

Characterization of Indoor Atmospheric Nitrogenous Chemicals in Poultry Farms

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Environmental Significance

Airborne nitrogenous chemicals (ANCs) are common pollutants in the poultry industry and threaten animals' welfare and producers' occupational health. It is important to characterize these chemicals in poultry farms, given that many of these ANCs are precursors of persisting ammonia pollution issues. Our research has discovered many ANCs in the farm and highlighted their partitioning between air, particle, and litter phases. Additionally, we reported the diurnal trend of uric acid - one of the major ANCs in the air. Our work has explored the

origin of ammonia pollution in a poultry farm, which can apply to other livestock facilities. At the same time, we have emphasized the implication of indoor air pollution on animals' welfare and producers' occupational health.

Abstract

Indoor air pollution is seen in poultry and many other livestock facilities. Small airborne nitrogenous chemicals (ANCs), such as ammonia and small amines, are common air pollutants in poultry farms. Elevated ANC concentration in poultry farms can significantly worsen the indoor air quality (IAQ) of the farm, which will affect animal productivity, animal welfare, and occupational health of producers. Recent studies have identified ammonia and small volatile organic pollutants in the farm. On the other hand, characterization of large ANCs, such as uric acid (UA) and large amines have rarely been reported, despite their being proposed as the major source of biological nitrogen waste. Our goal is to project a novel insight into nitrogen cycles in poultry farms. This project includes on-site time-resolved collections of ANCs using a particle-into-liquid-sampler (PILS), followed by chemical characterization by liquid chromatography-mass spectrometry (LC-MS) with a novel derivatization method. Over quantitative assessment of ANCs in the poultry farm, we discovered UA and suspended particles are correlated with changing animal behaviors. Phase partitioning of UA, ammoniacal species, and large amines was discovered among air, particle, and litter materials. The discovery of these indoor pollutants can be associated with the formation of dust particles and ammonia, and the results can benefit the poultry industry in solving persisting IAQ problems.

Introduction

Indoor Air Quality (IAQ) has gathered increasing attention from the public as it is becoming more relevant to public health and well-being.¹ Existing studies have focused on residential homes, which contain a complex mixture of emissions from humans,^{2,3} animal or biological

activities, and chemical processes.^{4,5} On the other hand, the workplace IAQ is as important as residential homes, as numerous contemporary occupations take place indoors.⁶ Governmental agencies have established workplace IAQ protocols to protect workers;⁷ however, the variation of different industries and occupations may cause unique air pollution, making it challenging to establish occupationally tailored standards. Especially for industries that have major sources of air pollution, workers can receive prolonged exposure to concentrations that exceed thresholds of exposure, threatening their productivity and occupational health.^{8,9}

The Department of Labor of the USA has identified common biological, chemical, and particulate pollutants in indoor commercial and institutional buildings; however, only general administrative and control guidance was provided.¹⁰ Managing workplace IAQ remains challenging due to the diversity of indoor environments, general benchmarks are not sufficient to resolve the needs of specific industries. For instance, elevated air pollution in office-like environments not only causes discomfort but also contributes to cardiovascular or respiratory diseases.^{11–14} Industries that usually involve indoor activities, such as the exhibition,^{15,16} entertainment,^{17,18} and beauty industries,^{19,20} are facing a problem with high volatile organic compounds (VOCs) exposure. Similarly, the poultry industry, which supplies eggs and chicken products for consumption, faces a challenging air pollution problem in indoor poultry facilities. Elevated levels of air pollutants are found in the farm, including carbon dioxide,²¹ ammonia (NH_3), particulates (PM_{10} , $\text{PM}_{2.5}$),²² and VOCs.²³ Concentrations of these pollutants have been a concern and are often associated with the productivity and welfare of chickens,^{24–26} but systematic study remains rare. Despite that air ventilation has always been a costly burden for producers,²⁷ many commercial poultry farms still suffer from heavily polluted air.^{28,29}

The major source of air pollutants in a poultry farm comes from chicken manure. Due to its low density, manure can be easily suspended by birds' activities.³⁰ Airborne nitrogenous chemicals (ANCs) are abundant in livestock facilities and are usually odorous or toxic.³¹ Small ANCs, such as methylamines, ethylamine, and NH_3 are among commonly identified

species, as they are highly volatile, concentrated, and odorous.^{29,32,33} NH_3 is a widely employed benchmark compound used as an indicator of IAQ in poultry facilities and in the guidance for animal care.³⁴ High concentration of NH_3 is found responsible for reduced body weight gain, calorie conversion, immune system in chickens.³⁵ However, the current literature and NH_3 control strategies often neglect the fact that the majority of NH_3 is not directly emitted from the birds. Instead, it is chemically produced via enzyme-assisted microbial decomposition of uric acid (UA).^{36,37} Understanding chemical processes occurring in indoor poultry facilities plays a crucial role in managing ongoing NH_3 pollution, as well as mitigating risks associated with farmers' health and animal welfare.

UA is a common biogenic nitrogenous chemical in animal and plant bodies,^{38,39} which is also rich in agricultural facilities and wastes. However, very few studies have confirmed its presence in the atmosphere, despite UA is predominantly excreted via manure and is responsible for major nitrogen emissions.^{34,40,41} Although non-volatile, UA can be exposed to producers and livestock via inhalation of dust particles.⁴² An elevated concentration of UA-rich particles can affect the dynamics of nitrogen cycles in the farm⁴³ and may trigger acute or chronic health problems in farmers and livestock. More importantly, UA gives rise to total ammoniacal nitrogen, which represents NH_3 gas and ammonium (NH_4^+) salts.⁴⁴ Due to enzyme-assisted microbial decomposition, UA can be converted to urea over a chain reaction, which eventually gives rise to CO_2 and NH_3 through hydrolysis.^{45,46} Studies have shown that UA contributes to over half of the total nitrogenous emission in poultry farms, as well as unclassified contributions from other nitrogenous species.^{47,48}

Other than UA, we propose that there would be many other organic ANC's in poultry facilities due to the nitrogen-rich environment and that these ANC's can also act as the precursors of inhalable small ANC's.⁴⁹ In the past, these compounds have been considered insignificant^{23,50,51} due to the limitations of analytical techniques.⁵² However, certain amines, such as cadaverine (CAD), putrescine (PUT), and guanine (GUA), are relevant to the meat and produce industries, as they are associated with the decay of proteins.^{53,54} Existing re-

view studies have also discussed them as part of the total VOCs in livestock facilities.⁵⁵ Quantitative analyses of these ANC_s in poultry facilities are rarely performed, and little is known about the behaviors and distributions of organic ANC_s. The driving force leading to the change of indoor ANC_s is yet to be reported by existing literature.

