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Research Paper

# Measuring and assessing the role of deep litter to estimate the ventilation rate using the $CO_2$ mass balance method

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Carbon dioxide (CO2) produced from manure is a necessary input when using CO2 as a naturally produced tracer gas to measure ventilation rate in naturally ventilated livestock buildings. This work compares different chamber calculation methods for measuring CO2 production from solid manure and evaluates the variability, affecting factors and potential contribution of CO2 emissions from manure to total CO2 production in the building. A total of 925 static chamber measurements were used to this aim, conducted in five dairy cattle and three goat houses. Linearity (R2) and curvature (convex or concave) were the main factors explaining differences among models. CO2 emission from manure was on average 20.86 g m-2 h-1, but it was very variable in spatial terms within the same measurement day (coefficient of variation = 48%), among different measurement days in the same farm (coefficient of variation = 30%) and among farms of the same animal type (coefficient of variation = 66% and 48% for dairy and goat farms, respectively). Manure height and temperature were directly correlated with manure CO2 emission (r = +0.36 and + 0.38, respectively). For goats, a prediction equation of CO2 emission was obtained using these two variables (R2 = 0.74). Solid manure had a relevant contribution to the total farm production and needs to be quantified in each case. Models to predict CO2 manure are not available at the moment and therefore, measuring manure contribution using chambers seems the best option according to the current knowledge.


## 1. Introduction

Measuring ventilation rate in animal buildings is essential to evaluate climate control systems and to quantify gas emissions. However, measuring ventilation rates in naturally ventilated buildings is particularly challenging because direct measurements of ventilation are not possible in practice. Therefore, indirect methods using tracers are used to estimate ventilation (Ogink et al., 2013). The carbon dioxide ($CO_2$)

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balance is a particular application of the tracer gas method. This gas is naturally produced in the building by the animals and their manure. It is also relatively easy to measure at the concentrations usually found on the farm. Therefore, it can be used as a tracer following the rules established by the International Commission of Agricultural and Biosystems Engineering (Pedersen et al., 2008; Pedersen & Sällvik, 2002).

Using the $CO_2$ balance method requires an accurate estimation of the $CO_2$ produced by the animals and their manure. Animal $CO_2$ production depends on the metabolic rate of the animal, which in turn depends on animal weight, production level and animal activity. This information can be obtained from studies on animal metabolism, and specific equations for different animal types are available to calculate this amount (Pedersen & Sällvik, 2002). However, the contribution of manure to total $CO_2$ production remains one of the most relevant uncertainty sources of this method, particularly when solid manure is accumulated over long times (Calvet et al., 2013).

The main pathways for $CO_2$ formation in manure are (1) the decomposition of urea into ammonia and $CO_2$, (2) the aerobic fermentation of manure, and (3) the anaerobic degradation of organic matter in liquid slurries (Philippe & Nicks, 2015). The amount of $CO_2$ produced by manure depends on a variety of interrelated factors. The manure type (solid or liquid) is a critical factor, because production pathways are different: in solid systems the aerobic fermentation (composting) is dominant, but in liquid systems a combination of anaerobic degradation and surface aerobic fermentation occurs (Hafner et al., 2013; Philippe & Nicks, 2015). Accumulation time and temperature also affect these emissions.

Manure is not expected to be a relevant source of $CO_2$ if it is frequently removed from the house. Pedersen et al. (2008) suggested that manure contributes to 10% in houses where the manure is stored for less than 3 weeks. For liquid manure in fattening pig housings, Zong et al. (2014) reported values around 3% of animal $CO_2$ for manure accumulations not deeper than 30 cm. In straw systems, higher $CO_2$ production rates are reported. Values about 20–40% were found for fattening pigs on litter (Philippe et al., 2007, 2012), but it has been reported that manure can produce as much $CO_2$ as the animals in their respiration (Jeppsson, 2000).

Using default $CO_2$ production from manure as a percentage of animal production may lead to relevant biases of the $CO_2$ balance method. Using site specific $CO_2$ production seems therefore necessary. This could be modelled from manure characteristics (type, amount, water content or temperature), but few data are available in literature to establish prediction models. Direct measurements seem therefore necessary, either using measurement chambers (Miles et al., 2006), comparing farm emissions with and without animals (Calvet et al., 2011), or measuring total farm production and detracting the calculated animal production (Philippe et al., 2007). Static chamber methods consist in enclosing a small surface for a short time, and therefore they may be used at any time with a small disturbance to animals. Inside the chamber, $CO_2$ concentration increases with time and the initial slope of this increase can be related to the emission rate. Linear (Miles et al., 2006) and quadratic (Sommer et al., 2004) models have been used for this calculation. The fundamental basis underlying gas diffusion in chamber methods suggests using an exponential model as described by Hutchinson and Mosier (1981) for nitrous oxide measurements. This model, however, is seldom referenced in literature when calculating $CO_2$ emissions from manure, since it is developed for the particular conditions of nitrous oxide emissions from soils. All these methods have been recently reviewed for nitrous oxide applications in soil measurements (Venterea et al., 2020) and can be adapted for $CO_2$ measurements from manure.

This study had two main objectives. First, to review, apply and compare the main models used to determine $CO_2$ emissions from manure when using static chambers. And second, to provide recommendations to calculate the contribution of manure to total $CO_2$ production in a livestock building and evaluate the factors involved in that process. After reviewing four emission calculation models for static chambers, we used a comprehensive set of static chamber measurements of $CO_2$ from solid manure, to evaluate the differences among them and the underlying causes for those differences. The set of static chamber measurements included several farms of two species (dairy cows and dairy goat farms) and repeated measurements (several measurement days and repeated measurements within a day). As a second step, the factors affecting those emissions were explored and the potential contribution to total $CO_2$ production was estimated.

## 2. Material and methods

### 2.1. Chamber measurements

Repeated measurements of $CO_2$ from manure were done in five dairy cow and three goat houses using deep-litter housing system in which animals are kept on a bedding of a mixture of manure and straw, woodchips or compost. Measurements were repeated in different days using the static chamber method. At each measurement day, static chamber measurements were repeated several times on different spots distributed randomly across the bedding to cover spatial variation (Table 1). All farms were situated in The Netherlands. A total of 50 measurement days were considered in this study (21 and 29 days for cows and goats, respectively). In each day, between 10 and 25 chamber measurements were conducted at different spots. In all, a total of 925 chamber measurements (457 and 468 for cows and goats, respectively) were evaluated. The stocking density was 1.2–1.3 $m^2$ for goats and 18.7 $m^2$ for cows. About 1.0–1.2 kg bedding material (straw) per goat was applied daily at goat farms 1 and 3, whereas at goat farm 2 the bedding application was 0.7 kg per goat per day. Part of the whole bedding material is removed every 2–3 months at goat farms 2 and 3. At goat farm 1 the removal frequency varied from every 4–5 weeks in the summer, to every 3 months in the winter.

