

BGD

11, 6519–6553, 2014

Trimethylamine emissions in animal husbandry

J. Sintermann et al.

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Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

⏪

⏩

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Abstract

Degradation of plant material by animals is an important transformation pathway in the nitrogen (N) cycle. During the involved processes, volatile reduced alkaline nitrogen compounds, mainly ammonia (NH_3) and aliphatic amines such as trimethylamine (TMA), are formed. Today, animal husbandry is estimated to constitute a main source of aliphatic amines into the atmosphere with TMA being the main emitted compound. Here, we show how the interaction between faeces and urine in animal production systems provides the primary source for agricultural TMA emissions. Excreted urine contains large quantities of urea and TMA-N-oxide, which are transformed into NH_3 and TMA, respectively, via enzymatic processes provided by microbes present in faeces. TMA emissions from areas polluted with urine-faeces mixture are on average in the order of 10 to 50 $\text{nmol m}^{-2} \text{s}^{-1}$. Released amines promote secondary aerosol particle formation in the agricultural emission plume. The atmospheric lifetime of TMA, which was estimated to be in the order of 30 to 1000 s, is determined by the condensation on aerosol particles.

1 Introduction

Atmospheric reduced nitrogen species, such as amines or ammonia (NH_3), are crucial for secondary aerosol particle formation as they constitute the alkaline counterparts to acidic vapours, such as sulphuric and nitric acid (Finlayson-Pitts and Pitts, 2000; Seinfeld and Pandis, 2006). Recent publications have suggested that aliphatic amines exhibit a potential for aerosol particle formation orders of magnitudes higher than that of NH_3 (Murphy et al., 2007; Kurten et al., 2008; Barsanti et al., 2009; Loukonen et al., 2010; Almeida et al., 2013). The influence of anthropogenically induced secondary particle formation has a potentially high impact on earth's radiative forcing (Lohmann and Feichter, 2005; IPCC, 2013) and human health (Pope et al., 2002). There is a large knowledge gap linking amine sources, their distribution, and secondary particle for-

BGD

11, 6519–6553, 2014

Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



mation (Andreae, 2013; Kulmala et al., 2013). Additionally, measurements of ambient amine concentrations are scarce. However, among the volatile aliphatic amine species, trimethylamine (TMA) release from agriculture constitutes the most dominant source and, consequently, TMA has been found to be the dominant gas phase aliphatic amine (for example compared to methylamine: MMA, and dimethylamine: DMA) at agricultural sites with a molar ratio TMA : NH₃ between 0.3 and 1 % at animal housings and feedlots (Hutchinson et al., 1982; Schade and Crutzen, 1995; Kuhn et al., 2011) (see also Kuwata et al., 1983; Grönberg et al., 1992; Kallinger and Niessner, 1999; Rappert and Müller, 2005; Schade and Crutzen, 1995; Filipy et al., 2006; Ngwabie et al., 2008; Blanes-Vidal et al., 2009; Feilberg et al., 2010; Ge et al., 2011; Trabue et al., 2011; Dawson et al., 2014). Based on this narrow range, Schade and Crutzen (1995) provided an estimate of the global aliphatic amine emissions by scaling with the more detailed assessed NH₃ emissions. Subsequently, it has been shown that the TMA-NH₃ analogy is not generally transferable to all agricultural emission stages, as stored cattle slurry is considerably TMA-depleted. The global agricultural amine source has been refined accordingly (Kuhn et al., 2011). Since cattle rumen content contains a very high proportion of volatile TMA, Kuhn et al. (2011) discussed the hypothesis that ruminants may exhale high concentrations of TMA by rumination. However, no measurements under real conditions have so far supported this hypothesis. The relevance of amine emissions to aerosol particle formation (Almeida et al., 2013) motivates this study to gain more information on the formation mechanism and emission of agricultural TMA. The focus lies on TMA emission pathways in dairy systems.

Measurements were performed at a cattle stable at the Federal Dairy Research Station in Posieux, Switzerland. Relying on a combination of different fast analysers (Proton Transfer Reaction-Time of Flight Mass Spectrometry: PTR-TOF, High Temperature-Chemical Ionisation Mass Spectrometry: HT-CIMS, Cavity Ring-Down Spectroscopy: CRD) the different possible TMA sources in animal housings were traced. In a further laboratory trial, TMA and NH₃ emissions from cattle urine, faeces, and a mixture of both were quantified by means of a dynamic chamber in combination with HT-CIMS

Trimethylamine emissions in animal husbandry

J. Sintermann et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

and CRD. We discuss the fate of the identified agricultural TMA emissions and their role in secondary aerosol particle formation.

2 Material and methods

2.1 Concentration measurements at the dairy cattle stable

5 Measurements were carried out in summer 2011 at a dairy cattle stable at the Federal Research Station in Posieux, Switzerland (7.10653° E, 46.7692° N; 640 m a.s.l.). The herd consisted of 60 dairy cows (average live weight 680 kg, average annual milk production 8500 kg cow⁻¹) milked twice a day: early in the morning and in the afternoon. Before and after milking, the animals spent approx. 3 to 5 h in the barn complex in an open-air waiting yard (surrounded at three sides by the stable building). During most of this time they had access to feeding stations providing them their concentrate ration of a cereal mix, offered in individual amounts based on current standardised current milk production and maintenance requirements. They had also access to feed bunks to consume a limited daily amount of maize silage. The remainder of the day the herd was on pasture. The yard's concrete floor was cleaned with automatic scrapers, usually within 1 h after the animals' presence. Based on HT-CIMS, PTR-TOF, and CRD (see Sect. 2.3), diurnal patterns of ambient trace gas concentrations in the outdoor stable yard were measured at 3 m height over 3.5 days. In addition, short period trials were pursued measuring close (within 10 cm) to the mouth of ruminating cattle. Measured target trace gas species were: NH₃, TMA, acetone, CH₄.

