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Nitrogen loss abatement from dairy cow excreta through urine and faeces separation: The effect of temperature and exposure period on NH_3 fluxes

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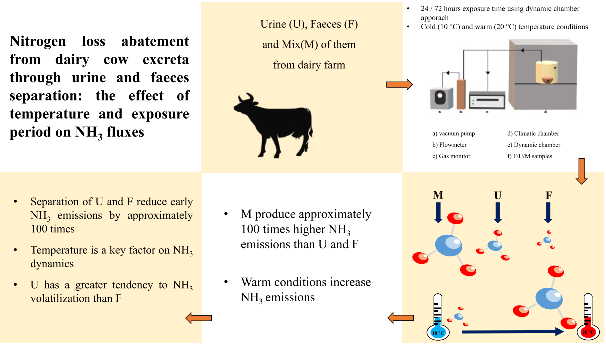
HIGHLIGHTS

- NH_3 emissions from dairy cattle excreta were monitored using a dynamic chamber.
- Faeces, urine and the mix of them were monitored at different exposure period.
- NH_3 emissions from the mix are almost 100 times higher than faeces and urine alone.
- Separation of dairy cattle excreta is an effective strategy to cut NH_3 emissions.
- Warm conditions promote NH_3 emissions.

GRAPHICAL ABSTRACT



Nitrogen loss abatement from dairy cow excreta through urine and faeces separation: the effect of temperature and exposure period on NH_3 fluxes

Urine (U), Faeces (F) and Mix(M) of them from dairy farm

- 24 / 72 hours exposure time using dynamic chamber apporach
- Cold (10 °C) and warm (20 °C) temperature conditions

- M produce approximately 100 times higher NH_3 emissions than U and F
- Warm conditions increase NH_3 emissions

- Separation of U and F reduce early NH_3 emissions by approximately 100 times
- Temperature is a key factor on NH_3 dynamics
- U has a greater tendency to NH_3 volatilization than F

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ABSTRACT

CONTEXT: Manure management is a crucial aspect warranting attention since a significant proportion of anthropogenic NH_3 emissions are derived from it. In dairy cattle, only 20 to 30% of the dietary N is retained in milk, while a relevant amount is excreted in urine and faeces. A substantial proportion (60 to 80%) of the urine-N of high-producing dairy cows exists as urea, which is rapidly converted to NH_4^+ by the urease enzyme, and volatilizes into the atmosphere as NH_3. While extensive literature studied the effects of technical solutions for cattle housing and waste storage, environmental parameters and manure treatments on NH_3 emission, limited information exists on its quantification under controlled conditions from isolated and mixed excreta.

OBJECTIVE: The aim of the study was to evaluate the NH_3 emission reduction potential from maintaining urine and faeces separate in dairy farming systems.

METHODS: NH_3 emissions from urine (U) and faeces (F) alone, as well as their mixture (M) were monitored under different environmental conditions (10 °C and 20 °C) and different exposure periods (24 and 72 h).

RESULTS AND CONCLUSIONS: The M samples had significantly higher NH_3 emissions (about 100 times higher) than U and F, both in cold and warm conditions and at all exposure periods. Significantly higher NH_3 emissions were measured from U, compared to F, after 48/72 h of exposure, probably linked to the higher pH and water content found in U. Separating urine and faeces (before contact) has the potential to reduce NH_3 emissions by about 99% during the first 24–72 h of air exposure in dairy cow management systems.

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SIGNIFICANCE: This potential approach should also be coupled with accurate urine management, under conditions that prevent environmental urease contamination. Such strategy could also be an effective solution to improve on-farm N reuse and to reduce the adoption of chemical fertilizers.

1. Introduction

When it comes to comparing dairy housing systems in terms of pollutant gaseous emission, the way that manure is handled represents a relevant aspect to consider. It is known that different manure management techniques can lead to different rates of emission from the housing system (Leytem et al., 2011; Owen and Silver, 2015) since the synthesis pathway of certain pollutant gasses produced in manure, such as greenhouse gasses (GHGs) or ammonia (NH_3), rely on the succession of specific conditions that may be favoured by a certain system (Montes et al., 2013). In the case of NH_3, average surface concentrations are generally very low in remote areas (<1 ppbv) but increase to over 25 ppbv in regions of intensive agriculture (Nair and Yu, 2020). The emission of this gas, however, is relevant given its toxicity. NH_3 is a precursor to the development of particulate matter with an aerodynamic diameter of less or equal to 2.5 μm ($PM_{2.5}$). As such, NH_3 is a major contributor to many environmental problems, being one of the main factors responsible for the acidification of the atmosphere and the eutrophication of soils and waters (Sanchis et al., 2019; Zhang et al., 2019). Once emitted, NH_3 is a reactive gas, combining primarily with sulphur and nitrogen oxides. NH_3 participates in global warming from the formation of its derivative, nitrous oxide (N_2O), which has a warming potential 273 times higher than CO_2 (IPCC, 2022). When considering the sources of emissions, about 70% of the anthropogenic production of NH_3 is derived from agricultural activities, primarily manure management (Sutton et al., 2013). In the case of dairy cattle under commercial conditions, only 20 to 30% of the dietary N is retained in milk (Spek et al., 2013) and the remainder is excreted through urine and faeces, with the consequent risk of being emitted into the atmosphere in the form of NH_3. In high-producing dairy cows, 60 to 80% of the total urinary N is represented by urea, which is rapidly hydrolysed to ammonium (NH_4^+) that volatilizes in the form of NH_3, thus representing the main source of NH_3 emitted from cattle manure (Hristov et al., 2011; Edouard et al., 2016). This conversion happens almost instantly when urine and faeces come in contact since the urea-degrading enzyme, urease, is found mostly in faeces (Muck, 1982; Hagenkamp-Korth et al., 2015), Therefore, when the urea present in urine comes in contact with the soil and faeces, NH_3 is rapidly synthesized and diffused to the manure surface, from where it is then released into the ambient air. This process is affected by many factors, including temperature and pH. The urea-N hydrolysis rate increases with temperature (Moraes et al., 2017). Instead, increasing pH from acid to either neutral or basic favours the point of equilibrium between NH_3 and NH_4^+, thereby promoting NH_3 volatilization (Sigurdarson et al., 2018). In housing facilities, NH_3 emissions are highly influenced by barn design, ventilation, bedding and manure removal, and storage (Hristov et al., 2011). Certain manure management systems are being developed to avoid this mixture or to reduce the derived NH_3 emissions in dairy barns (Galama et al., 2020) or pig facilities (Hansen et al., 2020; Koger et al., 2014). Emissions generated from different ratios of excreta, cleaning regimes, temperatures, pH, ventilation rates and slurry treatments were studied in cattle barns or storage tanks (Burchill et al., 2019; Hempel et al., 2023; Sanchis et al., 2019; Zilio et al., 2020). NH_3, N_2O and CH_4 emissions from cattle urine and faeces deposition on pastures in field conditions were also investigated (Mulvaney et al., 2008; da Silva Cardoso et al., 2016). However, NH_3 emission produced from isolated urine and faeces, respectively, compared to mixed excreta, has not been profoundly researched yet. In research carried out under laboratory conditions, cumulative NH_3 emissions from dairy cow excreta under controlled and constant temperature conditions were both studied as isolated samples (Vaddella et al., 2010) and compared to different bedding materials (Misselbrook and Powell, 2005). The effects of temperature variation on NH_3 emissions of cattle excreta deposited on simulated concrete flooring were assessed by Pereira et al. (2012). However, the separate emissions from urine and faeces, respectively, were not determined. Further, ammonia emissions from dairy cows' mixed excreta in relation to the lactation stage, feeding system and time of excretion were determined (Rodrigues et al., 2022), but the effects of temperature and excreta separation were not assessed. There is a lack of evidence on the magnitude of benefits deriving from preventing faecal-urine contact in dairy cows' farming under different environmental conditions. Nevertheless, such knowledge is crucial given its relevance to NH_3 volatilization. In this framework, the scope of our research was (i) to conduct a fully controlled experiment to determine the extent to which NH_3 emissions can be reduced by preventing the mixing of urine and faeces, compared with the reference situation in which the two matrices come into contact after deposition; (ii) to assess the effect of environmental temperature on the two scenarios.

