ORIGINAL PAPER

Effect of Acidic Oxidation of Swine Manure on Antibiotic-Resistant Bacterial Load, Unpleasant Odor, Veterinary Antibiotics, and Plant Nutrient Profile in the One-Health Perspective

Enrico Buscaroli[1] · Sonia Blasioli[1] · Monica Marianna Modesto[1] · Luciano Cavani[1] · Giampaolo Di Biase[1] · Donatella Scarafile[1] · Paolo Trevisi[1] · Paola Mattarelli[1] · Ilaria Braschi[1]

Abstract

Purpose Advanced oxidation processes are known for their ability to remove organic pollutants, pharmaceuticals, and bacteria from wastewater, yielding outstanding results. However, these processes requires high energy input, costly equipment, and specialized operators.This study proposes a simple acidic oxidation method (H_2O_2 at pH 3 for 24 h) for in-situ treatment of pig effluent, offering a low-cost alternative for environmental management.

Methods The treatment was applied to effluents from three Italian farms, and its effectiveness was evaluated against: (i) bacterial load and amoxicillin-resistant bacteria by total viable count on selective media, (ii) nine commonly used veterinary antibiotics by LC-MS, and (iii) foul odor emissions by e-nose. Additionally, the fertilizer effect of treated effluents was examined using a maize pot experiment.

Results The treatment fully eliminated amoxicillin-resistant bacteria, reduced total bacterial load, and enhanced antibiotic degradation compared to natural dissipation (-53% vs. -48% over 24 h). Odor emissions were reduced by 20–80%, depending on organic content. Fertilizing properties were preserved, with improved Cu, Fe, and S uptake in plants ($+25$ to $+32\%$).

Conclusions These results highlight a practical, effective method for treating pig sludge, reducing its environmental impact while maintaining agricultural value.

Highlights

- Simple acidic oxidative treatment for in-situ treatment of pig waste.
- Bacterial and amoxicillin-resistant load abated in treated pig effluents.
- Faster (53% compared to 48) veterinary antibiotic dissipation in treated effluents.
- 20–80% reduced unpleasant odor of treated pig effluents.
- Preserved fertility and reduced hazardousness allow agricultural utilization of treated waste.

✉ Sonia Blasioli
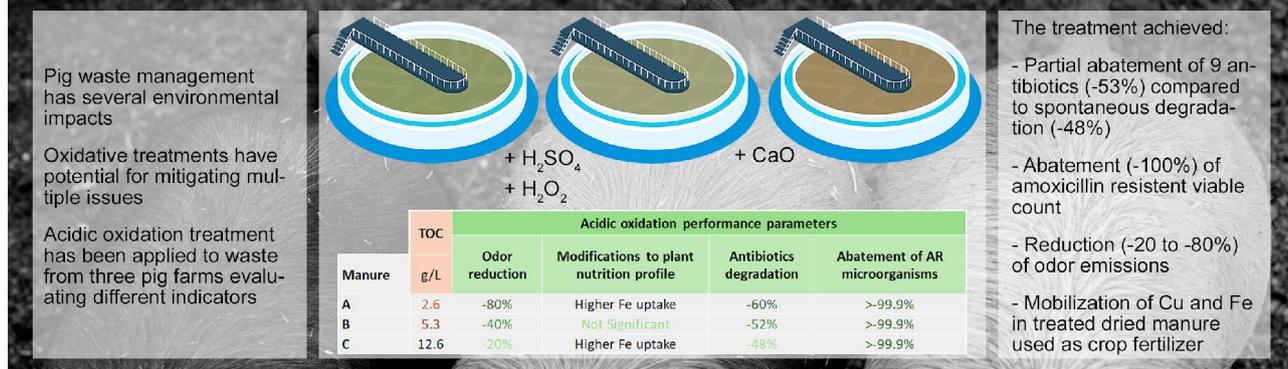sonia.blasioli@unibo.it

✉ Monica Marianna Modesto
monica.modesto@unibo.it

1 Department of Agricultural and Food Sciences, University of Bologna, Viale Fanin 40-50, I-40127 Bologna (BO), Italy

🙋 Springer

Graphical abstract

Effect of acidic oxidation of swine manure on antibiotic-resistant bacterial load, unpleasant odor, veterinary antibiotics, and plant nutrient profile in the one-health perspective

E. Buscaroli, S. Blasioli, M. M. Modesto, L. Cavani, G. Di Biase, D. Scarafile, P. Trevisi, P. Mattarelli, I. Braschi

Introduction

Antimicrobial resistance (AMR) of pathogens is a growing public health concern because it can reduce the effectiveness of antimicrobial drugs, leading to increased morbidity and mortality from pathologies that were previously treatable [1, 2]. Briefly, AMR mechanisms occur through exposition of pathogens to inhibitory concentration of antimicrobials, leading to the selection of population bearing antimicrobial resistance genes (ARGs), as well as through horizontal transfer of genetic material among microorganisms (i.e., integrons) [3, 4].

In the environment, genes and antibiotic resistance genes (ARGs) are frequently transferred among bacteria through horizontal gene transfer. In contrast, low concentrations of antimicrobial substances can induce selective pressure when their levels fall below a threshold known as the minimum inhibitory concentration (MIC) [5]. This situation commonly arises in the presence of certain anthropogenic waste, such as untreated wastewater from antimicrobial manufacturing plants, hospitals, and animal farms [6, 7].

The utilization of veterinary antibiotics (VA) in pig farming is a subject of growing concern in the developed world [1, 8]. Antibiotics play a vital role in maintaining animal health and productivity by preventing and treating infectious diseases [9]. However, their widespread and indiscriminate use in intensive pig production systems raises apprehensions regarding the emergence and dissemination of antibiotic-resistant bacteria, posing significant risks to both animal and human health [10]. The input intensity in pig farms, along with high stocking densities and the presence of several other stressors (such as chronic hunger, painful mutilations, and early weaning) [11], creates an ideal environment for the proliferation of bacterial pathogens. Consequently, the routine administration of VA as growth promoters or prophylactic agents has become commonplace in many countries [12, 13], leading to an increased selection of antimicrobial-resistant pathogen strains.

In the European Union, surveillance data on AMR is available, but data on animal farms is scarce and nonsystematic. VA have been frequently identified in livestock manure and in soil amended with manure where, in some cases, the concentrations have been found to be above MIC, roughly over 100 µg kg^{-1} [14, 15].

In Italy, the amount of VA sold in 2022 (last available data) has been estimated in 157.5 mg of active ingredient per kg of livestock, as indicated in the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) project report issued by the European Medicines Agency [16]. Penicillins, tetracyclines, sulfonamides, and lincosamides account for about 80% of the sold amount. In 2022, pork production in Italy accounted for 8.441 million pigs, about 1.2 million tons of slaughtered mass. Group prophylactic treatments are rather common in Italian farms and larger enterprises are more efficient in antibiotic use [17]. Despite the consistency of the sector and the relevance of the matter, assessments on antibiotic resistance impacts in Italian pig farms are scarce. Petrin et al. [18] assessed the spread of ARGs in environmental samples and pig excreta in two Italian farms. Bonvenga et al. [19] completely sequenced *Escherichia coli* genomes isolated from faecal and environmental samples in a pig farm in Piedmont region (Italy).

Clearly, the management of pig farm effluent plays an extremely important role in mitigating bacterial load, as well as the spread of ARG and VA traces [20, 21]. Among the most promising technologies, advanced oxidation processes (AOPs) are powerful techniques that can be employed in treating pig manure and eliminating several contaminants. AOPs use powerful oxidants to generate reactive oxygen species (ROS), such as hydroxyl radicals, that react with untargeted contaminants, breaking them down into less harmful substances. These processes are particularly effective at destroying persistent organic pollutants, such those antibiotics which are not easily removed by conventional wastewater treatment methods [22, 23]. Some examples of AOPs include ozone-based processes, hydrogen peroxide-based processes, Fe-based Fenton reactions, as well as non-Fe Fenton processes (e.g., in the presence of Cu as a catalyst), and many others. The degradation performance of Fenton processes in organic matter-rich wastewaters can be ameliorated by coupling Fenton process with UV-light, an energy source, ultrasonic waves (photo-, electro-, and sono-Fenton, respectively) or any combination of these [24]. Although AOPs processes have shown great promise for the abatement of VA and ARG, in most cases they are highly costly and not easy to operate. In addition, further research is needed to optimize and finalize these techniques for the treatment of animal effluents and ensure their environmental and economic sustainability. The mere effectiveness of any treatment in abating antibiotic concentration and ARG in animal effluents may not constitute a sufficient incentive for farmers to adopt such technology (in small farms especially). However, any additional benefit deriving from their adoption would contribute to increasing the return on the investment. An additional advantage could lie in the reduction of odor emissions, or the possibility to reuse the treated waste as crop fertilizer.

Swine farms produce significant odor emissions which may adversely impact nearby residents owing to the release of various volatile substances present in swine waste, including hydrogen sulfide, ammonia, volatile organic compounds, and particulate matter [25]. Prolonged exposure to these foul odors can result in respiratory irritations, such as coughing, wheezing, and shortness of breath. Moreover, the offensive odors can cause psychological distress, leading to reduced quality of life and overall well-being among affected individuals [26]. Odor emission management may be tackled with several techniques, such as food supplements, smart ventilation designs, and AOP treatment as well, mostly UV based [27, 28].

In addition, the utilization of animal excreta in agriculture serves as a crucial component of sustainable nutrient management practices, facilitating the recycling of elements back into the soil [29]. A safe and nutrient-rich material readily usable in agriculture should be obtained at the end of any waste management process. Rich in nitrogen, phosphorus, potassium, and other micronutrients, properly managed animal waste replenishes soil fertility, enhances crop yields, and mitigates the reliance on synthetic fertilizers [30]. Processes such as maturation or anaerobic digestion are widely employed to make nutrients more bioavailable [31, 32], reduce the content of pathogens and decrease ARG content as well [33]. It is essential that the final product distributed in the field complies with current regulations to ensure the protection of the environment, public health, and crop welfare [34], and any treatment performed on animal manures should aimed to this goal.

