# Ammonia and greenhouse gas distribution in a dairy barn during warm periods

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#### **KEYWORDS**

Ammonia, greenhouse gas, environmental monitoring, cows' behavior, barn management, housing system, climatic parameters

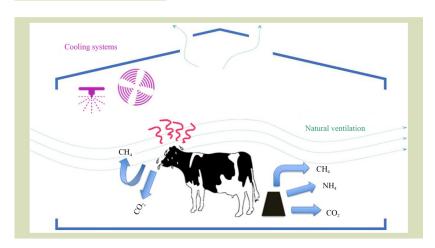
#### **HIGHLIGHTS**

- Environmental impacts in the dairy sector are mostly related to emissions of ammonia and greenhouse gases.
- Highest concentrations of these gases were in the center of the open barn during warm periods.
- Gas distribution varied vertically and horizontally, and differed between gases.
- Openings and the cooling systems increased indoor ventilation diluting these gases.
- Cleaning, milking and cooling practices affected cow behavior and altered diurnal gas patterns.

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#### **GRAPHICAL ABSTRACT**



#### ABSTRACT

This research aimed to quantify concentrations of ammonia (NH<sub>3</sub>), carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>), estimate emissions, and analyze the factors influencing them during warm periods in an open dairy barn equipped with two cooling systems in a Mediterranean climate zone. Gas distribution within the barn was observed to vary both vertically and horizontally, with the highest gas concentrations observed in the central area of the barn. NH<sub>3</sub>, CH<sub>4</sub> and  $CO_2$  ranged in 1.7–7.4, 7–18, 560–724  $\mu g \cdot g^{-1}$ , respectively. Natural ventilation through openings and the operation of cooling systems induced changes in indoor microclimate conditions, influencing cow behavior and, consequently, gas production. Gas concentrations were the highest at air velocities below 0.5 m·s<sup>-1</sup>. The highest concentration of NH<sub>3</sub> was observed when the temperature-humidity index (THI) was > 72 and ≤ 78; and CO<sub>2</sub> and  $CH_4$  concentrations were the highest with THI  $\geq$  72 and decreased with THI  $\leq$ 72. NH<sub>3</sub> concentrations when barn management included three daily milkings were higher than those measured when barn management was based on two daily milkings, and lower for CH<sub>4</sub> and CO<sub>2</sub>. NH<sub>3</sub> and CH<sub>4</sub> emissions were the highest during barn cleaning, while the lowest NH3 emissions occurred during activity of the cows (i.e., feeding, walking).

## 1 Introduction

The second mission of the recent National Recovery and Resilience Plan under the program Next Generation EU aims to promote actions, in line with the reduction of the greenhouse gas emissions proposed by the European Green Deal, to allow the ecological transition, by promoting sustainable agriculture and reducing the environmental pollution<sup>[1]</sup>. Animal husbandry is an important source of environmental concerns<sup>[2,3]</sup> due to the production of greenhouse gases (i.e., methane (CH<sub>4</sub>), nitrous oxide (N<sub>2</sub>O), and carbon dioxide (CO<sub>2</sub>)) as well as other atmospheric pollutants (i.e., ammonia (NH<sub>3</sub>)) responsible for negative impacts on the environment and human health<sup>[4–6]</sup>.

Global warming is posited to soon impact emissions from European dairy cattle. A specific study<sup>[7]</sup> based on an artificial neuronal network determined that NH3 and CH4 emissions are projected to increase by about 16 and 0.1 Gg per year, respectively, by the end of the century. Also, heat stress is anticipated to adversely affect both animal behavior and milk yield, as well as emissions, especially in areas characterized by a Mediterranean climate<sup>[7,8]</sup>. Of the primary measures to mitigate environmental impact, maintaining good animal health and welfare is recommended to keep emission levels low<sup>[9]</sup>. Indeed, the increase in emissions is influenced by the conditions of animal welfare, including heat stress or common cattle diseases, such as lameness, ketosis and mastitis<sup>[10]</sup>. In this context, the alleviation of heat stress in cows is pivotal[11-13], and monitoring the animals allows for the identification of various behaviors[14-16].

In Europe, dairy cows are predominantly housed in naturally ventilated dairy barns, and numerous studies in the literature have focused on emissions primarily in barns located in northern Europe<sup>[17–25]</sup>. Currently, estimates of emissions from the livestock sector originate from dairy gas emission models applied in northern European contexts, providing an average annual value<sup>[26]</sup>. In the Mediterranean context, emission estimation is based on emission factors established under various climatic and management conditions typical of northern European countries.

In the Mediterranean Basin, dairy barns typically feature an open building envelope. The natural ventilation is generally augmented by the use of cooling systems to enhance the ventilation rate in the barn, thereby mitigating the adverse effects of high temperatures on animal welfare<sup>[10,11,27]</sup>.

Drawing upon existing literature, numerous studies have

examined the impact of heat stress on cows in Mediterranean climates, specifically focusing on housing systems and barn management<sup>[28–30]</sup>. However, comparatively less attention has been devoted to examining the relationship between gaseous release and environmental drivers. Therefore, to advance research in this domain, it is essential to include environmental monitoring of gas concentrations, emission estimations and assessment of key influencing parameters, particularly during warm periods.

To address these gaps, this research involved studying the concentrations of NH<sub>3</sub>, CH<sub>4</sub>, and CO<sub>2</sub> during warm periods in an open dairy barn situated in a Mediterranean climate zone. It was hypothesized that climatic conditions, animal behavior and barn management influenced gas production. The objectives encompassed: (1) investigating gas distribution both horizontally and vertically; (2) identifying influencing factors affecting gas concentrations in relation to climatic conditions, barn management and animal behavior; (3) evaluating gaseous emissions and their influencing factors; and (4) providing specific data on concentrations and emissions dependent on the various factors considered, along with associated statistical information. The findings of this research are anticipated to offer valuable insights for researchers and stakeholders, contributing to the characterization of barn environments in the Mediterranean area.

