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Functional relationship of particulate matter (PM) emissions, animal species, and moisture content during manure application

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ABSTRACT

Livestock manure is recycled to agricultural land as organic fertilizer. Due to the extensive usage of antibiotics in conventional animal farming, antibiotic-resistant bacteria are highly prevalent in feces and manure. The spread of wind-driven particulate matter (PM) with potentially associated harmful bacteria through manure application may pose a threat to environmental and human health. We studied whether PM was aerosolized during the application of solid and dried livestock manure and the functional relationship between PM release, manure dry matter content (DM), treatment and animal species. In parallel, manure and resulting PM were investigated for the survival of pathogenic and antibiotic-resistant bacterial species. The results showed that from manure with a higher DM smaller particles were generated and more PM was emitted. A positive correlation between manure DM and PM aerosolization rate was observed. There was a species-dependent critical dryness level (poultry: 60% DM, pig: 80% DM) where manure began to release PM into the environment. The maximum PM emission potentials were 1 and 3 kg t^{-1} of applied poultry and pig manure, respectively. Dried manure and resulting PM contained strongly reduced amounts of investigated pathogenic and antibiotic-resistant microorganisms compared to fresh samples. An optimal manure DM regarding low PM emissions and reduced pathogen viability was defined from our results, which was 55–70% DM for poultry manure and 75–85% DM for pig manure. The novel findings of this study increase our detailed understanding and basic knowledge on manure PM emissions and enable optimization of manure management, aiming a manure DM that reduces PM emissions and pathogenic release into the environment.

1. Introduction

Particulate matter (PM), also known as atmospheric aerosol particles, is a complex mixture of microscopic liquid and solid particles suspended in the atmosphere (Cambra-Lopez et al., 2010). PM particles are classified by their aerodynamic diameter and the associated deposition in human airways into two main classes: respirable and nonrespirable particles. Particles > 10 µm are non-respirable and will be filtered out by the nose and upper airways (Anderson et al., 2012). Inhalable particles < 10 µm are typically subdivided into “coarse” (diameter 2.5–10 µm (= PM2.5-PM10)), “fine” (0.1–2.5 µm) and “ultrafine” (< 0.1 µm).

The smaller the particles are, the deeper they can penetrate into the respiratory tract and negatively affect human health. The World Health

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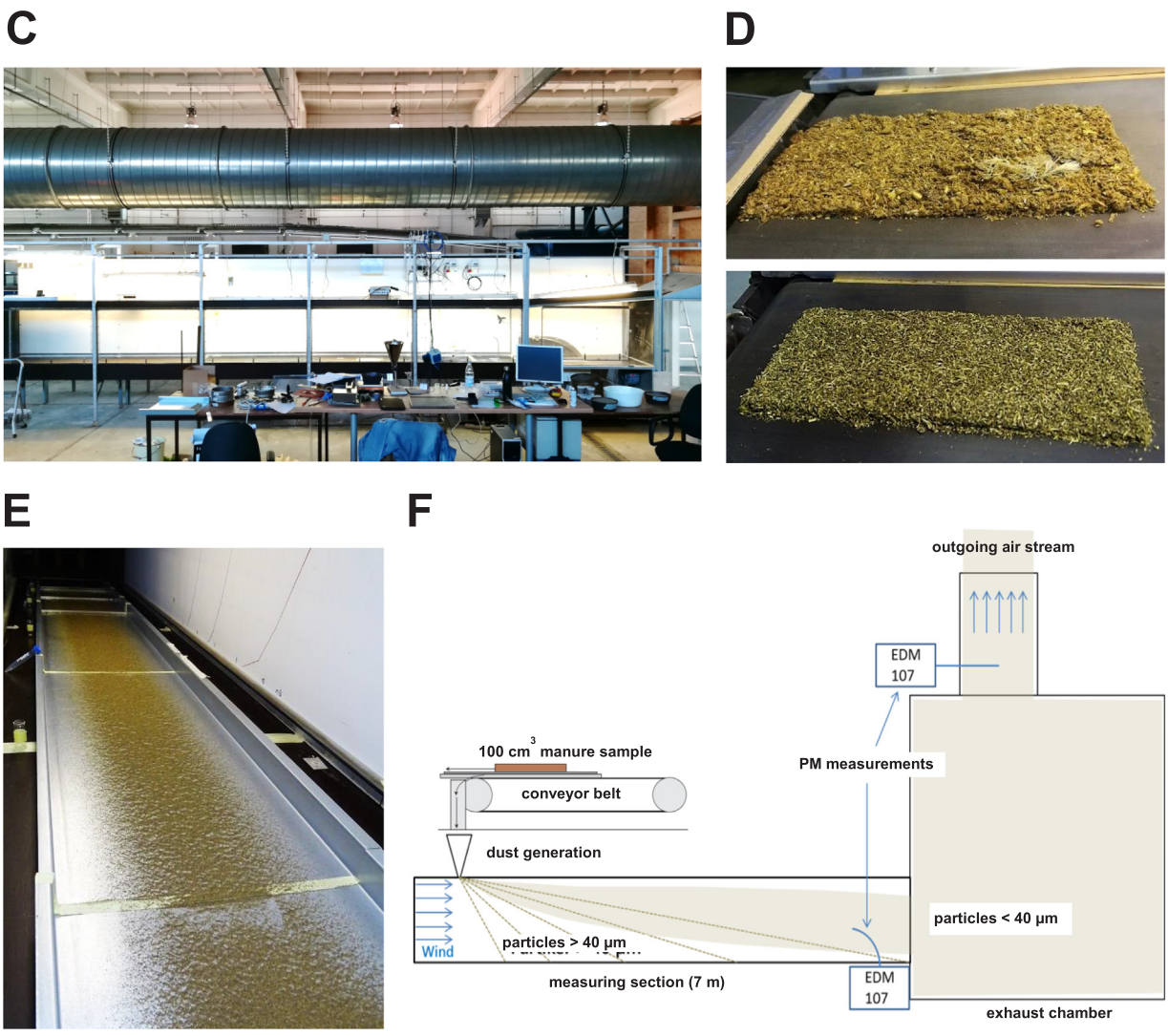

Fig. 1. PM emission measurements in the wind tunnel. (A) Photo of the stationary wind tunnel used for PM emission measurements. (B) Photos of sample material used for wind tunnel measurements - top: poultry manure; bottom: pig manure. (C) Photo of manure particle separation by size along the wind tunnel measuring section. (D) Schematic experimental setup of the wind tunnel for PM emission measurements.

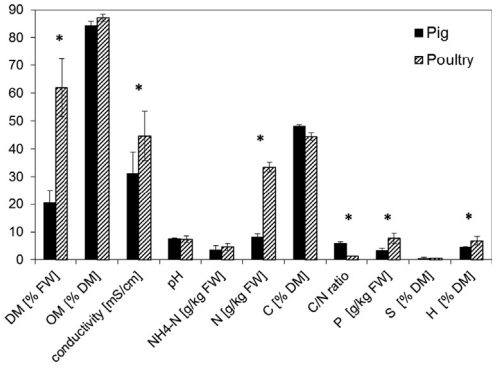

Fig. 2. Physical and chemical properties of used manure. Comparison of physical and chemical properties of poultry (black) and pig (striped) manure. Error bars are the standard deviation (SD) of five replicates. Asterisks mark statistically significant differences of *P < 0.05 by Student's *t*-test. Units are indicated in square brackets. Abbreviations: dry matter content (DM), organic matter content (OM), fresh weight (FW), ammonium-nitrogen (NH_4-N), nitrogen (N), carbon (C), phosphate (P), sulfur (S) and hydrogen (H).

Organization estimates that 90% of the world population breathe air containing high levels of PM and that PM air pollution contributes to approximately 4.2 Mio deaths each year (WHO, 2016), ranking it the 13th leading cause of mortality worldwide (Anderson et al., 2012). PM can lead to DNA mutations, heart attacks, cardiovascular and respiratory diseases, such as lung cancer and asthma (Burnett et al., 2014; Pozzer et al., 2017). A study investigating approx. 300 000 people in nine European countries revealed a 22% and 36% increase in lung cancer; when PM10 and PM2.5, respectively, increased by 10 µg m^{-3} (Raaschou-Nielsen et al., 2013). People have a higher risk of developing respiratory diseases when they are permanently exposed to > 5–30 µg PM m^{-3} (Burnett et al., 2014). To protect human health and the environment, the EU defined critical PM values in 2005: the daily average limit of PM10 was set to 50 µg m^{-3} of air with 35 exceedances allowed per year (Winkel et al., 2015). The annual average limits for PM10 and PM2.5 were set to 40 and 25 µg m^{-3} of air, respectively.

