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Spatial analysis of airborne bacterial concentrations and microbial communities in a large-scale commercial layer facility

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ABSTRACT

This study investigated the spatial distribution patterns of airborne bacterial concentrations and microbial community structures in a modern commercial layer facility housing approximately 50,000 laying hens equipped with advanced environmental control systems. Air samples were systematically collected at 50 strategically distributed locations using a six-stage Andersen microbial air sampler, while environmental samples (dust, manure, intestinal contents) were characterized using 16S rRNA gene sequencing.

Results demonstrated a distinct longitudinal gradient in airborne bacterial concentrations, progressively increasing from the air inlet (883±177 CFU/m^3) to exhaust fans (12,650±813 CFU/m^3), with a facility-wide mean concentration of 5,618±530 CFU/m^3. Spatial analysis revealed significant bacterial concentration heterogeneity, with elevated bacterial loads (>8,000 CFU/m^3) concentrated in central regions while peripheral areas maintained lower concentrations (<6,000 CFU/m^3). Taxonomic profiling identified Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidetes as predominant phyla across all sample types, with significant compartment-specific distribution patterns: Firmicutes dominated intestinal samples (72.9 %), Proteobacteria and Bacteroidetes were enriched in posterior dust and manure samples, while Acinetobacter exhibited highest abundance (19.90 %) in anterior dust. Differential abundance analysis demonstrated significant enrichment of fecal-associated bacteria (particularly Bacteroides and *Escherichia coli*) in posterior sampling locations, establishing direct correlations between environmental parameters and microbial dissemination patterns.

This comprehensive spatial-microbial analysis elucidates critical factors influencing bacterial dispersion within intensive poultry production environments, providing the empirical foundation for implementing concentration-based risk stratification management systems and targeted interventions to enhance biosecurity, minimize disease transmission, and optimize poultry health in commercial operations.

Introduction

The global poultry industry has expanded significantly in recent decades, establishing itself as a key contributor to meeting the increasing demand for affordable animal-derived protein (Astill et al., 2020; Kleyn and Ciacciariello, 2021). Modern commercial layer operations have increasingly adopted high-density housing for laying hens, incorporating advanced technological systems such as automated feeding, environmental regulation, and sophisticated ventilation mechanisms to maximize productivity and operational efficiency (Astill et al., 2020; Franzo et al., 2023; Kleyn and Ciacciariello, 2021). Although these technological advancements have greatly improved production outcomes, they have also introduced new challenges regarding environmental hygiene and biosecurity within poultry production facilities (Astill et al., 2020; Luiken et al., 2020).

Air quality in poultry facilities is crucial for both animal health and production performance. Poor air quality, characterized by elevated concentrations of airborne particulates and microorganisms, poses significant risks to poultry respiratory health, potentially leading to reduced growth rates, impaired immune responses, and increased disease susceptibility (Bist and Chai, 2022; David et al., 2015). Airborne bacteria within poultry houses can originate from various sources, including feather dust, feed particles, litter, and manure, acting as vectors for pathogenic microorganisms and posing risks to both avian and human health (David et al., 2015).

Ventilation systems play a critical role in regulating the indoor

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environment of poultry houses by controlling temperature, humidity, and air composition (Ivulic et al., 2022; Nguyen et al., 2022; Xu et al., 2022b). In particular, negative pressure ventilation systems, widely used in modern layer operations, are designed to expel stale air while introducing fresh air, thereby mitigating heat stress and reducing moisture accumulation. However, the efficacy of these systems in reducing airborne bacterial concentrations is influenced by factors such as airflow dynamics, stocking density, and housing structure configuration (Li et al., 2021).

A thorough understanding of the spatial distribution of airborne bacterial concentrations within poultry facilities is essential for optimizing ventilation strategies and enhancing biosecurity measures. Spatial variability can lead to localized areas of elevated bacterial loads, thereby increasing the risk of disease transmission among birds and posing occupational health risks to farmworkers (Xu et al., 2022b). Recent studies emphasize the importance of detailed mapping of airborne bacterial concentrations to identify critical control points and develop targeted interventions (de Rooij et al., 2019).

Despite the importance of these issues, data on the spatial distribution of airborne bacterial concentrations and the associated microbial community structures in large-scale commercial layer facilities with advanced environmental control systems are limited. Most existing research has been conducted in small-scale or experimental settings, limiting the applicability of findings to commercial operations (Li et al., 2021; Luiken et al., 2020; Osorio et al., 2023; Zhi et al., 2020). Moreover, the interactions between ventilation systems, housing design, and microbial dispersion are still inadequately understood. Addressing these knowledge gaps is crucial for improving air quality management and biosecurity in intensive poultry production systems. Our research aims to tackle these challenges by integrating spatial analysis of airborne bacterial concentrations with microbial community profiling using 16S rRNA gene sequencing, providing a comprehensive understanding of the factors driving microbial dispersion and enabling the development of evidence-based interventions to enhance poultry health and productivity.

Materials and methods

Experimental site and environmental control

This study was conducted at a state-of-the-art commercial layer facility in Sichuan province, China (31°60 N, 105°13′E), equipped with a modern negative pressure ventilation system (Fig. 1 and Figure S1). The poultry house measured 100 m in length and housed approximately 50,000 laying hens. The internal structure comprised four rows of cages, each containing eight tiers, with plastic manure belts positioned beneath each tier to facilitate daily manure removal. Each row was 2 m wide, and walkways approximately 1 m wide were located between the cage rows to facilitate management activities and sampling.

Air entered the poultry house through evaporative cooling pads mounted on the front wall. When ambient temperatures exceeded 30°C, the cooling system activated automatically to lower the temperature of incoming air. The cooled air flowed longitudinally through the poultry house and was expelled by 20 axial fans mounted on the rear wall (diameter: 1.25 m; model: XLXS; power: 1,100 W; airflow rate: 44,000 m^3/h). An automated environmental control system regulated temperature and humidity to maintain optimal conditions for bird growth, keeping the temperature below 26°C and maintaining a relative humidity of approximately 60 %. An automatic drinking system supplied water based on the hens' needs, and feed was provided at 08:30, 10:00, and 15:30 daily. Lighting was scheduled from 04:30 to 20:30, providing a photoperiod of 16 h of light and 8 h of darkness to support the hens' biological rhythms.

