

Article

Enteric Methane Emissions of Dairy Cattle Considering Breed Composition, Pasture Management, Housing Conditions and Feeding Characteristics along a Rural-Urban Gradient in a Rising Megacity

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Received: 23 September 2020; Accepted: 11 December 2020; Published: 13 December 2020



Abstract: Greenhouse gas emissions from livestock farming and in particular enteric methane (CH₄) from ruminants are criticized for being one of the main contributors to climate change. Different breeding, feeding and management strategies are tested to decrease these emissions, but a status quo analysis is also relevant to implement such measures. The present study aimed to analyze the concentration of CH₄ in air exhaled by dairy cows along a rural-urban gradient of Bangalore, India. Urban, mixed and rural areas were defined based on a survey stratification index (SSI) comprising build-up density and distance to the city center. Using a laser methane detector (LMD), CH₄ concentration was determined in 2-min spot measurements of exhaled air of 448 cows at three equally spaced visits between June 2017 and April 2018. Mean, maximum and CH₄ concentration per duration of the overall measurement, eructation and respiration bouts were calculated. For the overall mean and respiration bouts, CH₄ concentration was higher in cows from urban areas, which had also higher milk yield than cows from mixed and rural areas. Although no differences were found in terms of the intake level of fibrous diet components, the type of measurement location (indoor, half-outdoor or outdoor) and pasture access had an impact on CH₄ concentration. To our knowledge, this is the first study using the LMD on-farm and in an urbanizing environment. The LMD measurements show variations in enteric CH₄ emissions along the rural-urban gradient of Bangalore that reflect differences in dairy husbandry systems governed by the social-ecological context.

Keywords: dairy cattle; husbandry system; laser methane detector (LMD); methane emission; rural-urban gradient; survey stratification index

1. Introduction

In the past 30 years, world milk production has increased by more than 52% from 542 million tons in 1990 to 828 million tons in 2017. In the same period, India has tripled its milk production from 54 to 176 million tons, becoming the world's largest milk producer with 21% of the global production [1]. With more than 78 million dairy farms [2] that are mostly smallholdings with two to six cows [3],

India hosts 18% of the global dairy cattle population [1]. Moreover, urbanization in India has increased rapidly and the share of urban population has doubled in the past 60 years [4]. Urban and (peri-)urban agriculture have contributed to food security and covered the demand for food products in these areas, e.g., with regard to milk consumption, by keeping dairy cattle close to urban areas [5].

As a detrimental by-product, the livestock farming sector contributes 14.5% to the global greenhouse gas (GHG) emissions [6], whereby enteric methane (CH_4) emitted by ruminant livestock is the largest source (17%) of global anthropogenic CH_4 emissions [7]. GHG emissions are expected to increase by 35% until 2050, especially in developing countries due to animal population growth driven by increased demands of meat and dairy products [8].

Several techniques have been developed to measure enteric CH_4 emissions from ruminants. Among them, the respiration chamber is considered the reference method although it is neither portable nor suitable for recording a large number of animals [9]. Under practical farming conditions, the Sulphur hexafluoride (SF_6) tracer technique [10] has a high accuracy. Nevertheless, both CH_4 recording techniques are expensive and can interfere with animal behavior [9,11]. Sniffer methods like GreenFeed (C-Lock Inc., Rapid City, SD, USA) are portable and labor extensive, but require a certain extent of animal training [11]. The laser methane detector (LMD) technique was first suggested by Chagunda et al. [12] for large-scale trait recording in the field. Since then, several studies applied the methodology in ruminants [13–16]. Even though concerns exist in view of the repeatability and accuracy of the measurements [11,17], due to its portability, low cost, ease of handling, non-invasiveness and non-interference with animal behavior, the LMD is currently the most flexible and simple method for on-farm determination of enteric CH_4 emissions.

The predictions of CH_4 emission from ruminant livestock traits is another option to calculate GHG emissions. Ricci et al. [18] proposed a multiple equation approach including energy intake, physiological stage and the diet type to improve the precision of CH_4 predictions. Based on feeding characteristics and cow conformation traits, Yin et al. [19] predicted test-day methane emission via deterministic equations from cattle nutrition combined with stochastic simulations. Amount and quality of feed intake have a direct impact on enteric CH_4 emission [20]. The recorded CH_4 emission is also affected by the length of the time interval between cattle feeding and LMD recording [15,21]. Feed intake is associated with farm management practices and affected by environmental components. For example, season, ambient temperature and rainfall influence forage quality and possible access to pasture, and there is evidence that pasture-based dairy farming systems and confinement systems differ in enteric CH_4 output [22]. Heat stress may be higher when animals graze on pasture than when staying in the barn and this affects feed intake [23] as well as animal health [24]. Furthermore, Garnsworthy et al. [25] observed variations in CH_4 emissions depending on body weight (BW) and milk yield (MY) of dairy cows, whereby emissions were higher for cows with higher BW and MY. Although these effects are partly mediated by feed intake and feed composition, the higher metabolic rate of a heavier animal and/or an animal synthesizing more milk may also play a role [26]. Thus, enteric CH_4 emission is the result of a variety of partly coupled physiological, environmental and managerial factors (Figure 1). In the urbanizing Indian context, an important element that integrates both environmental and social aspects is farm location within a rural-urban interface [27]. The spatial location of a farm not only governs its physical access to resources such as pasture, but also plays a role concerning breed type kept, herd structure, housing space and housing structure [27]. These factors in turn shape the livestock management, and as a further consequence they influence productivity as well as CH_4 emissions.