2.2 Emission measurements with dynamic chambers

In order to quantify TMA and NH₃ emissions in a controlled environment, a dynamic chamber (Pape et al., 2009) was placed over different excretion substrates (see below) inside a climate controlled (20 °C, relative humidity of 60 %) well ventilated room. Given steady-state and no trace gas destruction or creation, such as chemical conversion

BGD

11, 6519–6553, 2014

Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

⏪

⏩

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



inside the chamber, the substrate chamber flux F_{cham} is derived by:

$$F_{\text{cham}} = \frac{Q}{A} \cdot \rho_d (c_{\text{cham}} - c_{\text{amb}}), \quad (1)$$

where Q is the purging air flow rate, A denotes the area of investigated surface inside the chamber, ρ_d is the density of dry air, and c_{cham} and c_{amb} represent the trace gas mixing ratios inside and outside of the dynamic chamber, respectively (Pape et al., 2009).

The cylindrical chamber with walls of PFA film had an enclosed headspace volume of 41 L and was continuously flushed with 60 L min^{-1} of ambient air. It was set up on a base into which a circular plastic sample container ($A = 0.049 \text{ m}^2$) was placed. c_{amb} and c_{cham} were measured for NH_3 and TMA by CRD, HT-CIMS, and impinger sampling combined with ion chromatography (IC) analysis.

On 9 February 2012, urine and faeces were collected upon excretion at the cattle stable in Posieux. Stored slurry from the slurry pit was also collected. The samples were sealed airtight, cooled, and brought to the laboratory within 6 h. The substrates were subdivided and recombined to a 2 : 1 urine-faeces mixture immediately before the first measurement. For this, 1 L subsamples were prepared in the sample containers. Both sets, the subsamples and remaining fractions, were stored in separate climate rooms under similar conditions throughout the entire experiment period of about 160 h. For measurements, each subsample was put inside the dynamic chamber for 30 to 60 min. After some minutes it was stirred for one minute. Average fluxes were calculated subsequently to stirring considering chamber equilibration time (with respect to NH_3 and TMA wall ad- and desorption effects), which was determined to be less than 5 min. pH values were monitored (pH-3310, WTW, DE). The pH rod was cleaned using demineralised water following each measurement. To estimate maximum emission potentials of the volatile pools of NH_3 and TMA, each substrate was additionally investigated at the beginning, in-between, and at the end of the whole measurement period by adding NaOH solution to 1 L aliquots from the parallel samples immediately before measurement until substrate pH exceeded 12. This way, dissolved TMA and NH_3 is

Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



supposed to be driven into gas phase. In-between any treatments the chamber lid was opened for a minimum of 10 min to let the chamber volume and surfaces equilibrate with the ambient concentration.

2.3 Concentration determination

2.3.1 At the dairy cattle stable

Concentrations of trace gas species were recorded on-line, with a combination of PTR-TOF for VOCs (including TMA) (Ionicon Analytik GmbH A; Jordan et al., 2009; Graus et al., 2010), HT-CIMS (based on PTR-MS, Ionicon Analytik GmbH, A) for NH₃ and TMA (Sintermann et al., 2011), and a CRD analyser for CH₄ (Los Gatos Research, CA, USA). Flexible inlet lines were used in order to sample at different locations. PTR-TOF and HT-CIMS sub-sampled from a common 24 m 1/2" OD PFA inlet line flushed with about 80 L min⁻¹. The inlet line was heated above 150 °C in order to achieve response times for NH₃ and TMA in the order of seconds (Sintermann et al., 2011). The CRD sampled through a parallel 1/4" OD PFA tube. For distinct TMA and NH₃ calibration events with PTR-TOF and HT-CIMS, impingers (64712-U, Supelco, USA) sampled air into 15 mL H₂SO₄ (0.1 mol L⁻¹) solution with an airflow rate of 0.6 nL min⁻¹. The solution was analysed offline by IC as described by Kuhn et al. (2011). Applying two impingers in series resulted in TMA and NH₃ breakthrough of less than 2 %, even at high concentration levels of approx. 800 ppb NH₃ and 10 ppb TMA. The HT-CIMS instrument was operated with $E/N \approx 121$ Td at 180 °C. With this setup, TMA is detected primarily at m/z 58 with an E/N -dependent fraction also occurring at m/z 59 (10–35 %), whereas acetone peaks at m/z 59 with a minor fraction at m/z 58 (15 %). This potential for cross-interference has been included as a range of possible values in the HT-CIMS concentration calculation for TMA. PTR-TOF and HT-CIMS were calibrated for TMA and NH₃, respectively, by impingers sampling ambient air in parallel ($n = 8$ for NH₃, $n = 6$ for TMA). The HT-CIMS zero offset was determined by zero air measurements, with air provided by an LN industries (Geneva, CH) permeation device. The

Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



PTR-TOF calibration setup for selected VOCs (as in Taipale et al., 2008) and concentration calculation procedures will be described in detail by Schallhardt et al. (2014).

2.3.2 Dynamic chamber measurements

The climate room $\text{NH}_{3\text{amb}}$ concentration was continuously monitored using a CRD instrument (Picarro Inc., CA, USA) with a 15 cm short 1/4" OD PFA inlet, sampling with 0.5 nL min^{-1} close to the dynamic chamber inflow. Additionally, $\text{NH}_{3\text{amb}}$ and TMA_{amb} concentrations were periodically determined with impingers (as outlined in Sect. 2.3.1). These data revealed that TMA did not accumulate in the climate room, hence TMA_{amb} was set constant; this also corresponded to HT-CIMS observations before and after the dynamic chamber measurements. The chamber headspace concentrations TMA_{cham} and $\text{NH}_{3\text{cham}}$ were continuously monitored at the center of the volume by means of HT-CIMS (operated similarly as during the animal stable concentration measurements) equipped with a 65 cm long 1/4" OD PFA inlet line, heated to 150 °C and flushed with 0.9 nL min^{-1} . In addition, the impinger system described in Sect. 2.3.1 was operated in parallel to the HT-CIMS inside the dynamic chamber for some occasions. The CRD and HT-CIMS were calibrated for NH_3 using gas from a standard bottle (50 ppm NH_3) down-mixed by a mass flow controller with zero-concentration bottled synthetic air. This zero air stream was guided through two large (0.25 L) impingers with H_2SO_4 solution (0.01 mol L^{-1}) and demineralised water, respectively, set up in a temperature regulated water bath in order to ensure gas relative humidity of 60% at 20 °C – the same as in the climate room. For NH_3 calibration, the NH_3 gas was mixed into this air stream. The air stream was supplied to the CRD or HT-CIMS over a T-piece with an overflow. Overflow impinger sampling with IC analysis was applied to check the calibration gas concentration. These calibrations were performed at least over 2.5 h to ensure sufficient equilibration time of the sticky NH_3 molecules with involved surfaces. TMA calibration factors for HT-CIMS were obtained by comparison with impinger samples that had been collected during parallel operation of HT-CIMS and impingers inside the dynamic cham-

BGD

11, 6519–6553, 2014

Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



ber. Applying two impingers in series resulted in less than 3% breakthrough even at mixing ratios of approx. 3 ppm NH₃ and 80 ppb TMA.