2. Material and methods

The laboratory experiments were conducted at the Department of Agriculture, Food, Environment and Forestry (DAGRI) laboratory, at the University of Florence (Italy). The experiments to analyse possible NH_3 flux variances from different types of dairy cow excreta under controlled conditions were performed over two months, from October to November 2021.

2.1. Sample collection and treatment

Samples were obtained from a commercial dairy farm located in a flat region in the Po Valley, Northern Italy (45°10′11.3" N 10°45′01.7″ E), during September 2021. The average temperature in the area was 20.4 °C at the time of sample collection (data from the nearest weather station). Three types of samples were analysed: faeces (F), urine (U), and an even mix of both matrices (M), consisting of 50% of urine and 50% of faecal material on a weight basis. The same ratio was used in previous research investigating NH_3 emissions from dairy cows (Misselbrook et al., 2005; Wheeler et al., 2007). U and F samples were obtained from a pool of 8 Holstein-Friesian cows. The group of lactating cows, involved in the experiment, was permanently housed in a cubicle barn with straw bedding. The animals were fed a Total Mixed Ration (DM basis: 17% corn silage, 21% grass silage, 8% alfalfa hay, 1% wheat straw, 29% ground corn, 9% soybean meal, 15% commercial pelleted concentrate). U and F samples were first taken directly from each cow to avoid contamination. All U samples were immediately collected and mixed, to obtain a homogeneous pool. The same procedure was adopted for F samples. A total of 32 aliquots were extracted from the U and F pools and 8 replicate samples of U and F were obtained, respectively. The residual 8 urine aliquots and the 8 faecal aliquots were accurately mixed to obtain 8 replicate samples of M. The 24 samples were instantly poured into sterile vacuum plastic bags and frozen at −20 °C after collection. Each replicate sample had a volume of 0.5 L.

2.2. Environmental conditions during the study

To study the effect of temperature on NH_3 fluxes from the three types of samples, two experimental scenarios (cold vs. warm) were established using a climatic chamber (KBF 115-230 V, Binder, Germany). The two environmental treatments were set up based on local average temperatures (T) and relative humidity (RH): “cold” treatment (T = 10 °C, RH = 70%) and “warm” treatment (T = 20 °C, RH = 70%). All three types of samples (F, U, M) were studied both under the “cold” and the “warm” treatments, respectively. For each combination (F_{cold}, F_{warm}, U_{cold}, U_{warm}, M_{cold}, M_{warm}), 4 replicates were examined.

The first three weeks of the study were dedicated to the analysis under “cold” conditions, and were followed by an additional three weeks in which the “warm” treatment was implemented.

2.3. NH_3 fluxes methodology

A dynamic flux chamber was designed to perform the NH_3 measurements inside the climatic chamber. The dynamic chamber was made from an airtight stainless-steel vessel (6 l, 16.5 cm tall, 13.5 cm diameter), which was perforated firstly on the wall to create an inlet entry of air coming from the climatic chamber, and secondly on the cover to generate an outlet. Two ports were fixed into each of the holes using pipe threads. The outlet port was attached to a 4.78 mm inner diameter polytetrafluoroethylene (PTFE) tube, which led to the outside of the climatic chamber. In the exterior, the tube was connected to a flowmeter, by which the airspeed was constantly regulated at 1.2 l min^{-1}. The flux was induced by the action of a vacuum pump (model VP30, LabTech, Italy) at the end of the circuit. Between the pump and the flowmeter, a stainless-steel union tee was added to allow the sampling of the outlet air. The experiment setup is depicted in Fig. 1.

NH_3 fluxes were measured using the dynamic chamber method, equipped with a photoacoustic multi-gas analyser (Photoacoustic Gas Monitor INNOVA 1512, Lumasense - DK). Air samples were collected from the outlet port of the dynamic chamber using a 6 mm (inner diameter) Teflon tube connected directly between the union tee and the gas analyser. Samplings were initiated immediately after closing the chamber and air samples were collected each hour for an exposure time of 24 h per substrate sample. Additionally, one sample for each treatments (F_{cold}, F_{warm}, U_{cold}, U_{warm}, M_{cold}, M_{warm}) was kept inside the chamber and analysed for a prolonged period (min: 48 h; max: 72 h).

All samples were thawed for approximately 24 h at +4 °C before the start of measurements. On the day of measurements, before the start of the experiment, the samples were placed in an oven at the same temperature as the climatic chamber for approximately 1 h. The Afterwards, the samples were extracted from the sealed plastic bag and poured into a plastic container (13 cm bottom diameter, 18.5 cm surface diameter and 6 cm tall) and the emitting surface was recorded. Moreover, each sample weight was recorded before and after the measurement period. The air outside the climatic chamber was also sampled and analysed using the same photoacoustic gas analyser to calculate NH_3 fluxes.

Temperature and relative humidity (RH) were measured at 1-h intervals throughout the experiment using portable loggers (HOBO MX2301, Onset Computer Corp., USA) placed inside both the climatic chamber and the dynamic chamber.

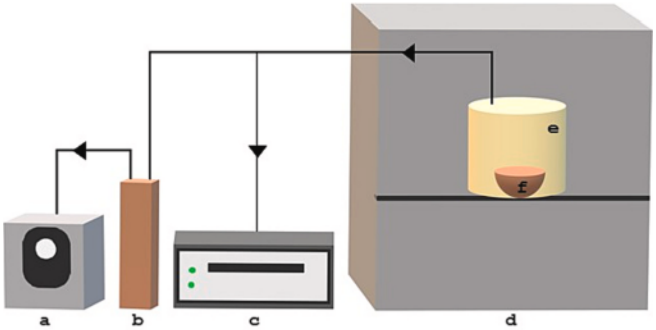

Fig. 1. Schematic setup of the experiment. a: vacuum pump; b: flowmeter; c: infrared photo-acoustic spectroscopy gas monitor; d: climatic chamber; e: dynamic chamber: f: container for the F/U/M samples. The airflow direction is indicated by arrows.