# 2 Materials and methods

#### 2.1 Main features of the dairy barn

The experimental activities included various trials in an opensided barn located in the province of Ragusa (Sicily, Italy). Climatic conditions of this area are categorized under Koppen climate classification as hot summer Mediterranean climate, where the warmest month has a mean temperature higher than 22 °C and the driest month in summer has average precipitation lower than 30 mm.

The barn was a free-stall dairy house with three boxes and 64 head-to-head cubicles (Fig. 1(a)). Each box is divided into distinct zones, encompassing a designated resting area, feeding space and service alleys. The building, measuring 55.5 m by 20.8 m, consisted of an open supported with pillars around its perimeter and along its central longitudinal axis, covered by a symmetric roof with a 7-m high ridge vent. The structure had a solid concrete floor, and the cubicles set in two rows bordered by concrete curbs and covered with a layer of sand. Natural ventilation was provided by roof openings and the absence of

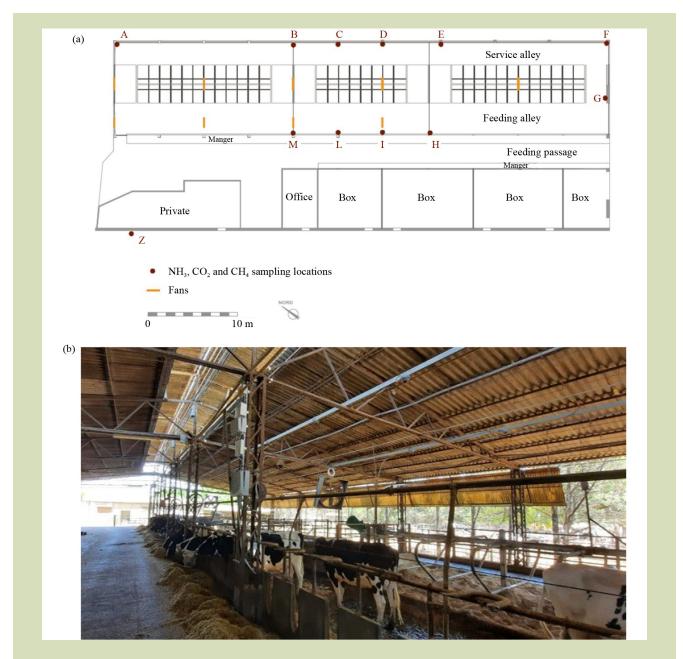


Fig. 1 Plan of the barn studied (a) and indoor view of the barn with two open sides (b).

three perimeter walls, while the SW side had a continuous wall with four small openings close to the calf boxes (Fig. 1(b)). Given the potential severity of heat stress during warm periods, the natural ventilation system was complemented by two cooling systems, incorporating fans and sprinklers in feeding and resting areas (Fig. 2). The position and height of the fans, positioned on a 20° tilt from the horizontal, are illustrated in Fig. 1 and Fig. 2. Specifically, the fans situated in the resting area were 1400 mm wide and facilitated a ventilation rate of 34,600 m<sup>3</sup>·h<sup>-1</sup>. The axis of rotation for these fans was 2.75 m above the floor, aligned with the longitudinal axis of the barn,

and the spacing between fans was 14 m. The misting system within the resting area comprised misters operating at a pressure of 200 kPa, delivering a rate of 1.01 L·min<sup>-1</sup> for each nozzle. These misters were positioned 2.9 m above the floor, spaced approximately 3.1 m apart along the longitudinal axis of the barn. In the feeding alley, semicircular (180°) sprinklers, operating at a pressure of 200 kPa and dispensing water at a rate of 2.57 L·min<sup>-1</sup>, were installed above the rack. These sprinklers were positioned 2 m above the floor, aligned with the longitudinal axis of the feeding alley, and spaced 1.9 m apart. Each axial fan in the feeding alley, in total five, had a

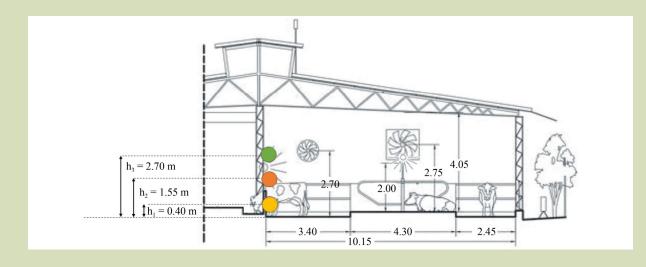


Fig. 2 Section view of the barn with the vertically distributed sampling points.

diameter of 900 mm and a ventilation rate of 22,250 m<sup>3</sup>·h<sup>-1</sup>. Positioned above the feeding alley, the rotation axis of these fans was 2.7 m above the floor, parallel to the longitudinal axis of the feeding alley, and with a 14-m separation between fans in the row.

#### 2.2 Description of the barn management

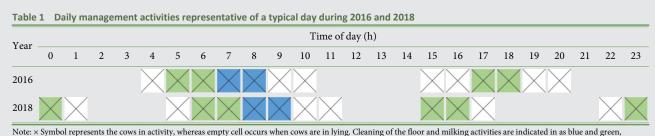
Barn management involves specific procedures associated with daily management activities, including feeding, barn floor cleaning, the frequency of daily milkings and the operation of the cooling system (Table 1).

Throughout the experimental activities, feed was delivered daily after the morning cleaning of the barn floor. The feed was available ad libitum to the cows via a feeding trough. Barn floor cleaning was conducted once per day in the early morning, lasting approximately 45 min, using a tractor equipped with a scraper blade. During this process, manure was moved to the manure storage area, located outside the barn. The frequency of daily milkings varied during the investigated period.