3 Results

3.1 Concentration measurements

3.1.1 Exhaling cow

To test the hypothesis that ruminating animals exhale TMA-enriched breath as the main TMA source, air was sampled close to the mouth of a ruminating cow. Figure 1 shows such an episode with high time resolution. Peaks in the acetone concentration time course mark collection of air enriched with the animal's respiration (Elliott-Martin et al., 1997; Turner et al., 2012). CH₄ is related to eructation and respiration. NH₃ and TMA concentrations in surrounding air were highly variable. They did not significantly correlate with the observed peaks in acetone and CH₄. The mean TMA concentration was 2.7 ppb with a maximum of 4.5 ppb while the average TMA : NH₃ ratio during the displayed period was 0.65% (see Sect. 3.1.2).

3.1.2 Diurnal variations at the outdoor stable area

Over a period of 3.5 days, trace gas concentrations were monitored in the open-air stable yard (Fig. 2). The animals were usually away on pasture, but were gathered twice a day at the yard in the early morning and around noon (grey bars in Fig. 2). Background concentration levels were approx. 8 ppb acetone, 7 ppm CH₄, 2 ppm NH₃, 6 ppb TMA, corresponding to 0.3% TMA : NH₃. The animals' presence immediately correlated with strongly elevated acetone and CH₄ concentrations (Figs. 2 and 3). In contrast, NH₃ and TMA concentrations, as well as the ratio, began to increase subsequent to the cow's arrival with a delay of around 2 h. However, their maximum average levels of approx. 4 ppm NH₃, 50 ppb TMA, and 1.5% TMA : NH₃ were reached when

BGD

11, 6519–6553, 2014

Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



the cattle were gone and persisted as long as the stable's concrete floor was polluted with an excrement layer. Highest 1 min TMA values were approx. 120 ppb and TMA : NH₃ peaked at 2.6 %. The activation of the floor scraper and water-cleaning of the floor (dashed line in Fig. 2) preceded declines in TMA and TMA : NH₃ levels.

Typically, the concentration peaks were more pronounced in the afternoon than in the morning. An interesting feature from Fig. 2 is the plateau of the TMA and NH₃ concentrations as well as their ratio observed on the afternoon of 1 August onwards. Under usual operation, the yard's floor is cleaned a short time after the cows leave the stable. This cleaning procedure did not take place that one afternoon and the excrement mixture remained on the floor until the next morning. During that entire episode, the elevated TMA concentration levels remained. Simultaneously measured wind speeds in the open-air waiting yard were always below 1 ms⁻¹ coming from variable directions. We therefore assume that the observed air concentrations are not mainly caused by variations of atmospheric transport capacity, but that the measured concentrations primarily scale with emission rates.

Figure 3 summarises the findings of Fig. 2 by scatterplots subdivided into values reflecting periods with and without cattle. Figure 3 shows that high levels of acetone and CH₄ correlate and are linked to direct emissions from the animals, whereas the occurrence of high NH₃ and especially TMA concentrations is decoupled from direct animal emissions.

3.2 Emission measurements

Figure 4 summarises the dynamic chamber emission measurements for NH₃ and TMA. NH₃ emissions were dominated by volatilisation from the urine-faeces mixture. After about 45 h, emissions began to decrease again. Direct faecal and urinary emissions played a minor role. pH values tended to increase with increasing emissions. In detail: faeces pH remained below 7 with a slight increase towards the end of the measurement period, mixture pH increased from 7.7 to 8.5 and declined down to 8.1 after 160 h, and urine pH rose from 8.5 to 9.5. Potential NH₃ emissions were in general much

larger than actual emissions, whereby the mixture exhibited the by far largest emission potential. NH_3 emissions from stored slurry ($0.3 \mu\text{mol m}^{-2} \text{s}^{-1}$) were lower than from the substrates, because the composition of stored slurry reflects a long term equilibrium.

TMA emissions behaved similar to NH_3 emissions. Only the urine-faeces mixture showed larger emissions during more than the first 120 h. The maximum flux during this period was $0.13 \mu\text{mol m}^{-2} \text{s}^{-1}$, and potential emissions were even higher. The following decrease down to $31.2 \text{nmol m}^{-2} \text{s}^{-1}$ remained far above the very small actual and potential TMA emissions from the stored slurry (0.73 and $1.3 \text{nmol m}^{-2} \text{s}^{-1}$ with 0.2 and 0.05% emission ratio, respectively). With the mixture, the TMA : NH_3 emission ratio was in the order of 1% ($0.3, 1.0, 0.9, 0.6, 0.7, 0.7\%$ for the periods in Fig. 4), consistent with the observations in Fig. 2. Faecal TMA emissions were continuously lowest, not exceeding $9.2 \text{nmol m}^{-2} \text{s}^{-1}$. Due to the acidic pH no significant emissions occurred, but also the emission potential of the volatile TMA pool was low. Until around 80 h, urinary TMA emissions remained low with only minor emission potential during the first 24 h. After 130 h, however, a very large TMA emission peak up to $1.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ was observed, which decreased again towards the following measurement interval. We think this peak was induced by contaminating the urine sample with some faecal residues on the pH electrode sometime after 88 h. During storage, corresponding bacterial action was likely to effectively convert the concentrated TMA precursor to TMA (see discussion Sect. 4.2). Considering that ambient temperatures are usually lower than the 20°C during our experiment (hence, more TMA will remain in the liquid phase) and assuming that in a real situation with a high stocking density there is a constant supply of excrements which mix and show an emission behaviour like the mixture in Fig. 4, we derive a typical average TMA emission rate of $10 \text{nmol m}^{-2} \text{s}^{-1}$ that we regard representative for ambient situations.