Bedding management for dairy farms consisted in general of daily tillage of the top layer with a cultivator to mix fresh faeces through the bedding and yearly renewal of the whole bedding with fresh material. Fresh material was sometimes added when needed during a year. At the farms using woodchips as bedding material an aeration system in the bedding floor provided air to the bedding to stimulate composting

**Table 1 – Measurement farms, bedding materials used and number of animals. The number of measurement days in each farm are shown, indicating in parenthesis the number of measurement days in Winter, Spring, Summer and Autumn, respectively. The total number of measurements the frequency of gas concentration measurements are also presented.**

| Farm | Bedding material | Number of animals | Number of days | Number of chamber measurements | Frequency of gas measurements (min) |
|---|---|---|---|---|---|
| Dairy 1 | Woodchips | 104 | 8 (3,1, 2, 2) | 171 | 3 |
| Dairy 2 | Woodchips | 88 | 3 (0, 2, 1, 0) | 67 | 3 |
| Dairy 3 | Woodchips | 90 | 6 (0, 2, 1, 3) | 134 | 3 |
| Dairy 4 | Compost | 9 | 2 (0, 0, 1, 1) | 46 | 3 |
| Dairy 5 | Woodchips | 16 | 2 (0, 0, 0, 2) | 39 | 3 |
| Goat 1 | Straw | 1050 | 8 (2, 3, 3, 0) | 138 | 2 |
| Goat 2 | Straw | 900 | 10 (2, 4, 3, 1) | 152 | 4 |
| Goat 3 | Straw | 900 | 11 (5, 1, 2, 3) | 178 | 4 |

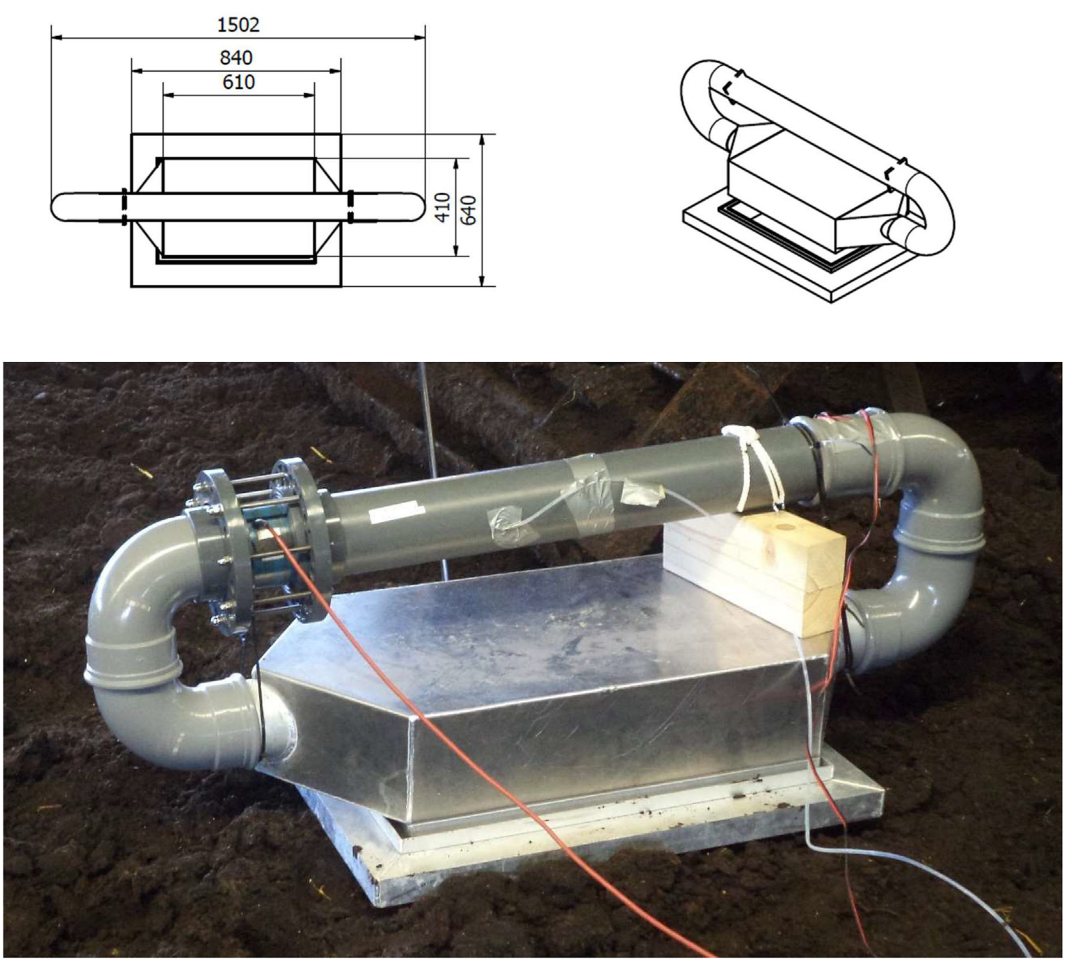


**Fig. 1 – Chamber used for the analysis: top view (upper left), isometric perspective (upper right) and chamber picture (bottom). Dimensions are expressed in mm.**

processes in order to increase the temperature in the bedding and keep the top layer dry.

A chamber made of stainless steel was used for all measurements (Fig. 1). The chamber covered an area of 41 × 61 cm (0.250 $m^2$) and enclosed a volume of 0.068 $m^3$. To create a properly mixed recirculating air flow of around 0.2 m $s^{-1}$ across the measured area a 12 V computer ventilator (Model KD1208PTB2, Sunon, Taiwan) was mounted in the PVC-duct connecting inlet and outlet. Gas samples were taken from this duct via a PE sampling tube (1/4″). At each spot, at least 4 gas concentration measurements were conducted after chamber was placed on the manure, to obtain a concentration increase curve. Gas concentrations were measured every 2–4 min as indicated in Table 1, and therefore we considered total accumulation times in each chamber measurement of 6–12 min. After a chamber measurement was finished, it was opened for some minutes for renewing the inside air and moving to another location inside the farm. Gas concentrations were measured using a photoacoustic gas monitor. An Innova 1312 (LumaSense Technologies, Ballerup, Denmark) was used at all dairy farms and goat farms 2 and 3. A Gasera ONE (Gasera, Turku, Finland) for goat farm 1. The gas analyzers used in this experiment were calibrated at the Wageningen University before the measurements. The air sampled from the measurement device was returned to the chamber to avoid renewal of the air enclosed in the chamber.