The aim of REFLUA project was to assess the feasibility of an easy-to-perform batch oxidative technique able to tackle multiple environmental issues associated with the management of pig effluents, in the context of the one-health perspective. In this paper, a simple oxidation treatment at acidic pH was applied on effluents of three different pig farms in a lab-scale experiment, serving as proof of concept for an easy to operate in-situ batch treatment. The abatement of total and resistant bacteria, as well as VA, was evaluated after 24 h of treatment. Finally, the reduction of odor emissions of treated manure was assessed using an electronic nose, and the plant nutrients profile investigated by plant growth trials.

Materials and Methods

Pig Manure Sampling, Processing, and Physico-Chemical Characterization

Three farms located in south-western Lombardia region (Italy) were selected as Reflua project partners due to their diverse pig production and management of pig effluents. Each farm oversees the entire lifecycle of pigs, from nursery to fattening, and differs in the type of manure treatment facility employed. On farms' request, they were anonymized and named farm "A", "B", and "C". Figures reported in the next three paragraphs are willingly approximative. A brief description of the manure management of the farms is reported as supporting information.

Throughout the spring of 2021, manure samples were collected from weaning barns, as this is a phase of pig life cycle where extensive antibiotic treatments are mostly administered. Pig manure samples were collected by submerging plastic bottles into collection wells, always closed, of weaning stables. This operation was repeated three times for each farm, with 2 weeks intervals. Following collection, the bottles were stored in the dark into a refrigerated container and promptly transported to the laboratory and stored

Table 1 Physical and chemical characterization of homogenized pig manure samples from weaning barns

Manure sample	pH	Conductivity (mS cm^{-1})	TOC (g L^{-1})	TN (g L^{-1})	Moisture (g kg^{-1})	Ash content (g kg^{-1} dw*)	Volatile solids (g kg^{-1} dw*)	Cu (µg kg^{-1})	Fe (µg kg^{-1})
Farm A	7.85±0.02	26.98±0.37	2.6±0.1	2.5±0.1	946.1±1.4	412±5	588±5	36±4	488±24
Farm B	5.44±0.01	12.89±0.01	5.3±0.9	1.2±0.1	955.4±7.7	242±5	758±5	39±3	546±52
Farm C	6.02±0.01	24.39±0.26	12.6±0.2	2.2±0.0	884.6±68.5	410±132	590±132	40±2	750±51

*dw at 105 °C

at -80 °C. A total of nine samples were obtained (three subsamples per farm). Then, subsamples of each farm were thawed and mixed to form a composite sample. To standardize the composition of each pig manure, the three composite samples were sieved at 2 mm, removing coarse particles such as insect larvae and food residues. Subsequently, the sieved samples were homogenized for 2 min at 9000 rpm by an Ultra Turrax homogenizer mixer (IKA-Werke, Staufen, DE).

The homogenized samples collected from farms A, B, and C were designated as "A", "B", and "C", respectively, and were characterized in terms of pH, electrical conductivity (EC), total organic carbon (TOC), total nitrogen (TN), moisture, dry residue, volatile solids and trace elements. Methods and conditions of the physical and chemical characterizations performed on manure samples are detailed as SI. Data of characterization is reported in Table 1.

Possible residual antibiotic concentration contained in swine samples, due to the therapeutic treatments occurred in the farms, was evaluated by LC-MS analysis as described in *Antibiotic chromatographic analysis Section*.

Acidic Oxidative (AOX) Treatment of Manure

Adapting the management operations commonly performed in urban wastewater treatment plans, manure acidification to pH 3 was performed by adding H_2SO and, at the end of the treatment, the pH was restored to the initial value by CaO to obtain, as a final product, $CaSO_4$, that is a valuable and palatable fertilizer.

The pH of homogenized pig manure samples (25 mL) was adjusted to a value of 3.0±0.1 by adding H_2SO_4 (ACS reagent, 95.0–98.0%, Sigma Aldrich, USA). Each sample was then immediately added with 85 µL of H_2O_2 (40% w/v, Carlo Erba Reagents, Cornaredo, Milano, Italy). After the bubbling ceased (approx. 30 s), the samples were closed and left to shake gently for 24 h in the dark, after that, the pH of each manure sample was quickly restored to the original value by adding known amounts of CaO (Carlo Erba, Italy). Sample aliquots of 50 mL each were withdrawn and immediately analyzed for microbial load.

Experiments were carried out in triplicate. Data was expressed as an average of three replications and standard deviations were calculated.

Microbiological Analysis of Manure Samples

The microbiological assessment was conducted on both treated and untreated manure samples, the latter being just gently shaken in parallel for 24 h as a control.

To assess the bacterial load of each manure, the total viable count (TVC) was measured on 50 mL aliquots of each liquid manure sample. Enumeration of TVC was achieved by pour plating technique. This was done by resuspending 1 mL of each manure sample in 9 mL of sterile saline and by inoculating, in triplicate, 0.3 mL tenfold serially diluted samples onto nutrient agar (Plate Count agar, Merck, Germany) for TVC or onto nutrient agar supplemented with amoxicillin (100 mg/1000 mL) for counts of amoxicillin-resistant bacteria. The inoculated nutrient Agar plates were incubated at different temperatures and conditions: (1) at 37 °C in both aerobiosis and anaerobiosis for 48 h for counting mesophilic bacteria, and (2) at 22 °C in both aerobiosis and anaerobiosis for 72 h to count environmental bacteria.

The anaerobic atmosphere was obtained using the Gas-Pak EZ Anaerobic Pouch system (BD). After incubation, only plates with 20–200 isolated colonies were enumerated. Observed colonies were counted and expressed as colony-forming units per ml (CFU/mL), including the standard deviation calculation.

Selection of VA and LC-MS Analysis in Manure Samples

VA selected for this study were part of the therapeutic plan adopted in the partner pig farms. Antibiotics and their main physico-chemical characteristics are listed, as supporting information, in Table SI1. Amoxicillin trihydrate, doxicycline hyclate, florfenicol, gamithromycin, lincomycin hydrochloride, marbofloxacin, oxytetracycline dihydrate, tiamulin, and tilmicosin were provided as certified standards by A2S Analytical Standard Solutions (France). All standards were in powder form except for tiamulin that was in oil form.

Stock solutions of antibiotics were prepared by dissolving 1 mg of substance in 10 mL of MilliQ® water (in the case of amoxicillin) or in 10 mL of CH_3OH (all other substances, methanol for chromatographic analysis, Sigma

Aldrich, USA) to obtain a concentration of 100 mg L^{-1} for each substance.

Spontaneous Dissipation Tests of VA in Untreated Manure

Homogenized untreated pig manure samples (25 mL) were simultaneously spiked with antibiotics by adding 0.25 mL of both stock solutions, obtaining an initial concentration of 1 mg L^{-1} for each antibiotic to work with values far enough from the analytical limit of quantitation (LOQ $\leq$ 200 μg L^{-1}, as reported in the following *Antibiotic chromatographic analysis* Section), The spiked amount was set similarly for all the antibiotics to make easier the comparison among their dissipation trends.

The spiked manure samples were kept close and in the dark to mimic the conditions experienced by VA residues inside collection wells of weaning stables. Sample aliquots (2 mL each) were withdrawn after 2 and 24 h from spiking, then immediately frozen at -20 °C till LC-MS analysis. Experiments were carried out in triplicate. Data were expressed as the average of three replications and standard deviations were calculated.

VA Abatement in Manure by AOX Treatment

Homogenized pig manure samples (25 mL) were adjusted to pH 3.0 by H$_2$SO$_4$, then spiked with antibiotics (1 mg L^{-1} each), immediately added with 85 μL of H$_2$O$_2$ and finally managed as already described in *Acidic oxidative (AOX) treatment of manure* Section. After 24 h, sample aliquots of 2 mL each were withdrawn and immediately frozen till LC-MS analysis. Experiments were carried out in triplicate. Data were expressed as the average of three replications and standard deviations were calculated.

The antibiotic residues in the treated manure samples were compared to those remaining after 24 h in untreated manure samples, produced as already described in *Spontaneous dissipation tests of antibiotics in untreated manure* Section, as a control.

VA Chromatographic Analysis

All frozen treated and untreated manure samples were delivered to Laemme Group laboratories (Torino, Italy) for LC-MS analysis. The samples were thawed at RT before antibiotic extraction and analysis. Briefly, antibiotic extraction from manures was performed by adding 5 mL of acetonitrile to 5 g of manure sample. The samples were shaken for 10 min in a horizontal shaker at RT and further sonicated for 10 min. After centrifugation at 1000 RCF for 10 min, the supernatant was collected and purified with 250 mg

Bondesil C18 bulk sorbent. Extracts were filtered through 0.45 μm PVDF syringe filters and stored at -20 °C until analysis. The recovery efficiencies of antibiotics in manure samples were 77% (amoxicillin), 84% (doxycycline), 101% (florfenicol), 87% (gamithromycin and lincomycin), 103% (marbofloxacin), 89% (oxytetracycline), 78% (tiamulin), and 88% (tilmicosin).

The extracts were analysed by a UHPLC Ultimate 3000 liquid chromatograph coupled with a high-resolution Q-Exactive Focus mass spectrometer (Thermo Fisher Scientific, USA) as detailed, as supporting information, in table SI2. The Limit Of Quantitation (LOQ) of the antibiotics was 100 μg L^{-1} of manure, except for 150 μg L^{-1} for lincomycin and marbofloxacin, and 200 μg L^{-1} for gamithromycin.

