



Deep litter removal frequency rate influences on greenhouse gas emissions from barns for beef heifers and from manure stores



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ABSTRACT

The emission of greenhouses gases (GHG) from ruminant production systems needs to be reduced. This can be achieved partly by better manure management, particularly for deep litter (DL) systems. Two contrasting removal frequency rates (1×, every 63.5 ± 3.5 days; and 3×, every 23.1 ± 1.5 days) were compared in a DL system for Belgian blue double-muscled heifers, focusing on CO₂, CH₄ and N₂O emissions from the barn and during two manure storage periods, one mainly in autumn and the other mainly in winter. No significant effect ($p = 0.447$) of manure removal frequency on total GHG emissions was observed (1×: 10.2 ± 3.5; 3×: 8.7 ± 2.2 kg CO₂ eq. kg⁻¹ live weight gain). The manure contributed significantly to total GHG emissions (average of 38.9 ± 8.0% of CO₂ eq.), emissions from the barn (4.0 ± 0.7%) and manure store included (34.9 ± 8.7%). Higher emissions (time 4.8 in CO₂ eq.) from manure were observed when it was stored during the warmer period than the colder one. Large variations in emission pattern with the manure removal frequency rates were also observed, leading potentially (not measured) to higher emissions from the 1× treatment than the 3× treatment for a longer storage period than the one tested in this experiment (63 ± 1 days). Given the experimental choices, the variations in emission pattern observed indicated that mitigation options for GHG emissions from the barn and manure store related to manure removal frequency depend on manure storage duration and that keeping deep litter manure in barns without intermediate storage before spreading should be investigated. These options need to be confirmed through emission measurement during and after manure spreading in order to avoid a trade-off between emission stages. The relevance of such options in terms of agronomical concerns needs to be confirmed.

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1. Introduction

The effects of human activities on climate change through greenhouse gas (GHG) emissions have been internationally recognised (Intergovernmental Panel on Climate Change and Stocker, 2014) and are the subject of global agreements (UNFCCC, 2015). Estimations indicate that agriculture, forestry, land use and land-use change are the source of about 25% of the GHG globally; about 50% of this is from agriculture (Smith et al., 2014), with nitrous oxide (N₂O) and methane (CH₄) being the main contributing gases. In Europe, 45% of the total emissions come from animal husbandry. About 70% of these animal-related emissions originate

from cattle systems and 25% from manure in barns or in storage (Freibauer, 2003). N₂O and CH₄ emissions from animal husbandry depend on the microbiological degradation of organic matter. The processes leading to these GHG emissions, however, are influenced by many factors, including O₂ availability, temperature, pH and the amount and characteristics of degradable organic matter (Webb et al., 2012). The diversity of agricultural practices used in herd or manure management is associated with variations in these factors, leading potentially to large differences in GHG emissions from animal husbandry. In a cattle barn, the main source of GHG is the animal itself through the direct emission of CH₄ due to enteric fermentation (Amon et al., 2001; Olesen et al., 2006). Apart from these direct emissions, however, gaseous compounds are also released by manure in the barn, as well as during its storage outside the barn and during or after spreading (Petersen et al., 2013; Snell et al., 2003; Webb et al., 2012; Zhang et al., 2005). The

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importance of considering both barn and storage emissions has been stressed by Külling et al. (2002).

In Wallonia, Belgium, beef and dairy cattle rearing produces mainly solid manure (more than 50%, as indicated by the N distribution in manure (NIR, 2015)). So far as we know (no inventory available), a large proportion of the solid manure is deep litter manure (DL) that is present in facilities for all calves, all fattening bulls and many dairy cows and suckler cows. In this particular manure management system, “faeces or droppings and urine are mixed with large amounts of bedding (e.g., straw, sawdust, wood shavings) and accumulated over a certain time on the floors of buildings housing any type of livestock or poultry” (Pain and Menzi, 2003) before being spread or stored prior to spreading. In DL systems: (1) a large amount of bedding material can be supplied (e.g., 2.9 kg of straw per m² per day; Kapuinen, 2001a), increasing the potential amount of nutrients (e.g., C and N) lost as environmentally damaging compounds; and (2) the particular manure characteristics influence organic matter decomposition, at least in the barn, resulting in self-heating, with the manure therefore reaching high temperatures (up to 60 °C; Kapuinen, 2001b), which is related to variations in gaseous emissions from the manure (Husted, 1994; Webb et al., 2012). High emissions of CH₄, N₂O, NH₃ and CO₂, or a trade-off between the emissions of these gases in the barn and during storage, are therefore likely and can be modified through manure management. Choices such as (1) amount of organic material added as litter, (2) frequency of manure removal from the barn and (3) type of bedding material (Petersen et al., 2013) therefore need to be investigated with regard to their impact on manure characteristics and the gaseous emissions from those systems, ideally including the barn, storage and spreading stages. For manure storage outside the barn, it has been reported that emissions of N₂O and CH₄ (Chadwick, 2005; El kader et al., 2007; Webb et al., 2012) from solid manure are a function of manure management that modifies degradation processes, depending on external conditions (e.g., ambient temperature) or manure characteristics (e.g., density, chemical composition). These management options include: amount of straw supplied (Yamulki, 2006); composting (Amon et al., 2001; El kader et al., 2007); and solid manure covering and compaction (Chadwick, 2005). Other management systems leading to seasonal changes in storage ambient conditions and the resulting variations in emissions (Husted, 1994; Mathot et al., 2012) need to be explored at regional level and to include particular climate and management conditions in order to identify efficient GHG emission mitigation options (Sommer et al., 2009). In this respect, in the DL system, as suggested by emission factors

from the national GHG inventory (IPCC, 2006), the frequency of removal of solid manure from the barn should be investigated. More information is also needed on CO₂ emissions from solid manure in the barn in order to use total CO₂ emissions as tracer gas for estimating air flows in naturally ventilated barns (Ngwabie et al., 2009).

We set up an experiment that sought to measure N₂O, CH₄ and CO₂ emissions in a barn and during manure storage from beef heifers raised in a DL system (fully strawed barn), based on two removal frequency rates of the manure accumulated in the barn during two climatic periods. The aim of the experiment was to observe the effects of simple manure management options on GHG emissions in DL systems. Variable manure removal frequency could influence manure characteristics and storage conditions and therefore potentially modify GHG emissions. Nutrient flows were also studied in order to validate the observations.

2. Materials and method

This experiment was performed at the same time (and followed the same procedure) as those described by Mathot et al. (2012). Their paper provides a full description of the materials and method. Only the main principles and the particularities of the present experiment are reported here.

The trial was conducted during the 2009–2010 cattle housing period at Libramont (49°55′43″N; 5°21′37″E; altitude 487 m) in Belgium in experimental barns (Fig. 1). The aim was to test the effect of two rates (1× and 3×) of the removal of deep litter manure (DLM) in an experimental DL barn system on GHG emissions and nutrient cycling. Typically, this type of barn is characterised by the accumulation of solid manure below the animals for a fairly long period (up to 6 months), followed by the total removal of the DLM and its storage before it is spread on soil.

The trial was performed over two periods: P1 and P2. During these periods, we raised Belgian blue double-muscle heifers fed with an identical concentrate-rich diet. P1 began in autumn, on 16 November, and P2 in winter, on 8 February, with the solid manure stored outside mainly during the winter in P1 and during the spring in P2 (Fig. 2).

2.1. Barn and storage facilities

The trials were conducted in two experimental loose barns, measuring 25 m², with a fully strawed area (16.2 m²) (Fig. 1). The barns were air tight and mechanically ventilated (1030 m³/h), with regulation and flow measurements as described by Mathot et al.