Specifically, cows were milked twice per day in 2016 and 2022 and three times per day in 2018. Milking was organized in three sessions, one for each group of cows in a pen. The cooling system remained inactive during milking and barn floor cleaning, with fans and sprinkles activated only when air temperatures exceeded 22 and 27 °C, respectively. Fans were turned off during sprinkling to minimize water dispersion. Various sprinklers management strategies were implemented during data acquisition (see Section 2.5).

# 2.3 Measurements of gas concentrations, climatic parameters and animal routine

Gas concentration monitoring was performed by a photoacoustic analyzer (INNOVA, LumaSense Technology A/S, Ballerup, Denmark). The instrument, comprising a Multigas Monitor 1412i linked to a Multipoint Sampler 1409/12, collected data from multiple locations. Configured based on the spatial distribution of the sampling locations (SLs), the sampler system featured inlet channels connected to tubes for gas sampling. The use of AISI-316 stainless steel and



Note: × Symbol represents the cows in activity, whereas empty cell occurs when cows are in lying. Cleaning of the floor and milking activities are indicated in as blue and green respectively.

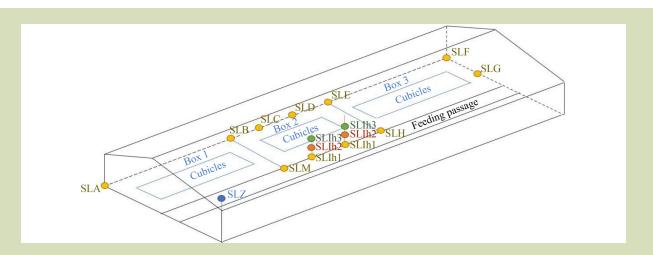
PTFE (polytetrafluoroethylene) tubes minimized sample adsorption[31]. Air filters were attached to tube ends at each SL to minimize particle intake into the sampler. Installed in the barn, the system continuously measured NH<sub>3</sub>, CH<sub>4</sub> and CO<sub>2</sub> concentrations. Instrument detection limits were 0.2 μg·g<sup>-1</sup> for NH<sub>3</sub>, 0.4 µg·g<sup>-1</sup> for CH<sub>4</sub>, and 1.5 µg·g<sup>-1</sup> for CO<sub>2</sub>. Instrument calibration and air filter replacement preceded each experiment. During the experiments, two sampling configurations were used to acquire data. In detail, in the first configuration INNOVA measured gas concentrations at 12 horizontally-distributed SLs in the barn (Fig. 1(a)). All SLs were located 0.40 m above the floor, except for SLZ that was set above the roof and upwind to measure outdoor concentrations. In the second configuration, gas concentrations were acquired at different vertically distributed SLs (i.e., 0.40 m above the floor at SLh1, close to the upper bar of the feeding rack, 1.55 m above the floor at SLh2 and close to the fans in the feeding alley, 2.70 m above the floor at SLh3) located in the central area of the barn (i.e., the same vertical axis as SL-L and SL-I) (Fig. 2). The position of SLs in both configuration are represented in the Fig. 3. In all the experiments, the sampling interval was 15 min.

Air relative humidity (RH) and temperature sensors (Rotronic Italy s.r.l., Milano, Italy) and anemometers (WindSonic, Gill Instruments Ltd., Lymington, UK) continuously recorded indoor parameters at the barn center and outdoor parameters above the roof (i.e., air RH, air temperature, wind direction and wind speed). In detail, platinum thermo-resistance air temperature sensors (Pt 100 Ohm at 0 °C) with a measurement

range from -40 to +60 °C with a precision of  $\pm 0.2$  °C (at 20 °C) were used. The hygrometer utilized was a transducer, featuring a sensitivity of  $\pm 0.04\%$  RH °C<sup>-1</sup> and a precision of  $\pm$  2% (at 20 °C). To mitigate potential inaccuracies caused by direct radiation, these sensors were placed inside a shelter. Indoor air velocity and direction were gauged by sensors located within the building at the central box of the barn, positioned about 2.0 m above the floor. Wind speed and direction sensors were situated outside the building at the ridge vent above the roof. The anemometers used were two-dimensional sonic sensors measuring velocity from 0.01 to 60 m·s<sup>-1</sup> with precision of  $\pm 2\%$ (at 12 m·s<sup>-1</sup>) and resolution of 0.01 m·s<sup>-1</sup>. The direction measurement ranged from 0° to 359° with a precision of ±3% (at 12 m·s<sup>-1</sup>) and resolution of 1°. A data-logger CR10X (Campbell Scientific, Shepshed, Loughborough, UK) recorded the values of indoor and outdoor air temperature, RH, air velocity and direction, wind speed and direction at five-second intervals. Every 5 min, the data-logger computed average values, storing them in memory locations. Additional information on cow behavior and barn management was obtained through a video recording system by using 10 cameras (Kon.Li.Cor, Ecosearch, Perugia, Italy) positioned 4 m above the floor.

#### 2.4 Data analyses

Data acquired in the experimental tests conducted during warm periods from 2016 to 2022 were processed and organized into distinct data sets. In addition to variables collected by instruments (i.e., gas concentrations, air velocity, air



**Fig. 3** 3D model of the barn with the position of sampling locations (SLs) in the two configurations. The configuration related to the first experiment was based on the monitoring of the gases at SLs located 0.40 m above the floor (i.e., all yellow SLs and the blue SLZ outside the barn). The second configuration acquired data in the central sampling poles in Box 2. The distance between the floor and the SLs was 0.40 m (i.e., yellow SLs), 1.55 m (i.e., orange SLs) and 2.70 m (i.e., green SLs) for SLh1, SLh2 and SLh3, respectively.

temperature and RH), specific indices (i.e., temperature-humidity, cow lying, cow standing and cow feeding indices) and emissions calculated via  $\rm CO_2$  mass balance were derived using climatic sensors and the video recording system.