The objective of this study is to project novel insights into nitrogen cycles in indoor poultry farms. Firstly, this study will demonstrate a new method for time-resolved collection and quantitation of ANC_s. Secondly, the distribution of ANC_s in different phases (air, particles, and litter) will also be evaluated. Thirdly, using the UA as an example, the correlation between ANC_s and IAQ parameters will be addressed with the aid of aerosol monitoring instruments. By reporting results obtained from a campaign in local commercial poultry farms, this work will assess the origin of IAQ issues in poultry farms, as well as provide a comprehensive evaluation of airborne ANC_s, which can play a key role in terms of air quality, occupational health, agricultural productivity, and animal welfare in the livestock facilities.

Material and Methods

Chemicals and Materials

MiliQ water used in this study was made by a Thermo-Fisher Scientific Barnstead™ E-Pure™ Ultrapure Water Purification System. HPLC grade acetonitrile, boric acid (>99.5%), formic acid (98-100%), ammonium hydroxide (NH₄OH) solution (28% NH₃ in water), uric acid (>99%), guanine (98%), allantoin (>98%), urea (99.0-100.5%), p-toluenesulfonyl chloride (TsCl) (>99%) were purchased from Sigma-Aldrich. Sodium hydroxide pellets were purchased from Fisher Chemical.

Two solvents were prepared for sample collection and extraction. A 0.25 M sodium formate buffer was prepared by dissolving boric acid solids in MiliQ water, with its pH then adjusted to 9.0 by NaOH. A 0.1% formic acid solution (pH = 2.7) was prepared by dissolving

pure formic acid in MiliQ water. These two solutions are herein referred to as the basic buffer and the acidic buffer, respectively, to be used in subsequent steps.

Instrumentation

Aerosol samples were collected by a particle-into-liquid sampler (PILS) (Model 4001), and an auto collector manufactured by Brechtel Inc. The aerosol monitoring instrument was an optical particle counter (OPC) (Model 11-C) manufactured by Grimm Inc. The primary chemical analysis instrument was the Thermo-Fisher Accela HPLC system and Thermo-Fisher LTQ-XL mass spectrometer, operated in ESI-positive mode. The column for LC separation was a Phenomex Luna Omega polar C-18 column, dimension 150 mm \times 2.1 mm \times 3 μ m. An Orbitrap high-resolution mass spectrometer (Thermo-Fisher Exactive Orbitrap) was also used for the determination of exact mass.

Sample Collection and Treatment

Figure 1 is a schematic for approaches taken to measure ANCs in indoor poultry facilities. Functionality tests of all instruments involved in Figure 1 were carried out preliminary in the Poultry Research Center (PRC) at the University of Alberta. The farm had floor pen housings for a small flock of 70 birds. Commercial farm samples involved in this study were collected on a farm located near Camrose, Alberta, Canada (Figure S1). The farm was a completely indoor, free-run, organic table egg farm. The barn we sampled was home to 8000 birds at approximately 60 to 70 weeks of age. In the commercial farm, on-site instrument testing and trial sample collection were performed between November 2022 and March 2023. Results shown in this study were collected in April 2023. During the winter season, Air ventilation in the barn was usually minimized to combat cold outdoor air. Lighting in the barn is governed by incandescent light bulbs which are covered by red plastic covers. According to farmers, red light can reduce the anxiety of chickens. Collected field samples were analyzed on the same day in the lab. A sketch of the barn can be found in Figure S2

in the SI.

Gas samples were collected using a homemade impinger driven by a diaphragm pump, the gas flow rate was controlled by an Allicat Mass Flow controller at 0.7L/min. At the upstream pump inlet, a 0.2 μ m Watman filter was installed to remove incoming particles. The acidic buffer described above was used to maximize the collection efficiency of NH₃ gas.

Real-time particle profiles were collected by the OPC. Time-resolved chemical profiles were collected by the PILS and its corresponding auto collector. An activated charcoal gas denuder was installed upstream of the instrument inlet to remove gas phase species. Particle samples were collected using the basic buffer. Through preliminary trials, we discovered that a better solubility of most of the ANCs was achieved using the basic buffer; however, it may compromise the collecting efficiency of NH₄⁺. The solvent was driven by a peristaltic pump at a rate of 0.3 mL/min, the resulting solution was injected directly into a 1.8-mL autosampler vial every 2 min. There were also occasions when these samples were collected in a 12-mL vial every 20 minutes.

Chicken litter samples were collected by hand-picking chicken litter from five random locations inside the farm. The five samples were pooled by shaking in a 20-mL glass vial upon collecting. A portion of the litter was weighed and extracted by the basic buffer during the sample treatment in the lab. We noted that the litter sample was a mixture of bedding materials and chicken manure (Figure S3 in the SI), the process would not efficiently extract the bedding material as it is mostly wood pellets. Hence, we assume that all chemicals obtained in the extracted solution come from chicken manure.

Derivatization and Chemical Analysis

Derivatization was carried out directly inside the autosampler vial. The derivatization method was developed according to Rudnicka et al.⁵⁶ All the samples were mixed with 0.052 M TsCl solution in acetonitrile and prepared in the basic buffer. The derivatization takes at least two hours in a 50 °C water bath. All derived samples were analyzed via LC-

ESI-MS, and the details and settings regarding this instrument are listed in Section S2 in the SI.

TsCl is known to be selective towards R-NH and R-OH functional groups, forming sulfonamides and sulfonates via nucleophilic tosylation.^{57,58} We did not experience interferences from any sulfonates, since our basic condition would favor their detosylation reaction, while sulfonamides remained stable.⁵⁹⁻⁶¹ Thus sulfonates were excluded from our chromatography, making our method very selective towards sulfonamides. All detected TsCl-derived species were firstly isolated via background subtraction in the high-resolution orbitrap MS, with a proposed molecular formula. Then their identities were further confirmed by comparing them with the derivatives of commercially available standards.