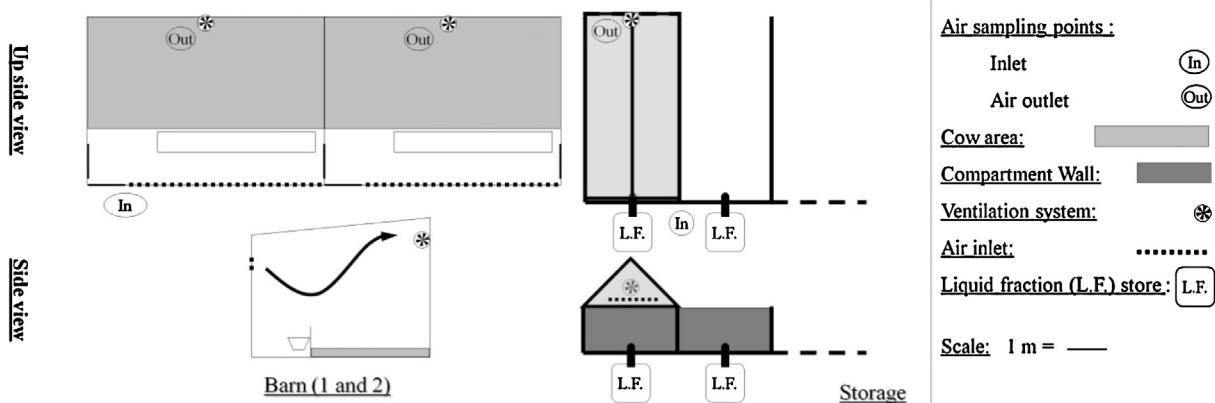


Fig. 1. Barn and manure storage facilities representation. The solid manure storage facility had four compartments, one for each treatment × period (Fig. 2), with a surface area of 11.4 m². The compartments were delimited by a 1.2 m-high concrete wall and were each equipped with a liquid fraction collecting system (1 m³ tank).

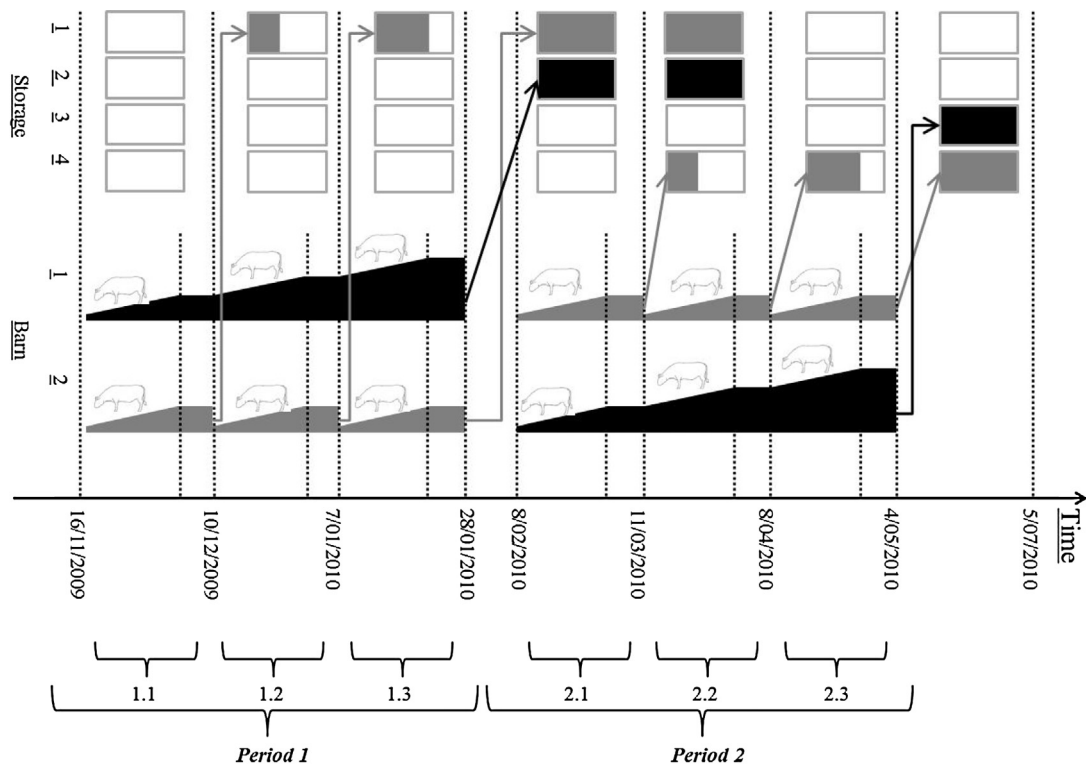


Fig. 2. Timeline of the experiment and location of the manure as a function of the treatments (1×: one removal during the experimental phase, in black; 3×: three removals, in grey).

(2012), representing air renewal every 4.8 min. The solid manure was retained by a wooden beam and the trough was elevated in line with the accumulated manure height in order to facilitate feeding for the cattle.

2.2. Cattle characteristics and feeding

Two groups of four Belgian blue double-musled heifers, obtained from a commercial farm, were constituted and each group was assigned to an experimental barn. The groups were identical in terms of average weight (about 350 kg) and age (15 ± 0.7 months) at the beginning of the experiment (Table 1). The heifers were fed *ad libitum* with a diet composed, on a dry matter (DM) basis, of 44.4% grass silage and 55.6% commercial concentrate for young cattle. The diet (Table 2) was balanced to meet cattle requirements as detailed in the Dutch bovine feeding system (Ministry of Agriculture, 1990; Tamminga et al., 1994; Van Vliet, 1997). Feed was supplied once a day (9 am). The heifers were

familiarised with their feed over 14 days before the beginning of the trial. They were kept in the experimental barns until the removal of the manure, except on days when only manure gaseous emissions were measured in the barns; on those days, the heifers were kept in an adjacent commercial barn, in their two groups, and continued to receive the experimental diet.

2.3. Manure management

Two contrasting manure management systems were compared. In the first one, we removed the manure three times (3×) during each period of the trial (i.e., about every 20 days). In the second one, we removed the manure only once (1×), at the end of the trial (Fig. 2). The two trial periods (P1 and P2) were therefore each divided in three sub-periods. Period durations were chosen so that the trial could be conducted over one winter with the same animals. This ensured that in the 1× treatment, enough manure (at least 30 cm) accumulated below the animals, thus stabilizing its

Table 1
Starting dates, manure removal dates, duration of manure accumulation in the barns and in storage compartments, animal weight (mean (standard error of the mean), $n=4$), cattle barn/group attribution as a function of treatment (Treat.), period (Per.) and manure removal (Rem.).

Treat.	Per.	Rem.	Starting date	Barn #/cattle group	Cattle ($n=4$) initial live weight (kg head ⁻¹)	Duration of accumulation in the barn (days)	Manure removal (date)	Storage duration (days)
1X	1	1	16/11/2009	1/A	349 ± 13	66	28/01/2010	64
		1	8/02/2010	2/B	438 ± 12	73	4/05/2010	62
3X	1	1	16/11/2009	2/B	347 ± 11	22	10/12/2009	113
		2				26	7/01/2010	85
		3				18	28/01/2010	64
	2	1	8/02/2010	1/A	441 ± 12	29	11/03/2010	116
		2				26	8/04/2010	88
		3				18	4/05/2010	62

Table 2

Diet composition and characteristics (mean (standard error of the mean, n=4)).

