



Sheep excreta cause no positive priming of peat-derived CO₂ and N₂O emissions



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ABSTRACT

Large areas of peatlands in Germany and the Netherlands are affected by drainage and high nitrogen deposition. Sheep grazing is a common extensive management activity on drained peatlands, in particular on nature protection areas. However, input of easily mineralisable material such as sheep excrements could enhance degradation of soil organic carbon (C_{org}), thereby increasing the effect of these ecosystems on national GHG budgets. Thus, a microcosm experiment on the influence of sheep excreta on GHG emissions from a histic Gleysol with strongly degraded peat was set up. The ¹⁵N and ¹³C stable isotope tracer technique was used to partition sources of CO₂ and N₂O. Labeled sheep faeces and urine were obtained by feeding enriched material. Undisturbed soil columns were treated with surface application of urine, faeces or mixtures of both in different label combinations to distinguish between direct effects and possible priming effects. Incubation was done under stable temperature and precipitation conditions. Fluxes as well as ¹⁵N and ¹³C enrichment of N₂O and CO₂, respectively, were measured for three weeks. Addition of sheep excreta increased emission of total CO₂ in proportion to the added carbon amounts. There was no CO₂ priming in the peat. No effect on CH₄ and N₂O was observed under the aerobic experimental conditions. The N₂O–N source shifted from peat to excreta, which indicates negative priming, but priming was not significant. The results indicate that sheep excreta do not significantly increase GHG emissions from degraded peat soils. Considering the degraded peatland preserving benefits, sheep grazing on peatlands affected by drainage and high nitrogen deposition should be further promoted.

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1. Introduction

Sheep grazing on peatlands is a common practice (Germer, 2006), which aims at maintaining grass and heath and preventing growth of birch and shrubbery, in particular when soils are drained. Sheep grazing preserves an open landscape, which offers habitats and ecological niches for rare species. Moreover, it directly affects the greenhouse gas (GHG) balance of a peatland by plant biomass export, CH₄ emissions from enteric fermentation and GHG released from faeces or urine patches. Excreta are hotspots of biological activity. In a Mongolian grassland GHG emissions (CO₂, N₂O, CH₄)

were 20% higher from urine and fresh faeces patches than from unaffected soil (Ma et al., 2006).

N₂O emissions on peat soils from artificial urine patches emitted maximal 50 mg N₂O–N m⁻² d⁻¹ mainly via nitrification in a study by Koops et al. (1997). In the Netherlands 1.5–9.9% of the nitrogen in faeces and urine N input was emitted as N₂O from a peat grassland (Velthof et al., 1996). It is unknown whether the reported N₂O fluxes originated entirely from the excreta or whether additional nitrogen was mobilized from the peat soil by priming. Priming occurs if easily degradable substances added to soils activate or hamper microbial activity. Positive priming increases carbon and/or nitrogen mineralization from soil organic matter compared to a control treatment. Negative priming reduces emissions and soil organic matter decomposition (Kuzuyakov et al., 2000).

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Drainage induces peat decomposition which results in high CO₂ emissions (Smith and Conen, 2004). Decomposition of pure peat is slow, but in previous incubation studies (Reiche et al., 2010; Hahn-Schoeffl et al., 2011) CO₂ emissions drastically increased when labile, energy-rich substrates were present. Again it is unknown whether the reported fluxes originated entirely from the labile plant-derived material mixed with the peat in the laboratory (Reiche et al., 2010; Hahn-Schoeffl et al., 2011) or whether additional carbon was mobilized from the peat soil by priming. Positive or no priming effects have been observed in peat soil after the addition of substrates typical for plant debris (e.g. glucose, oxalic acid, etc.; Hamer and Marschner, 2002).

Stable isotopes (¹³C and/or ¹⁵N) are commonly used to study priming effects in soil. Bol et al. (2000) traced cattle dung-derived carbon in a temperate grassland using ¹³C natural abundance measurements. After 150 days only around 10% of cattle dung C remained in the upper 5 cm of soil. Angers et al. (2007) observed significant CO₂ emissions from pig slurry but no priming effect in mineral soil. In general, very few studies have investigated priming effects or the fate of excreta C or N and studies have exclusively been performed in mineral soils (Monaghan and Barraclough, 1993; Bol et al., 2000). So far, priming effects of excreta have not been studied in organic soils.

Sheep excreta contain active microbes, labile substances and a C/N ratio narrower than the peat. Consequently, they have a strong potential for positive priming in peat soil. We hypothesize that

1. Sheep excreta increase emissions of CO₂, CH₄ and N₂O from peat soil.
2. Sheep excreta induce a positive carbon and nitrogen priming in peat soil and trigger CO₂ and N₂O release from the peat.

Therefore, we studied the influence of sheep excreta on GHG emissions from a degraded peat soil using stable isotope tracers to partition sources and determine possible priming effects.

2. Material & methods

2.1. Experimental design

A microcosm study under constant temperature and moisture conditions was performed with pure peat and sheep excreta amendments to test the hypotheses. Sheep excreta were applied on the surface of undisturbed peat columns from a nutrient-poor peat grassland. One excretion event by one sheep was simulated by surface application of faeces, urine, or faeces plus urine. Priming effects and source attribution of CO₂ and N₂O to peat soil versus excreta were studied by tracing ¹³C and ¹⁵N signals in isotopically double labeled (¹³C and ¹⁵N) excreta. GHG emissions were measured for 21 days.

Seven different treatments were used in the microcosm experiment (Table 1). For all treatments C4 labeled excreta were applied with or without ¹⁵N enrichment. Pure peat columns (=pure peat)

Table 1
Overview on experimental treatments.

Treatments ^a	Denomination in the text
Control 1: peat	<i>Pure peat</i>
Control 2: ¹⁴ N faeces + sand	<i>¹⁴N faeces plus sand</i>
Peat + ¹⁵ N faeces	<i>¹⁵N faeces</i>
Peat + ¹⁵ N urine	<i>¹⁵N urine</i>
Peat + ¹⁵ N faeces + ¹⁵ N urine	<i>¹⁵N faeces plus ¹⁵N urine</i>
Peat + ¹⁴ N faeces + ¹⁵ N urine	<i>¹⁴N faeces plus ¹⁵N urine</i>
Peat + ¹⁴ N faeces + ¹⁴ N urine	<i>¹⁴N faeces plus ¹⁴N urine</i>

^a For C: C3 signal of peat, C4 signal of faeces and urine.

were used as control columns for the background levels of CO₂, N₂O and CH₄ fluxes. A second control containing C4 faeces with natural abundance ¹⁵N signature on annealed sand (= ¹⁴N faeces plus sand) was used for plausibility checks. Peat was amended with faeces or urine or both faeces and urine as combined signal using ¹⁵N faeces or ¹⁵N urine (= ¹⁵N faeces, ¹⁵N urine, ¹⁵N faeces plus ¹⁵N urine, respectively). To distinguish between faeces and urine unlabeled faeces were combined with labeled urine (= ¹⁴N faeces plus ¹⁵N urine). Peat amended with unlabeled ¹⁴N faeces and ¹⁴N urine (= ¹⁴N faeces plus ¹⁴N urine) served as a control treatment to check for eventual isotopic effects on the priming effect.

2.2. Sheep excreta labeling

For the production of double labeled excreta a male sheep was housed in a metabolism cage and fed with highly enriched ¹⁵N tracer substance and corn silage. Corn is a C4 plant (corn silage δ¹³C –12.5 ± 0.12‰), which results in a natural abundance ¹³C label of the corn silage compared to the peat material with its δ¹³C signal of C3 vegetation (peat δ¹³C –28.1 ± 0.15‰). The daily diet contained 2.8 kg corn silage (54.7% dry matter content), 18 g NH₄Cl and 10 g urea where NH₄Cl was enriched to 95% ¹⁵N. Diet enrichment was about 15 atom% ¹⁵N. After two weeks of adaptation to the fodder combination, labeled excreta were collected for 10 days. Faeces and urine were collected separately two to three times a day, bulked and frozen at –19 °C until use. At the start of the labeling period, N-unlabeled faeces and urine were collected from the same sheep fed with C4 corn silage and unlabeled NH₄Cl.