We have selectively quantified UA and ammoniacal compounds in our sample with external standards. UA calibration was done in the basic buffer, and a five-point calibration ranging from 0 to 400 μ M was completed via serial dilution, with the R^2 value greater than 0.9990. ammoniacal calibration was done by derivatizing diluted NH_4OH solution and constructing a six-point calibration curve ranging from 0 to 20 mM via serial dilution, with an R^2 value greater than 0.9990. These calibration curves were reviewed monthly by measuring a known concentration of standard solutions.

Quality Control

Although the PILS is designed to collect particles, some gas phase chemicals can be collected even with a gas denuder installed. To identify the bias from break-through gaseous chemicals, we have performed a joint calibration between the PILS, an NH_3 analyzer (Model 17i, ThermoFisher), and a scanning mobility particle sizer (SMPS, TSI inc.) which consist of a diffusion mobility analyzer (Model 3080) and a condensation particle counter (Model 3775). During this experiment, we measured a stable source of ammonia and ammonium bisulfate particles in replicates. At the same time, the gas removal efficiency of the PILS gas denuder was verified. This experiment also has the standard error of PILS determined (6.7%), which

194 serves as error bars in the following quantitation in this study. Detailed information about
195 this experiment can be found in Section S3 in the SI.

196 PILS collected only 6% of gas-phase NH_3 without the denuder, as compared to the NH_3
197 analyzer. With the denuder mounted, the collected NH_3 concentration was below the limit
198 of detection (LOD, 20 ppb, gas phase equivalent). We have also compared the collection
199 of dimethylamine (DMA), which has a lower LOD (0.25 ppb), and discovered that only
200 0.3% of the gas was collected with the PILS denuder mounted. Hence, we considered that
201 breakthroughs of gaseous ANCs during our particle collection were not significant.

202 On the other hand, the PILS has demonstrated its capability to collect fine particles.
203 While the literature has shown that the collection efficiency between 30 nm and 10 μm is
204 greater than 97%,⁶² a portion of salt particles generated in this experiment was less than
205 30 nm, which was outside of the working range of PILS. We noted that the NH_3 analyzer
206 is also capable of measuring ammonium salts, as its internal catalyst can also convert NH_4^+
207 into NO/NO_2 . As a result, the PILS has only 61% NH_4^+ collection efficiency compared to
208 the NH_3 analyzer in this specific intercomparison. Higher efficiency can be achieved when
209 the size of particles is larger according to the working fundamentals of the PILS.^{62,63} As will
210 be addressed later, most particles in the commercial poultry facility were larger than 30 nm,
211 therefore the collection efficiency of NH_4^+ particles would be higher than our intercomparison
212 experiment.

213 A recovery test of UA was performed by spiking a UA standard solution with a known
214 concentration into a bedding material extract. This test aimed to examine the efficiency
215 of derivatization. Two sets of samples were prepared for this test: one had five replicates
216 of non-spiked bedding extract, and the other had five replicates of UA-spiked extract. The
217 recovery value obtained was $72.7\% \pm 11.5\%$. Additionally, we have also performed a stability
218 test of the derived sample to account for the sequence queueing time on the autosampler.
219 This was done by repetitively analyzing the same derived standard compound over time. The
220 results of this control experiment are shown in Section S3, and the corresponding correction

to the sample degradation has been applied to our time-resolved data series.

Results and Discussion

Identification of ANCs in Different Phases

With the aid of high-resolution mass spectrometry (resolution greater than 50,000), our untargeted analysis has detected 15 ANCs, with 10 of them identified, and the other five remain unidentified. Section S4 in the SI has summarized the proposed identities of these ANCs. TsCl derivatives are identified by their unique isotopic profiles. The presence of sulfur in the TsCl-derivative generates a unique peak profile at the mass of $[M+2]^+$ position. Due to the mass of $[^{34}\text{S}-^{32}\text{S}]$ being smaller than $2\times[^{13}\text{C}-^{12}\text{C}]$, the $[M+2]^+$ position will have a split peak, with the lighter peak refers to $[M(^{34}\text{S})]^+$, and the heavier peak refers to $[M(^{13}\text{C}_2)]^+$ or $[M(^{14}\text{C})]^+$. Additionally, as the natural abundance of ^{34}S is higher than ^{14}C or ^{13}C , the former peak will be more intense.⁶⁴ By assuming the only source of sulfur is TsCl in the chromatograph, TsCl derivatives are identified. Details about this identification method can be found in Section S4. We further confirmed a certain number of ANCs by referring to commercial standards, those are NH_3 , dimethylamine (DMA), GUA, UA, PUT, and CAD. We have also identified a trace peak of urea and allantoin (ALA), which are proven intermediates in the UA decomposition mechanism.^{45,46} Although it has not been detected, trimethylamine, a tertiary amine, is found abundant in livestock facilities.^{31,32,65,66} It is absent in our sample because the TsCl cannot react with any tertiary amines due to the lack of active amino groups.

With these ANCs identified, we evaluated the phase distribution of them among three phases (gas, particle, and litter) via targeted analysis. We observed that the presence of ANCs is different among the three phases, with the litter phase having the greatest variety of compounds, while not all ANCs are present in the gas or particle phase. We propose that this observation is likely due to the result of partitioning of these compounds. According

to Figure 2A, highly volatile ANC_s were found in the gas phase, while most of the other compounds are absent, likely due to lack of volatility, leading to their gaseous concentrations below LOD. To support this argument, we constructed a model that predicts the fraction of a chemical in the air phase under two different particle concentrations in Figure 2B. The details about the model can be found in SI Section S5. When the tested species are allowed to reach equilibria between the gas phase and particle phase, NH₃ and DMA would exclusively be present in the gas phase. A fraction of CAD and PUT would also enter the gas phase, however, their concentration in our sample is not detectable. These trends were consistent between the two particle concentrations (5 and 20 mg/m³) chosen to represent typical particle concentrations in the indoor poultry facility.