Diet characteristics									
DM (g/kg)	Ash (g kg ⁻¹ DM)	dOM	CP	DVE	VEM	OEB	NDF	ADF	ADL
703 ± 4.4	95.2 ± 2.7	697.4 ± 2.7	147.5 ± 1.0	77.9 ± 2.9	918.8 ± 8.2	18.0 ± 0.9	395.1 ± 7.6	240.9 ± 4.1	32.7 ± 0.3

Diet composed of 44.4% grass silage and 55.6% concentrate on a DM basis. DM, dry matter; CP, crude protein (Kjeldahl N × 6.25); dOM, digestible organic matter; NDF, ADF and ADL, the fibre fractions (derived from Van Soest et al., 1991); DVE, digestible feed and microbial true protein in the small intestine; VEM, energy; OEB, degraded protein balance (nutritive value of forage, following the Dutch system; Van Vliet, 1997 and Tamminga et al., 1994) determined using NIRS predictions as proposed by De Boever et al. (1996).

temperature, after a period of increase (Kapuinen, 2001b), whereas, in the sub-periods, corresponding to manure accumulation in the 3× treatment, with a manure height of 15–20 cm (Kapuinen, 2001b), temperature did not increase too much. Temperature rises signal the beginning of organic matter degradation processes that could lead to large and variable gaseous emissions. At the end of each sub-period, the animals were removed from the barns for 2 days in order to measure the gaseous emissions from the manure alone in the barns on the first day and then remove that manure on the second day. Once removed, the solid manure was stored in a concrete compartment (Fig. 1). The manure from 3× was stored in one compartment for each trial period, with freshly removed manure added to the already stored manure. For both treatments, the manure was kept in these storage facilities for 64 and 62 days after the last removal of solid manure from the barns for P1 and P2, respectively (Table 1 and Fig. 2). Straw was supplied daily, with a target rate of 1 kg 100 kg⁻¹ animal live weight (LW) except for the first 3 days in the empty barn, sub-period included, when it was supplied at a rate of 2 kg 100 kg⁻¹ animal LW. The daily animal LW and live weight gain (LWG) were estimated by weighing them at the beginning and end of the periods.

2.4. Data collection for balance calculation

The procedures used for characterising the manure in storage (weight, temperature, density and composition), the feed (weight and composition) and the LWG (weight and composition) and for calculating the N and C balances were the same as those used by Mathot et al. (2012). The bulk density of the manure in the barns was estimated by dividing the solid manure weight by its measured volume. The volume was calculated by multiplying the area by the mean thickness of the manure layer measured at 12 points. The solid manure temperature in the barns was also measured at 12 points (30 cm deep) at the end of each sub-period. The input (In) of the system (cattle intake + straw) and the output of the barns (Out 1: DLM and LWG) and of the entire system (barn+storage) (Out 2: DLM, LWG and liquid fraction) were calculated in order to estimate the overall and intermediate element balance (Table 4).

The N and C balances were calculated in the barns, in storage and for the whole system (barn + storage), as described in Table 3, using the mass flow approach (Haas et al., 2002).

2.5. Gas emission measurement

2.5.1. Barns

Gas emissions were calculated on a daily basis by summing the hourly emissions. The hourly emissions were calculated by subtracting the incoming gas from the outgoing gas from the barns. Incoming and outgoing gases were calculated on an hourly basis using averaged (n=4) gas concentrations multiplied by the average air fluxes measured with the full size anemometer in the mechanical ventilation system (Fancom[®]) and corrected for air density. The concentrations of a given gas were measured with a 1312 photoacoustic multi-gas analyser (Lumasense Technologies SA, Ballerup, Denmark) configured as described by Mathot et al. (2012). Total emissions in the barns with cattle were calculated using the relationships between emissions expressed per barn per day and time (days) when the cattle were inside the barn (Fig. 3). The integration over time of this relationship gave the total amount of emissions from the cattle over the trial periods. In order to estimate the relative proportion of emissions from the cattle and manure separately in the barns, in both treatments, the gas emissions were measured using the same approach as that used when the cattle were in the barns. The manure emissions in these barns were therefore measured over 2 consecutive days, three times per trial period, with the last measurement just before manure removal. The daily emissions from the manure alone were averaged, and this value was multiplied by the number of days of the experiment with the cattle in the barns (Fig. 3). This value was then subtracted from the total emissions with manure and cattle in order to estimate the CO₂ and CH₄ emissions due to the cattle. For the N and C balances at barn level, emissions on days with manure alone in the barns were added to the emissions when the cattle were in the barns.

2.5.2. Solid manure storage

During manure storage, gaseous (CO₂, CH₄, N₂O) emissions were measured using the system and methodology described by

Table 3

Balance calculation equations and abbreviations.

Parameter		Formula
Barn and system input (In)	=	straw + ingestion (feed input – feed refusal)
Barn output (Out 1)	=	solid manure (barn) + cattle live weight gain
System output (Out 2)	=	solid manure (storage) + liquid fraction (storage) + live weight gain (barn).
Barn losses	=	In – Out 1
Storage losses	=	Out 1 – Out 2
System losses	=	In – Out2

Table 4
Cattle, input (straw and feed) and manure characteristics as a function of treatment and trial period (x = mean; sem = standard error of the mean).

			1× ^f				3×				Treatment
			P1 ^g		P2		P1		P2		p ^h
			x	sem	x	sem	x	sem	x	sem	
Cattle ^a	Age	(day)	510	43	628	69	540	69	598	43	0.980
	LW ^c	(kg head ⁻¹)	385	20	479	27	381	19	479	18	0.500
	LWG ^d	(kg)	247		286		247		300		0.500
In	Straw	(g DM kg ⁻¹ LWG)	3.3		4.1		3.6		4.1		0.519
	FC ^e	(kg DMI kg ⁻¹ LWG)	8.0		9.7		8.0		9.4		0.588
DLM ^b (Out 1)		(kg 100 kg ⁻¹ LW d ⁻¹)	5.77		5.31		6.14		5.96		0.171
	DM	(g kg ⁻¹ FM)	235	5	275	9	244		273		0.642
	pH		8.15	0.17	7.87	0.11	8.57		8.02		0.275
	Ash	(g kg ⁻¹ DM)	157	4	145	4	156		147		0.668
	C	(g kg ⁻¹ DM)	428	2	434	2	429		433		0.668
	N	(g kg ⁻¹ DM)	30.6	1.7	29.7	2.2	28.3		27.9		0.075
	N-NH3	(g kg ⁻¹ DM)	5.29	0.63	5.72	0.66	4.16		5.87		0.586
	C/N		14.2	0.8	15.1	0.9	15.4		15.7		0.199
	DLM (Out 2)	(kg 100 kg ⁻¹ LW d ⁻¹)	5.39		3.71		5.24		3.20		0.318
	DM	(g kg ⁻¹ FM)	197	5	263	13	193	6	272	19	0.822
pH		8.09	0.09	8.60	0.04	8.15	0.11	8.62	0.05	0.580	
Ash	(g kg ⁻¹ DM)	184	7	233	15	235	22	268	12	0.011	
C	(g kg ⁻¹ DM)	415	4	390	8	389	11	372	6	0.011	
N	(g kg ⁻¹ DM)	29.5	1.2	33.9	2.2	31.5	2.0	35.6	4.9	0.512	
N-NH3	(g kg ⁻¹ DM)	4.53	0.60	3.92	0.76	3.16	0.40	2.42	0.48	0.017	
C/N		14.2	0.6	11.8	0.7	12.8	1.0	11.9	1.8	0.536	
LF (Out 2)	(kg 100 kg ⁻¹ LW d ⁻¹)	1.43		0.21		1.89		0.32		0.351	

^a For cattle, when the sem represents the variability among the animals (n=4).

^b For manure, the sem represents the variability among the samples (n=8).

^c LW: live weight.

^d LWG: live weight gain over the whole period. DMI: dry matter ingested by the heifers. DLM: deep litter manure, Out 1: removed from the barn, Out 2: after the outside storage period. LF: liquid fraction released from the DLM stored outside.

^e FC: feed conversion ratio.

^f 1× and 3× are respectively the less (1 time) or more (3 times) frequent manure removal frequency rates from the barn.

^g P1 and P2 are the two periods when the trials were conducted.

^h Result as p value of the statistical analysis for the treatment effect.

Mathot et al. (2012). In brief, it was based on large dynamic chambers, each completely covering a concrete storage compartment (Fig. 1). Measurements were taken every 3.7 days, on average. Total emissions were calculated using the same procedure as for the barns. The trapezoidal rule was used to estimate daily emissions on days without measurements. Emissions over the period were calculated by adding up the daily emissions of the manure heap under consideration. Between measurement periods, the measuring hoods were removed and the solid manure heaps remained uncovered in order to be exposed to normal temperature, rainfall and humidity conditions.