PM can be directly emitted (primary PM) or can be formed indirectly in the atmosphere (secondary PM) by transformation of gaseous emissions (e.g., sulfur oxide, nitrogen oxide, methane, ammonia) (Pozzer et al., 2017). Sources of primary PM can be natural or anthropogenic. Natural sources of PM are volcanoes, forest fires, desert dust storms, and aerosolized sea salt. Anthropogenic sources of PM include combustion in mechanical and industrial processes, burning of

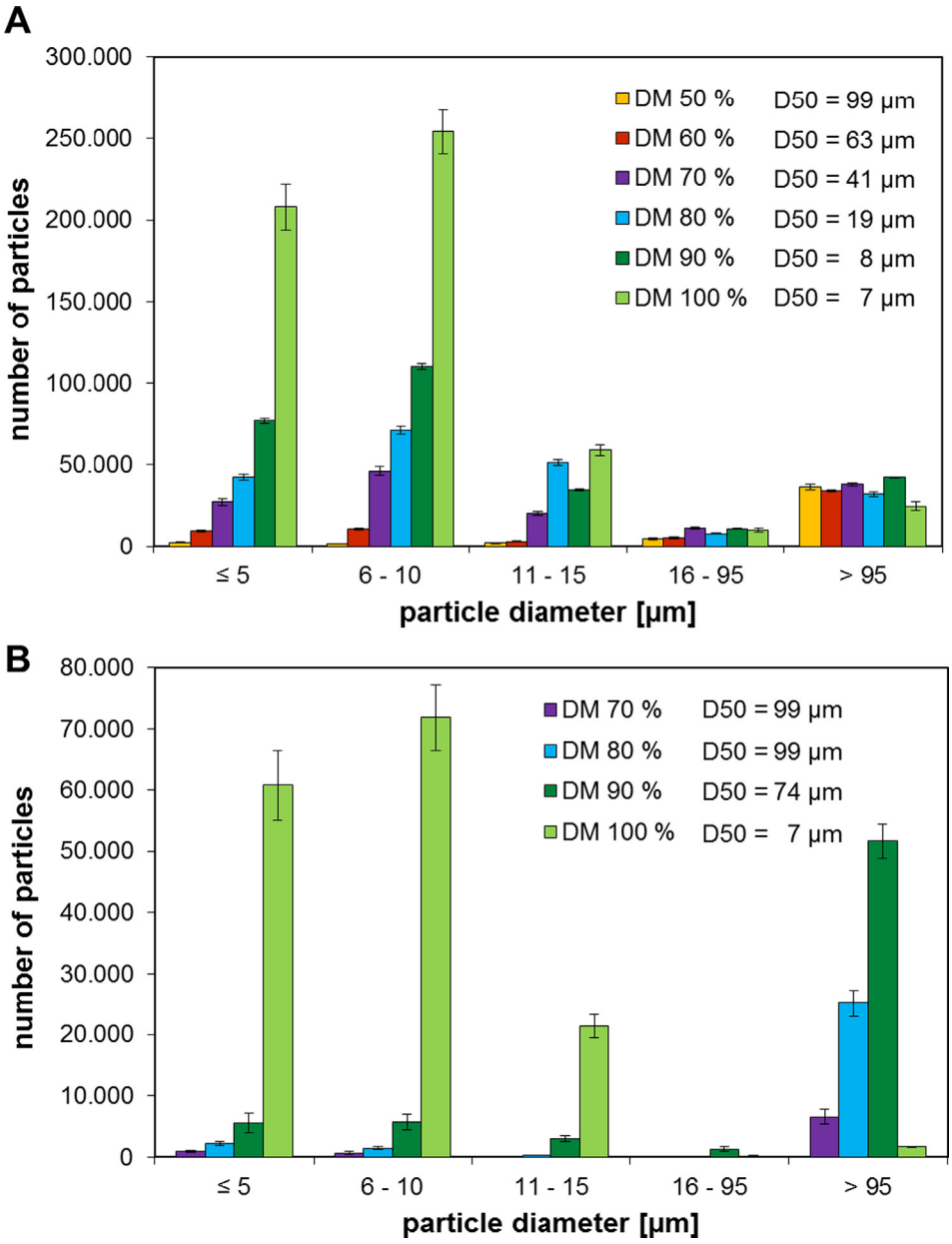

Fig. 3. Manure particle size distribution (PSD). Number of particles in different size classes per 100 cm^3 poultry (A) and pig (B) manure with different dry matter contents (DM). Error bars are the standard deviation (SD) of ten replicates. D50 is the median particle size for every DM level.

fossil fuels, vehicle emissions, tobacco smoke and agriculture. Primary and secondary agrarian PM contributes approx. 20% to the total dust-associated emissions and is thus the largest European PM2.5 polluter (Aarnink & Ellen, 2007; Lelieveld et al., 2015). Direct PM emissions in agriculture originate mainly from livestock production, manure management, fertilizer use and occur due to field cultivation and consecutive wind erosion from agricultural land (Funk et al., 2008; Takai et al., 1998). Agricultural emissions of PM2.5 are mainly induced by secondary particle nucleation of ammonia emissions (Lelieveld et al., 2015).

Most agrarian PM originates from poultry and pig houses, which are responsible for 50% and 30%, respectively, of the total European agricultural PM emissions (Cambra-Lopez et al., 2010). Livestock houses can significantly affect the air quality in the surrounding environment, because in areas with intensive animal production, an increased PM10 prevalence has been observed (McEachran et al., 2015). It has been known for decades that poultry husbandry is connected with strongly elevated PM levels (up to 200-fold higher compared to ambient air); followed by pig houses, whereas dairy husbandry emits the least amount of airborne dust (Aarnink and Ellen, 2007; Lai et al., 2014; Winkel et al., 2015).

PM from livestock husbandry consists of up to 90% organic matter (e.g., feathers/hairs/skin, feed, litter, feces) and is biologically active; therefore, it is called bioaerosol (Cambra-Lopez et al., 2010). Bioaerosols can carry hazardous materials such as odors and irritant gases (e.g., ammonia), bioactive components (antibiotics, endotoxins) and microorganisms (viruses, bacteria, fungi) (Mostafa et al., 2016). Antibiotic-resistant bacteria are of particular interest worldwide, because conventional animal production relies on the extensive use of veterinary pharmaceuticals, especially antibiotics (Hamscher et al., 2003; Singer et al. 2016). This leads to a high selective pressure in favor of resistant bacteria (e.g., methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and vancomycin-resistant enterococci (VRE)) in the animal gastrointestinal tract, which are released to the environment by excretion (McEachran et al., 2015).

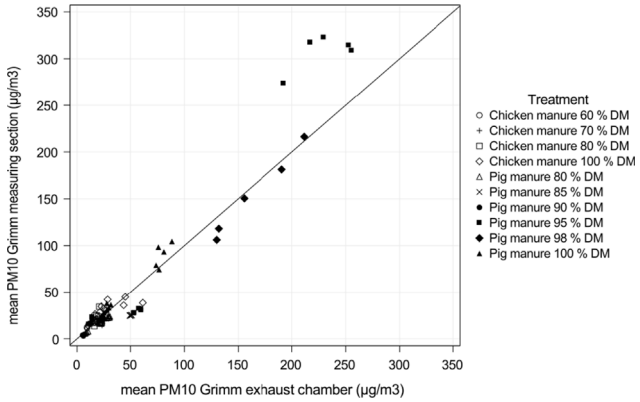

Fig. 4. Comparison of PM concentrations in the measuring section and exhaust chamber. Mean PM10 concentrations during manure application measured by GRIMM EDM 107 at two different locations within the wind tunnel (schematically shown in Fig. 1D).