For C and N measurement, faeces were dried at 105 °C and measured on an elemental analyzer LECO TruMac CN. C and N of urine were measured in the liquid phase on an elemental analyzer LECO TruSpec CN to avoid NH₃ volatilization. ¹⁵N and ¹³C abundance were measured in dried faeces and acidified urine on a Thermo Fisher Scientific Delta plus with a coupled elemental analyzer (CE Instruments FLASH EA 1112) and a ConFlo II interface.

The C/N ratio of faeces was 22 in accordance with previously reported values (Floate and Torrance, 1970). More of the excreted N was found in faeces than in urine, which reflects the N-adapted diet. Sheep faeces and urine were enriched by up to 13.2 atom% ¹⁵N and 9.6 atom% ¹⁵N, respectively. δ¹³C of faeces and urine showed a clear C4 signal of –13.1 ± 0.1‰ (1.09 atom% ¹³C) and –13.3 ± 0.2‰ (1.09 atom% ¹³C), respectively. Further parameters are shown in Table 2. Enrichment of ¹⁵N was sufficient in both excreta to enable the detection of priming effects during incubation.

2.3. Microcosm experiment

For the microcosm experiment intact soil cores were extracted from a histic Gleysol in Lower Saxony, Germany (for detailed site description see Leiber-Sauheitl et al., 2014). The upper 5 cm of the soil surface including vegetation and roots were removed to avoid experimental artifacts due to decaying plant material. Subsequently, 20 cm deep soil cores were extracted by hammering a stainless steel sampling device enclosing a plexiglass cylinder (30 cm high, 15 cm diameter) into the soil.

In the laboratory, these undisturbed peat columns were installed in a microcosm system (Hantschel et al., 1993). The headspace was flushed throughout the experiment with 10 mL min⁻¹ of synthetic air (20% O₂, 80% N₂, 400 ppb N₂O). For measurement of flow rates, a flowmeter was switched automatically from one column to the next at regular intervals so that each column was measured every 12 h. N₂O was included in the synthetic air to allow for measuring possible N₂O uptake into the peat column.

Table 2
C and N content, ^{15}N and ^{13}C signature of faeces, urine and peat soil. Average C and N content per column.

	Urine	Faeces	Urine + faeces	Peat soil
Total C (%)	0.30 ± 0.15 ^a	39.0 ± 0.13 ^a	–	27.0 ± 1.6 ^a
Total N (%)	0.35 ± 0.03 ^a	1.77 ± 0.03 ^a	–	0.90 ± 0.04 ^a
Atom% ^{15}N				
^{15}N labeled	9.64 ± 0.19 ^a	13.17 ± 0.19 ^a	–	–
^{15}N unlabeled	0.381 ± 0.005 ^a	0.3689 ± 0.0002 ^b	–	0.3677 ± 0.0004 ^a
Atom% ^{13}C	1.09106 ± 0.00009 ^a	1.0911 ± 0.0002 ^c	–	1.0749 ± 0.0002 ^a
Average C content (g)	–	–	–	508
Average N content (g)	–	–	–	17
C/N	0.85	22.0	14.8	29.9

^a n = 3.

^b n = 11.

^c n = 12.

Suction plates with –130 hPa pressure were installed at the lower end of the columns to adjust the water content to 63 vol.% (equivalent to 80% water filled pore space (WFPS) according to a pre-experiment). Irrigation of 50 mL d⁻¹ (=2.8 mm d⁻¹) with 0.001 M CaCl₂ started two weeks before the incubation to equilibrate water contents in the columns and to avoid drying of the soil surface. Incubation temperature was 15 °C.

After this equilibration period, tracer was added to the top of the microcosms corresponding to hot spot conditions of faeces and urine input in natural systems. Excreta were added moist and unconsolidated in order to have maximal decomposition rates during the experiment (Floate, 1970). To simulate C and N input representative for field conditions, C or N amounts were applied representative of one excretion event of sheep. This led to different application rates per treatment. Following the procedure in Ma et al. (2006) 33 g coarsely shredded fresh faeces and 40 mL urine were applied onto the peat columns as single treatments or in combination. This is equivalent to 6.79 g C m⁻² and 7.92 g N m⁻² for urine and 335.01 g C m⁻² and 15.20 g N m⁻² for faeces additions (Table 2). Properties of faeces and urine and isotopic signatures are given in Table 2. The difference in C input between faeces and urine treatments was large (faeces C: urine C = 49: 1), while the N application was relatively similar with a ratio of faeces N: urine N = 2: 1. The quality of the carbon and nitrogen substrates also differed considerably between faeces and urine treatments.

A pre-test indicated that the peat had a high turnover during the first few days after application. A field experiment of excreta or slurry application used an observation period of 16 days (Eickenscheidt et al., 2014). Accordingly, the duration of the experiment was set to 21 days.

2.4. Analyses

The headspace of each soil column was sampled for the concentration of N₂O, CO₂, CH₄ and the isotopic signature of N₂O via headspace vials (20 mL and 120 mL, respectively), which were interconnected to the outgoing air tubes. N₂O, CO₂ and CH₄ concentrations were also measured in empty control columns to check and correct for concentrations present in the stream of artificial air before passing the column. After excreta application the gas phase was sampled with diminishing frequency, daily to weekly, as the experiment progressed. Gas measurements were carried out on a Shimadzu GC-2014 gas chromatograph with a FID and an ECD (Loftfield et al., 1997). N₂O isotopic signature was measured on an isotopic ratio mass spectrometer (Delta Plus, Thermo Scientific, Germany) coupled with a PreCon Interface with double needle.

Carbon isotope ratio $\delta^{13}\text{C}$ of sample gas was measured with a cavity ringdown analyzer (Picarro G1101-i Analyzer, Picarro Inc. Sunnyvale Ca. USA). For this, sample gas was taken from the vent of

the flowmeter of the microcosm facility. The sample gas was diluted with 20 mL min⁻¹ N₂ to keep CO₂ concentration inside the measurement range of the instrument. Diluted sample gas entered the instrument via a T-piece to allow the instrument to draw sample gas with its immanent flow rate (ca. 20 mL min⁻¹). The analyzer takes a measurement every 13 s. After switching from one soil column to the next, constant CO₂ concentration and $\delta^{13}\text{C}$ values were achieved after approx. 5 min. $\delta^{13}\text{C}$ value was calculated as average of the last 10 measurements before switching to the next column. Thus, the switching interval was set to ca. 8 min. For further calculation 30 s running averages of $\delta^{13}\text{C}$ were used.

Ammonia (NH₃) emissions from urea degradation were measured daily in urine treatments in the first week after application and afterward once at the end of the experiment. Urine treatments were equipped with two vials containing 50 mL 0.05 M H₂SO₄ each to trap NH₃. NH₃ was determined as N content in the acid on a Skalar Continuous-Flow Analyzer, consisting of San++ analysis system with a SA 1050 Random Access Auto Sampler. It should be noted that the air flow rate was optimized for N₂O and CO₂ flux measurement and not for NH₃ flux measurement.

The mass flow of dissolved organic carbon (DOC), dissolved organic nitrogen (DON) and mineral nitrogen (N_{min}) from each column was sampled every four days in the suction flasks. Cumulative C and N losses by leaching were calculated according to Bol et al. (2000).

After the experiment each peat column was divided into a 0–5 cm and a 5–20 cm depth increment. For each depth increment, gravimetric water content, mineral nitrogen content (NH₄⁺ and NO₃⁻) according to VDLUFA (1997), bulk soil C, ^{15}N and C/N ratios were determined. Microbial biomass was determined in the depth increments by chloroform fumigation extraction (CFE) with 0.05 M K₂SO₄ (modified after Brookes et al., 1985; Joergensen, 1995). The chloroform obviously also reacted with the peat so that microbial biomass could not be determined via carbon analysis but only by nitrogen analysis. An aliquot of the extract was used for N content determination on a Mitsubishi Total Nitrogen Analyzer TN-100 with Auto Liquid Sampler and Auto Sample Injector ASI-100. 10 mL of each sample were freeze dried and ^{15}N was determined on a Thermo Fisher Scientific Delta plus with a coupled elemental analyzer (CE Instruments FLASH EA 1112) and a Conflo II interface.

