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Research Article

Detection and High-Throughput Microbial Analysis of Particulate Matter in Houses and Downwind Areas of Duck Farms

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Particulate matter (PM) and the microorganisms of duck houses may have negative impacts on animal and human health. During 2021-2022, PM2.5 and PM10 inside and outside the duck house were sampled with a built-in air sampler in Tai'an City, Shandong Province, and the diversity and abundance of microorganisms within the PM were analyzed by macrogenomic and absolute sequence analysis. The results showed that PM2.5 and PM10 concentrations in the house and at downwind points exceeded the short-term (24 h) guideline of the global air quality guidelines (AQG). Macrogenome sequencing showed that the microbial composition of the PM2.5 samples was dominated by bacteria (exceed 85%); a total of 1316 bacterial genera and 110 fungal genera were identified in PM2.5 samples from duck house 1 in winter, which were much higher than the results of amplicon sequencing method reported before, and relatively high levels of the pathogenic bacteria (Coccidioides immitis, etc.) and the conditionally pathogenic bacterium (Rothia nasimurium) were identified at the species level. Absolute quantitative sequencing detected conditionally pathogenic bacteria and allergens at high levels in PM10 samples: Corynebacterium (5.6×10^7 copies/g), Aerococcus (9.9×10^6 copies/g), Alternaria (3.3×10^6 copies/g), and Aspergillus (8.3×10^5 copies/g). Moreover, Corynebacterium was the highest content of PM10 in summer and PM2.5 samples in winter, and its pathogenicity and potential threat should be noted. The diversity and relative abundance of microorganisms were similar in the duck house and at the downwind point. The results showed that the microorganisms in the house environment have a greater influence on the air environment around the downwind point and may pose a public health risk to the staff and the surrounding area.

1. Introduction

In recent years, aerosol research has received increasing attention with the development of air pollution. Aerosol is defined as a dispersed system of tiny particles suspended in a gaseous medium, either solid or liquid. Bioaerosols, which include bacteria, fungi, viruses, and chemical toxins suspended in the air, are important components of atmospheric particulate matter such as PM2.5 and PM10 and account for 25% of total aerosols [1]. PM2.5 is able to penetrate deep into the ends of small bronchial tubes and alveoli, which can cause damage when the body's immunity is low or when the level of pathogenic microorganisms is high [2, 3]. Short-term or long-term exposure to PM2.5/PM10 can lead to respiratory and cardiovascular diseases [4, 5].

Most studies on particulate matter and bioaerosols in poultry houses focused on the environment of chicken sheds [6–10], with fewer studies on the environment of duck sheds, and the qualitative analyses of microbial constituents in particulate matter at different sampling points have been limited to the genus level, and there is also lack of quantitative analyses for the microbial constituents, which does not reflect the true absolute abundance of microbial constituents in the particulate matter samples.

The conventional approach to microbial flora analysis is amplicon sequencing, a technique that uses suitable universal primers to amplify 16S rDNA/18S rDNA/ITS hypervariable regions or functional genes of environmental microorganisms and then sequence the amplified products, but this technique can only achieve genus-level resolution [11].

Macrogenome sequencing is the high-throughput sequencing of the total DNA of all microorganisms in environmental samples using next-generation high-throughput sequencing (NGS) technology. On the basis of analyzing microbial diversity, population structure, and evolutionary relationships, it can be used to further explore the functional activities of microbial groups, interactions, and relationships with the environment and to discover potential biological significance. Compared with amplicon sequencing, macrogenome sequencing can identify microorganisms down to the species level. Therefore, macrogenome sequencing has a high advantage in the process of species identification.

Accu16S™/ITS™ absolute quantitative sequencing is a technology that combines qPCR absolute quantitative and conventional amplicon sequencing into a single technology, the key is that it can resolve the absolute copy number of each species in the sample, which can reflect the true number of microorganisms in the sample and the true differences between the samples in the group, whereas the conventional 16S/ITS amplicon sequencing methods can only resolve the composition of species in samples and their relative abundance. Therefore, absolute quantitative analysis can better reflect the true status of the bacterial/fungal communities in the samples relative to quantitative analysis.

The aim of this study was to determine the distribution, species, and levels of microorganisms in the air inside and outside the duck house by macrogenomic and absolute quantitative sequencing analyses and to explore their hazardous risks. These results can help to manage environmental aerosol pollution in duck houses, protect animal and human health, and provide a reference for further research on air pollutants and bioaerosols in poultry farms.

2. Material Methods

2.1. Duck Farm Sampling. In this study, samples were collected from two typical commercial Cherry Valley duck farms in the rural area of Tai'an City, Shandong Province, China, during 2021-2022. Winter samples of PM2.5 were collected from duck house 1, which is about 850 m^2 in size and has a capacity of about 9,500 Cherry Valley ducks, and the duck house is well ventilated with a closed net-flat-breeding method. In summer, PM10 samples were collected from duck house 2, which covers an area of about 270 m^2 and can accommodate about 3,000 Cherry Valley ducks. The duck house is well ventilated, and semienclosed net-flat-breeding is used. Both duck houses are equipped with two exhaust fans on the wall opposite the entrance and excellent ventilation.

2.2. Sampling Points. Air was sampled 1 meter above the ground at different locations on the duck farm in winter and summer. Air sampling points were included inside the house (A) and downwind point (B, about 5 meters from the boundary), and three replicate samples were collected from each sampling point. The samples of inside house (A) were collected at the center and entrance of the duck house [10].

2.3. Particulate Matter Collection. An integrated ambient air particulate sampler (ZR-3920, Qingdao Zhongrui Intelligent Instrument, Qingdao, China) was used to collect PM (PM2.5/PM10) on a glass fiber filter membrane with a typical aerosol retention rate of 99.9%. The airflow rate was set to 100 L/min; the sampling height was 1 m, and the sampling time was 24 h. The particulate matter concentration ($\mu g/m^3$) was calculated according to the formula $C = (W1 - W0)/(t \times F)$, where t and F are the collection time and airflow rate of the samples, respectively, $W0$ is the weight of the filter blank (weighed on a microbalance) before sampling, and $W1$ is the weight of the filter and PM after sampling. Temperature, humidity, CO_2, and NH_3 concentrations were also recorded during winter sampling.

2.4. Endotoxin Collection. Endotoxin was collected using an AGI-30 collector at an airflow rate of 12.5 L/min and a drive time of 30 min. The sampling medium was 50 mL of water without a heat source, and three replicate samples were collected.

2.5. Sequencing Analysis. The filter membrane with PM samples was cut into 1 cm^2 pieces, frozen in liquid nitrogen, and stored in the refrigerator at -80°C. The PM2.5 samples were entrusted to Beijing Prime Biotechnology Company to perform macrogenome sequencing analysis; the PM10 samples were entrusted to Shanghai Tian Hao Biotechnology Company to perform absolute quantitative sequencing analysis.

2.6. Data Analysis. Data analysis was mainly performed using Microsoft Excel, GraphPad Prism (8.0.1) and SPSS (21.0). All samples were analyzed for differences in diversity indices between groups based on the Wilcoxon rank sum test with p value < 0.05 as the screening threshold for significance of differences and multiple hypothesis testing corrected for p value using the Bonferroni method (FDR), which was used to assess whether there was a significant difference in species diversity between groups. Species with significant differences in abundance between groups were obtained using the linear discriminant analysis (LDA) effect size method (LEfSe) with $|LDA| > 2$ and $p < 0.05$ as the difference screening threshold. The no-metric multidimensional calibration method (NMDS) was applied to analyze between- or within-group differences in the samples.

3. Results

3.1. Particulate Matter Detection and Environmental Monitoring in Duck House. The average concentrations of samples at different sampling points for PM2.5 and PM10 are shown in Table 1. The average environmental monitoring data of the duck house in winter is shown in Table S1, in which the endotoxin concentration was $25.26 \pm 1.04 \times 10^3$ EU/m^3.