Manure is an excellent fertilizer, due to high amounts of major plant nutrients (e.g., nitrogen, phosphorus, and potassium) and increasing the organic matter content of the soil. The global amount of annually generated animal manure and their application to agricultural lands is constantly increasing due to rising livestock production (Blaustein et al., 2015). Dungan, 2010 found that animal manure, containing drugs and resistant bacteria, significantly contributes to airborne PM emergence when it is used for fertilization of arable land. Many bioaerosol studies have been published on PM from animal production facilities; however nearly none have investigated emissions during manure application or the associated threats to environmental and human health (Jahne et al., 2015; Dungan, 2010). Field spreading of manure potentially poses a twofold enhanced health risk by elevated PM levels and the distribution of pathogenic bacteria in the regional area (Dungan, 2012). Thus, it is of utmost importance to obtain basic information and detailed knowledge about the relationship between PM aerosolization rate and manure moisture content, as well as between manure PM particle size and mass distribution during manure application. Such findings are important for decision-making and evaluation of PM emission control strategies and for assessing the potential impact on public health and the surrounding environment. Therefore, the objective of this study was to investigate the functional relationship between manure dry matter content (DM), treatment, animal species (pig and poultry), PM emission rate, and survival of antibiotic-resistant and pathogenic bacteria.

2. Materials and methods

2.1. Manure material

Poultry manure was collected from three farms in Germany. At all farms, broilers were kept under similar conventional conditions in a floor housing system with wood shavings as bedding material and dry feed. Pig manure was collected from two farms in Germany with conventional housing conditions (slatted floors). Farm 1 manure was a slurry mixture from sows and piglets, in contrast to farm 2, where manure was derived from the slurry of fattening pigs.

In all experiments, fresh poultry and pig manure with different DMs were tested. In this context, 'fresh' means manure directly collected in animal houses. For poultry manure, also the influence of manure treatment was tested. The two investigated manure treatments were 'stored' (heaped and older than 3 months) and 'composted' (stored manure that was mixed once per week).

The 100% DM weight of all samples was determined through total drying at 105 °C. Than weights of desired DM levels were calculated. Manure samples with different DMs were generated by gently drying the samples in a 35 °C incubator to maintain microbial activity until they reached the specific weight. For all experiments, manure with 50–100% DM from poultry and with 70–100% DM from pigs was used. Therefore, all manures used in this study were of solid consistence and obtained by drying. Below 50% and 70% DM, manure was too wet and sticky, leading to aggregate formation, which prevented proper measurements from being performed.

2.2. Physical and chemical parameters

All manure samples were analyzed for DM, organic matter content (OM), electric conductivity, pH value, ammonium-nitrogen (NH_4-N), nitrogen (N), carbon (C), phosphate (P), sulfur (S) and hydrogen (H) concentrations.

To analyze the DM, the sample was weighed before and after drying for 24 h at 105 °C; the difference allowed calculation of initial DM. OM was determined by ashing the DM sample in a muffle furnace for 5 h at 550 °C and calculating the weight difference. The pH value was measured with a standard pH meter (WTW pH 3210, Weilheim, Germany).

Analysis of total N and NH_4-N contents was conducted by steam distillation according to Kjeldahl using KjelMaster K-375 (BÜCHI Labortechnik GmbH, Essen, Germany) following the manufacture's instructions. P content was determined by photometric flow injection analysis (FIA) using samples obtained after Kjeldahl disintegration (ISO 15681-1). Elemental analyses for C, S and H were performed by combining high-temperature combustion and gas chromatography using an elemental analyzer vario EL (Elementar Analysensysteme GmbH, Langenselbold, Germany).

2.3. Particle size distribution analyses

The particle size distribution (PSD) of 100 cm^3 homogenized manure with different DMs was examined by dynamic image analysis (100 pictures per s) using a PartaAn 3001L 3D analyzer (Microtrac Inc., Montgomeryville, USA). A high speed digital camera takes multiple images of each particle and reconstructs a 3D projection, including information about particle length, width, thickness, perimeter, and area. Particles from 3 to 100 µm, in 1 µm steps, according to their area equivalent diameter, were counted. For graphical presentation, particle counts of different sizes were summarized as indicated (Fig. 3). The total particle numbers of the 16–95 µm class were divided by 16 because the size range of this class is 16-fold higher than that of the other classes. Ten replicates were measured for every DM level.

2.4. PM emission measurements under controlled conditions

PM emissions released from solid manure were measured under controlled conditions in a standardized and reproducible approach using a stationary wind tunnel (Institute of Soil Landscape Research in Müncheberg) (Funk et al., 2008; Funk et al., 2019). The measuring section of the wind tunnel had a length of 7 m and a cross-section area of 0.7 × 0.7 m (Fig. 1). The wind speed was set to 3 m s^{-1}.

The wind tunnel acted as a gravitational cross-flow separator (particles fall vertically into a horizontally directed air stream) according to standardized particle size analysis (DIN 66118). The particle size separation was based on the air stream-dominated drift of fine particles and free-fall trajectories of coarse particles (Fig. 1C). This particle separation ensured that only particles < 40 µm reached the end of the wind tunnel measuring section (Fig. 1D), where a GRIMM EDM 107 dust analyzer (GRIMM Aerosol Technik GmbH & Co. KG, Ainring, Germany) was installed for continuous counting of PM particles in a size range of 0.25–32 µm. A second GRIMM EDM 107 was placed within the outgoing air stream (schematically shown in Fig. 1D). The sampling airflow rate was 1.2 L min^{-1} and the measuring interval was 6 s.

For measurements of PM emissions, 100 cm^3 of dried manure material (Fig. 1B) was applied at the beginning of the measuring section on