E-nose Analysis of Manure Samples

The analysis of the volatile mixture released by the manure samples was carried out with a commercially available portable e-nose (PEN3, Airsense Analytics GmbH, Germany). PEN3 consists of a sampling apparatus, a detector unit containing the sensor array, and a pattern recognition software (WinMuster v.1.6.2) for data recording. The sensor array contains 10 metal oxide semiconductor sensors working in the 150–500 °C range of temperature, which is not adjustable by the operator, to ensure a correct classification and identification of volatile species. Each sensor (Sn) responds to a class of organic compounds: S1 and S3 are specific for the aromatic compounds' detection, S4 for hydrogen, S5 for aromatics and aliphatics, S7 and S9 for sulfur- and chloroorganic compounds, respectively, and S10 for methane and aliphatics. Sensors S2, S6, and S8 respond to a broad range of organic substances. The sensor response is expressed as resistivity (Ω). Conditions of sample collections are detailed in SI.

All samples were analyzed in quintuplicate. Principal Component Analysis (PCA) [35] was used for the statistical analysis of data using a covariance matrix to build the PCA plot. The software WinMuster v.1.6.2 was used for chemometrics analysis.

Plant Growth Pot Trials

A pot trial using washed sand and maize (Zea mays L. CV KWS Eldorado 105) was set up using 8 different treatments: negative control CK- (no nutrient addition), positive control CK+ (50% Hoagland solution), untreated pig manure samples from farms A, B and C, and AOX treated samples, called A_AOX, B_AOX, and C_AOX, respectively. The same amount of N was added to each pot (excluding negative control) as follows: all the pig manure samples were diluted with MilliQ® water to reach a concentration of 100

mg L^{-1} of total N, and then 150 mL of these solutions were added to the pots, thus resulting in 15 mg N pot^{-1}. Additional information on N dosage is reported as supporting information in table SI3.

Plants were grown for 3 weeks in a climate chamber under controlled conditions: 16 h, 25 °C, 70% relative humidity (RH) during the day; 8 h, 13 °C, 70% RH during the night. Soil was kept at 60% water holding capacity during the experiment by weighing the pots every other day and adding, if necessary, water. Four replicates were prepared for each treatment and organized with a completely randomized block design.

At the end of the trial, maize plants were collected for biomass assessment and concentration of micro- and macro-nutrients analysis as detailed as supporting information (see Table SI3).

The shoot N uptake (N_{upt}) per pot was calculated as follows:

$N_{upt}(mg\ pot^{-1}) = Shoot\ Dry\ Matter\ (g) \times Shoot\ N\ concentration\ (mg\ g^{-1}dw)$

The apparent N recovery (ANR) was calculated and expressed as a percentage of the total N applied (N_{app}) to by the fertilizer:

$ANR\ (\%) = 100 \times (N_{upt}treatment - N_{upt}negative\ control) / N_{app}$

Data handling and statistical analysis were performed using R version 4.3.1 [36]. Pot experiments followed a completely randomized design, and one way analysis of variance (ANOVA) was carried out. The results are presented as means of our replicates ± standard errors (SE). The ANOVA assumptions were verified through Bartlett's test for homogeneity of variances and Shapiro-Wilk's test for normality of distributions. Post hoc HSD Tukey's test was performed to investigate differences between treatments when ANOVA returned a significant global test. The significance of all tests was assessed at $\alpha = 0.05$. Data are expressed on oven dried basis.

Results and Discussion

Physico-Chemical and Microbiological Characterization of Manure

As reported in Table 1, the parameters of the manure samples collected at the three farms indicate wide variations in the values of pH (5.44–7.85), conductivity (12.89–26.98 mS cm^{-1}), total organic carbon (2.6–12.6 g L^{-1}), and total nitrogen (1.2–2.5 g L^{-1}). Such differences could mainly depend on the different dietary uptakes. Creep-feeding is a settled practice that consists of introducing solid animal food during the breastfeeding phase for furnishing nutritional support to the piglets [37]. The variety of animal foods present on the market could affect the manures' pH as well as TOC and TN values. As expected, pig effluents were essentially composed of water, as confirmed by values of moisture ($\geq$88%), with a composition of the residue at 105 °C $\leq$41% of ash content and $\leq$76% of volatile solids.

No detectable amount of antibiotics was measured in the manure samples, with the exception of doxycycline, whose residual concentration in manures A, B and C resulted to be detectable but lower than the LOQ (see *Antibiotic chromatographic analysis Section*), namely, 0.042, 0.172 and 0.104 mg L^{-1}, respectively.

Concerning the microbiological assessment of the effluents, 22 and 37 °C growth conditions were selected as indicative of environmental and clinical relevance bacteria, respectively. As reported in Table 2, the average bacterial load of the three matrices resulted high for both aerobic and anaerobic species growing either at 22–37 °C. Except for anaerobic bacteria growing at 22 °C in manure C (TVC = 3.8 log CFU mL^{-1} of manure), the bacterial loads ranged between 4.8 and 5.8 log CFU mL^{-1}, thus indicating highly biologically active environments.

Amoxicillin-resistant bacteria were assessed because of the common use of this antibiotic in pig production. The TVC of resistant bacteria, ranging between 3.5 and 5.6 log_{10} CFU mL^{-1}, partially accounted for the total bacteria in the pig manures with a few exceptions. In fact, amoxicillin-resistant aerobic and anaerobic bacteria growing at 22 and 37 °C represented a significant fraction of the total bacteria

Table 2 Total viable count (TVC) assessment of pig manure samples from weaning barns. All data are mean expressed as log_{10} colony forming unit per mL (CFU mL^{-1}) of manure. Standard errors were always two orders of magnitude lower than their mean

Manure sample	TVC aerobic bacteria	TVC Amoxicillin resistant aerobic bacteria	TVC anaerobic bacteria	TVC Amoxicillin resistant anaerobic bacteria	TVC aerobic bacteria	TVC Amoxicillin resistant aerobic bacteria	TVC anaerobic bacteria	TVC Amoxicillin resistant anaerobic bacteria
	22 °C				37 °C			
Farm A	5.4	3.7	5.3	3.7	5.1	5.0	5.7	5.5
Farm B	5.7	5.3	4.8	4.2	5.7	5.6	5.6	5.6
Farm C	5.1	4.2	3.8	3.5	5.2	3.9	5.4	4.9

in manure B, and a similar trend was observed for the aerobic resistant bacteria growing at 37 °C in manure A.

Microbial Abatement by AOX Treatment

Figure 1 shows the microbial counts of total and amoxicillin-resistant bacteria, both aerobic and anaerobic, kept at 22 and 37 °C, in 24 h treated and untreated manure samples (red and gray dots, respectively) from farms A, B, and C.

In farm A, control manure samples showed a total viable bacteria count of about 10^5 CFU mL^{-1}. The single treatment resulted effective in killing all aerobic and anaerobic bacteria growing at 22 °C, including the amoxicillin-resistant ones. A similar figure was observed for bacteria grown at 37 °C, whereas only a biocidal effect was detected on the total anaerobic component that underwent a 2-log reduction in viable counts.

In Farm B, control manure samples showed total aerobic and anaerobic bacteria counts of about 10^5 CFU mL^{-1}. The treatment decreased by 3-log the total viable counts of anaerobic bacteria growing at both 22 and 37 °C. Differently, the aerobic bacterial growth at both temperatures was unaffected by the treatment. Otherwise, the treatment resulted effective in killing aerobic and anaerobic amoxicillin resistant bacteria, able to grow at both 22 and 37 °C.

In farm C, total viable counts of aerobic and anaerobic bacteria at 22 and 37 °C treated samples showed an average 1-log reduction respect to control samples. However, the treatment resulted effective in killing all amoxicillin resistant aerobic and anaerobic bacteria at 22 and 37 °C.

Generally, the observed total viable bacterial load reduction (for both anaerobic or aerobic bacteria growing at 22–37 °C) was highest in manure A and lowest in manure C. These figures suggest a matrix-dependent effect, but a microbiota-dependent effect cannot be excluded. A complete microbiota population characterization via high throughput sequencing techniques should elucidate this aspect in further tests.