Table 1: Average concentration of PM at different sampling points.

Parameters	PM2.5 ($\times 10^2 \mu g/m^3$)	PM10 ($\times 10^2 \mu g/m^3$)
A	2.31 ± 0.87	1.37 ± 0.54
B	1.04 ± 0.25	0.63 ± 0.25

The data denote mean ± standard error of the mean.

3.2. Biodiversity Analysis of Particulate Matter in Duck House Environment

3.2.1. Macrogenome Sequencing Analysis. Microbial communities inside and outside the duck house were analyzed by macrogenome sequencing. In order to examine the distribution of gene numbers among specified samples (groups) and to analyze the gene common and unique information among different samples (groups), a Venn diagram was drawn. The results showed that the number of genes unique to house (A) was 59380 and to downwind point (B) was 135351, and the number shared by the two sampling points was 855851 (Figure S1). The alpha diversity reflects the diversity of microbial communities within the samples, and the diversity of individual samples can reflect the abundance of the species and the diversity of species within the samples. The alpha diversity index showed no significant differences in species abundance and diversity among sampling sites in the duck house environment (Table S2). The house (A) samples contained 85.29% bacteria, 0.02% eukaryotes, 0.01% archaea, 0.48% viruses, and 14.2% undefined; the downwind point (B) samples contained 85.18% bacteria, 0.09% eukaryotes, 0.01% archaea, 0.58% viruses, and 14.14% undefined (Figure S2).

A total of 86 phyla were identified at the phylum level, and the dominant phyla in the house and downwind sites were consistent, with the top five phyla being Actinobacteria, Firmicutes, Proteobacteria, Bacteroidetes, and Chlamydiae, with Actinobacteria having the highest relative abundance at 65% and 55%, followed by Firmicutes (33%, 38%) and Proteobacteria (1.7%, 4.3%) (Figure 1(a)). At the genus level, a total of 1583 genus were identified, and the dominant genera in the house and downwind sites were consistent, with the top 5 dominant genera being Corynebacterium, Jeotgalicoccus, Staphylococcus, Rothia, and Aerococcus, with Corynebacterium having the highest relative abundance at 65% and 57%, followed by Jeotgalicoccus (6.5%, 6%) and Staphylococcus (4.4%, 5.2%) (Figure 1(b)). At the species level, a total of 7681 species were identified, and the top five species in the house and downwind sites were Rothia nasimurium, Corynebacterium stationis, Corynebacterium xerosis, Jeotgalicoccus halophilus, and Lactobacillus aviarius, with Rothia nasimurium having the highest relative abundance at 13.4% and 13.2%, respectively, followed by Corynebacterium stationis (10.4%, 8.2%) and Corynebacterium xerosis (8.0%, 7.9%) (Figure 1(c)).

NMDS analysis can reflect inter- or intragroup differences of samples, and the results showed that samples collected at downwind sites are clustered together, indicating that they had similar communities; compared with downwind sites, samples in the house were relatively dispersed (Figure S3). Based on the annotation results and abundance information of the genes in the Comprehensive Antibiotic Research Database (CARD), the top five resistance genes were aminoglycosides (APH(6)-Id and APH(3″)-Ib), fluoroquinolones (Neisseria gonorrhoeae gyrA conferring resistance to fluoroquinolones and Staphylococcus aureus pare conferring resistance to fluoroquinolones), and quinolones (AAC(3)-IV) (Figure S4).

3.2.2. Bacterial Diversity Analysis by Matter Macrogenome Sequencing. Airborne bacterial communities inside and outside the duck house were further analyzed by macrogenomic sequencing. At the phylum level, a total of 68 phyla were identified, and the dominant bacterial phyla in the house and the downwind point were consistent; the top 5 bacterial phyla were Actinobacteria, Firmicutes, Proteobacteria, Bacteroidetes, and Chlamydiae, with Actinobacteria having the highest relative abundance at 65.2% and 55.9%, followed by Firmicutes (32.7%, 38.5%) and Proteobacteria (1.7%, 4.3%) (Figure 2(a)). At the genus level, a total of 1316 genera were identified; the top 5 dominant genera in the house were Corynebacterium, Jeotgalicoccus, Staphylococcus, Rothia, and Aerococcus, with Corynebacterium (65.3%) having the highest relative abundance, followed by Jeotgalicoccus (6.5%) and Staphylococcus (4.4%); the top 5 dominant genera at downwind sites were Corynebacterium, Jeotgalicoccus, Staphylococcus, Rothia, and Psychrobacter, with Corynebacterium (57.6%) having the highest relative abundance, followed by Jeotgalicoccus (6.1%) and Staphylococcus (5.2%) (Figure 2(b)). At the species level, a total of 7065 strains were identified and the top 5 dominant strains in the house and downwind sites were Rothia nasimurium, Corynebacterium stationis, Corynebacterium xerosis, Jeotgalicoccus halophilus, and Lactobacillus aviarius, with Rothia nasimurium having the highest relative abundance at 13.6% and 13.4%, followed by Corynebacterium stationis (10.6%, 8.4%), Corynebacterium xerosis (8.1%, 8.0%), Jeotgalicoccus halophilus (7.6%, 6.6%), and Lactobacillus aviarius (1.9%, 2.0%) (Figure 2(c)).

3.2.3. Fungal Diversity Analysis by Macrogenome Sequencing. The airborne fungal communities inside and outside the duck house were further analyzed by macrogenome sequencing. At the phylum level, a total of eight fungal phyla were identified such as Ascomycota, Basidiomycota, Mucoromycota, Chytridiomycota, Zoopagomycota, Blastocladiomycota, Microsporidia, and Cryptomycota, with Ascomycota (91.9%, 93.4%) having the highest relative abundance, followed by Basidiomycota (4.4%, 4.2%) and Mucoromycota (2.9%, 1.8%) (Figure 3(a)). At the genus level, a total of 110 genera were identified, and the top 5 dominant genera in the house were Talaromyces, Alternaria, Friedmanniomyces, Verticillium, and Coccidioides, with Talaromyces (29.2%) having the highest relative abundance, followed by Alternaria (12.3%) and Friedmanniomyces (8.1%); the top 5 dominant genera at the downwind point were Talaromyces, Alternaria, Friedmanniomyces, Acidomyces, and Aspergillus, with Talaromyces (42.7%) having the highest relative abundance, followed by Alternaria (11.9%), Friedmanniomyces (11.1%),