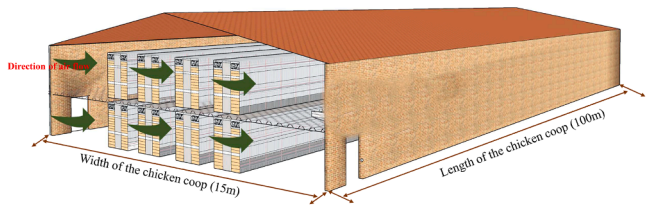

Fig. 1. Three-dimensional illustration of the poultry house structure, showing the chicken cages' layout, air flow direction, and overall dimensions (width: 15 m, length: 100 m). The airflow direction is indicated by arrows to demonstrate the ventilation system within the poultry house.

Air sampling and bacterial concentration analysis

Airborne bacterial concentrations in the poultry house were determined using a six-stage Andersen microbial air sampler (model S6, Beijing Senlong Technology Development Co., Ltd.). Sterile Tryptic Soy Agar (TSA) plates containing 15 mL of medium (Qingdao Hope Bio-Technology Co., Ltd.) were loaded into each stage of the sampler. The sampler operated at a flow rate of 28.3 L/min, enabling the collection of airborne bacterial particles of various sizes, which impacted and deposited onto the agar plates. Sampling was conducted for 1 min per site, resulting in a total air volume of 28.3 L per sampling point.

Following air sampling, plates were incubated at 37°C for 24 h to allow the formation of bacterial colonies, which were enumerated as colony-forming units (CFUs). Airborne bacterial concentration was calculated using the equation:

$$C = \frac{1000N}{Q \times t}$$

where C represents the airborne bacterial concentration (CFU/m^3), N is the total number of colonies observed on all plates, Q is the airflow rate of the sampler (28.3 L/min), and t is the sampling time (1 min).

Sampling point distribution and data collection

To evaluate the spatial distribution of airborne bacterial concentrations, 50 sampling points were established within the poultry house using a grid-based layout (Fig. 2A). Sampling points were evenly distributed along five walkways, with ten points per walkway, spaced uniformly from the evaporative cooling pads to the exhaust fans. Sampling was conducted at a height of 1.5 m, corresponding to the human breathing zone, ensuring consistency across data points. Each sampling cycle lasted 10 min, consisting of 1 min of air sampling, 5 min for relocating the sampler, and 4 min for sample preparation to ensure continuous and efficient sampling.

Collection of environmental samples and 16S rRNA gene sequencing

To characterize the microbial communities within the poultry house environment, four types of samples were collected: front-end dust (FAB), back-end dust (BAB), manure (Mnure), and intestinal content (IC). The specific sampling sites are illustrated in Fig. 2B. All samples were subsequently subjected to 16S rRNA gene sequencing for microbial analysis.

Dust sampling

Dust samples were collected from areas near the evaporative cooling pads (front-end) and exhaust fans (back-end). Sterile gauze or swabs were used to wipe surfaces covering approximately 1 m^2. The collected samples were transferred into sterile centrifuge tubes, immediately placed on ice, and transported to the laboratory within 2 h for storage at −80°C until DNA extraction.

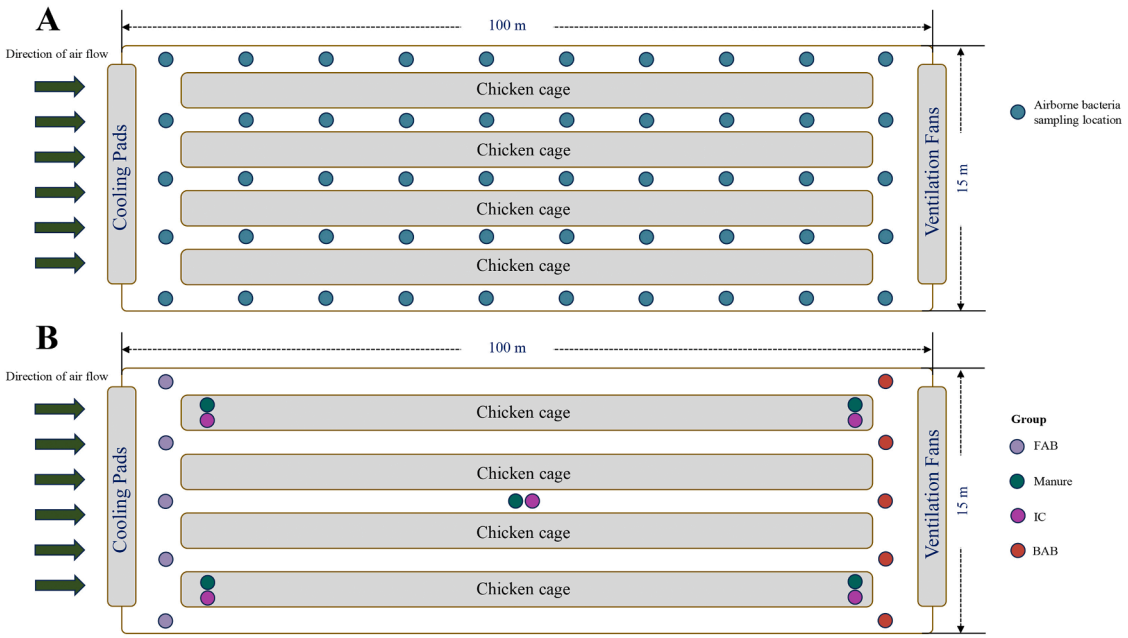

Fig. 2. Schematic layout of the poultry house and the sampling locations for airborne bacteria. (A) Overview of the poultry house, indicating cooling pads, ventilation fans, chicken cages, and sampling locations for airborne bacteria. (B) Distribution of different sampling groups, including FAB, Manure, IC, and BAB, within the poultry house. The direction of airflow and sampling points are represented to illustrate the environmental context and spatial organization of sampling efforts.

Manure and intestinal content collection

Fresh manure samples were randomly collected from manure belts beneath the cage tiers using sterile scoops. Approximately 10 g of each sample was placed in sterile bags and kept refrigerated during transport to the laboratory within 2 h, then stored at −80°C.

Three healthy hens were randomly selected from designated locations within the poultry house and were humanely euthanized via carbon dioxide asphyxiation. Ileal contents were obtained post-dissection and placed into sterile centrifuge tubes for transport to the laboratory, where they were stored at −80°C.