2.5. Calculations

The fractions of excreta and soil derived N₂O–N and CO₂–C were calculated with the following equations. For two sources (urine + soil, faeces + soil), the measured amount of N₂O or CO₂ (M_a) consisted of excreta derived (M_{excreta}) N₂O or CO₂ fraction and a soil derived (M_{soil}) fraction by Eq. (1).

$$M_a = M_{\text{excreta}} + M_{\text{soil}} \quad (1)$$

M_a – measured amount of N₂O [μmol] or CO₂ [mmol]

M_{excreta} – excreta (faeces or urine) derived N₂O [μmol] or CO₂ fraction [mmol]

M_{soil} – soil derived N₂O [μmol] or CO₂ fraction [mmol]

Isotope signals of the background air ($at\%_{\text{gas,back}}$), of the column headspace ($at\%_{\text{gas,lab}}$) and of the added excreta ($at\%_{\text{excreta}}$) were used to calculate the faeces and urine derived fractions of the N₂O or CO₂ emissions (Amelung et al., 1999) by Eq. (2).

$$M_{\text{excreta}} = \frac{at\%_{\text{gas,lab}} - at\%_{\text{gas,back}}}{at\%_{\text{excreta}} - at\%_{\text{gas,back}}} \times M_a \quad (2)$$

$at\%_{\text{gas,back}}$ – $at\%^{15}\text{N-N}_2\text{O}$ or $at\%^{13}\text{C-CO}_2$ of background air
 $at\%_{\text{gas,lab}}$ – $at\%^{15}\text{N-N}_2\text{O}$ or $at\%^{13}\text{C-CO}_2$ of column headspace
 $at\%_{\text{excreta}}$ – $at\%^{15}\text{N}$ or $at\%^{13}\text{C-CO}_2$ of excreta (faeces or urine)

For three sources (urine + faeces + soil), the measured amount of N₂O (M_a) consisted of a faeces derived (M_{faeces}), a urine derived (M_{urine}) and a soil derived (M_{soil}) N₂O fraction. The urine derived fraction ($M_{\text{urine}'}$) was calculated via the ¹⁴N faeces plus ¹⁵N urine treatment evaluated with Eq. (3).

$$M_{\text{soil}} = \frac{M_a \times (at\%_{\text{gas,lab}} - at\%_{\text{faeces}}) - M_{\text{urine}'}}{at\%_{\text{gas,back}} - at\%_{\text{faeces}}} \times (at\%_{\text{urine}} - at\%_{\text{faeces}}) \quad (3)$$

$M_{\text{urine}'}$ – urine derived N₂O fraction [μmol] determined via ¹⁴N faeces plus ¹⁵N urine treatment.

$at\%_{\text{faeces}}$ – $at\%^{15}\text{N}$ of added faeces tracer

$at\%_{\text{urine}}$ – $at\%^{15}\text{N}$ of added urine tracer

Priming effects for C and N were calculated as priming factors (PF; Bol et al., 2003) by Eq. (4).

$$PF = \frac{M_{\text{soil,treat}}}{M_{\text{soil,control}}} \quad (4)$$

$M_{\text{soil,treat}}$ – soil derived CO₂-C or N₂O-N in treatment with excreta application

$M_{\text{soil,control}}$ – soil derived CO₂-C or N₂O-N in pure peat control

2.6. Statistical analyses

All statistics were performed using R (Version 3.1.2; R Core Team, 2014). If not indicated differently, mean values are always given with ±1 standard deviation. The significance limit was set to 0.05. ANOVA tables were calculated for cumulated total, source specific gas fluxes, extractable microbial N and N_{min}. Subsequently, TukeyHSD was used to test for the treatment wise significance of differences. Linear models were applied in order to determine the significance of the ¹³C enrichment of the different treatments in comparison to *pure peat*. Two sided t-tests of soil derived C or N fluxes of the different treatments versus the control (= *pure peat*) were performed in order to determine the significance of priming.

3. Results

3.1. Total CO₂ and CH₄ fluxes

All treatments were identified as significant sources of CO₂ throughout the experiment (Fig. 1). Cumulative CO₂-C emissions were in the range of 20–60 g within 21 days. In accordance with the different amounts of C added, cumulative CO₂-C fluxes of ¹⁵N faeces and ¹⁵N faeces plus ¹⁵N urine were significantly higher than from *pure peat*, ¹⁵N urine and ¹⁴N faeces plus sand.

CO₂ fluxes from the *pure peat* treatment were stable over the course of the experiment at about 1.15 g CO₂-C m⁻² d⁻¹ and variance between replicate columns was small (Fig. 1a). This suggests that environmental conditions were stable during incubation and that differences in fluxes can be attributed to the excreta amendment. Levels of CO₂ fluxes varied between treatments but variance between replicates was also large in some of the excreta treatments, in particular in urine treatments. The lowest fluxes were found in the *faeces on sand* and *pure peat* treatments ranging from 0.48 to 1.44 g CO₂-C m⁻² d⁻¹ (Fig. 1g and a). In total, *urine* exhibited fluxes of a similar magnitude to *pure peat* but its fluxes were higher on the first day after the application (Fig. 1d). The *faeces* treatment nearly doubled fluxes from *pure peat* and *urine* treatments (1.68–3.6 g CO₂-C m⁻² d⁻¹; Fig. 1c) over the course of the experiment. Treatments with *faeces plus urine* showed highest fluxes (2.4–5.28 g CO₂-C m⁻² d⁻¹;

Fig. 1b, e and f) with pronounced variability in time and between replicates.

CH₄ emissions ranged from 4.1 × 10⁻⁸ to 9.4 × 10⁻⁴ g m⁻² d⁻¹ with a mean flux of 3.3 × 10⁻⁵ g m⁻² d⁻¹ over the course of the experiment and all treatments. CH₄ fluxes were hence negligible in all treatments and no treatment effects were observed (data not shown).

3.2. Excreta derived CO₂-C emissions

Excreta derived CO₂ fluxes were significantly different from *pure peat* except for the ¹⁵N urine treatment (p < 0.001). 26.7% (±7.8%) of urine C, 9.3% (±1.5%) of faeces C and 12.9% (±2.3%)/13.2% (±1.9%)/15.4% (±3.5%) of faeces plus urine C (three treatments with different label combinations) were mineralized to CO₂ after 21 days (Fig. 2a). ¹³C enrichment in CO₂ was small but significant for all treatments as compared to background, at least for the first sampling dates.

Excreta CO₂-C emissions from treatments with combined application of urine and faeces were slightly higher than the prediction by a mixing model (9.7% of applied C) based on the results from separate application. Urine induced a small positive priming of faeces carbon.

