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Insights into Physiochemical and Biological Characteristics of Pig Manure During Anaerobic Digestion and Sheep Manure During Composting

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Abstract: The physiochemical and biological properties of animal manures are crucial factors in resource utilization. Herein, the physiochemical and biological characteristics of pig manure during anaerobic digestion and sheep manure during composting were investigated. The animal manures were rich in heavy metals. Zn was the most abundant heavy metal, in the range of 586.9–2069 mg/kg in the animal manures. After anaerobic digestion, the contents of cellulose, hemicellulose, and lignin increased by 59.97%, 6.90%, and 171.81%, respectively, while the contents of NH_4^+-N, NO_3^--N, total nitrogen, total phosphorus, and K decreased by 5.50–48.27% in the pig manure. The contents of NH_4^+-N, NO_3^--N, total phosphorus, and K increased by 20.56–61.82% in the sheep manure after composting. The contents of all heavy metals increased in the compost, especially the Zn content which increased by 145.6%. Potential pathogenic bacteria including *Pseudomonas*, *Clostridium sensu stricto* 1, *Acholeplasma*, *Tissierella*, and *Halomonas* were abundant in the animal manures. Composting could inactivate pathogenic bacteria in the animal manures well, while a large number of pathogenic bacteria still remained in the digestate if the solid retention time was short in anaerobic digestion. The findings would be helpful for understanding the characteristics of animal manures and developing effective treatment and resource utilization technologies.

Keywords: digestate; compost; heavy metal; microbial community; pathogenic bacteria

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1. Introduction

With the development of intensive farming, the productivity of animal husbandry has greatly improved in China [1]. However, the environmental pollution caused by a large amount of animal manure has become increasingly prominent [2,3]. The distribution of livestock and poultry breeding in different regions is uneven, especially in provinces with large breeding areas where there is not enough matching farmland, resulting in poor utilization of animal manure [4,5]. The total emission of animal manure is approximately 3.9 billion tons per year in China [6]. Based on the statistical breeding number, the average emissions of pig and sheep manure are approximately 9800 and 1800 t/d, respectively [1]. Animal manure is rich in organic matter and nutrients such as nitrogen and phosphorus, but it also contains a large amount of heavy metals as well as pathogenic bacteria, chemical additives in feed, and antibiotics [5,7]. The discharge of animal manure without effective treatment not only affects the ecological environment, but also hinders the sustainable development of the ecological breeding industry [5].

Composting and anaerobic digestion are the main treatment methods for animal manure [8,9]. Composting is considered a resource technology of animal manure that can convert organic matter into more-stable compounds with certain humic properties [10]. Composting has the advantages of low investment and simple operation. However, the problems of nitrogen loss, odor and greenhouse gas emissions, the high bioavailability of heavy metals, antibiotic residue, the potential risks of antibiotic resistance genes, and the low organic matter humus content have to some extent limited the industrial promotion of composting [11]. Anaerobic digestion is a technology that converts organic matter into biogas for energy recovery under anaerobic conditions [12]. But, due to the presence of stubborn structures such as wood fibers and biomass fibers in animal manure, traditional anaerobic fermentation techniques often have a lower treatment efficiency [12,13].

The physiochemical properties of manure vary with animal types due to differences in dietary structure and intestinal physiology, which lead to varied functional microorganisms, thereby affecting the selection of treatment methods [14]. Different animal manures have different compositions and biodegradability, which have a significant impact on composting operations, including the selection of expansion agents, aeration rate, odor control, composting time, and product quality [15]. Wang et al. [15] found that among the chicken, cow, and pig manures, the chicken manure had the highest dissolved organic carbon and dissolved total nitrogen, and took a longer time to compost than the others. Among animal manures, pig and chicken manures contain more biodegradable organic matter than others [16]. The proportion of lignocellulose in animal manure varies greatly owing to differences in the feed and digestibility of livestock and poultry [17]. Compared with pig, chicken, and rabbit manures, the fiber content is much higher in cow manure, which can slow down the biodegradation of cow manure in anaerobic digestion due to the complicated structure of lignocellulose [16]. The NH_4^+-N concentration in the feces of herbivores is significantly lower than that of omnivores, due to the dietary quality and the degradability of protein in manure types [16], which can mitigate the inhibitory effect of NH_4^+-N on anaerobic digestion [18,19]. Mineral elements and antibiotics are often added to feed for the growth and disease prevention of livestock, resulting in antibiotic and heavy metal residues and inhibiting the biodegradation of organic matter in manures [20,21]. Additionally, the composition of animal manures may contain foreign materials such as sawdust, lime, and other substrates, which is largely affected by the bedding materials used in livestock farms [11]. Therefore, fully understanding the physiochemical and biological properties of animal manures and their treatment products would be helpful to comprehend the composition change and the mechanisms during the treatment process, and thereby provide theoretical references for developing their treatment and resource utilization technologies.

The aim of this study was to explore the physiochemical and biological characteristics of manures and their change during anaerobic digestion and composting, and to provide a guidance for the resource utilization and biotreatment engineering of animal manures. Different animal manures, i.e., pig and sheep manure, and different treatment methods, i.e., anaerobic digestion and composting, were investigated. The physiochemical properties of pig manure during anaerobic digestion and sheep manure during composting, including the components, nutrients, and heavy metals were characterized. The microbial communities in the manures and their treatment products (i.e., digestate and compost) were identified and their relationships with physiochemical variables were estimated.