2.6. Data and statistical analysis

The GHG emissions were calculated in terms of CO₂ equivalent (CO₂ eq.) by summing N₂O and CH₄ emissions multiplied by their global warming potential over 100 years (298 and 34, respectively; Intergovernmental Panel on Climate Change and Stocker, 2014).

The results were related to the total animal LWG produced in the barns in order to prevent cattle performance interfering with the treatment comparison and to reflect the efficiency of the system in producing LW. The LWG values used were the total LWG values calculated for the whole period. The LWG on days when the cattle were outside the barns (for measurement of emissions from manure alone) was subtracted, using average daily gain calculated over the whole period. Emissions were also presented per head per day in order to observe the emission dynamic over time.

Two-ways analysis of variance (ANOVA) procedures were used to test the effects of the treatments (A) as fixed factors, with period

(B) as the random factor in the observations (Y). As there was no repetition, the treatment effects were tested against the interaction period × treatment (E) as the basis of comparison, as recommended by Dagnelie (2011), using the model $Y_{ij} = M + A_i + B_j + E_{ij}$, where M is the average of the observations.

The averaged values were presented with the standard error of the mean and, where necessary, the number of data used for their calculations (\pm sem, n).

3. Results

3.1. Animal performance, manure production and composition

No significant effect ($p > 0.05$) of the manure removal frequency was observed on animal performance, feed conversion ratio (FC), straw supply, fresh (Out 1) solid manure amount or solid manure composition (Table 4). Animal LWG was similar for both systems, with an average over the two periods and treatments of 270 ± 19 g $100 \text{ kg}^{-1} \text{ LW d}^{-1}$. Straw was supplied at a rate of 851 ± 18 g $100 \text{ kg}^{-1} \text{ LW d}^{-1}$. Despite the slightly higher amount of straw in 3× in order to provide adequate conditions for cattle to lie down, there was no effect of manure management on the level of straw input per unit of LWG ($p = 0.519$, Table 4). This was probably due to the lower additional amount in 3× than in 1× and the slight variation observed in animal LW between treatments. This amount of straw was similar to that used in a previous study by Jeppsson (1999) on DLM systems ($830 \text{ g DM } 100 \text{ kg}^{-1} \text{ LW d}^{-1}$), but very different to that used by Kapuinen (2001a) (870 ± 60 vs $2.900 \text{ kg DM m}^{-2} \text{ d}^{-1}$). Some differences were observed for the solid manure composition

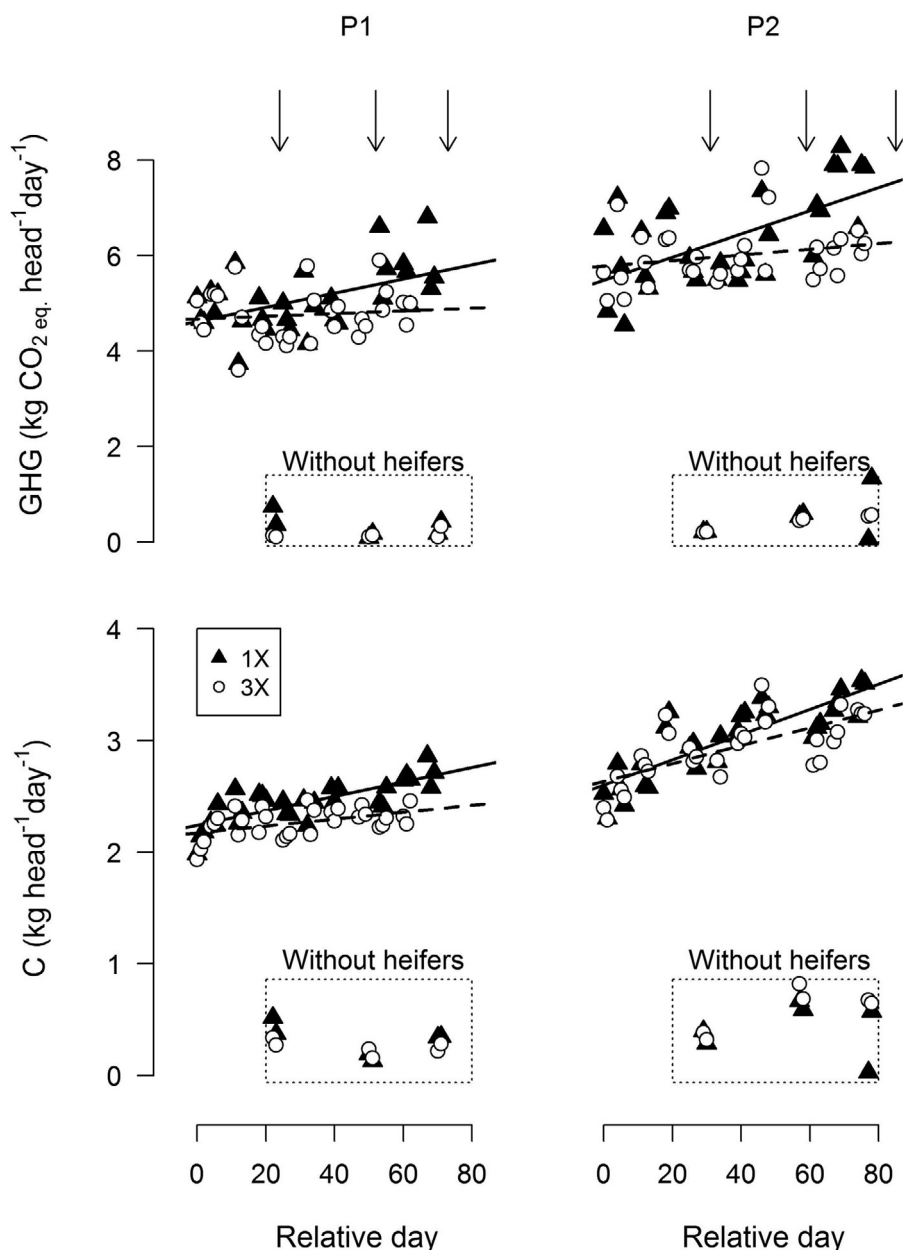


Fig. 3. GHG and C emissions ($\text{kg head}^{-1} \text{d}^{-1}$) in the barns by treatment and period as a function of the relative time since the beginning of the period. 1 \times and 3 \times are respectively the less (1 time) or more (3 times) frequent manure removal frequency rates from the barn. P1 and P2 are the two periods when the trials were conducted. Linear regressions were calculated by considering only measurements with the heifers in the barns. The measurements of emissions without the heifers in the barns are in the dotted border boxes. \blacktriangle and $-$ relate to 1 \times , and \circ and $--$ relate to 3 \times . Linear regression characteristics for GES were: 1XP1, $4.62 + 0.0148 \times x = y$, $p = 0.004$; 3XP1, $4.68 + 0.0028 \times x = y$, $p = 0.575$; 1XP2, $5.48 + 0.0242 \times x = y$, $p < 0.001$; and 3XP2, $5.77 + 0.0044 \times x = y$, $p = 0.193$. Linear regression characteristics for C were 1XP1, $2.24 + 0.0064 \times x = y$, $p < 0.001$; 3XP1, $2.17 + 0.0031 \times x = y$, $p < 0.001$; 1XP2, $2.60 + 0.0112 \times x = y$, $p < 0.01$; and 3XP2, $2.64 + 0.0080 \times x = y$, $p < 0.001$.

at the end of the trial (Out 2), with higher C and total ammoniacal N (TAN) concentrations and lower ash concentrations in the 1 \times treatment ($p < 0.05$).