BGD

11, 6519–6553, 2014

Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

⏪

⏩

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



4 Discussion

4.1 TMA in exhaled air

Kuhn et al. (2011) hypothesised oral TMA volatilisation from ruminating cattle as the main pathway for the generally enhanced TMA concentrations and emissions from agriculture. This might be due to TMA enriched rumen content (pH = 6.5) being mixed with saliva of high pH (pH = 8). In our measurements close to a ruminating cow's mouth, air influenced by the animal's respiration was characterised by short-term concentration peaks of acetone and CH₄ (Fig. 1). Acetone, present in the blood, mainly originates from exhalation where it occurs at ppm level (Dobbelaar et al., 1996; Elliott-Martin et al., 1997; Spinhirne et al., 2003; Turner et al., 2012). CH₄ is created in the rumen as a by-product of enteric fermentation (Russell and Wallace, 1997) and is released during eructation and exhalation (Martin et al., 2012). CH₄ release from the rumen dominates compared to release via the lung (<http://c-lockinc.com/data.php>). Elevated concentrations of CH₄ and acetone are thus directly linked to the animal's respiration as can also be seen in Figs. 2 and 3. In order to support the hypothesis by Kuhn et al. (2011), strongly increased TMA : NH₃ values should be observed when measuring close to a ruminating cow's mouth and when cattle stay in the stable yard. This was not evident from our measurements (Fig. 1). In fact, TMA concentration levels and TMA : NH₃ ratios began to rise most strikingly only subsequent to the animals' presence in the yard (Fig. 2), which suggests that the main source for agricultural TMA volatilisation and high TMA : NH₃ ratios cannot be oral release from ruminating cows. The amount of volatilised TMA during rumination is insufficient to yield the observed elevated TMA concentration episodes. This may be explained by limited gas exchange between mouth and atmosphere.

BGD

11, 6519–6553, 2014

Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



4.2 Characteristics of TMA volatilisation

Periods with highest TMA concentrations and TMA : NH₃ ratios were found subsequent to the animals being in the stable yard and correlated with the existence of an excrement layer on the ground (Fig. 2). The TMA and NH₃ concentrations in the afternoon exceeded those during morning. The higher temperatures promote the volatilisation of the alkaline constituents since solubility is decreased and the hydrolysis of urea is accelerated leading to an increase of the pH-value. When the excrement layer was removed by a scraper and cleaned with water, the emissions and hence concentrations subsided. On one occasion (1 August) cleaning was omitted until the next morning resulting in persistently high TMA levels. Our dynamic chamber emission measurements (Fig. 4) demonstrate how TMA is most rapidly and efficiently released from a mixture of urine and faeces whereas urine and faeces alone exhibited rather small emission rates. Elevating the substrate's pH to > 12 ("volatile emission pool") showed that very little TMA for volatilisation was available in urine and faeces alone. Hence, there is most likely a TMA precursor which is converted efficiently by the mixing of urine and faeces. The sudden massive TMA emission peak from urine after 135 h suggests that this precursor is contained within the urine. Several publications (see Sect. 4.3) show that TMA-N-oxide (TMAO) is excreted in urine by different mammals. We analysed urine from two Australian lactating cows for TMA and TMAO content using an established method (Johnson, 2008). Results reveal two to four times more TMAO than TMA (762–1121 μmol TMAO L⁻¹ vs. 251–386 μmol TMA L⁻¹). This fraction likely varies from animal to animal depending on diet, health, and lactation status. Humans, as another example, excrete much more TMAO than TMA with urine (TMA : TMAO < 4%; Lee et al., 2010). TMAO was found already at the beginning of the twentieth century in animal tissues (Suwa, 1909). It appears to have manifold physiological functions and is, for example, an important compound in marine organisms for cellular osmotic and pressure regulation (Samerotte et al., 2007) and protein stabilisation (Bennion and Daggett, 2004; Sarma and Paul, 2013). The decomposition to TMA is responsible for

Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



the smell of decaying fish (Barrett and Kwan, 1985). The ratio of TMA : TMAO in human urine is, for example, used as a diagnostic tool for a disturbed metabolism (Cashman et al., 2003; Lee et al., 2010).

TMAO converting microbes are mainly abundant in the faeces. We propose that the dynamic chamber urine sample was contaminated by bacteria from the faeces some-time after 88 h, probably through an insufficiently cleaned pH rod. The urine emis-sion peak occurs for TMA but not for NH_3 , which demonstrates that different microbial species might have been responsible for the TMA creation than are for NH_3 formation by urea hydrolysis. Alkaline conditions are required to make TMA available for volatilisa-tion. Urea hydrolysis to NH_3 increases pH and provides the conditions for TMA release into the gas phase.

4.3 The fate of TMA in ruminants

To the best of our knowledge a picture of ruminant TMA formation, cycling, and volatil-isation, complementing the schemes given by Bain et al. (2005) and Rappert and Müller (2005), has not yet been compiled. We attempt this with Fig. 5 schematically demonstrating the fate of NH_3 and TMA in ruminants. As already indicated by Schade and Crutzen (1995), the primary source for TMA is the rapid microbial degradation of choline (Davies, 1936; Eddy, 1953; Neill et al., 1978; Zeisel et al., 1989; Bain et al., 2005; Wang et al., 2011; Craciun and Balskus, 2012) taken up with the feed (Pinotti et al., 2002). In addition, rumen microorganisms might metabolise TMA from dietary supply of carnitine, betaine, and TMAO (Davies, 1936; Mitchell et al., 1979; Zhang et al., 1999). The TMA is further used for methanogenesis. This removal process be-comes saturated (Neill et al., 1978; Patterson and Hespell, 1979; Barrett and Kwan, 1985; Pinotti et al., 2002; Padmanabha et al., 2013). Rumen TMA has been found to accumulate with a molar ratio of dissolved TMA : NH_3 of 1 : 1.7 to 1 : 3.1 (Kuhn et al., 2011). Like NH_3 , TMA diffuses through the rumen wall into plasma and blood stream (Bain et al., 2005). In the liver, the NH_3 is converted to urea as a detoxification mech-anism (Meijer et al., 1990; Walt, 1993). Similarly, TMA acts detrimentally (Guest and

Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Varma, 1992; Rappert and Müller, 2005) while liver cells are capable to enzymatically oxidise TMA to TMAO (Baker and Chaykin, 1962; Baker et al., 1963; Gut and Conney, 1993; Bennett et al., 2013). Saliva is known to contain urea serving as additional NH₃ source in the rumen when being recycled (Wallace et al., 1997). Likewise it seems reasonable that also TMAO, dissolved in the saliva is being returned to the rumen, where it would be reconverted to TMA and then recycled back again to TMAO.