NH_3 fluxes F_{amm}, expressed in mg · m^{-2} · h^{-1}, were calculated hourly as:

$$F_{amm} = \frac{(C_{in} - C_{ex}) \bullet Q}{A} \tag{1}$$

where C_{in} is NH_3 concentration (mg · m^{-3}) measured at the outlet of the dynamic chamber, C_{ex} is ambient NH_3 concentration (mg · m^{-3}) measured outside the dynamic and the climatic chamber, Q is the airflow rate through the dynamic chamber (m^3 · h^{-1}) and A is the emitting surface (m^2).

2.4. Laboratory analysis

One sample from each pool of F, U and M (250 ml) was sent to an external laboratory to assess the chemical composition. The samples were analysed to determine pH, total solids (TS), total Kjeldhal nitrogen (TKN), and total ammoniacal nitrogen (TAN) using standard methods (Rice and Bridgewater, 2012).

2.5. Statistical analysis

Statistical analyses were performed using R 4.0.3 (R Core Team, 2020). To assess the effect of the environmental conditions, the type of sample and a linear mixed model for repeated measures (package “lme4”) was performed. The model included the fixed effects of environmental conditions (T = 10 °C, 20 °C), type of sample (F, U, M), number of hours since the start of the experiment (0–6 h, 6–12 h, 12–18 h, 18–24 h) and their respective interactions. Additionally, repetitions were entered into the model as a random effect. Normality and homoscedasticity of variance were visually evaluated with residuals plots. The estimated means for each combination of treatments and pairwise comparisons using the Tukey method were calculated with the package “emmeans”. The significance level was $p < 0.05$.

For samples where NH_3 concentrations were measured >24 h, fluxes were calculated hourly according to (1). Data were presented graphically and through descriptive statistics.

Cumulative NH_3 fluxes were analysed separately for F, U, and M. Fixed effect linear models were constructed for each type of sample, including the effect of environmental conditions (T = 10 °C, 20 °C) and the number of hours since the start of the experiment, as covariate. The interaction effect between the two variables was included in the model.

3. Results and discussion

3.1. Effect of material on NH_3 volatilization

Weight loss of the samples after the 24 h experiment was higher in the “warm” treatment (F_{warm} = 2.93 ± 0.57 g; U_{warm} = 3.66 ± 0.66 g; M_{warm} = 5.36 ± 0.52 g) than in the “cold” (F_{cold} = 1.05 ± 0.94 g; U_{cold} = 1.11 ± 1.06 g; M_{cold} = 1.51 ± 0.85 g). Weight loss was also higher in the M samples than F and U. The analysis of the chemical characteristics of the three types of samples (Table 1) showed that U had the highest pH, followed by M and F, respectively. The pH, together with temperature

Table 1
Chemical characteristics of the samples used in the experiment.

Type of sample	pH	TKN	TAN	TAN/TKN	TS
		%N on the original sample	%N on the original sample	%	%
Faeces	5.7	0.30	0.081	27	11.72
Urine	7.6	0.70	0.071	10	ND
Mix	7.0	0.57	0.328	58	8.68

TKN, total Kjeldahl nitrogen; TAN, total ammoniacal nitrogen; TS, total solids.

have been shown to play a crucial role in the equilibrium between volatile NH_3 and non-volatile NH_4^+ in solution, which are coupled in equilibrium by a dissociation reaction (Moraes et al., 2017). At either a neutral or basic pH, NH_3 is formed and lost through volatilization. Among the three types of samples, U displayed the highest TKN, while in M, total N was slightly lower. The lowest total N content was found in F. However, when considering ammoniacal nitrogen, M had the highest content, exceeding the TAN of F and U by more than threefold. Accordingly, the TAN/TKN ratio peaked in the M, showing that ammoniacal nitrogen accounts for nearly 60% of total N.

The impact of manure management on NH_3 emissions has already been studied by many authors (Dai and Karring, 2014; Hou et al., 2015; Holly et al., 2017; Mohankumar Sajeev et al., 2018), where it was shown that the main source of NH_3 emissions from livestock farming is derived from the action of the enzyme urease, which is found in bacteria present in the solid fraction of manure and in soils. This enzyme catalyses the hydrolysis of urea to NH_3 and carbonic acid (H_2CO_3) in a very short period, implying that most of the NH_3 emissions would have been produced before the manure is even applied to the field (Sigurdarson et al., 2018). In dairy cattle, the inefficient conversion of N from the feed into milk results in 70 to 80% of the feed–N intake being excreted through manure, mainly in the form of urea (Bristow et al., 1992). About 75% of urinary N is present in this form (De Boer et al., 2002; Bristow et al., 1992), whereas the small fraction of faecal N is mostly found as undigested organic N compounds. Therefore, as urine provides most of the urea that will be hydrolysed by the ureases present in faeces, the bulk of manure NH_3 emissions are produced when the excreta are mixed in the barn.

According to existing literature, the faeces-urine ratio in dairy cows can range from 1.1 to 2.4 and this proportion can be affected by several factors including weather conditions (Morse et al., 1994), lactation stage and feeding (Smith and Frost, 2000). In several controlled experiments, mixtures of cow faeces and urine were prepared to determine NH_3 emissions. The utilized amounts differed among studies: Vaddella et al. (2010) used an F—U ratio of 1.68:1, in Elzing & Monteny (1997) and Dai & Karring the ratio was 1.5:1, in Rodrigues et al. (2022) 1.7. When the proportions of faecal material and urines vary in a mixture, the maximum values of NH_3 emissions generated also vary (Wheeler et al., 2007). In the study by Wheeler et al. (2007), NH_3 emissions from slurry mixtures with different proportions of F and U were measured. They concluded that in the 50:50 ratio urease concentration is not a limiting factor, resulting in the highest emission rate for the longest time, thus expressing the maximum emission potential.

Results from the present study corroborated previous findings of Vaddella et al. (2010) and Pereira et al. (2012) showing that NH_3 volatilization as a consequence of the urease activity upon urinary N, resulted in 100 times higher fluxes than when both excreta remain separated (Table 2). Nevertheless, urease activity isolated from the urea input has not been widely studied. The effect that the mixture of urine and faeces has on the formation of NH_3 has been addressed previously. However, the magnitude of these emissions, when compared to those produced from unmixed materials, were not known previously. Despite the lack of studies on dairy cow manure addressing this issue, some work has been conducted on pig slurry. Lachance et al. (2005) found a 50% reduction in NH_3 emissions during the first 3 days of storage in the pit by separating urine and faeces directly under the slats in comparison with the conventional manure management system. Similarly, Panetta et al. (2005) evaluated the NH_3 emission when pig urine and faeces were stored separately, finding that this segregation reduced the NH_4^+-N concentrations by 78% in slurries. In accordance with these findings, our experiment highlighted that NH_3 volatilization in mixed materials exceeded one hundred times that recorded in the separate faeces and urine samples, respectively (Table 2). Initially, U had the greatest TKN levels, but in the absence of F, the synthesis of NH_4^+-N was significantly reduced. Potentially, the adoption of innovative manure management systems preventing the mixing of urine and faeces could lead to a decrease in NH_3 volatilization up to 99%. Interestingly, the effect of separating cow urine and faeces on NH3 emissions was studied previously (Vaddella et al., 2010). The authors observed low NH_3 emissions solely from faecal samples over a 3-week period. However, sole urine samples showed delayed but detectable NH_3 emissions. This was because urease is considered ubiquitous in the environment and the eventual contamination of urine is inevitable, while without urease, urea degradation is considered negligible (Sigurdarson et al., 2018). Avoiding the contact between urine and faeces has the potential to efficiently limit NH_3 emissions within the barn, with positive effects on the air quality inside the building for both humans and animals.