3.2. Greenhouse gas emissions

3.2.1. Emissions in the barns

CO_2 , N_2O and CH_4 emission were measured regularly in the barn, with or without cattle, and in the manure store throughout the experiment in order to calculate total C and greenhouse gas emissions from the whole system and from manure and cattle separately.

3.2.1.1. With heifers. There was a significant ($p < 0.05$) increase in C emissions per heifer over time in the barns and higher emissions in P2 than in P1 (Fig. 3). For GHG, the trend was less clear, with a significant relationship only between emissions at barn level and time for the 1 \times treatment in both periods ($p < 0.05$). With regard to LWG, there was no significant difference between N_2O , CH_4 or total GHG emissions with manure management (Table 5); on average, however, CO_2 emissions ($8.1 \pm 0.1\%$) were higher ($p < 0.05$) for 1 \times than for 3 \times . For both treatments, GHG emissions were due mainly to CH_4 emissions (i.e., 1 \times : $99.0 \pm 0.6\%$; 3 \times : $99.1 \pm 0.2\%$), whereas total C emissions were due mainly to CO_2 emissions (i.e., 1 \times : $95.4 \pm 0.1\%$; 3 \times : $95.5 \pm 0.1\%$).

Table 5

Gaseous emissions in the barns, in storage and in total, nutrient balance and key ambient (temperature) and manure parameters (temperature, density).

			1× ^b		3×		Treat.
			P1 ^c	P2	P1	P2	p ^d
Barn	External temperature	(°C)	0.4	4.6	0.4	4.6	–
	Temperature in barn	(°C)	6.1	9.9	5.4	10.2	0.758
	DLM temperature ^a	(°C)	16.7 ± 1.7	22.7 ± 2.9	14.3 ± 0.5	25.5 ± 3.8	0.951
	DLM density ^a	(kg FM m ⁻³ DLM)	976	965	960	950	0.021
	C input (In)	(g kg ⁻¹ LWG)	5075	6160	5225	5992	0.964
	N input (In)	(g kg ⁻¹ LWG)	210	257	213	248	0.703
	N ₂ O (1) total	(g kg ⁻¹ LWG)	0.07	0.35	0.12	0.20	0.699
	CH ₄ (2) total	(g kg ⁻¹ LWG)	161	189	149	170	0.144
	CO ₂ total	(g kg ⁻¹ LWG)	9212	10821	8509	10030	0.038
	GHG (1 and 2) total	(g CO ₂ eq. kg ⁻¹ LWG)	5484	6536	5092	5843	0.172
	N ₂ O (1) manure	(g kg ⁻¹ LWG)	0.18	0.42	0.16	0.27	0.447
	CH ₄ (2) manure	(g kg ⁻¹ LWG)	8.77	4.65	8.32	9.41	0.604
	CO ₂ manure	(g kg ⁻¹ LWG)	1213	1235	1222	2074	0.493
	GHG (1 and 2) manure	(g CO ₂ eq. kg ⁻¹ LWG)	350	284	331	399	0.605
Storage	External temperature	(°C)	1.7	13.3	0.2	10.6	0.177
	DLM temperature ^a	(°C)	40.0	59.3	33.4	47.9	0.168
	DLM density	(kg FM m ⁻³ DLM)	578	643	811	775	0.172
	N ₂ O (1)	(g kg ⁻¹ LWG)	1.5	6.3	1.3	4.2	0.455
	CH ₄ (2)	(g kg ⁻¹ LWG)	24	160	29	113	0.737
	CO ₂	(g kg ⁻¹ LWG)	2054	4614	2809	6409	0.097
	GHG (1 and 2)	(g CO ₂ eq. kg ⁻¹ LWG)	1247	7300	1391	5115	0.542
	C output (Out2)	(g kg ⁻¹ LWG)	1989	2042	1781	1693	0.158
Total	N output (Out2)	(g kg ⁻¹ LWG)	167	197	167	180	0.523
	N ₂ O (1)	(g kg ⁻¹ LWG)	1.6	6.6	1.5	4.4	0.471
	CH ₄ (2)	(g kg ⁻¹ LWG)	184	349	178	283	0.437
	CO ₂	(g kg ⁻¹ LWG)	11266	15435	11318	16438	0.467
	GHG (1 and 2)	(g CO ₂ eq. kg ⁻¹ LWG)	6731	13837	6482	10958	0.445

^a Measured at each removal of the manure from the barns. For the 3× treatment, the density is the mean of measured values of the three removals.

^b 1× and 3× are respectively the less (1 time) or more (3 times) frequent manure removal frequency rates from the barn.

^c P1 and P2 are the two periods when the trials were conducted.

^d Result as p value of the statistical analysis for the treatment effect.

3.2.1.2. Without heifers. The emissions from the solid manure accumulated beneath the animals, quantified when the heifers were not in the barns, are shown in Fig. 3. They varied greatly, variation coefficients ranging from 39 to 96% for GHG and from 27 to 109% for C emissions across treatment × period values, but without ($p > 0.05$) any increase trend over time. The calculated total emissions from the manure in the barns were estimated, using a daily emission average, to be 0.26 ± 0.08 g N₂O kg⁻¹ LWG, 7.8 ± 1.5 g CH₄ kg⁻¹ LWG and 1436 ± 301 g CO₂ kg⁻¹ LWG, without any significant effect of manure management ($p > 0.05$). These emissions amounted to $158 \pm 28\%$ of the total N₂O, $4.7 \pm 0.8\%$ of the total CH₄ and $14.8 \pm 1.9\%$ of the total CO₂ emitted when the heifers were in the barns, without a treatment effect ($p > 0.480$). The GHG kg⁻¹ LWG emissions from manure alone were driven mainly by CH₄ ($76.6 \pm 10\%$), whereas for C emissions the main driver was CO₂ ($98.5 \pm 0.4\%$). Manure densities (Table 5) in the barns were quite high and differed significantly between treatments, with higher values for 1× (971 ± 6 kg of fresh DLM m⁻³) than for 3× (955 ± 5 kg of fresh DLM m⁻³). The DLM temperature did not change with treatment, averaging 19.8 ± 3.7 °C.

3.2.1.3. Heifers. Estimations of the direct CH₄ and CO₂ emissions from the cattle were based on the difference between total emissions at barn level and estimated total emissions by DLM alone (Table 4). Extrapolated to a yearly basis, the cattle emitted, on average, 56.4 ± 3.4 kg CH₄ head⁻¹. Using the methodology proposed by IPCC (2006), the fraction of gross energy in feed converted to methane (Y_m, in%) was, on average, $5.5 \pm 0.2\%$, without a manure management effect ($p > 0.05$).

3.2.2. Emissions from the solid manure store

Similar amounts of manure were produced (Table 4) and stored in the concrete facilities. Gaseous emissions from the heaps were

measured over 13–24 h day⁻¹ for 29 and 30 days for P1 and P2, respectively. The total emission amounts were not influenced by manure management ($p > 0.05$, Table 5). On average, the emissions were 3.33 ± 1.68 g N₂O kg⁻¹ LWG, 81 ± 47 g CH₄ kg⁻¹ LWG and 3971 ± 1377 g CO₂ kg⁻¹ LWG. For P2, however, the emissions were far higher than for P1, irrespective of the gas (on average, by a factor of: 3.7 ± 0.3 for N₂O; 5.3 ± 0.7 for CH₄ and 2.3 ± 0.1 for CO₂). The emissions varied strongly with time (Fig. 4). The highest CH₄ and CO₂ emissions were observed after a few days for CH₄ and directly after DLM deposition for CO₂ (Fig. 4) and they then decreased with time, apart from 1X P2. For that treatment × period, CH₄ emissions unexpectedly remained at a high level for a long period (62 days; i.e., until the end of the trial). No clear emission patterns were observed for N₂O. Methane was the main contributor to GHG emissions, accounting for 64.5%, 74.4%, 71.4% and 75.3% of these emissions, but contributing marginally to total C emissions, accounting for 3.1%, 8.7%, 2.8% and 4.6% of these emissions for 1× P1, 1× P2, 3× P1 and 3× P2, respectively.