Urine derived CO₂ emissions followed an exponential decay function (Fig. 3) with a half-life of 0.7 days. Consequently, no significant contribution of urine carbon to CO₂ emissions was detected after four days. The dynamics is consistent with fast hydrolysis of urea in urine. The expected cumulative emission from complete hydrolysis would be 0.12 g C and the measured value was 0.11 ± 0.02 g urine C. In contrast, the contribution of faeces C to total

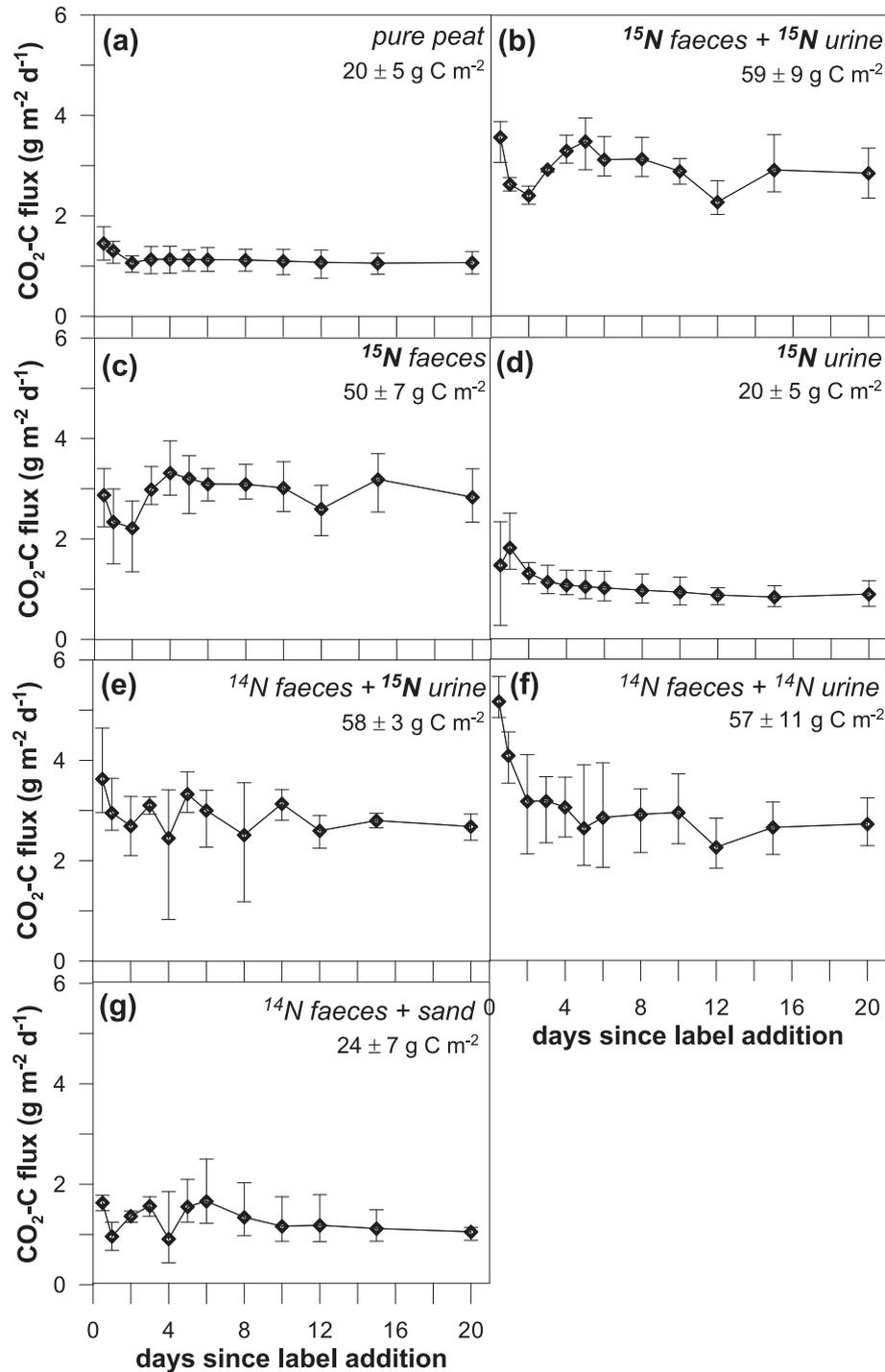


Fig. 1. Time series of CO_2 fluxes since labeling of *pure peat* (=background; a) and different ^{15}N labeled treatments (b–g). Bars represent minimum and maximum values of replicates. Cumulated C fluxes of each treatment over 21 days are indicated in $\text{g C m}^{-2} \pm$ standard deviation of the replicates.

CO_2 fluxes lasted over the three weeks of the experiment as indicated by significant ^{13}C enrichment in CO_2 ($\delta^{13}\text{C} - 15.8\text{‰}$) at the end of the experiment where *pure peat* showed a $\delta^{13}\text{C}$ of -26.2‰ . Thus, faeces decomposition was not completed at the end of the incubation.

Flux rates of excreta derived $\text{CO}_2\text{-C}$ followed linear or exponential trends with small fluctuations during the sampling period depending on treatment. Variations among columns of the same treatment were small (see Appendix Fig. S1a–c).

3.3. Soil derived $\text{CO}_2\text{-C}$ emissions

During the incubation 0.01% ($\pm 0.00\%$) to 0.07% ($\pm 0.03\%$) of the soil C was mineralized (Fig. 2b). Soil CO_2 contributed more than 85% to the total fluxes in the ^{15}N *urine* treatment and 38% in the ^{15}N *faeces* treatment. This difference is primarily a consequence of the different amounts of C added (Fig. 6).

Priming factors indicate mostly non-significant negative priming effects for all treatments (Table 3). Significantly less soil C was

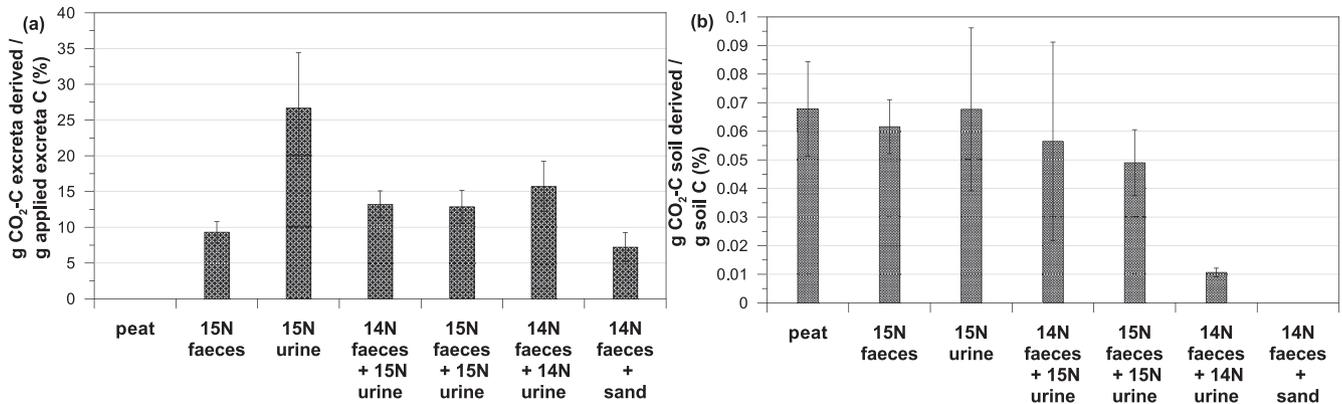


Fig. 2. Proportion of excreta C (a) and of soil C (b) mineralized to CO₂ during 3 weeks (mean ± standard deviation of the replicates; n = 4).

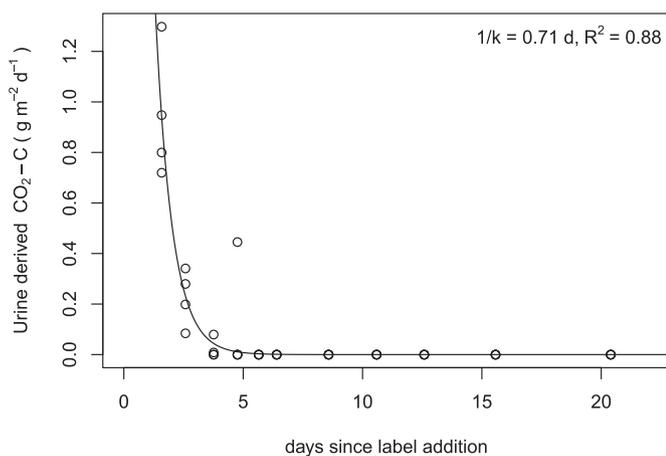


Fig. 3. Mineralization kinetics for urine carbon (flux $\sim c1 \times \exp(-k \times \text{time})$ with $c1 = 8.73 \pm 2.99 \text{ g m}^{-2} \text{ d}^{-1}$ and $k = 1.41 \pm 0.21 \text{ d}^{-1}$) derived from the ¹⁵N urine treatment (n = 4).

converted to CO₂ if urine and faeces were present: Two of the three urine plus faeces treatments (¹⁴N faeces plus ¹⁴N urine, ¹⁴N faeces plus ¹⁵N urine) exhibited significantly lower soil C derived respiration than pure peat ($p < 0.05$ and $p < 0.01$, respectively) and the other treatment (¹⁵N faeces plus ¹⁵N urine) exhibited the same trend ($p = 0.05$). ¹⁵N urine as well as ¹⁵N faeces exhibited no significant difference to pure peat, although there was also a trend towards lower soil C derived respiration.