DNA extraction, PCR amplification, and sequencing

DNA from the environmental samples was extracted using the QIAamp DNA Stool Mini Kit (QIAGEN) according to the manufacturer's protocol. Dust samples, including gauze or swabs, were processed directly in lysis buffer, whereas 0.5 g of each manure and intestinal content sample was used. DNA quantity and quality were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) and 1 % agarose gel electrophoresis. DNA samples were stored at −20°C until further use.

The V3–V4 hypervariable regions of the bacterial 16S rRNA gene were amplified using specific forward (5′-CCTACGGGNGGCWGCAG-3′) and reverse primers (5′-ATTACCGCGGCTGCTGG-3′). PCR reactions were performed in 25 μL volumes containing 12.5 μL of 2 × PCR Master Mix (TaKaRa, Japan), 1 μL of each primer (0.2 μM), 10 ng of template DNA, and sterile water. The PCR program consisted of an initial denaturation at 98°C for 2 min, followed by 30 cycles at 98°C for 15 s, 55°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 5 min.

PCR products were verified on 2 % agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA). Purified amplicons were quantified using a Qubit 2.0 fluorometer (Invitrogen, USA), pooled in equimolar concentrations, and sequenced on an Illumina MiSeq platform (Illumina, USA) with 2 × 300 bp paired-end reads by a professional sequencing service provider (e.g., Beijing Novogene Bioinformatics Technology Co., Ltd.).

Bioinformatics and statistical analysis

Raw sequencing reads were subjected to quality control using Trimmomatic v0.39, and paired-end reads were merged using FLASH v1.2.11. Operational taxonomic units (OTUs) were clustered at 97 % similarity using QIIME2 v2022.2, and the taxonomic assignment was performed using the SILVA 138 database with a naive Bayesian classifier. Alpha diversity indices, including observed species, Chao1 richness, Shannon diversity, and Simpson diversity, were calculated to assess microbial richness and diversity. Beta diversity was analyzed using principal coordinate analysis (PCoA) based on Bray-Curtis distances to compare microbial community composition across different sample types. Group differences in microbial community structures were assessed using analysis of variance (ANOVA) and the Kruskal-Wallis test.

Significantly different taxa between groups were identified using MetaStat, and false discovery rate corrections were applied to obtain q-values. Linear discriminant analysis effect size (LEfSe) was further employed to identify taxa with significant differences among groups. All statistical analyses were conducted using R v4.2.0 (R Foundation for Statistical Computing, Vienna, Austria), with significance defined as $P < 0.05$. Visualizations were generated using ggplot2 v3.3.5. MATLAB 2022a (The MathWorks, USA) was used to visualize the spatial distribution of airborne bacterial concentrations in the poultry house, employing piecewise cubic interpolation to generate surface and contour maps. Moreover, the raw sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1224969.

Results

Spatial distribution of airborne bacterial concentrations

Airborne bacterial concentrations were measured at 50 sampling points throughout the poultry house, providing a detailed spatial profile (Fig. 3). Bacterial concentrations exhibited a significant longitudinal gradient, increasing from the air inlet (evaporative cooling pads) to the air outlet (exhaust fans) (Fig. 5). Concentrations ranged from 883 ± 177

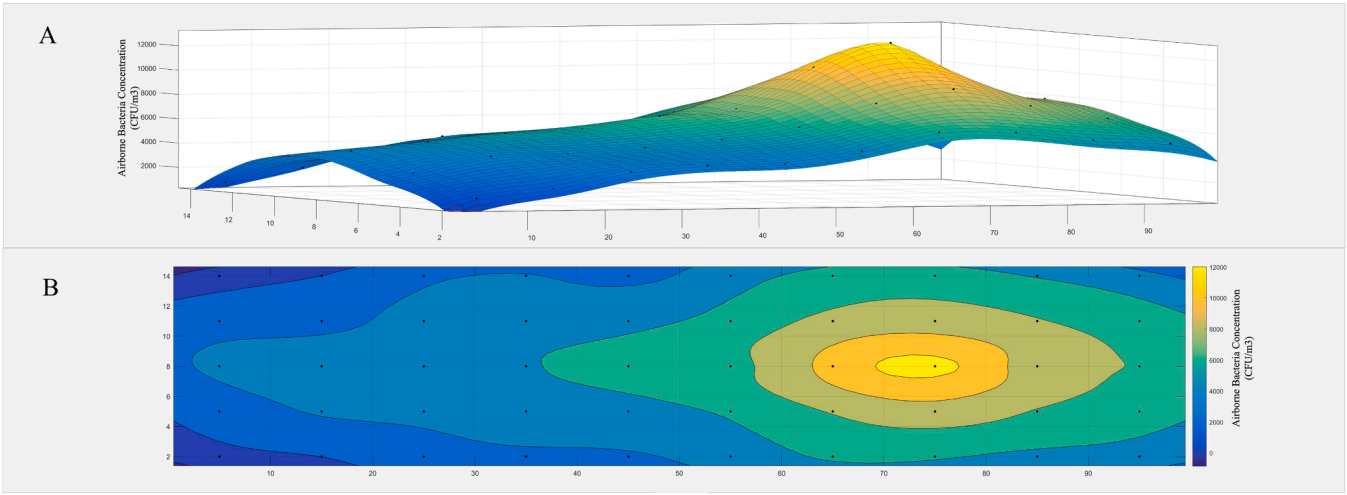

Fig. 3. Spatial distribution of airborne bacterial concentration (CFU/m^3) in a poultry house. (A) Three-dimensional surface plot illustrating bacterial concentrations across different locations. (B) Contour map showing the distribution and intensity of airborne bacterial concentrations, highlighting areas of higher concentration in the central regions.

to 12,650 ± 813 CFU/m^3, with the highest concentration observed at the eighth sampling point on the third walkway, 75 m from the cooling pads. The average concentration across the poultry house was 5,618 ± 530 CFU/m^3.

Statistical analysis revealed significant differences in bacterial concentrations among the walkways ($P < 0.05$). The data indicate significant variations in bacterial concentration across different spatial locations within the poultry house, with a notable peak observed in the central to rear regions, reaching concentrations between 10,000 and 12,000 CFU/m^3. In contrast, the concentration is considerably lower, generally below 6,000 CFU/m^3, at the periphery of the poultry house (Fig. 3A). The contour map further confirms this trend, highlighting a distinct region of high bacterial concentration (>8,000 CFU/m^3) in the central area, with concentrations decreasing gradually towards the outer sections (Fig. 3B). This pattern may be attributed to the activity of poultry, airflow dynamics, and the spatial distribution of cleaning measures within the poultry house. The elevated concentration in the central area is likely associated with increased poultry activity, which leads to the disturbance of bacteria and subsequent aerosolization into the surrounding air.