3.2.3. Total emissions

The N₂O, CH₄ and CO₂ emissions ranged from 1.6 to 6.6 g kg⁻¹ LWG, from 184 to 349 g kg⁻¹ LWG and from 11.3 to 16.4 kg kg⁻¹ LWG, respectively (Table 5). Despite substantially more emissions of N₂O (factor of 1.28 ± 0.21), CH₄ (factor of $1.13 \pm 0.10\%$), and therefore GHG (factor of 1.15 ± 0.11), the gaseous emissions from 1× did not differ significantly from 3×. Emissions from the barns accounted for 5.7 ± 0.9 , 71.2 ± 8.3 , 72.0 ± 4.4 and $65.1 \pm 8.7\%$ of the total N₂O, CH₄, CO₂ and GHG emissions, whereas emissions from manure in the barns and in storage were estimated to account for 101–107% of the N₂O, 17.6–47.1% of the CH₄, 29.0–51.6% of the CO₂ and 23.7–54.8% of the total GHG emitted. For all gases and their combination in GHG, the emissions were higher in P2 than in P1 (from a factor of 1.4 ± 0.2 for CO₂ to a factor of 3.7 ± 1.0 for N₂O).

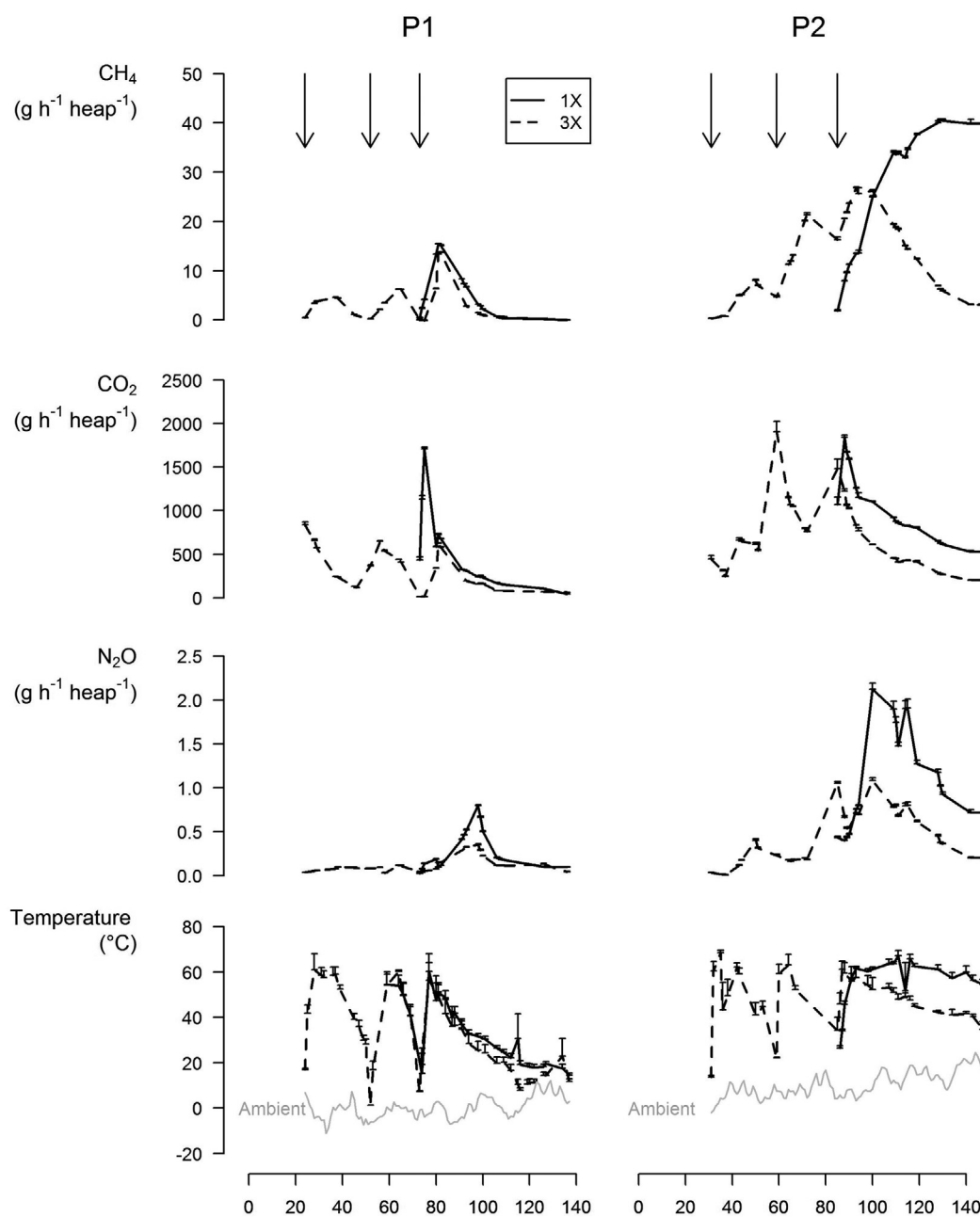


Fig. 4. Emissions of CH_4 , CO_2 and N_2O and heap temperature in DLM during storage by treatment \times period, as a function of time. 1 \times and 3 \times are respectively the less (1 time) or more (3 times) frequent manure removal frequency rates from the barn. P1 and P2 are the two periods when the trials were conducted. Arrows indicate the day of manure removal from the barns. Relative days are the number of days from the start of the experiment in the barns. Error bars represent the standard error of the mean of hourly emissions ($n = 13\text{--}24$). The grey line in the temperature figure is the mean external temperature.

3.3. Nitrogen and carbon balances

On average, straw for litter accounted for $10.4 \pm 0.2\%$ of N inputs and $31.1 \pm 0.4\%$ of C inputs in the systems (In). There was no significant effect of manure management on the C and N inputs and outputs, expressed per kg LWG ($p > 0.05$, Table 4). On average, the C and N inputs were 5613 ± 384 and $232 \pm 17 \text{ g kg}^{-1}$ LWG, respectively. The C and N outputs (Out 2) were 1876 ± 117 and $178 \pm 10 \text{ g kg}^{-1}$ LWG and the losses were 3737 ± 402 and $53.4 \pm 8.4 \text{ g kg}^{-1}$ LWG, respectively, calculated using mass balance (Table 3; i.e., emissions not considered). On average, about $46.5 \pm 2.1\%$ and $3.1 \pm 1.0\%$ of the C and N entering the system were lost at barn level and $66.3 \pm 3.2\%$ and $23.2 \pm 2.2\%$ during both stages (barn and

storage, Out 2), not taking manure management effects into account because not significant ($p > 0.05$). Manure management did, however, significantly ($p = 0.003$) influence the C losses/C stored ratio ($1\times = 0.32 \pm 0.08$; $3\times = 0.46 \pm 0.08$), but not ($p = 0.412$) the N losses/N stored ratio (0.24 ± 0.03) during DLM storage.

Finally, most of the C ($90 \pm 1\%$) and N ($79 \pm 2\%$) remaining in the system at Out 2 (animal LWG + liquid fraction + DLM) was in the DLM (Fig. 5). Only very small amounts of C and N migrated towards the manure liquid fraction, seeped from the storage facilities ($0.2 \pm 0.1\%$ and $1.2 \pm 0.6\%$, respectively), whereas animal LWG mobilized $3.3 \pm 0.1\%$ of C inputs and $14.7 \pm 1.0\%$ of N inputs. No manure management effect was observed in these distributions ($p > 0.05$).