3.4. DOC export

Cumulative DOC export ranged from 10.5 ± 1.3 to $15.6 \pm 1.7 \text{ g C m}^{-2} (21 \text{ days})^{-1}$. DOC export was generally high and similar in magnitude and dynamics among the treatments. Peat was apparently the most important source of DOC. DOC loss was equivalent to 0.04%–0.06% of the total C stock in the columns (peat plus excreta). Hence, DOC contributed to total C export equivalent to 17%–25% of the gaseous C emissions.

3.5. Total N₂O and NH₃ fluxes

Cumulative N₂O–N emissions ranged from 0.2 to $3.3 \text{ g N m}^{-2} (21 \text{ days})^{-1}$ and did not differ significantly between treatments. N₂O emissions from treatments with peat were between 7.2×10^{-3} and $9.6 \times 10^{-3} \text{ g m}^{-2} \text{ d}^{-1}$ (Fig. 4a–f). Lower emissions were observed in the ¹⁴N faeces plus sand treatment (3.6×10^{-4} to

$7.2 \times 10^{-4} \text{ g m}^{-2} \text{ d}^{-1}$; Fig. 4g). Similar to the respiration results, N₂O emissions from pure peat exhibited a low standard deviation (SD) of up to $3.7 \times 10^{-3} \text{ g m}^{-2} \text{ d}^{-1}$ (Fig. 4a). Treatments with excreta varied more among the replicates (SD increasing in the order ¹⁴N faeces plus sand < pure peat < ¹⁵N faeces < ¹⁵N urine; SD of up to $1.0 \times 10^{-2} \text{ g m}^{-2} \text{ d}^{-1}$). Towards the end of the experiment, N₂O emissions tended to increase except for the ¹⁵N faeces treatment where N₂O remained constant (Fig. 4c). In the ¹⁴N faeces plus ¹⁴N urine treatment N₂O emissions were comparable to the pure peat treatment and variance between replicates was low (up to $2.4 \times 10^{-3} \text{ g m}^{-2} \text{ d}^{-1}$; Fig. 4f).

Low NH₃ emissions were observed in all urine treatments ($< 1.2 \times 10^{-7} \text{ g m}^{-2} \text{ d}^{-1}$). This is consistent with fast infiltration of urine, the daily irrigation and the low soil pH (3.9), which all suppress NH₃–N losses (Black et al., 1987; Clough et al., 1996).

3.6. Excreta derived N₂O emissions

N₂O–N of the column headspace was highly enriched in all treatments with ¹⁵N excreta addition (up to 8 atom% ¹⁵N) which reflects the high turnover of excreta nitrogen. Large variability among the replicates (Fig. 4) in total as well as source-specific N₂O–N fluxes masked treatment effects so that no significant differences could be detected. Except for urine derived N₂O–N in the ¹⁴N faeces plus ¹⁵N urine treatment, excreta derived N₂O–N differed non-significantly, but tended to decrease in the order ¹⁵N urine > ¹⁵N faeces plus ¹⁵N urine > ¹⁴N faeces plus ¹⁵N urine > ¹⁵N faeces. Soil derived N₂O–N was highest in the pure peat and ¹⁵N faeces treatment.

The proportion of excreta N mineralized to N₂O during 21 days was highest in the ¹⁵N urine treatment ($66\% \pm 60\%$) and lowest in the ¹⁵N faeces treatment ($6.7\% \pm 6.7\%$; Fig. 5a). In urine plus faeces treatments 37.5% ($\pm 13.9\%$; ¹⁴N faeces plus ¹⁵N urine) and 35.5% ($\pm 26.0\%$; ¹⁵N faeces plus ¹⁵N urine) of the added ¹⁵N was emitted as N₂O which was in the range expected from a mixing model based on the results from separate application (45.8%).

Flux rates of excreta derived N₂O–N followed linear or exponential trends during sampling period depending on treatment and the respective column. For ¹⁵N faeces, trends between single columns varied strongly. In contrast, for ¹⁵N urine trends of all columns were very similar (See Appendix Fig. S1d–g).

3.7. Soil derived N₂O emissions

The amount of soil N mineralized to N₂O ranged from $0.29\% \pm 0.21\%$ – $0.56\% \pm 0.32\%$ (Fig. 5b). Priming factors (Table 3) of ¹⁵N faeces were slightly above one, indicating a small positive

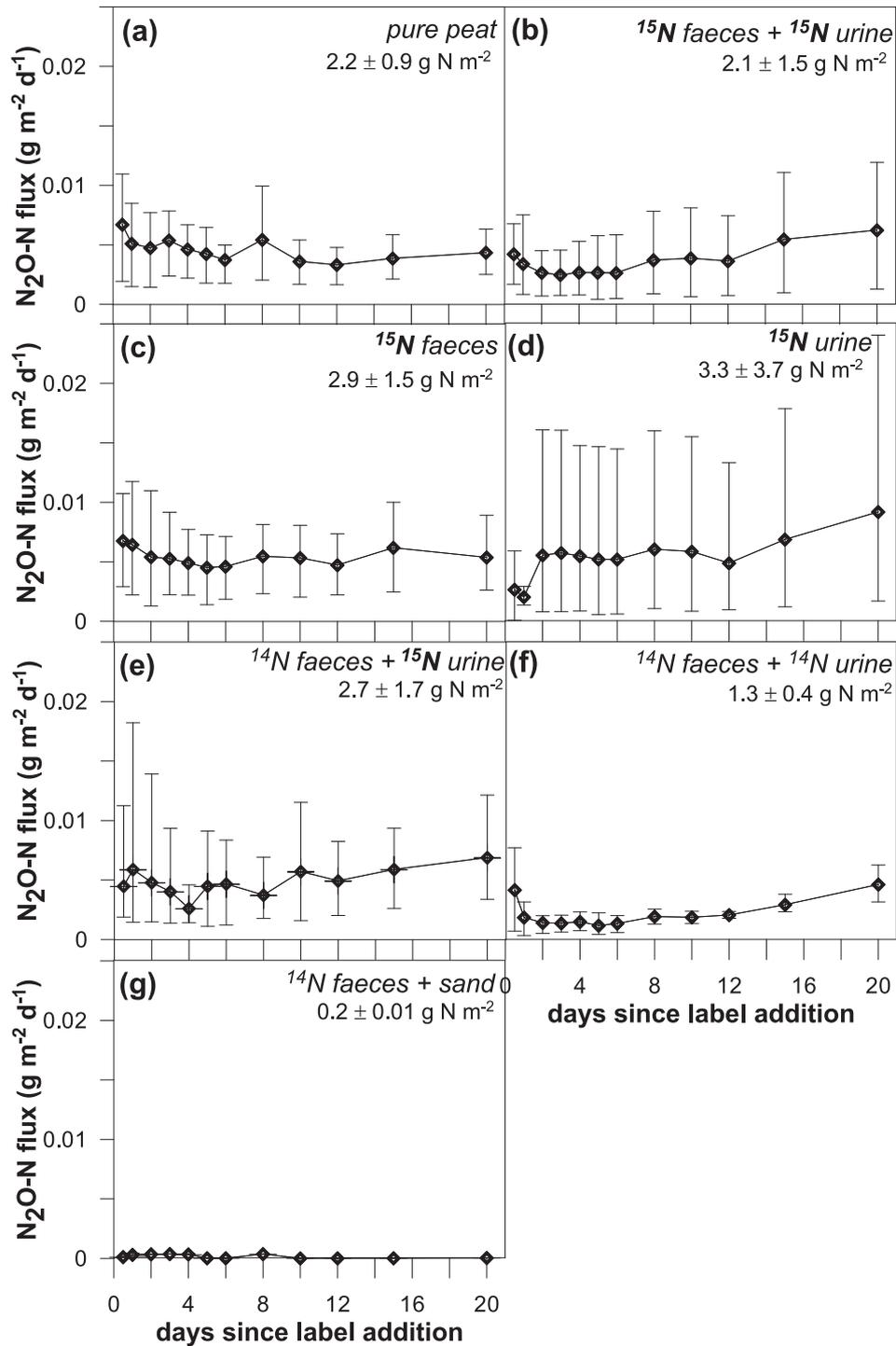


Fig. 4. Time series of N_2O fluxes since labeling of *pure peat* (=background; a) and different ^{15}N labeled treatments (b–g). Bars represent minimum and maximum values of replicates. Cumulated N fluxes of each treatment over 21 days are indicated in $\text{g N m}^{-2} \text{d}^{-1} \pm$ standard deviation of the replicates.