In the particle phase, the volatile DMA is absent, while ANC_s with less volatilities are present. UA has the most intense peak, which has suppressed responses from others. Four ANC_s are detected in the particle sample, suggesting that these compounds are major forms of nitrogenous compounds in suspended dust and can be exposed to producers and chickens via inhalation. The model result (Figure 2B) supported this observation by showing that minimal DMA is expected to be in the particle phase, regardless of the concentrations of particles. The only discrepancy between the model and observation is NH₃, which the model predicts that it is predominantly present in the gas phase. However, we note that the K_{oa} values used in the model were simulated based on the neutral forms of the compounds (i.e., NH₃). It is known that acid-base equilibria can significantly affect the partitioning of compounds like NH₃. The particle-phase ammoniacal signal is likely attributed to ammonium salts in the dust. In comparison with gas and dust samples, chicken litter contains the greatest variety of ANC_s. The MS signal of UA in this phase is very high, exemplifying that the source of UA is the litter. Volatile ANC_s are also found in the litter, such as the DMA, PUT, and CAD. They are likely dissolved in the water contained by the litter, dissolved in the litter's organic matter, or trapped within the air space within litter particles. Urea and ALA are exclusively detected in the litter. The detection of these two compounds indicates

that the litter is the reaction site of uric acid decomposition. In other words, the litter serves as a persistent source and reservoir of NH_3 in the barn.

Distribution of Nitrogenous Species in Each Phase

In the previous section, ANC_s have shown a distribution profile among three phases. Here, we quantified those ANC_s to add further details to the mentioned distribution. Gas-phase ANC concentrations were calculated based on the total volume of air sampled by the impinge. ANC percentages in the particle phase were calculated based on the amount quantified by LC-MS calibration and then related to the total particle mass monitored by OPC. The litter phase was calculated based on the dry mass of the litter. For litter samples, we used dry mass to calculate the mass percentage, while the extraction of litter was performed with fresh litter, this is considering that the loss of volatile amine during drying is inevitable. Anion molar percentages were shown in pie charts, and their molarity was determined by the US EPA colorimetric method⁶⁷ carried out by the Natural Resource Analytical Laboratory (NRSL) at the University of Alberta. We did not carry out cation analysis according to the scope of the study as well as the limited instrument availability. Therefore, we assumed all anions were counter ions to the pool of ammonium that we detected via TsCl derivatization. To discover the molar distribution of different salts, charge ratios between ions are considered. For instance, ammonium phosphate has a higher molarity percentage than its mass percentage, since the ratio between two ions is 1 to 3. The calculated molar distribution of anions is shown in pie charts on both panels. This result can only serve as a preliminary estimation, as the presence of multiple-charged cations (Such as Ca^{2+} , Mg^{2+} , and Al^{3+}) would affect the molar distribution of anions.

According to the anion molar distribution of ammonium salts between the particle and the litter, their distribution has shown some correlations. Note that we have only quantified five anions, and other anions, such as bisulfates or biphosphates, cannot be detected with our method. Hence our reported value might be underestimated and serve as a preliminary

quantitation. Regarding the pie chart of Figure 3A, phosphate in suspended particles has shared the largest molar fraction, followed by chloride, sulfate, and nitrate. In comparison with the particle phase, the pie chart of Figure 3B describes the anion distribution in the litter. Here, phosphate has a dominant molar fraction over the other salts, followed by nitrate and sulfate. As a result, the chicken litter contains a very high concentration of phosphate, due to direct excretion by chickens via manure,⁶⁸ raising the phosphate content in the litter. The high fraction of phosphates in particles is likely coming from the suspension of litter, either due to air circulation or the motion of animals. Interestingly, chloride is the second-most abundant anion in particles, it does not share a similar fraction in litter, suggesting alternative sources of chlorides other than chicken manure. The analysis of anions in the particle and litter indicated a significant inhalable exposure to elevated phosphate salts by animals and workers, which may lead to phosphate toxicity problems.⁶⁹

According to Figure 3A, the gas phase contains a ppm-level of ammonia and DMA, while this concentration can be variable according to the ventilation: weaker ventilation in cold weather may concentrate gaseous ANCs. In the particle phase, we obtained mass fractions of each ANC based on the total particle mass (TPM), which was obtained by the OPC with an hourly average of 19 mg/m³. Ammonium salts occupied more than 18% of the TPM, followed by 1.43% of UA. However, this concentration was 1.29% in the litter sample (Figure 3B), which is more than 10 times less than that in particles. Therefore, ammonium salts in dust particles did not necessarily come from litter. We propose it is due to the high CO₂ concentration in the farm air, which has acidified suspended particles⁷⁰ and leads to the repartitioning of ammonia gas into particles. However, this proposal needs further research to be confirmed. In comparison, the litter sample had a higher mass concentration of UA (2.64%) than that in particles, suggesting that the only source of airborne UA is the resuspension of the litter. As a result, dust particles in a poultry farm not only come from litter bedding but also a product of the repartitioning of indoor ammonia. Exposure to these ammonium-rich particles may lead to an increasing concentration of acids in the respiratory

system.⁷¹ The consequence of this mechanism includes respiratory acidosis, which may lead to the acidification of blood pH.⁷²

The dominating concentration of UA in the chicken litter (Figure 3B) suggests a direct excretion from birds. On the other hand, a relatively lower ammonium concentration suggests that it is a secondary product from UA decomposition.^{45,46} Thus, the atmospheric concentrations of NH_3 are likely dependent on the fraction of UA in the litter. CAD and DMA have higher fractions in the litter than other phases. 0.32% of the litter is occupied by CAD, making it the third-most dominant nitrogenous chemical. This indicates CAD may be directly excreted by birds, rather than being a secondary compound. DMA has the lowest mass ratio among all ANCs, which is 0.011%.

The comparison of nitrogenous species with other literature is made in Table 1. The indoor environment of a commercial poultry farm is very dynamic, and the concentration of pollutants is often governed by the activity of chickens, ventilation, and farm infrastructures. Hence it is very challenging to find a representative concentration even for the most commonly measured air pollutants (i.e., NH_3 and PM). As shown in Table 1, Our gas-phase NH_3 is within the range of literature reported value, and lower than the concentration stated by regulations (10 ppm).^{73,74} In the particle phase, our ammonium measurement is within the same order of magnitude as the reported value, but more than 3 times higher. It is likely due to the different farm conditions between research, as ours only contains one farm. Additionally, our measurement has included active periods for chickens, which caused a larger amplitude of standard deviation. Apart from ammoniacal chemicals, there is a lack of quantitative analysis on all other ANCs, such as UA, DMA, CAD, and PUT, making our study the first to report their concentrations in an indoor poultry facility.

