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Longitudinal analysis of broiler cecal microbiome: unraveling the species level dynamics across age and sex

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ABSTRACT

This study investigated the longitudinal dynamics of the cecal microbiome in male and female broiler chickens from hatch (d 0) to market age (d 42) using full-length 16S rRNA gene sequencing via Oxford Nanopore Technology. Microbial composition, diversity, and differential abundance were assessed across four time points (d 0, 14, 28, and 42). Alpha diversity analyses showed no significant differences between male and female within the same age group. The longitudinal comparisons revealed significantly lower diversity on d 0 compared to later timepoints in both male and female. Beta diversity analyses using Bray-Curtis and Jaccard metrics demonstrated distinct microbial community clustering at d 0 and a major compositional shift by d 14. PERMANOVA confirmed age as a significant factor influencing microbial composition. However, both sex and age influenced the dominant bacterial species. On d 0, *Enterococcus faecalis* was the most abundant in both males (58.57%) and females (61.35%). Whereas on d 42, *Romboutsia ilealis* (14.12%) became the predominant species in female and *Negativibacillus massiliensis* (9.7%) in males. In addition to the variation in the dominant species, differential abundance analysis revealed that bacterial species such as *Clostridium celatum, C. disporicum, C. jeddahitimonense,* and *C. saudiense*, had significantly higher abundance at d 0 compared to later time points in both males and females. Whereas bacterial species such as *Bittarella massiliensis, Turicibacter bilis,*and *Turicibacter sanguinis* had significantly higher abundance at d 28 and 42. Furthermore, core microbiome analysis showed microbial richness plateaued at approximately 330-350 species from d 14 onward. Our results indicate that early microbial communities are more sex-specific and they gradually converge over time probably due to extrinsic environmental factors.

Introduction

In the United States, according to USDA data, a total of 9 billion broilers is raised annually yielding a total of 47.0 billion pounds of meat (USDA, 2025). As the broiler industry is expanding each year due to rising demand of meat, it is also facing several challenges due to increasing intensive farming (Gržinić et al., 2023; Brothers et al., 2023). In the last decade, there have been several regulation changes regarding restraining the usage of antibiotics in animal diets to control the further spread of antimicrobial resistance (FDA, 2012, 2013; European Medicines Agency, 2018; Page et al., 2021). The changes in the regulation regarding antibiotic growth promoters sparked the initiative for searching for an alternative that can enhance growth performance of broilers. A healthy gut is key for increased production as healthy ceca could extract up to 10% higher energy (Hegde et, al., 1982; Jozefiak et al., 2004). The gastrointestinal tract (**GIT**) of poultry consists of a diverse microbial community that plays a valuable role in the host's health, physiology, immunity, and nutrition (Wei et al., 2013; Diaz Carrasco et al., 2019). Among the different segments of the GIT, the ceca harbour the highest number of bacteria (10^{10}-10^{11} cells/g) and a diverse microbial community, which is pivotal in nutrient absorption, digestion, and overall host health (Sergeant et al., 2014). These microbiomes can elicit different interactions with the hosts, which could be pathogenic or beneficial to poultry gut health, suggesting the importance of studying the cecal microbiota in poultry (Kim et al., 2019).

With the recent advancement of sequencing technology, it is now possible to analyze the taxonomy classification and abundance analysis of extensive bacterial populations. Sequencing a specific segment of the 16S rRNA gene using next-generation sequencing (**NGS**) technology such as Illumina has been a widely employed method for identifying

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microbial communities (Walters et al., 2011). However, for its short-read generating capabilities, ~450 bp of 16S rRNA covering only one/two hypervariable regions (V3-V4, V4-V5), predicting a species-level resolution of the bacterial community is difficult (Shin et al., 2016; Cha et al., 2023). The third-generation sequencer MinION from Oxford Nanopore Technologies (ONT) is capable of sequencing the entire 16S rRNA, which can be used to identify species-level bacterial identification. Previously, due to its higher error rate on base calling (Goodwin et al., 2015), ONT had not been used frequently, but with the recent improvement of nanopore chemistry, such as the R10.4.1 flow cell, ONT has significantly enhanced the sequencing accuracy, achieving an average of 99% accurate full-length reads (Zhang et al., 2023). This advancement has led to precise species-level identification, which is crucial to understanding the microbial composition and function (Matsuo et al., 2021).