Microbial community structure

A total of 2,611 OTUs were identified, with 99.46 % (2,597 OTUs) successfully annotated against the SILVA 138 database. Annotation rates at different taxonomic levels were as follows: phylum (96.59 %), class (92.68 %), order (86.98 %), family (76.79 %), and genus (47.11 %), indicating high coverage of microbial diversity.

Alpha diversity analysis

Alpha diversity analysis of cecal microbial communities revealed significant differences among groups. Shannon and Simpson diversity indices indicated that the BAB group had significantly lower diversity and evenness compared to the IC and FAB groups ($p < 0.05$). The p-values for the Shannon index were 0.010 (BAB vs. IC) and 0.020 (BAB vs. FAB), while for the Simpson index, they were 0.0008 and 0.0043, respectively. No significant differences were found between the IC, BAB, and Manure groups or between the FAB and Manure groups ($p > 0.05$) (Fig. 4).

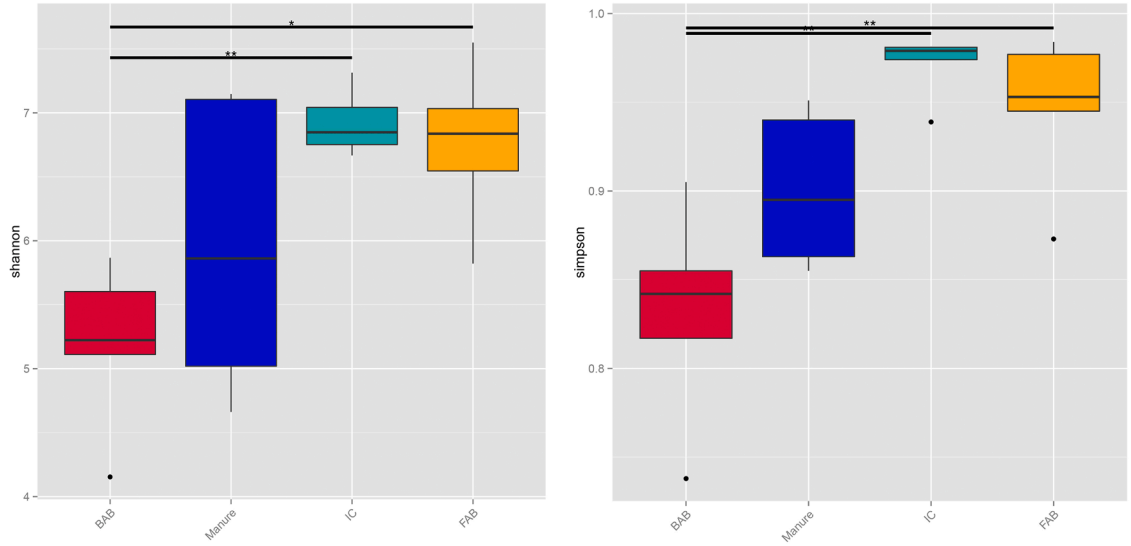

Fig. 4. Box plots showing the Shannon diversity index (left) and Simpson diversity index (right) for different sample types, including BAB, Manure, IC, and FAB. The plots illustrate variations in microbial diversity and evenness across different sample types.

Beta diversity analysis

Beta diversity analysis using Bray-Curtis-based PCoA demonstrated significant differences in microbial community composition among the groups (Fig. 5). PC1 and PC2 explained 40.80 % and 20.05 % of the total variation, respectively. Samples from BAB, Manure, IC, and FAB groups were distinctly separated along the PC1 and PC2 axes, with the IC group showing the most pronounced separation, indicating unique microbial community characteristics in the intestinal samples.

Microbial community composition

At the phylum level, Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidetes were predominant. Firmicutes and Proteobacteria accounted for 26.6 %–72.9 % and 20.5 %–52.2 % of total abundance, respectively (Fig. 6). Firmicutes had the highest relative abundance in the IC group (72.9 %) and lower proportions in other groups, while Proteobacteria peaked in the BAB group (52.2 %) but decreased significantly in the IC group (20.5 %). Bacteroidetes had a relative abundance of 16.4 % in the BAB group, with lower proportions in the other groups (1.5 %–15.3 %). Actinobacteria were more abundant in the FAB group (16.7 %) compared to other groups (3.1 %–4.0 %).

At the genus level, significant differences were observed among groups. Unidentified Enterobacteriaceae were dominant across all groups, with the highest abundance in the BAB group (35.77 %) and the lowest in the IC group (3.35 %). *Acinetobacter* had the highest abundance in the FAB group (19.90 %), emphasizing its significance in this group. Additionally, *Bacteroides* was more abundant in the BAB group (13.50 %), indicating its dominant role in this group (Fig. 7).

Differential analysis of microbial taxa

MetaStat analysis identified significant differences in microbial composition among sample groups. Firmicutes had a significantly higher abundance in the IC group compared to other groups ($p < 0.05$), indicating its dominance in the intestinal environment. Proteobacteria were more abundant in the Manure and BAB groups ($p < 0.05$), while Bacteroidetes and Acidobacteria showed higher abundance in the Manure group ($p < 0.05$), possibly reflecting the high organic matter content (Fig. 8).

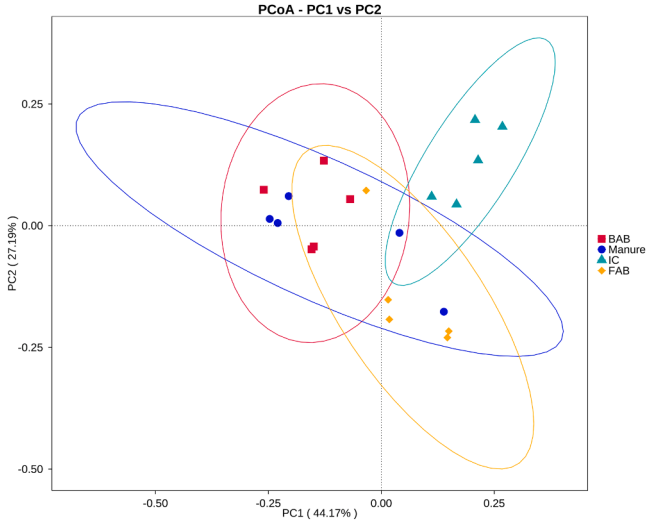

Fig. 5. Principal coordinate analysis (PCoA) plot based on Bray-Curtis dissimilarity, showing the microbial community composition among different sample types, including BAB, Manure, IC, and FAB. Each point represents a sample, and the ellipses indicate 95 % confidence intervals for each sample type.