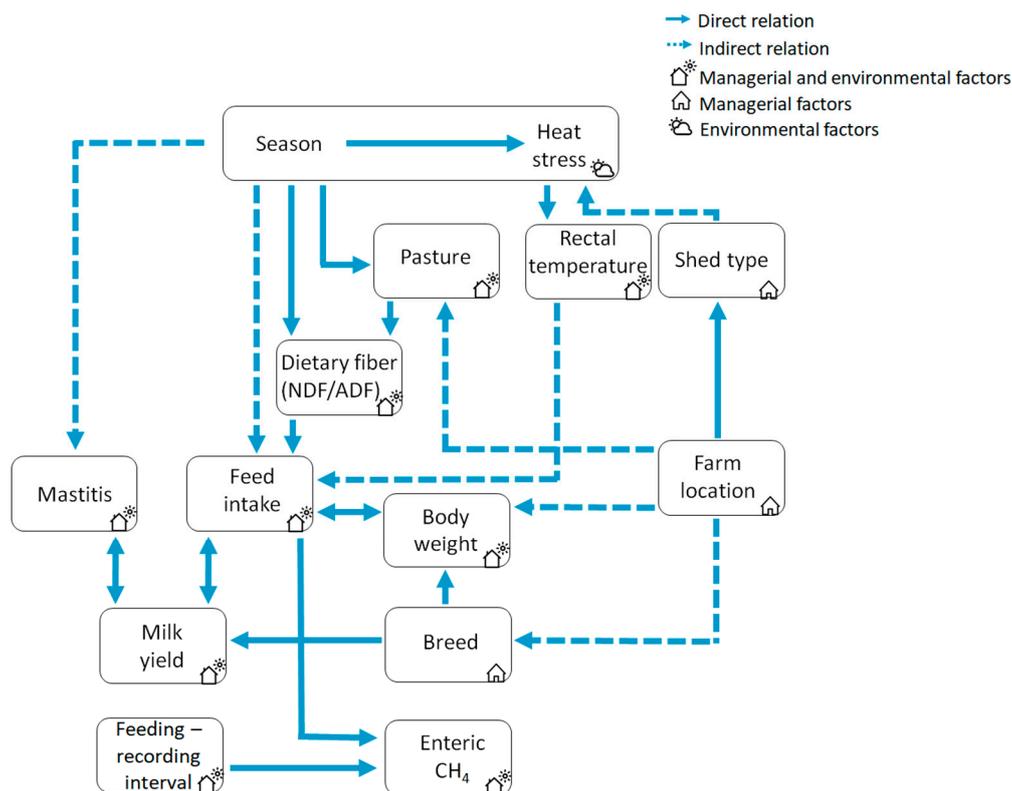


Figure 1. Conceptual framework depicting the relations of spatial, managerial and environmental factors with enteric methane emission across a rural-urban interface.

The combination of social and environmental components within a so-called social-ecological system has recently been proposed for livestock farming systems' classification [28]. Rising megacities are hotspots of complex and dynamic social-ecological systems, because they are very vulnerable to environmental and anthropogenic hazards, and their social-economic components are more diverse than those of smaller urban areas [29]. In consequence, the aim of the present study was an assessment of the social-ecological responsiveness of enteric CH₄ emissions along a rural to urban gradient, choosing the Indian megacity of Bangalore with its challenging environmental conditions, social complexity and large (peri-)urban dairy production [5] as the research site.

2. Materials and Methods

2.1. Farm Selection and Description

The study was conducted in Bangalore, southern India, where the population has doubled in the past 15 years and is expected to grow to over 12 million inhabitants by 2021 [30]. Dairy farms were sampled from 31 different villages located along a northern and southern transect in Bangalore (Figure 2). In order to study the influence of ecological gradients in combination with human-animal interaction, a survey stratification index (SSI) was developed by Hoffmann et al. [31] for each geographical location within the mapped area. The SSI (between 0 and 1) considered the built-up density (houses and infrastructures) and the distance to the city center. In the current study, the SSI considered three SSI clusters: "urban" (SSI < 0.3), "rural" (SSI > 0.5), and "mixed" (SSI: 0.3–0.5). A two-step approach was applied to select the 119 dairy farms. In a first step, villages were selected semi-randomly, considering pre-defined percentages per SSI. In a second step, 20 to 30% of dairy cow herds were randomly selected per village (300 herds), based on the latest vaccination list for foot-and-mouth disease. To ensure continued, though minimal, participation in milk production, only dairy cow herds with two or more dairy cows were sampled. The original 300 dairy cow herds were reduced to 119 herds after being

clustered into four groups based on coordinates for herd location, feeding strategies and predominant herd genotypes [27,32]. Each farm was visited three times between June 2017 and April 2018 in intervals of four months, resulting in 835 individual measurements at cow-level from 448 milking cows. The average herd size comprised 3 milking cows and ranged from 2 to 12 cows per farm.

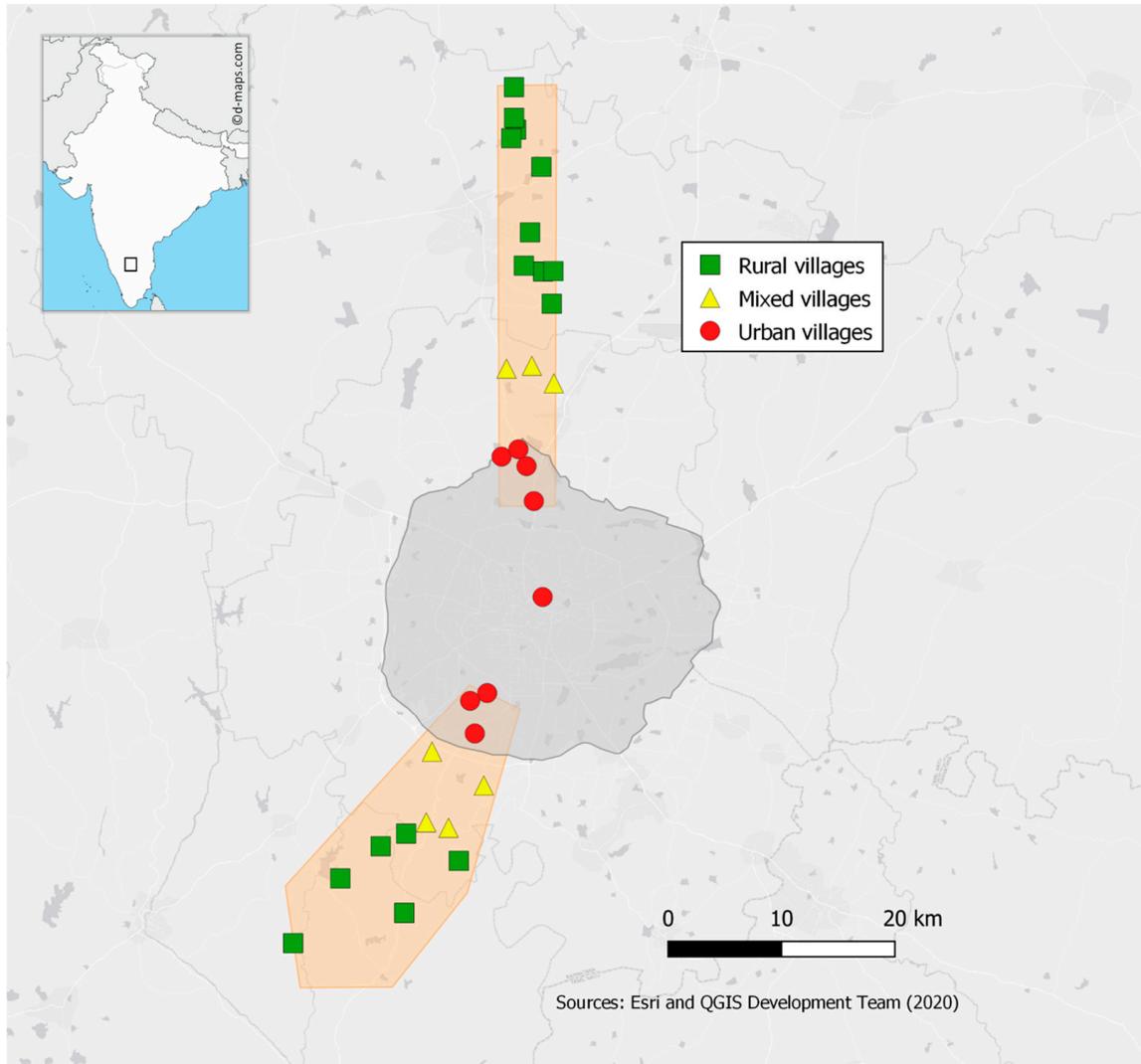


Figure 2. Map of Bangalore depicting the 31 sampled settlements. The dark grey area represents the urban city zone. The orange contours indicate the northern and southern transects.