Gut microbiota plays a significant role in production performance, such as growth rate and feed efficiency. Additionally, sex-linked variations may be associated with different microbial composition and diversity between male and female birds, which might be influenced by different hormonal, genetic, and environmental factors (Lee et al., 2017; Cui et al., 2021). Although numerous studies have previously explored the impact of age and sex on microbiome composition of the broiler ceca, the microbiome at the species-level composition remains unexplored. Understanding the microbiome composition at the species-level is crucial because previously studied genus-level classifications alone were unable to capture the full potential of the microbial taxa, as the single genus *Clostridium* harbours both beneficial bacteria, such as *Clostridium butyricum,* and the pathogenic one, *Clostridium perfringens*. Therefore, this study aimed to comprehensively assess the impact of age and sex on the microbial dynamics at the species-level of broiler ceca.

Materials and methods

Study design and sample collection

A total of 70 1-day-old Ross YP × 708 broilers were obtained from the Aviagen Hatchery in Albertville, AL, whereas fertilized eggs were sourced from Aviagen research farms in Albertville AL. Based on sex, birds were divided into two pens, each with an area of 2.79 m^2 in dimensions and a concrete floor. Pens were bedded with soft white wood shavings > 5 cm and < 10 cm in depth. At placement, the experimental room temperature was set to 32°C, then decreased gradually to 21°C on day 21 and maintained for the remaining of the experiment. One hour of dark period was provided until day 7 and from day 8 onwards 6 hours of darkness and 18 h of light maintained for the rest of the experiment. Diet (maize-soy-based) was formulated at Auburn University's feed mill following the nutrient and energy specification recommended by Aviagen (2022). Chicks had *ad libitum* access to feed and water throughout the experimental period from day 0 to 42. On days 0, 14, 28, and 42, six birds from each sex were euthanized via exposure to carbon dioxide (CO_2). Following euthanasia, a necropsy was performed to collect the cecal contents. The samples collected were transported to the lab and stored at −20°C until DNA was extracted. All procedures for bird management were approved by the Institutional Animal Care and Use Committee of Auburn University (IACUC Approval No. 2024-5384).

DNA extraction and full-length 16S rRNA sequencing

The QIAamp PowerFecal Pro DNA Kit (Cat. No. 51804) (Qiagen, Germany) was used according to the manufacturer's protocol for the extraction of the genomic DNA. PCR amplifying the full-length 16S rRNA V1-V9 hypervariable region was conducted following the methods described by Poudel et al. (2025). Following, PCR Native Barcoding Kit 96 V14 (SQK-NBD114.96) was used for the library preparation following the manufacturer's protocol. The final library was measured using the Qubit dsDNA high-sensitivity assay kit (Invitrogen), and 200 femtomoles (fmol) of the final library were sequenced using FLO-MIN114 flow cells equipped with R10.4 nanopores in MinION (Oxford Nanopore Technologies, Oxford, United Kingdom). Reads with lengths less than 800 bp and more than 2000 bp were removed to avoid non-specific reads, and raw reads quality having a quality score (Q score) >10 was maintained. The *Gallus gallus* (GCF_016699485.2) reference genome was used to exclude the host reads from the data. Raw reads were demultiplexed and base called using MinKNOW (24.11.10) with Super High Accuracy. A total of 5,129,172 reads were generated with mean read sequences of 89985.47 per sample (S Tables 5 and 6). The FASTQ sequencing data were analyzed using the EPI2ME platform using the 16S workflow of the tool that uses the NCBI 16S database for taxonomic classification. Species-level taxonomic classification was predicted with a minimum percent identity and coverage threshold of 90%.

Statistical analysis

The taxonomic data file was analyzed and visualized using R version 4.4.2 (2024-10-31). A rarefaction curve was generated using the vegan package (Oksanen et al., 2025). Phyloseq v1.50.0 was used to visualize microbial abundance, normalization, and background correction (McMurdie and Holmes, 2013; Poudel et al., 2025). Sequencing depth was standardized to 4,000 reads per sample via rarefaction, with samples failing to meet threshold or containing fewer than 5 taxonomic species were excluded from downstream analysis. Alpha diversity matrices (Shannon, Richness, Pielou evenness, and Chao1) were estimated, and statistical analysis was conducted between the sexes (male vs female) within the same age group and between the age groups separately for both males and females. The Shapiro-Wilk test was used to check the normality of the alpha diversity matrices data, and either ANOVA or Kruskal-Wallis test was performed, followed by Tukey's HSD or Dunn's multiple comparison test.