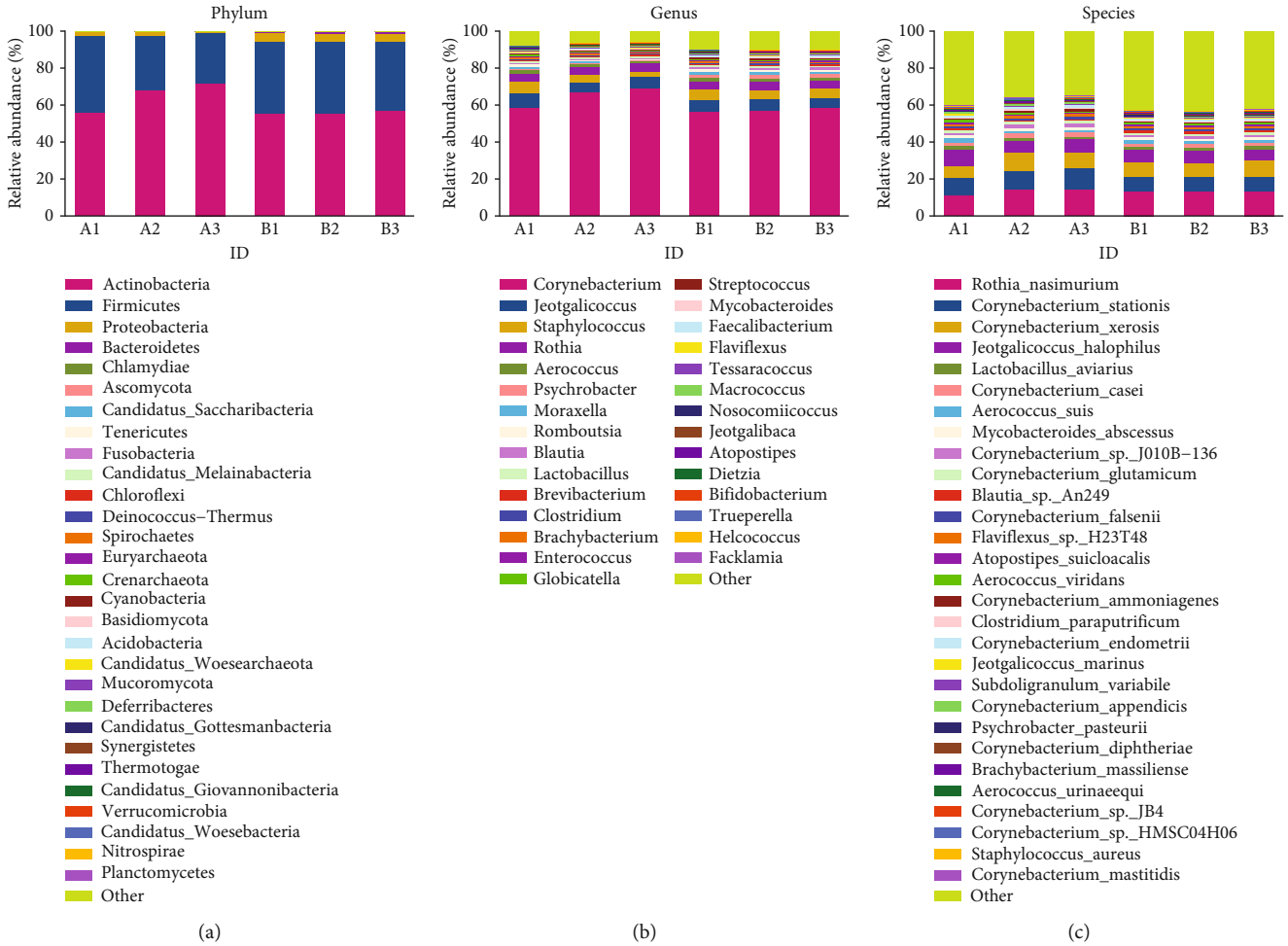

Figure 1: Relative abundance of microorganisms at the (a) phylum, (b) genus, and (c) species levels for PM samples in the duck house (A) and downwind point (B).

and Aspergillus (3.4%) (Figure 3(b)). At the species level, a total of 157 species were identified, and the top 5 dominant species in the house were Talaromyces islandicus, Verticillium longisporum, Friedmanniomyces endolithicus, Coccidioides immitis, and Phlebia centrifuga, with Talaromyces islandicus (15.7%) having the highest relative abundance, followed by Verticillium longisporum (13.9%), Friedmanniomyces endolithicus (10.9%), Coccidioides immitis (6.7%), and Phlebia centrifuga (4.6%); the top five dominant species at downwind sites were Talaromyces islandicus, Friedmanniomyces endolithicus, Verticillium longisporum, Coccidioides immitis, and Acidomyces richmondensis, with Talaromyces islandicus (25.2%) having the highest relative abundance, followed by Friedmanniomyces endolithicus (15.8%) and Acidomyces richmondensis (6.1%) (Figure 3(c)).

3.3. Quantitative Analysis for the Biodiversity of Particulate Matter in Duck House Environment

3.3.1. Quantitative Analysis of Bacterial Diversity by Absolute Quantitative Sequencing. The composition and content of airborne bacteria for PM10 samples inside and outside the duck house were analyzed by the Accu16S™ bacterial absolute quantification method. Based on the diversity indices of each sample, it was possible to test whether there was a significant difference in the alpha diversity of samples between groups. The indices showed no significant differences in bacterial abundance and diversity between sampling points in the duck house (Figure S5). In terms of operational taxonomic unit (OTU) interaction, there were 773 OTUs unique to the house (A) samples and 496 OTUs unique to the downwind point (B) samples, and the two sampling points combined had 552 OTUs (Figure S6).

At the phylum level, a total of 23 phyla were identified, and the top 5 dominant phyla in the house and downwind sites were Firmicutes, Actinobacteria, Proteobacteria, Bacteroidetes, and Acidobacteria. The relative abundance of Firmicutes was 48.9% and 60.6%, the absolute abundance was 6.5×10^7 copies/g and 2.3×10^7 copies/g, followed by Actinobacteria and Proteobacteria. Relative abundance of Actinobacteria was 41.6% and 35.7%, and the absolute abundance was 6.2×10^7 copies/g and 1.6×10^7 copies/g; the relative abundance of Proteobacteria was 11.2% and 7.6%, and the absolute abundance was 1.6×10^7 copies/g and 3.4×10^6