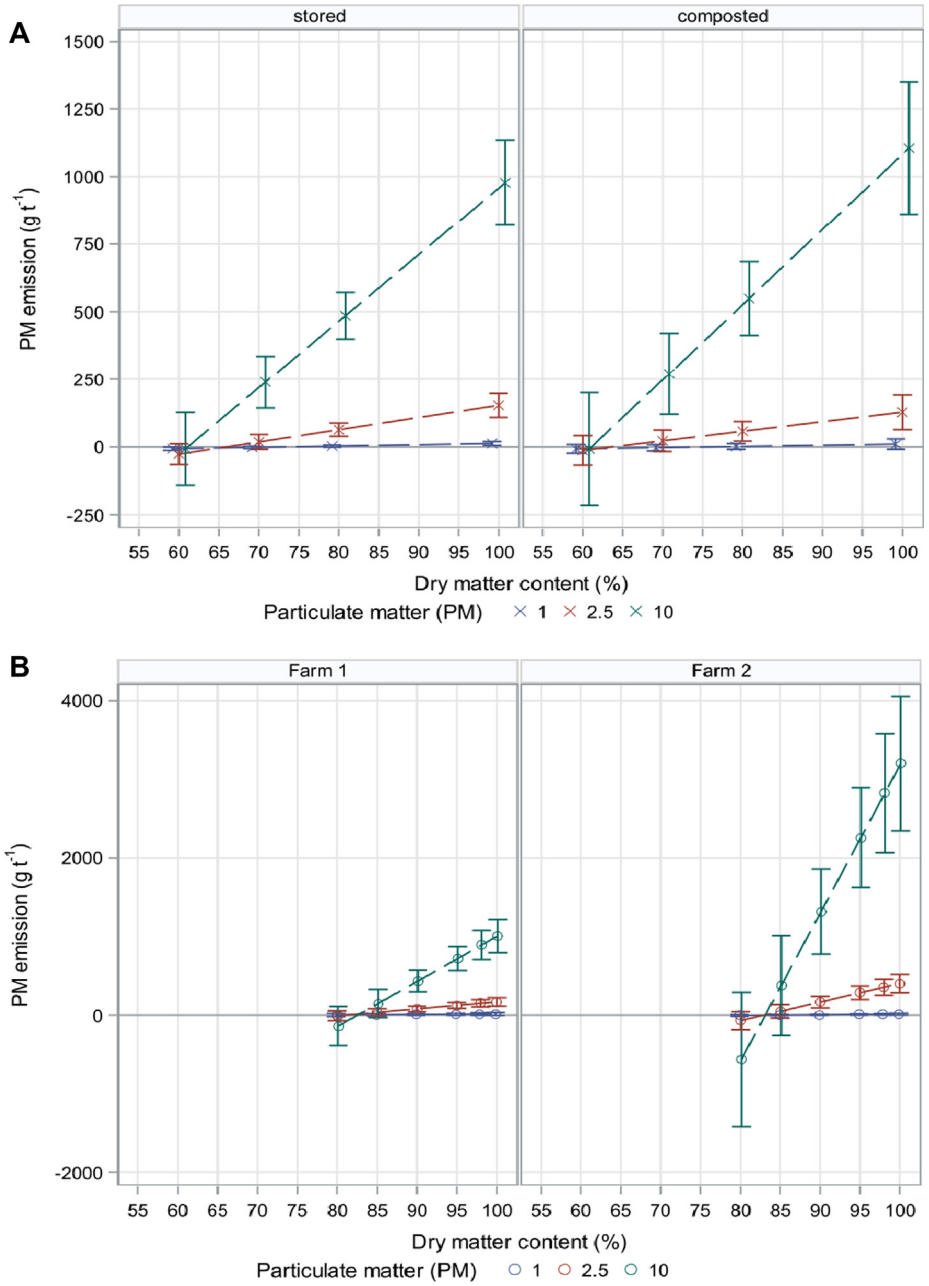

Fig. 5. Overview of manure PM emissions in dependence on DM, treatment and animal species. Means of PM release (g of PM per t of applied manure) for class PM1 (blue), PM2.5 (red) and PM10 (green) measured in the wind tunnel during solid poultry (A) and pig (B) manure application. Error bars represent the 95% confidence interval of at least five replicates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

top of the wind tunnel by a conveyor belt with a speed of $4 * 10^{-4}$ m s^{-1}, resulting in a mean sample application of 0.07 g s^{-1} (Fig. 1D). Approx. 8.5 min were needed to apply the whole sample to the wind tunnel. The PM background concentration was measured 10 min before and after each sample, averaged and subtracted from the manure PM measurements. At least five replicates were measured for every tested species and DM combination.

The PM emission rate from GRIMM counts was calculated in several steps (for equations and details see Funk et al. (2008) and Funk et al. (2019)). First, the mean PM emissions per minute were multiplied by the measurement time, the air volume and a correction factor of 0.36 for the spatial distribution of the dust cloud in the wind tunnel to calculate the emitted PM (µg per m^3 of air). Then, this value was divided by the manure mass used to obtain the emitted PM per g of substrate, which was then subjected to further statistical analysis.

CMD (count median diameter), MMD (mass median diameter), standardized number and mass fraction were calculated according to Lai et al., 2014.

2.5. Statistics

All data were statistically analyzed with SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

A linear model was used to estimate the influence of PM size, DM, animal species, treatment (poultry manure) and farm system (pig manure) on PM emissions per g of substrate. PM size class and manure treatment or farm system were handled as fixed class factors, while DM content was a linear regression covariable. The interaction between DM content and PM size class, as well as the variance heterogeneity between manure treatment or farm system and PM size class, were taken

Table 1
PM particle numbers for different manure DMs. Mean particle counts (n cm^{-3} of air) of wind tunnel measurements for different manure DMs categorized into four indicated size classes. Abbreviations: Standard error (SE) and count median diameter (CMD). "mean %" is the proportion of the indicated size class of the total particle number.

Species	Treatment	DM (%)	0.25–1.0 µm	SE	1.0–2.5 µm	SE	2.5–10 µm	SE	10–32 µm	SE	CMD µm	SE
Poultry	stored	60	33	2	0.48	0.17	0.094	0.032	0.000	0.002	0.437	0.000
		70	115	3	0.20	0.24	0.046	0.038	0.007	0.014	0.415	0.000
		80	108	3	0.22	0.36	0.067	0.059	0.008	0.013	0.412	0.000
		100	109	5	0.39	0.91	0.125	0.180	0.013	0.019	0.412	0.000
	composted	60	106	3	0.84	0.23	0.129	0.037	0.000	0.002	0.422	0.001
		70	213	7	0.21	0.18	0.040	0.037	0.006	0.014	0.401	0.001
		80	93	4	0.30	0.69	0.076	0.080	0.012	0.02	0.412	0.000
		100	159	5	0.42	0.61	0.155	0.127	0.017	0.021	0.408	0.001
	mean		**108**	**4**	**0.34**	**0.30**	**0.090**	**0.050**	**0.010**	**0.010**	**0.410**	**0.000**
	mean %		**99.2**		**0.32**		**0.078**		**0.007**			
Pig	farm 1	80	359	5	0.13	0.38	0.009	0.013	0.000	0.002	0.394	0.000
		90	82	3	0.10	0.10	0.020	0.027	0.002	0.007	0.415	0.001
		95	123	5	0.20	0.38	0.046	0.079	0.015	0.023	0.406	0.002
		100	149	5	0.36	0.81	0.096	0.151	0.014	0.024	0.406	0.001
	farm 2	80	255	5	0.08	0.06	0.009	0.012	0.000	0.001	0.405	0.000
		90	285	6	0.12	0.22	0.019	0.037	0.004	0.013	0.400	0.000
		95	340	14	2.34	3.51	1.757	1.512	0.135	0.106	0.392	0.001
		100	376	8	0.77	1.45	0.317	0.379	0.041	0.045	0.393	0.000
	mean		**270**	**5**	**0.16**	**0.38**	**0.030**	**0.060**	**0.010**	**0.020**	**0.400**	**0.000**
	mean %		**99.8**		**0.05**		**0.007**		**0.001**			

into account.

In Fig. 2, significant differences between means were determined by Student's *t*-test (* $P < 0.05$), and the mean variability of data is indicated by standard deviation (SD).

In Fig. 5, estimated values are depicted as means with 95% confidence intervals as indicators of variation.

2.6. Microbiological analyses

For the collection of microorganisms attached to PM particles, two different samplers with indicated air flow rates (in brackets) were used: 1) a six-stage viable Andersen cascade impactor (Thermo Fisher Scientific, Franklin, Massachusetts, US; serial number: S-0072) (28.3 L min^{-1}) and 2) a DYCOR XMX-CV (Dycor Technologies Inc., Edmonton, Canada) (530 L min^{-1}).

For microbiological analysis, 20 g of fresh or dried manure was filled into plastic bags and diluted 1:10 in liquid LB media. The sample was homogenized with a stomacher (Bagmixer 400, Interscience, France) and incubated for 30 min at room temperature. From the air collected samples, 27 ml of liquid LB media was added to 3 ml of PBS containing the collected PM particles and directly used or incubated for 24 h at 37 °C. The obtained suspensions were 10^1- to 10^6-fold diluted afterwards.

To determine the amount of total cultivable bacteria, dilutions were plated onto blood agar and for the detection of *Enterococci* on Kanamycin aesculin azide (KAA) agar (both Fisher Scientific GmbH, Schwerte, Germany). Quantification of antibiotic-resistant and pathogenic bacteria (*Clostridioides difficile (C. diff), Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella* spp., *Campylobacter* spp., methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant *Enterococcus* (VRE)) was performed on commercially available species-specific selection media. For the detection of *Enterobacteriaceae* in general, samples were plated on MacConkey Agar (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) and for extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* on MacConkey Agar supplemented with 1 mg L^{-1} cefotaxime. Colony-forming units (CFUs) of *C. diff* were counted after incubation for 48 h at 37 °C and without oxygen, and for all others after incubation for 24 h at 37 °C. Confirmation of taxonomic classification of grown pathogenic and antibiotic-resistant bacteria was performed by MALDI-TOF MS.

3. Results and discussion

3.1. Chemical and physical manure properties

Poultry and pig manure differ in consistency and composition. Poultry manure is a mixture of mainly feces, urine, feathers and litter and is compared to pig manure dry and solid, whereas unprocessed pig slurry is liquid and more homogeneous. To determine the similarities and fundamental differences of the two manure types, especially of the material used in this study, several physical and chemical parameters were examined (Fig. 2).