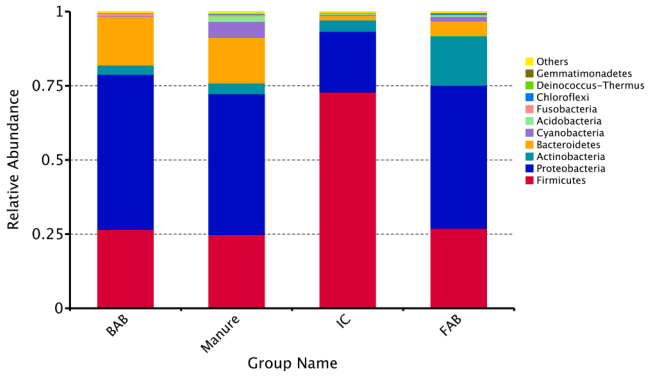

Fig. 6. Relative abundance of bacterial phyla across different sample types, including BAB, Manure, IC, and FAB. The stacked bar chart shows the proportional representation of major bacterial phyla, including Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes, and others. Each bar represents the average relative abundance for a given sample type, illustrating differences in community composition across groups.

At the genus level, unidentified Enterobacteriaceae, *Bacteroides*, and *Citrobacter* were significantly more abundant in the BAB group compared to other groups ($p < 0.05$), potentially associated with increased manure exposure and hen activity density. Conversely, Lachnospiraceae and Agathobacter were more abundant in the IC group ($p < 0.01$), indicating a preference for the intestinal environment. *Faecalibacterium* and *Intestinimonas* were more abundant in the Manure group ($p < 0.05$), indicating their preference for manure-rich environments (Fig. 9). LEfSe analysis confirmed the differential enrichment of taxa between the FAB and BAB groups, with Actinobacteria and related taxa enriched in the FAB group, while Bacteroidetes and Enterobacteriaceae, including *Bacteroides gallinaceum* and *Escherichia coli*, were enriched in the BAB group, likely due to increased manure exposure (Fig. 10). These results demonstrate significant community structure differences among groups and emphasize the role of environmental conditions, such as manure exposure and airflow patterns, in selectively enriching microbial taxa. Such insights are critical for optimizing poultry house management practices and mitigating pathogen spread.

Discussion

This study provides a comprehensive analysis of the spatial distribution of airborne bacterial concentrations and microbial community structures within a large-scale commercial layer facility equipped with advanced environmental controls. The findings reveal significant spatial variability in airborne bacterial loads and distinct microbial communities associated with different ecological niches in the poultry house. These results carry important implications for poultry health management, environmental control strategies, and biosecurity measures in intensive poultry production systems.

Spatial distribution of airborne bacterial concentrations

The observed longitudinal gradient in airborne bacterial concentrations, increasing from the air inlet (evaporative cooling pads) to the air outlet (exhaust fans), corresponds to the airflow patterns characteristic of negative pressure ventilation systems. Similar trends have been reported in previous studies, where elevated bacterial concentrations were observed near exhaust areas due to the accumulation and transport of airborne particles along the ventilation flow (Cambra-López et al., 2010; Chen et al., 2022; Li et al., 2019). The highest airborne bacterial concentration was recorded at the back end of the poultry house, indicating that as air moves through the facility, bacteria accumulate, carrying particulates from sources such as manure belts and bird activity zones.

Dividing the poultry house into three zones based on bacterial

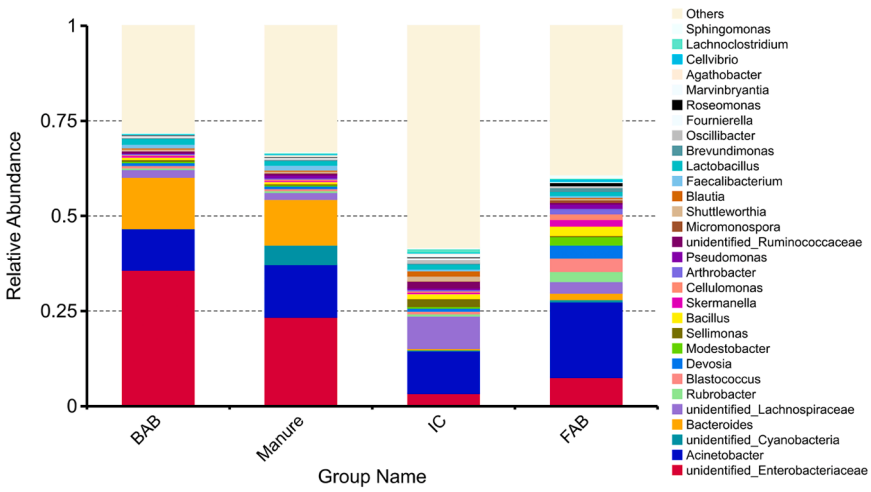

Fig. 7. Relative abundance of bacterial genera across different sample types, including BAB, Manure, IC, and FAB. The stacked bar chart shows the proportional representation of major bacterial genera. Each bar represents the average relative abundance for a given sample type, highlighting variations in microbial community structure at the genus level among groups.

concentrations—low (<4,000 CFU/m^3), transition (4,000-8,000 CFU/m^3), and high (>8,000 CFU/m^3)—provides a practical framework for targeted environmental management in commercial operations. This spatial stratification enables the systematic identification of critical monitoring points, assessment of air disinfection efficacy, and prioritization of ventilation system modifications. The significant differences between zones ($p < 0.05$) provide quantitative benchmarks for evaluating intervention outcomes while maintaining operational simplicity for production implementation. The higher bacterial concentrations observed in the central regions and near the exhaust fans may be attributed to increased bird density and activity, as well as the convergence of airflow-carrying particulates from multiple sources. These findings highlight the need for differential ventilation strategies and localized interventions to mitigate airborne bacterial loads in critical areas.