Dust and Chemical Correlation

To explore the correlations of ANCs with other conditions, including the lighting in the farm and common IAQ parameters, we conducted a case study on April 13, 2023. This

date was selected due to several reasons. Firstly, the outdoor temperature was mild, so the ventilation rate in the farm was close to its annual median rate. Secondly, the producers planned to start removing birds from the farm on this date. This was a unique opportunity to observe how chickens' activity would directly affect the airborne compounds. Additionally, this opportunity also allowed us to study the diurnal cycle of IAQ in the farm in a relatively short sampling period, considering our instrumental capacity.

In addition to concentrations of particles and ANCs, we have also evaluated the size distribution of particles in the atmosphere, considering the PILS has a minimum size requirement of particles (30 nm) for optimal collection efficiency. According to data from the OPC, we obtained a 2-D contour plot of particle concentrations in different size bins, ranging from 0.25 μm to 32 μm . This plot can be found in Section S6 in the SI. According to the contour, we confirmed that most particles in the farm atmosphere were greater than 0.25 μm . Hence, our PILS was working in its optimal conditions and the collection efficiency was higher than the value (61%) stated previously. However, limited by instrument availability, we were unable to perform an on-site evaluation of the collection efficiency.

Figure 4A shows the time profile of UA and the TPM measured by the PILS-LCMS and the OPC, the shading of the background indicates the change in lighting conditions in the barn. The concentration of UA and TPM were plotted against each other to elucidate their correlations (Figure 4B). We differentiated our sampling period into three zones: daytime, sunset, and nighttime, and each of them represents different light conditions. Farm lights had the maximum output during the daytime (white zone) and were gradually dimmed during the sunset period (light grey zone). In the nighttime, no lights were on inside the farm (dark grey zone). During the daytime, TPM fluctuated around $3 \times 10^4 \mu\text{g}/\text{m}^3$ while the concentration of UA can be as high as $500 \mu\text{g}/\text{m}^3$. The mass percentage of UA among the TPM is about 1.5%, which agrees with the results presented in Figure 3. According to our on-site observations, most of the birds were gathering on the ground during the daytime and were in direct contact with the floor chicken litter. Motions of birds will suspend dust from

the litter bedding. Thus, an elevated concentration of both UA and TPM was observed. Fluctuations in TPM can be due to the local activities of chickens, giving rise to plumes of TPM arriving at the instrument. When there was a major event, for instance, chickens were agitated around 19:00, both TPM and UA concentration saw a significant surge that is about 5 times higher.

During the sunset period, chickens started relocating to upper “layers”, which were made of steel frames and served as the sleeping places of birds. As the steel frame could not retain a lot of litter particles, the motions of chickens cannot resuspend litter particles, leading to a reduction of both PM and UA particles. When the night arrived, chickens fell asleep within a short time and could seemingly remain asleep. The concentration of UA and TPM would remain at a low level until the next morning. However, as the producers were in the process of removing the flock from the farm, sleeping birds were awakened. Thus, a rise in UA and TPM after 19:00 was observed. The time profile of TPM exhibited multiple sharp peaks, which were not observed during the daytime. It is likely caused by localized and sporadic bird activities induced by farmers. The UA profile has shown rather a single broad peak than multi-peaks, due to the reduced PILS sampling frequency at night.

The correlation between the PILS and the OPC results ($R^2 > 0.8$) is plotted in Figure 4B. These two instruments were co-located during measurement, and within the concentration ranges of UA and TPM observed, the two instruments were in good agreement. The regression value indicates that 1) TPM is a major carrier of airborne UA – which agrees with discussions in previous sections, and 2) The fluctuating concentration of airborne UA reflects the changing chicken activities on the farm. This agreement also confirms that UA shares a relatively stable ratio in airborne particles, which again implies that airborne UA has a consistent source, e.g., the suspension of manures.

Conclusions

Our project has demonstrated a hitherto most detailed exploration of airborne nitrogenous chemicals (ANCs) inside a commercial poultry farm. Various organic and inorganic ANCs have been identified and quantified by this study, while most of them have never been evaluated in existing research. Nitrogenous species share a major proportion of chemicals in commercial poultry farms. Elevated concentrations of these chemicals can directly reduce indoor air quality. Hence putting the producers' occupational health at risk. More importantly, birds' welfare, productivity, and the cost-effectiveness of investments made to farm ventilation will also diminish.

While existing research usually focuses on small volatile compounds,^{21,23} our results demonstrated the presence of a large variety of ANCs and ammonium salts in the farm air. ANCs are key components in the nitrogen cycle in poultry farms, at the same time, serving as the precursors of ammonia. ANCs also demonstrate a variable distribution between three indoor phases. In the gas phase, ammonia and DMA were quantified, and concentrations were comparable with existing literature.^{21,32} In the particle phase, ammonium concentration was significantly higher than litter, which implies a result of the repartition of gaseous ammonia into particles. Large organic ANCs such as UA were also found in airborne particles. These organic ANCs could be inhaled directly or serve as reservoirs of NH_3 , as they can undergo microbial decomposition. Litter bedding is found to be the reservoir of many ANCs in other phases, it can also potentially be the reaction site of bacterial-assisted UA decomposition, which contributes to the majority of indoor NH_3 .

Our time-resolved measurements have provided explicit and novel relationships between animal activity, total suspended particles, and individual inhalable chemicals. This observation implies that 1) a significant difference between day and night TPM and ANC concentration was observed, 2) spikes of both TPM and ANC corresponded to events that caused intense animal activity, 3) the strong agreement between the TPM and the ANC time profiles were detected. Prolonged exposure to airborne ANCs and dust particles by

chickens will not only threaten their wellness but may also compromise the effectiveness of investments. Events that are causing acute rises in airborne ANC_s can also put farmers' health at risk when proper personal protection equipment is absent.