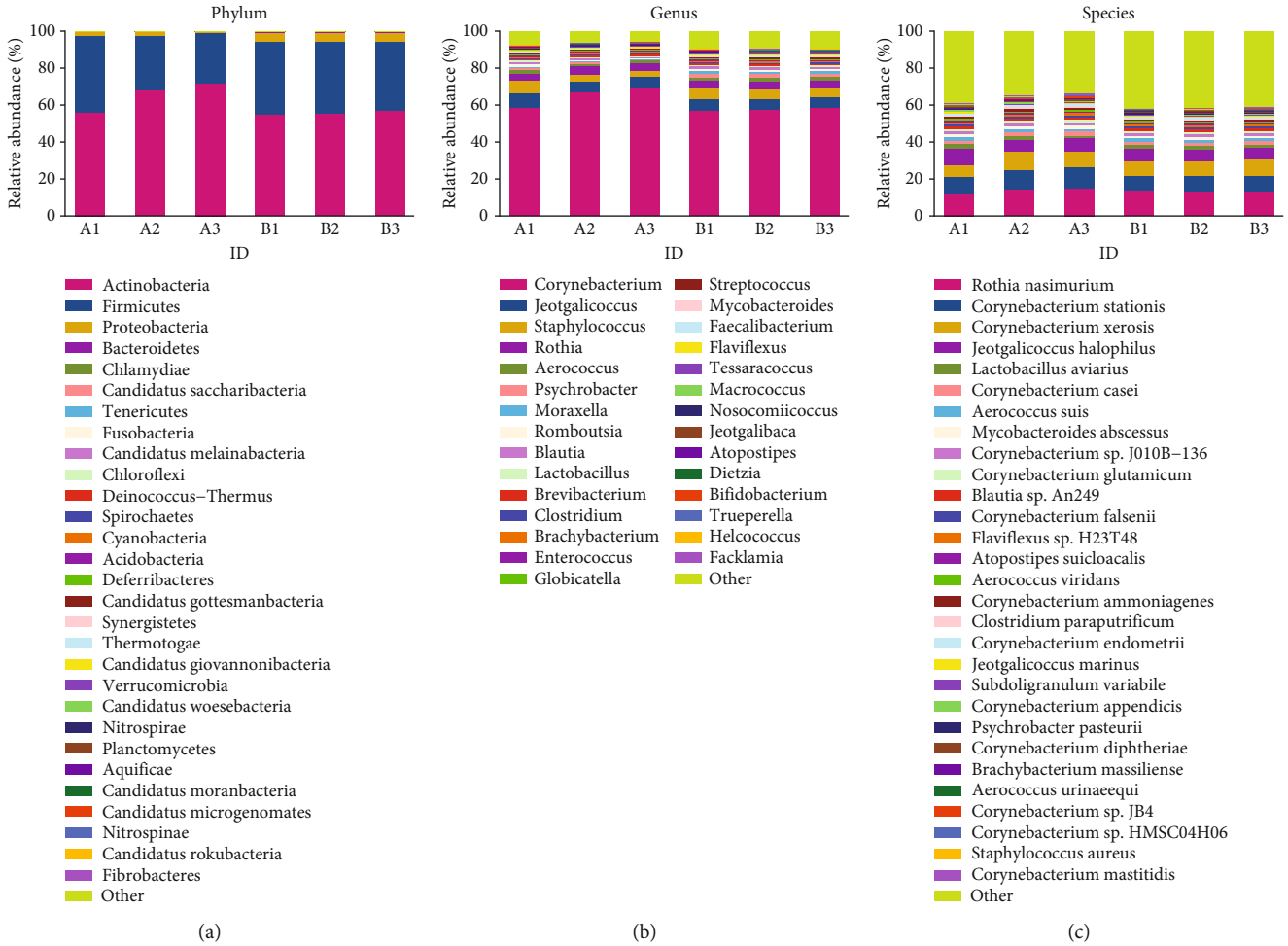

FIGURE 2: Relative abundance of airborne bacteria at the (a) phylum, (b) genus, and (c) species levels for PM samples in the duck house (A) and at the downwind point (B).

copies/g, respectively (Figure 4(a)). At the genus level, a total of 307 genera were identified. The top 5 dominant genera in the house were Corynebacterium, Romboutsia, Aerococcus, Psychrobacter, and Staphylococcus, with Corynebacterium (33.9%, 5.6×10^7 copies/g) having the highest abundance, followed by Romboutsia (9.3%, 1.4×10^7 copies/g) and Aerococcus (6.7%, 9.9×10^6 copies/g); the top 5 dominant genera at downwind sites were Corynebacterium, Romboutsia, Aerococcus, Jeotgalicoccus, and Facklamia, with Corynebacterium (23.7%, 1.5×10^7 copies/g) having the highest abundance, followed by Romboutsia (15%, 6.8×10^6 copies/g) and Aerococcus (5%, 2.3×10^6 copies/g) (Figure 4(b)).

Further LEfSe analysis was performed to identify biomarkers of airborne bacteria in different sample sets. Differential bacterial genera with significantly higher abundance in the house (A) samples were Gemmobacter, Soonwooa, Propioniciclava, and Shinella compared to the downwind point samples; and differential bacterial genera with significantly higher abundance in the downwind point (B) samples were Bacillus and Pontibacter (Figure 5).

3.3.2. Quantitative Analysis of Fungal Diversity by Absolute Quantitative Sequencing. The composition and content of airborne fungi for PM10 samples inside and outside the duck house were analyzed by AccuITS™ absolute quantification of fungi. Based on the diversity indices of each sample, it was possible to test whether there was a significant difference in the alpha diversity of samples between groups. There were no significant differences in fungal diversity and abundance among the different sampling points of the duck house (Figure S7). In terms of OUT interactions, there were 659 species of OTUs unique to the house (A) sample and 218 species of OTUs unique to the sample from the downwind point (B), and the two sampling points combined had 251 species of OTUs (Figure S8).

At the phylum level, a total of eight phyla were identified, and the top five dominant phyla both in the house and at downwind sites were Ascomycota, Basidiomycota, Mucoromycota, and unidentified. The relative abundance of Ascomycota was 54.8% and 70%, and the absolute abundance was 1.2×10^7 copies/g and 9.7×10^6 copies/g; this was followed by Basidiomycota with the relative abundance of 43.6% and 28.2% and the absolute abundance of 8.5×10^6 copies/g and 4.9×10^6 copies/g in the house and downwind sites, respectively (Figure 6(a)). At the genus level, a total of

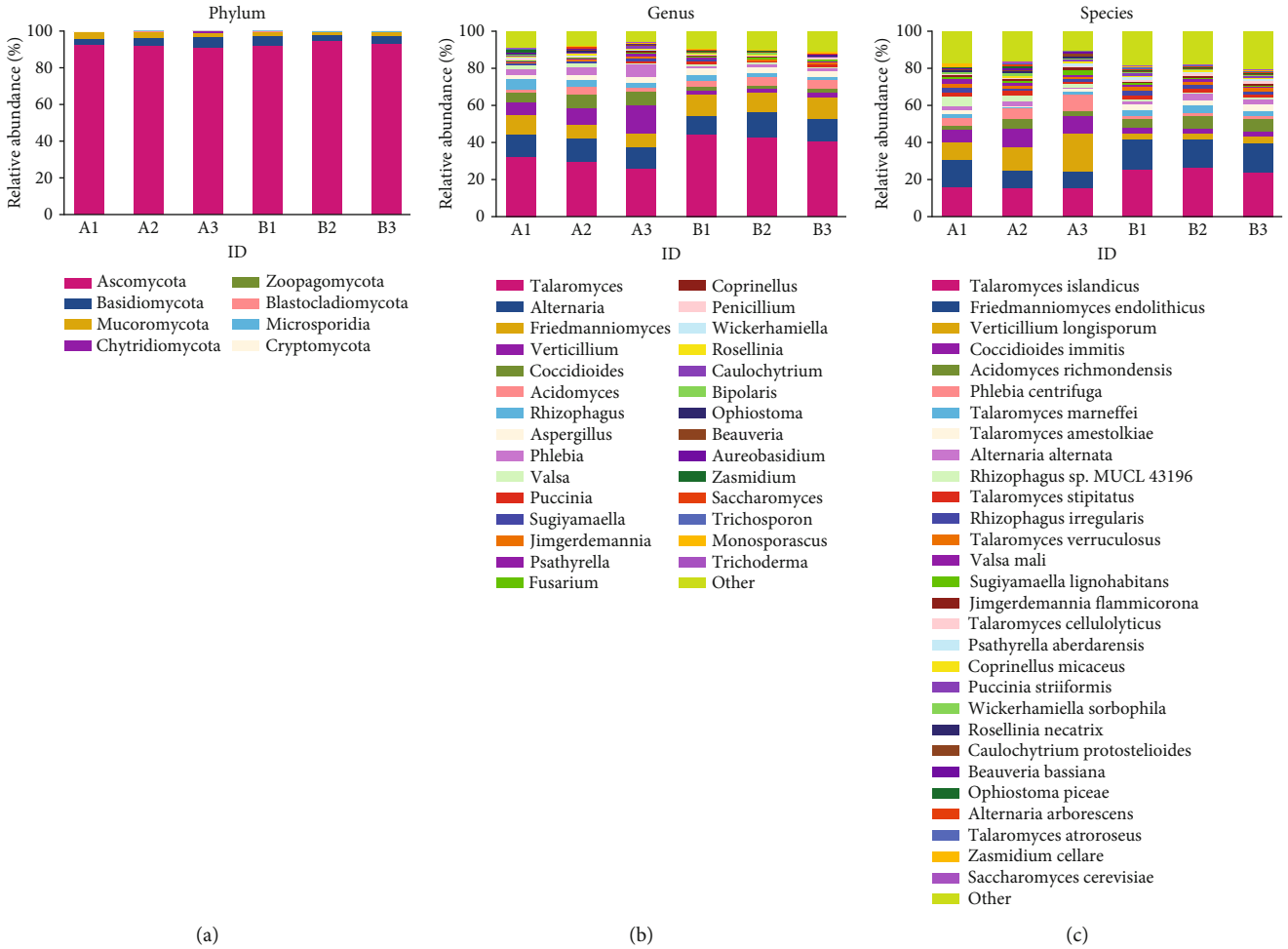

Figure 3: Relative abundance of airborne fungi at the (a) phylum, (b) genus, and (c) species levels for PM samples from inside (A) and downwind points (B) of the duck house.