Several studies have found that a large TMA fraction from ingested precursor substances had been excreted in form of urinary TMAO with various mammals (Davies, 1936; Norris and Benoit, 1945; Al-Waiz et al., 1987; Dacosta et al., 1990; Zhang et al., 1999). Once excreted, microorganisms reduce TMAO to TMA, subsequently convert TMA to DMA, MMA, and finally to NH₃ under aerobic conditions (Barrett and Kwan, 1985; Kim et al., 2001; Rappert and Müller, 2005). Microorganisms are known to feed on TMAO anaerobically as well (Kim et al., 2001; Baraquet et al., 2006). This behaviour generally appears to be similar to urea hydrolysis and both processes are responsible for the rapid occurrence of high NH₃ and TMA volatilisation rates when urine and faeces come into contact with one another (Fig. 4). The combination of both substrates is relevant since most urea- and TMAO-converting microorganisms are present in the intestine. For example, *E. coli* is found in the intestine and also occurs in excreted faeces where it can survive over weeks (Fremaux et al., 2008; Callaway et al., 2009; Hanajima et al., 2011) and it can process TMAO to TMA under aerobic as well as anaerobic conditions (Gon, 2000; Baraquet et al., 2006; Ansaldi et al., 2007). Furthermore, TMA degradation occurs as stored slurry exhibits only minor TMA concentrations and emission potential (Sect 3.2; Kuhn et al., 2011). This is consistent with the observation that a variety of microorganisms are capable to efficiently degrade methylamines, including TMA, in the presence and absence of oxygen (Colby and Zatman, 1973; Colby et al., 1979; Patterson and Hespell, 1979; Jang et al., 1999; Rappert and Müller, 2005; Ho et al., 2008; Ferguson et al., 2008; Chen et al., 2011; Yang et al., 2013).

BGD

11, 6519–6553, 2014

Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

⏪

⏩

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



4.4 TMA emission measurements in the context of literature

There are aliphatic amine emissions related to poultry, sheep, horses, pigs, and cattle (Schade and Crutzen, 1995; Schade and Ngwabie, 2005). Emission flux measurements of amines are rare. Denmead et al. (1974) identified the occurrence of amine emissions by application of a micrometeorological gradient technique over an alfalfa pasture grazed by 200 sheep. They found indications of varying contributions of amine emissions to the total gaseous reduced nitrogen flux. In a finishing pig stable, Feilberg et al. (2010) determined TMA emissions by monitoring stable exhaust ventilation rate and air concentrations. They found a TMA flux of 41 mg h^{-1} corresponding to $6 \text{ nmols}^{-1} \text{ pig}^{-1}$ (however, there was also a slurry pit in the stable). Laubach et al. (2013) performed micrometeorological mass balance flux measurements of NH_3 near a cattle herd with a stocking density of $150 \text{ animals ha}^{-1}$ on grassland and found additional (unquantified) emission fractions of amines. Downwind of a cattle feedlot with $840 \text{ animals ha}^{-1}$, Hutchinson et al. (1982) determined NH_3 and amine emissions from vertical concentration gradient measurements. The largest measured TMA emission constituted about $14.5 \text{ nmol m}^{-2} \text{ s}^{-1}$. The authors do not provide further direct information on determined TMA fluxes. Based on the given information, their TMA : NH_3 emission flux ratio was between 0.3 and 0.5%. Assuming an average ratio of 0.4% and using the tabulated NH_3 emission rates results in a mean (\pm standard deviation) TMA emission of $10.9 \pm 5.3 \text{ nmol m}^{-2} \text{ s}^{-1}$. This value compares well with the $10 \text{ nmol m}^{-2} \text{ s}^{-1}$ derived from our dynamic chamber experiments. It can be expected that the feedlot's high stocking density resembles conditions similar to situations in animal housings rather than, for example, grazing cattle. Excrements cover the surface with high chances of urine and faeces interactions while there is ongoing supply. Thus, the measurements by Hutchinson et al. (1982) represent a setting comparable to our stable environment and the dynamic chamber emission trial.

With micrometeorological field measurements (eddy covariance and gradient), Kuhn et al. (2011) demonstrated that stored cattle slurry applied to grass- and cropland had

BGD

11, 6519–6553, 2014

Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



only minor TMA emissions of up to $0.3 \text{ nmol m}^{-2} \text{ s}^{-1}$ with TMA : NH_3 ratios of maximum 0.1 %. Direct analysis of the slurry TMA and NH_3 content confirmed the low TMA emissions. Twigg et al. (2011) determined TMA emissions following 6 month old cattle-slurry applied to grassland by scaling NH_3 emissions from the used eddy covariance method (Whitehead et al., 2008) with the observed ambient TMA : NH_3 concentration ratio (TMA determined by PTR-MS using a theoretical calibration factor). This yielded TMA emission rates of up to $3.6 \text{ nmol m}^{-2} \text{ s}^{-1}$ and a 31 h average emission rate of $0.69 \text{ nmol m}^{-2} \text{ s}^{-1}$. The concentration ratio TMA : NH_3 was on average 0.38 % and a maximum emission rate ratio of 0.74 % was derived. These values are higher than expected from the results of this study and that by Kuhn et al. (2011). Nevertheless, we suggest that there is sufficient experimental and theoretical (Sects. 3.2 and 4.3) support for our observed low TMA emissions from stored slurry.

Currently, there are roughly 1.4×10^9 cattle animals on earth (FAO, 2013). Assuming that a surface between 0.5 and 5 m^2 associated with each animal constantly emits 10 to $30 \text{ nmol m}^{-2} \text{ s}^{-1}$, yields a global emission of 0.003–0.093 Tg TMA-N yr^{-1} . The upper estimate is in a similar order of magnitude as the assessment of cattle emissions by Schade and Crutzen (1995) (0.083–0.167 Tg TMA-N yr^{-1}) refined by Kuhn et al. (2011) (0.035–0.053 Tg TMA-N yr^{-1}).