### 2.2. Comparison of calculation methods

Placing a static chamber on top of an emitting (manure) surface results in an increase of $CO_2$ concentration inside the chamber as a function of time. The emission rate (g $m^{-2}$ $h^{-1}$) is then calculated as the initial slope of this curve (expressed in g $m^{-3}$ $h^{-1}$) multiplied by the chamber volume ($m^3$) and divided by the manure surface enclosed ($m^2$). Four calculation methods for the initial slope were compared for each chamber measurement: (1) a simple linear method using 3 measurement points; (2) an exponential model with 3 measurement points using the equation by Hutchinson and Mosier (1981) (HM-model); (3) an exponential model using 3 measurement points fitted by numerical approximation; and (4) the HMR model suggested by Pedersen et al. (2010) and Venterea et al. (2020) using four measurement points.

The linear model assumes a constant increase of concentration inside the chamber during the first minutes. This model was applied calculating the slope (concentration vs. time) by linear regression of the first three measurements. The $R^2$ coefficient was obtained as a quantitative indicator of the linear relationship.

The model by Hutchinson and Mosier (1981) assumes that the emitting surface acts as a barrier to diffusion, causing a concentration gradient between the emitting source and the chamber air space. If the air is properly mixed, gas concentration inside the chamber is expected to follow an exponential Equation (1) as follows:

$$C(t) = C_{max} - (C_{max} - C_0) \times e^{-kt} \quad (1)$$

where C(t) is the concentration variation expressed in g $m^{-2}$ $h^{-1}$ as a function of time (t, expressed in hours); $C_{max}$ is the theoretical maximum concentration reached in the chamber after a long enclosure time (g $m^{-2}$ $h^{-1}$), $C_0$ is the initial concentration in the chamber (g $m^{-2}$ $h^{-1}$) and k is the decay factor ($hour^{-1}$).

The application of this model has to be understood in practical terms: the objective of this fitting is not defining an exponential curve with just three points, but to obtain the initial slope of an increasing concentration curve, which is needed to calculate the emission. The parameters of this equation ($C_{max}$ and k) can be calculated mathematically from 3 consecutive measurement points ($C_0$, $C_1$ and $C_2$) if time between measurements is constant (Eqs. (2) and (3)).

$$C_{max} = \frac{C_1^2 - C_2 \times C_0}{2C_1 - C_2 - C_0} \quad (2)$$

$$k = \frac{1}{t} Ln\left(\frac{C_1 - C_0}{C_2 - C_1}\right) \quad (3)$$

The slope at time = 0 of the exponential model can be obtained by derivation of Eq. (1) and substitution of $C_{max}$ and k by Eqs. (2) and (3), respectively. Thus, emissions (g $m^{-2}$ $h^{-1}$) can be calculated mathematically as indicated by bib_hutchinson_and_mosier_1981Hutchinson and Mosier (1981), considering the surface area A ($m^2$) and the chamber volume (V, $m^3$) (Eq. (4)):

$$E = \frac{V \times (C_1 - C_0)^2}{A \times t(2C_1 - C_2 - C_0)} \times Ln\left(\frac{C_1 - C_0}{C_2 - C_1}\right) \quad (4)$$

Although this equation provides results as long as the concentration curve is raising ($C_2 > C_1 > C_0$), the authors indicate that results are valid only if that curve is convex (the increase $C_1$–$C_0$ is higher than $C_2$–$C_1$).

The exponential model was also calculated by fitting numerically the three first data to the exponential curve. This fitting was done using the Solver add-in of MS Excel, setting the squared residuals between modelled and observed values as a target variable to be minimized. Emission was calculated from the initial slope of the fitted curve. The HMR model (Version 1.0.2, available at https://cran.r-project.org/web/packages/HMR/index.html) developed by Pedersen et al. (2010) was applied using the add-on pack of the R statistical program (https://www.r-project.org).

Comparisons between models were done considering the HMR model as the reference method, as suggested by Venterea et al. (2020). Differences were expressed in relative terms to this method and the variables explaining these differences were explored considering the data distributions (linearity and curvature) observed in practice.

### 2.3. Variation factors and contribution of litter to total emissions

By performing chamber measurements at different spots in each measurement day and averaging the results of these measurements, the spatial variation of the $CO_2$ production from the bedding (solid manure) was obtained. As different farms were considered and several (2–11) measurement days were done in each farm, within and between farm variability was also evaluated. A descriptive data analysis was conducted to explore the variability of emissions from manure, both within one measurement day (spatial variation) and among different measurement days (temporal variation). This variability was represented using a box plot for each farm and measurement day.

Unfortunately, not all measurement variables (manure height and manure temperature at different heights) were available for all measurements. For dairy cattle farms, no information on bedding depth was available and most temperature data referred to 20 cm depth (except for the days when litter depth was lower than that value). For dairy goats, manure height was registered, and temperature was measured at two heights (surface and bottom of manure layer). Therefore, these potentially affecting variables could be evaluated partially and only with the available data. Relationships between $CO_2$ production and these variables were explored using a pairwise correlation analysis, using individual chamber measurements.

Daily means of $CO_2$ production from the animals were estimated following the calculation procedures indicated by

Pedersen & Sällvik (2002) and Pedersen et al. (2008). Average animal weight and milk production were used for Holstein cows (664 kg live weight, 30 kg day$^{-1}$ milk) and Saanen goats (80 kg live weight, 3.5 kg day$^{-1}$ milk) in those farms during the experiments. This resulted in 1453 and 10,642 g of $CO_2$ per animal and day for goats and cows, respectively. Finally, $CO_2$ from the manure was related to the animal using the surface area per animal in each measurement day.