priming effect, which was not significant. There was a tendency towards a negative priming effect as indicated by results from the ^{15}N faeces plus ^{15}N urine and the ^{15}N urine treatments in comparison to *pure peat* treatment.

3.8. Extractable microbial N

Extractable microbial N did not differ significantly between treatments. Considering the complete columns, the amount of extractable microbial N per g dry soil ranged from $35 \pm 30 \mu\text{g N/g}$

dry soil (*pure peat*) to $48 \pm 18 \mu\text{g N/g}$ dry soil (^{15}N faeces plus ^{15}N urine) with large scatter among replicates (Table 4). Extractable microbial biomass was higher in the top layer (0–5 cm: $42\text{--}60 \mu\text{g N/g}$ dry soil) than in the bottom layer (5–20 cm: $11\text{--}36 \mu\text{g N/g}$ dry soil; data not shown). More extractable microbial N was observed in ^{15}N faeces plus ^{15}N urine than in ^{15}N urine, ^{15}N faeces or *pure peat* in 0–5 cm. In the 5–20 cm layer higher extractable microbial N was found in ^{15}N faeces plus ^{15}N urine than in ^{15}N urine or in *pure peat*.

Unfumigated samples (=exchangeable N) indicated a higher enrichment than fumigated ones (=exchangeable N plus microbial

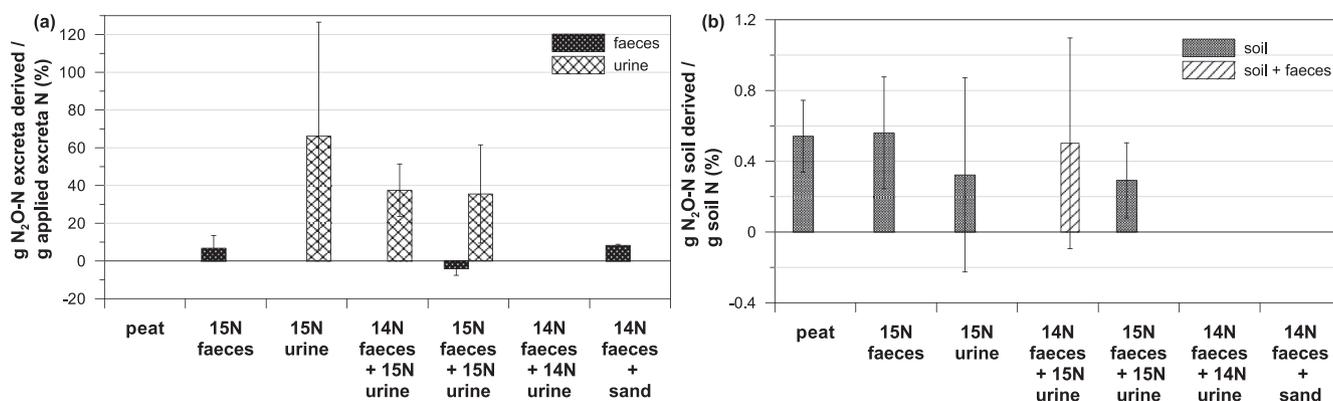


Fig. 5. Proportion of excreta N (a) and soil N (b) mineralized to N_2O during 3 weeks (mean \pm standard deviation of the replicates; $n = 4$).

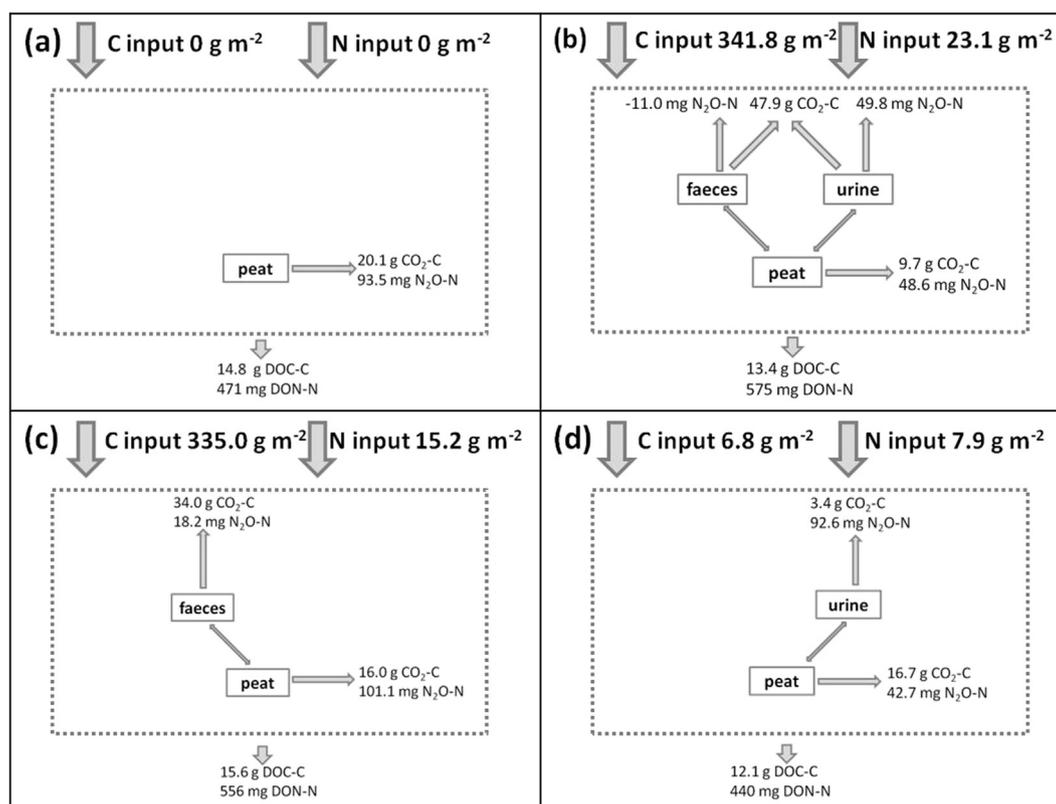


Fig. 6. Source specific carbon and nitrogen release ($g\ C$ or $mg\ N\ m^{-2}$) of different treatments over 21 days: (a) pure peat, (b) ^{15}N faeces and ^{15}N urine (for CO_2-C emissions mean value of all faeces plus urine treatments), (c) ^{15}N faeces and (d) ^{15}N urine. DOC-C and DON-N refer to emissions from total column. On top of each figure (a–d) amounts of C and N input per column are indicated. The dotted boxes represent one microcosm system.

Table 3

Priming factors (PF) of soil CO_2 and N_2O calculated according to Bol et al. (2003). Values >1 indicated positive priming, values <1 indicate negative priming. Standard deviations were calculated using Gaussian error propagation. p values were derived from two sided t-tests treatment vs. control (=pure peat).