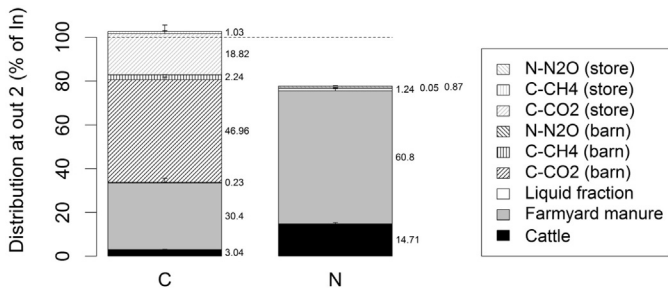


Fig. 5. Distribution of C and N at the end of the trial (Out 2), relative to the inputs.

4. Discussion

4.1. Emissions from the cattle

The estimated emissions from cattle alone (56.4 ± 3.4 kg CH_4 head $^{-1}$) extrapolated to one year were very close to 62 kg head $^{-1}$ year $^{-1}$, the value used in the national inventory report for bovine females older than 1 year (NIR, 2015). The Y_m calculated ($5.5 \pm 0.2\%$) corresponded to the Y_m (5.7%) reported for the same diet by Mathot et al. (2012) and was lower than the current value recommended for such animals in the inventory (6.5%; IPCC (2006)), but can be explained by the diet (Doreau et al., 2011) proposed for the animals.

4.2. High and variable emissions from manure

The emissions from manure were very variable and contributed significantly to the total emissions, mainly due to emissions during storage. The emissions during manure storage were far higher (factor of 16 ± 7 for CH_4 , 6.7 ± 2.4 for N_2O and 4.6 ± 1.1 for CO_2) than those observed by Mathot et al. (2012) in a tied stall system during an experiment conducted at the same time as ours, with similar animals receiving the same feed and using the same methodology. The only difference lay in the production of semi-solid manure instead of solid and accumulated manure. With regard to N_2O emissions, those from storage accounted for $1.14 \pm 0.35\%$ (0.52–2.02%) and, when emissions from the barns were included, for $1.22 \pm 0.36\%$ (0.61–2.12%) of the total N excreted as calculated according to N balance at cattle level (ingested-retained). These emission factors are similar to those commonly used for inventories (IPCC, 2006) considering solid storage (0.5%) or unmixed deep bedding (1%), including the uncertainty factor (time 2). For the storage period, when expressing the N_2O emissions as a percentage of N stored, they were between 0.48 and 1.88%, corresponding more to farmyard manure (0.0–2.3 N_2O as a percentage of N stored) than deep litter (0.0–0.3 N_2O as a percentage of N stored), according to the review conducted by Webb et al. (2012). As estimated, over the whole period, the emissions from the manure in the DL barn were not negligible compared with those from the store. For N_2O , CH_4 and CO_2 , they amounted to $9.3 \pm 1.6\%$, $19.5 \pm 7.9\%$, and $40.4 \pm 7.1\%$ of the emissions during storage respectively. In addition, they were stable over time showing that the rise in CH_4 emissions from the barn over time was not necessarily due to an increase in emissions from manure, but rather to variations in emissions from the cattle over time. When extrapolated for the whole barn period, the N_2O emissions from the manure alone in the barn were about 1.5 times higher than those from the barn with the cattle over the same period. This led to the ratio of total N_2O emissions from manure alone over the whole trial (barn and store) to N_2O emissions from manure and the barn with cattle being higher than 100% (see Section 3.2.3). This observation might cast doubt on the accuracy of

the results. There are at least two explanations for the difference in emissions with and without heifers in the barns. The first is the calculation method, which was based on measurements at the end of the period and thus supposed to be representative of the whole period. The second is the accuracy of N_2O gas concentration measuring technique, which had to measure very small (in the order of 10 ppb) increases in concentration compared with ambient concentration (about 300 ppb), considering its sensitivity to other gases in the barn (Hassouna et al., 2013).

For methane, 13.0 – 54.3 g CH_4 kg $^{-1}$ volatile solid excreted (VS) was emitted. The average value of 31.1 ± 10.1 g CH_4 kg $^{-1}$ VS was higher than the default value calculated for proposed emission factors by IPCC (2006) for deep bedding over a long accumulation period (>1 month at 10°C , 20.5 g CH_4 kg $^{-1}$ VS). On average, 0.040 ± 0.020 g CH_4 (minimum: 0.010 for $3 \times \text{P1}$ and maximum: 0.099 for $1 \times \text{P2}$) was emitted per kg fresh manure stored and per day of storage for 0.018–0.026 g CH_4 per kg fresh manure stored and per day reported by Chadwick (2005). As for N_2O , given the findings reported by Webb et al. (2012), CH_4 emissions from the manure in our experiments (2.1 ± 0.5 CH_4 as % of C stored) corresponded more to farmyard manure (0.5–9.7 CH_4 as a percentage of C stored) than to DL manure (0.00–0.03 CH_4 as a percentage of C stored). These high emissions were probably linked to the high digestibility of the feed used (Møller et al., 2014), as well as to the high temperature ($45 \pm 7.1^\circ\text{C}$) that, associated with anaerobic conditions (average density of 0.70 ± 0.05 t FM/m 3), favoured CH_4 production in manure heaps (Moral et al., 2012). The high daily emissions could also be explained by shorter storage duration than that used by Chadwick (2005, more than 80 days), who reported low rates of CH_4 emission at the end of the storage period.

A significant amount of CH_4 and CO_2 was attributed to the DLM in the barns even when the manure temperature was relatively low ($19.8 \pm 2.6^\circ\text{C}$). This low temperature could be explained by the high density of the manure (0.96 ± 0.01 t FM m $^{-3}$) because of the relatively low amount of straw added as litter, as well as pressure from the animals, leading to anaerobic fermentation in the manure, which released less heat than the aerobic processes. The amount of straw and its characteristics can modify the decomposition process (e.g., Jeppsson, 1999; Kapuinen, 2001a; Yamulki, 2006) and therefore significantly different emissions can be expected at the barn and storage levels, depending on the manure (and straw) management. For example, Jeppsson (1999) reported CO_2 emissions from litter in barn emissions ranging from 576 to 2016 g CO_2 m $^{-2}$ day $^{-1}$, whereas in our experiment they ranged from 225 to 526 g CO_2 m $^{-2}$ day $^{-1}$. Given the positive relationship between the CH_4 and CO_2 emissions observed in our trials ($[\text{CH}_4 \text{ [g m}^{-2} \text{ d}^{-1}]] = -3.6 + 0.0062 \times [\text{CO}_2 \text{ [g m}^{-2} \text{ d}^{-1}]]$, $p < 0.001$, $r^2 = 0.524$), higher emissions of CH_4 in barns could occur, but this depends on the shift from anaerobic to aerobic fermentation processes and its consequences (e.g., temperature variation). The correlation between emissions from manure in barns and in storage has also to be checked. Finally, on average, the manure accounted for $14.4 \pm 2.0\%$ of the total C emissions in the barns. The use of C balance and CO_2 concentration as tracer gas for gas flow measurement at the barn level should therefore take into account this amount so as not to underestimate gaseous losses.

There was no significant effect of manure management on total gaseous emissions during storage. Patterns of emission differed strongly, however. Peaks of emissions rates (for both CH_4 and N_2O) were observed three times and were lower in $3 \times$ than in the one higher peak in $1 \times$. The peaks in emission rate and temperature could be explained by self-heating organic matter degradation processes, organic matter that is heterogeneous in terms of the oxygen availability and temperature that occur within a few weeks of fresh manure being deposited in storage, leading to CO_2 , N_2O and

CH₄ emissions (El kader et al., 2007; Sommer and Møller, 2000). The amount of organic matter supply was lower per deposit in 3× than 1×, which led to lower emissions rates after the manure deposit. For manure storage during the cooler period (P1), the two patterns observed ended with similar emissions and low emission rates before the end of the storage period. No treatment effect would therefore be expected for the longer storage period. The pattern changed in the second period of the trial (P2). At the end of the storage period, the CH₄ and N₂O emission rates in 1× were still relatively high compared with 3×, as well as being higher when accumulated over the total storage period, but the lack of repetition meant this could not be confirmed statistically. Given the likely pattern of emissions from manure heaps (Wolter, 2004), however, over a longer storage period CH₄ and N₂O emissions from 1× would be expected to be higher than from 3×. The high emission rates for 1× at the end of the storage period, at least for CH₄, accord with the higher mean temperature in the 1× heap (50 ± 10 °C) than in the 3× heap (41 ± 7 °C) during storage periods and with the temperature pattern (Fig. 4), which remained high for 1× until the end of the storage period. In addition, depending on DLM composition at the end of the storage period, C concentration tended to remain higher for 1× than for 3× (Table 4), indicating that organic matter degradation was still likely, leading to more gaseous compound emissions. Therefore, if stored longer, higher emissions with the 1× manure removal frequency rate than the 3× rate would be expected. From this second period it appears that, in warmer conditions, the lower removal frequency rate from the barn could induce significant increases in GHG emissions from stored manure due to modifications in the degradation pattern caused by the higher amount of fresh organic matter stored in one deposit.