#### 2.4.1 Temperature-humidity index

The temperature-humidity index (THI) is a parameter representing the heat stress for cows under specific conditions of temperature and RH the animal is exposed to<sup>[32]</sup>. Based on the literature<sup>[33,34]</sup>, the equation applied for hot climate conditions was:

THI = 
$$(1.8 \times T_{db} + 32) - (0.55 - 0.55 \times RH \div 100) \times (1.8 \times T_{db} - 26)$$

where,  $T_{db}$  is the dry bulb air temperature (°C) and RH is the air RH (%).

Armstrong<sup>[35]</sup> defined many ranges of THI that are representative of specific heat stress condition for cows: THI  $\leq$  72 represents no stress conditions; 72 < THI  $\leq$  78 represents a low thermal stress; 78 < THI < 84 represents thermal stress condition; and THI  $\geq$  84 represents conditions of emergency. In this study, gas concentrations associated to THI  $\geq$  84 were not recorded. Gas concentrations measured in this study were grouped under these three ranges of THI.

# 2.4.2 Indices related to animal behavior and barn management

Cow behavioral indices (i.e., cow lying, cow standing and cow feeding indices) were determined using the video recording system. A skilled operator applied the scan sampling method for image visual assessment, consistent with previous studies<sup>[36,37]</sup>. This involved counting the number of cows exhibiting specific animal behaviors from the video-recorded images with a sampling frequency of 15 min. The cow behavioral index for feeding, lying and standing behaviors was then computed by taking the ratio of animals exhibiting a particular behavior to the total number of animals<sup>[28]</sup>. Based on cow behavioral indices and barn management, distinct groups of animal behavior were identified: (1) cow activity, encompassing standing, eating and walking; (2) cow lying in the resting area; and (3) cows walking in the feeding and service alleys in preparation for floor cleaning.

#### 2.4.3 Emission estimation

Hourly  $NH_3$  and  $CH_4$  emissions were estimated by applying the  $CO_2$  mass balance method, generally used for naturally ventilated dairy buildings<sup>[38]</sup>. The ventilation rate was calculated as:

$$Q = (P_{CO_2} \times N) / (C_{CO_2 \text{in}} - C_{CO_2 \text{out}})$$
 (2)

where,  $P_{CO_2}$  represents the excretion rate of  $CO_2$  from one cow (g·h<sup>-1</sup> per cow), N is the number of cows inside the building, Q is the ventilation rate calculated according to the  $CO_2$  balance (m³·h<sup>-1</sup>),  $C_{CO_2in}$  is the hourly average concentrations of the gas inside the barn computed by using the four SLs at the center of the barn (i.e., SLL, SLM, SLI, SLH), and  $C_{CO_2out}$  is the value outside the building acquired at SLZ, respectively (g·m<sup>-3</sup>).

The CO<sub>2</sub> excretion rate was calculated by using the following equations<sup>[39]</sup>:

$$qt = 5.6 \times m^{0.75} + 1.6 \times 10^5 \times p^3 + 22 \times y$$
 (3)

$$CF = 4 \times 10^5 \times (20 - T_i)^3 + 1 \tag{4}$$

$$q_{cor} = q_t \times CF \tag{5}$$

$$P_{\text{CO}_2} = 0.299 \times q_{\text{cor}} \tag{6}$$

where,  $q_t$  is the total heat production (W),  $q_{cor}$  is the corrected value of the total heat production (W), m is the average mass of the cows (kg per cow), p is the number of days after insemination (d), y is the milk yield (kg·d<sup>-1</sup>),  $T_i$  is the temperature inside the barn (°C), and CF is the temperature correction factor. The milk yield, average mass, and days of pregnancy of animals were 32 kg·d<sup>-1</sup>, 650 kg per cow and 135 days, respectively.

The emission rate of NH<sub>3</sub> and CH<sub>4</sub> was estimated by using the equation:

$$E_{t} = Q \times (C_{in} - C_{out}) \tag{7}$$

where,  $E_t$  is the emission rate of the gas  $(g \cdot h^{-1})$ , Q is the ventilation rate calculated according to the  $CO_2$  balance method  $(m^3 \cdot h^{-1})$ ,  $C_{in}$  is the average concentrations of the gas  $(g \cdot m^{-3})$  (i.e.,  $NH_3$ ,  $CH_4$ ) inside the barn computed by using the four SLs at the center of the barn (i.e., SL-L, SL-M, SL-I, SL-H), and  $C_{out}$  is the value recorded outside the building at SL-Z.

The equation for emissions expressed per livestock unit (LU) in  $g \cdot h^{-1} \cdot LU^{-1}$  was:

$$E = (E_t \times LU)(N \times m)^{-1}$$
 (8)

where, LU is equal to 500 kg as a cow mass reference value [40]. This parameter was employed due to variations in cow weight across herds. Consequently, emissions are measured not on a per-animal basis but rather on a per-LU basis.

#### 2.5 Statistical analyses

Measured data and computed indices underwent various statistical analyses by using Microsoft Excel and Minitab.

Groups of gas concentrations and emissions were examined by one-way analysis of variance (ANOVA) with a significance level of p < 0.05, followed by Tukey's post hoc test. Throughout all experiments, the number of observations consistently surpassed the minimum required for statistical significance.

The initial analysis examined gas distribution using NH<sub>3</sub>, CH<sub>4</sub> and CO<sub>2</sub> concentrations recorded at different horizontally and vertically distributed SLs with a 15-min sampling frequency. Two one-way ANOVA were performed for each gas. In the first, the gas distribution was evaluated across various horizontally positions of the SLs. Gas concentrations at three groups of SLs were examined: SL H1 for SLs in the central area (SLH, SL-Ih1, SL-Lh1 and SLM,); SL\_H2 for SLs at the perimeter (SLB, SLC, SLD and SLE); SL\_H3 for SLs at the corners (SLA, SLF and SLG). Subsequently, these groups were differentiated using Tukey's honestly significant difference test at p < 0.05 (post hoc test). In the second, the gas concentration was analyzed at different vertically distributed SLs by using three groups: SL\_V1 for gas concentrations acquired at SLs near the floor (SL-Ih1 and SL-Lh2, 0.40 m above the floor), SL\_V2 for gas concentrations acquired close to the upper bar of the feeding rack,1.55 m above the floor (SL-Ih2 and SL-Lh2, 1.55 m above the floor) and SL\_V3 for gas concentrations acquired close to the fans in the feeding alley, 2.70 m above the floor (SL-Ih3 and SL-Lh3, 2.70 m above the floor) (Fig. 3).