Treatment	PF (CO_2)	PF (N_2O)
^{15}N faeces	0.79 ± 0.21 (p = 0.18)	1.08 ± 0.11 (p = 0.84)
^{15}N urine	0.83 ± 0.27 (p = 0.30)	0.46 ± 0.09 (p = 0.26)
^{15}N faeces + ^{15}N urine	0.66 ± 0.19 (p = 0.05)	0.52 ± 0.07 (p = 0.13)
^{14}N faeces + ^{15}N urine	0.58 ± 0.23 (p < 0.05)	n. d.
^{14}N faeces + ^{14}N urine	0.21 ± 0.05 (p < 0.01)	n. d.

N) with 6 atom% ^{15}N versus 4 atom% ^{15}N (data not shown). During chloroform fumigation not only microbial cells can be lysed, but ammonium or old cell wall fragments of the peat substrate could also have been released (Miltner et al., 2012), causing dilution of the ^{15}N label due to their low ^{15}N content. Apparently, chloroform fumigation does not allow a robust quantification of microbial biomass in peat soil.

3.9. Mineral nitrogen

There were significant differences in mineral N (N_{min}) contents of soil between treatments (p < 0.0001). N_{min} was dominated by ammonium, which contributed about 96% and 83% to N_{min} in the top and bottom layer, respectively (Table 4). As expected, N_{min} was

Table 4
Extractable microbial N and NO_3^- , NH_4^+ , mineral N and DON contents after 21 days.

Treatment	Extractable microbial N (mg N/g dry soil)	NO_3^- (mg N per column)	NH_4^+ (mg N per column)	N_{min} (mg N per column)	DON (g N m^{-2} in 21 days)
Pure peat	0.04 ± 0.03	2.49 ± 0.64	18.13 ± 6.83	20.63 ± 3.30	0.47 ± 0.04
^{15}N faeces	0.04 ± 0.02	2.28 ± 2.64	34.19 ± 7.41	36.47 ± 10.40	0.56 ± 0.06
^{15}N urine	0.04 ± 0.03	5.41 ± 8.19	102.66 ± 56.96	108.07 ± 36.29	0.44 ± 0.03
^{15}N faeces plus ^{15}N urine	0.05 ± 0.02	2.48 ± 2.17	71.63 ± 24.98	74.11 ± 11.67	0.57 ± 0.07
^{14}N faeces plus ^{15}N urine	n. d.	0.69 ± 1.23	72.93 ± 29.65	73.59 ± 14.90	0.46 ± 0.11
^{14}N faeces plus ^{14}N urine	n. d.	6.39 ± 10.04	165.38 ± 33.68	171.77 ± 36.54	0.52 ± 0.09

higher in the top than in the bottom layer. Lowest N_{min} contents were found in the *pure peat* and ^{15}N *faeces* treatment.

3.10. DON

Cumulative DON export was similar in magnitude and dynamics among all treatments and ranged from 0.4 ± 0.03 up to 0.6 ± 0.07 g N m^{-2} over 21 days (Table 4). Among 0.04%–0.08% of the total N content of the columns (peat plus added excreta N) was lost by DON export. DON export was three to four times higher than the gaseous nitrogen loss as N_2O .

Fig. 6 gives an overview of the observed cumulative carbon and nitrogen export during the experiment.

4. Discussion

4.1. Carbon losses after excreta application on peat soil

We confirmed our first hypothesis that sheep excreta increase emissions of CO_2 from peat soil, but found no effect on CH_4 under aerobic conditions. Indeed, CO_2 emissions increased proportionally to the amount of carbon added as excreta. We rejected, however, our second hypothesis and found no stimulation of peat mineralization by excreta application.

The magnitude and dynamics of CO_2 emissions from excreta applied to peat soil in the microcosm experiment agree with previous findings. In a field experiment on Mongolian mineral soils, Ma et al. (2006) also observed no significant difference in CO_2 fluxes between plots with urine amendment and control plots, but between plots with faeces and control plots.

Mineralization of urine C to CO_2 was completed within a day whereas mineralization of faeces C would have continued for longer than the 21 days of our experiment. The 10% of faecal C mineralized within 21 days in the peat soil of our experiment is similar to that in mineral soils (Kristiansen et al., 2004), which seems to indicate that mineralization of faeces on the soil surface is not influenced strongly by the soil and its microbial community.

The CO_2 release from urea may have been entirely due to the physical process of hydrolysis or by a combination of physical and microbial processes. Around 80% of total N in sheep urine is present in the form of urea (Bristow et al., 1992), which dissociates in water into CO_2 and NH_3 . After excreta application, easily available C compounds are preferentially degraded by microorganisms. Subsequently more complex compounds are used according to their utilizability (urine derived > faeces derived > soil derived) (Kuzakov and Bol, 2006). As soon as the easily available substances are consumed, CO_2 fluxes decline to background level (*pure peat*) as observed in the ^{15}N *urine* treatment (Fig. 1d). As proposed by Blagodatskaya et al. (2009), the input of easily available substrate – here sheep urine – could have stimulated fast growing r-strategists whereas slowly available substrate – here sheep faeces – might

have shifted the microbial community to slow growing K-strategists. This could explain the different release patterns for total $\text{CO}_2\text{--C}$ in urine and faeces treatments (Fig. 1).

The incubated peat was a significant CO_2 source of about 1.15 g C m^{-2} d^{-1} . Although the most intensively rooted surface peat had been removed before extracting the soil cores the peat still contained the deeper roots of grasses and herbs. The CO_2 release from the incubated peat tended to be lower than from the same soil during field measurements (Leiber-Sauheitl et al., 2014). Ecosystem respiration measured at around 15°C in the grassland site ranged from 6 to 11 g C m^{-2} d^{-1} . About half of ecosystem respiration can be attributed to autotrophic respiration from the vegetation and the other half is soil respiration (Schulze et al., 2009). Plant roots, in turn, roughly contribute 35–50% to soil respiration (Silvola et al., 1996; von Arnold et al., 2005). Using these rough assumptions we can estimate a soil derived CO_2 source of 20–30% of measured ecosystem respiration, equivalent to 1.2–3.3 g C m^{-2} d^{-1} . The field site included the intensively rooted surface peat and may have been drier than the incubated peat, which likely explains its slightly higher soil derived CO_2 emission.

Peat mineralization is a much stronger CO_2 forming process than SOC mineralization in mineral soils. Adding relatively small amounts of extra carbon on the surface of the peat column as excreta may have little effect on microbial activity unless there are strong energy or nutrient limitations. The incubated peat (*pure peat*) emitted 39 mg C kg^{-1} d^{-1} in aerobic conditions. The CO_2 release is higher than in anaerobically incubated peat, where topsoil layer peat and peat with roots emitted CO_2 of up to 28 mg C kg^{-1} d^{-1} (Hahn-Schoefl et al., 2011). Decomposition is much faster in aerobic than in anaerobic conditions. The peat in our incubation seems relatively inert and thus could be expected to have the potential for positive priming by fresh carbon sources. Nevertheless, we found no mechanism that would suggest a risk of increased peat mineralization by sheep excreta. In contrast to studies on mineral soils (Shand et al., 2002; Shand and Coutts, 2006), DOC loss was not enhanced by excreta application on peat (Fig. 6). Obviously, the degraded peat was the prevailing source of the massive DOC loss in our experiment.

4.2. Nitrogen losses after excreta application on peat soil

We must reject our hypothesis that sheep excreta increase emissions of N_2O from peat soil, at least for the degraded peat soil in our study, but cannot rule out a risk of elevated N_2O emissions. In contrast, we found a source shift of N_2O from peat to excreta derived nitrogen.

N_2O emission levels in our incubation exceeded emissions observed in the field after urine (Clough et al., 1996) and slurry application (Eickenscheidt et al., 2014). We observed high background N_2O emissions from the peat (Fig. 6). This contrasts with these two field studies and with the field observations at the

grassland site from which the incubated soil originated. At this site mean and maximum field N_2O emissions were $3.4 \times 10^{-4} \text{ g N m}^{-2} \text{ d}^{-1}$ and $2.2 \times 10^{-3} \text{ g N m}^{-2} \text{ d}^{-1}$, respectively (Leiber-Sauheitl et al., 2014). N_2O emission levels in our incubation are in the upper range observed in field studies of peat grassland soils (Leppelt et al., 2014).

