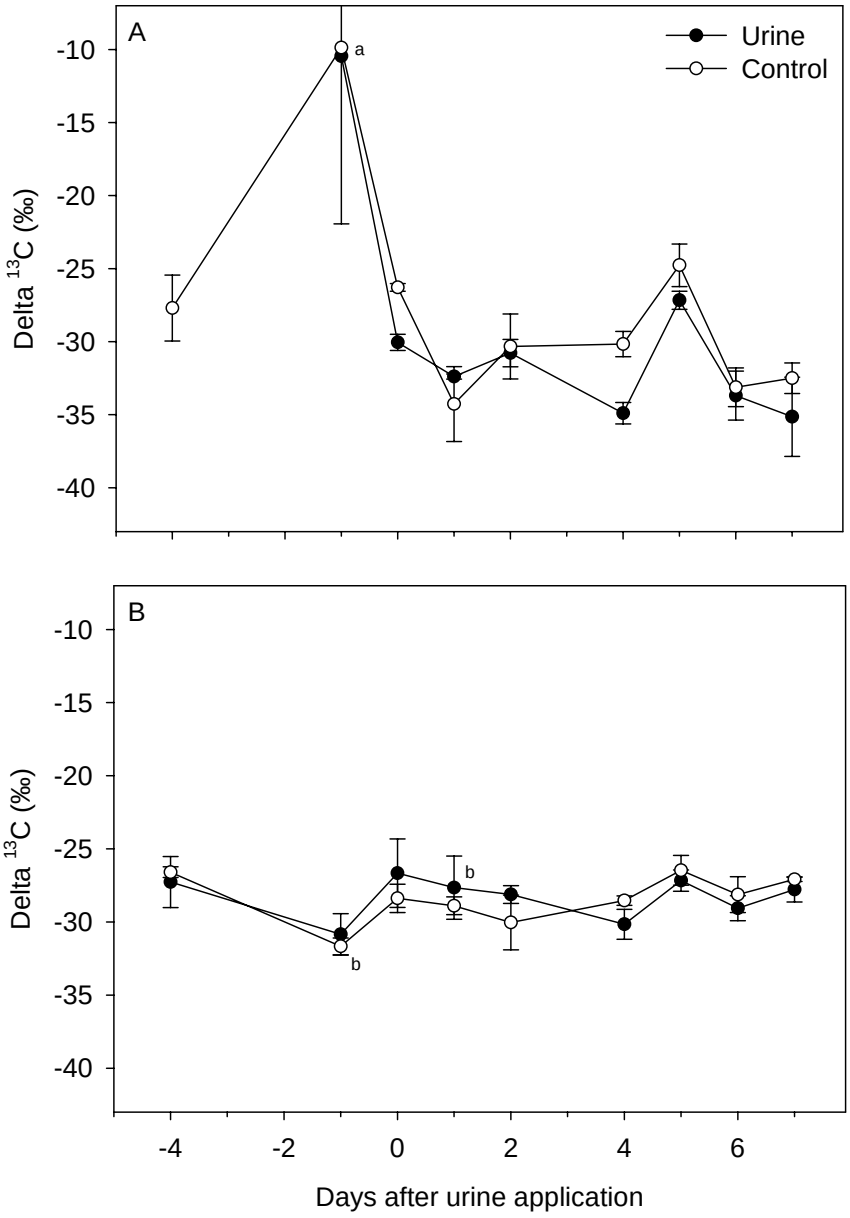
Figure 5. Delta ^{13}C of dissolved organic C (DOC), soil organic matter (SOM) and CO_2 evolved by microbial respiration (MR) and soil respiration (SR) determined at the start of labelling and on day 2 of the first and second experiment, as well as $\delta^{13}C$ of root and shoot determined at the start of labelling and on day -6 of the second experiment; n = 4-6; means ± SE.