2. Materials and Methods

2.1. Experimental Materials

The pig and sheep manures and their treatment products including compost and anaerobic digestate used in this study were taken from Zhejiang Province (Zhejiang) and Xinjiang Uygur Autonomous Region (Xinjiang), China (Figure 1). The pig manure samples were collected from three pig farms with a yield of 10,000–20,000 head/year in Quzhou city, Zhejiang Province, and labeled as QZP1, QZP2, and QZP3. In a 480 t/d anaerobic digestion project of pig manure in Quzhou city, the pig manure samples were taken from the influent tank at three different times, mixed, and labeled as ADM. The anaerobic digestate separated by solid–liquid separation using a centrifuge at 4000 rpm was withdrawn from a dumping site and labeled as ADD. The centrifugal effluent was collected from the collection tank. The chemical oxygen demand (COD), NH_4^+-N, and total nitrogen concentrations were 8839–9557, 2176–2385, and 2775–2958 mg/L in the centrifugal effluent, respectively. The anaerobic digestion project was operated in a continuously stirred tank reactor at 37–38 °C with a TS concentration of ~12% and a solid retention time of 22–25 d. The sheep manure samples were taken from farms with a yield of 15,000–40,000 sheep per year in Yingjisha and Jiashi Regions in Xinjiang, and labeled as XJS and JSS, respectively. The compost sample was collected from the sheep manure composting factory without the addition of any structuring agent in Jiashi Region and labeled as JSC. The composting factory was operated in an aerobic composting tank reactor with a temperature above 60 °C for ~7 d and then manure was composted in static windrows at room temperature for 20–30 d. The composting products meet the quality criteria of Microbial Organic Fertilizers (NY884-2021 [22]) in China. In each sampling site, three samples were collected and mixed for subsequent analysis of their physiochemical and biological characteristics. Some samples were stored at −80 °C for molecular biology analysis. The remaining samples were air-dried and used for the determination of physiochemical characteristics.

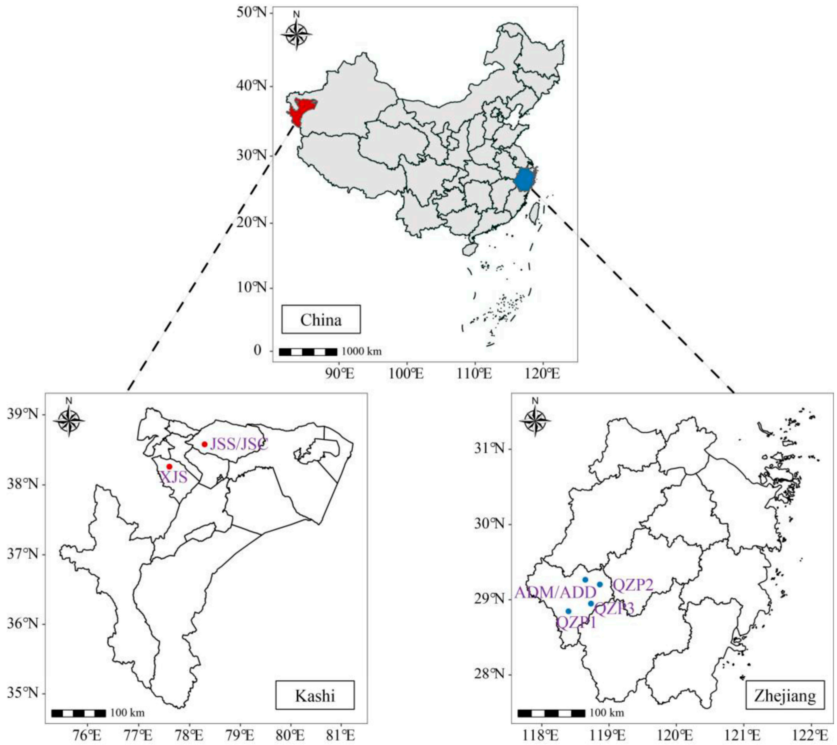

Figure 1. Sampling sites in this study.

2.2. Analytical Methods

The pH value was detected using a pH meter (Mottlertolledo, PE20K, Zurich, Switzerland). The water content and total solid (TS) content were determined using the drying method at 105 °C. The volatile solid (VS) content based on TS was determined by burning at 600 °C for 3 h. The contents of total nitrogen, NH_4^+-N, NO_3^--N, total phosphorus, and total sulfur were determined by the standard methods described previously [23]. The contents of cellulose, hemicellulose, and lignin were determined using the Van Soest method [24]. The contents of metal including K, Pb, Ni, Cr, Cu, Cd, Zn, and Hg, and As were detected using inductively coupled plasma–atomic emission spectrometry (ICPOES, Thermo iCAP 6000, Waltham, MA, USA) [25].

2.3. DNA Extraction, PCR Amplification, and Sequencing Analysis

Genomic DNA was extracted from the pig and sheep manures and their treatment products including the compost and anaerobic digestate samples using the E.Z.N.A.™ soil DNA extraction kit (Omega Bio Tek, Inc., Norcross, GA, USA). DNA extracted from the three samples collected in each sampling site was mixed and stored at −80 °C for subsequent molecular biology analysis. The V4 region of the bacterial 16S rRNA gene was amplified with the barcode primer 515F/806R. PCR amplification was performed as described by He et al. [26]. The PCR products were sent for MiSeq sequencing on the Illumina PE300 platform at Shanghai Meiji Biomedical Technology Co., Ltd. (Shanghai, China). The sequencing data were filtered, merged, and clustered to obtain operational taxonomic units (OTUs) as described previously [26].