On the contrary, a complete abatement of amoxicillin-resistant anaerobic and aerobic bacteria growing at both temperatures was observed in all the treated manure samples. This evidence could be explained by the higher susceptibility of antibiotic-resistant bacteria to environmental stressors, such as ROS actions and acidic conditions, with respect to non-resistant ones. In fact, the high metabolic effort spent by resistant bacteria to maintain resistance genes [10] might have gone to the detriment of their general defense from environmental agents.
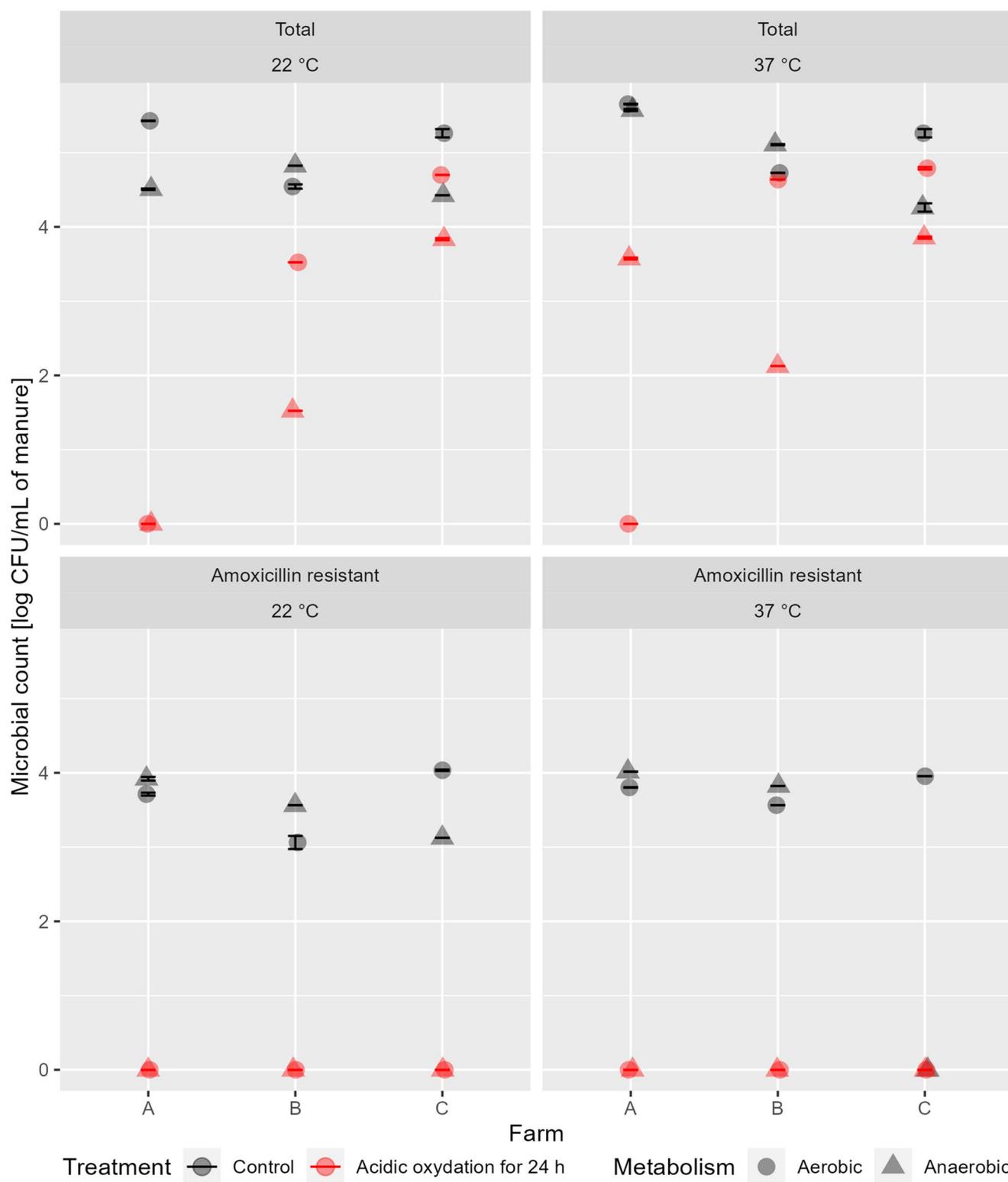
VA Spontaneous Degradation Tests

The spontaneous degradation of VA in pig manures was investigated on untreated samples spiked with 1 mg L^{-1} of each antibiotic at 2 and 24 h after spiking. Doxycycline had a slightly higher initial content due to its presence in the unaltered samples (namely 1.042, 1.172 and 1.104 mg L^{-1} in manure A, B, and C, respectively).

The VA concentration chosen for carrying out the investigation was higher than that typically detected in environmental conditions for certain antibiotics such as lincomycin but was similar, if not lower, for other ones such as oxytetracycline and doxycycline, generally, present in extremely high concentrations in pig farm fecal samples [38, 39].
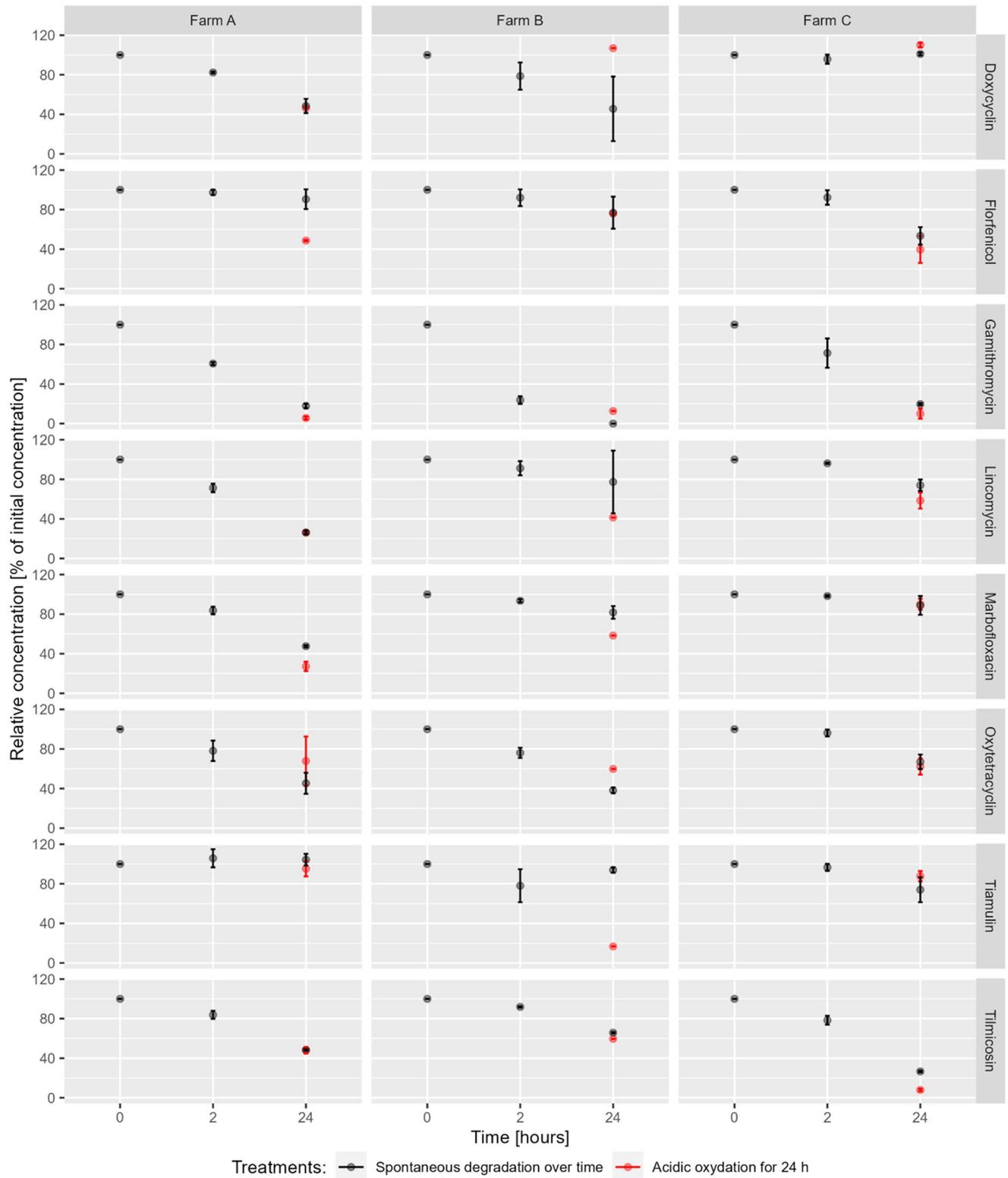
Figure 2 shows, as black dots, the antibiotics' spontaneous dissipation after 2 and 24 h in the manure samples. Amoxicillin was omitted in the figure because it was not detected in all the samples already after 2 h from spiking. Its known fast disappearance in animal waste is mainly due to hydrolysis [40] according to the Veterinary Substances Database – VSDB. Doxycycline antibiotic showed a similar dissipation trend in manures A and B: in sample A the molecule was degraded by -20% at 2 h and up to -50% at 24 h, whereas no reduction was observed in manure C. Florfenicol was found quite persistent in manure A (-10% after 24 h), underwent slow dissipation in manure B (-20% at 24 h), and halved in manure C samples over the same period. A fast degradation was observed for gamithromycin in all the three effluents: after 24 h since treatment, its residue was −80% in A and C, and no residue was detected in sample B. The dissipation was also rapid for lincomycin in manure A (-70% at 24 h), whereas it was slower in samples B and C (20–25% of reduction at 24 h). Marbofloxacin concentration halved after 24 h in manure A, slowly declined in B (-20%), and remained stable in sample C. Oxytetracycline spontaneously degraded until its concentration halved at 24 h in A and B (-50 and −60%, respectively), while the degradation was slower in C manure (-30%). Tiamulin resisted spontaneous degradation in all the samples although a slight decrease (-25%) was observed in manure C at 24 h. Finally, the degradation of tilmicosin after 24 h in the three manures followed the order B<A<C, with degradation of -30, -50, and −70%, respectively.

A general fast dissipation (DT50<1 d) of amoxicillin, gamithromycin, oxytetracyclin, and tilmicosin was observed in the three pig manures. Owing to the wide pH range covered by our manure samples (5.44–7.85, Table 1), it seems reasonable to generalize their low persistence in any kind of pig manure. On the contrary, the dissipation rate of the other antibiotics (namely, doxycycline, florfenicol, lincomycin, marbofloxacin, and tiamulin) widely varied among the manure samples, thus indicating strong dependence on

Fig. 1 Microbial counts of total (top panels) and amoxicillin resistant (bottom panels) bacteria in **A**, **B**, and **C** farms effluents treated with acidic oxidation for 24 h (red data points) and their respective 24 h controls (black data points). Effluents have been incubated both at 22 °C and 37 °C (left and right panels, respectively)

Fig. 2 VA degradation occurred spontaneously (black dots) and under AOX treatment (red dots) in manures **A**, **B**, and **C** for 24 h. Black and red dots are mean values ($n = 3$), and error bars represent standard errors

the matrix context. Among the substances investigated, tiamulin was the most persistent and its insolubility in water (see SI1) could reasonably be considered the main impediment to microbial degradation. In fact, insoluble substances are scarcely bioavailable for microbial transformations. These pieces of information on antibiotic persistence in pig manure are of utmost importance to evaluate their eventual input to soils through manure spreading and, from there, their potential leaching to water bodies.