4.5 Emissions of reduced N-species from agriculture and their role in secondary aerosol particle formation

Nucleation of a few-molecule-cluster is the initial step of secondary aerosol formation. Aliphatic amines and NH_3 have been identified as the neutralising components of secondary aerosol formation, which is initiated by sulphuric acid (Kulmala et al., 2013). While there is a consensus that amines are important for nucleation (Andreae, 2013; Kulmala et al., 2013), the differences between various amines regarding particle formation are not known in detail (Paasonen et al., 2012). Hence, it is assumed that aliphatic amines behave similarly and, explicitly, that TMA and DMA have similar

Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



nucleation enhancement potentials. Comparing modelling results under varying atmospherically relevant conditions to experimental results from seven different measurement sites, Paasonen et al. (2012) suggest that TMA provides the rate-limiting step for atmospheric particle formation. The presence of aliphatic amines has been shown to enhance formation rates more than thousand-fold compared to the ternary NH_3 -sulphuric-acid-water system, already at very low mixing ratios (e.g. in the case of 5 ppt of DMA compared to 250 ppt of NH_3) (Almeida et al., 2013). The quantum chemical modelling and experiments conducted by Almeida et al. (2013) resemble pristine atmospheric conditions considering DMA. The enhancing effect of TMA on secondary particle formation has been found in other modelling studies (Murphy et al., 2007; Kurten et al., 2008; Paasonen et al., 2012). Measurements of aerosol number concentration upwind, within, and downwind a cattle farm have demonstrated a threefold increase in particle concentration indicating secondary particle formation (Lammel et al., 2004).

Nucleation rates are higher with an aliphatic amine than with NH_3 and therefore, if for example DMA was present, it would be expected to enhance the particle formation. To what extent this happens is dependent on sulphuric acid and amine concentrations. When the amine concentration exceeds that of sulphuric acid, the nucleation rates observed by Almeida et al. (2013) will occur. If the amine concentration is lower than that of sulphuric acid, it is likely that NH_3 also neutralises the remaining sulphuric acid-water-clusters, and that the formation rate will be between the one determined by NH_3 (lower limit) and amine (upper limit). Typical sulphuric acid mixing ratios range between 0.005 to 5 ppt (Eisele and Tanner, 1993; Petäjä et al., 2009; Almeida et al., 2013). Unlike sulphuric acid, which is formed by oxidation of sulphur dioxide in the atmosphere (Boy et al., 2005), amines are emitted directly from their sources (this study; Ge et al., 2011). While the sulphuric acid gets re-supplied continuously, the availability of gaseous amines for particle formation is further determined by their overall atmospheric residence time. It is influenced by dry deposition, oxidation by radicals – mainly the hydroxyl radical (OH) (TMA lifetime due to OH: 4.6 to 7.7 h; Ge et al., 2011) – and condensation on existing particles. The lifetime due to condensation is the reciprocal

Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



of the condensation sink, which is determined by the aerosol number size distribution. Based on the calculation method by Kulmala et al. (2012), we derive a TMA lifetime due to condensation in the range of 30 to 1000 s: half a minute for the case of a number size distribution (aerosol load) as found in the plume of a German cattle farm (Schneider et al., 2008), several minutes in cleaner environments such as in a boreal forest in Finland (Dal Maso et al., 2007). Hence, the removal of gas-phase TMA is dominated by the condensation sink. Cattle stables and feedlots are emission hot-spots for NH_3 , TMA, and also aerosol particles. The particles originate from both primary emissions as well as secondary formation. Given a lifetime of 60 s due to the condensation sink, TMA and other aliphatic amines cannot travel far in the gas phase. For example, a plume with 3 ms^{-1} wind speed would advance 1.5 km within 8.4 min and the initial 10 ppb TMA would be reduced down to 1 ppt. In addition, mixing of that plume with cleaner air would dilute the air parcel by roughly 1 : 100 and TMA concentrations would have fallen below values typical for sulphuric acid. Consequently, gas-phase TMA could determine aerosol particle formation relatively close to the source while the further away NH_3 begins to dominate the formation.

At the Finnish boreal forest research site in Hyytiälä there is experimental evidence that particle formation events coincide with episodes of increased ambient aliphatic amine concentrations (Kieloaho et al., 2013). The prevailing ambient conditions and commonly observed low pre-existing particle concentrations at the forest site differ strongly from those of intensely populated intensive agricultural regions in e.g. central Europe, surrounded by anthropogenic activities leading to higher aerosol loading.

5 Conclusions

To advance the understanding of agricultural TMA emission sources and their strength, we carried out detailed trace gas concentration measurements at a cattle stable as well as in laboratory experiments assessing TMA and NH_3 emissions from excretion substrates. TMA is not exhaled from rumination in significant amounts but is formed from

excreted TMAO by microbial action. The TMAO originates from rumen TMA which is oxidised by the liver. TMAO is mainly present in urine while microbes, providing the enzymatic conversion, are primarily contained in faeces. Hence, under usual conditions, a urine-faeces mixture will yield high TMA emission rates which could explain observed elevated ambient TMA concentrations at agricultural facilities. For TMA volatilisation, a situation with alkaline pH values is required; this occurs with urine-faeces mixtures due to bacterial urea hydrolysis into NH_3 . The formed TMA seems to be further microbially degraded to NH_3 resulting in comparably small TMA emissions from stored slurry. Derived average TMA emission rates from urine-faeces mixtures are in the order of $10 \text{ nmol m}^{-2} \text{ s}^{-1}$ which roughly agrees with current global agricultural TMA emissions assessed by scaling with NH_3 emission inventories.

Once emitted, gas phase TMA participates in nucleation with a high potential efficiency for secondary particle formation, but also undergoes condensation onto existing aerosol particles. Depending on the prevailing particle number size distribution, the corresponding atmospheric lifetime is estimated to range from 30 to 1000 s, with the first value being probable for agricultural areas. This confines the high particle formation potential of agricultural TMA to its source regions with dwindling direct impact of agricultural emissions at increasing distance. Close to agricultural sources there are relatively high NH_3 concentrations in addition to abundant amines. In the vicinity of farms, amines will be the key neutralising component in the ternary sulphuric acid-water-base nucleation and can enhance the formation of new aerosol particles by a factor of a thousand compared to aerosol formation with NH_3 under otherwise equal conditions. As the air mass is transported further away from the source, the amines are depleted and NH_3 must become the primary neutralising agent. For more direct evidence of agriculturally enhanced secondary aerosol particle formation by amines, simultaneous measurements of gas-phase amines, NH_3 , sulphuric acid, and particle number size distributions should be performed up- and downwind of animal husbandry farms.