The second analysis examined climatic conditions, comparing gas concentrations and emissions at different air velocities (i.e., low air velocity of  $\leq 0.5~\text{m}\cdot\text{s}^{-1}$  and high air velocity of  $>0.5~\text{m}\cdot\text{s}^{-1}$ ). In particular, a one-way ANOVA was conducted for each gas, dividing the data into two groups: gas concentrations measured during low air velocity of  $\leq 0.5~\text{m}\cdot\text{s}^{-1}$  and gas concentrations recorded at high air velocity of  $>0.5~\text{m}\cdot\text{s}^{-1}$ . Another ANOVA for each gas focused on emissions and the two groups identified for the statistical analyses were emissions measured during low air velocity of  $\leq 0.5~\text{m}\cdot\text{s}^{-1}$  and gas emissions recorded at high air velocity of  $>0.5~\text{m}\cdot\text{s}^{-1}$ .

The third analysis examined concentrations and emissions under varying barn management, including comparisons related to cooling system modes (i.e., activation/deactivation of the sprinklers in the feeding alley) and number of daily milkings (i.e., two or three). Specifically, a one-way ANOVA was conducted for each gas by using two groups: gas concentrations acquired when the sprinklers in the feeding alley were switched on and gas concentrations acquired when the sprinklers in the feeding alley were switched off. Another one-way ANOVA for each gas was executed using gas

emissions acquired for the two groups mentioned above. Also, a one-way ANOVA was utilized to examine gas concentrations of NH<sub>3</sub>, CH<sub>4</sub>, and CO<sub>2</sub> within two groups, corresponding to gas concentrations recorded during two and three daily milkings.

The fourth analysis examined concentrations and emissions under various animal welfare conditions. An individual ANOVA was conducted for each gas, taking into account the gas concentrations obtained from three THI groups: THI  $\leq$  72 for the absence of heat stress, 72 < THI  $\leq$  78 indicating a low risk of thermal stress for cows and 78 < THI < 84 representing thermal stress. Additionally, a separate one-way ANOVA was performed for emissions of each gas for three THI groups.

The final analysis examined gas concentrations and emissions associated with cow behavior. Specifically, one-way ANOVA for each gas was performed considering the following three groups: gas concentrations measured during activity, gas concentrations recorded during lying, and gas concentrations acquired during activity of cows when the floor of the barn was cleaned. Another one-way ANOVA for each gas was performed by using the following groups: emissions estimated during activity, emissions estimated during lying and emissions estimated during activity of the cows when the floor of the barn was cleaned.

# 3 Results

Throughout the observation period (i.e., 2016–2022), mean values of air temperature, RH, and air speed ranged in 16.8–28.8 °C, 35.1%–86.5% and 0.22–2.09 m·s<sup>-1</sup>, respectively. One-way ANOVA revealed that the spatial distribution of NH<sub>3</sub>, CH<sub>4</sub> and CO<sub>2</sub> in the barn was non-uniform both horizontally and vertically. The results of the statistical analyses on the distribution of gas concentrations are presented in Table 2.

The results concerning the distribution of gas concentrations exhibited significant differences (p < 0.001) with changes in the SLs position, both vertically and horizontally. Specifically, for horizontally distributed SLs close to the floor, the central sampling locations showed the highest concentrations of NH<sub>3</sub>, CH<sub>4</sub> and CO<sub>2</sub>, while the lowest gas concentrations were measured when the SLs were positioned around the perimeter of the barn. Additionally, CH<sub>4</sub> values significantly varied across all SLs, with the highest concentrations near the fans and the lowest gas concentrations near the upper bar of the feeding rack, CO<sub>2</sub> concentrations recorded near the upper bar of the feeding rack were significantly higher than those acquired in

Table 2 Statistical analyses performed for groups of NH<sub>3</sub>, CH<sub>4</sub> and CO<sub>2</sub> concentrations measured at different horizontal (i.e., SLs in the central area, SLs at the perimeter and SLs at the corner) and vertical (i.e., SLs near the floor, SLs near the upper bar of the feeding rack and SLs near the fans) SLs in the barn

Groups	Horizontal distribution of SLs located near the floor		Vertical distribution of SLs in the central area of the barn			
Groups	Gas concentrations (μg·g <sup>-1</sup> )	SD	Gas concentrations (μg·g <sup>-1</sup> )	SD		
	SLs in the central	SLs in the central area		SLs near the floor		
NH <sub>3</sub>	7.4 <sup>a*</sup>	2.4	3.5 <sup>a</sup>	1.2		
$CH_4$	15 <sup>a</sup>	6	11 <sup>b</sup>	11		
CO <sub>2</sub>	724 <sup>a</sup>	124	594ª	79		
	SLs at the perim	SLs at the perimeter		SLs near the upper bar of the feeding rack		
NH <sub>3</sub>	3.4 <sup>b</sup>	0.8	1.7 <sup>b</sup>	0.5		
$CH_4$	8 <sub>p</sub>	3	9с	7		
CO <sub>2</sub>	597 <sup>b</sup>	43	560 <sup>b</sup>	50		
	SLs at the corners		SLs near the fans			
NH <sub>3</sub>	1.8°	0.4	1.7 <sup>b</sup>	0.6		
$CH_4$	7 <sup>b</sup>	3	18 <sup>a</sup>	15		
CO <sub>2</sub>	580 <sup>b</sup>	33	599 <sup>a</sup>	84		

Note: Each gas in a specific position (i.e., horizontal or vertical) has a specific color. \*Group means with a specific color followed by the same letter are not significantly different within each gas and distribution.

the other vertically distributed SLs, and NH<sub>3</sub> concentrations measured near the floor were significantly higher at the barn center than concentrations measured in other vertically distributed SLs. Also, NH<sub>3</sub> concentrations decreased from the floor to the roof of the barn. These findings align with those depicted in Fig. 4 and Fig. 5, illustrating the daily trend of gas concentrations at different SLs.