On average, manure A was slightly more active than B and C in dissipating the nine antibiotics considered (-52.4, -46.8, and −43.9%, respectively) in a 24 h time lapse.

VA Abatement by AOX Treatment

Briefly, in Fenton reaction, hydrogen peroxide produces ROS under acidic conditions in the presence of Fe ions, or Cu ions in not-Fe Fenton process, as a catalyzer. The acidic pH, usually in the range 2–4, allows maintaining Fe and Cu ions in soluble forms.

In this study, the Fe and Cu levels "naturally" contained in the manure samples (Table 1) were exploited to catalyze the H_2O_2 transformation with a ratio of $H_2O_2/Fe + Cu \geq 1.4$ mol/mol.

In Fig. 2, the antibiotic residues observed after 24 h treatment in the three manure samples are reported as red dots. Doxycyclin reduction in sludge A was similar to that observed spontaneously (black dots), while in sludges B and C there was no reduction at all. In this case, the treatment efficacy against antibiotics resulted to be worse than that observed in doxycycline natural degradation. This behavior was not surprising in that doxycycline is known for its high chemical stability and its biotic degradation pathway [41]. The ROS produced by treatment, as well as the acidic conditions, are known to be active against organic xenobiotics (e.g. our antibiotics) but also against microbial membranes [42]. The massive oxidation of organic structures, produced by investigated conditions, showed a double effect: on one side, the abiotic degradation of antibiotics, and, on the other, a strong decrease in their biotic degradation due to the abatement of microorganisms, previously described.

Florfenicol residual amount at 24 h treatment was 48.7, 75.9, and 39.7% in effluents A, B and C samples, respectively. Only in manure A, the treatment performed better than the spontaneous degradation. Conversely, gamithromycin almost completely degraded in all three samples (5.7, 12.7, and 10.0% of initial amount persisted in A, B and C, respectively), similarly to spontaneous degradation control tests at 24 h.

Residues of lincomycin (26.0, 41.3, and 58.6% persisting after treatment in manure A, B and C, respectively), marbofloxacin (27.1, 58.3, and 90.0%), oxytetracycline (67.9,

59.8 and 62.3%) and tilmicosin (47.9, 59.6, and 8.83%) after treatment were similar to those persisting over 24 h spontaneous dissipation. Finally, tiamulin amount persisted after 24 h treatment was 95.2, 16.7 and 87.1% (manure A, B and C, respectively). Only in sample B, the tiamulin final concentration was consistently lower (red dots) than the spontaneous degradation control (black dots).

The treatment performed differently among the three manure types. Including amoxicillin, the antibiotic degradation followed the order: manure A (-60%) > manure B (-52%) > manure C (-48%). The different results could be related to the TOC values of the manure samples: low in manure A (2.6 ± 0.1 mg kg^{-1}), medium in manure B (5.3 ± 0.9 mg kg^{-1}), and high in manure C (12.6 ± 0.2 mg kg^{-1}) (Table 1). In fact, the ROS produced by the treatment can massively degrade any organic substance, and the higher the organic matter level in the aqueous solution, the lower is the antibiotic abatement efficiency.
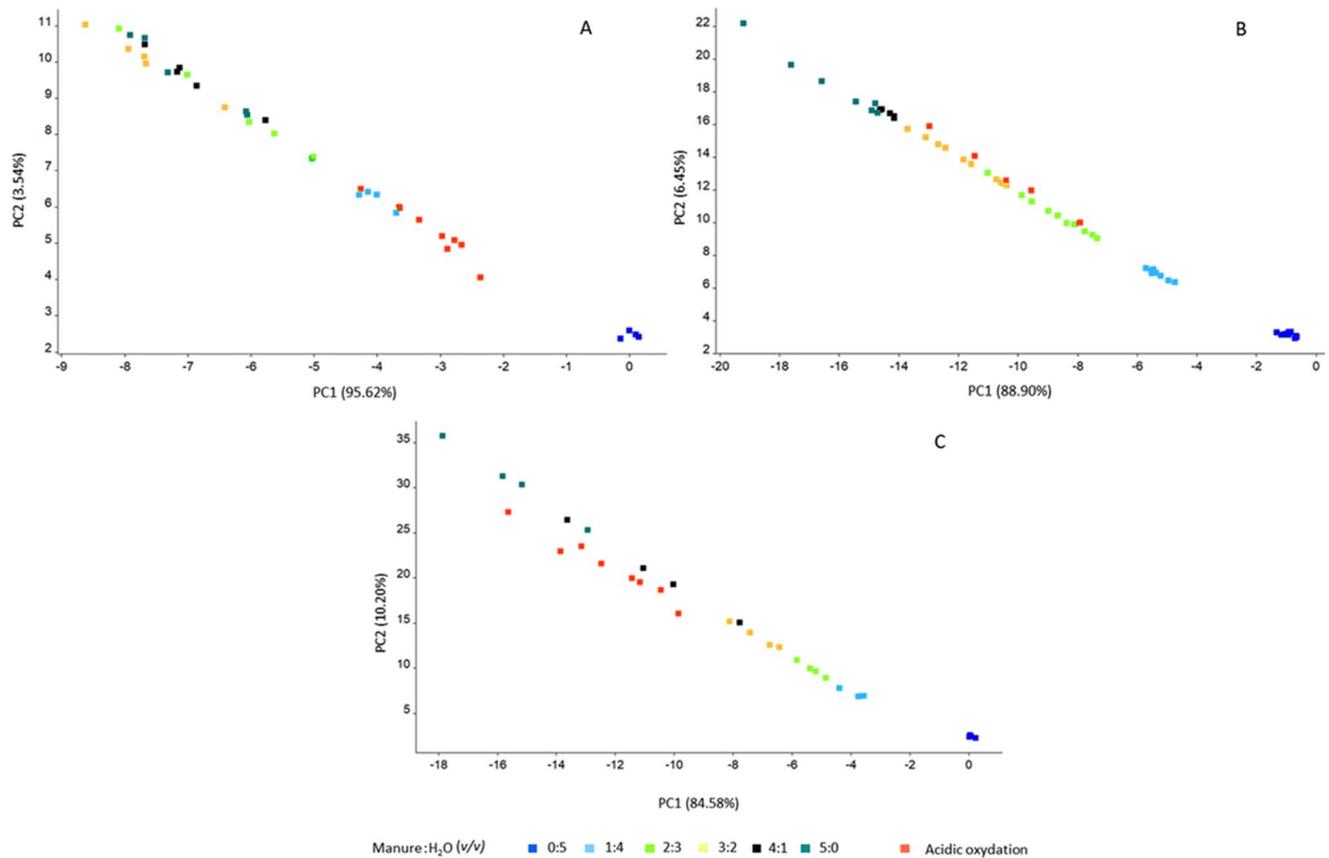
On average, the reduction of the selected VA, including amoxicillin, after 24 h treatment showed a slightly better performance (-53%) compared to the spontaneous reduction observed in the untreated manure over the same period (-48%). This finding is particularly significant given that the drastic abatement of microorganisms obtained by the treatment was expected to slow down the biotic degradation. Therefore, the simultaneous abatement of VA residues, bacterial populations, and antibiotic-resistant species, operated by the treatment, could be considered additional benefits to contain the antibiotic-related environmental issues.

Odorous Emission Abatement by AOX Treatment

The composition of the volatile fraction of pig manure is well documented. The typical smell of this organic matrix is due to a mixture of volatile organic acids, aromatics, nitrogen and sulfur compounds, as well as aldehydes [43]. The effect of ozone and H_2O_2-based treatment on these volatile substances is also well acknowledged in literature [44].

In our study, the odor of the treated manure samples was perceived as strongly reduced with respect to the controls. To overcome the measurement of specific volatile components profile in our samples and to assess the general variation of odor emission between treated and untreated manure from each farm, for the first time an e-nose analysis was set-up.

First of all, the three untreated control samples were diluted with increasing amounts of distilled water according to manure: water ratios of 5:0, 4:1, 3:2, 2:3, 1:4, and 0:5 (v: v). The six step dilution was necessary to create an odor scale from the most intense (pure manure, manure: water 5:0 v: v) to no odor (pure water, manure: water 0:5). For each manure, the samples representing the whole dilution

Manure:H₂O (v/v)   ■ 0:5   ■ 1:4   ■ 2:3   ■ 3:2   ■ 4:1   ■ 5:0   ■ Acidic oxydation

Fig. 3 Score plot in the plane defined by the first two principal components of volatile compounds from **A**, **B** and **C** manures at different dilution levels. The response of volatiles emitted by AOX treated manures is also reported as light red pots

scale, from pure manure to pure water, were analyzed by e-nose. Figure 3 shows the score plot of the principal component analysis (PCA) of the sensorial data collected on the control samples at different levels of dilutions (blue dots: distilled water; teal: undiluted manure sample; azure, lime, yellow and black: diluted manure samples, from most to the least diluted sample) as well as the treated manure samples (red dots). The score plots were built using the first two principal components which explained 99.16, 95.35, and 94.78% of total data variance for A, B and C sample manures, respectively.

For all the manure samples, the variables related to the sensors specific for the detection of hydrogen (S4), sulfur- and chloro-organic compounds (S7 and S9, respectively), methane and aliphatics (S10), as well as broad range S2 sensor variable for A and C manure samples only, were excluded from the PCA analysis because their signals smoothed over the differences among samples. Based on the sensors selected, the volatile compounds mainly responsible for the discrimination among samples were the aromatic and aliphatic fractions. The relationship among the selected Sn variables, and their influence on the data analysis system,

is reported in supporting information (Figure SI3) as loading plot.