Temperature and humidity were recorded at every farm visit using a portable weather station (HAMA 87,682 LCD THERMO-/HYGROM. TH-200). The average temperature during the visits was 26.7 °C, ranging from 15.2 to 35.9 °C. Humidity on the farms ranged from 20 to 88%, with an average of 54.7% across farms and visits. The temperature-humidity index (THI) per farm and visit was calculated considering temperature (T) and relative humidity (RH) and applying the NRC [33] formula:

$$\text{THI} = (1.8 \times T + 32) - (0.55 - 0.0055 \times \text{RH}\%) \times (1.8 \times T - 26)$$

In Bangalore, access of cattle to pasture depended on farm location and varied with season, farmer's management strategy and health status of the cow. In this regard, at every visit, the pasture access (Past: yes, no) was recorded for each animal. Percentages of measurements with access to pasture in the three SSI clusters are listed in Table 1. None of the farms had ad libitum access to water, however, they offered water at milking time (15 L approx. twice a day) along with the concentrates.

Table 1. Percentage of measurements per breed, pasture access (Past), measurement location (Loc), average milk yield (MY) and body weight (BW) for each survey stratification index (SSI) cluster.

SSI Cluster	No. of Measurements	Breed (%)			Past (%)		Loc (%)			MY (L/day)	BW (kg)
		Exotic	Crossbreed	Native	No	Yes	Indoor	Half-Outdoor	Outdoor		
Urban	191	51.3	45.6	3.1	29.3	70.7	46.1	16.7	37.2	11.5	392.6
Mixed	250	62.0	31.2	6.8	34.4	65.6	32.0	11.2	56.8	10.5	382.8
Rural	394	68.3	31.5	0.2	62.7	37.3	22.6	29.7	47.7	10.4	379.1

2.2. Cattle Production and Conformation Traits

Milk yield (MY), heart girth (HG) and body condition score (BCS) were recorded for all cows. BCS was subjectively scored on a linear scale ranging from 1 (thin) to 5 (fat) in increments of 0.25 [34]. Out of 448 cows, 285 were exotic breeds (Holstein Friesian and Jersey), 13 were native breeds (Zebu cattle, mainly of the breed Hallikar) and 150 were first and multigenerational crossbreeds (exotic × native). Due to significant correlations between CH₄ and BW in previous studies [25], BW of cows at recording was predicted based on the conformation traits HG, BCS, age, breed and pregnancy status, according to the following equation [35]:

$$BW = \exp(-7.35492 + (2.55408 \times \ln(\text{HG})) + (0.04043 \times \ln(\text{age})) + (\beta_1 \times \text{BCS}) + (\beta_2 \times \text{breed}) + (0.024741 \times \text{pregnant}) + 0.08317)$$

where HG = heart girth measured in cm; age = age at measurement (in month); β_1 = linear regression coefficient for BCS; BCS = body condition score; β_2 = regression coefficient for breed; breed = exotic, native or crossbred; pregnant = 1 (pregnant) or 0 (non-pregnant).

The average lactation number was 2.9 and the maximum lactation number was 12. On average, daily MY, BCS, and BW from the 835 measurements were 10.67 L, 2.65 points, and 383.29 kg, respectively. Average daily MY and BW in the three clusters are displayed in Table 1.

2.3. Methane Spot Measurements

The CH₄ concentration in the exhaled air per cow was measured using the LMD, model Laser Methane mini [36] considering windless environmental conditions, so that wind did not disturb the CH₄ measurements. The measurement location (Loc) was classified into outdoor, indoor and half-outdoor according to the type of shed, in which CH₄ emissions from individual cows were measured: the indoor class depicted a closed room with four walls and a roof, with or without ventilation (Figure 3a); The outdoor class was assigned when cattle were kept on an open field or in a shed without walls (Figure 3c); Half-outdoor was in-between the outdoor and the indoor classification, e.g., a shed with only two walls where air could freely circulate (Figure 3b). Percentage of measurements per measurement location for each SSI cluster is shown in Table 1.

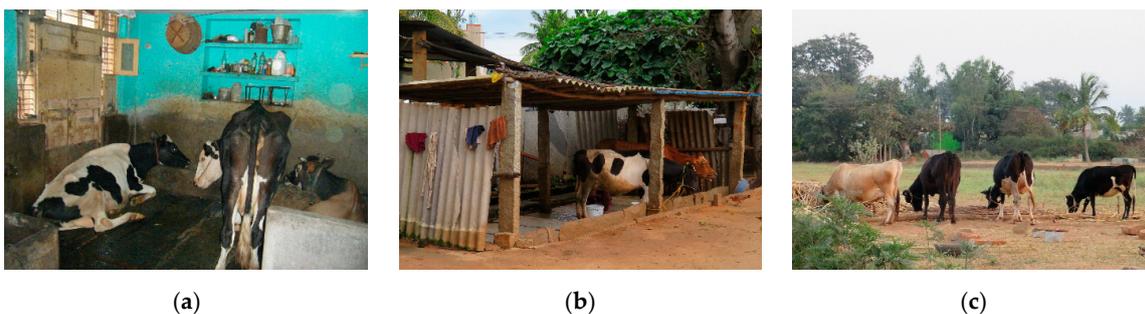


Figure 3. Measurement location for methane emissions: (a) Indoor, (b) half outdoor and (c) outdoor location.