Apart from the effect of manure management, there was also strong evidence that the storage period influenced emissions. Whatever the gas, emissions were always higher in P2 than in P1 (e.g., P1: 14 ± 1 and P2: 48 ± 6 g CH₄ kg⁻¹ VS stored). Although other factors could explain this, it seems that factors influencing the temperature and thermophilic microorganisms of the heap could be the drivers of this period effect (Wolter, 2004). Compared with P1, the temperature of the manure in P2, whatever the stage of the experiment (barn or storage), was higher (Table 5), indicating faster organic matter degradation, leading to more emissions in P2 than in P1. These variations in manure temperature could be due partly to different ambient temperatures (P1: about 1 °C; P2: about 12 °C) that probably cooled down the manure in P1 more rapidly than the manure in P2. Depending on an increase in cattle size and intake, however, the effect of a higher amount of manure produced cannot be excluded (P1: 6.0 ± 0.15; P2: 7.9 ± 0.5 t). For both P1 and P2, the height of the heap (1.20 m) was considered representative of the size of commercial manure heaps stored on the edge of fields. We therefore assumed that the variability observed partly reflected the variability encountered under real manure storage conditions.

The observations and the procedure chosen (storage duration and period) did not lead to conclusions on the effect of manure removal frequency on total GHG emissions. Given the emission patterns and the period effect, however, hypotheses on the GHG mitigation options for barns and manure storage can be proposed. Obviously, without modification of the barn phase, a reduction in manure storage duration would reduce emissions from stored manure. This mitigation options should be preferably considered for the second period for practical reason (spreading possibilities) and efficiency (higher emission during warmer periods). With this in mind, one option is the direct spreading of manure after the barn phase (i.e., just after the third removal of manure in 3× and the manure removal in 1×). In this case, the total emissions from the manure stored outside were calculated to be 0 and 1850 g

CO₂eq.kg⁻¹ LWG for 1× and 3×, respectively. For the whole period, barn and cattle emissions included, GHG emissions would be reduced by a factor of 2.1 for 1× and 1.4 for 3× during P2. This suggests that keeping such manure beneath animals in barns is a better option than more frequent removal when the plan is to spread the manure at the time of the last removal of manure from the barn (i.e., when manure storage outside the barn can be avoided because it can be spread directly). In contrast, depending on the emission patterns, if the manure is kept in storage for a long time (longer than in this trial) during the warm period, higher emissions from 1X are likely. Based on the GHG emission rates from the manure in the barn and store, whatever the removal frequency (1× or 3×), another option could be to keep the manure in the barn until spreading, instead of removing it at the end of the barn period, and thus avoid the need for intermediate storage. More work needs to be done on this option of leaving the manure cold and compressed in barn, instead of partially aerating it, which lead to self-heating and to higher emissions. Whatever the best manure management option proposed, however, we accounted only for GHG emissions from the barns and manure store in a particular system and under specific climatic conditions. Before drawing conclusions from the results and applying them to all DL systems, it is therefore necessary at least to check that (1) the mitigation option does not induce higher GHG emissions from manure after its application on soil; (2) this option is agronomically appropriate; (3) it does not lead to an unwanted increase in the emission of other gases, such as NH₃, in the whole manure management system depending on, for example, variation in amount and composition at spreading of manure; and (4) variation in other management factors, such as the amount of straw for bedding, do not greatly modify emission patterns and amounts.

4.3. Ranking of accuracy with C balances

In these trials, for practical reasons the gaseous emissions from the liquid fraction produced in storage were not measured. Given the amount of C and N involved (estimated to be about 0.2% C and 1.2% N remaining in the system after storage; Fig. 5), however, this was assumed to be negligible. C recovery in the system (Output/Input, C-CO₂ and C-CH₄ emissions included in the output) was 103 ± 4%, on average, without any treatment effect (Fig. 5). In the barns (Out 1), the measured C emitted as C-CH₄ and C-CO₂ accounted for, on average, 111 ± 4% of the default in the C balance, without any treatment effect (p > 0.05). This surplus suggests an overestimation of about 10% in the gaseous emissions in the barns. The average recovery for manure storage (Out 2) was 99 ± 10%, but with large variations (from 80% for 3× P1 to 111% for 1× P2). Overall, the ratio of C emissions measured/C lost was, on average, 107 ± 5%, with a minimum value of 97% for the 3× P1 treatment and a maximum value of 111% for the 3× P2 treatment. These balances were well within the few published values for C (e.g., Moral et al., 2012; Wolter, 2004). No N recovery estimation was possible because NH₃ and N₂ emissions were not estimated. NH₃ would have been useful for investigating other environmental problems, such as eutrophication and acidification and for a discussion on N balance. As reported by Mathot et al. (2012), however, interference in NH₃ concentration measurement prevented the use of NH₃ emissions measurements, a problem also reported by Hassouna et al. (2013) and Edouard et al. (2016), who estimated NH₃ emissions within a selected time range of measurement, an option not chosen in the present study because it was considered too uncertain in terms of material, experimental procedure and observations. When adjusting gaseous emissions proportionally to the default of C recovery at the storage and barn levels, the main conclusion remains the

same: there was no manure management effect on total (barn and storage) or partial gaseous emissions. This adjustment led, on average, to lower ($-6 \pm 2\%$) total emissions for CH₄, CO₂ and GHG and similar emissions for N₂O ($+1 \pm 7\%$). When corrected for C recovery, the total emissions were 1.52, 5.93, 1.79 and 4.23 g N₂O kg⁻¹ LWG, 168, 314, 181 and 254 g CH₄ kg⁻¹ LWG, 10306, 13927, 11757 and 14708 g CO₂ kg⁻¹ LWG for 1 × P1, 1 × P2, 3 × P1 and 3 × P2, respectively. For inventory calculations, variation in emissions depending on C balances could be used for ranking uncertainty even when there is uncertainty about C concentration measurements (Vedrenne et al., 2008) in manure. For GHG emission inventory purposes, however, uncertainty about GHG emissions estimated through C recovery (less than 10%) was low compared with that related to variation between manure management, period (influencing ambient conditions) or assumptions about storage duration based on variation in gaseous emission patterns.

5. Conclusion

In deep litter systems, emissions from manure at the barn or storage levels can contribute significantly to emissions at the production system level. There are ways of modifying the emissions from solid manure in barns and storage using simple management procedures, such as modifying the frequency with which manure is removed from barns. In this respect, the mitigation option could focus on storage duration and place during warm periods. Differences in GHG emission were observed between the two manure removal frequency rates tested, but it was not possible to determine the best one because their ranking changed with outside manure storage duration. Spreading the manure just after its removal from the barn, however, is a promising option in terms of avoiding intermediate storage by keeping manure in the barn when degradation is slow as indicated by the moderate manure temperature. Finally, estimating the C recovery is useful to rank uncertainty of the emissions observed. In our trial it was relatively low compared with the changes observed and expected with variation in manure management.

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