321 genera were identified, and the top 5 dominant genera in the house were Alternaria, Cladosporium, Trametes, Mycosphaerella, and Coprinellus, in which the genus with highest abundance was Alternaria (11.1%, 3.3×10^6 copies/g), followed by Cladosporium (10.7%, 2.2×10^6 copies/g) and Trametes (8.9%, 1.8×10^6 copies/g); the top 5 dominant genera at downwind sites were Cladosporium, Mycosphaerella, Talaromyces, Schizophyllum, and Alternaria, with Cladosporium (17.6%, 2.6×10^6 copies/g) having the highest abundance, followed by Mycosphaerella (11.9%, 1.7×10^6 copies/g) and Talaromyces (11%, 1.6×10^6 copies/g) (Figure 6(b)). The percentage of Aspergillus was 4.0%, with a concentration of 3.3×10^5 copies/g.

Further LEfSe analysis was performed to identify biomarkers of airborne fungi in different sample groups. The differential fungal genera with significantly higher abundance in the house (A) samples were Ganoderma, Chaetosphaeronema, Schizopora, and Resupinatus compared to the downwind point samples; and the differential fungal genera with significantly higher abundance in the downwind point (B) samples were Gibberella, Xeromyces, Pseudogymnoascus, and unidentified (Figure 7).

4. Discussion

China is a large poultry farming country in the world. In 2020, the number of commercial meat ducks in China reached 4.683 billion, with an increase of 4.855 billion in 2021 [12]. High concentrations of microbial aerosols are produced during the process of rearing meat ducks, which contain a number of pathogenic bacteria that pose a major threat to human and animal health [13]. The average concentration of PM2.5 samples collected in the winter house (A) in this study was $2.31 \pm 0.87 \times 10^2\ \mu g/m^3$, which was higher than the PM2.5 concentrations in duck houses reported by Wu et al. ($1.1 - 1.6 \times 10^2\ \mu g/m^3$), while the average concentration of PM10 in the summer house (A) was $1.37 \pm 0.54 \times 10^2\ \mu g/m^3$, which was lower than the results reported by Wu et al. [14]. According to the global air quality guidelines set by the World Health Organization (WHO), the short-term (24 h) AQG level for PM2.5 is less than 15 $\mu g/m^3$, and the short-term (24 h) AQG level for PM10 is less than 45 $\mu g/m^3$; the relative risk of nonaccidental mortality per 10 $\mu g/m^3$ of 24-hour average PM2.5 is 1.0065%, and per 10 $\mu g/m^3$ of 24-hour average, the relative risk of

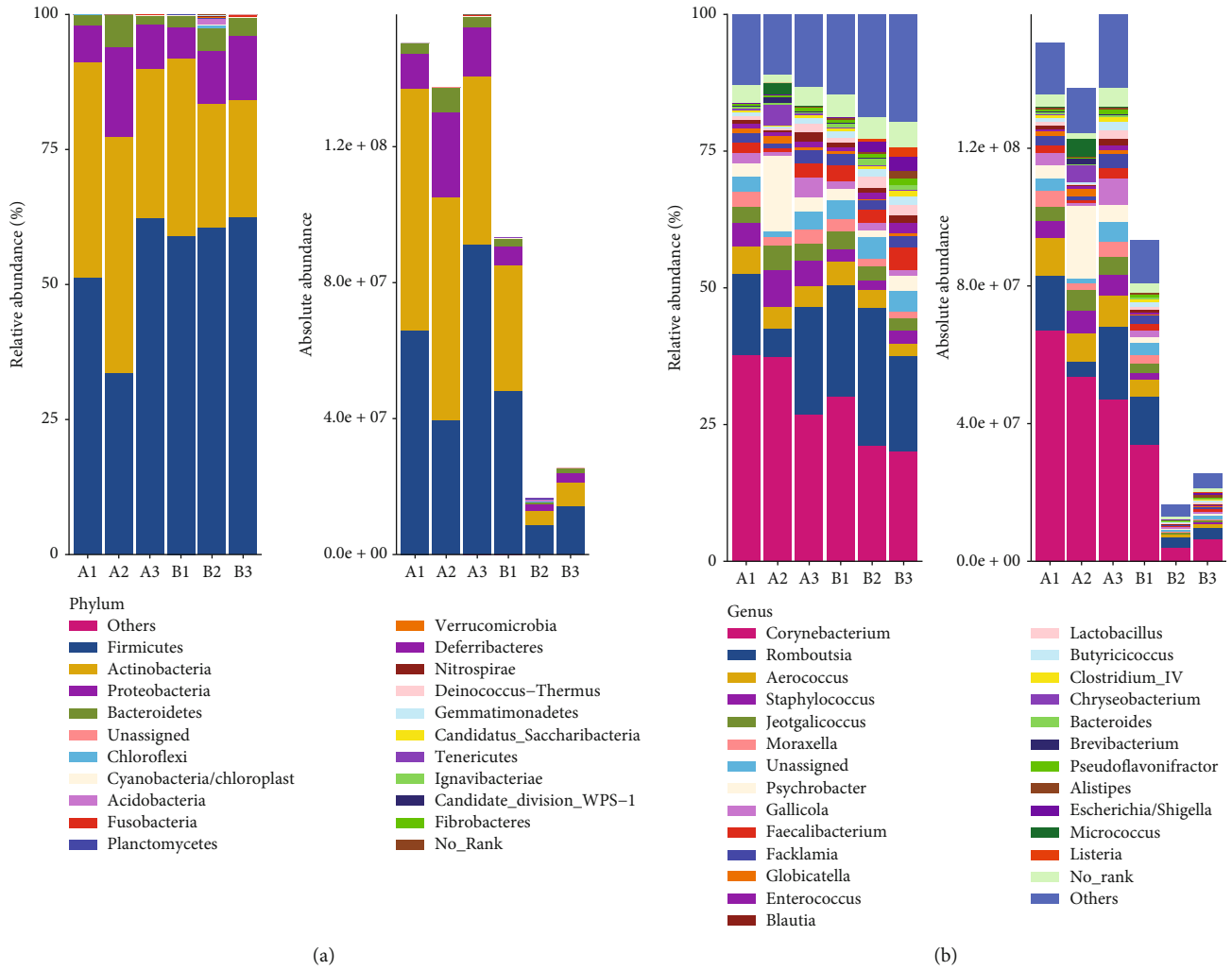

Figure 4: Relative and absolute abundance of airborne bacteria at the (a) phylum and (b) genus levels for PM samples in the duck house (A) and downwind point (B).

nonaccidental mortality for PM10 was 1.0041% [15]. The results of this study were approximately 15 and 3 times the short-term standards for PM2.5 and PM10, and prolonged exposure to such high concentrations of PM can be detrimental to human health and pose a threat to the health of animals.

In addition to the effects of airborne aerosols on human and animal health, the effects of airborne endotoxins on humans and animals cannot be ignored. Endotoxins can cause impaired lung function and respiratory symptoms in humans [16, 17]. Residents living near livestock farms exposed to high levels of endotoxin are more likely to have reduced lung function and increased prevalence of asthma [18, 19]. In a dispersion modeling study, a significant increase in asthma was associated with high levels of endotoxin when an endotoxin exposure model was used [20]. Endotoxin induces pulmonary hypertension in reared broilers [21]; differences in its concentration and mode of application reduce or increase specific antibody responses in poultry [22, 23]. Prolonged exposure to airborne endotoxin of high concentrations affects components of the immune system and respiratory tract of broilers, which in turn may affect disease susceptibility [24]. In this study, the endotoxin level of duck house was $25.26 \pm 1.04 \times 10^3$ EU/m^3, which exceeds the no-effect level for human health (100 EU/m^3) and even exceeds the recommended criterion for causing pneumonia in humans (2,000 EU/m^3) [25]. Therefore, being chronically exposed in such environments can greatly affect worker and poultry health. Conventional microbial cultures can only detect certain pathogenic microorganisms and reveal only a small fraction of the aerosol [26], greatly limiting species and quantitative analysis [27]. Many of the sequences obtained by conventional amplicon sequencing cannot be annotated to the species level and do not reflect the true number of microorganisms in the samples. Therefore, in this study, we used macrogenome sequencing technology and Accu16S™/ITS™ absolute quantification methods to analyze the environmental PM2.5 in winter duck house 1 and PM10 in summer duck house 2, respectively.