The LMD was programmed to record the CH₄ concentration in narrow intervals of 0.1 s. To capture the exhaled air, the green laser guide light from the LMD was pointed at the animal's nostrils for 2 min, implying a single dataset per measurement including 1200 CH₄ concentration values. Measurements were taken once per cow and visit, by milking time and at least 2 h after feeding. Thus, the mean time interval from cow feeding to the CH₄ recording was 3.85 h with a standard deviation of 3.56 h. The LMD automatically identified and recorded errors in the reflectance of the (invisible) laser beam occurring at any 0.1 s. These erroneous values were manually deleted from the data set upon processing. The recorded unit for CH₄ concentration was ppm × meter, but since the distance between the nostrils of the animal and LMD was exactly 1 m, all values were expressed in ppm. A distance range laser was used to ensure the 1 m distance between the nostrils and the LMD.

In a first step, all CH₄ measurements were corrected for the background environmental CH₄ at the measurement location. The minimum CH₄ concentration for each individual measurement and visit was set as environmental CH₄, which was subtracted from all other values of the respective data set. Afterwards, the corrected measurements were categorized into mini-peaks and mini-troughs [15]. Only mini-peaks were kept for further calculations, and the remaining measurements were assumed to represent a mixture of two normal distributions with different means and variances, which correspond to two physiological paths of CH₄ emissions in ruminants, i.e., respiration and eructation of CH₄ [37,38]. Therefore, the CH₄ concentrations were separated into lower (the one with lower mean) and upper (the one with greater mean) normal distributions according to thresholds defined at 10% cumulative probabilities of the upper normal distribution for every individual and visit [15]. The thresholds for every individual measurement were estimated based on the “mixtool” package in R [39]. Measurements lower than the thresholds were defined as respiration CH₄ and the remaining measurements as eructation CH₄. The average share of eructation CH₄ was 77.18% and for respiration CH₄ 22.82% in line with previous studies [37,38]. Means (absolute values) of overall CH₄ (AllMean, including respiration and eructation measures), respiration CH₄ (RespMean) and eructation CH₄ (ErucMean) were calculated by averaging the measurements per individual and visit according to the three definitions. Thereafter, the interval between starting and ending the LMD measurement was calculated and termed “measurement duration”. The sum of all mini-peaks for every individual and visit was further divided into the respective measurement duration to calculate overall CH₄ (AllMinute, including respiration and eructation measures), respiration CH₄ (RespMinute) and eructation CH₄ (ErucMinute). Finally, maximum respiration CH₄ (RespMax) and eructation CH₄ (ErucMax) were determined for each measurement. Descriptive statistics for the eight CH₄ traits are listed in Table 2.

Table 2. Descriptive statistics of the defined methane emission traits.

CH ₄ Trait	# Farm	# Cow	# Measurement	Methane Emission (ppm)			
				Mean	SD	Min.	Max.
AllMean	119	448	835	42.8	34.92	8.2	351.7
AllMinute	119	448	835	3969.0	2446.35	771.1	23,785.2
RespMean	119	448	835	16.0	17.21	1.0	160.3
RespMax	119	448	835	48.4	81.40	1.0	900.0
RespMinute	119	448	835	879.9	1249.82	0.5	12,873.9
ErucMean	119	448	835	108.1	148.28	9.7	1425.0
ErucMax	119	448	835	410.2	371.99	31.0	4152.0
ErucMinute	119	448	835	3089.0	2253.80	106.0	22,098.0

= Number of; SD = Standard Deviation; Min = minimum value; Max = maximum value; AllMean = average all methane; Allminute = all methane per minute; RespMean = average respiration methane; RespMax = maximal respiration methane; RespMinute = respiration methane per minute; ErucMean = average eructation methane; ErucMax = maximal eructation methane; ErucMinute = eructation methane per minute.

2.4. Nutrition Monitoring Subsample

Nutritional monitoring was carried out in 27 out of the 119 selected herds, with 8 herd visits in intervals of 6 weeks during the study period as described by Reichenbach [32]. The green fodder component included tall African maize (*Zea mays*) and Napier’s hybrid grass (*Pennisetum glaucum* × *P. purpureum*). Urban dairy producers additionally used organic wastes (vegetable and fruit peels) for feeding their cattle. Dry forages were mostly straw of finger millet (*Eleusine coracanal*) and dried maize. Concentrated feed contributed to 27–28% of the daily dry matter (DM) intake. On average, the cattle stayed on pasture for 5.9 ± 2.3 h [32]. Feed type and quantitative feed intake of each individual cow in these herds were recorded. Fodder samples were analyzed for neutral detergent fiber (NDF) concentration according to Van Soest et al. [40] at the National Institute of Animal Nutrition and Physiology (NIANP) in Bangalore. Organic waste, pasture biomass, and green and dry forages had an average fiber concentration of 482, 669, 675 and 708 g NDF kg⁻¹ DM, respectively [32]. Descriptive statistics for the eight CH₄ traits as determined in the nutrition subsample are listed in Table 3.

Table 3. Descriptive statistics of methane emission from 27 farms with nutrition information.

CH ₄ Trait	# Farm	# Cow	# Measurement	Methane Emission (ppm)			
				Mean	SD	Min.	Max.
AllMean	27	78	146	37.2	25.00	8.2	119.2
AllMinute	27	78	146	3645.0	2110.67	905.2	11,353.7
RespMean	27	78	146	13.3	12.86	1.0	74.8
RespMax	27	78	146	48.6	89.58	1.0	721.0
RespMinute	27	78	146	891.0	1207.36	0.5	7550.2
ErucMean	27	78	146	114.2	174.42	10.1	1271.2
ErucMax	27	78	146	489.1	537.96	39.0	4152.0
ErucMinute	27	78	146	2754.1	1993.99	106.0	10,747.6

= Number of; SD = Standard Deviation; Min = minimum value; Max = maximum value; AllMean = average all methane; Allminute = all methane per minute; RespMean = average respiration methane; RespMax = maximal respiration methane; RespMinute = respiration methane per minute; ErucMean = average eructation methane; ErucMax = maximal eructation methane; ErucMinute = eructation methane per minute.

2.5. Statistical Models

A linear mixed model implemented in R package “lme4” [41] was applied to analyze the CH₄ traits. The basic statistical model was:

$$y_{ijklmno} = \mu + \left(\frac{MY}{BW} \times 100\right)_i + B_j + THI_k + FasT_l + F_m + cow_n + e_{ijklmno} \quad (1)$$

where $y_{ijklmno}$ = logarithm transformation of the CH₄ traits; μ = overall mean effect; $\left(\frac{MY}{BW} \times 100\right)_i$ = fixed effect for MY/BW ratio in liter per kg multiplied by 100; B_j = fixed effect for breeds (exotic, crossbreed and native); THI_k = fixed effect for temperature-humidity index; $FasT_l$ = fixed effect for the time interval from cow feeding until the CH₄ recording (in hours); F_m = random farm effect; cow_n = random cow effect for repeated measurements; $e_{ijklmno}$ = random residual effect.