## 3. Results

### 3.1. *Comparison of data treatment methods of chamber measurements*

Comparison between measurement methods is presented in Fig. 2. The reference method was the HMR model using 4 points. Compared to this reference, both the HM model and the exponential model using only 3 points were coincident on average terms (average deviation less than 1% in both cases). The standard deviation of individual differences with the reference method was 20% and 15% for the HM model and exponential using 3 points, respectively (Fig. 2, top and middle). In absolute terms, the standard deviation of differences between methods were 3.10 and 2.38 g m$^{-2}$ h$^{-1}$. Compared to the reference, the linear model using the first three points underestimated results by 28% (Fig. 2, bottom). In absolute terms, using the linear model involved a bias of −6.28 g m$^{-2}$ h$^{-1}$ and added a random error of 6.92 g m$^{-2}$ h$^{-1}$. In Fig. 2 (bottom) it is also evident that the linear model never overestimates emissions compared with the HMR model. The reason for this is that the HMR model provides a linear solution in concave curves and overestimation of linear models are therefore not possible (see also Fig. 3).

The differences found between models can be explained by the linearity and curvature of the measured points. Four different situations were detected, which are exemplified in Fig. 3. Figure 3a represents all situations in which linear models underestimate the measured emissions compared to the HM or other exponential models. It can be observed that

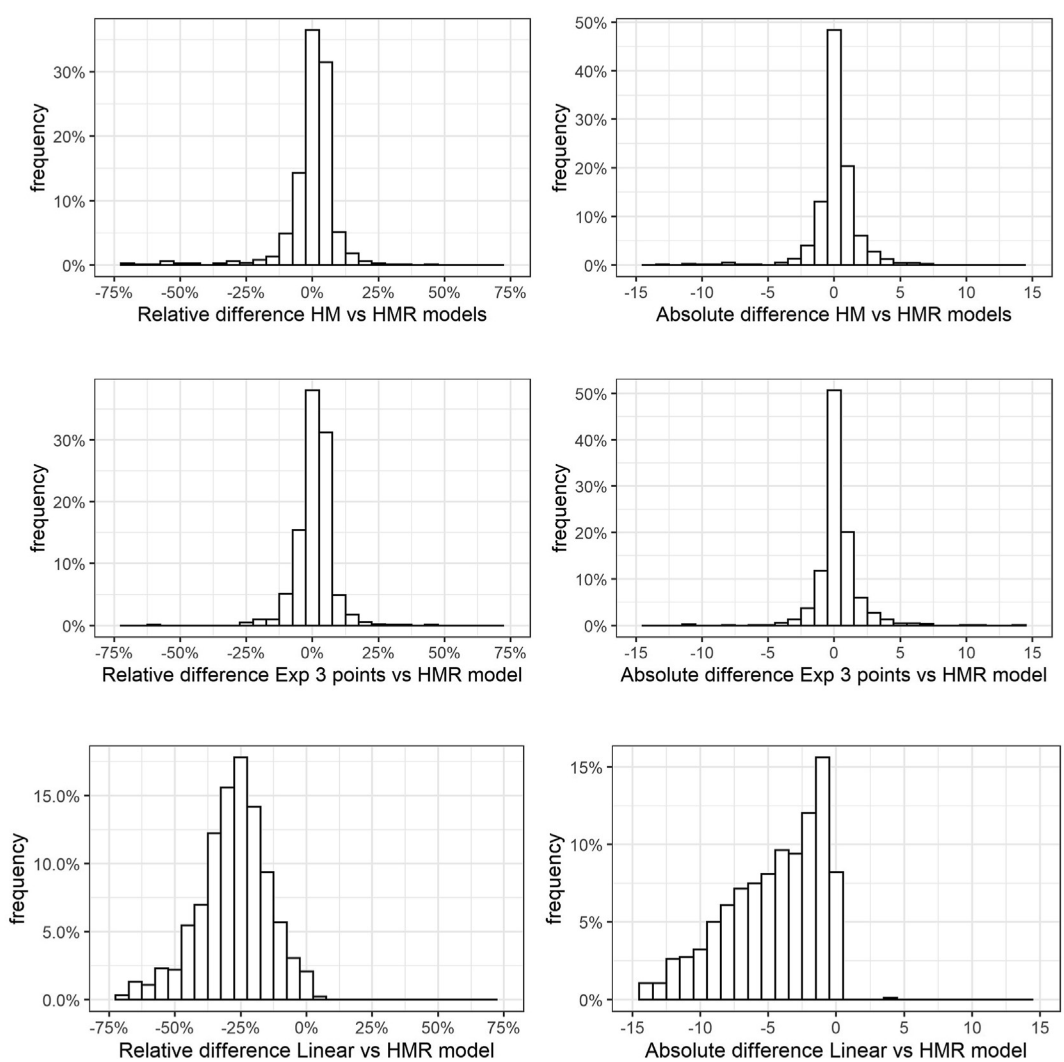


**Fig. 2 – Frequency histogram of differences with respect to the reference HMR model using the Hutchinson and Mosier (HM) model (top), exponential model using tree points (middle) and linear model (bottom). Relative and absolute differences are presented in the left and right histograms, respectively. Both cows and goats chamber measurements are included.**