Overall, our study has provided new insights into air pollutants that can be associated with the formation of NH₃ gas. According to discoveries made by this work, resolving indoor air pollution in poultry housings may benefit from taking a different approach. First, controlling NH₃ formation in poultry facilities requires a better picture of the entire nitrogen cycle. As demonstrated in this work, many ANC_s are involved in the nitrogen pool, likely making a variable degree of contribution to NH₃ production. Thus, the removal of ANC precursors in the environment would be beneficial, and future studies should explore technologies that can make this possible. Second, our study, for the first time, demonstrated the importance of chemical partitioning of ANC_s inside the farm. In other words, pollutants can be distributed unevenly among the gas, particle, and surface phases. A better understanding of this distribution may lead to new strategies of ventilation and waste treatment that can remove specific pollutants in a more targeted manner.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest Statement

No conflict of interest was declared.

Author Contribution Statement

Xinyang Guo: Led the project, Identified all carbonyl compounds in the sample, built up experimental procedures, processed all data, and wrote the manuscript.

Rowshon Afroz: Helped to dispatch instruments on-site.

Shuang Wu: Constructed model prediction, and wrote model-related details in the SI.

Kimberly Wong: Helped construct calibration curves.

Joey Saharchuk: Involved in the intercomparison of instrument.

Hans Osthoff: Involved in the intercomparison of instrument.

Ran Zhao: The PI, oversaw the entire project with advice and proofread the manuscript.

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Other Statements

Ethics approval and patient consent statements do not apply to the study. This study does not contain any reproduced materials from other sources.

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Tables

Table 1: Comparison of nitrogenous species with other literature

| Chemicals | Gas | | Particle | | Litter | |
|-----------------------------|---------------------------------|-----------|---------------------------------|--------------------------|--------------------------|-----------|
| | Literature | This work | Literature | This work | Literature | This work |
| $\text{NH}_3/\text{NH}_4^+$ | $6.55 \pm 2.2 \text{ ppm}^{21}$ | 5.40ppm | $5.45\% \pm 1.53\%^{75}$ | $18.41\% \pm 7.76\%$ | $0.78\% \pm 0.92\%^{76}$ | 1.29% |
| DMA | $<0.22 \text{ mg/m}^3^{32}$ | 0.047ppm | N/A | Below LOD | N/A | 0.011% |
| UA | N/A | Below LOD | N/A | 1.43% | $2.6\%-3.0\%^{77}$ | 2.64% |
| Total Particle | N/A | N/A | 0.168-9.61 mg/m^3^{29} | 7.2-36.8 mg/m^3 | N/A | N/A |

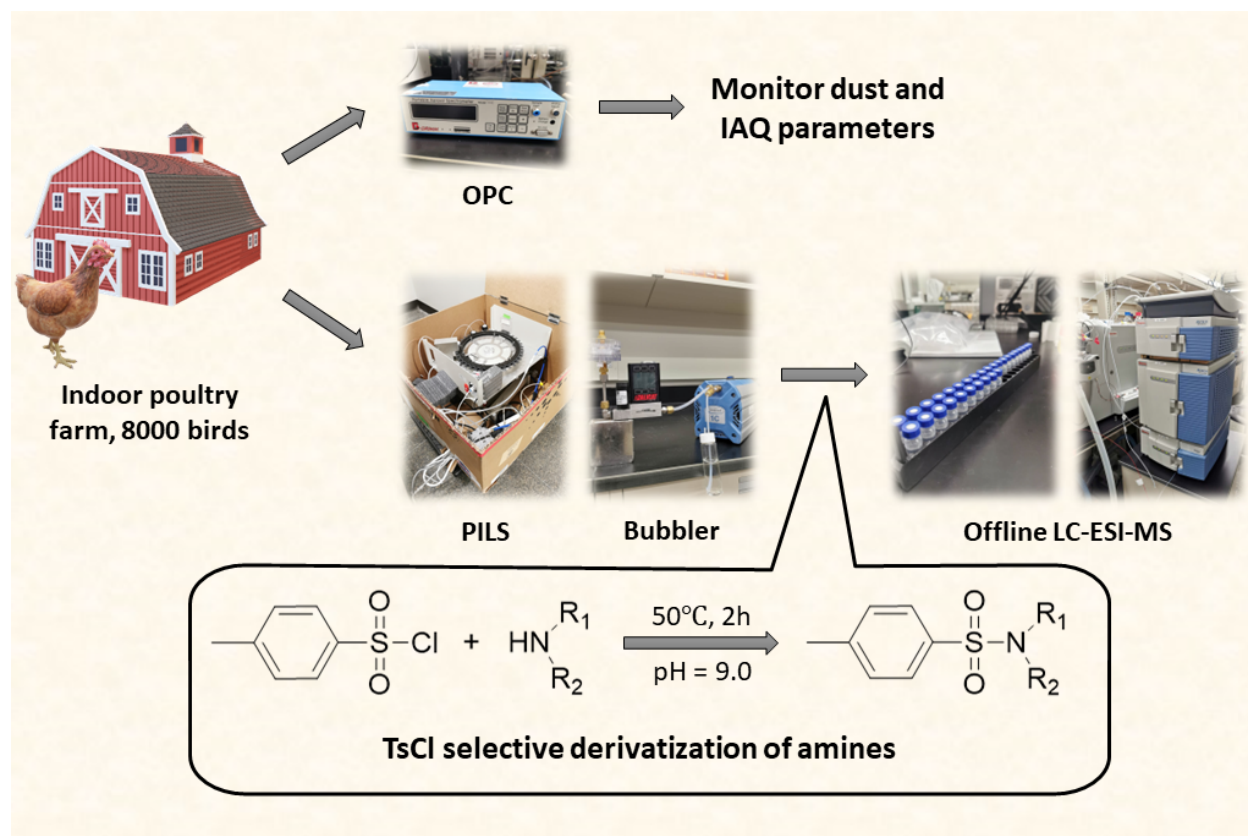


Figure 1: Layout of sample collection, derivatization, and analysis.