Following the model 1, three different variables, i.e., SSI cluster, pasture access and measurement location, were included one by one into the basic statistical model:

$$y_{ijklmnop} = \mu + \left(\frac{MY}{BW} \times 100\right)_i + B_j + THI_k + FasT_l + TestEff_m + F_n + cow_o + e_{ijklmnop} \quad (2)$$

where $TestEff_m$ = included the fixed effects SSI cluster (rural, mixed, urban), Past (yes, no) and Loc (outdoor, half-outdoor and indoor) in consecutive runs.

For the nutrition monitoring subsample, one additional variable was included as $TestEff_m$ in model 2, where $TestEff_m$ = level of NDF intake per metabolic weight ($MW = BW^{0.75}$; in g NDF/kg MW) on the day of visit. Two NDF intake levels were distinguished: high (NDF ≥ 94 g/kg MW, $n = 71$ measurements) and low (NDF < 94 g/kg MW, $n = 75$ measurements).

The R package “emmeans” [42] was applied to estimate least square means (LSMeans) of levels within different effects.

3. Results

3.1. Basic Environmental Factors on CH₄ Concentrations

From the basic statistical model 1, the MY/BW ratio significantly influenced AllMean (Table 4). Except for RespMinute and ErucMax, an increase in the MY/BW ratio was associated with an increased CH₄ concentration. The fixed effect THI significantly influenced the eructation CH₄, i.e., ErucMean, ErucMinute and ErucMax (Table 4). However, ErucMean and ErucMax decreased with THI and ErucMinute had a positive regression coefficient of 0.02. With the prolongation of the time interval from feeding to CH₄ recording, AllMean, ErucMean and ErucMax decreased significantly. The fixed effect breed was not significant for the CH₄ traits. However, the exotic breed had the lowest values for

all CH₄ traits, except for ErucMinute. The crossbred showed highest LSMs for ErucMean and for the respiration CH₄, including RespMean, RespMax and RespMinute (Table 4).

Table 4. Regression coefficients of milk yield to body weight ratio (MY/BW), temperature humidity index (THI) and time interval from feeding to methane recording (FasT) and least square means of breed levels for methane emission (CH₄) traits.

CH ₄ Trait	Fixed Effects					
	MY/BW ¹	THI ¹	FasT ¹	Breed ²		
				Exotic	Crossbred	Native
AllMean	0.03 *	−0.00 ^{ns}	−0.01 *	33.19 ^a	33.33 ^a	35.38 ^a
AllMinute	0.02 ^{ns}	−0.01 ^{ns}	−0.01 ^{ns}	3328.17 ^a	3351.99 ^a	3550.51 ^a
RespMean	0.03 ^{ns}	−0.01 ^{ns}	−0.00 ^{ns}	10.09 ^a	10.88 ^a	10.81 ^a
RespMax	0.03 ^{ns}	−0.00 ^{ns}	−0.02 ^{ns}	19.80 ^a	23.63 ^a	21.48 ^a
RespMinute	−0.01 ^{ns}	0.01 ^{ns}	0.00 ^{ns}	275.74 ^a	344.81 ^a	284.45 ^a
ErucMean	0.02 ^{ns}	−0.03 ^{**}	−0.03 ^{**}	61.50 ^a	70.33 ^a	70.31 ^a
ErucMax	−0.01 ^{ns}	−0.04 ^{***}	−0.04 ^{***}	296.92 ^a	305.91 ^a	358.25 ^a
ErucMinute	0.04 ^{ns}	0.02 *	−0.00 ^{ns}	2406.74 ^a	2242.53 ^a	2313.07 ^a

¹ = regression coefficient; ² = LSMs; ns = not significant; * = *p*-value < 0.05; ** = *p*-value < 0.01; *** = *p*-value < 0.001; Different letters in the same row indicate significant difference between breed levels (*p*-value < 0.05); AllMean = average all methane; Allminute = all methane per minute; RespMean = average respiration methane; RespMax = maximal respiration methane; RespMinute = respiration methane per minute; ErucMean = average eructation methane; ErucMax = maximal eructation methane; ErucMinute = eructation methane per minute.

3.2. Impact of Measurement Location on CH₄ Concentrations

The location at which the LMD measurement took place also had an effect on CH₄ concentrations. LSMs for AllMean, AllMinute, RespMean, RespMax, RespMinute, ErucMean and ErucMinute were significantly higher (*p*-value < 0.05) in the indoor class, followed by the half-outdoor and the outdoor classes (Figure 4). For ErucMax, the CH₄ concentration was 356.45 ppm in half-outdoor and decreased to 313.05 ppm in indoor and to 308.78 ppm in outdoor classes. No differences were detected between locations for ErucMax. Significant differences in CH₄ between indoor and outdoor classes were observed for AllMean (16.13 ppm), AllMinute (870.26 ppm/minute), RespMean (7.70 ppm), RespMax (14.19 ppm), RespMinute (131.52 ppm/min), ErucMean (25.47 ppm) and ErucMinute (587.39 ppm/min).

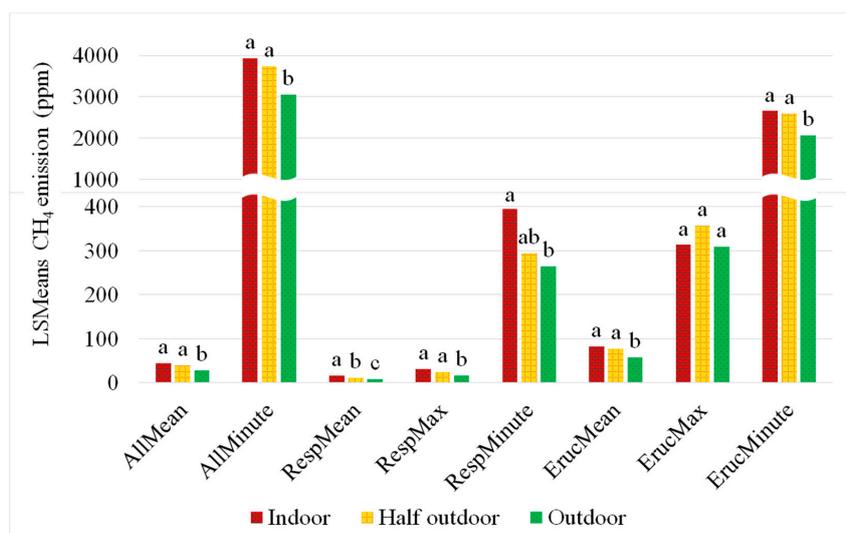


Figure 4. Least square means (LSMeans) for the methane traits in indoor, half-outdoor and outdoor classes. Different letters on bars indicate significant differences (*p*-value < 0.05).