Table 2

Least squares mean (LSM) and standard errors (SE) of NH_3 fluxes ($mg \cdot m^{-2} \cdot h^{-1}$) produced by the type of samples × climatic conditions × hours from the start of the experiment.

		Hours from the start of the experiment			
		0–6	6–12	12–18	18–24
Environmental conditions	Type of sample	LSM SE	LSM SE	LSM SE	LSM SE
10 °C	Faeces	0.89^{a} 5.77	0.86^{a} 5.52	0.80^{a} 5.96	0.79^{a} 6.97
	Urine	1.50^{a} 5.77	1.55^{a} 5.52	1.51^{a} 5.96	1.52^{a} 6.97
	Mix	179.92^{b} 5.77	193.68^{a} 5.52	191.96^{a} 5.96	183.34^{ab} 6.97
20 °C	Faeces	3.30^{a} 5.77	2.88^{a} 5.52	2.83^{a} 5.96	3.18^{a} 6.97
	Urine	5.77^{a} 5.77	5.18^{a} 5.52	5.38^{a} 5.96	6.04^{a} 6.97
	Mix	365.22^{ab} 5.77	364.49^{b} 5.52	373.94^{a} 5.96	350.84^{c} 6.97

[a,b]Significant differences among columns (hours) within environmental conditions and type of sample ($P < 0.05$).

3.2. Temperature-dependent fluxes

The results of the linear mixed model applied on NH_3 fluxes ($mg \cdot m^{-2} \cdot h^{-1}$) are reported in Table 2. An increase from 10 °C to 20 °C ambient temperature, at constant relative humidity, produced an increase in NH_3 fluxes for all three types of materials ($F_{cold} = 1.00$, $F_{warm} = 3.05$, $p = 0.78$; $U_{cold} = 1.52$, $U_{warm} = 5.59$, $p = 0.60$). However, differences were only significant for the M samples ($M_{cold} = 187.23$, $M_{warm} = 363.62$, $p < 0.001$). In both environmental conditions, NH_3 fluxes for the mix of faeces and urine were significantly higher ($p < 0.001$) than fluxes from faeces and urine alone, while no significant differences were detected in NH_3 fluxes between F and U, respectively.

When examining the dynamics of F under “cold” conditions, NH_3 fluxes were constant during the first 24 h from deposition (Fig. 2a), with a small variation among repetitions. Under “warm” conditions, the variability in NH_3 fluxes among repetitions increased, particularly for one sample where volatilization was high immediately after deposition and progressively decreased over time (Fig. A1, Annex 1). However, in most samples, fluxes were steady for the entire duration of the experiment. The observed dynamics corroborated those found in the literature, which report a certain amount of NH_3 emission from F. Despite a lower N content and pH than in U, F was shown to contain a higher content of bacteria capable of promoting urease-catalysed hydrolysis of ammonium N (Marini and Van Amburgh, 2005).

The volatilization of NH_3 from U samples revealed an increasing trend during the first 24 h from the start of the experiment (Fig. 2b), with substantial homogeneity at 10 °C and increased variability among samples at 20 °C. The temperature effect appeared to almost double NH_3 fluxes in “warm” conditions since temperature governs the equilibrium between volatile NH_3 and non-volatile NH_4^+ (Moraes et al., 2017) by increasing the dissociation of NH_4^+-N to NH_3-N and thus enhancing NH_3 volatilization (Hristov et al., 2011). Higher emissions observed in U