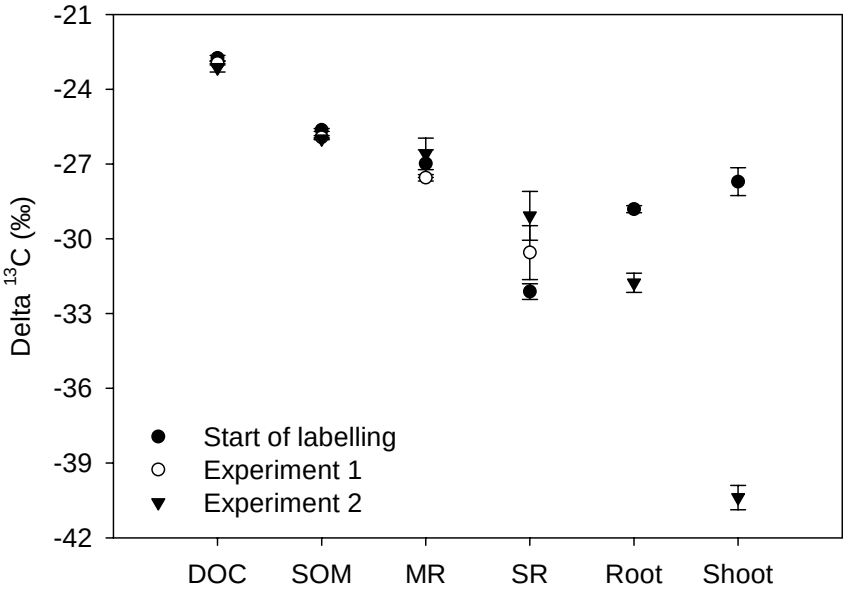
Figure 6. (A) Denitrifying enzyme activity, DEA, and (B) nitrifying enzyme activity, NEA, in the urine treatment and the control on day -1, 2 and 7 after the first urine application event; n = 2-3; means ± SE.

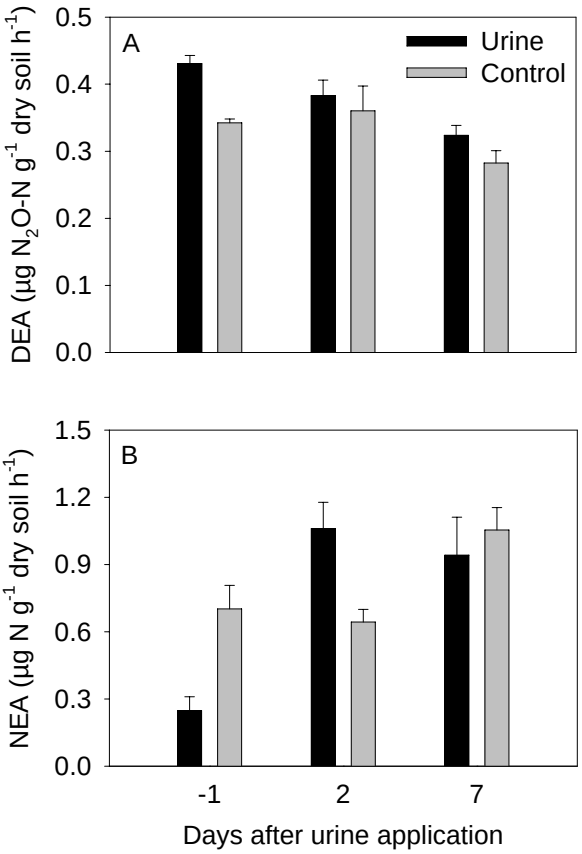

Experiment 1
Experiment 2
Irrigation (mm)
0
5
10
15
20
25
30
-4
-2
0
2
4
6
Days after urine application


A
Urine
Control
B
100
80
60
40
20
0
-20
-4
-2
0
2
4
6
N2O emission (µg N2O-N m-2 h-1)
Days after urine application


A
Urine
Control
B
CO2 emission (mg CO2-C m-2 h-1)
300
250
200
150
100
50
0
-4
-2
0
2
4
6
Days after urine application


A
Urine
Control
a
-10
-15
-20
-25
-30
-35
-40
Delta ^{13}C (‰)
B
b
b
-10
-15
-20
-25
-30
-35
-40
Delta ^{13}C (‰)
-4
-2
0
2
4
6
Days after urine application


-21
-24
-27
-30
-33
-36
-39
-42
Delta 13C (‰)
Start of labelling
Experiment 1
Experiment 2
DOC
SOM
MR
SR
Root
Shoot


A
Urine
Control
DEA (µg N2O-N g-1 dry soil h-1)
0.0
0.1
0.2
0.3
0.4
0.5
B
NEA (µg N g-1 dry soil h-1)
0.0
0.3
0.6
0.9
1.2
1.5
-1
2
7
Days after urine application