3.3. Impact of Access to Pasture on CH₄ Concentrations

Pasture access was an important factor, because LSM means for AllMean, AllMinute, RespMean, RespMax, RespMinute, ErucMean and ErucMinute were significantly higher (p -value < 0.05) for cows having no access to pasture (Figure 5). The differences between CH₄ concentrations in cows with and without access to pasture were 6.01 ppm for AllMean, 541.26 ppm/min for AllMinute, 3.26 ppm for RespMean, 7.96 ppm for RespMax, 132.71 ppm/min for RespMinute, 19.00 ppm for ErucMean, and 55.63 ppm for ErucMax.

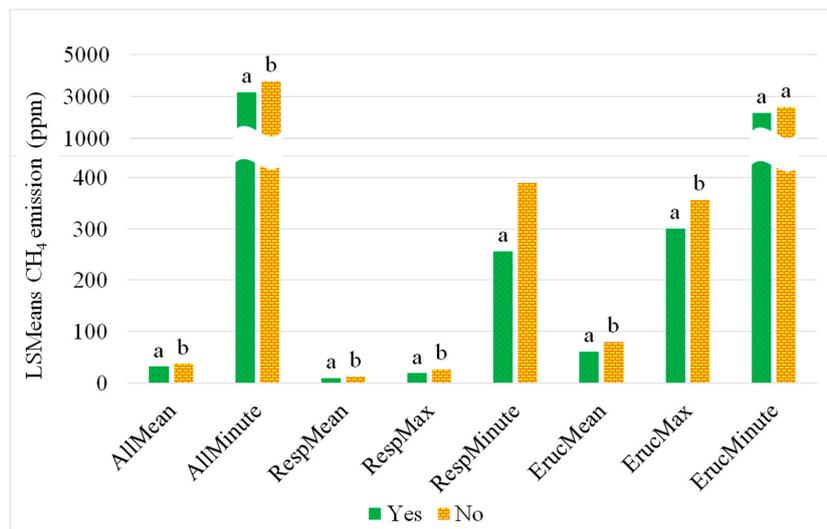


Figure 5. Least square means (LSMeans) for the methane traits in classes with (Yes) and without (No) access to pasture. Different letters on bars indicate significant differences (p -value < 0.05).

3.4. Impact of Fibre Intake on CH₄ Concentrations

No significant impact on the CH₄ traits was identified with respect to the daily NDF intake level, even though LSM means for all CH₄ traits were numerically higher at the high NDF intake level (Figure 6). The differences in CH₄ concentrations between cows with high and low NDF intake level were 3.93 ppm for AllMean, 425.03 ppm/min for AllMinute, 1.40 ppm for RespMean, 4.64 ppm for RespMax, 49.84 ppm/min for RespMinute, 16.64 ppm for ErucMean, 71.34 ppm for ErucMax, and 85.22 ppm/min for ErucMinute.

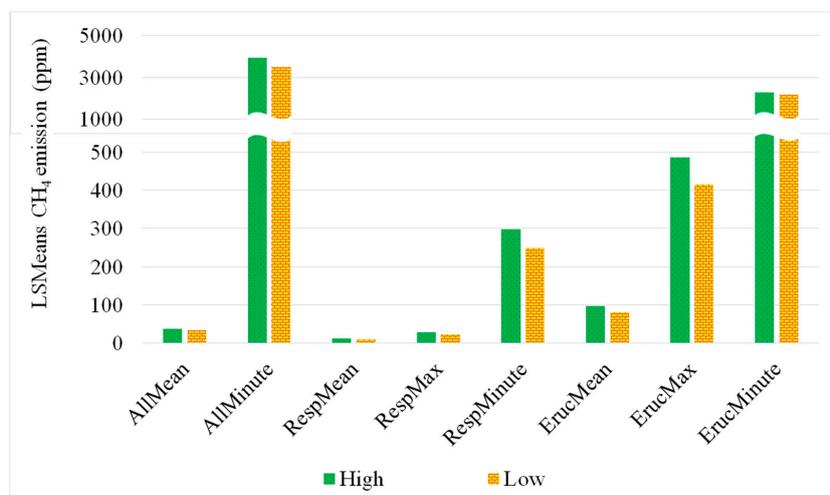


Figure 6. Least square means (LSMeans) for the methane traits subjected to a high (High) and a low (Low) neutral detergent fiber intake level.

3.5. Impact of SSI Cluster on CH₄ Concentrations

LSMeans for AllMean, AllMinute, RespMean, RespMax, RespMinute, ErucMean and ErucMinute were highest in the urban areas, followed by the rural and the mixed areas. For ErucMax, the highest CH₄ concentration was observed in the mixed areas (Figure 7). The fixed effect SSI cluster had a significant impact (p -value < 0.05) between urban and mixed areas on both AllMean and RespMean while between urban and rural areas impact was only significant on AllMean. The differences in CH₄ concentrations between urban and mixed areas were 10.31 ppm for AllMean and 3.89 ppm for RespMean.

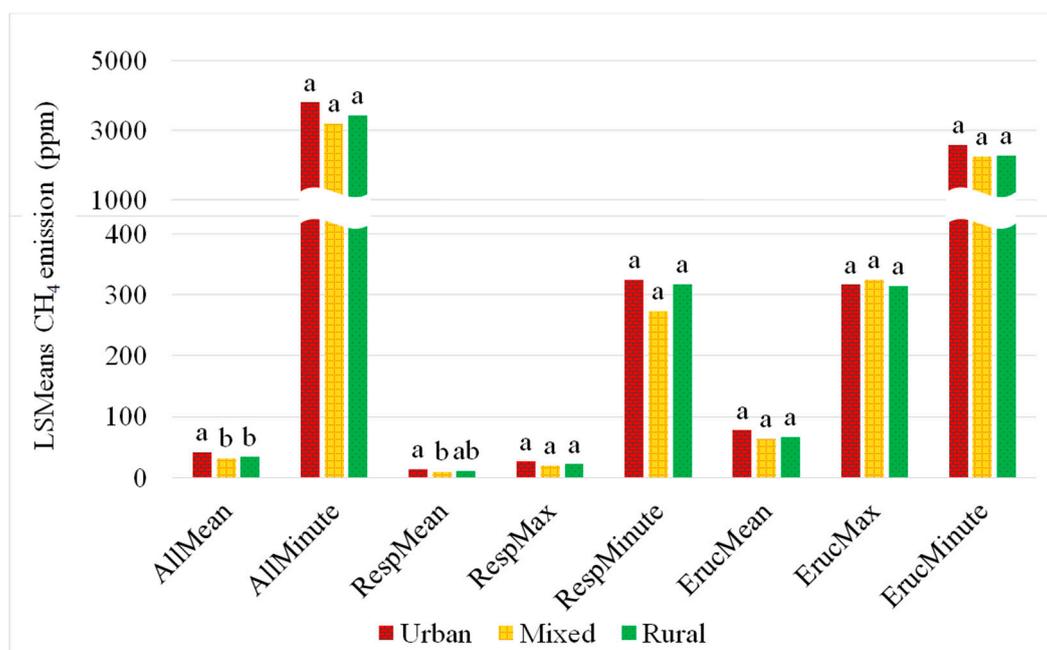


Figure 7. Least square means (LSMeans) for the methane traits across three location clusters classified by the survey stratification index. Different letters on bars indicate significant differences (p -value < 0.05).