2.4. Data Analyses

The Pearson correlation and Mantel test between the physiochemical variables and microbial communities were performed using the ggplot2 package of R (Version 4.2.1). Canonical correlation analysis (CCA) was conducted to analyze the influence of the physiochemical variables on microbial community structure using CANOCO (Version 5). A significant statistical analysis was conducted on the relationship between the physiochemical variables and the microbial community using Monte Carlo tests. Heatmap plots were produced using the OmicStudio tools (https://www.omicstudio.cn, accessed on 1 April 2025).

3. Results and Discussion

3.1. Physiochemical Properties of Animal Manures

3.1.1. Pig Manures

The pH value was 6.90–7.79 in the experimental pig manures (Figure 2). All of the water contents of the pig manures were above 87% owing to the mixtures of pig urine and flushing water used to maintain the proper hygiene of livestock housing during intensive pig production [27]. The TS contents of the pig manures were 6.90–12.34%. The VS contents of the pig manures were in the range of 70.07–81.18%. The contents of cellulose, hemicellulose, and lignin in the pig manures were 7.06–17.11%, 15.31–26.70%, and 4.52–10.20%, respectively. The total nitrogen contents of the pig manures were similar and fluctuated within 17.02–20.22 g/kg. The NH_4^+-N content was 6.09–7.62 g/kg in the pig manures. The NO_3^--N contents in the pig manures were 31.01–44.77 mg/kg, The contents of total phosphorus were 54.60–76.42 mg/kg in the pig manures. The total sulfur content was 6.50–9.71. The K content ranged from 23.60 to 62.75 g/kg in the pig manures. Among the detected heavy metals in the pig animal manures, the highest content was Zn, in the range of 568.6–2069 mg/kg, followed by Cu, Cr, Ni, Pb, and Cd, and Hg was below the detection limit in all of the pig animal manures. All the contents of the heavy metals and

As were below the Limitation Requirements of Toxic and Harmful Substances in Fertilizers in China [28], except for the Cd content (3.12 mg/kg) in QZP2. Among all the detected physicochemical properties, all the values of ADM, except for As and Pb, were in the ranges of the pig manure samples taken from the three pig farms in Quzhou city, indicating that ADM could represent the mixture of pig manures from different pig farms in Quzhou city.