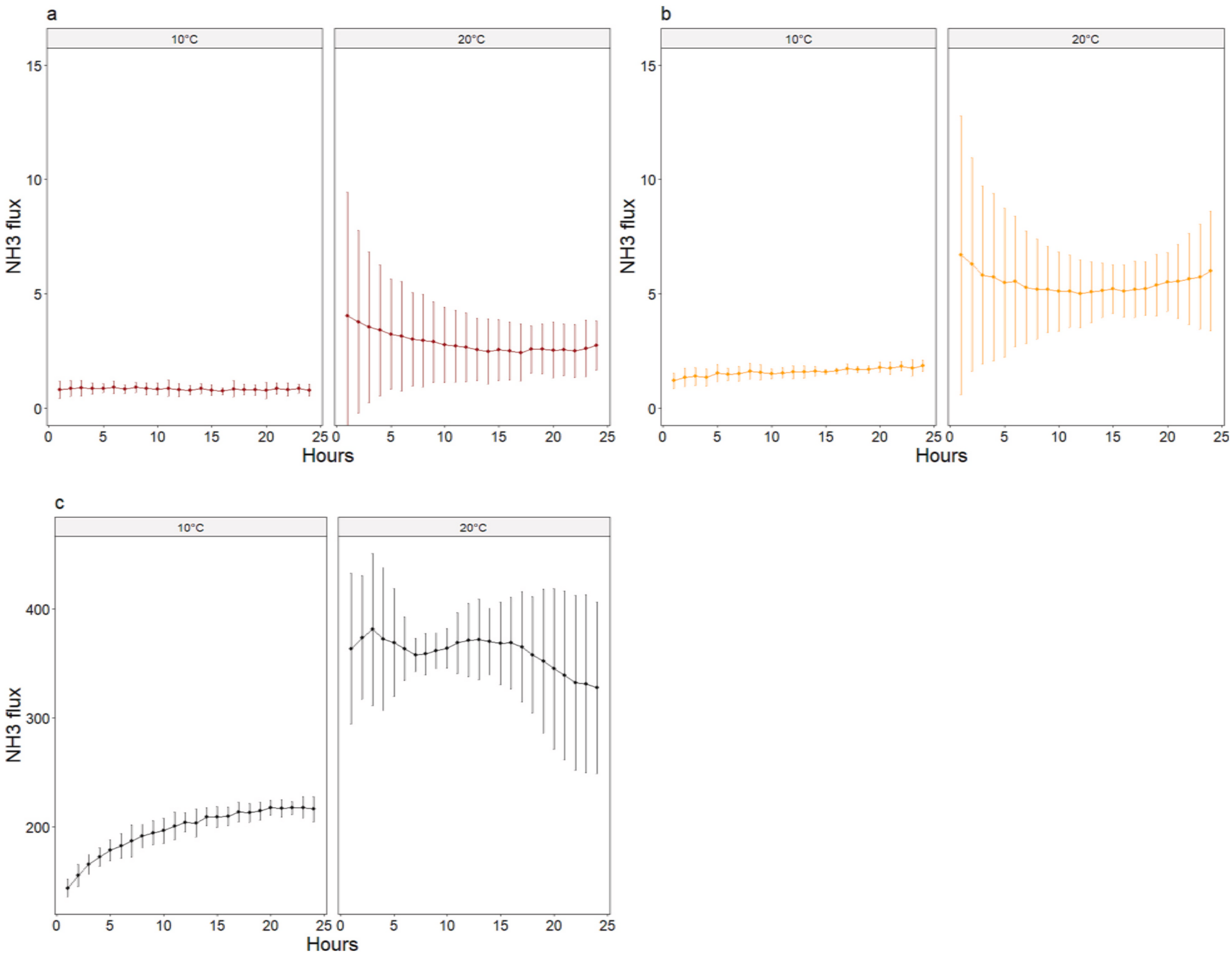

Fig. 2. Average NH_3 fluxes ($mg \cdot m^{-2} \cdot h^{-1}$) over 24 h with 95% confidence intervals in the two environmental treatments (10 °C and 20 °C) over time (hours). a: faeces, b: urine, c: mix.

were supported by the higher TKN compared to that of F, despite the similar TAN content between the two sources.

When mixing urine and faeces (M), an increase in NH_3 fluxes over time was evident under "cold" conditions. At 20 °C, the trend was variable among samples and peaked, on average, around the 3rd hour from the start of the experiment. The present results supported previous work, showing a peak in NH_3 fluxes from an even mix of cattle faeces and urine within 3 h from deposition on a simulated concrete floor (Pereira et al., 2012). Regardless of the emission trends, we observed that mixing urine and faeces triggered a huge increase in NH_3 emissions compared to U and F alone (Fig. 2c). This is in accordance with available literature (Vaddella et al., 2010; Pereira et al., 2012), reporting a clear effect on NH_3 emissions when mixing urine and faeces, due to ureolytic microorganisms contained in faeces triggering urease-catalysed hydrolysis of ammonium N in urine. As mentioned previously, NH_3 volatilization in the present study, as a consequence of the urease activity upon urinary N, resulted in fluxes approximately 100 times higher than when both excreta were separated. These values were shown to be comparable to those measured by Pereira et al. (2012) on mixed excreta of lactating cows at similar temperatures.

Environmental temperature is a factor affecting urease activity. Urease activity has been shown to increase exponentially above 10 °C, with a very low dissociation of NH_4^+ to NH_3 rate below that threshold (Powell et al., 2008; Balcells et al., 2020). NH_3 volatilization also depends on the equilibrium between its presence in the liquid and gas phases. As this equilibrium is strictly temperature-dependent, higher temperatures would also increase NH_3 losses by decreasing its solubility and by increasing the conversion of TAN to NH_3 (Monteny and Erisman, 1998; Meisinger and Jokela, 2000). The present results corroborated these findings. A positive correlation was found between temperature increase and NH_3 emissions. The significant interaction between the type of material and air temperature showed that NH_3 emissions were higher at 20 °C than at 10 °C for the M samples ($p < 0.001$) (Table 2). However, for the F and U samples the threshold for statistical significance was not attained despite the tendency towards higher emissions during the warm period. The results were in agreement with Denmead (1983), who predicted an increase in NH_3 losses by a factor of almost 3 for each 10 °C increase in temperature. Further, the meta-analysis carried out by Sanchis et al. (2019) also showed a linear growth in NH_3 emissions with a rise in air temperature, with an increment of 1.47 g NH_3 cow^{-1} and day per degree raised. One of the limitations of our study lies in the range of temperatures we tested (10 °C and 20 °C). Specifically, based on our results, it would be interesting to repeat the experiment by increasing the range of temperatures to determine the trend of NH_3 emissions as temperature rises (beyond 20 °C), also in the view of climate change.

3.3. Volatilization of NH_3 over time

The analysis of flux variation over time (hours from the start of the experiment) revealed significant differences ($p < 0.05$) only for the hour intervals in the M samples (Table 2). NH_3 volatilization from M peaked, on average, between the 6th and 18th hour, respectively after the exposition, after which a decline was evident. This is in accordance with previous studies conducted on other dairy by-products (digestate and slurries) in open field conditions (Carozzi et al., 2012; Verdi et al., 2018; Verdi et al., 2019). This tendency appeared in both the “cold” and “warm” treatments. The variations in NH_3 fluxes over time for the three types of samples in the two environmental treatments are depicted in Fig. 2.

In samples where NH_3 fluxes were measured for over 48 h, the maximum values were recorded for M samples, thereby confirming that observed over the 24 h-period experiment (Table 3). Considering the F and U sources, the maximum NH_3 flux in “warm” conditions was substantially higher in U (20.57 $mg \cdot m^{-2} \cdot h^{-1}$) than in F (7.62 $mg \cdot m^{-2} \cdot h^{-1}$). Although the TAN content between the two sources was similar, the increased flux in U may have been attributable to the higher TKN in U which could have fostered NH_3 emissions. As discussed by Vaddella et al. (2010), NH_3 emissions from urine starting after 48 h could be due to environmental urease contamination of the medium. This explains the delayed triggering of emissions from the start of the experiment. As in the 24 h-experiment, the temperature effect was observed to favour the emission of NH_3 in U.

In “cold” conditions, we observed that NH_3 fluxes from F decreased from the beginning of the experiment onward (Fig. 3a). As already discussed, the NH_3 emission dynamics occurs during the first stages (hours) after exposure. In contrast to urine, there is a lower tendency to volatilization in faeces, attributable to (among other factors) the lower pH that inhibits NH_3 emissions compared to other mediums (Dai and Karring, 2014). In “warm” conditions, F showed a decrease in NH_3 emissions, which then increased during the second day. However, this peak was observed for a short period and at the end of the second day, emissions decreased again. From the third day until the end of the experiment, emissions increased again (Fig. 3b). A slow dynamic of NH_3 volatilization from faeces was also detected by Vaddella et al. (2010), which was possibly attributed to the decomposition of organic N by heterotrophic bacteria.

Due to its higher tendency to NH_3 volatilization, U showed an increasing trend in both temperature conditions. In “cold” conditions, at the threshold of 10 °C, NH_3 emissions from U increased regularly during the entire duration of the experiment with low, albeit higher emissions than F. In “warm” conditions, NH_3 emissions from U decreased during the first 24 h of the experiment. From the second day until the end of the experiment, we observed a rising emissions trend causing significantly higher NH_3 fluxes, corroborating previous studies reporting emission increases with a factor of 3 every 10 °C rise in temperature (Denmead, 1983; Powell et al., 2008; Balcells et al., 2020). Accordingly, Vaddella et al. (2010) found that NH_3 emissions from urine, never exposed to faeces, become relevant after 2–3 days. The authors explain this delay as a consequence of the time needed for urine to receive an adequate quantity of urease from the environment to start the massive catalyses of urea hydrolysis. Due to the higher pH and water content (Table 1), U was more prone to NH_3 losses.