4. Discussion

4.1. Methane Emission Traits

In some studies, the spot exhaled air samples were transformed to diurnal CH₄ productions (in g/d) based on linear regression models [43,44], or by considering the ratio between CH₄ and CO₂ concentrations from each visit in automatic milking systems [45]. However, the transformations are only accurate for constant CH₄ and CO₂ concentrations throughout the testing day, and for climatic conditions at 25 °C with 1 atmosphere (101.3 kPa) pressure (the density of CH₄ is 665.7 mg/L under these conditions). These assumptions can easily be violated because the CH₄ and CO₂ concentrations definitively vary during the day [46], and temperature and pressure also differ across visits and farms. Therefore, only the CH₄ concentration as measured by the LMD, in parts per million (ppm), was used in the present study. This information can reflect relative differences in CH₄ concentrations between farms applying different dairy cattle management systems along the rural-urban gradient in Bangalore.

Van Engelen et al. [46] measured CH₄ concentrations from 1508 Dutch Holstein Friesian cows with infrared sensors installed in automatic milking systems and reported that the mean CH₄ concentration per visit ranged between 11 and 2073 ppm (mean = 254 ppm). In comparison, AllMean obtained from LMD ranged from 8.25 to 351.75 ppm (mean = 42.85 ppm), which is significantly lower than AllMean reported by van Engelen et al. [46]. However, the CH₄ concentrations, including all, respiration, and eructation CH₄, from steers measured with an LMD [15] are comparable to our findings. The methods applied for measuring CH₄ output can be a reason behind the varying CH₄

emission values. Differences between seasons, sheds and diets contribute to further variations in CH₄ and impede the comparison of data from different studies [47].

4.2. Body Weight and Milk Yield

Given the diversity in body size and weight of the sampled genotypes in this study, the positive correlation between MY and BW [48] would have overemphasised breed differences in MY. Additionally, the imbalanced numbers of cows of different genotypes biased the analysis of MY effects on CH₄ concentration. Therefore, MY was corrected and expressed in liters per kg BW. In this study, AllMean is used as the reference value to represent a full exhalation–inhalation cycle [11,12]. The positive regression coefficient of 0.03 for MY/BW (p -value < 0.05) agrees with the findings from Garnsworthy et al. [43], who found that CH₄ emissions were positively associated with MY. Cows on urban dairy farms had higher MY and BW than cows on mixed and rural farms. Usually, CH₄ per unit of product, i.e., liter of milk, decreases with increasing MY [49,50]. When considering AllMean to calculate CH₄ per liter of milk, urban areas still showed the highest CH₄ emission (4.62 ppm/L) followed by rural areas (3.92 ppm/L), while mixed areas had the lowest CH₄ emission per liter of milk (3.88 ppm/L). In this regard, farms in the mixed areas seemed to be more efficient in terms of methane output per unit of product (liter of milk).

4.3. Time Interval from Feeding to CH₄ Recording

In line with the decreasing CH₄ concentrations with the increasing time interval from feeding to CH₄ recording reported by Chagunda [21] and Ricci et al. [15], the time interval from feeding to CH₄ recording always had negative regression coefficients for all CH₄ traits. Actually, CH₄ emissions are higher after feeding when more substrate is available for rumen fermentation and more hydrogen is available for methanogenesis [51] and it decreases over time when the substrate (feed) is degrading and undegraded feed is getting scarce.

4.4. Breed

Swamy and Bhattacharya [52] stated that Indian indigenous cattle had higher CH₄ emission per unit of milk than crossbred cattle. In the present study, native cows had highest CH₄ emissions for AllMean, AllMinute and ErucMax (Table 4), although no significant difference to the other breeds existed. Chagunda et al. [49] compared cattle enteric CH₄ between groups of low and high genetic merit for milk fat and protein content in combination with low and high forage intake and concluded that breeding for cows with high genetic merit was associated with a decrease in enteric CH₄ emissions per liter of milk. However, the number of native cows in our sample was very small ($n = 13$) compared to exotic ($n = 448$) and crossbreed cows ($n = 285$) since native cows are not primarily used for dairy production but for fieldwork. In urban areas, since there is no field to cultivate, the native cows are used as dairy cows. For this reason, we found a higher percentage of native dairy cows in urban and mixed than in rural areas (Table 1).

4.5. Heat Stress and Measurement Location

Heat stress is associated with a reduction in the DM intake in ruminants [23] and consequently leads to a reduction in enteric CH₄ emissions [20]. Even though in this study there was no significant impact of THI on AllMean (Table 4), a decrease in emissions of all CH₄ traits was associated with increasing THI. Along the rural–urban gradient, THI on farms located in rural areas (72.85) was higher than on farms in the remaining areas, i.e., urban (70.19) and mixed (69.94). In urban areas, due to the building density, cattle are predominantly kept in the basement or ground floor or in front of the house under a well-insulated roof. In rural areas, only a simple wooden structure with some hay on top is used as a shed, offering little protection against heat stress. As a consequence, the increase in THI in rural areas might be due to the scarcity of shadow and heat insulation.

Independent of the rural-urban gradient, highest CH₄ concentrations were measured in the indoor barns, followed by the half-outdoor and the outdoor sheds (Figure 5). As stated above, in the densely populated urban areas, limited space is available for cattle, forcing farmers to keep their cows indoors. As a consequence, 46.07% of the CH₄ measurements in urban areas were conducted indoors, compared to 22.59% of indoor CH₄ measurements in rural areas (Table 1). According to Ngwabie et al. [53], indoor air temperature is negatively correlated with daily CH₄ emissions, because with the higher temperatures that prevail in (poorly ventilated) indoor conditions the feed intake decreases, resulting in lower CH₄ emissions. However, in our study, the highest THI was found for half-outdoor locations (72.70) which were the most common in rural areas (Table 1). Besides, Ngwabie et al. [47] also showed that CH₄ concentrations in indoor barns were higher than in outdoor barns and that ventilation rate had an inverse linear relationship to the CH₄ concentrations. Thus, the ventilation determined by shed type has more influence on measured CH₄ emissions relative to dry matter intake than the THI.