Acid soils like our peat soil have high $\text{N}_2\text{O}/\text{N}_2$ production ratios and thus high potential N_2O emissions (Mørkved et al., 2007; Liu et al., 2010). The peat substrate in our incubation, however, had a C/N ratio of 29, which is close to the threshold of 30–35 for very low N_2O formation (Leppelt et al., 2014). Thus, soil properties are inconclusive for explaining the relatively high N_2O emission levels in the incubation.

N_{min} content in the incubation experiment exceeded the values found in the field, which were almost exclusively present as ammonium as well (Leiber-Sauheitl et al., 2014). Absence of plants, and consequently no plant N-uptake, might have resulted in a stronger N_{min} accumulation. The laboratory results highlight that a peat soil can quickly switch from low to high N_2O emissions when N_{min} contents rise and environmental conditions are favorable for N_2O producing processes of the N cycle, such as denitrification or nitrifier denitrification.

Urine application indicated a risk of elevated N_2O emissions, but the variability in the treatments was too high for a robust statement. The source partitioning, however, clearly indicated a source shift from peat to urine in N_2O formation. A similar, but less pronounced shift in N substrate was induced by faeces.

The fraction of N_2O –N emitted per unit of applied N is defined as the emission factor (IPCC, 2006). The source partitioning traced by isotopic label resulted in extreme emission factors, which exceed default emission factor of IPCC (2006) of 2% of N as N_2O from grazing animal excreta by an order of magnitude. Field studies on mineral soils estimated an emission factor of 0.4% of N from cattle dung patches and of <0.1–3.8%, with 1.5% as overall mean for urine (Oenema et al., 1997). After the application of cattle excreta to a Scottish grassland on mineral soil, Bell et al. (2015) reported N emission factors of urine (1.1%) and dung (0.2%) in summer which were in all experiments lower than the IPCC default of 2%. N emission factors smaller than 1% were also found for cattle slurry application on organic soils (Eickenscheidt et al., 2014). This strong discrepancy in emission factors, however, is no contradiction. The field studies and the IPCC emission factors refer to apparent N application effects by comparing total emissions from sites with excreta with emissions from control sites. This approach ignores the substrate shift from soil N to excreta N, which was the main effect in our experiment. Calculating apparent emission factors in line with IPCC (2006) results in near zero emission factors in our experiment as well.

4.3. Priming effects for C and N

We reject our priming hypothesis and found instead no or negative carbon and nitrogen priming in peat soil by sheep excreta.

CO_2 from excreta was additive to CO_2 from the peat with no obvious interaction. We found, however, a clear source shift from soil to sheep excreta. This finding contrasts with results for a mineral soil. Ma et al. (2013) found a positive priming effect on soil C decomposition by sheep faeces application on two Mongolian grassland soils. The contrasting effects could be a consequence of dryer conditions in the Mongolian study, carbon limitation of decomposition or a different microbial community in the mineral soil as opposed to the degraded peat soil in our study.

The C addition to peat soil was a mixture of pulse inputs (easily degradable substances in faeces and urine such as urea or amino acids) and continuous inputs (more recalcitrant substances in

faeces such as lignin) as defined by Kuzyakov (2010). In a first step bacteria decompose easily degradable substances whereas afterward fungi produce extracellular enzymes to degrade more stable soil organic matter (Kuzyakov, 2010). Fungi would stimulate positive priming effects while bacteria would induce no or negative priming by preferential feeding on energy-rich substrate. Given the methodological difficulty of extracting microbes from peat soil we could not distinguish bacteria from fungi. Nevertheless our findings suggest that the excreta have stimulated bacterial activity, which preferentially fed on easily degradable substances, and bacterial nitrification and denitrification. Of course, a non-negligible number of microorganisms were also added with the excreta, in particular with the faeces (Kristiansen et al., 2004; Ritz et al., 2004).

4.4. Field application of lab results

Sheep grazing is often conducted on peatlands protected for nature conservation with a low stocking density. The surface area of a sheep faeces patch is roughly below 0.005 m^2 and that of a sheep urine patch (wetted area) about 0.03 m^2 (=one excretion event; Oenema et al., 1997). A typical stocking density of 0.5 livestock units per hectare, equivalent to 5 ewes would result in about $50 \text{ kg ha}^{-1} \text{ a}^{-1}$ of N and $750 \text{ kg ha}^{-1} \text{ a}^{-1}$ of C input by excreta (Haenel et al., 2014). During one sheep excreta application event in our experiment, 34% of N as urine and 66% as faeces were added (Table 2 and Fig. 6). Jones and Woodmansee (1979) found 70% of the total excreted N in urine and 30% in faeces. With a recommended maximal herd size of 1100 sheep (Germer, 2006), an area of 33 m^2 and 5.5 m^2 is covered with urine and faeces patches, respectively. Urine and faeces would affect 3.3% and 0.5% per grazed hectare, respectively. Possible, but according to our results negligible priming effects would be constrained to a small area.

Compaction by sheep trampling may help conserve the peat. In a laboratory study the presence of sheep trampling led to smaller CO_2 fluxes independent of the trampling intensity (Clay and Worrall, 2013). This came from the damaged vegetation and from an increase in surface bulk density which affected the connectivity of the pore space. It is to be expected that soil conditions become more anaerobic and therefore peat is less degraded. Soil compaction by trampling could, however, cause increased wetness, which in turn may increase N_2O or CH_4 emissions. This was not tested in the microcosms. Compaction mainly occurs when stocking density is high and long periods of grazing are conducted on a grassland on wet peat soil. However, this risk is minimized by grazing recommendations on grasslands in nature conservation areas where low stocking density and short periods of grazing are mandatory.

As we already indicated for priming effects low stocking densities minimize impact of sheep excreta to a small area. Therefore, the risk of increased N_2O and CH_4 emissions due to water logging should also be constrained to a small area. Consequently, wetness induced by compaction should not alter our conclusions.

Worrall and Clay (2012) have developed a model, which uses submodels of the physical impact of grazing, biomass production, energy usage in sheep, and peat accumulation in combination with IPCC emission factors (IPCC, 2006) to estimate the greenhouse balance in a near-natural peatland in dependence of grazing intensity. They estimated an average GHG emission from the peat soil of about $166 \text{ kg CO}_2\text{-eq. yr}^{-1} \text{ ewe}^{-1}$. Their results indicate that, depending on the GHG sink of the peat soil, grazing intensities between 0.2 and 1.7 ewes ha^{-1} can result in a neutral greenhouse balance. In Germany and the Netherlands, however, peat soils are usually drained for forage production and grazing. In such a situation where peat is constantly lost by aerobic decomposition, the greenhouse gas balance of sheep grazing on drained peat is always a net CO_2 source.

So far, there are no comparable field experiments that would allow immediate conclusions for practice. Nevertheless, the mechanisms and trends we observed agree with field observations on peat soils in other studies and their interpretation, but disagree with findings in mineral soils. We therefore suggest that our laboratory results can be generalized for grazed peat soils.

In total, sheep grazing will slow down peat soil degradation by small negative carbon priming and compaction by trampling.

5. Conclusions

In the microcosm experiment, sheep excreta did enhance CO₂ emissions proportionally to the amount and type of C added. In contrast, N₂O and CH₄ emissions were not affected by excreta addition. Methane emissions were very small and so not relevant during excreta decomposition. Sheep excreta caused no or small negative priming on peat decomposition and led to a source shift of the nitrogen released as N₂O from peat to labile N forms in excreta. We found no mechanism that would suggest a risk of increased peat mineralization by sheep excreta. Sheep excreta cover a small fraction of the grazed area and exert no or slightly negative carbon priming. Sheep also compact the soil by trampling. Overall, sheep grazing can be expected to slow down the degradation of drained peat soil.

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at <http://dx.doi.org/10.1016/j.soilbio.2015.06.001>.

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