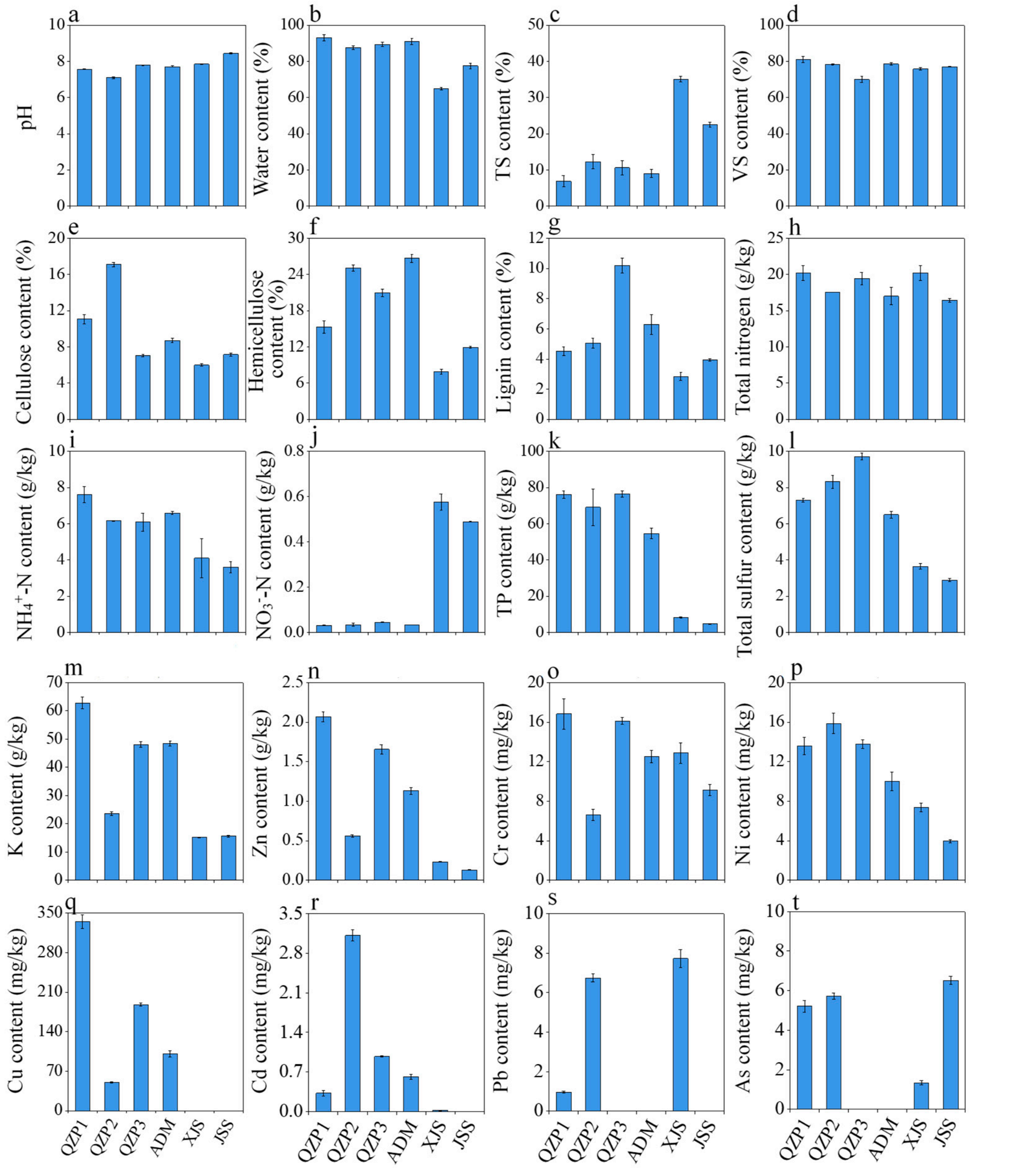

Figure 2. The physiochemical properties of animal manures including pH (**a**), water content (**b**), TS (**c**), VS (**d**), cellulose (**e**), hemicellulose (**f**), lignin (**g**), total nitrogen (**h**), NH_4^+-N (**i**), NO_3^--N (**j**), TP (**k**), total sulfur (**l**), K (**m**), Zn (**n**), Cr (**o**), Ni (**p**), Cu (**q**), Cd (**r**), Pb (**s**), and As (**t**). TP, total phosphorus.

3.1.2. Sheep Manures

Compared with the pig manure, sheep manure had a higher pH value of 7.86–8.44, while the water contents were lower at 64.86–77.46% (Figure 2). The TS and VS contents of sheep manures were 22.54–35.14% and 75.85–77.05%, respectively. Since the feed and digestion efficiency of animals were variable, the proportion of lignocellulose in the pig and sheep manures varied greatly, with them being mainly composed of hemicellulose [29]. The sheep manures had a lower content of lignocellulose, with contents of cellulose, hemicellulose, and lignin of 6.01–7.15%, 7.86–11.96%, and 2.84–3.95%, respectively, relative to the pig manures, which were similar to the values reported by Zhu et al. [30]. The total nitrogen of sheep manures (16.46–20.22 g/kg) was close to that of pig manures. However, the NH_4^+-N contents were 3.60–4.09 g/kg and the NO_3^--N contents were 489.5–576.2 mg/kg, being 32.84–52.76% lower than that in the pig manures and 10.93–18.58 times that in the pig manures, respectively. This may be because the lower water content of the sheep manures could prompt oxygen diffusion, thereby increasing nitrification. The total phosphorus and total sulfur contents were 4.67–8.14 g/kg and 2.89–3.65 mg/kg in the sheep manures, respectively. The heavy metal contents in the sheep manures were less than those in the pig manures, except for Cr, which were mainly ascribed to the use of pig feed supplements for growth-stimulating and antimicrobial effects [31]. The contents of K, Zn, Cr, Ni, and As were 15.14–15.62 g/kg, 131.1–233.7 mg/kg, 9.13–12.87 mg/kg, 3.95–7.38 mg/kg, and 1.34–6.51 mg/kg, respectively. Cu and Cd were below the detection limit in the sheep manures. The Pb content in XJS was 7.73 mg/kg, while it was not detected in JSS.

3.2. Variation in Physiochemical Properties of Animal Manures During Anaerobic Digestion and Composting

3.2.1. Pig Manure During Anaerobic Digestion

After anaerobic digestion, the pH value of animal manure decreased to 6.90, which might be because a large amount of ammonium and alkaline substances were released into the anaerobic digestion solution, thereby leading to a decrease in the pH value of the digestate. The water content of the digestate (ADD) was 83.23% (Table 1). Compared to the original manures, the VS content of digestate was 72.94% with a decrease of 1.85%. This indicated that a large amount of organic matter was accumulated in the digestate. Animal manure is rich in lignocellulose with a high content of ~50% due to a fiber-rich diet [32]. Lignocellulose is resistant to anaerobic degradation owing to its recalcitrance and physiochemical complexity [33]. In the anaerobic digestion process, the contents of cellulose, hemicellulose, and lignin in the animal manure mixture were 8.73%, 26.70%, and 6.30%, respectively. The contents of cellulose, hemicellulose, and lignin in the anaerobic digestate were 13.96%, 28.54%, and 17.13%. Compared with ADM, the contents of cellulose and hemicellulose increased by 59.97% and 6.90%, owing to the corresponding decrease in the total amount of anaerobic digestion residue. It was presented by the high increase of 171.81% in the lignin content in ADD, due to the fact that lignin is a recalcitrant compound and mainly accumulated in the anaerobic digestate. Compared with the lignin content, the variation in the hemicellulose content of manure during anaerobic digestion suggested that a part of the hemicellulose in the animal manure could be decomposed during anaerobic digestion. A similar result was obtained by Shen et al. [34], who found that almost no lignin was decomposed during anaerobic digestion due to the complicated structure of lignin, while a part of the hemicellulose and cellulose was degraded. After the degradation of organic matter during anaerobic digestion, the contents of NH_4^+-N, NO_3^--N, total nitrogen, total phosphorus, and K all decreased by 5.50–48.27% in the animal manures. Compared with the original animal manures, the contents of heavy metals including Cd and Cr decreased by 0.71–16.05%, while the contents of Cu, Zn, and Ni increased by 7.11–31.27%. This may be because the decrease in pH value in the digestate might lead to the release of some heavy metals into the liquid and reduce the contents of some heavy metals in the digestates. However, most of the heavy metals were accumulated in the digestate. Zheng et al. [35] also reported that the contents of heavy metals were considerably higher in the digestate than those in the liquid portion because the heavy metals tended to be in an insoluble form after anaerobic digestion.

Table 1. The physiochemical variables in the animal manures and their change rates (i.e., increasing or decreasing percentage) after anaerobic digestion and composting.