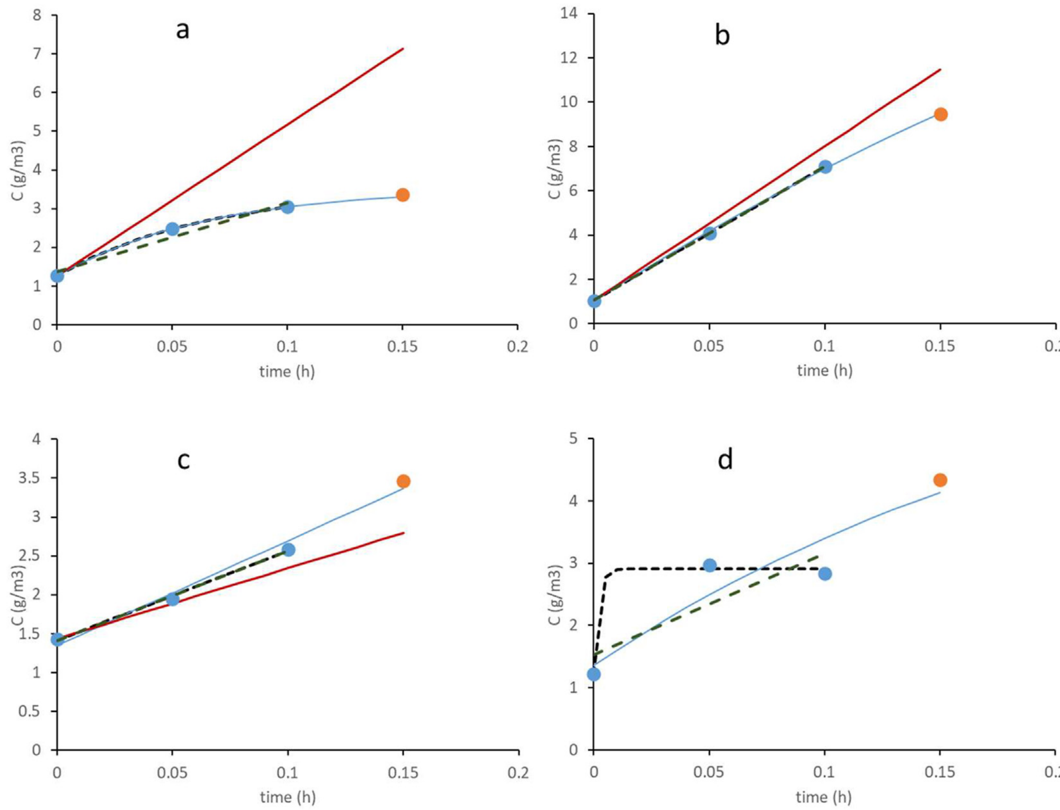


**Fig. 3 – Examples of possible situations according to linearity and concavity of the fist three measurement values. a. convex growing curve (Farm Dairy 1, day 4 of measurement); b. Linear growing curve (Farm Dairy 3, day 2 of measurement); c. concave growing curve (Farm Dairy 5, day 9 of measurement), d. convex decreasing curve (Farm Dairy 1, day 3 of measurement). Blue points represent the first three measurements. The orange point is the fourth measurement. Red solid line is the initial slope value provided by the HM model. The green dashed line is the fitting to a linear model. The black dahsed line is the fitting to an exponential curve obtained by numerical methods using 3 points. The blue solid line is the fitting exponential curve using 4 points. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)**

the linear slope is always lower than the initial slope of the exponential model, thus leading to underestimation of the linear model. The difference between the linear and the HM model was higher as the curvature increases. In practice, this situation can be identified with measured data because $C_2 > C_1 > C_0$ and $(2C_1–C_2 - C_0) > 0$. As the concentration curve becomes linear (Fig. 3b), linear and exponential methods are more coincident, thus leading to lower differences. This situation occurs when measured values follow the rules $C_2 > C_1 > C_0$ and $(2C_1–C_2 - C_0) \sim 0$. In about 9% of measured data, however, the curve was concave ("upward" curve) as in Fig. 3c. In those cases, the HM model provides the initial slope, which is lower than the linear and numerically solved exponential models, which in turn become coincident. This situation can be identified as those measurement datasets in which $C_2 > C_1 > C_0$ and $(2C_1–C_2 - C_0)<0$. The overestimation of the linear model with respect to the HM model is higher as the curvature increases. Finally, very few measurements resembled Fig. 3d, in which increasing and decreasing measurements alternate ($C_2 < C_1$ or $C_1 < C_0$). This situation can be considered an invalid measurement because the HM model provides an error output, while linear and exponential methods diverge drastically. In Fig. 3(a, b and c) it is also appreciable that adding a fourth point of the model (which is considered the reference in this study) does not change drastically the results, with respect to the reference HM method, and this change depends on whether the fourth point changes in one sense or the other the initial slope of the model.

The divergence between the linear and HM model depended on the linearity of the measured concentration data. More specifically, this divergence can be predicted by using the $R^2$ coefficient of the linear regression between gas concentration and time (Fig. 4). The values corresponding to Fig. 3a are those leading to underestimations of the linear model. Values near to $R^2 = 1$, as those represented by Fig. 3b, lead to coincident values. The cases represented by Fig. 3c would lead to overestimation when using linear models, but in this case this model seem to be more appropriate, while the HM model is suspect for underestimation (see Fig. 3c).

### 3.2. *Variation factors*

Considering all measurements, average $CO_2$ emission from litter was 20.86 g $m^{-2}$ $h^{-1}$. A wide variation of emissions was found among locations, but also within one location in different days and within one day (Fig. 5 and Table 2).

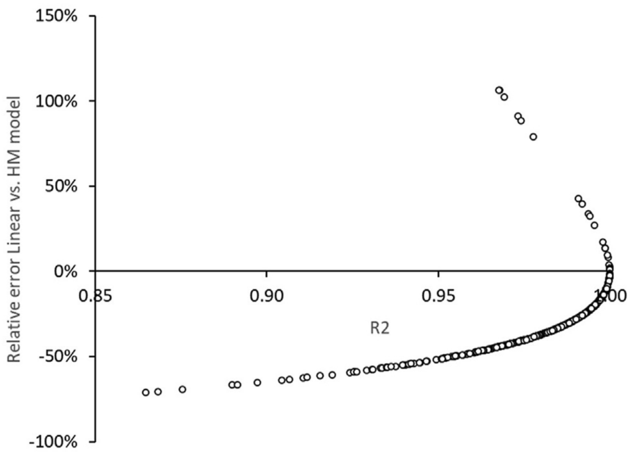


**Fig. 4 – Relationship between $R^2$ of the concentration vs. time curve of the three first measurements and the relative error between the linear and the HM model.**

Individual chamber measurements ranged from 0 to 120 g $m^{-2}$ $h^{-1}$. When aggregated to farm measurement day, average emissions ranged between 3.75 and 66.36 g $m^{-2}$ $h^{-1}$. An important effect of the farm and the measurement day was found. Some farms (Goat 1, Dairy 2 and Dairy 4) had consistently emissions lower than the average, while most measurement days in Dairy 1, Dairy 3 and Dairy 5 exceeded the average.

The spatial variation of emission within each measurement day was high. The coefficient of variation of measurements within a measurement day was on average 48% (range between 17% and 120%). In some farms, a high variability within the same building was found because of different manure status. When considering average daily measurements within the same farm, a high temporal variability is also obtained (Table 2). The coefficient of variation of repeated measurements within the same farm was on average 30% (ranging from 8% in farm Dairy 5 to 54% in farm Dairy 3). When aggregating values per farm, a high variation was also found among farms within the same animal species (coefficient of variation of 66% and 48% for dairy and goat farms, respectively).

As shown in Fig. 6, both temperature and manure depth were positively correlated with $CO_2$ production from manure on a surface basis. Based on the measurements from goat farms only, a significant correlation coefficient of +0.36 between manure depth and $CO_2$ production was obtained. Using both data from cattle and goats, it was found that temperature at manure bottom was also positively correlated with $CO_2$ production ($r = +0.38$). Among farm types, the correlation was particularly high in the goat farms ($r = +0.67$).