As shown in Fig. 3, for all samples, the points on the score plot were linearly distributed in space as the concentration of manure decreased. In sample A, the discrimination could be appreciated in the range of dilution 2:3 − 0:5 manure: water ratio. At lower dilutions (from manure: water ratios 3:2 to 5:0), it was not possible to unambiguously discriminate the samples. In samples B and C, the responses of the sensors to different dilutions were sufficiently discriminated along the whole dilution scale (from 5:0 to 0:5 ratios).

Once the odor scale was built, the treated samples were analyzed and represented as red dots in Fig. 3. In manure A (Fig. 3A), the treated samples fell in the same spatial region as the most diluted control samples (manure: water 1:4 ratio), thus confirming a substantial reduction in odorous molecules of about −80% after treatment. In B treated manure (Fig. 3B), the odor reduction was approximately −40%, as the red dots fell between the manure: water ratios of 3:2 and 2:3 samples. Finally, in treated sample C, the reduction was about −20% since, as observed, the red dots mainly overlapped the cluster of untreated samples with a dilution ratio of 4:1 manure: water.

Table 3 Height, shoot dry weight, root dry weight and shoot/root ratio of maize plants grown in sand with negative control (CK-), positive control (CK+), untreated pig manure from farms A, B, C and treated pig manure from farms A (A_AOX), B (B_ AOX), C (C_ AOX). Data are reported as means and standard error (dw=dry weight). F=equality test of variance. MSE=mean standard error. Different letters indicate statistically different values within each column at HSD Tukey test ($P<0.05$). NS, *, **, *** not significant, or significant at $P\leq0.05$, 0.01, 0.001, respectively

Factor	Height (cm)	Shoot (g dw pot^{-1})	Root (g dw pot^{-1})	Shoot/ Root ratio
CK-	43.3±0.8 b	0.451±0.033 c	0.478±0.011	0.94±0.05 c
CK+	54.6±1.0 a	0.796±0.048 a	0.609±0.011	1.30±0.06 ab
A	52.4±0.7 a	0.640±0.046 ab	0.517±0.052	1.26±0.11 ab
A_AOX	53.6±0.6 a	0.659±0.030 ab	0.546±0.015	1.21±0.06 ab
B	52.4±1.1 a	0.645±0.060 ab	0.529±0.029	1.21±0.06 ab
B_AOX	52.9±0.8 a	0.676±0.034 ab	0.488±0.030	1.39±0.04 a
C	50.3±3.0 a	0.573±0.073 bc	0.517±0.049	1.10±0.07 bc
C_AOX	51.9±1.8 a	0.645±0.045 ab	0.493±0.028	1.31±0.04 ab
F_{7-21}	5.68***	7.94***	1.63^{NS}	6.34***
MSE	1.5	0.048	0.03	0.06

Table 4 Shoot C and N, root C and N of maize plants grown in sand with negative control (CK-), positive control (CK+), untreated pig manure from farms A, B, C and treated pig manure from farms A (A_AOX), B (B_ AOX), C (C_ AOX). Data are reported as means and standard error (dw=dry weight). F=equality test of variance. MSE=mean standard error. Different letters indicate statistically different values within each column at HSD Tukey test ($P<0.05$). NS, *, **, *** not significant, or significant at $P\leq0.05$, 0.01, 0.001, respectively

Factor	Shoot C (mg g^{-1} dw)	Root C (mg g^{-1} dw)	Shoot N (mg g^{-1} dw)	Root N (mg g^{-1} dw)
CK-	38.4±0.5 ab	33.4±0.3	1.70±0.07 b	0.90±0.04 b
CK+	39.4±0.3 a	31.5±0.7	2.53±0.09 a	1.17±0.03 a
A	37.6±0.4 ab	31.3±1.3	2.04±0.10 b	1.07±0.06 ab
A_AOX	37.2±0.4 b	32.1±0.9	2.04±0.06 b	1.04±0.02 ab
B	37.6±0.3 ab	33.0±0.3	2.06±0.15 b	1.09±0.06 ab
B_AOX	36.9±0.2 b	32.7±0.9	1.82±0.04 b	1.08±0.05 ab
C	37.1±0.6 b	31.7±0.7	2.03±0.09 b	1.05±0.04 ab
C_AOX	37.7±0.6 b	33.8±0.3	2.09±0.10 b	1.13±0.07 a
F_{7-21}	4.33**	1.39^{NS}	7.01***	2.86*
MSE	0.5	0.8	0.09	0.05

As expected, the efficacy of odor abatement in the treated manure samples followed the trend of bacteria abatement as already described in *Microbial abatement by AOX treatment Section* (manure A > manure B > manure C). In fact, bacteria are known to be the main responsible organisms for producing noxious volatile compounds (namely, indole, scatole, phenols, cresole, formaldehyde, acetaldehyde, butanal) in pig manure [43]. Therefore, the higher the bacteria abatement the better was the odorous molecules abatement in pig manures.
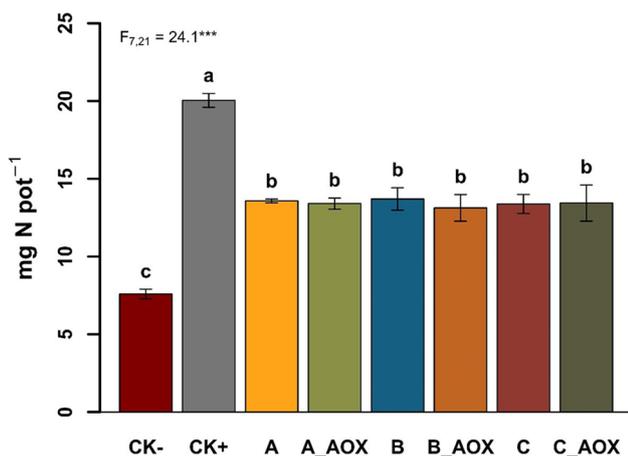
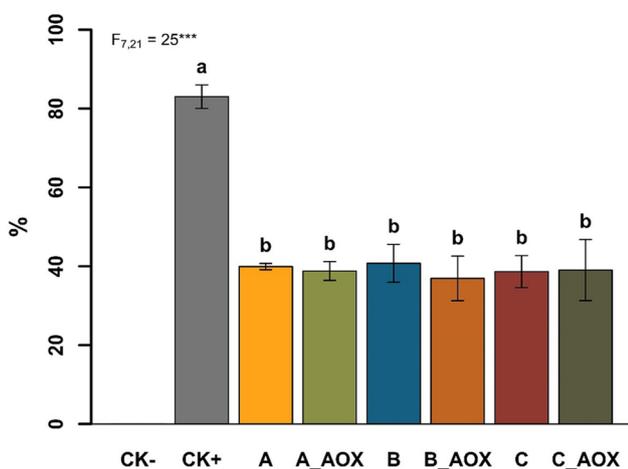## Pot Growth Trials on Substrate Enriched by AOX Treated Manure

Table 3 summarizes the growth parameters of maize plants grown in substrates enriched with untreated and AOX treated manure from the three different farms. All three effluents (either untreated or treated) produced plants of average height between 50.3 (C) and 53.6 cm (A_AOX) not significantly lower than the positive control CK+ (54.6 cm). As expected, negative control CK- resulted in plants of significantly lower height (43.3 cm). A similar result is observed for shoot biomass: all manure-enriched theses (apart from C) produced slightly less biomass than CK+ (0.796 g), but the difference was not significant. Pots C only produced 0.573 g of shoot biomass, somewhat in between the other theses and the negative control (0.451 g). Conversely, root weight differences were never significantly different among treatments. Finally, the shoot/root index, which is an indicator of nutrient stress, favored B_AOX (1.39) and penalized C (1.10), with all the other theses and the positive control in-between these two values and the negative control scoring lowest (0.94).

Overall, considering maize plant carbon content, differences among the theses were very low (Table 4). With respect to shoots, negative and positive controls were not significantly different under Tukey's pairwise comparison (mean CK- and CK+ shoot C content was 38.4 and 39.4 mg g^{-1} dw, respectively), and all effluent-enriched pots had similar shoot C content means (between 36.9 and 37.7 mg g^{-1} dw). Even more so, shoot carbon content was not significantly different among the control theses at all.

On the other hand, shoot or root nitrogen content showed a clear difference between negative (1.70 mg g^{-1} shoot dw and 0.90 mg g^{-1} root dw) and positive (2.53 mg g^{-1} shoot dw and 1.17 mg g^{-1} root dw) control. Either considering shoot or root nitrogen content, the effluent-enriched pots scored between CK- and CK+, with little to no differences among them. This is also confirmed by the results shown in Fig. 4, where total nitrogen uptake for each thesis is represented in a bar graph. Negative control maize plants took up from substrate 7.59 mg of N per pot on average, while positive control took up 20.04 mg of N per pot (corresponding to 83% of N added by fertilizing, Fig. 5). All maize plants from manure-enriched pots took up 13.1 to 13.7 mg of N per pot on average, corresponding to an apparent N recovery (ANR) of 36.9 to 40.8% of nominal N added. All manure-based theses total nitrogen uptake was significantly different from both negative and positive control but not significantly different from each other.