The transverse symmetry observed in bacterial concentrations suggests that the housing design and ventilation system produce relatively uniform airflow patterns across the width of the facility. However, the higher concentrations detected in the central regions compared to the sides could result from the cumulative effects of bird activity and manure accumulation in these areas. This observation aligns with the findings by Xu et al. (2022b); Yang et al. (2018), and Yan et al. (2023), who reported that central zones in poultry houses often have elevated levels of airborne particulates and microorganisms due to higher stocking densities.

Microbial community structures and diversity

The high annotation rates of operational taxonomic units (OTUs) at various taxonomic levels indicate that 16S rRNA gene sequencing effectively captured the microbial diversity in the poultry house environment. The predominance of Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidetes at the phylum level is consistent with prior studies investigating microbial communities in poultry environments. These phyla encompass many bacteria associated with the poultry gastrointestinal tract, environmental sources, and potential pathogens (Awasthi et al., 2018; Crippen et al., 2022; Song et al., 2021).

Alpha diversity analyses revealed significant differences among sample groups. The lower Shannon and Simpson diversity indices in the back-end dust (BAB) group compared to the intestinal content (IC) and front-end dust (FAB) groups suggest that the microbial community in the BAB samples is less diverse and more dominated by specific taxa. This may be due to the accumulation of bacteria associated with manure and bird excretions near the exhaust fans, leading to the enrichment of certain bacterial groups (Galler et al., 2021; Xu et al., 2022b; Yan et al., 2023). In contrast, the higher diversity observed in the FAB and IC groups reflects a more heterogeneous microbial environment at the air inlet and within the intestinal tract.

Beta diversity analyses further supported the distinct microbial community compositions among different sample types. The clear separation of the IC group in the principal coordinates analysis (PCoA) plot underscores the unique microbiota of the poultry intestinal tract, influenced by host-specific factors and dietary inputs. The differentiation between the FAB and BAB groups emphasizes the impact of environmental conditions and spatial location within the poultry house on microbial community structures.

Microbial community composition and environmental influences

At the genus level, the dominance of unidentified Enterobacteriaceae in the BAB group, alongside their lower abundance in the IC group, suggests that these bacteria are predominantly associated with environmental contamination, particularly in areas with increased manure exposure. The higher abundance of *Acinetobacter* in the FAB group may be attributed to their prevalence in soil and water sources, which may enter the facility through the evaporative cooling pads (Jung and Park, 2015).

The enrichment of *Bacteroides* in the BAB group is indicative of fecal contamination, as these bacteria are common inhabitants of the gastrointestinal tract and are excreted in feces (Wei et al., 2013; Xiao et al., 2016). Firmicutes, particularly Lachnospiraceae, and Agathobacter, in the IC group, align with their known roles in the gut microbiota, contributing to nutrient metabolism and maintaining intestinal health (Wang et al., 2021; Zhou et al., 2021).

Environmental conditions such as manure exposure, airflow patterns, and hen activity significantly influence the selective enrichment of microbial taxa. The increased abundance of Proteobacteria and Bacteroidetes in the BAB and manure groups reflects the nutrient-rich environment and organic matter content, which support the growth of these bacteria (Delgado-Baquerizo et al., 2017; Fierer et al., 2012). These findings underscore the interconnectedness of environmental management practices and microbial dynamics within poultry facilities.