In goat farms, where both temperature and manure depth were available, a statistically significant linear regression of $CO_2$ production depending on manure temperature and depth was obtained (Fig. 7). Using average daily data ($n = 29$), the following equation was found: $CO_2 = -18.8 + 1.12 \times T + 0.15 \times D$ ($p < 0.001$, $R^2 = 73\%$), where $CO_2$ is expressed in g $m^{-2}$ $h^{-1}$, T is the temperature expressed in °C and D is the depth expressed in cm. This equation covered a temperature range between 16 and 50 °C, and manure depths between 5 and 75 cm. This range covered 95% all measured data. A farm effect on temperature and depth was detected, which affected the average $CO_2$ emission levels of each farm.

### 3.3. *Contribution of litter to total emissions*

Table 2 shows the $CO_2$ production by manure on animal basis and the relative amount of $CO_2$ from litter in comparison with animal respiration. On an animal basis, cattle manure produced on average about 10 times more $CO_2$ than goats (8675 vs 816 g $animal^{-1}$ $day^{-1}$). However, similar to production on a surface basis, a very high variation was found. Cattle manure produced between 2460 and 16,450 g $animal^{-1}$ $day^{-1}$, while goats manure production ranged between 384 and 1162 g $animal^{-1}$ $day^{-1}$.

Based on the weight and production data, $CO_2$ production from the animals was calculated to be 10,624 and 1452 g $animal^{-1}$ $day^{-1}$. On average, cow manure produced 82% of animal respiration $CO_2$ (range between 23% and 155%). Goat manure produced on average 56% of $CO_2$ compared to animal respiration (range from 26% to 80%).

## 4. Discussion

This work provides insight on chamber measurement calculations for $CO_2$ contribution of manure and explores production variability, affecting factors and potential contribution to total $CO_2$ production on the farm. We found that most static chamber measurements followed an exponential curve, although a small proportion of them showed a linear increase in concentration. Theoretically, the initial part of a static chamber measurement is expected to be linear, but as exponential increases are found and considering the concentration measurement interval (2–4 min) it seems that emission rate is high compared to the chamber size. Our results suggest that linearity ($R^2$) and curvature (estimated as $2C_1-C_2-C_0$) can be used as a rapid evaluation criterion when using three measurement points. In most cases, measurement curves are convex ("downward" curve), and the formula suggested by Hutchinson and Mosier (1981) is the a valid calculation option. However, particular care must be taken in concave curves, when $2C_1-C_2-C_0 < 0$, since that formula underestimates real emissions. Similar situations as those described in Fig. 3 were also described by Pedersen et al. (2010) in nitrous oxide emission estimations from soils. Accordingly, these authors suggested flexibility mechanisms in data analysis, using either exponential or linear models according to the shape of the concentration curve. However, special care must be taken in concave concentration curves and they must be carefully assessed.

Establishing a selection criterion is not easy because almost-linear datasets provide no relevant error. However, in this study the underestimation of the Hutchinson and Mosier model was higher than 15% in concave curves with $R^2 < 0.998$. Using this percentage as a selection criterion would leave out the analysis about 5% of the measured dataset used in this study. Another option would be using the linear model in concave curves (those fulfilling $2C_1-C_2-C_0 < 0$), which were 8% of measurements in this study. This flexibility criterion is also suggested in a recent review in which a gold standard of