The dry matter content (DM) revealed the proportion of solid ingredients in a sample. Fresh pig manure showed a DM of approx. 20% and poultry manure of approx. 60%. Thus, the DM of poultry substrate was 3-fold higher than that of pig manure. The main reasons for the DM variation between poultry and pig manure are differences of the digestive system functioning and the use of bedding material for poultry, but not for pigs. There was no fundamental difference in the organic matter content (OM), which was approx. 85% of dry matter for both manure types. The electrical conductivity, and hence the proportion of soluble salts, was slightly higher for poultry than for pig manure. There were no significant differences for pH values (approx. 7.5 = neutral) and ammonium-nitrogen (NH_4-N) (approx. 4 g kg^{-1} fresh weight) for both manure types. The proportion of the macroelements carbon (C) and nitrogen (N) and their quotient are important indicators for the fertilizer quality of the manure. Poultry and pig showed nearly no variation in C amounts but a strong difference in N values, which caused a higher C/N ratio for pig manure (5.9) than for poultry manure (1.3). Phosphorus (P) is a key driver in plant metabolic processes and therefore another important macroelement in fertilizers. Poultry manure contained significantly more P (7.7 g kg^{-1} FW) than pig manure (3.3 g kg^{-1} FW). There was almost no variation of sulfur (S) (0.5% of DM) between the two manure types. Hydrogen (H), one of the four basic elements, was found in a marginally higher amount in poultry manure.

3.2. Manure particle size distributions (PSDs)

To determine whether and how the particle size composition of solid manure differs between pigs and poultry and to investigate the influence of different DMs, PSDs of those manures were measured. For

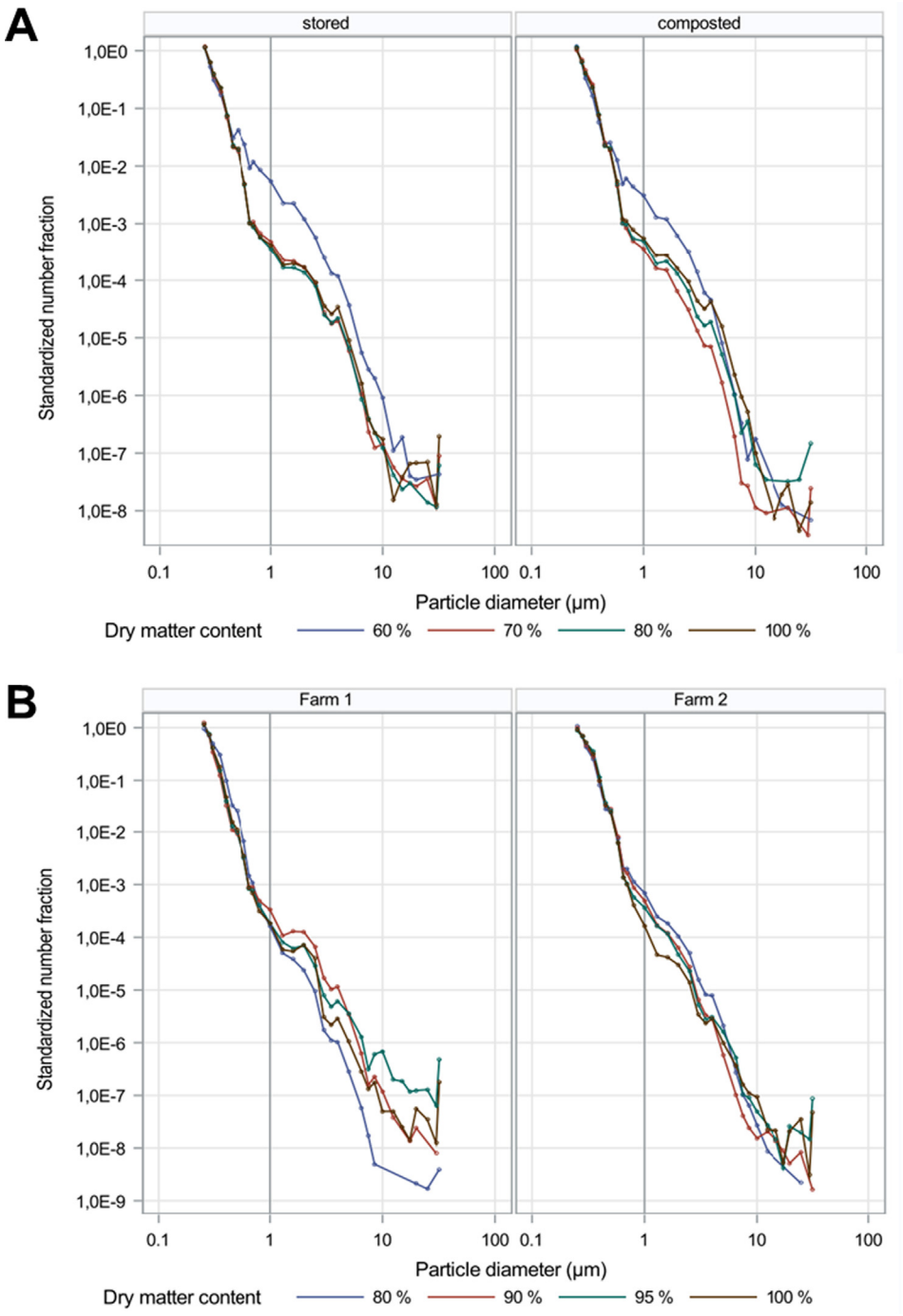

Fig. 6. Manure PM particle size distribution by particle numbers. Standardized PM particle number fraction from poultry (A) and pig (B) manure with different dry matter contents (DMs) in dependence of particle size (both at log10 scale).

poultry, manure with 50–100% DM was tested and for pigs, manure with 70–100% DM was investigated.

In general, samples of both species with low DMs showed fewer particles than dryer samples, and these were larger in size (Fig. 3). On the other hand, samples with high DM were composed of many particles, which were smaller in size. The negative correlation of DM and particle size was confirmed by the D50 value, which is the median particle size diameter of every DM class. This correlation can be explained by enhanced coagulation of particles at high moisture contents, where small PM particles will aggregate and bind to larger particles when DM is low (Aloyan et al., 1997). Consequently, the higher the manure DM has been, the lower the median particle size was. Therefore, dry manure is characterized by a high PM emission potential.

There was a species-specific DM threshold, above which manure began to dissociate into more PM particles (< 10 µm), rather than large particles. These thresholds were between DM 60–70% for poultry manure (Fig. 3A) and between DM 90–100% for pig manure (Fig. 3B). Interestingly, the abundance of large particles > 95 µm was highly similar for every DM of poultry manure, but notably different for pig manure. Thus, differences in particle numbers were limited to small particle classes (< 15 µm) for poultry manure, whereas for pig manure, particle numbers of every size class were different between various DM levels.

Table 2
PM particle masses for different manure DMs. Mean particle masses (mg m^{-3} of air) of wind tunnel measurements for different manure DMs categorized into four indicated size classes. Abbreviations: Standard error (SE) and mass median diameter (MMD). "mean %" is the proportion of the indicated size class of the total particle mass.