Fig. 4 Nitrogen uptake for maize shoot fertilized with different pig manure: negative control (CK-), positive control (CK+), untreated pig manure from farms A (**A**), B (**B**), C (**C**), and treated pig manure from farms A (A_AOX), B (B_AOX), C (C_AOX). Data are reported as means and standard error (dw=dry weight). F=equality test of variance. Different letters indicate statistically different values within each column at HSD Tukey test ($P \le 0.05$). NS, *, **, *** not significant, or significant at $P \le 0.05$, 0.01, 0.001, respectively

Fig. 5 Apparent nitrogen recovery for maize shoot fertilized with different pig manure: negative control (CK-), positive control (CK+), untreated pig manure from farms A (**A**), B (**B**), C (**C**), and treated pig manure from farms A (A_AOX), B (B_AOX), C (C_AOX). Data are reported as means and standard error. F=equality test of variance. Different letters indicate statistically different values within each column at HSD Tukey test ($P \le 0.05$). NS, *, **, *** not significant, or significant at $P \le 0.05$, 0.01, 0.001, respectively

Maize uptake of the other macronutrients P and K is shown in Table 5. The P uptake showed no significant difference among any thesis whatsoever. In any case, P and K taken up by maize plants peaked in CK+ pots (1.02 and 6.01 mg pot^{-1}, respectively) and had the lowest values in CK- pots (0.92 and 3.49 mg of P and K per pot, respectively), while

all manure-enriched pots had intermediate means with no significative difference among them.

Mesonutrients S, Ca, and Mg, as well as micronutrients Fe and Cu, are also reported in Table 5. Interestingly, S, Fe, and Cu uptake was slightly higher in pots added with AOX treated manure with respect to pots added with untreated manure. In fact, maize plants in pots enriched with farm A manure took up 0.81 mg of S, 3.74 µg of Cu, and 82.9 µg of Fe per pot, while maize plants in pots enriched with A_AOX manure took up 1.03 mg of S (+27.1%), 4.87 µg of Cu (+30.2%), and 131 µg of Fe (+58%) per pot. Similar improvement was observed for B and C labelled samples. The uptake of plants in pots B was 0.95 mg of S, 5.09 µg of Cu and 105 µg of Fe per pot, while B_AOX plants uptake was 1.13 mg of S (+32.6% significantly more), 5.21 µg of Cu and 143 µg of Fe per pot. Similarly, C-AOX plants took up +32% of S, +25% of Cu, and +85.9% of Fe with respect to control C plants per pot.

Concerning the increase of sulfur uptake, this was reasonably due to H_2SO_4-based pH correction on treated sludge, which enriched the sludge of said element. In the case of Cu and Fe, the reasons for a higher metal uptake need a deeper and specific investigation. Nevertheless, several interdependent effects induced by the acidic oxidative treatment can be considered responsible for such a higher plant uptake. First, the treatment acidity (pH 3) might have solubilized more Fe and Cu from the organic and inorganic components of manure with respect to their bioavailability in control sample. Secondly, the relevant amount of calcium ions introduced in the manure samples in form of CaO for pH restoration might have occupied a large part of cation exchange capacity of the matrix, impeding Cu and Fe to be re-adsorbed or complexed again, and thus enhancing their bioavailability. Lastly, the H_2O_2-based oxidation might have partially destroyed the organic moieties able to complex metals (e.g., carboxylic, amino, hydroxylic groups) [45], thus improving their bioavailability in the treated manure.

Conclusions

Swine manure poses environmental challenges due to its high bacterial load, including antibiotic-resistant strains, the presence of antibiotics, and unpleasant odors, which complicate its use as fertilizer. This study presents a simple acidic oxidative treatment, designed for in-tank application during the weaning phase—when antibiotic use is highest and manure volume lowest—to improve manure safety for agricultural reuse.

In three manure types differing in pH and total carbon, veterinary antibiotics dissipation varied by matrix, with amoxicillin fully degraded and tiamulin persistent,

Table 5 Phosphorous (P), sulfur (S), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu) and iron (Fe) uptake of maize plants grown in sand with negative control (CK-), positive control (CK+), untreated pig manure from farms A, B, C and treated pig manure from farms A (A_AOX), B (B_AOX), C (C_AOX). Data are reported as means and standard error (dw = dry weight). F = equality test of variance. MSE = mean standard error. Different letters indicate statistically different values within each column at HSD Tukey test ($P < 0.05$). NS, *, **, *** not significant, or significant at $P \le 0.05$, 0.01, 0.001, respectively

Factor	P (mg pot^{-1})	S (mg pot^{-1})	K (mg pot^{-1})	Ca (mg pot^{-1})	Mg (mg pot^{-1})	Cu (µg pot^{-1})	Fe (µg pot^{-1})
CK-	0.92 ± 0.05	0.57 ± 0.03 d	3.49 ± 0.25 b	3.16 ± 0.18 b	1.58 ± 0.08 c	2.28 ± 0.21 d	102 ± 5 ab
CK+	1.02 ± 0.06	1.23 ± 0.09 a	6.01 ± 0.40 a	5.32 ± 0.22 a	3.44 ± 0.13 a	4.45 ± 0.23 ac	107 ± 8 ab
A	0.88 ± 0.05	0.81 ± 0.01 cd	4.52 ± 0.45 ab	4.51 ± 0.28 ab	2.60 ± 0.10 b	3.74 ± 0.15 c	82.9 ± 10 b
A_AOX	0.95 ± 0.06	1.03 ± 0.04 ac	4.14 ± 0.33 ab	5.05 ± 0.10 a	2.76 ± 0.07 ab	4.87 ± 0.16 ac	131 ± 4 a
B	1.02 ± 0.08	0.95 ± 0.06 bc	4.64 ± 0.60 ab	4.70 ± 0.37 a	2.76 ± 0.20 ab	5.09 ± 0.44 ab	105 ± 13 ab
B_AOX	1.13 ± 0.05	1.26 ± 0.05 a	4.47 ± 0.40 ab	5.15 ± 0.23 a	2.88 ± 0.15 ab	5.21 ± 0.10 a	143 ± 11 a
C	0.93 ± 0.05	0.88 ± 0.03 c	4.00 ± 0.83 ab	4.23 ± 0.42 ab	2.45 ± 0.20 b	3.97 ± 0.19 bc	75.3 ± 6 b
C_AOX	1.04 ± 0.08	1.16 ± 0.07 ab	4.47 ± 0.40 ab	4.68 ± 0.28 a	2.74 ± 0.17 b	4.98 ± 0.27 ab	140 ± 9 a
F_{7-21}	1.53^{NS}	18.8***	2.28*	5.68***	12.5***	15.7***	7.43***
MSE	0.06	0.05	0.48	0.29	0.15	0.25	9.3

highlighting the importance of understanding their environmental fate. A single 24-hour treatment reduced bacterial load (100% for amoxicillin-resistant strains), antibiotics residues (-53% vs. -48% from natural dissipation), and odor (-50%). Abatement efficiency was inversely related to organic content. Where needed, treatment repetition can enhance performance.

The preserved fertility of the treated effluent, together with a meliorative effects on S, Cu and Fe uptake for maize, as a model plant, makes this animal waste more valuable for its use as a fertilizer or soil amendment.

Although further research is needed to assess the feasibility, efficacy, financial sustainability, and scalability of the proposed acidic oxidative treatment, the obtained results pave the way to a more general assessment of animal manure that is comprehensive of the numerous environmental issues connected with these matrices in the one-health perspective.

Declarations

References

1. Ben, Y., Fu, C., Hu, M., Liu, L., Wong, M.H., Zheng, C.: Human health risk assessment of antibiotic resistance associated with antibiotic residues in the environment: A review. Environ. Res. (2019). https://doi.org/10.1016/j.envres.2018.11.040
2. Hernando-Amado, S., Coque, T.M., Baquero, F., Martínez, J.L.: Defining and combating antibiotic resistance from one health and global health perspectives. Nat. Microbiol. (2019). https://doi.org/10.1038/s41564-019-0503-9
3. Holmes, A.H., Moore, L.S.P., Sundsfjord, A., Steinbakk, M., Regmi, S., Karkey, A., Guerin, P.J., Piddock, L.J.V.: Understanding the mechanisms and drivers of antimicrobial resistance. Lancet. (2016). https://doi.org/10.1016/S0140-6736(15)00473-0

4. Larsson, D.G.J., Flach, C.-F.: Antibiotic resistance in the environment. Nat. Rev. Microbiol. (2022). https://doi.org/10.1038/s41579-021-00649-x

5. Tello, A., Austin, B., Telfer, T.C.: Selective pressure of antibiotic pollution on Bacteria of importance to public health. Env Health Persp. (2012). https://doi.org/10.1289/ehp.1104650

6. Ezzariai, A., Hafidi, M., Khadra, A., Aemig, Q., El Fels, L., Barret, M., Merlina, G., Patureau, D., Pinelli, E.: Human and veterinary antibiotics during composting of sludge or manure: Global perspectives on persistence, degradation, and resistance genes. J. Haz Mat. (2018). https://doi.org/10.1016/j.jhazmat.2018.07.092

7. Wohde, M., Berkner, S., Junker, T., et al.: Occurrence and transformation of veterinary pharmaceuticals and biocides in manure: A literature review. Environ. Sci. Eur. **28**, 23 (2016). https://doi.org/10.1186/s12302-016-0091-8

8. Van Boeckel, T.P., Pires, J., Silvester, R., Zhao, C., Song, J., Criscuolo, N.G., Gilbert, M., Bonhoeffer, S., Laxminarayan, R.: Global trends in antimicrobial resistance in animals in low- and middle-income countries. Science. (2019). https://doi.org/10.1126/science.aaw1944

9. Charlier, J., Barkema, H.W., Zadoks, R.N.: Disease control tools to secure animal and public health in a densely populated world. Lancet Planet. Health. (2022). https://doi.org/10.1016/S2542-5196(22)00147-4

10. Checcucci, A., Trevisi, P., Luise, D., Modesto, M., Blasioli, S., Braschi, I., Mattarelli, P.: Exploring the animal waste resistome: The spread of antimicrobial resistance genes through the use of livestock manure. Front. Microbiol. (2020). https://doi.org/10.3389/fmicb.2020.01416

11. Albernaz-Gonçalves, R., Olmos, G., Hötzel, M.J.: My pigs are Ok, why change? – animal welfare accounts of pig farmers. Animal. (2021). https://doi.org/10.1016/j.animal.2020.100154