Table 3
Descriptive statistics of NH_3 fluxes ($mg \cdot m^{-2} \cdot h^{-1}$) produced by the type of samples in the two environmental regimes (10 °C and 20 °C), measured for >48 h. For the F_{cold} sample, fluxes at 70 h could not be determined.

Type of material	Climatic conditions	NH_3 fluxes Average ± SD	Min	Max	Hours
Faeces	10 °C	1.33 ± 0.28	0.98	2.17	48
	10 °C	–	–	–	70
	20 °C	3.16 ± 1.21	1.89	5.32	48
	20 °C	3.45 ± 1.38	1.89	7.62	70
Urine	10 °C	1.76 ± 0.29	1.05	2.23	48
	10 °C	1.94 ± 0.37	1.05	2.52	67
	20 °C	6.14 ± 1.59	4.82	12.36	48
	20 °C	8.52 ± 4.57	4.82	20.57	69
Mix	10 °C	209.95 ± 25.43	137.95	239.26	48
	10 °C	217.60 ± 24.21	137.95	239.85	69
	20 °C	341.09 ± 18.40	292.01	356.61	48
	20 °C	347.95 ± 18.99	292.01	375.29	68

Regarding the variation in NH_3 flux from M over time during the “cold” treatment, NH_3 increased during the first 24 h (Fig. 2) and reached a maximum level of emissions in <72 h after exposure (Fig. 3). Then a decrease in emissions was detected from that moment onward. Applying these results to real emissions happening in the barn, we can assume a very rapid NH_3 volatilization in the housing system. A different result was obtained in warm conditions, in that fluxes significantly exceeded those in the cold conditions over the entire duration of the experiment. However, examining the NH_3 emission tend, it was reduced for about 15 h after the beginning of the measuring time, before sustaining a growth trend that persisted until the end of the sampling time (Fig. 3). As mentioned before, temperature plays a key role in terms of NH_3 hydrolysis, meaning that its activity will be limited in cold temperatures. This could explain the constant reduction in emissions in the cold season samples after approximately 50 h from the beginning of the test, and the rapid increase in NH_3 volatilization from warm season samples that would have continued progressing beyond the three-day measurement period. The high temperatures would favour possible urease activity present in the faeces, triggering a prolongation of urea degradation after the first days of urine and faeces mixture.

Dai and Karring (2014) compared the urease activity in faeces and in manure from swine and cattle, determining the rates of NH_3 formation at different urea concentrations. While finding clear evidence of differentiation in the urea hydrolysis rate between animal species, they did not focus on the variation of emission from both types of samples used. Instead, they showed that the TAN formation rate decreases significantly with reaction time (0.45 mM/min at 5 min vs. 0.22 mM/min at 20 min), despite not providing measurements over a longer period of time. Instead, Van der Stelt et al. (2007) performed a longer manure NH_3 volatilization experiment, performing gas measurements for 223 days, showing an increasing emission trend in warm temperatures (20 °C) similar to those obtained in the present study. Volatilization under cold conditions (5 °C) was also measured, providing lower and more constant emissions over time.

The analysis of cumulative NH_3 fluxes (Fig. 4) confirmed the effect of environmental temperature on fostering NH_3 emissions in all treatments. According to the fitted linear models (Table 4), the interaction between environmental conditions and time of exposure was significant for the three sample types ($p < 0.001$), confirming that the dynamic of NH_3 fluxes over time is temperature-dependent and that emission increases linearly over time. F showed the lowest cumulative NH_3 emissions due to the lower TKN, water content and pH, which made the sample less sensitive to NH_3 volatilization losses. NH_3 fluxes from U were significantly higher than F, with emissions almost doubling throughout the 24 h-experimental period in “warm” conditions. Again, this was due to the effect of temperature in swaying the equilibrium between volatile NH_3 and non-volatile NH_4^+ towards volatile NH_3 (Moraes et al., 2017). Similarly, over the 24-h period, the variability in flux measurements in the replicates of both in F and U treatments increased with environmental temperature. As observed for average fluxes (Fig. 2 and Fig. 3), M was shown to produce significantly higher cumulative NH_3 emissions than F and U in both “cold” and “warm” conditions. Cumulative emissions from M after 24 h of exposure were 100 times higher than the separated sources. Variability was observed to be clearly reduced, probably due to the mixing effect of the two samples. In order to thoroughly assess the dynamics of NH_3 emissions and provide insights into the NH_3 fluxes from different types of substrates, further experiments should focus on evaluating NH_3 emissions over extended periods (on a monthly/seasonal/yearly scale).