Species	Treatment	DM (%)	0.25–1.0 µm	SE	1.0–2.5 µm	SE	2.5–10 µm	SE	10–32 µm	SE	MMD µm	SE
Poultry	stored	60	1.88	0.02	3.55	0.08	6.92	0.18	10.88	4.95	27.86	3.50
		70	3.48	0.01	1.77	0.15	5.81	0.38	307.94	30.83	31.40	0.07
		80	3.35	0.01	2.18	0.22	9.27	0.50	460.44	34.02	31.41	0.06
		100	3.49	0.03	3.96	0.63	15.44	1.15	722.69	45.11	31.37	0.03
	composted	60	4.42	0.02	6.04	0.09	7.31	0.14	5.57	2.76	17.82	5.73
		70	6.69	0.01	1.56	0.11	5.07	0.34	360.28	34.18	31.41	0.09
		80	2.96	0.03	3.01	0.55	10.94	0.68	774.04	52.44	31.39	0.05
		100	4.92	0.04	4.46	0.48	23.61	1.23	877.62	52.43	31.23	0.03
	mean		**3.49**	**0.02**	**3.28**	**0.18**	**8.29**	**0.44**	**410.36**	**34.10**	**31.38**	**0.07**
	mean %		**0.82**		**0.77**		**1.95**		**96.46**			
Pig	farm 1	80	11.93	0.02	1.15	0.34	0.81	0.11	36.25	13.95	25.66	5.36
		90	2.16	0.01	0.86	0.05	1.82	0.15	53.46	14.92	30.76	0.23
		95	3.37	0.02	2.16	0.23	7.30	0.59	596.90	37.54	31.16	0.02
		100	4.21	0.03	4.30	0.55	16.46	1.33	758.80	54.01	31.48	0.05
	farm 2	80	4.46	0.11	1.35	0.04	1.68	0.07	1.71	0.99	16.15	5.01
		90	5.02	0.14	1.26	0.07	1.66	0.15	87.58	13.44	31.22	0.15
		95	12.93	0.06	14.32	1.13	114.72	5.72	2628.98	118.06	31.14	0.03
		100	12.65	0.03	9.10	0.95	50.43	2.79	1645.89	81.30	31.25	0.04
	mean		**4.74**	**0.03**	**1.76**	**0.29**	**4.56**	**0.37**	**342.24**	**26.23**	**31.15**	**0.10**
	mean %		**1.34**		**0.50**		**1.29**		**96.87**			

3.3. Correlation of manure PM emissions and DM

In the field, PM emissions from manure application are strongly dependent on wind speed and direction, which are permanently changing under natural conditions. Therefore, to obtain standardized results independent of wind fluctuations, the amounts of PM release from pig and poultry manure with different DMs were measured in a stationary wind tunnel (Fig. 1) under controlled conditions.

Within this wind tunnel, two aerosol spectrometers were installed, one at the end of the measuring section and one within the outgoing air stream (schematically shown in Fig. 1D). PM emissions (particles of 0.25–32 µm) were detected at two different locations to exclude position-dependent variations of measurements. The amounts of PM emergence detected at both wind tunnel locations were nearly identical, except for one sample (pig manure with 95% DM) (Fig. 4). Therefore, PM measurements within the wind tunnel measuring section and within the exhaust chamber can both be used for further analyses and do not differ significantly.

Stored and composted poultry manures of 60–100% DM as well as pig manure from two different farms of 80–100% DM were analyzed (Fig. 5). There was a species-specific critical manure dryness level where solid manure began to aerosolize PM into the air, which was lower for poultry manure (DM 60%) than for pig manure (DM 80%). PM emissions from stored and composted poultry manure (Fig. 5A) were nearly identical, with a maximum PM10 emission potential of approx. 1 kg fine dust per ton of spread manure. Therefore, in this case, manure treatment didn't influence the amount of released PM; probably because the drying process had the dominant effect over the manure treatment on the PM aerosolization rate.

However, PM emissions from dried pig manure of two different farms varied considerably (Fig. 5B). Maximum PM10 emission potentials for farm 1 (sows and piglets) and farm 2 (fattening pigs) were approx. 1 kg t^{-1} and 3 kg t^{-1} of applied manure, respectively. This 3-fold variation was presumably based on different developmental ages of the pigs and the associated maturity of the digestive system, and different types of feed. Sows and piglets received feed with a high proportion of proteins and crude fiber, whereas feed of fattening pigs contained a higher fat content. In conclusion, feeding conditions and developmental age seem to influence the PM emission potential of manure, which is in agreement with PM emission observations from animal houses (Aarnink & Ellen, 2007; Cambra-Lopez et al., 2010). Confirming our results, it has been shown previously that PM emissions from fattening pig houses were higher than those form sow houses (Winkel et al., 2015; Lai et al., 2014). Furthermore, our findings identified a strong positive correlation between manure DM and PM release independent of animal species, farm or manure treatment.

Poultry manure, as it is used for field fertilization, has a DM of approx. 65% (Fig. 2). According to our results, the PM10 emissions of poultry manure with 65% DM are approx. 120 g t^{-1} of applied manure (Fig. 5A). Assuming that 5–15 t of poultry manure will be applied per hectare of agricultural land (Griffiths, 2004; Hamscher et al. 2002), this leads to PM10 emission factors of 0.6–1.8 kg ha^{-1}, which is equivalent to a PM10 concentration of approx. 3 500 µg m^{-3} of air during poultry manure application. PM10 emission values inside poultry houses were approx. 1 100 (Lai et al., 2014) and 1 800 µg m^{-3} of air (Winkel et al., 2015). In conclusion, PM10 aerosolization rate during poultry manure application is roughly twice as high as inside poultry houses.

PM pollution from both sources, poultry houses and manure application, exceeds WHO and EU health thresholds (20 and 50 µg PM10 per m^3 of air, respectively) by far and therefore can be classified as strongly harmful to health and environment. However, in contrast to poultry housings, which generate high levels of PM the whole year, manure application is restricted to a few days per year. Agricultural fields are typically fertilized twice per year, once in spring and once in autumn. Therefore we estimate that the contribution of manure application to annual PM air pollution is negligible low compared to animal housings. The distribution area of PM in the environment after manure application, associated levels of PM concentration and whether populations living in the vicinity of arable land are endangered, should be further clarified with the help of dispersion models.

3.4. Detailed characterization of manure PM emissions

To obtain detailed insights into PM particle size distributions during solid manure application, particle concentrations measured during wind tunnel experiments (wind speed 3 m s^{-1}) were analyzed in detail for particle numbers and particle masses.

Particle numbers of dust originating from poultry and pig manure application were very similar and nearly exclusively within the PM1 class (> 99%) (Table 1). The mean count diameter (CMD) for manure PM from both species was approx. 0.4 µm. Fig. 6 shows the standardized number fractions of particles measured for the different manure