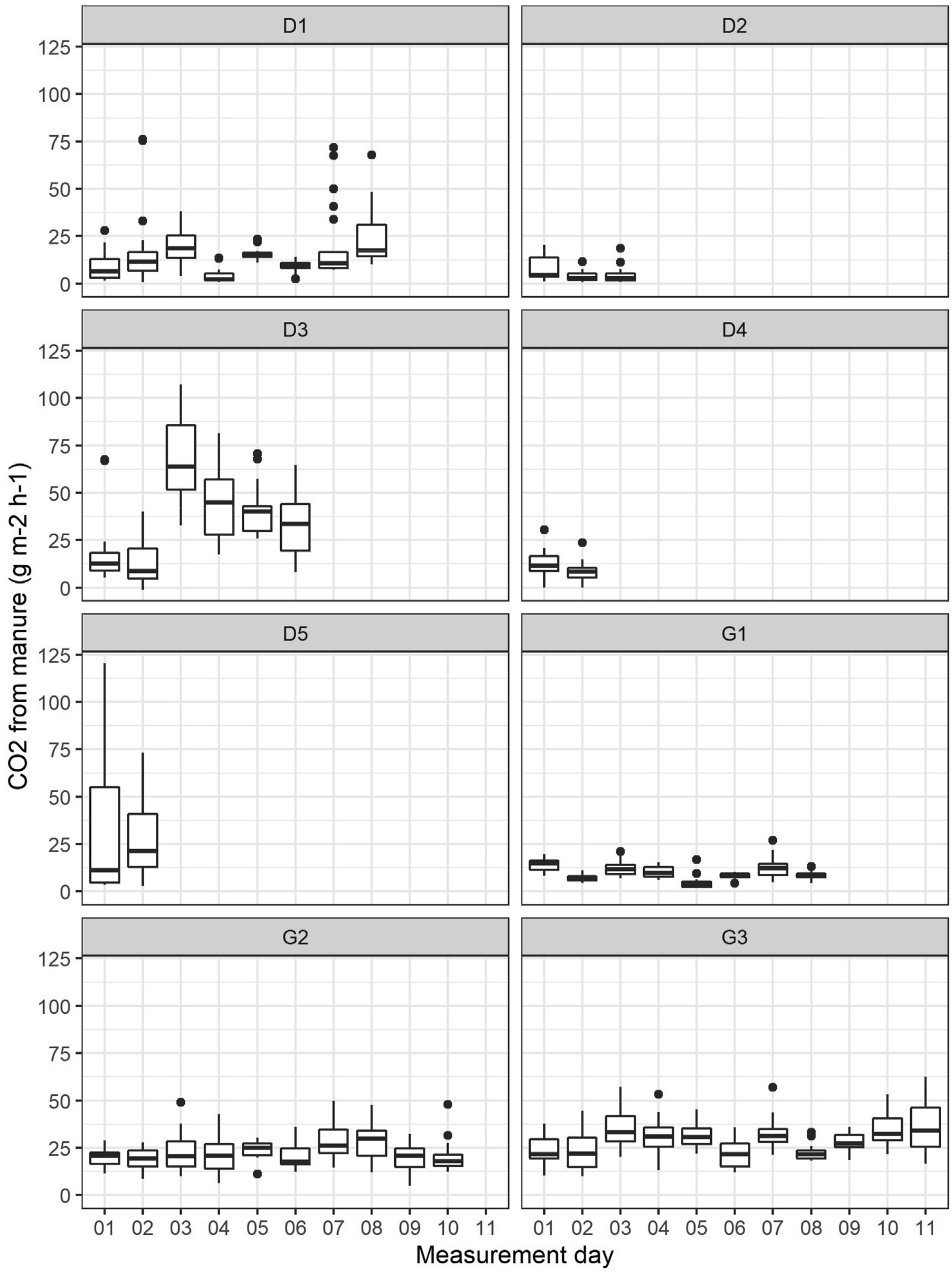


**Fig. 5 – Box plot of manure $CO_2$ emissions according to the farm and measurement day. D1 to D5 are dairy farms and G1 to G3 are goat farms.**

chamber measurements is suggested for nitrous oxide (Venterea et al., 2020). According to these authors, a shift from the exponential to the linear model can be done for concave curves, thus avoiding underestimation of the Hutchinson and Mosier model. These authors also suggest using four or more measuring points, and for that reason we used the HMR model using four points as a reference. However, our study shows that, in average terms, only minor effects on $CO_2$ production are found when considering four points instead of three.

Differences between nitrous oxide from soils and $CO_2$ measurements from manure are evident: higher concentrations and shorter measurement times are used in the latter, which facilitates $CO_2$ measurements. However, some similar problems may occur, for example those related to imperfect chamber sealing or horizontal flux through the manure substrate. These effects may be expected to be even more relevant than in soils because of the porosity of the solid manure. For all these reasons, the person on

**Table 2 – Average $CO_2$ production from the litter expressed per square meter and hour, per animal and day and as a percentage of the expected $CO_2$ produced by the animals. Standard deviation between day means is also provided as ± values).**

| Location | Referred to area (g $CO_2$ $m^{-2}$ $h^{-1}$) | Referred to animal (g $CO_2$ $animal^{-1}$ $day^{-1}$) | Relative to respiration (%) |
|---|---|---|---|
| Dairy 1 | 15.04 ± 6.64 | 6794 ± 3014 | 64 ± 28% |
| Dairy 2 | 5.48 ± 2.58 | 2460 ± 1156 | 23 ± 11% |
| Dairy 3 | 36.65 ± 19.84 | 16,450 ± 8903 | 155 ± 84% |
| Dairy 4 | 11.31 ± 2.68 | 5077 ± 1203 | 48 ± 11% |
| Dairy 5 | 28.06 ± 2.34 | 12,595 ± 1052 | 119 ± 10% |
| Goat 1 | 9.69 ± 3.27 | 384 ± 129 | 26 ± 9% |
| Goat 2 | 22.83 ± 3.13 | 904 ± 124 | 62 ± 9% |
| Goat 3 | 29.34 ± 5.65 | 1162 ± 224 | 80 ± 15% |

charge of processing the data has a relevant role and must evaluate individual measurements before considering them as valid or not.

Measured values in this study (average 20.86 g $m^{-2}$ $h^{-1}$) are in the range of values reported in literature. Jeppsson et al. (2000) found $CO_2$ emission values between 24 and 87 g $m^{-2}$ $h^{-1}$ for young cattle and attributed the variation among measurements the effect of litter type, manure accumulation temperature and water content. Vac et al. (2013) measured $CO_2$ emissions from manure in a sheep and a cattle farm and reported values of 11.5 and 45 g $m^{-2}$ $h^{-1}$, respectively. Borhan et al. (2013) measured $CO_2$ emissions from manure in calves after manure accumulation during 28 days and found between 25 and 30 g $m^{-2}$ $h^{-1}$ regardless the use of bedding or not. Despite the values obtained in our study are in comparable ranges to those reported in the literature, it seems that particular farm conditions affecting bedding conditions are determining factors for manure $CO_2$ emission.

In this study we used data available from previous projects and therefore the number of measurement days and farms was not balanced. This may cause potential limitation in the interpretation the variation factors. As expected, positive correlations were found with manure depth and temperature at manure bottom. For goats, a statistically significant prediction model was obtained, which demonstrates the quantitative effect of those variables. However, prediction in practice must be used with caution, because the model obtained may not be representative of farm conditions different to those in this study. $CO_2$ production from solid manure is the result of a complex interaction between organic matter composition, oxygen availability, humidity, mass and