Environmental factors are also very important in influencing the microbial community of poultry house and

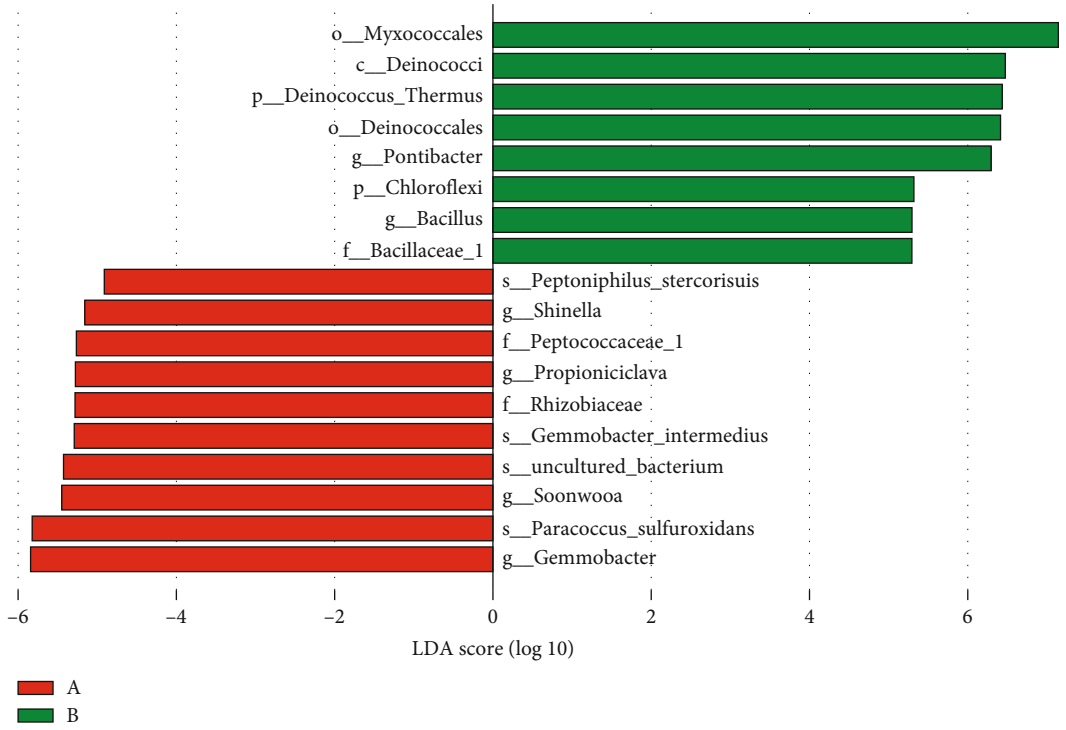

Figure 5: LDA of bacterial taxa of PM samples from inside the duck house (A) and downwind point (B). Bars of different colors indicate different species in different groups with LDA scores (log10) greater than 2 and with significantly higher abundance in the group, and the length of the bars represents the size of the LDA score value.

are key factors affecting the environment for poultry production [28, 29]. Some studies have shown a positive correlation between temperature and PM concentration [30], but this is under the same ventilation conditions, and in general, the ventilation is significantly better in summer than in winter, so usually, the air pollutants of the duck house in summer will be significantly lower than that in winter, and in the present study, the PM concentration in summer was lower than that in winter, which is consistent with the results of Dong et al. [31]. It has been shown that when the relative humidity is in the range of 37-97%, the effect of relative humidity on microbial activity in bioaerosols is minimal [32]. The relative humidity in the present study was 53.30 ± 3.06% during the winter season, which was in the range of 37-97% and should had little effect on the microbial activity in the house. Elevated CO_2 concentrations can have an impact on poultry performance, with higher CO_2 negatively affecting the performance of turkey poults [33]. The International Commission of Agricultural and Biosystems Engineering has established a maximum CO_2 concentration of 3,000 ppm in general production facilities and 2,500 ppm in poultry production facilities [28]. In the present study, the CO_2 concentration in winter was 1217 ± 5.57 ppm, which is within the threshold range. High concentrations of NH_3 in poultry houses can adversely affect poultry health and production performance and increase mortality [34, 35]. The winter NH_3 concentration in this study was 2.10 ± 0.10 ppm, which was lower than the recommended threshold for human exposure of 25 ppm [36] and lower than China's Environmental Quality Standard for Livestock and Poultry Farms (13 ppm, NY/T 388-1999).

The influence of environmental factors on the concentration of aerosols, the diversity and abundance of microbial communities in poultry houses, and the health and production performance of poultry are comprehensive and complex; in addition to the influence of temperature, humidity, and the concentration of CO_2 and NH_3, it may also be related to the wind speed, as well as other air pollutants, etc. Therefore, we should strengthen the management of environmental conditions in poultry houses to ensure the health of poultry and human beings and also enhance the comprehensive research related to the influence of environmental factors on aerosol concentration and microbial communities in poultry houses.

Macrogenome sequencing can reveal the overall community status in the environment. The analysis of PM2.5 samples from the duck house1 environment in winter showed that compared with the samples from the downwind point, the samples in the house were relatively dispersed, and there was a difference within the group, which may be related to the more dispersed location of the sample collection in the house; the environmental microbial community inside and outside of the house was mainly dominated by bacteria, and their percentage was all over 85%, which was in line with the results of Zhai et al. [37]. This shows that the microscopic organisms of PM2.5 particles in duck house were dominated by bacteria, and we should increase the study of bacteria in the