Indexes	Anaerobic Digestion			Composting		
	ADM	ADD	Change Rate (%)	JSS	JSC	Change Rate (%)
pH	7.72	6.90	−10.63	8.44	8.46	0.24
Water content (%)	90.99	83.23	−8.53	77.46	39.93	−48.45
TS (%)	9.01	16.77	86.08	22.54	60.07	166.49
VS (%)	78.57	72.94	−7.16	77.05	70.38	−8.66
Cellulose (%)	8.73	13.96	59.97	7.15	7.02	−1.85
Hemicellulose (%)	26.70	28.54	6.90	11.96	9.56	−20.11
Lignin (%)	6.30	17.13	171.81	3.95	4.28	8.42
Total nitrogen (g/Kg)	17.02	13.94	−18.06	16.46	14.63	−11.11
NH_4^+-N (g/Kg)	6.59	3.41	−48.27	3.60	4.55	26.32
NO_3^--N (mg/Kg)	0.03	0.02	−24.28	489.54	0.79	61.82
Total phosphorus (g/Kg)	54.60	51.60	−5.50	2.89	5.63	20.57
Total sulfur (g/Kg)	6.50	6.93	6.65	4.67	2.21	−23.61
K (g/Kg)	25.81	48.38	−46.66	15.62	25.23	61.53
Zn (mg/Kg)	1212.30	1131.80	7.11	131.13	322.07	145.60
Cr (mg/Kg)	12.42	12.51	−0.71	9.13	9.87	8.11
Ni (mg/Kg)	13.14	10.01	31.27	3.95	3.95	0.12
Cu (mg/Kg)	126.52	100.44	25.97	—	—	/ [b]
As (mg/Kg)	— [a]	—	/	6.51	6.74	3.55
Cd (mg/Kg)	0.52	0.62	−16.05	—	—	/
Pb (mg/Kg)	8.85	—	/	—	0.48	/

Note: [a], data were lower than the detection limit; [b], no data.

3.2.2. Sheep Manure During Composting

After composting, the VS content of sheep manure was 70.38% with a decrease of 8.66%, and the content of hemicellulose and cellulose decreased by 20.11% and 1.85%, respectively, while the content of lignin increased by 8.42% (Table 1). This result indicated that some hemicellulose and cellulose in the sheep manure could be degraded during aerobic composting, while lignin was difficult to degrade and mainly accumulated in the compost. Compared with anaerobic digestion, more hemicellulose, cellulose, and lignin were degraded during the composting process. After composting, the contents of NH_4^+-N, NO_3^--N, total phosphorus, and K in the manure increased by 20.56–61.82%, while the total nitrogen decreased by 11.11%, mainly due to ammonia volatilization during the degradation of organic matter [36]. Compared with anaerobic digestion, more nutrients of animal manures including N, P, and K were deposited in the compost. The contents of heavy metals including Ni, As, and Cr increased by 0.12–8.11%, while the Zn contents increased by 145.6%. Pb and Cd were detected in the compost with the contents of 0.005–0.48 mg/kg, despite being below the detection limit in the manure. Similarly, Zheng et al. [8] found that the contents of heavy metals increased by 1–3 times in animal manure after composting, which was more obvious than in anaerobic digestion. Therefore, the issue of heavy metal accumulation in animal manure in the composting process should be noted and effective measures taken to reduce the environmental risk of composting.

3.3. Cluster Analysis of Physiochemical Properties of Animal Manures and Their Treatment Products

Heatmap analysis was performed to explore the relationship between the physiochemical properties of manures and their treated products. The physiochemical properties of the sheep and pig manures, respectively, clustered together (Figure 3), indicating that the physiochemical properties of manures varied with the animal type. The physiochemical properties of sheep manures were separated from those of the compost, while the physiochemical variables of pig manures and their digestate were grouped together. This suggested that the physiochemical properties of pig manures were similar to their digestate, while they were significantly different from the compost in the sheep manures. This may be because more organics of manure might be degraded in composting than in anaerobic digestion. A similar result was obtained by Kong et al. [37] who found the physiochemical variables of chicken manures were separated at different composting times.

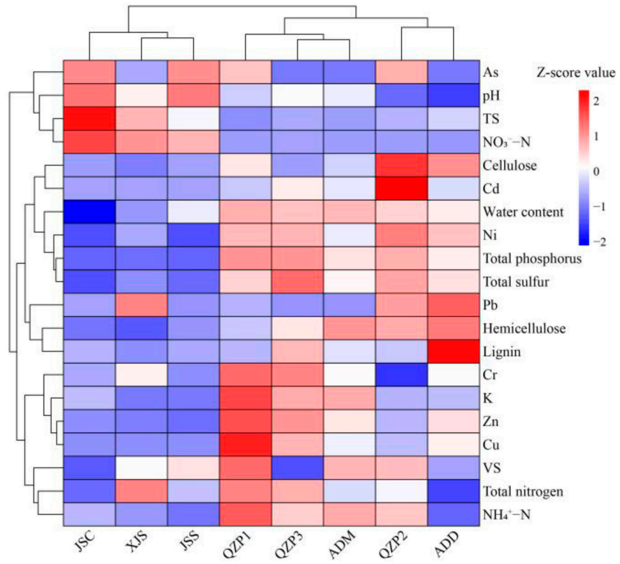

Figure 3. Heatmap of the physiochemical variables in the animal manures and their treatment products after anaerobic digestion and composting.