12. Lekagul, A., Tangcharoensathien, V., Yeung, S.: Patterns of antibiotic use in global pig production: A systematic review. Veterinary Anim. Sci. (2019). https://doi.org/10.1016/j.vas.2019.100058

13. Sodhi, K.K., Kumar, M., Balan, B., Dhaulaniya, A.S., Shree, P., Sharma, N., Singh, D.K.: Perspectives on the antibiotic contamination, resistance, metabolomics, and systemic remediation. SN Appl. Sci. (2021). https://doi.org/10.1007/s42452-020-04003-3

14. Menz, J., Olsson, O., Kümmerer, K.: Antibiotic residues in livestock manure: Does the EU risk assessment sufficiently protect against microbial toxicity and selection of resistant bacteria in the environment? J. Haz Mat. (2019). https://doi.org/10.1016/j.jhazmat.2019.120807

15. Österberg, J., Wingstrand, A., Nygaard Jensen, A., Kerouanton, A., Cibin, V., Barco, L., Denis, M., Aabo, S., Bengtsson, B.: Antibiotic resistance in Escherichia coli from pigs in organic and conventional farming in four European countries. PLOS ONE. (2016). https://doi.org/10.1371/journal.pone.0157049

16. EMA: *Sales of veterinary antimicrobial agents in 31 European countries in 2022 Trends from 2010 to 2022, Thirteenth ESVAC report.* (2023)

17. Scali F., Santucci G., Maisano A.M., Giudici F.,… Alborali G.L. (2020) The Use of Antimicrobials in Italian Heavy Pig Fattening Farms. *Antibiotics*. https://doi.org/10.3390/antibiotics9120892

18. Petrin, S., Patuzzi, I., Di Cesare, A., Tiengo, A., Sette, G., Biancotto, G., Corno, G., Drigo, M., Losasso, C., Cibin, V.: Evaluation and quantification of antimicrobial residues and antimicrobial resistance genes in two Italian swine farms. Env Poll. (2019). https://doi.org/10.1016/j.envpol.2019.113183

19. Bonvegna, M., Tomassone, L., Christensen, H., Olsen, J.E.: Whole genome sequencing (WGS) analysis of virulence and AMR genes in Extended-Spectrum β-Lactamase (ESBL)-Producing Escherichia coli from animal and environmental samples in four Italian swine farms. Antibiotics. (2022). https://doi.org/10.3390/antibiotics11121774

20. Pinto Jimenez, C.E., Keestra, S., Tandon, P., Cumming, O., Pickering, A.J., Moodley, A., Chandler, C.I.R.: Biosecurity and water, sanitation, and hygiene (WASH) interventions in animal agricultural settings for reducing infection burden, antibiotic use, and antibiotic resistance: A one health systematic review. Lancet Planet. Health. (2023). https://doi.org/10.1016/S2542-5196(23)00049-9

21. Zhao, S., Chang, Y., Liu, J., Sangeetha, T., Feng, Y., Liu, D., Xu, C.: Removal of antibiotic resistance genes and mobile genetic elements in a three-stage pig manure management system: The implications of microbial community structure. J. Envir Manage. (2022). https://doi.org/10.1016/j.jenvman.2022.116185

22. Azuma, T., Usui, M., Hayashi, T.: Inactivation of Antibiotic-Resistant Bacteria in wastewater by Ozone-Based advanced water treatment processes. Antibiotics. (2022). https://doi.org/10.3390/antibiotics11020210

23. Zalewska, M., Błażejewska, A., Czapko, A., Popowska, M.: Pig manure treatment strategies for mitigating the spread of antibiotic resistance. Sci. Rep. (2023). https://doi.org/10.1038/s41598-023-39204-4

24. Walling, S.A., Um, W., Corkhill, C.L., Hyatt, N.C.: Fenton and Fenton-like wet oxidation for degradation and destruction of organic radioactive wastes. Npj Mater. Degrad. (2021). https://doi.org/10.1038/s41529-021-00192-3

25. Ni, J.-Q., Robarge, W.P., Xiao, C., Heber, A.J.: Volatile organic compounds at swine facilities: A critical review. Chemosphere. (2012). https://doi.org/10.1016/j.chemosphere.2012.04.061

26. Nie, E., Zheng, G., Ma, C.: Characterization of odorous pollution and health risk assessment of volatile organic compound emissions in swine facilities. Atmos. Environ. (2020). https://doi.org/10.1016/j.atmosenv.2019.117233

27. Lee, M., Koziel, J.A., Murphy, W., Jenks, W.S., Chen, B., Li, P., Banik, C.: Mitigation of odor and gaseous emissions from swine barn with UV-A and UV-C photocatalysis. Atmosphere. (2021). https://doi.org/10.3390/atmos12050585

28. Maurer, D.L., Koziel, J.A.: On-farm pilot-scale testing of black ultraviolet light and photocatalytic coating for mitigation of odor, odorous VOCs, and greenhouse gases. Chemosphere. (2019). https://doi.org/10.1016/j.chemosphere.2019.01.086

29. He, Z., Pagliari, P.H., Waldrip, H.M.: Applied and environmental chemistry of animal manure: A review. Pedosphere. (2016). https://doi.org/10.1016/S1002-0160(15)60087-X

30. Wezel, A., Casagrande, M., Celette, F., Vian, J.-F., Ferrer, A., Peigné, J.: Agroecological practices for sustainable agriculture. A review. Agron. Sustain. Dev. (2014). https://doi.org/10.1007/s13593-013-0180-7

31. Grigatti, M., Petroli, A., Ciavatta, C.: Plant phosphorus efficiency from Raw and composted Agro- and Bio-Waste anaerobic digestates. J. Soil. Sci. Plant. Nutr. (2023). https://doi.org/10.1007/s42729-023-01274-8

32. Sciubba, L., Cavani, L., Grigatti, M., Ciavatta, C., Marzadori, C.: Relationships between stability, maturity, water-extractable organic matter of municipal sewage sludge composts and soil functionality. Environ. Sci. Pollution Res. (2015). https://doi.org/10.1007/s11356-015-4611-7

33. Qian, X., Gu, J., Sun, W., Wang, X.-J., Su, J.-Q., Stedfeld, R.: Diversity, abundance, and persistence of antibiotic resistance genes in various types of animal manure following industrial composting. J. Haz Mat. (2018). https://doi.org/10.1016/j.jhazmat.2017.11.020

34. Criteri e Norme Tecniche Generali per La Disciplina Regionale Dell'utilizzazione Agronomica Degli Effluenti Di Allevamento e Delle Acque Reflue Di Cui All'art: 112 Del. Decreto Legislativo

(2016). 3 Aprile 2006 n. 152, Nonché per La Produzione e l'utilizzazione Ag

35. Beebe, K.R., Pell, R.J., Seasholtz, M.B.: Chemometrics: A practical guide. In: Journal of Chemical Information and Computer Sciences, vol. 38. Issue 6). Wiley (1998)

36. R core team: *R: A language and environment for statistical computing.* (4.3.1.). R Foundation for Statistical Computing. (2023). https://www.r-project.org/

37. Barnett, K.L., Kornegay, E.T., Risley, C.R., Lindemann, M.D., Schurig, G.G.: Characterization of creep feed consumption and its subsequent effects on immune response, scouring index and performance of weanling pigs. J. Anim. Sci. (1989). https://doi.org/10.2527/jas1989.67102698x

38. Marutescu, L.G., Jaga, M., Popa, M.: Insights into the impact of manure on the environmental antibiotic residues and resistance pool. Front. Microbiol. (2022). https://doi.org/10.3389/fmicb.2022.965132

39. Rasschaert, G., Van Elst, D., Colson, L., Herman, L., de Carvalho Ferreira, H.C., Dewulf, J., Decrop, J., Meirlaen, J., Heyndrickx, M., Daeseleire, E.: Antibiotic residues and Antibiotic-Resistant Bacteria in pig slurry used to fertilize agricultural fields. Antibiotics. (2020). https://doi.org/10.3390/antibiotics9010034

40. Elizalde-Velázquez, A., Gómez-Oliván, L.M., Galar-Martínez, M., Islas-Flores, H., Dublán-García, O., SanJuan-Reyes, N.: Amoxicillin in the Aquatic Environment, Its Fate and Environmental Risk. In *Environmental Health Risk - Hazardous Factors to Living Species*. InTech. (2016). https://doi.org/10.5772/62049

41. Redelsperger, I.M., Taldone, T., Riedel, E.R., Lepherd, M.L., Lipman, N.S., Wolf, F.R.: Stability of Doxycycline in feed and water and minimal effective doses in Tetracycline-Inducible systems. J. Am. Assoc. Lab. Anim. Sci. (2016). http://www.ncbi.nlm.nih.gov/pubmed/4943619

42. Abdelshafy, A.M., Neetoo, H., Al-Asmari, F.: Antimicrobial activity of hydrogen peroxide for application in food safety and COVID-19 mitigation: An updated review. J. Food. Prot. (2024). https://doi.org/10.1016/j.jfp.2024.100306

43. Zicari, G., Soardo, V., Rivetti, D., Cerrato, E., Russo, D.: Livestock farming and atmospheric emissions. Igiene E Sanità Pubblica. **69**(4), 445–457 (2013)

44. Vega, E., Martin, M.J., Gonzalez-Olmos, R.: Integration of advanced oxidation processes at mild conditions in wet scrubbers for odourous sulphur compounds treatment. Chemosphere. (2014). https://doi.org/10.1016/j.chemosphere.2014.02.061

45. Stevenson, F.J.: Humus Chemistry: Genesis, Composition, Reactions. In *Journal of Chemical Education* (2nd ed., Vol. 72, Issue 4). (1995). https://doi.org/10.1021/ed072pA93.6