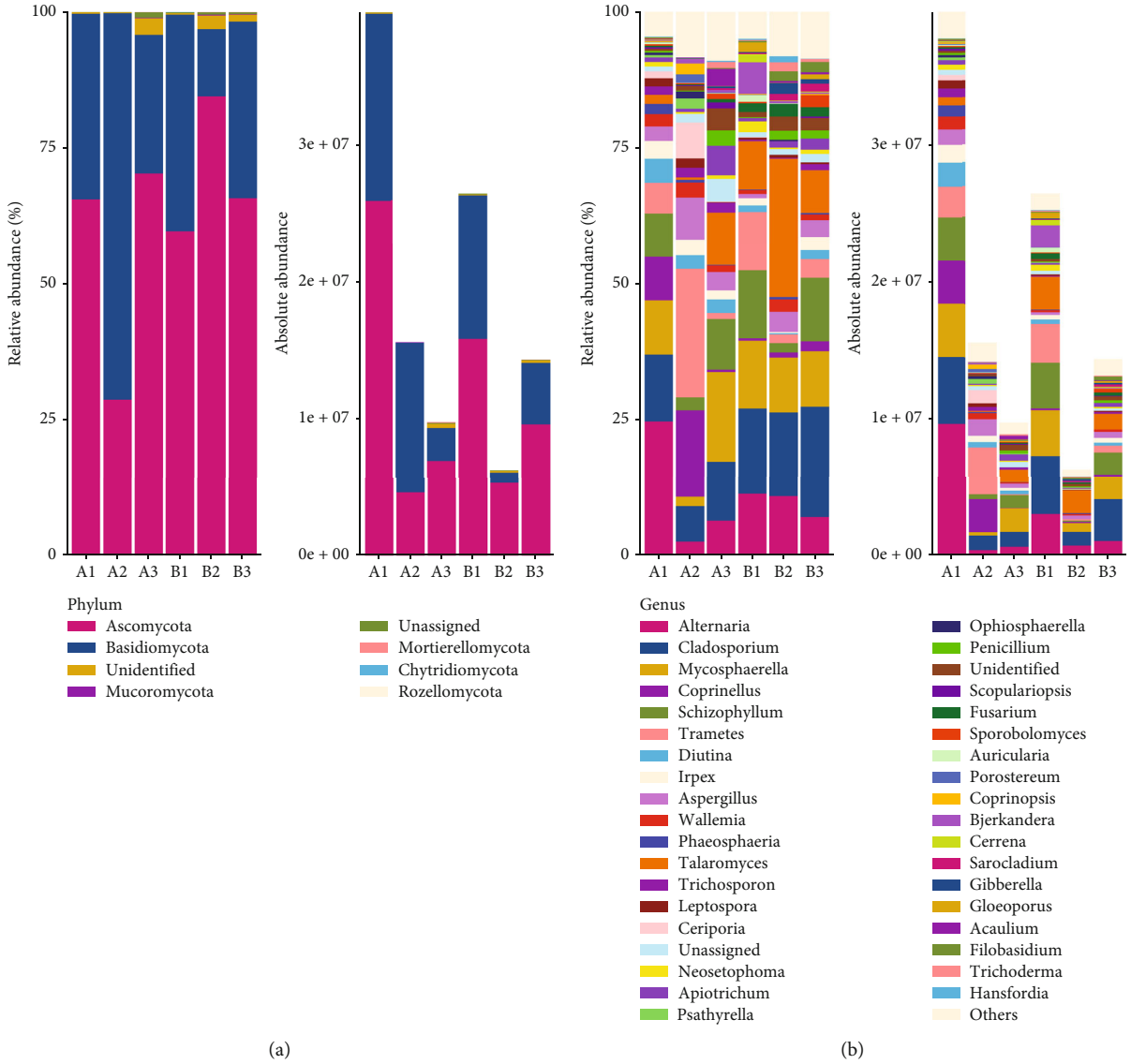

Figure 6: Relative and absolute abundance of airborne fungi at the (a) phylum and (b) genus levels for PM samples from inside (A) and downwind points (B) of the duck house.

duck house environment, especially those species that are threats to animals and humans.

PM in poultry houses is mainly particles of biological origin, as well as particles from feed, skin, and manure [38], with feces being the main source of aerosols in poultry houses [39]. Previous studies have shown that the most abundant bacterial phyla in particulate matter of duck house are Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes [14]. The present study showed that the top four phyla in the PM2.5 samples were Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes, which were consistent with the above reports, and the top four dominant bacterial phyla in the PM10 samples were Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes, which were the same species although the ranking order was different. The dominant phyla in the chicken gut microbiota were reported to be Firmicutes (60%), Bacteroidetes (22%), and Proteobacteria (17%) [40, 41]; and the dominant phyla in the duck gut microbiome were Firmicutes, Bacteroidetes, and Actinobacteria [42] and Firmicutes, Proteobacteria, and Bacteroidetes [43]. It was inferred that duck house aerosols were mainly derived from duck feces.

A total of 1,316 bacterial genera and 110 fungal genera were identified in the ambient PM2.5 samples from the winter duck house 1, and 7,065 bacterial and 157 fungal species were identified. 293 bacterial genera and 183 fungal genera were identified by Wu et al. Bacterial species identified in this study were much more abundant at the genus level than those identified by Wu et al. [14]. This discrepancy should be related to the different means of analysis, and the macrogenome is the whole genome sequencing analysis, while amplicon sequencing only detects a small segment of the genome, and the macrogenome is much more recognizable than amplicon sequencing of bacteria, and therefore, more species of bacterial groups can be found. The top five common bacteria at the genus level for both were Corynebacterium,

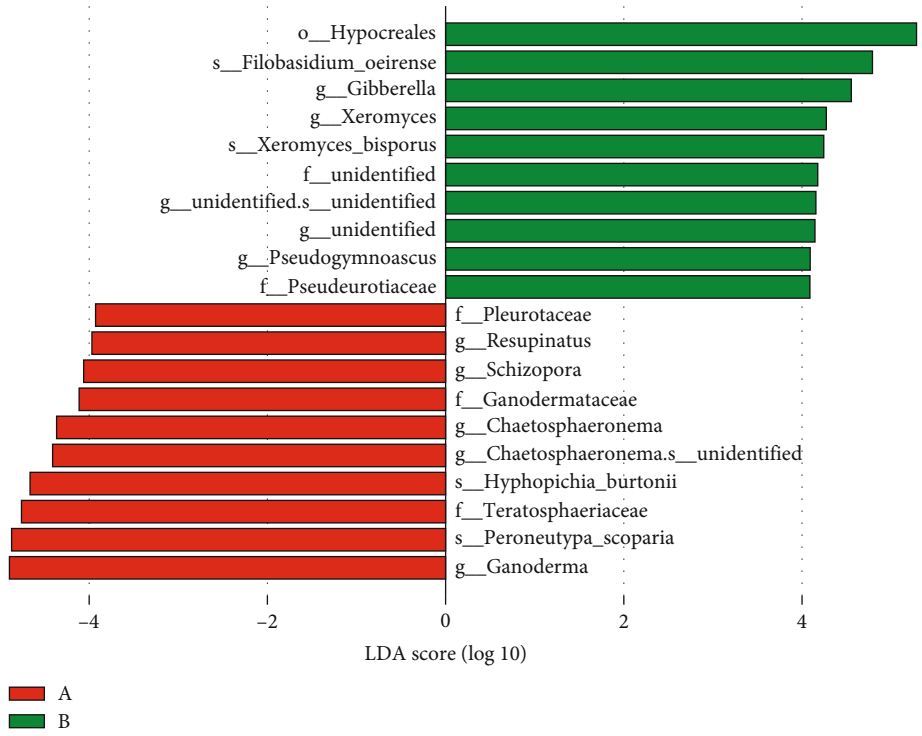

Figure 7: LDA of fungal taxa of PM samples from inside the duck house (A) and downwind point (B). Bars of different colors indicate different species in different groups with LDA scores (log10) greater than 2 and with significantly higher abundance in the group, and the length of the bars represents the size of the LDA score value.

Jeotgalicoccus, and the shared fungus Alternaria, suggesting that the genera prevalent in duck house were Corynebacterium, Jeotgalicoccus, and Alternaria.

A total of 307 bacterial genera and 321 fungal genera were identified in the environmental PM10 samples from the summer duck house 2, which was higher than the 261 bacterial groups and 180 fungal florae identified at the genus level by Wu et al. [14], but the difference was not significant because the methods used to detect the genes were both 16S/ITS. In the top five ranked genera, a common bacterial genus detected by the two studies was Corynebacterium, and the common fungi were Cladosporium and Alternaria, suggesting that Corynebacterium, Cladosporium, and Alternaria may be prevalent in PM10 samples from duck houses.

In this study, some conditionally pathogenic bacteria were identified and quantified at the genus level. Corynebacterium accounted for 33.9% of the PM10 samples in the duck house 2 during summer, amounting to 5.6×10^7 copies/g, and accounted for an even higher proportion of the PM2.5 samples in winter, amounting to 65.3%, which indicates that this genus of bacteria is absolutely dominant in the duck house. The results of ambient aerosol detection in duck houses reported by Wu et al. showed the dominance of Corynebacterium (35.94%) in PM10 samples [14], and the results of the present study showed higher values. Corynebacterium has a high isolation rate in infections of the upper respiratory tract, lungs, etc. It may also cause infections in immunocompromised hosts [44, 45], and certain species of Corynebacterium cause diphtheria, which triggers bacteremia and pneumonia complications [12].