3.4. Microbial Community Structure in Animal Manures and Their Treatment Products

MiSeq sequencing was conducted to identify the microbial community structure in the animal manures and their treatment products. Among the experimental manures, the highest OTU number was detected in ADD (1401), and the lowest OTU number was observed in ADM (240) (Figure 4a). The average OTU number was similar in the pig and sheep manures in the range of 784–827. After anaerobic digestion and composting, the OTU number increased in the digestate and compost of animal manures (1016–1402). The Shannon index was higher in the pig manures (3.62–4.89) than in the sheep manures (3.14–3.90) (Figure 4b). There was not much change in the Shannon index of the microbial community in the animal manures after anaerobic digestion and composting.

Firmicutes, *Proteobacteria*, *Bacteroidota*, *Halobacterota*, and *Halanaerobiaeota* were the main phyla in the manures and their treatment products, accounting for 82.17–99.81% of the total sequencing reads (Figure 4c). The same dominant taxonomic phyla were also found in animal manure studies using composting [38,39]. Among them, *Firmicutes* and *Proteobacteria* were the most abundant phyla in the manure samples. *Firmicutes* was the most abundant phylum in JSS (68.13%), JSC (71.50%), QZP2 (80.56%), and ADD (34.10%). *Proteobacteria* was the most abundant phylum in ADM (38.88%). Some members of *Firmicutes* are hydrolytic and acidifying bacteria that can produce extracellular enzymes such as protease and cellulase and prompt the decomposition of protein and cellulose [40]. Some members of *Proteobacteria* can produce laccase and β-glucosidase and enhance the degradation of lignocellulose [41]. The higher abundances of *Firmicutes* and *Proteobacteria* indicated that the degrading microorganisms of cellulose, sugar, and protein dominated in the manures and their treatment products. *Bacteroidetes* can degrade cellulose and polysaccharides into organic acids [42], and were also found to be abundant in the manures and their treatment products with relative abundances of 9.81–42.69%. Compared with the sheep manures, the relative abundances of the three major phyla of *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* were more similar in the pig manures, indicating the synergistic effect of different species was stronger in the pig manures than in the others.

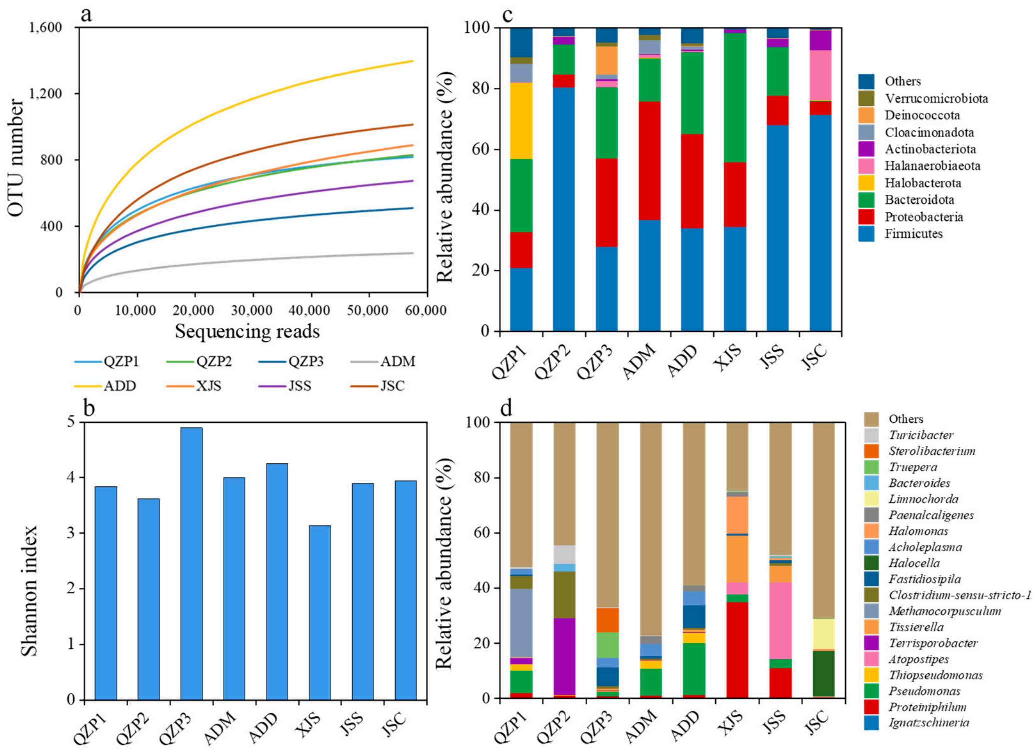

Figure 4. Operational taxonomic unit (OTU) number of sequencing reads (**a**), Shannon index (**b**), and relative abundance of bacteria at the phylum (**c**) and genus (**d**) levels in the animal manures and their treatment products after anaerobic digestion and composting.

The dominant genera in the manures and their treatment products varied with the animal type. *Proteiniphilum* is mainly responsible for the degradation of protein and amino acids [43]. Compared with the pig manures, the relative abundance of *Proteiniphilum* was higher in the sheep manures (11.20–35.01%) (Figure 4d). The relative abundance of *Pseudomonas* was higher in QZP1, ADM, and ADD than in the others. *Thiopseudomonas* can metabolize pollutants such as sulfur and acetate, with nitrate used as the electron acceptor [44,45]. The relative abundance of *Thiopseudomonas* was 0.04–3.16% in the pig manures, which was higher than in the others. The relative abundance of *Atopostipes* was 4.36–27.79% in the sheep manures, while it was low in JSC (i.e., the compost of sheep manure) (0.31%). This might be because *Atopostipes* is often detected in the mesophilic period of composting [46]. The microbial community varied significantly in the compost relative to the original manure. Similarly, Wang et al. [47] also found that the dominant microorganisms changed with time during composting. Compared with the composting process, the dominant microorganisms in the anaerobic digestate (ADD) were more similar to those in the pig manure (ADM), likely due to the same exposure to anaerobic conditions.