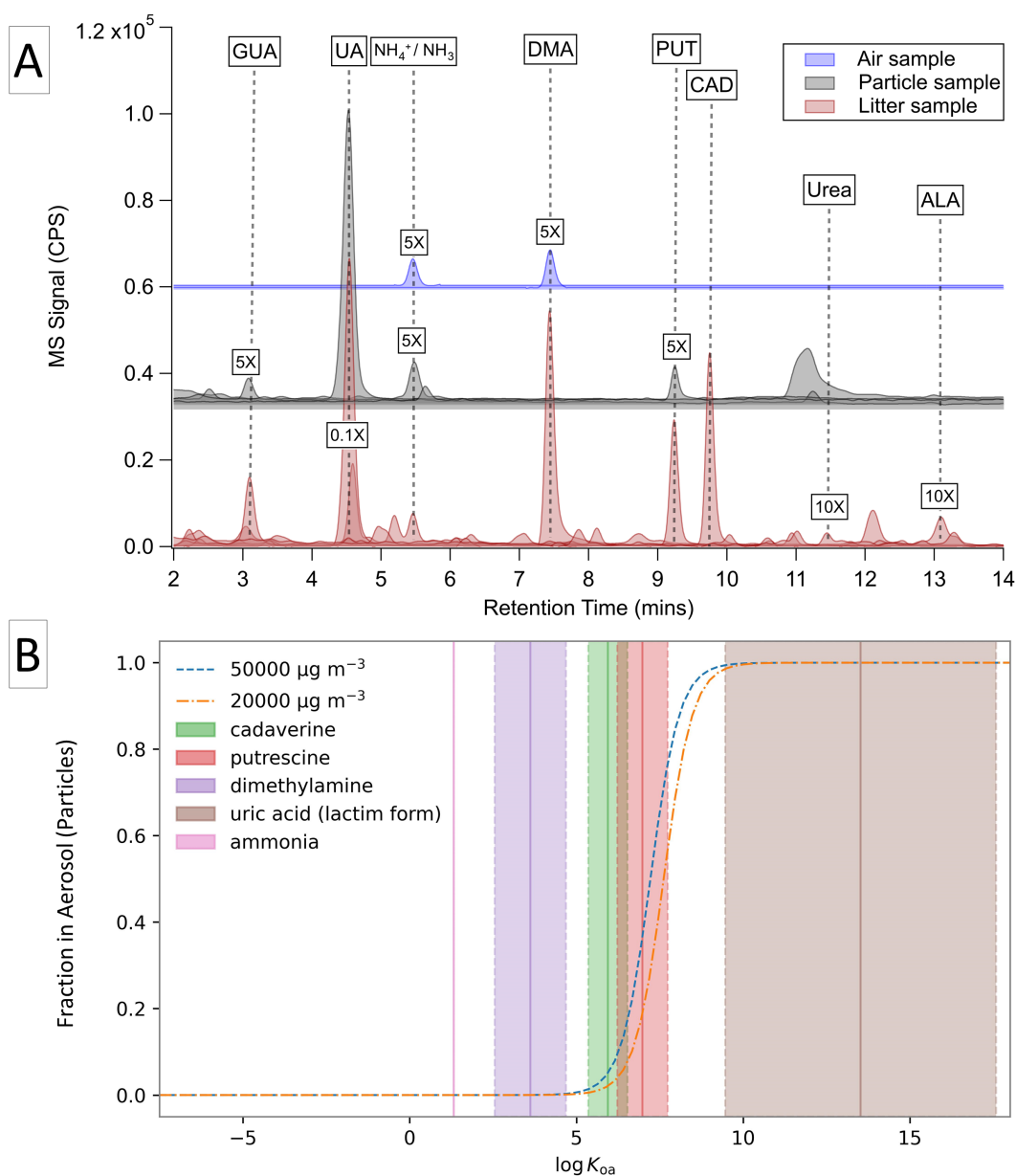


Figure 2: Identification of ANCs in air, particle, and litter phases, A) Extracted ion chromatogram of identified ANCs, certain peaks are scaled for better visualization; B) Aerosol-air equilibria of target compounds. The calculated fraction in aerosol under two selected concentrations of aerosol was a function of $\log K_{oa}$. Shaded regions represent the predicted range of $\log K_{oa}$ values for the compounds, while the solid lines in the center represent the predicted $\log K_{oa}$ values.