Implications for poultry health and environmental management

The findings of this study have practical implications for improving

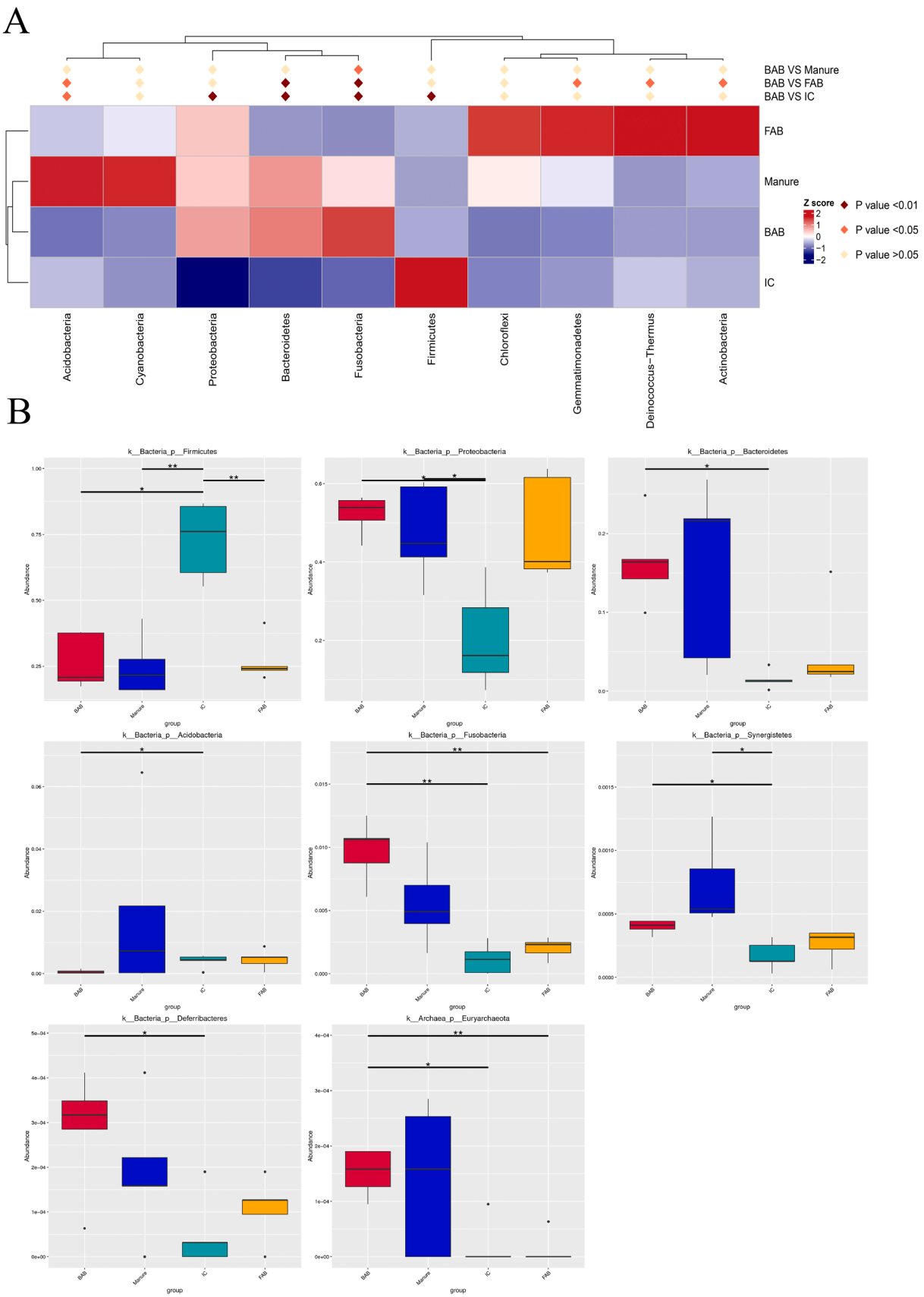

Fig. 8. (A) Heatmap illustrating the differential abundance of major bacterial phyla across different sample types, including BAB, Manure, IC, and FAB. The color intensity represents Z-scores, with red indicating higher abundance and blue indicating lower abundance. Significance levels between groups are indicated by symbols ($p < 0.05$, $*p < 0.01$). (B) Box plots showing the relative abundance of selected bacterial phyla across different sample types. Each box represents the distribution of abundance within each group, with statistical significance indicated by bars ($p < 0.05$, $*p < 0.01$).

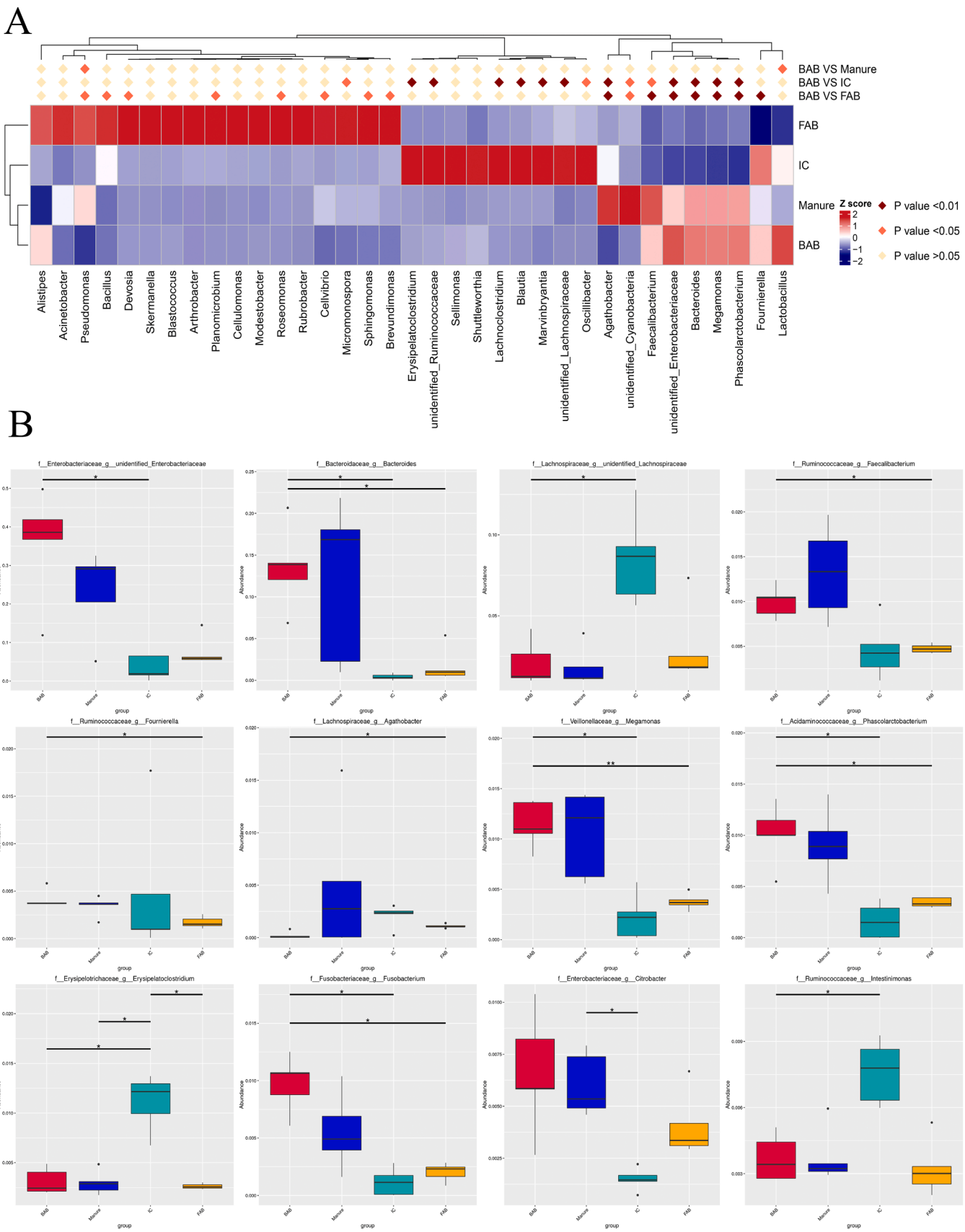

Fig. 9. (A) Heatmap showing the differential abundance of major bacterial genera across different sample types, including BAB, Manure, IC, and FAB. The color intensity represents Z-scores, with red indicating higher abundance and blue indicating lower abundance. Significant differences between groups are indicated by symbols ($p < 0.05$, $^{*}p < 0.01$). (B) Box plots depicting the relative abundance of selected bacterial genera across different sample types. Each box represents the distribution of abundance for each genus within a group, with statistical significance indicated by bars ($p < 0.05$, $^{*}p < 0.01$).