The impact of driving forces on gas concentrations and emissions is illustrated in Table 3 and Table 4, respectively. The findings demonstrate the statistical significance of how climatic conditions, barn management, animal welfare and animal behavior influence gas concentrations and emissions. Notably, in all results, the p value consistently remained below 0.05.

Specifically, NH<sub>3</sub>, and CH<sub>4</sub> and CO<sub>2</sub> concentrations were significantly influenced by different air velocities (p < 0.001). Notably, gas concentrations were highest at air velocities below 0.5 m·s<sup>-1</sup> whereas they were lowest when air velocities exceeded 0.5 m·s<sup>-1</sup>.

Differences in the management of the cooling system in the barn resulted in significant differences in  $CH_4$  concentrations. Specifically,  $CH_4$  concentrations were lower when the sprinklers were switched off in the feeding alley compared to those measured when the sprinkler system was activated.

The number of daily milkings produced significant effects on gas concentrations for all the gases (p < 0.001). NH $_3$  concentrations when barn management included three daily milkings were higher than those measured when barn management was based on two daily milkings. In contrast, CH $_4$  and CO $_2$  concentrations when barn management was based on three daily milkings were lower than with two daily milkings.

Given the observed significant influence of air velocity on gas concentrations, groups of animal welfare and behavior data were studied at air velocity  $\leq 0.5 \text{ m} \cdot \text{s}^{-1}$  to mitigate the impact of these parameters on gas concentrations.

Analysis of gas concentrations across the THI ranges indicated that THI significantly affected NH<sub>3</sub>, CO<sub>2</sub> and CH<sub>4</sub> concentrations (p < 0.05). The highest NH<sub>3</sub> concentration was observed with 72 < THI  $\leq$  78. CO<sub>2</sub> and the highest CH<sub>4</sub> concentrations with THI  $\geq$  72, decreasing with THI  $\leq$  72.

The results also revealed a significant difference between CO<sub>2</sub> and CH<sub>4</sub> concentrations measured during cow activity and cow lying. It was also observed a significant difference between NH<sub>3</sub> concentrations recorded during cow lying, cow activity and floor cleaning, with the highest gas concentrations recorded during the latter operation in the barn.

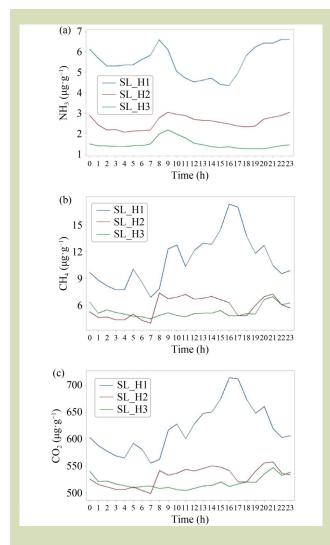


Fig. 4~ Hourly trend of gas concentrations of  $\text{NH}_3$  (a),  $\text{CH}_4$  (b), and  $\text{CO}_2$  (c) computed at three different horizontal position. The blue, red and green lines show the hourly mean value of gas concentrations recorded at central SLs (i.e., SL\_H1), at perimeter SLs (i.e., SL\_H2) and corner SLs (i.e., SL\_H3), respectively.

According to the findings presented in Table 4, climatic conditions, barn management, animal welfare and animal behavior also influenced gas emissions. The statistical analyses demonstrated that both NH<sub>3</sub> and CH<sub>4</sub> emissions were significantly influenced by air velocity (p < 0.001). Specifically, NH<sub>3</sub> and CH<sub>4</sub> emissions were higher with an air velocity of  $\leq 0.5 \text{ m} \cdot \text{s}^{-1}$  than with an air velocity of  $> 0.5 \text{ m} \cdot \text{s}^{-1}$ .

For the management of the cooling system, NH<sub>3</sub> and CH<sub>4</sub> emissions were significantly influenced by the activation of sprinklers in the feeding area. Specifically, lower CH<sub>4</sub> emissions in the barn were observed when the sprinklers were switched

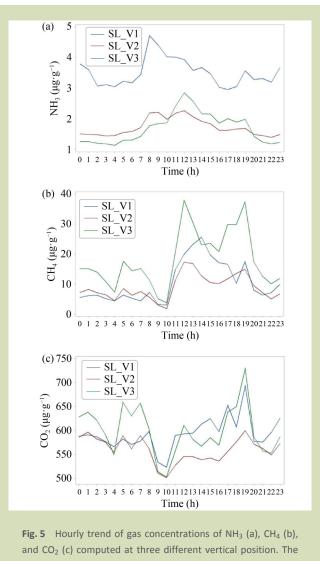


Fig. 5 Hourly trend of gas concentrations of NH $_3$  (a), CH $_4$  (b), and CO $_2$  (c) computed at three different vertical position. The blue, red and green lines show the hourly mean value of gas concentrations measured at SLs located 0.40 (i.e., SL\_V1), 1.55 (i.e., SL\_V2) and 2.70 m (i.e., SL\_V3) above the floor, respectively.

off in the feeding alley compared to when the sprinkler system was activated. Conversely, NH<sub>3</sub> emissions were higher when the sprinklers were switched off in the feeding alley compared to when the sprinkler system was activated.

Significant differences were observed in  $NH_3$  and  $CH_4$  emissions across the THI ranges.  $NH_3$  emissions at the three THI intervals were all significantly different from each other. The highest and the lowest  $NH_3$  and  $CH_4$  emissions occurred with  $THI \leq 72$  and 78 < THI < 84, respectively.