3.5. Potential Pathogenic Bacteria in the Animal Manures and Their Treatment Products

Pathogenic bacteria in animal manure pose a severe risk of spreading zoonotic diseases. To better understand the variation in pathogenic bacteria during anaerobic digestion and composting, the abundance of potential pathogenic bacteria in the animal manures and their treatment products was analyzed. *Pseudomonas*, *Enterococcus*, *Clostridioides*, *Lactobacillus*, *Streptococcus*, *Staphylococcus*, *Ignatzschineria*, *Tissierella*, *Clostridium sensu stricto* 1, *Acholeplasma*, *Halomonas*, and *Bacteroides* were the main genera in the sheep and pig manures to which potential pathogenic bacteria are affiliated (PB genera) [48–51], accounting for 6.78–33.23% of the total sequencing reads (Figure 5). *Pseudomonas*, *Clostridium sensu stricto* 1, and *Acholeplasma* were the main PB genera in the pig manures with a relative abundance of 5.86–20.02%. After anaerobic digestion, the relative abundance of the three PB genera increased from 14.31% to 24.31%, indicating that a large number of potential pathogenic bacteria might remain after anaerobic digestion. Similarly, Qi et al. [52] reported the pathogenic bacteria such as *E. coli*, *Salmonella*, *Enterococcus*, and *Campylobacter Salmonella* were abundant in the digestates, especially in mesophilic anaerobic digestion. The highly abundant PB genera remaining in the digestate might be because the T_{90} values of these bacteria (i.e., the time required for a 90% reduction in viable counts) in the digestion tanks were shorter than their solid retention time (22–25 d) in this study [49]. *Pseudomonas*, *Tissierella*, and *Halomonas* were the main PB genera in the sheep manures with a relative abundance of 9.90–33.06%. After composting, the relative abundance of the three PB genera decreased from 9.90% to below 1%, suggesting that the potential pathogenic bacteria could significantly reduce owing to the high temperature of above 60 °C during composting [53]. Ravindran et al. [54] reported that composting could also inactivate pathogens due to severe environmental conditions such as temperature, pH, and nutrient levels. Taken together, the main PB genera showed that composting could inactivate pathogenic bacteria well, while a large number of pathogenic bacteria remained in the digestate if the hydraulic retention time was shorter than the T_{90} values of the pathogenic bacteria in anaerobic digestion. Thus, digestates from anaerobic digestion plants should be further disposed of such as by composting to inactivate pathogenic bacteria before they can be applied to agricultural land.

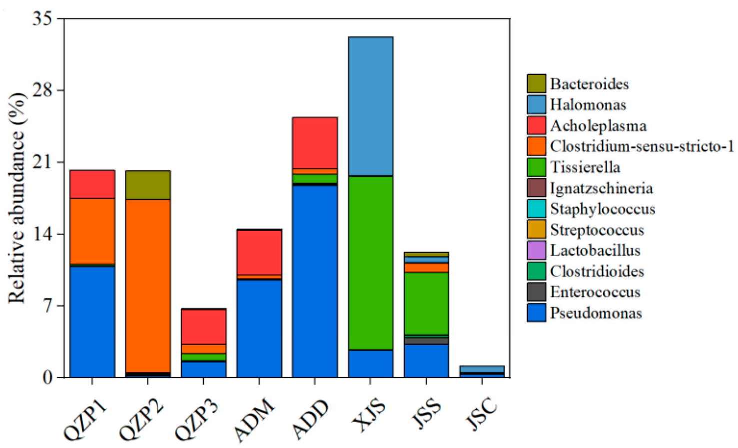

Figure 5. Relative abundance of potential pathogenic bacteria in the animal manures and their treatment products after anaerobic digestion and composting.

3.6. Relationship Between Physiochemical Properties and Microbial Communities in Animal Manures and Their Treatment Products

In order to understand the correlation between the physicochemical properties and the microbial communities in the animal manures and their digestate and compost samples, correlation analysis and Mantel tests were conducted (Figure 6a). Among the measured physicochemical variables, pH, water content, TS, NO_3^--N, total phosphorus, and total sulfur were related to many variables in the samples. The pH value was significantly positively correlated with total nitrogen and NO_3^--N, and significantly negatively correlated with cellulose, hemicellulose, and Pb in the manures and their treatment products. This may be because a high pH value, especially in alkaline conditions, might increase the accumulation of heavy metals in the manures and their treatment products [55]. The degradation of cellulose and hemicellulose can release a large amount of H^+, and lead to a decrease in the pH value [31,56]. TS was significantly positively correlated with NO_3^--N, and significantly negatively correlated with total phosphorus, total sulfur, Zn, and Ni. Since TS is the main contributor to the exchange or dissolution of metals in the system [19], a high correlation has been observed between the organic matter and heavy metals in the manures. Total phosphorus was significantly positively correlated with water content, cellulose, total sulfur, Zn, Ni, and Cu, and significantly negatively correlated with TS. This may be because total phosphorus was often found together with cellulose, total sulfur, Zn, Ni, and Cu in the animal manures. Liu et al. [57] also found a significant positive correlation between phosphorus and metals due to phosphorus mineralization by the metals. Total sulfur was significantly positively correlated with water content, Zn, Ni, Cu, and Cd, and significantly negatively correlated with TS and NO_3^--N. Compared with the physiochemical variables, the correlation of the heavy metals with the microbial communities was relatively weaker except for Zn, Ni, and Cu. This may be mainly because the contents of heavy metals in animal manure vary with feed and additives [30]. Total sulfur was significantly positively correlated with water content, Zn, Ni, Cu, and Cd, and significantly negatively correlated with TS and NO_3^--N. There was a significantly positive correlation between Cu and Zn in the manures and their treatment products, which were both positively correlated with K, Cr, and Ni. This result might be because Cu and Zn mainly came from the use of animal feed supplements for growth-stimulating and antimicrobial effects [30]. TS, water content, NO_3^--N, total sulfur, total phosphorus, and Ni could significantly influence the microbial communities in the manures and their treatment products.