The research activity should consider innovative solutions to reduce

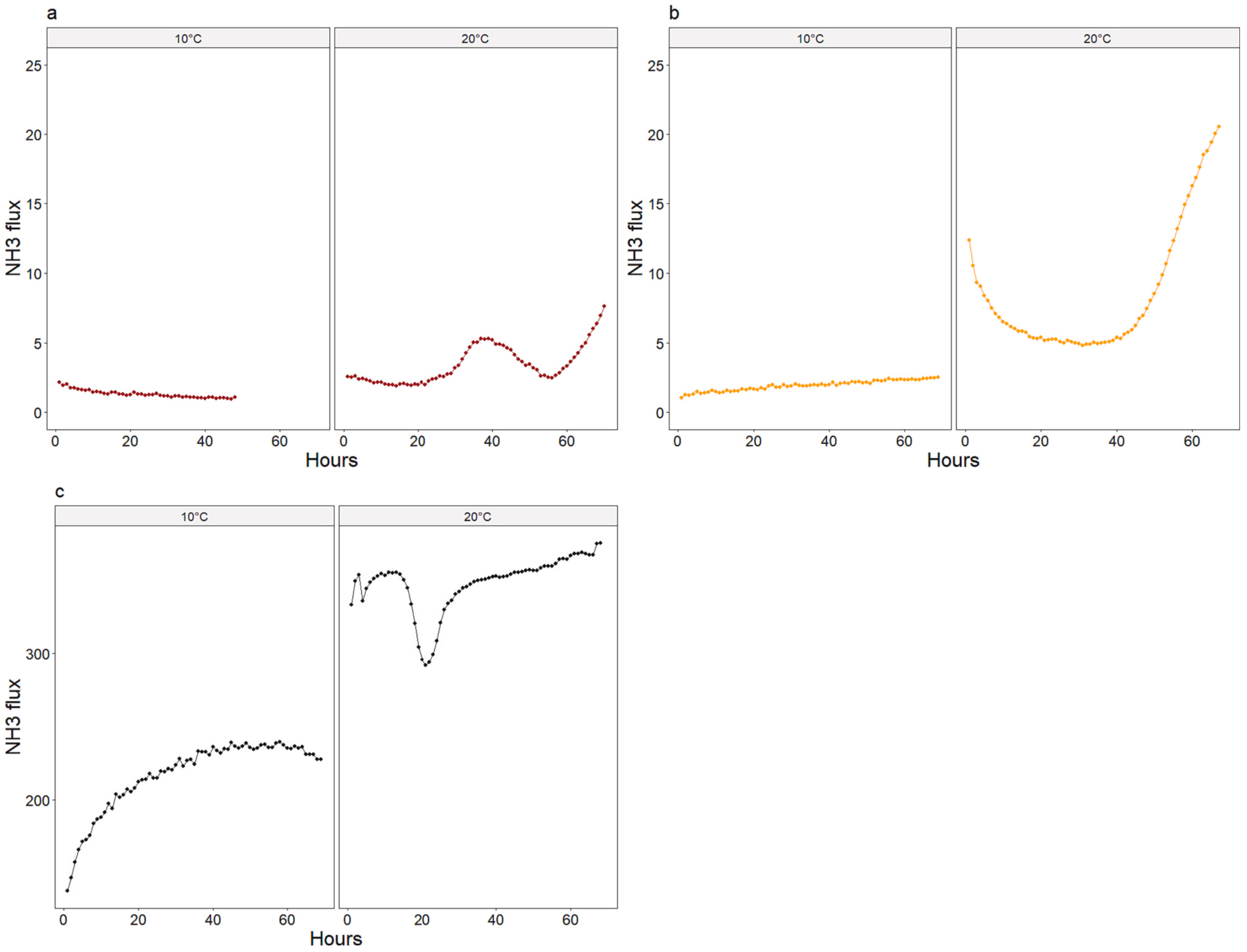

Fig. 3. NH_3 fluxes (mg · m^{-2} · h^{-1}) recorded for >48 h in the two environmental treatments (10 °C and 20 °C) over time (hours). Experimental observations are plotted as dots. a: faeces, b: urine, c: mix.

NH_3 emissions from barns. These solutions should aim to stabilize barn by-products to reduce NH_3 emissions into the atmosphere. Adopting urease enzyme inhibitors, using innovative materials (e.g. biochar), or applying physical-chemical treatments such as substrate acidification could provide valuable insights for end users (farmers).

4. Conclusions

Mixing faeces and urine, as normally happens in livestock buildings and manure storages, triggers NH_3 volatilization. In the present study, the emissions generated were 100 times higher than those originating from the individual matrices. Agriculture is required to produce efficient strategies for reducing nitrogen losses, thereby enhancing the capacity for reusing on-farm resources. NH_3 emissions from faeces and urine alone are low, and in the case of faeces almost negligible. Thus, the ideal situation would be to avoid the contact between urine and faeces. This emphasizes an interesting potential strategy to reduce NH_3 emissions within barns, with consequent positive effects for the air quality inside the buildings for both humans and animals. From the present study, the effect of temperature on NH_3 emissions was clear. NH_3 emissions increased in both M and sole U and F sources upon increasing temperature beyond the threshold of 10 °C. Under warm conditions (20 °C), NH_3 emissions almost doubled compared to values at 10 °C. The volatilization of NH_3 is influenced by the balance between its presence in liquid and gas phases, respectively. This balance is temperature-dependent, in that higher temperatures lead to increased NH_3 losses. Hence, higher temperatures decrease NH_3 solubility in liquid and increase the conversion of TAN to NH_3. Therefore, urine must be carefully managed in view of its high TAN content and optimal pH to produce NH_3. Although urine is low in bacteria responsible for urease-catalysed hydrolysis, the risk of contamination is high as urease is considered ubiquitous in the environment. The main limitation of this study is the reduced exposure period investigating the early NH_3 emissions. Further studies should investigate NH_3 emissions in longer periods by simulating storage conditions on the farm level. Preventing contact between faeces and urine is an effective strategy to reduce early NH_3 emissions from livestock systems, especially in barns. This amounts to a potential decrease in NH_3 emission losses by >99%. Implementing separation techniques that ensure limited cross-contamination between faeces and urine requires solutions to be operated during elimination events. Recent research attempted to train calves and piglets at using separate areas for defecation and urination, as tested by Dirksen et al. (2020) and Tillmanns et al. (2022), or at using devices dedicated urine collection during elimination events, similarly to the CowToilet (Hanskamp), as reviewed in Galama et al. (2020). While recognizing that novel solutions require to be studied, such a strategy could contribute towards addressing the requirements of the European Green Deal, which calls for an increase in the reuse of resources and a reduction in the adoption of mineral fertilizers. This approach has the potential to serve as an efficient solution for enhancing the on-farm recycling of N, while simultaneously decreasing the need for synthetic external inputs, in the pursuit of sustaining soil fertility and yields.