4.6. Pasture Access and Fibre Intake

The main factor driving CH₄ production is feed intake. Nevertheless, reducing intake has not been considered as a strategy of CH₄ reduction because of concerns in decreasing animal productivity [54]. However, modulating fibre content in diets and grazing management have been successfully used as mitigation strategies [55,56]. Grazing strategies employed to mitigate CH₄ emissions are often related to forage quality, which means type and maturity of the fodder. Thus, feeding higher quality forage facilitates CH₄ mitigation [57].

Pasture-based dairy farming systems and confinement systems differ in the amount of CH₄ produced [22]. In a tropical environment, the access of cows to pasture changes with season as well as with farm location, cow breed, lactation stage and health status of the cow. In Bangalore, more than half of the cows (53.41%) had access to pasture, although the percentage of pasture access decreased in the dry season when the availability of vegetation declined. Lower CH₄ concentrations were determined in cattle with pasture access, which accounted for 70.68% of cows in urban areas (Table 1). Access to high quality pasture ensures lower CH₄ emissions from grazing animals [58], but due to the increasing population size and build-up density, the quality of inner-urban pasturing sites (vacant building plots, organic waste dumps, roadsides) in Bangalore was very variable. In urban areas, local shops and neighbours also supply farms with organic waste (vegetable and fruit peels) as cow feed [32]. In mixed and rural areas, cows mostly received Napier grass, maize stover and finger millet straw, as well as concentrate feed. In the nutrition subsample, we used the quantitative threshold of 94 g NDF/kg MW to separate fibre intake levels—the qualitative equivalent of this threshold is 600 g NDF/kg DM. Similar diets, in terms of NDF concentration, were also reported by Ali et al. [59] for heifers in Kenya (ca. 700 g NDF/kg DM) although recommendations for the diets of high-producing dairy cows in peak lactation range from 250 to 400 g NDF/kg DM [60–62]. However, no impact of the intake level of fibre (low vs. high NDF) on CH₄ concentration was detected in our study, even though ruminants emit more CH₄ when ingesting a fibrous diet [63].

4.7. Rural-Urban Gradient

Results from the present study unveiled impacts of SSI on CH₄ emission. Although the SSI was constructed based on spatial information on a neighbourhood's building density and distance to the city centre [31], it was also able to capture differences in management on the dairy farms. Compared to mixed and rural areas, higher CH₄ concentrations were found for cows kept in urban areas. Urban dairy production is common in India [5] because of the strong market demand for milk products and higher milk prices in urban areas [27]. Furthermore, due to Indian culture and tradition, cattle are endeared and respected animals and are commonly allowed to graze or roam freely around in the whole city where they are often able to consume good quality vegetable residues from dumpsites. Access to quality pasture is associated with a decrease in CH₄ emissions [56], thus, the higher accessibility to pasture in the mixed areas along with their highest percentage of outdoor locations may explain the

lower CH₄ emission in mixed as compared with urban areas. In rural areas, apart from a higher number of outdoor measurement locations, the low productivity (MY) of the cattle and the higher THI might contribute to lower CH₄ emissions than in urban areas.

These examples demonstrate that social-ecological conditions influence farm management of dairy cattle along the rural-urban gradient in Bangalore and lead to variations in CH₄ emissions. On one hand, in urban areas, dairy cows benefited from better management and high quality pasture, thus having a higher MY and BW [32]. On the other hand, urban dairy farms are characterized by indoor sheds, lower THI, less heat stress, and improved hygienic and health conditions [27]. Although these conditions generally reduce CH₄ emissions, LMD spot measurements in urban locations yielded the highest values. Here, the measurement location type (poorly ventilated indoor barn with higher background CH₄ concentration versus well ventilated outdoor barn with lower background CH₄ concentration) may have overridden the positive effects of an improved cattle management in urban areas. In our study, we considered THI (which takes into account temperature and relative humidity) as an environmental factor that may influence the CH₄ measurement. However, Chagunda et al. [64] additionally considered atmospheric pressure, wind speed and wind direction to correct outdoor LMD emissions. Thus, the CH₄ concentration determined in outdoor locations of Bangalore may not represent the real values and rather underestimate these due to ignorance of the aforementioned atmospheric variables.

The impact of a rural-urban gradient on CH₄ emissions displays the relevance of the underlying factors, namely herd management and environmental conditions, as shaped by the (progressing) city environment. An index summarizing those factors, such as the SSI, can therefore be used as a social-environmental feature in which human/management effects and environmental effects (e.g., THI) are expressed together.

5. Conclusions

This study is a pioneer in the use of the LMD for on-farm methane emission measurements of dairy cattle in an urbanizing environment. The LMD technique showed variations in the CH₄ emissions along the rural-urban gradient of Bangalore. The additional consideration of SSI in CH₄ emission contributed to capturing variations in dairy husbandry systems that are variations associated with social-ecological conditions. Except for the fibre content in the diet (NDF), which was inconclusive due to the small sample size, the remaining effects, i.e., milk yield, body weight, the time interval between feeding and CH₄ measurement, pasture access and SSI cluster, as well as THI and measurement location (type of shed), significantly affected CH₄ emissions. Higher concentrations of CH₄ were associated with cows in urban areas with higher milk yield, kept in poorly ventilated indoor sheds, but with lower THI. Outdoor measurement locations and the high heterogeneity of farm management worked in favor of the rural farms' CH₄ emissions. Variations in individual CH₄ emissions indicate the potential for reduction, e.g., via breeding and feeding strategies. Nevertheless, some disturbing factors as identified in the present study suggest a standardisation and improvements of LMD-based CH₄ measurements.

Author Contributions: Conceptualization and methodology, S.K. and E.S.; data collection, A.P. and M.R.; data curation and investigation, A.P.; software, T.Y.; validation, S.K. and T.Y.; resources, R.B. and P.K.M.; writing—original draft preparation, A.P.; writing—review and editing, S.K., M.R. and E.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the German National Science Foundation, DFG, grant number KO 3520/5-1 and SCHL 587/6-1.

Acknowledgments: The authors gratefully acknowledge the kindness, patience and help of the dairy farmers and field assistants involved in the fieldwork, the logistic and scientific support offered by our Indian partners at the ICAR-National Institute of Animal Nutrition and Physiology (ICAR-NIANP) and the financial support provided by the German National Science Foundation, DFG, through grant number KO 3520/5-1 and SCHL 587/6-1, and by the Indian Department of Biotechnology (DBT), through grant number DBT/IN/German/DFG/14/BVCR/2016, as part of the Indo-German consortium of DFG Research Unit FOR2432/1 and DBT (The Rural-Urban Interface of Bangalore: A Space of Transitions in Agriculture, Economics, and Society). We also thank Regina Roessler for her statistical support with R.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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