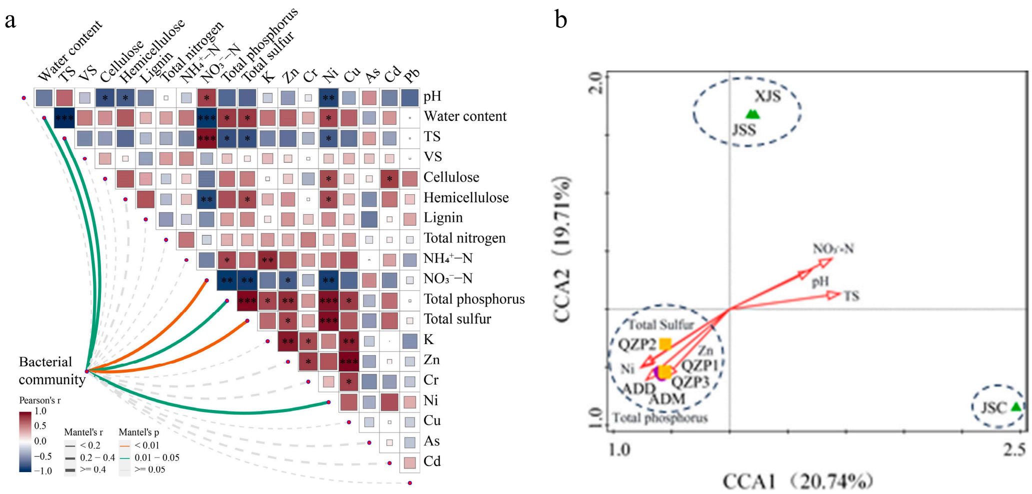

Figure 6. Correlation analysis of the physiochemical variables and their Mantel test analysis for the microbial community (**a**), and their canonical correlation analysis (CCA) (**b**) in the animal manures and their treatment products after anaerobic digestion and composting. Edge width corresponds to Mantel's r value. Pairwise correlations of these variables are shown with a color gradient denoting Pearson's correlation coefficient. * represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$.

CCA analysis of the physiochemical variables of animal manures and their treatment products and the microbial communities showed that the microbial communities in the pig and sheep manures were, respectively, clustered together (Figure 6b). Although the ADM, QZP1, QZP2, and QZP3 manures were collected from different pig farms, the microbial communities grouped together. This indicated that the microbial communities in animal manures vary with animal type, owing to the difference in animal feed. The microbial communities in ADM and ADD clustered together, while they were separated in JSS and JSC. This suggested that the microbial communities in the pig manures and their treatment product (i.e., digestate) in the anaerobic digestion process were more similar than those in the composting process. This might be because the manure and its treatment product were both exposed under anaerobic conditions during anaerobic digestion, while it was exposed under aerobic conditions in the composting process.

4. Conclusions

The physiochemical and biological properties of manures and their treatment products varied with animal type. The animal manures were rich in heavy metals. Compared with the sheep manures, the contents of Zn, Cu, and Ni were higher in the pig manures. The physiochemical properties of animal manures differed from the products of their anaerobic digestion and composting. After anaerobic digestion, a larger amount of nutrients including N, P, and K were released into the solution and resulted in a decrease in NH_4^+-N, NO_3^--N, total nitrogen, total phosphorus, and K contents in the digestate. Although the contents of Cd and Cr decreased slightly, most of the heavy metals were accumulated in the digestate. After composting, the contents of NH_4^+-N, NO_3^--N, total phosphorus, and K increased, while the total nitrogen decreased a little. Compared with the digestate, more nutrients of animal manures including N, P, and K were deposited in the compost. However, the contents of all heavy metals increased after composting, which was more obvious than in anaerobic digestion. The issue of heavy metal accumulation in animal manure in the composting process should be noted and effective measures taken to reduce the environmental risk of composting. There were some potential pathogenic bacteria in the animal manures. Composting could inactivate pathogenic bacteria in the animal manures well, while a large number of pathogenic bacteria remained in the digestate if the hydraulic retention time was shorter than the T_{90} values of the pathogenic bacteria in anaerobic digestion. Thus, digestates should be further disposed of by methods such as composting to inactivate pathogenic bacteria before they can be applied to agricultural land. Since there were few samples, the characteristics of pig and sheep manures only represented the status of the special areas in this study. Future studies such as those including a wide range of sampling and coverage areas, including different feeding and treatment parameters, and liquid samples from their treatment processes, should be conducted to better understand the physiochemical and biological characteristics of animal manures and biotreatment products, and to provide a guidance for resource utilization and biotreatment engineering for animal manures.

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