BGD

11, 6519–6553, 2014

Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

⏪

⏩

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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- 30

Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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**Trimethylamine
emissions in animal
husbandry**

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Jordan, A., Haidacher, S., Hanel, G., Hartungen, E., Märk, L., Seehauser, H., Schottkowsky, R., Sulzer, P., and Märk, T. D.: A high resolution and high sensitivity proton-transfer-reaction time-of-flight mass spectrometer (PTR-TOF-MS), *Int. J. Mass Spectrom.*, 286, 122–128, doi:10.1016/j.ijms.2009.07.005, 2009. 6524

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**Trimethylamine
emissions in animal
husbandry**

J. Sintermann et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

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**Trimethylamine
emissions in animal
husbandry**

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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**Trimethylamine
emissions in animal
husbandry**

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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**Trimethylamine
emissions in animal
husbandry**J. Sintermann et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[◀](#)[▶](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

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BGD

11, 6519–6553, 2014

**Trimethylamine
emissions in animal
husbandry**

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



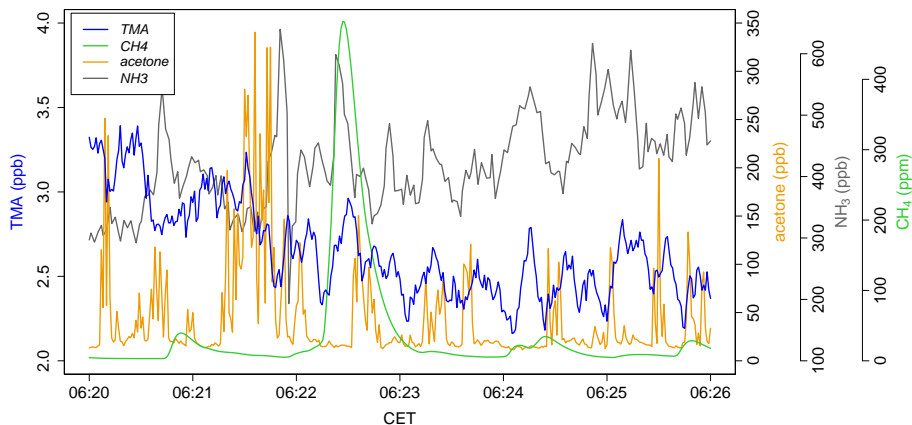


Fig. 1. High resolution time series of acetone, NH_3 , and TMA (8s moving average) during measurements in front of the mouth of a ruminating cow at the outdoor stable yard on 28 July 2011; the average TMA : NH_3 ratio for this period was 0.65 %.

Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

⏪

⏩

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



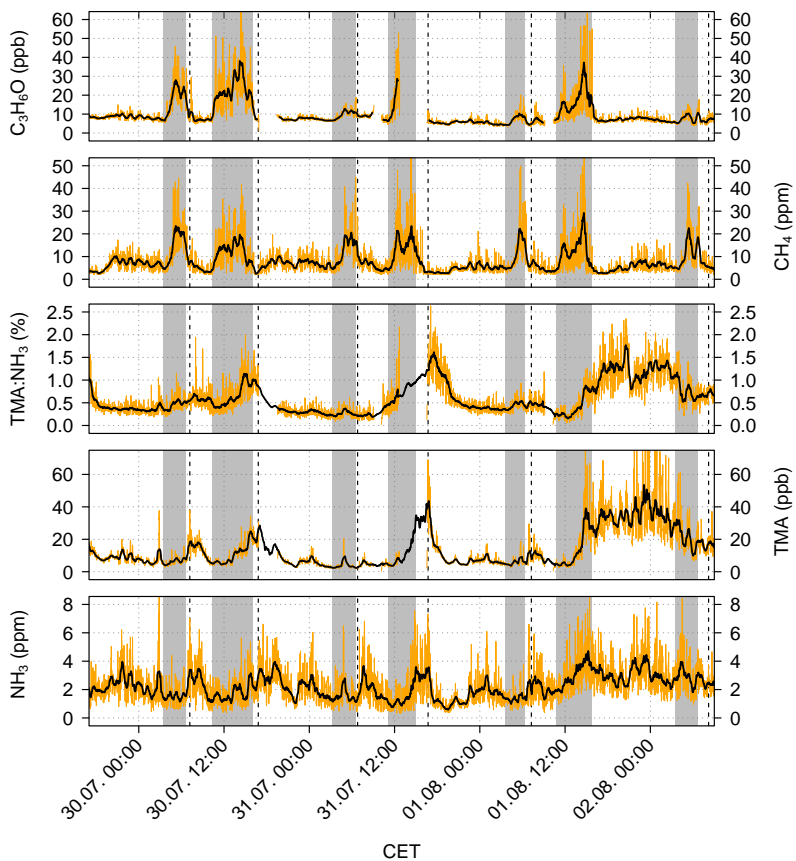


Fig. 2. One-minute (orange) and half-hour moving average (black) trace gas concentrations measured in the stable outdoor yard; the presence of the animals is indicated with grey bars; floor scraping is represented by vertical dashed lines.