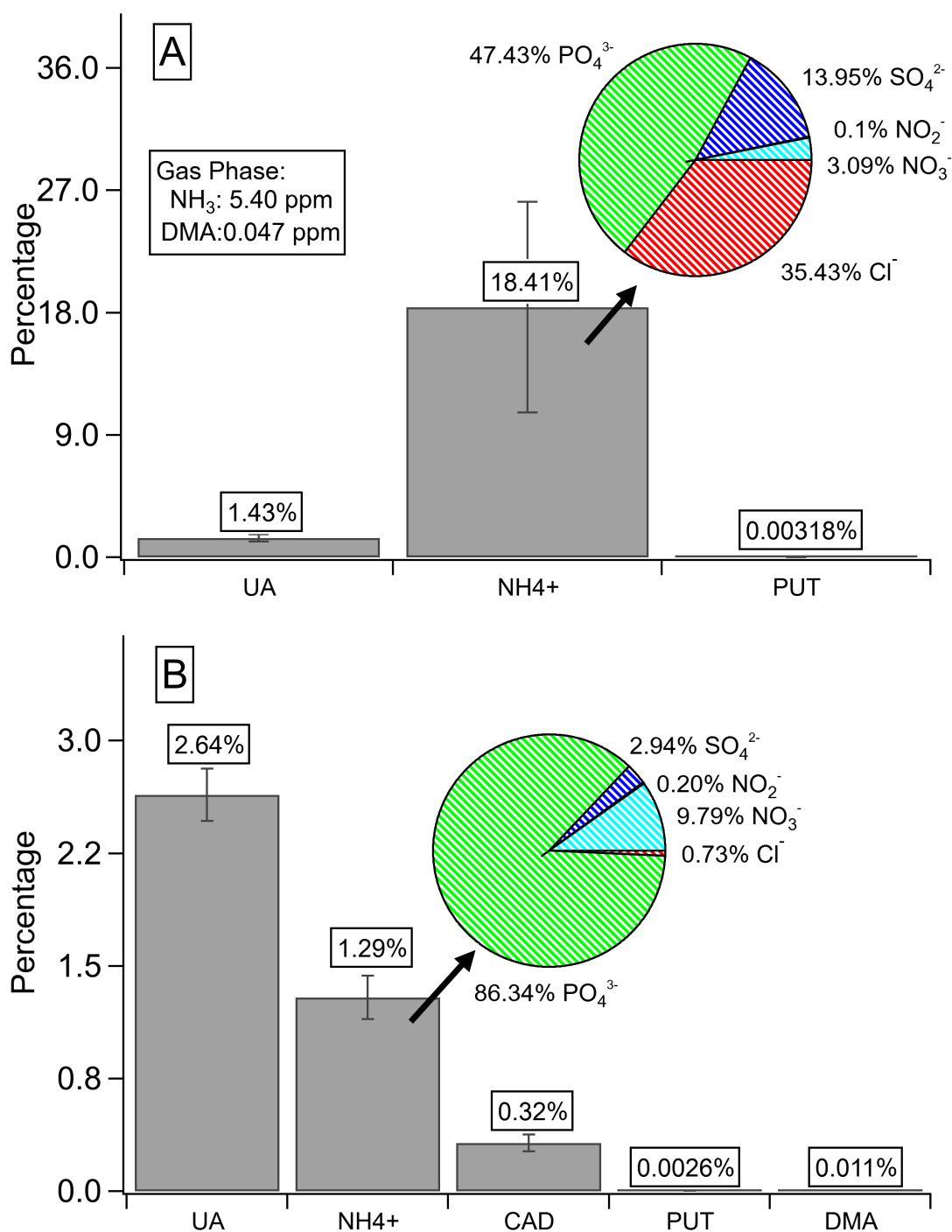


Figure 3: Distribution of nitrogenous species in A) particle phase and gas phase, B) litter phase (dry mass). Only NH_3 and DMA were detected in the gas phase, and their concentrations are shown as an inset in A). The y-axis represents mass percentages of ANCs, and pie charts represent the calculated molar percentage of ammonium salts.

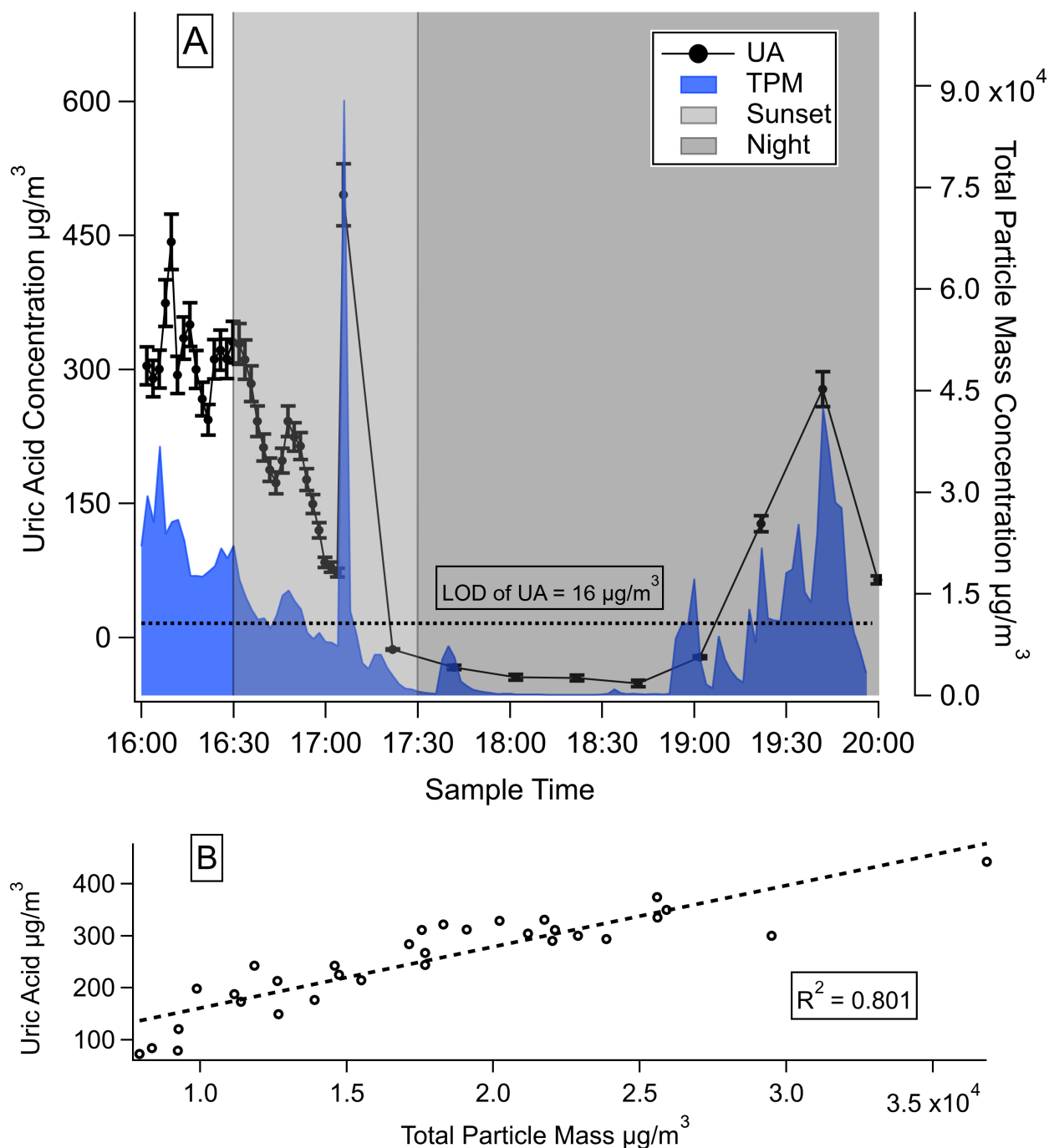


Figure 4: Time-resolved measurement of particles in the poultry farm, A) Time series of UA and TPM; B) Correlation plot between two sets of data. Error bars for UA in A) represent the standard deviation of PILS collection (6.7%) obtained from quality control experiments. The LOD of UA in particles is $16 \mu\text{g}/\text{m}^3$ represented by the dashed line in panel A.