Based on the one-way ANOVA results, NH<sub>3</sub> and CH<sub>4</sub> emissions were influenced by animal behavior. Notably, the

highest  $NH_3$  and  $CH_4$  emissions occurred during barn cleaning, while the lowest  $NH_3$  emissions occurred during activity of the cows.

## 4 Discussion

The most commonly used method for estimating emissions in naturally ventilated dairy barns is the CO<sub>2</sub> mass balance method<sup>[38]</sup>. Recent published studies have focused on identifying strategies to enhance its application<sup>[17,18]</sup>. Since gas concentrations are relevant parameters influencing estimation outcomes of the CO<sub>2</sub> mass balance method, a better understanding on gas variability is essential for explaining gas emission releases. Specifically, based on the applied model, the ratio between the measured outdoor and indoor concentrations differences of the tracer gas (CO<sub>2in</sub>-CO<sub>2out</sub>) and gas pollutants (i.e., NH<sub>3in</sub>-NH<sub>3out</sub> and CH<sub>4in</sub>-CH<sub>4out</sub>) elucidates how specific factors could increase/reduce emissions. Variations in gas concentrations significantly affect emission estimation.

The outcomes of this research contribute to the knowledge base for analyzing gas concentration production in an open dairy barn during warm periods. In the Mediterranean climate, these dairy barns are characterized by an open structure, and farmers implement specific barn management practices that enhance animal welfare and modify indoor microclimatic conditions, with effects on distribution of gaseous concentrations and related emissions.

Consistent with published studies reporting that the gas distribution is non-uniform in dairy barns [19,21,41,42], the results of this study confirmed that a non-uniform distribution of gas concentrations was also observed in the open barn under study. Distribution was influenced by the barn topology, the openings and the orientation of the building. The absence of the perimeter walls, building orientation for natural ventilation along the prevailing wind direction, and fans activation during warm periods diluted and flushed concentrations, reducing gas levels. The barn management (i.e., cooling system, number of milking sessions, and floor cleaning) affected gas concentrations, especially when a management practice increased animal activity. In fact, when the cooling system of the feeding alley was operated, cow increased feeding at the trough and thus animal activity increased; this resulted in an increase of the concentrations at the center of the barn. Also, the non-uniform vertical distribution of NH<sub>3</sub>, CH<sub>4</sub> and CO<sub>2</sub> concentrations was connected to the effect of fans that removed air from the feeding rack area to the outside along the longitudinal axis of the barn<sup>[18]</sup>. In fact, the tilt angle of the fans directed air from the upper bar of the feeding rack to the exterior of the barn. These findings are consistent with other studies<sup>[43]</sup> indicating that NH<sub>3</sub> concentrations decreased from the bottom to the top in similar open housing systems. However, a different vertical distribution of gas concentrations was found in other studies[42-44]. In the study of Mendes et al.<sup>[44]</sup>, NH<sub>3</sub> increased, and CO<sub>2</sub> decreased from bottom to top in a mechanical ventilated dairy barn. Sahu et al.[42] identified the highest NH3 concentrations at the top height (2.7 m) in a naturally ventilated dairy barn, with no significant differences in CO2 and CH4 concentrations at the bottom and center heights. In the barn under study, the presence of fans led to increased air dilution from the middle to the top of the barn. Another contributing factor to this dilution effect along the vertical distribution is the activation of the sprinkler system during the warmest hours of day. Indoor conditions were altered by the added water on the floor, which, through diluting urine in puddles, reduced NH3 concentrations in the air, as reported by Baldini et al.<sup>[5]</sup>.

Barn management practices (e.g., the activation of cooling systems, and the number of milking sessions per day) influenced gas concentration levels and related emissions due to the effects on cow behavior (e.g., time spent at lying increased when the sprinkler system at the feeding alley was not operated due to the activation of the sprinklers in the resting area) (Table 2 and Table 3). Notably, the highest NH<sub>3</sub> concentrations and emissions were generally linked to barn floor cleaning and cow activity. The barn floor cleaning operation resulted in the highest NH3 concentration and emissions because, during this process, the mixing of urea and faces in the barn reached its peak during the day. This resulted from the combined effect of the tractor performing the cleaning of the barn floor and increased animal activity. These findings are consistent with a published study<sup>[45]</sup> suggesting that the mix of urea and feces contributes to the production of NH<sub>3</sub> during the day. Another influencing factor related to barn management is the number of milkings per day<sup>[46]</sup>. The addition of one extra milking per day led to increased NH3 concentrations in the barn (as given in Table 2) due to increased cow activity, as cows were fed at the trough before and after each milking, thus increasing their activity at the trough.

The intensification of thermal stress throughout the day, resulting in increased respiratory activity, explained the highest CH<sub>4</sub> and CO<sub>2</sub> production values predominantly recorded during the daylight hours. According to the literature, cows increase heat dissipation under stressful conditions by spending more time standing and less time lying<sup>[29,47]</sup>. In the barn under study, the duration of time spent lying increased