Staphylococcus may be involved in inflammation, human skin infections, bone and joint infections, and food poisoning in chickens and humans [46, 47]. Lei et al. successfully constructed mouse skin abscesses using a 5×10^8 CFU S. aureus model [48]. Staphylococcus accounted for 3.9% of the PM10 samples in this study, amounting to 5.8×10^6 copies/g, and ranked second in the winter PM2.5 samples at 4.4%, which was the dominant bacterium, posing an infectious risk to both animals and humans.

Aerococcus accounted for 6.3% of the PM10 samples, amounting to 9.4×10^6 copies/g, and 1.5% of the winter PM2.5 samples, and it was not among the top ten genera in the results of Wu et al. [14], which is not consistent with the results of this study. Aerococcus is widely distributed in the natural environment, including other sources such as soil, air, and water, and members of the genus are thought to be associated with urinary tract infections [49, 50]. In addition, Aerococcus pneumoniae acts as a conditional pathogen that may cause bacteremia, sepsis, septic arthritis, and endocarditis when animals are trauma-prone and immunocompromised [51]. Liu et al. tested the toxicity of A. viridans in mice with 5.0×10^7 CFU, and only 10% of the mice survived after 4 days with a high mortality rate [52].

Fungi in animal houses also often pose a risk to human and animal health. Alternaria is a common human allergen that can cause asthma and bring great harm [53]. Alternaria accounted for 11.1% of the PM10 samples in this study, amounting to 3.3×10^6 copies/g and 12.3% of the PM2.5 samples in winter. In the study of Wu et al., the percentage of Alternaria in PM10 samples was 8.03% [14], which was lower

than the concentration of Alternaria in this study. Ahn et al. constructed a mouse model of sinusitis using 2×10^5 spores of Alternaria [54]. Therefore, less than 1 g of Alternaria in PM10 particulate matter is enough to cause sinusitis in mice.

Aspergillus is a kind of major fungal allergen and an opportunistic pathogen whose spores cause respiratory diseases such as invasive pulmonary aspergillosis [55, 56]. In this study, Aspergillus accounted for 4.5% of the PM10 samples, amounting to 8.3×10^5 copies/g, and accounted for 2.6% of PM2.5 samples. The previous results showed that Aspergillus accounted for 14.21% of PM10 samples and 9.9% of PM2.5 samples [14], which is significantly different from the detection results of the present study. The effect on cytokine release from mouse lung epithelial cells was assessed using 3×10^5 cells/ml Aspergillus fumigatus, and the result showed that Aspergillus fumigatus conidia induced T helper 2 (Th2) cytokine profiles in lens epithelial cells (LECs) [57]. A successful model of invasive pulmonary aspergillosis (IPA) infection was constructed by scholars using a final concentration of 6×10^4 CFU of A. fumigatus [58]. Thus, 10^4 copies are reached after every 0.1 g of PM10 particles is breathed in, reaching an infectious dose that triggers Aspergillosis in animals. The Aspergillus in the duck houses tested in this study is still generally high and poses a risk to the respiratory health of the animals and humans in the house.

Fusarium causes many diseases, including nail fungal diseases [59], bone and joint infections [60], fungal keratitis [61], and wound infections [62, 63], and its spores are also important respiratory allergens [64], with Fusarium accounting for 0.3% of PM10 samples, amounting to 6.6×10^4 copies/g, and 0.6% of winter PM2.5 samples. The dominant genus in the results of Wu et al. is consistent with the results of the present study [14]. Tarabishy et al. constructed a mouse model of Fusarium keratitis using 1×10^4 conidia/ml 2 μL [65]. It did not require 1 g of PM10 particulate matter to cause keratitis in mice.

Bioaerosols containing pathogens are responsible for disease transmission [66, 67]. Therefore, it is critical to understand the composition of microorganisms in ambient particulate matter (PM) from duck houses and to identify potential pathogens and allergens. Previous microbial identification of air PM samples has been limited to the genus level, but the pathogenicity of different species of bacteria in the same genus varies greatly and often fails to clarify their pathogenic risk. Macrogenome sequencing technology, which allows for the detection of the microbial composition of samples at the species level, can provide greater clarity on the species present in the duck house environment that are potential threats to humans and animals.

For example, the opportunistic pathogen Rothia nasimurium was first in the PM2.5 samples at 13.6%, and it can infect animals such as dogs, pigs, ducks, rabbits, and geese. The bacterium was isolated from captive chickens and geese for the first time and was found to be multidrug resistant by drug sensitivity test and animal regression test, resulting in severe hair loss in chickens and geese [68, 69]. Staphylococcus aureus (0.46%) is a kind of pathogen that can cause primary infections in humans or animals, leading to severe clinical conditions such as bacteremia and sepsis [70]. It is ubiquitous in nature and can be found in air, water, dust, and human and animal excreta, causing bacterial infections in humans and animals. Furthermore, air pollution is the main route of transmission to animal populations [71]. Staphylococcus aureus can invade the body when the skin and mucous membranes of the body are damaged, and the infection manifests as suppurative inflammation of the skin, tissues, and organs in mild cases, while in severe cases, it appears as pneumonia, pericarditis, endocarditis, and septicemia, and if the disease continues to develop, it can lead to death [72].

The results of diversity index showed that the differences in diversity and relative abundance between the indoor and downwind were not significant, which was consistent with the research results of airborne bacterial communities in chicken houses by Xu et al. [10]. Bioaerosols and particulate matter from the house are easily transmitted to downwind sites through the ventilation system and natural winds, so the particulate matter samples from the indoor and downwind sites showed relatively similar communities, which also suggests that bioaerosols and particulate matter from the aviary may pose a health risk to the duck farm workers and the neighboring residents.

5. Conclusions

This is the first study in which aerosol particles inside and outside the duck house were sampled and analyzed by macrogenome sequencing technology and Accu16S™/ITS™ absolute quantitative methods. The results showed that the concentrations of PM2.5 and PM10 particulate matter in the duck house and at downwind points exceeded the short-term (24 h) guideline of the global air quality guidelines. Macrogenomic sequencing of PM2.5 samples identified relatively high levels of causative agent (Coccidioides immitis) and conditional causative agent (Rothia nasimurium) at species level. Absolute quantitative sequencing of PM10 samples detected certain conditional pathogenic bacteria with high content at the genus level: Corynebacterium, Aerococcus, Alternaria, Aspergillus, and Fusarium. The diversity and relative abundance of microorganisms were similar in the duck house and at the downwind point. The results showed that particulate matter and its microorganisms of the sampled duck house may pose public health risk to the staff and the surrounding area, and the management of ambient air pollution in the duck house should be enhanced; it also provides a reference for further study of air pollutants and bioaerosols in poultry farms.

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

Conflicts of Interest

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

Authors' Contributions

Zhengxiu Qu did the conceptualization, methodology, investigation, and writing of the original draft. Ning Li did the conceptualization and methodology. Zhiyun Guo did the methodology and investigation. Jing Li did the investigation. Xiaoyang Lv did the investigation. Yinling Cui wrote and reviewed the manuscript. Hairong Wang did the conceptualization, methodology, and supervision and wrote and reviewed the manuscript. Tongjie Chai did the conceptualization, methodology, and supervision and wrote and reviewed the manuscript.

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Supplementary Materials

Figure S1: number of genes (Venn graph). Figure S2: relative abundance of PM samples at the domain level in duck houses (A) and downwind points (B). Figure S3: NMDS of samples in duck house (A) and downwind point (B). Figure S4: overview of resistance genes. Figure S5: the boxplot of bacterial diversity index of PM samples. Figure S6: comparative Venn diagram of bacteria OTUs in PM samples in duck house (A) and downwind point (B). Figure S7: the boxplot of fungi diversity index of PM samples. Figure S8: comparative Venn diagram of fungi OTUs in PM samples in duck house (A) and downwind point (B). Table S1: sampling information of duck house environment in winter. Table S2: alpha diversity index statistics. *(Supplementary Materials)*

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