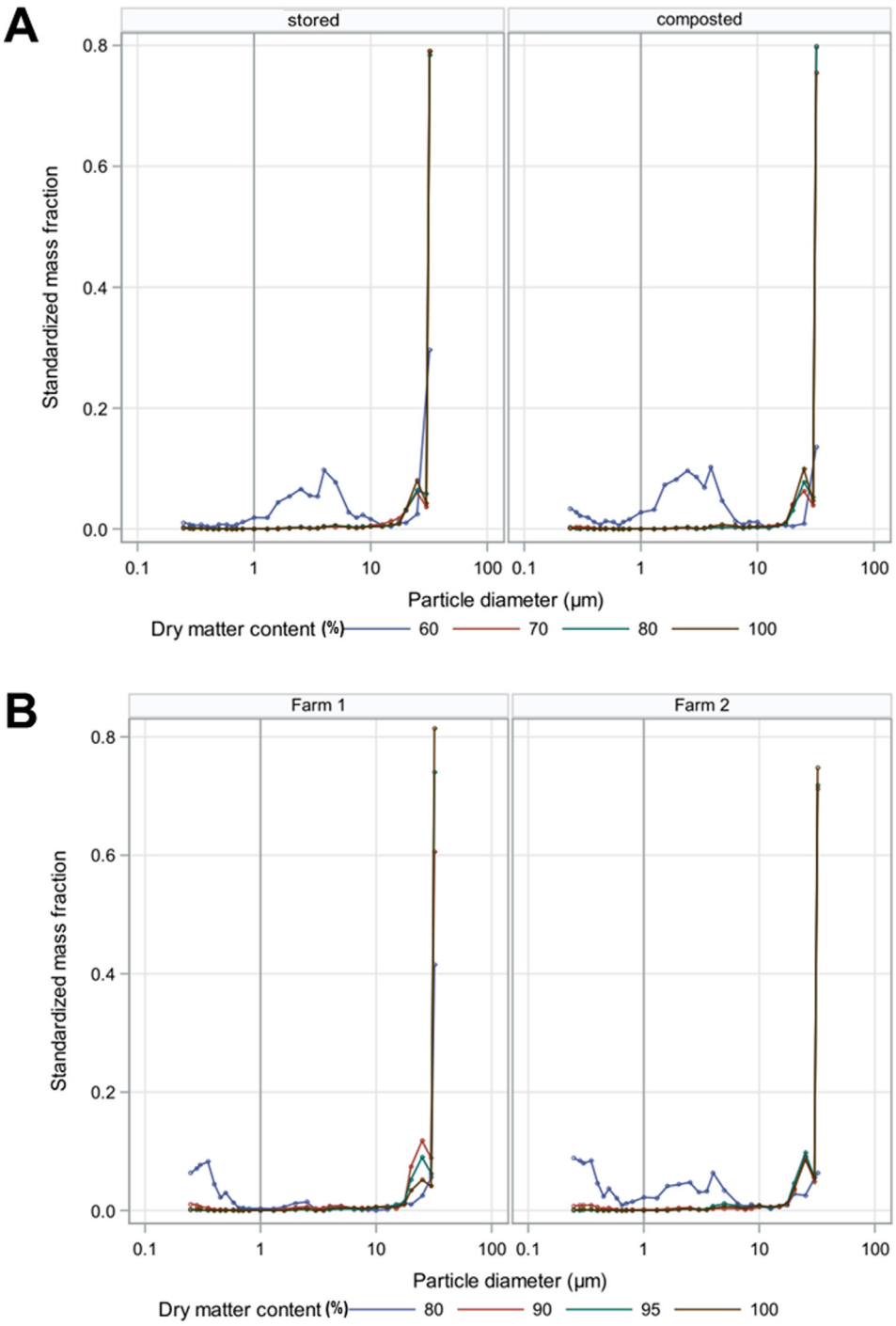

Fig. 7. Manure PM particle size distribution by particle mass. Standardized PM particle mass fraction from poultry (A) and pig (B) manure with different dry matter contents (DMs) in dependence of particle size (at log10 scale).

species, DM and treatment combinations. For all categories, the largest number of particles was in the size range of 0.25–0.30 µm. The PM particle distributions from poultry and pig manure, for the sample with the highest moisture content (DM 60% and 80%, respectively), varied slightly from other DMs. For PM from poultry manure, there was an enrichment of particles between 0.8 and 5 µm and for pig manure from farm 1 (sows and piglets) there was a significant decrease in particles larger than 8 µm. In general, the number of detected particles decreased sharply with increasing particle size, which can be explained by a faster deposition of larger particles due to a higher weight.

Particle mass distributions from poultry and pig manure were dominated (> 96%) by particles > 10 µm and therefore outside the PM range (Table 2 and Fig. 7). The mean mass diameter (MMD) for manure PM from both species was approx. 31 µm. Similar to the count distributions, samples with the highest moisture content showed a slightly different distribution to the other DMs. For PM from poultry manure with 60% DM, there was a mass peak for particles of 1–8 µm, and pigs exhibited a higher mass for particles < 0.8 µm or < 8 µm, depending on the farm, and a mass decrease for particles of approx. 30 µm. The higher the DM of the sample, the higher the mass of the largest particle class (> 32 µm) was, probably due to the presence of more aerosolized particles with increasing DM.

In summary, particle counts during manure application were highest (> 99%) in the size classes < 1.0 µm, while particle mass was

Table 3
Microbiological characterization of manure and resulting PM. Mean frequency of microorganisms in fresh and dried manure used for wind tunnel experiments (in colony forming units (CFUs) per g of manure) and of microorganisms associated with manure PM collected during wind tunnel experiments (in CFUs per m^3 air). Gram-positive bacterial classes are marked with "+" and gram-negative classes with "−". Abbreviations: extended-spectrum beta-lactamase (ESBL), *Clostridioides difficile (C. diff)*, methicillin-resistant *Staphylococcus aureus* (MRSA), not determined (n.d.) and limit of quantification (LOQ).

manure		Cultivable bacteria	Enterococci$^+$	*Enterobacteriaceae*$^-$	ESBL-producing *E. coli*$^-$	*C. diff*$^+$	MRSA$^+$
poultry	fresh	10^9	10^8	10^6	10^5	10^3	–
	dried	10^6	10^4	10^2	–	n.d.	–
	PM	10^5	10^2	10^2	–	–	–
pig	fresh	10^9	10^7	10^7	10^5	–	10^4
	dried	10^8	10^5	10^6	10^4	–	< LOQ
	PM	10^2	–	–	–	–	–

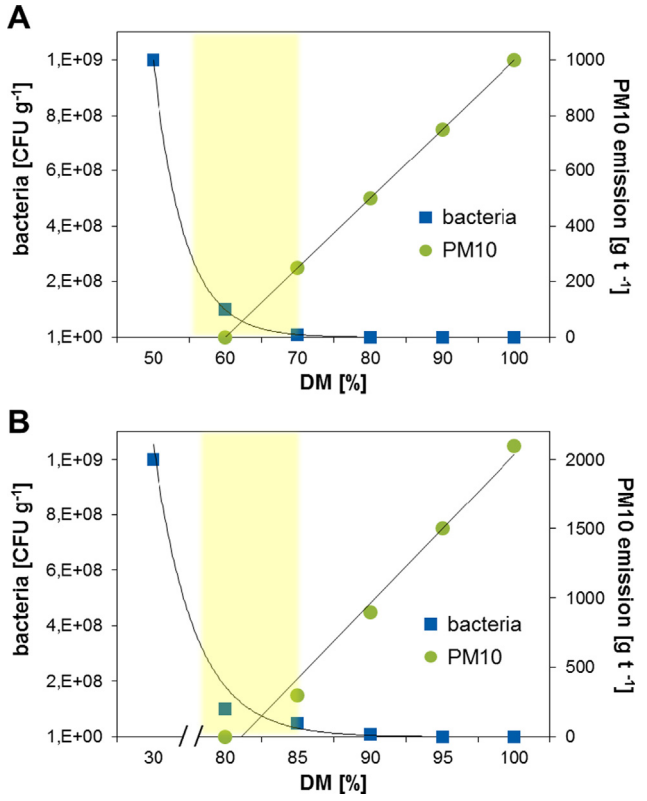

Fig. 8. Optimal manure DM in consideration of microbiological risk and PM emission potential. Identification of best poultry (A) and pig (B) manure DM interval (yellow) for highest bacterial reduction (blue squares) and lowest PM emission potential (green circles). Abbreviation: colony forming unit (CFU). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

highest (> 97%) in size classes > 10 µm. Meaning that only a small number of large particles made up almost the whole weight When comparing this with particle analyses from inside of animal houses, the distribution for particle counts was similar (87% in PM1) but different for the particle masses (97% in PM10) (Lai et al., 2014). This finding suggests that particles aerosolized during manure application are heavier than particles in livestock buildings.

Interestingly, although the sample material was identical, the analysis of the particle mass distribution differed from the particle number distribution. Both approaches lead to different results, perhaps because the ratio of particle size and mass of PM particles is not equal to 1. For PM particles, it is assumed that the density is higher than 1 mg mm^{-3}, depending on their origin (Cambra-López et al., 2011). For poultry manure, a shape factor between 1.15 and 1.45 and for pig manure between 1.36 and 2.03 has been reported (McCrone, 1993; Mostafa et al., 2016; Zhang, 2004). Thus, PM particles originating from pig manure seemed to have a higher density than PM particles from poultry manure.

3.5. Prevalence and survival of microorganisms in manure and in resulting PM

PM emissions can negatively influence public health by inhalation, either through the fine particles themselves or by harmful attachments associated with the PM particles, such as pathogenic microorganisms. Therefore, the abundance of bacteria in fresh and dried manure, as well as in PM derived from manure were determined, with a special focus on pathogenic and antibiotic-resistant bacteria.

Amounts of all cultivable bacteria, fecal indicator bacteria (*Enterococcus* spp. and *Enterobacteriaceae*), selected bacterial pathogens *(L. monocytogenes, P. aeruginosa, C. diff, Salmonella* spp., *Campylobacter* spp.), and antibiotic-resistant bacteria (VRE, MRSA and ESBL-producing *E. coli)* were quantified in each sample.