Trimethylamine emissions in animal husbandry

J. Sintermann et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



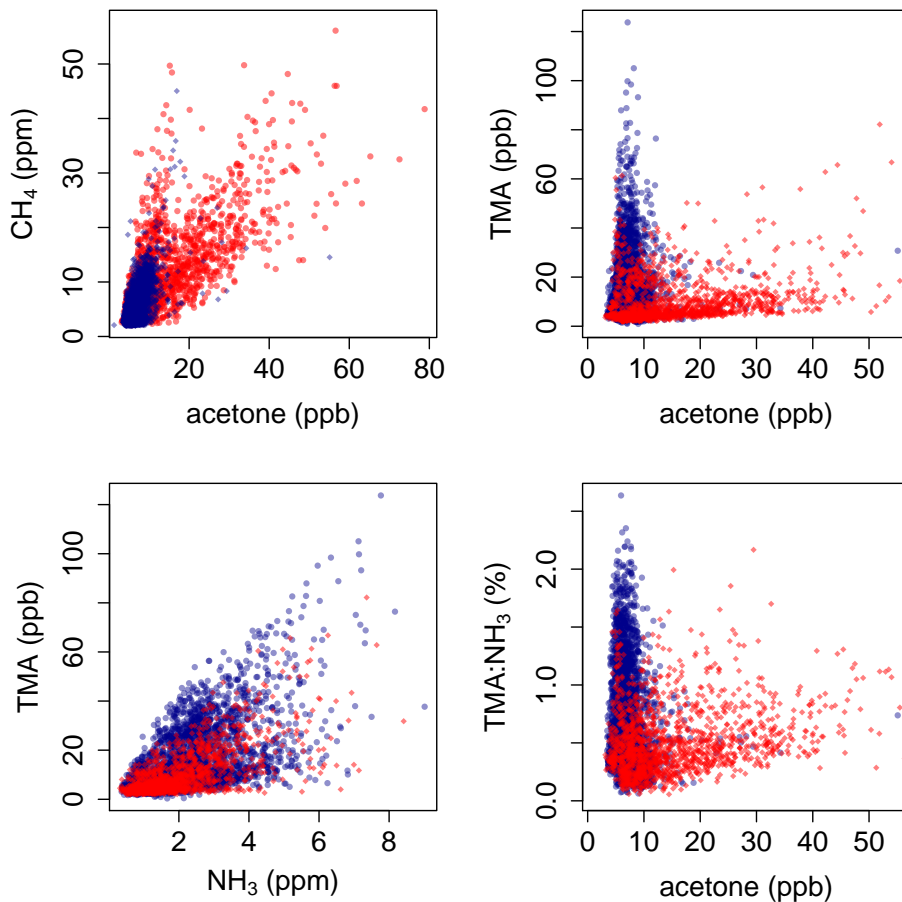


Fig. 3. Scatterplots of CH₄/TMA/TMA : NH₃ vs. acetone and NH₃ (1 min values) for the period 29 July 17:00 to 2 August 9:00 (also shown in Fig. 2); red circles = measurements during presence of cows, blue diamonds = in absence of cows.

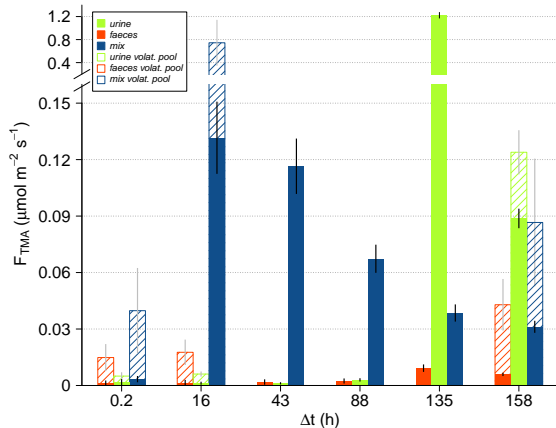
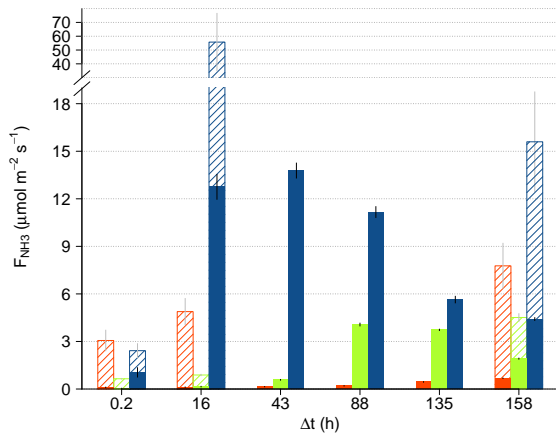


Fig. 4. Dynamic chamber NH₃ and TMA emission rates from samples of urine, faeces, and their mixture, as well as emission rates from parallel samples with pH raised > 12 (“volatile emission pool”).

Trimethylamine emissions in animal husbandry

J. Sintermann et al.

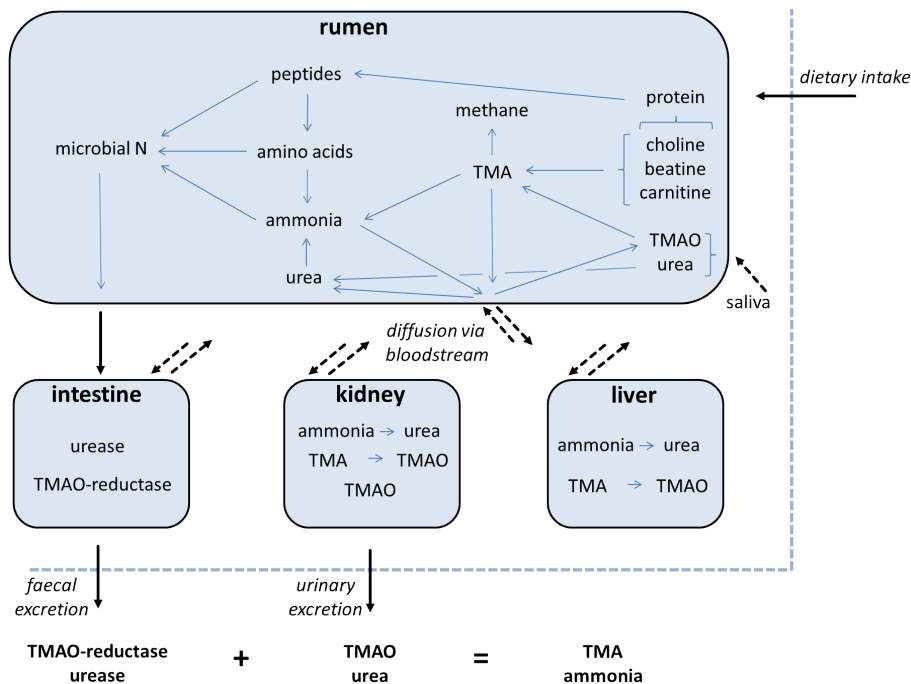


Fig. 5. Main pathways of TMA and NH₃ production by the ruminant system (with the focus on TMA).

[Title Page](#)

[Abstract](#) [Introduction](#)
[Conclusions](#) [References](#)
[Tables](#) [Figures](#)

⏪ ⏩
◀ ▶

[Back](#) [Close](#)

[Full Screen / Esc](#)

[Printer-friendly Version](#)

[Interactive Discussion](#)