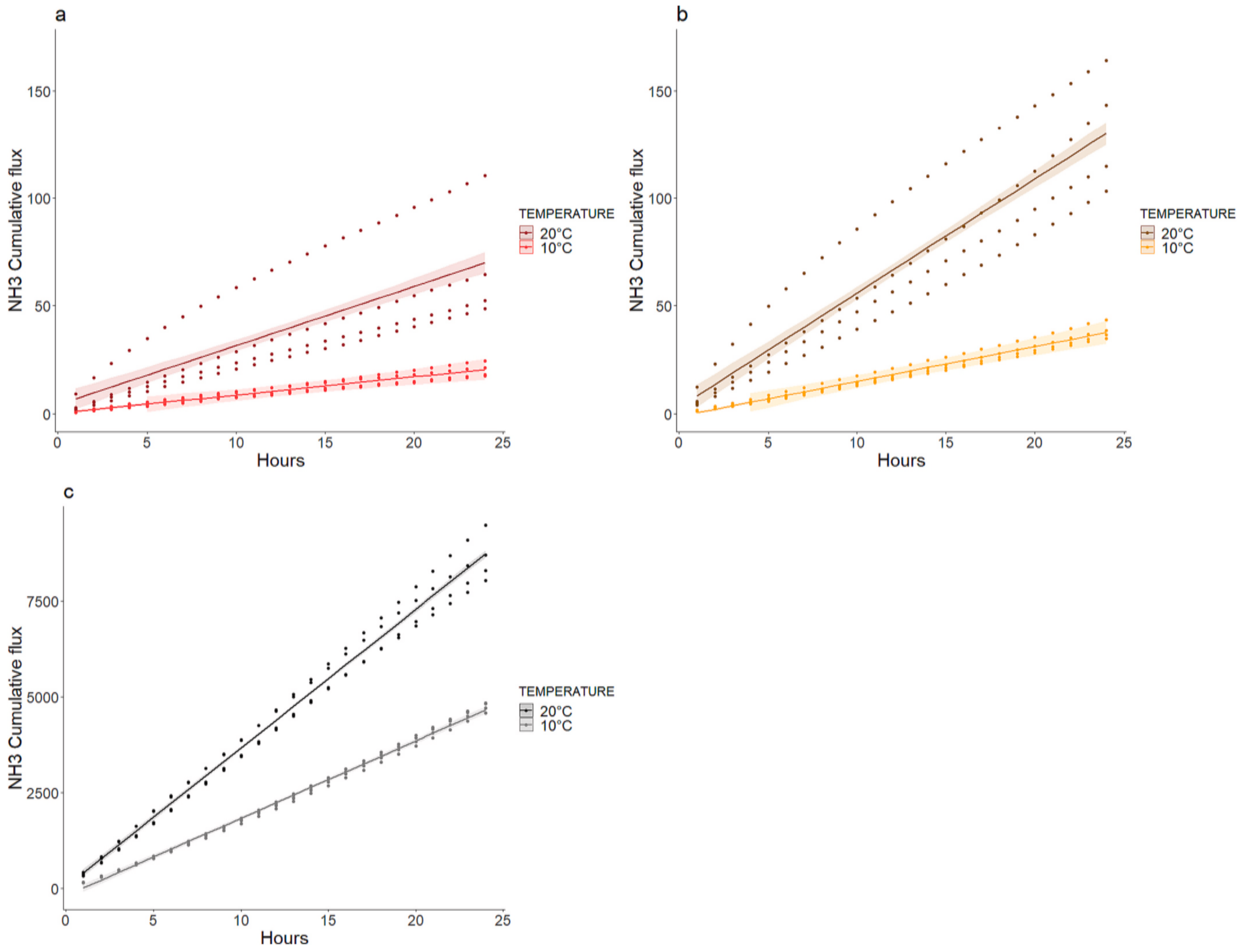

Fig. 4. Regression lines for NH_3 cumulative fluxes (mg · m-2) with 95% confidence intervals in the two environmental treatments (10 °C and 20 °C) over exposure time (hours). Original data points are depicted as points. a: faeces, b: urine, c: mix.

Table 4
Coefficient estimates, S.E. and R^2 from the linear regressions fitted to NH_3 cumulative fluxes (mg · m^{-2}) of the three types of samples (F, U, M).

Type of sample	Treatment	Regression coefficient	Coefficient estimate	Standard error	R^2
Faeces	10 °C	Intercept	0.168	3.677	0.723
		Slope	0.844	0.257	
	20 °C	Intercept	4.133	2.600	
		Slope	2.744	0.182	
Urine	10 °C	Intercept	−1.25	3.959	0.888
		Slope	1.616	0.277	
	20 °C	Intercept	2.970	2.799	
		Slope	5.296	0.196	
Mix	10 °C	Intercept	−190.754	68.326	0.991
		Slope	201.939	4.782	
	20 °C	Intercept	47.458	48.314	
		Slope	361.860	3.381	

CRediT authorship contribution statement

Valentina Becciolini: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft. **Lorenzo Leso:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Writing – review & editing. **Esperanza Fuertes Gimeno:** Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Giuseppe Rossi:** Resources, Writing – review & editing. **Matteo Barbari:** Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. **Anna Dalla Marta:** Resources, Supervision. **Simone Orlandini:** Project administration, Resources, Supervision, Writing – review & editing. **Leonardo Verdi:** Investigation, Methodology, Project administration, Writing – original draft.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Leonardo Verdi reports article publishing charges was provided by University of Florence.

Data availability

Data will be made available on request.

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Appendix A. Annex 1

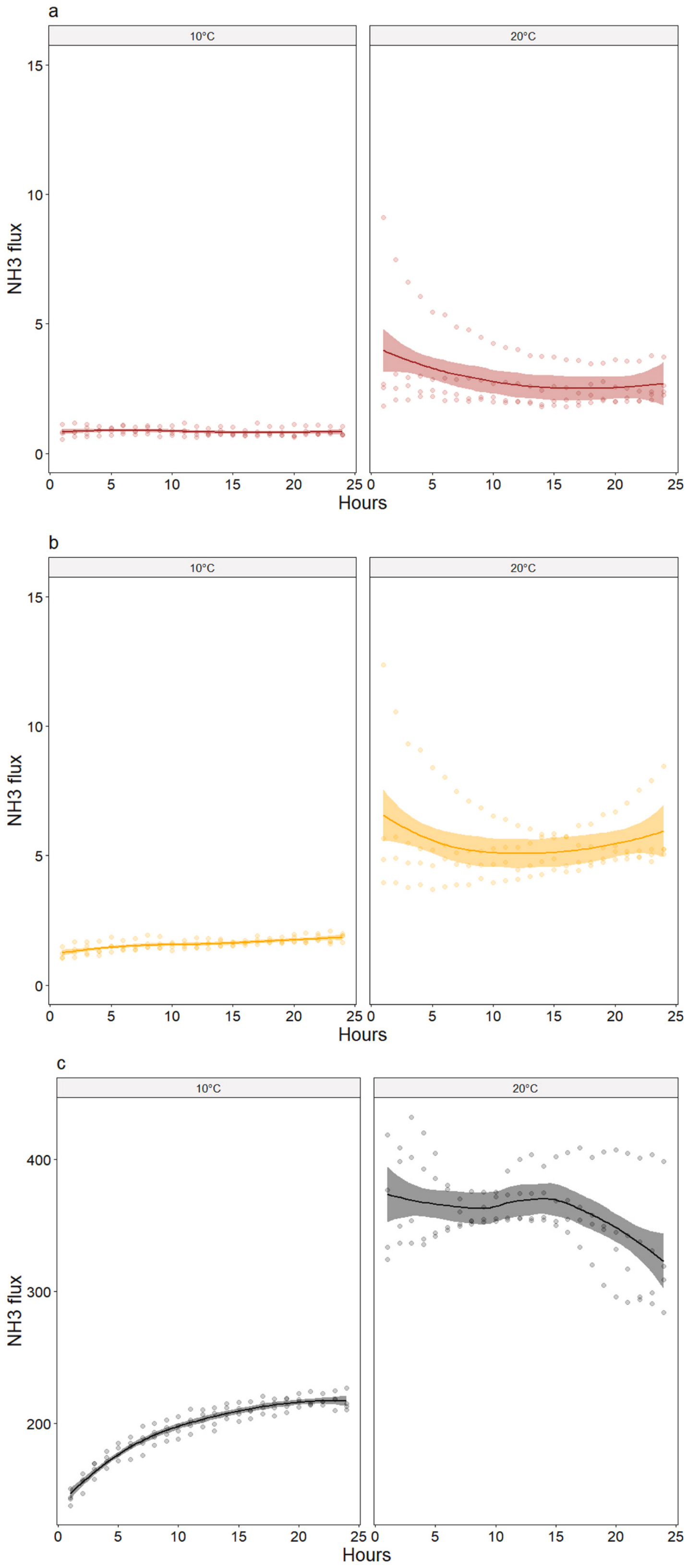

Fig. A1. NH_3 fluxes ($mg \cdot m^{-2} \cdot h^{-1}$) in the two environmental treatments over time (hours). Original data points and smoothing spline with 95% C.I. a: faeces, b: urine, c: mix.

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