The abundance of total bacteria in fresh manure was approx. 10^9 CFU g^{-1} (Table 3), but can be even higher (> 10^{11} CFU g^{-1}), as shown by the study of Jahne et al. (2015). *L. monocytogenes, P. aeruginosa, Salmonella* spp., *Campylobacter* spp. and VRE were not detected in any sample. *C. diff* was exclusively found in poultry manure and MRSA only in pig manure, confirming observations from previous studies (Rodriguez et al., 2016; Rosen et al, 2018; Voss et al, 2005). ESBL-producing *E. coli* were equally represented in both manure types (10^5 CFU g^{-1}), but survived better in dried pig manure (10^4 CFU g^{-1}) than in dried poultry manure (not detectable). In general, drying of manure led to a strong bacterial reduction of 3–4 log levels (1.000–10.000 -fold) in poultry manure and of 1–2 log levels (10–100 -fold) in pig manure. Thus, bacteria in pig manure may survive drying better than bacteria in poultry manure. An even stronger reduction of analyzed microorganisms was observed for PM obtained from manure. In this instance, a decrease of several magnitudes up to no viability for the investigated bacteria was observed.

Feces of livestock animals, grown up under intensive management conditions, contain increased microbial concentrations with elevated levels of zoonotic pathogens, which can be transmitted to the environment upon excretion (Dungan, 2010; Dungan, 2012, Blaustein et al., 2015). Bacteria detected in connection with farming animals are mostly gram-positive, predominantly of the *Staphylococcus* and *Streptococcus* genera, while gram-negative bacteria make up a proportion of < 10% (Cambra-Lopez et al., 2010). Zucker et al., 2000 found a proportion of gram-negative bacteria between 0.02% and 5.2%, depending on livestock species. The gram-negative bacterial flora was mainly composed of three families: *Enterobacteriaceae*, *Pseudomonadaceae* and *Neisseriaceae*.

65% of antibiotics produced globally (65,000 of 100,000 t) are administered to animal husbandry (Singer et al., 2016). Such extensive use of veterinary pharmaceuticals in livestock production not only leads to high amounts of antibiotic-resistant bacteria, but also to significant pharmaceutical concentrations (hormones, antibiotics, antihelminths) in manure and farm-associated PM (Hamscher et al., 2003; Singer et al.,

2016). In the EU and USA, 4.7 and 10 Mio kg, respectively, of antibiotics were consumed in conventional animal farming each year (Hamscher et al., 2002; McEachran et al., 2015). Thus, extensive amounts of antibiotics and resistant bacteria will be spread on agricultural land and in the environment during manure application (Hamscher et al., 2002; Schmitt et al., 2006). Fecal bacteria normally die off rapidly upon excretion due to temperature change, humidity decrease, and oxygen increase (Bradford et al., 2013; Dungan, 2010; Thomas et al., 2019). However, manure fertilization increases the organic matter content of the soil, which results in an enhanced survival rate of bacteria (Acosta-Martinez et al., 2015). Veterinary pharmaceuticals and antibiotic-resistant bacteria contained in manure are notably stable and can persist in the soil > 1 year after field fertilization (Boxall et al., 2006; Friese et al., 2013; Hartmann et al., 2012). Therefore, the supply rate of antibiotics by organic fertilization can be higher than the degradation rate, causing an accumulation of antibiotics in the soil. These drugs can be taken up by plants or surface water and may pose a threat to animal and human health by consumption. Especially relevant for this study is the observation that antibiotic concentrations were highly enriched (0.35 mg kg^{-1} tetracycline and 1.44 mg kg^{-1} chlortetracycline) in dried manure aggregates at the soil surface of a fertilized field, e.g., by drying off liquid manure through sunlight (Hamscher et al., 2002). Showing that solid manure aggregates arise naturally during manure application and present hotspots of high antibiotic concentrations, which could lead to biodiversity reduction of soil bacteria.

Measurements by Burrows et al., 2009a have shown that the mean aerial concentrations of bacteria over land were > 10^4 CFU m^{-3}. Bacterial abundance in the air was more than one order of magnitude higher (> 10^5 CFU m^{-3}) after manure application (Jahne et al., 2015), verifying the aerosolization of microorganisms during organic fertilizer spreading. Microorganisms seem to prefer certain particle sizes for adhesion. Jones & Harrison, 2004 reported a primary attachment of bacteria to PM particles > 3 µm; Madsen et al., 2018 have observed that human-pathogenic MRSA and *Staphylococcus aureus* from pig farms were mainly associated with PM particles of 7–12 µm. Bacteria naturally occur not as single cells, but prefer to build aggregates to protect themselves to harmful environmental influences (e.g. dryness, UV radiation, heat, etc.). Thus, it is not surprising that microorganisms are primary attached to coarse PM particles (2.5–10 µm). On the other hand, the larger the particles, the shorter the distance that they can be transported by wind and their dispersion area. Adhesion to PM particles allow microorganisms atmospheric residence times up to weeks and wind-driven transports over long distances (Burrows et al., 2009b, Maki et al., 2019). It has been reported that intercontinental PM and bacterial movements, such as from Africa to America (Florida), distance approx. 6 500 km, or from Africa to Europe (Spain), distance approx. 3 000 km, are possible (Griffin, 2007; Hervàs et al., 2009). Over 25% of airborne atmospheric particles are assumed to consist of microorganisms and organic matter (Jones & Harrison, 2004). Bacterial pathogens and endotoxins from animal manure can be absorbed by dust particles and stay airborne for long periods an travel several miles, potentially exposing residents to elevated levels of livestock-related PM and microorganisms (Schultz et al., 2019).

3.6. Optimal manure DM range

By summarizing the results of PM emission measurements and microbiological analyses, an optimal DM interval for high bacterial reduction and low PM release, can be defined (Fig. 8, yellow area). Both aims are in opposite relationship to each other. A high manure DM is effective for pathogenic reduction, but causes an elevated amount of PM emissions and *vice versa*. Therefore, the optimal DM range is a tradeoff between reducing the amount of viable microorganisms and low PM release. For poultry manure this range is between 55% and 70% DM and is therefore perfectly overlapping with the DM of unprocessed manure (approx. 65%, Fig. 2). The optimal range for pig manure would be between 75% and 85% DM, which is considerably higher than the natural DM of pig slurry (approx. 20%, Fig. 2). Hence, drying of pig manure would be required to achieve the optimal dry matter range identified in our study.

4. Conclusions

Since manure field application represents an uncharacterized aerosolization process in regard to livestock-associated PM emissions, our study investigated the functional relationship of manure moisture content, treatment and species on PM concentrations and properties. Simultaneously, the abundance of pathogenic and antibiotic-resistant bacteria in manure and in resulting PM was studied. Our results show that solid manure application represents a serious dust emission source with a high PM emission potential. A strong positive correlation between manure DM and PM emergence was found. The survival of harmful bacteria in dried manure and in resulting PM was strongly reduced compared to fresh manure. To obtain the optimal dry matter range (poultry manure: 55–70% DM, pig manure 75–85% DM), poultry manure does not need any further processing and pig manure would have to be dried. Drying manure is advised to reduce transport costs when farmers have to export manure to other regions because of regulations under the Nitrate Directive or to effectively reduce pathogenic and antibiotic-resistant microorganisms in the manure. For conditions where the drying of manure is advised, our study can give valuable information on the level of dry matter content that should be achieved. The findings of this research can help to understand relevance and dynamics of PM emissions during manure field application, to improve manure management strategies and to estimate the risk for health and environment.

CRediT authorship contribution statement

Tina Kabelitz: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Christian Ammon:** Data curation, Formal analysis, Investigation, Resources, Software, Validation, Visualization, Writing - original draft. **Roger Funk:** Conceptualization, Funding acquisition, Supervision. **Steffen Münch:** Investigation, Methodology, Resources, Writing - original draft. **Oliver Biniasch:** Investigation, Methodology, Resources, Writing - original draft. **Ulrich Nübel:** Conceptualization, Funding acquisition, Supervision, Writing - original draft, Writing - review & editing. **Nadine Thiel:** Investigation, Methodology, Resources. **Uwe Rösler:** Conceptualization, Funding acquisition, Supervision. **Paul Siller:** Investigation, Methodology, Resources, Writing - original draft, Writing - review & editing. **Barbara Amon:** Conceptualization, Writing - original draft. **André J.A. Aarnink:** Conceptualization, Writing - original draft. **Thomas Amon:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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