Groups	Concentrations (μg·g <sup>-1</sup> )	SD	Concentrations (μg·g <sup>-1</sup> )	SD	Concentrations (μg·g <sup>-1</sup> )	SD
Climatic conditions	Low air velocity of $\leq 0.5$		High air velocity of > 0.5			
NH <sub>3</sub>	8.8a*	2.2	6.6 <sup>b</sup>	1.2		
CH <sub>4</sub>	15 <sup>a</sup>	8	9ь	4		
$CO_2$	778 <sup>a</sup>	157	705 <sup>b</sup>	80		
Barn management	Cooling system on		Cooling system off			
NH <sub>3</sub>	7.7 <sup>a</sup>	2.4	7.8a	2		
$\mathrm{CH_4}$	16ª	6	12 <sup>b</sup>	5		
CO <sub>2</sub>	736 <sup>a</sup>	124	745 <sup>a</sup>	104		
Barn management	Three milkings per day		Two milkings per day			
NH <sub>3</sub>	8.8 <sup>a</sup>	2.5	7.4 <sup>b</sup>	2.9		
CH <sub>4</sub>	12 <sup>b</sup>	10	15ª	8		
CO <sub>2</sub>	704 <sup>b</sup>	96	721 <sup>a</sup>	144		
Animal welfare	THI ≤ 72		72 < THI ≤ 78		78 < THI < 84	
NH <sub>3</sub>	8.3 <sup>b</sup>	1.6	10.3ª	2.1	8.2 <sup>b</sup>	2.4
CH <sub>4</sub>	12 <sup>c</sup>	5	18 <sup>b</sup>	7	23ª	20
$CO_2$	702 <sup>c</sup>	54	854 <sup>b</sup>	160	943a	169
Animal behavior	Lying		Activity		Activity of the cows during floor cleanin	
NH <sub>3</sub>	7.4 <sup>c</sup>	0.8	9.0 <sup>b</sup>	2.3	10.6ª	1.4
$CH_4$	10 <sup>b</sup>	4	18ª	9	18ª	10
$CO_2$	690°	50	852ª	168	775 <sup>b</sup>	138

Note: \*Group means followed by the same letter are not significantly different within each row. In detail, within each row there are groups of climatic conditions (i.e., low air velocity, and high air velocity), management of the cooling system (i.e., cooling system on, and cooling system off), number of daily milkings (i.e., 2 and 3), animal welfare (i.e.,  $THI \leq 72$ ,  $TIH \leq 78$ , and  $TIH \leq 78$ 

during the hottest hours of the day due to the activation of the cooling system in the cubicles<sup>[27,48]</sup>. However, peaks in these gases may be linked to the rumination activity, which could be influenced by the routine of the cows (e.g., number of milking sessions). The daily fluctuations in NH<sub>3</sub> and CH<sub>4</sub> emissions were influenced by the management of fans and sprinklers, exerting effects on indoor climatic conditions. Specifically, when the sprinkler system was turned off, higher NH<sub>3</sub> emissions were observed<sup>[5]</sup> due to the diminished presence of the water in the puddles. Additionally, during this period, cow behavior was influenced by the distinct management of the cooling system; in detail, time spent lying increased, and time spent feeding decreased, resulting in a reduction of CH<sub>4</sub> emissions during the investigated period (Table 3 and Table 4).

# 5 Conclusions

In this study, statistical elaborations on experimental data proved that gas concentration monitoring and emissions estimations in open dairy barns during warm periods are impacted by various factors.

Through an examination of gas concentrations and emissions, environmental conditions, animal behavior and welfare, specific operations control (e.g., number of milkings, cleaning frequency of the barn floor, activation of sprinkler systems and enhancing ventilation with fans) could yield precise information for reducing environmental impacts and enhancing animal welfare and health.

The findings of this study pinpoint locations in the barns with elevated gas concentrations, where gas concentrations should be monitored, and contribute to guide monitoring efforts to verify threshold limits and investigate the effectiveness of mitigation measures. In addition, the outcomes showed that this barn topology exhibits unique features altering distribution of gas concentrations and emissions compared to other dairy barn topologies. Consequently, it is crucial to assess whether

Groups	Emissions $(g \cdot LU^{-1} \cdot h^{-1})$	SD	Emissions (g·LU <sup>-1</sup> ·h <sup>-1</sup> )	SD	Emissions (g·LU <sup>-1</sup> ·h <sup>-1</sup> )	SD
Climatic conditions	Low air velocity of $\leq 0.5$		High air velocity of > 0.5			
NH <sub>3</sub>	$7.04^{a^*}$	3.3	5.25 <sup>b</sup>	1.91		
CH <sub>4</sub>	7.66 <sup>a</sup>	1.09	6.86 <sup>b</sup>	1.36		
Barn management	Cooling system on		Cooling system off			
NH <sub>3</sub>	4.77 <sup>b</sup>	1.95	5.9 <sup>a</sup>	1.96		
CH <sub>4</sub>	5.85 <sup>a</sup>	1.2	5.52 <sup>b</sup>	1.28		
Animal welfare	THI ≤ 72		72 < THI ≤ 78		78 < THI < 84	
NH <sub>3</sub>	6.18 <sup>a</sup>	1.47	4.37 <sup>b</sup>	1.62	3.02 <sup>c</sup>	1.21
CH <sub>4</sub>	6.19 <sup>a</sup>	1.01	5.98 <sup>b</sup>	0.73	5.11 <sup>b</sup>	2.07
Animal behavior	Lying		Activity		Cleaning of the barn	
$NH_3$	5.40 <sup>a</sup>	1.41	3.73 <sup>b</sup>	1.82	6.14 <sup>a</sup>	2.36
CH <sub>4</sub>	5.69 <sup>b</sup>	1.01	5.85 <sup>b</sup>	0.98	6.61 <sup>a</sup>	0.97

Note: \*Group means followed by the same letter are not significantly different within each row. In detail, within each row there are groups of climatic conditions (i.e., low air velocity, and high air velocity), management of the cooling system (i.e., cooling system on, and cooling system off), animal welfare (i.e.,  $THI \le 72$ ,  $72 < THI \le 78$ , and 78 < THI < 84), animal behavior (i.e., lying and movement of the cows during the cleaning of the floor).

existing protocols are applicable to this specific barn topology through dedicated studies.

In advancing knowledge in this field, further investigations into gas concentrations and emission factors related to this barn topology across different seasons and their influencing parameters are recommended. Exploring additional parameters not addressed in this study (e.g., circadian rhythms) could provide deeper insights. By accumulating more knowledge about this barn topology, strategies for emissions reduction can be refined, leveraging the distinctive features of the barn for enhanced effectiveness.

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#### Compliance with ethics guidelines

Provvidenza Rita D'Urso, Claudia Arcidiacono, and Giovanni Cascone declare that they have no conflicts of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

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