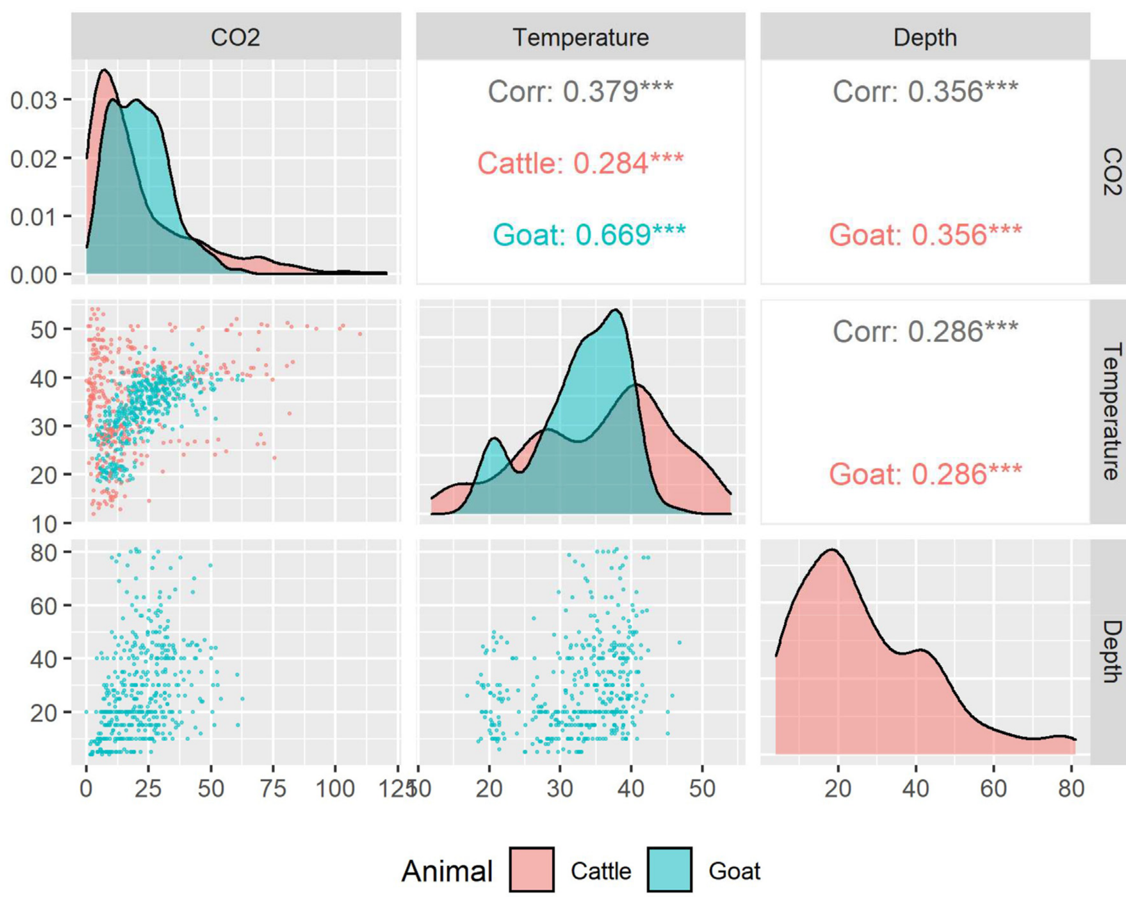


**Fig. 6 – Relation between $CO_2$ emission (g $m^{-2}$ $h^{-1}$), manure temperature at 20 cm depth (Temp, °C), and manure depth in cm (only for the goat farms). Data are presented separately by animal type. The density function of each variable is shown in the diagonal. Correlation coefficients are shown in the upper right part of the panel. Correlations in grey indicate the aggregated values without distinguishing between animals. Scatterplots are presented in the bottom left part. *** denotes statistical significance at $p < 0.001$.**

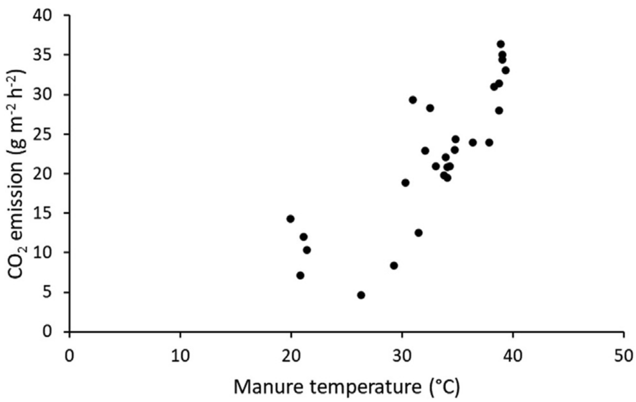


**Fig. 7 – Relationship between manure temperature and $CO_2$ emissions from litter in goat farms. Daily average values are provided (n = 29).**

porosity, which are very variable in practical conditions (Sanchis-Sebastiá et al., 2019). Unfortunately, manure mass, porosity and humidity are difficult to be assessed on real time basis, thus making predictions difficult. Measurements were distributed throughout all year, thus providing a complete representation of environmental variables. However, management options seemed to be more influencing, as evidenced by the high differences among farms.

When data was analysed by season, the highest emissions were found in autumn (26.2 g m$^{-2}$ h$^{-1}$). On the contrary, lower emissions were found in winter, spring and summer (19.9, 20.8 and 17.9 g m$^{-2}$ h$^{-1}$, respectively). Although manure accumulation was not consistently recorded through the experiment (only measured in the goat farms), manure is normally accumulated for longer times in winter, when land applications are restricted. Despite expecting higher manure accumulations in winter, this study found that winter had significantly lower manure temperature (31.4 °C) compared to spring, summer and autumn (35.5, 34.8 and 34.2 °C, respectively; $p < 0.001$). It seems that different temperature and manure accumulation throughout the year, together with other factors not registered in this study, are causing the seasonal variation of average $CO_2$ emissions.

This study confirms that $CO_2$ from manure in bedding systems is relevant and must be considered specifically when using the $CO_2$ method to calculate ventilation rates. The need of measuring different farms is supported by the results, because a high variation between farms was obtained, but also among measurement days within the same farm. The average contribution of manure (82% and 56% of animal respiration for cattle and goats, respectively) and the wide variations among farms and measurement days suggests that manure plays a relevant role in the $CO_2$ balance method in solid manure systems, which must not be overlooked.

This $CO_2$ contribution can be calculated when a quantitative model considering the variables involved in the process is available. With the existing data, however, it seems that developing such model is object of future research. Until this model is developed, it is evident that using the $CO_2$ balance method in real farm conditions requires direct measurement of manure contribution using chambers. Our results also show a high spatial variability in $CO_2$ production from the litter which is also observed in the previous literature (Vac et al., 2013). Within a measurement day, the coefficient of variation of $CO_2$ measurements from litter was on average 48%. This indicates that repeated chamber measurements are needed to characterise manure contribution, and a sample size of 20 measurement points would lead to an expected measurement error of ±21% (95% confidence interval). However, the coefficients of variation in our study ranged from 19% to 117%, which would involve an average daily measurement error of ±9% and ±50%, respectively (95% confidence interval). Therefore, an accurate assessment is convenient to explore the potential heterogeneity of litter before planning a measurement campaign.

## 5. Conclusions

Four data processing options were evaluated for chamber measurements of $CO_2$ emitted from solid manure at farm conditions. Linearity and curvature of concentration measurements are suggested as preliminary selection criterion to evaluate conflicting chamber measurements. The HMR model using four points is suggested to be used as a reference, while exponential models using 3 points can also provide reliable results.

$CO_2$ emission from manure was very variable within a measurement day in the same farm, among different measurement days in the same farm and among farms. Manure depth and temperature were identified as affecting variables and a prediction model of $CO_2$ was produced using these variables. However, extrapolation to different conditions can be misleading due to the effect of other related variables.

Solid manure contributes to a relevant share of animal respiration, and therefore its contribution to the total barn production can not be ignored in the $CO_2$ balance method to estimate ventilation rate. A manure contribution of 10% of animal production in current equations (Pedersen & Sällvik, 2002) underestimates the actual $CO_2$ production from manure and ignores the variation between and within farms. Prediction models are not available at the moment, and therefore this option needs future research. Alternatively, measuring manure contribution using chambers is the best option according to the current knowledge.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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