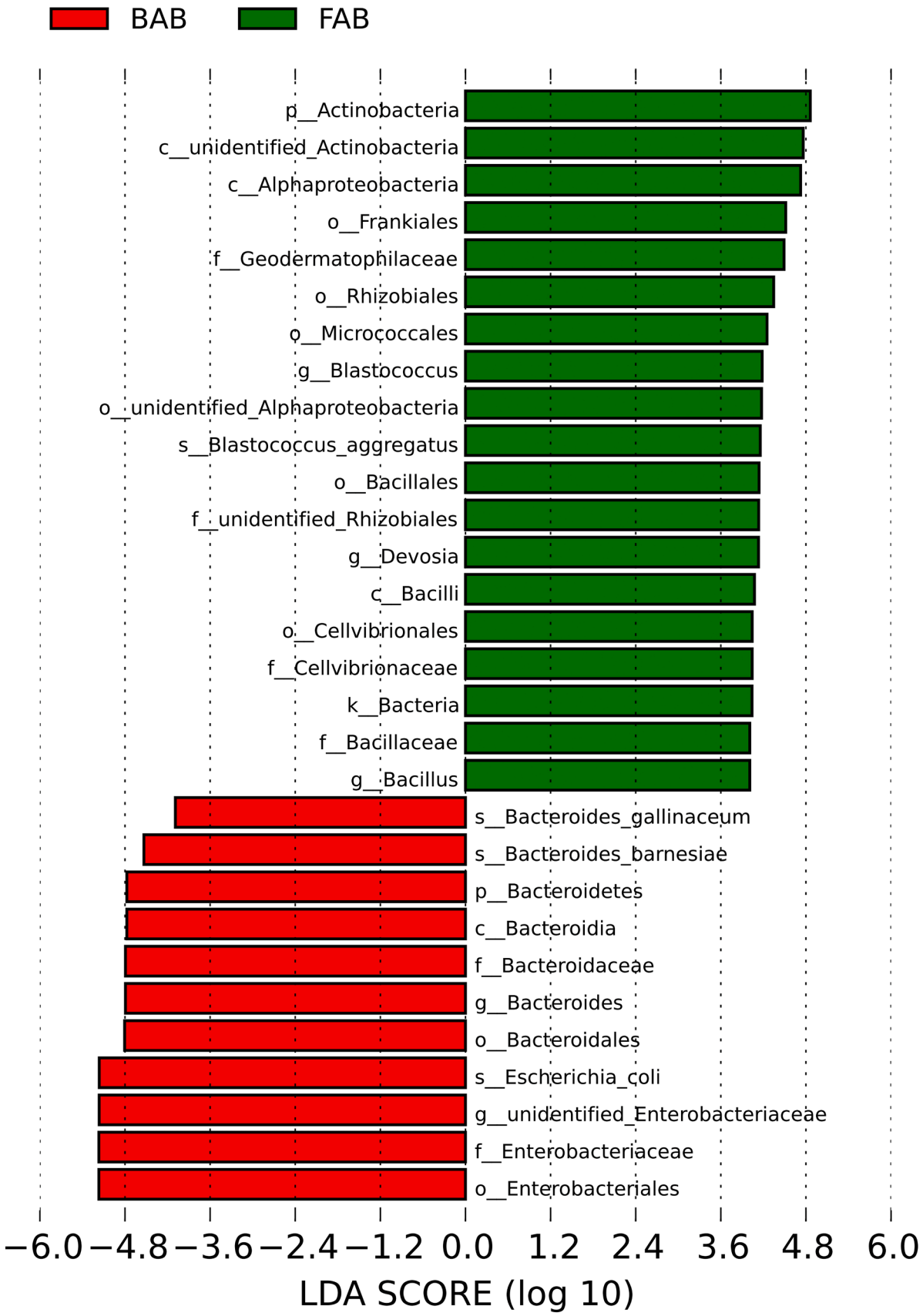

Fig. 10. Linear discriminant analysis (LDA) Effect size (LEfSe) analysis showing the bacterial taxa differentially enriched between BAB and FAB sample types. Red bars represent taxa significantly enriched in BAB, while green bars represent taxa enriched in FAB. The length of each bar indicates the LDA score (log 10), which reflects the effect size of the respective taxa in distinguishing between the two groups.

air quality and reducing the risk of disease transmission in poultry houses, particularly through implementation of targeted air purification systems in high-concentration zones and optimization of manure removal frequencies based on spatial risk assessment. The identification of zones with elevated bacterial concentrations suggests that targeted interventions, such as enhanced ventilation, air filtration, or localized disinfection, could mitigate airborne bacterial loads in critical areas. Adjusting ventilation rates and airflow patterns to minimize the accumulation of airborne bacteria near exhaust fans may help reduce the dissemination of potentially pathogenic microorganisms. Prioritizing manure management to disrupt fecal-derived pathogen reservoirs (e.g., Bacteroides, Enterobacteriaceae) can collectively mitigate zoonotic and avian pathogen dissemination, as demonstrated in analogous studies (Ström et al., 2018).

Understanding the microbial community structures associated with different environmental samples provides insights into potential sources of contamination and transmission pathways. The presence of fecal-associated bacteria in airborne dust near the exhaust fans emphasizes the importance of effective manure management and regular cleaning protocols to reduce environmental contamination. Implementing strategies to minimize dust generation, such as utilizing bedding materials that reduce particulate matter or installing dust suppression systems, may further improve air quality within the facility (Hofstetter et al., 2022; Mostafa and Buescher, 2011; Xu et al., 2022a).

Conclusion

This study elucidates distinct spatial patterns of airborne bacterial distribution in commercial layer facilities, characterized by a significant concentration gradient (883±177 to 12,650±813 CFU/m^3) increasing from air inlet to exhaust areas. We identified three distinct zones based on concentration levels: low (<4,000 CFU/m^3) near inlets, transition (4,000-8,000 CFU/m^3) in mid-facility, and high (>8,000 CFU/m^3) near exhaust fans, creating a comprehensive spatial risk map for poultry environments. Microbial community profiling revealed niche-specific taxonomic signatures, with Firmicutes dominating intestinal samples (72.9 %), Proteobacteria prevalent in back-end dust (52.2 %), and specific enrichment of fecal-associated bacteria (Bacteroides, Escherichia coli) in high-concentration zones.

This research contributes three substantive advancements to avian science: (1) establishment of quantitative thresholds for airborne bacterial concentration zones in modern layer facilities, providing objective parameters for environmental monitoring; (2) demonstration of direct correlations between spatial distribution patterns and microbial community structures, validating the biosecurity significance of ventilation system design; and (3) development of an integrated spatial-microbial analytical framework applicable across poultry production systems. The concentration-based risk stratification model we propose represents a paradigm shift from generalized to targeted intervention strategies, enabling precise biosecurity measures that maximize resource efficiency while minimizing disease transmission.

Declaration of competing interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2025.105021.

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