For beta diversity analysis, read count data were normalized by Cumulative Sum Scaling (CSS), and Bray-Curtis and Jaccard distance metrics were used to analyze the dissimilarity between the communities across different sexes and ages, and the data were visualized using PCoA (Principal coordinate analysis). Additionally, PERMANOVA was performed using the "adonis2" function and pairwise comparison was done using the "pairwise adonis2" function (Oksanen et al., 2025). The groupwise dispersion was further evaluated by calculating centroid distances using "betadisper" function followed by ANOVA and permutation tests (Oksanen et al., 2025).

Differentially abundant taxa among different age and sex were performed in DESeq2 using a likelihood ratio test (LRT) model, with size factors estimated using "poscounts" method. Pairwise contrasts were computed for all age groups using Wald tests (Love et al., 2014). P-values from both the global LRT and pairwise tests were adjusted for multiple comparisons using the Benjamini-Hochberg (BH) procedure, and taxa with FDR < 0.05 were considered statistically significant. Significant taxa were visualized using boxplots generated from normalized counts. Furthermore, the "Venn Diagram" package was used to identify the core microbiome at the species level for both sexes across each age group (Chen and Boutros, 2011).

Results

Microbiome diversity in ceca of broilers across age and sex

While comparing the alpha diversity metrics between the sexes (male and female) within the same age group (d 0, d 14, d 28, and d 42, male vs. female), there were no significant differences in bacterial communities' richness and evenness between the sexes (S Figure 1). Whereas, while comparing the metrics longitudinally, d 0 had significantly lower diversity ($p < 0.05$) across all metrics in both males and females that shifts after d 0 and remains stable for d 14, 28 and 42, (Figs. 1 and 2)

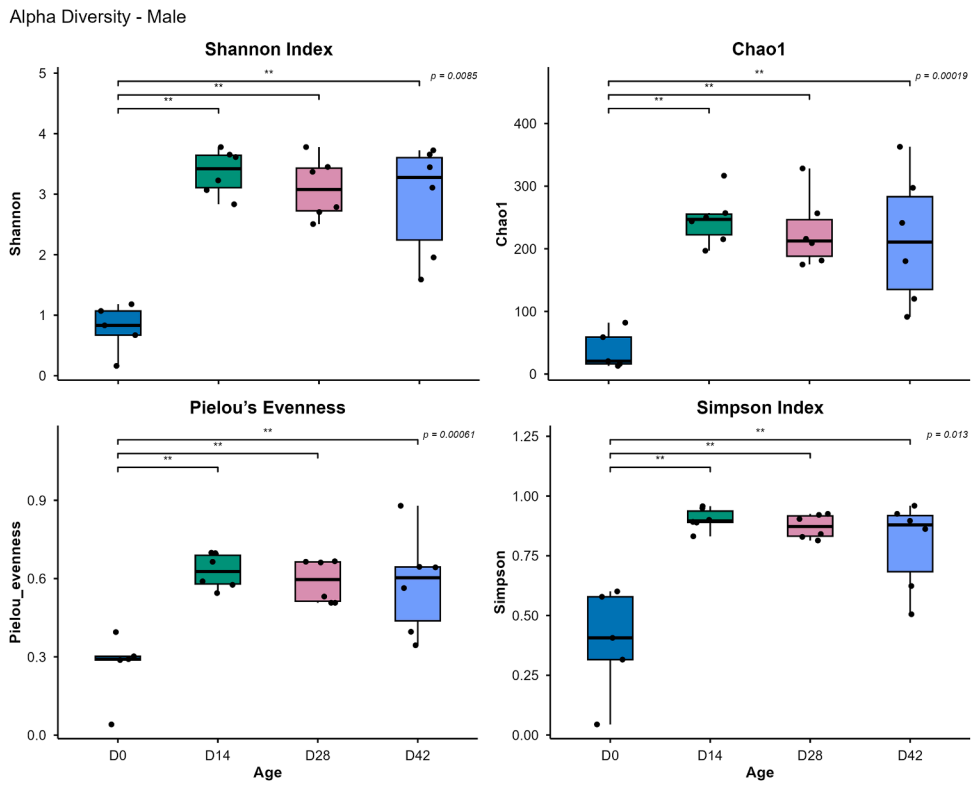

Fig. 1. Alpha diversity metrics for cecal microbiota across age groups of male broilers.
The figure consists of four boxplots presented representing distinct alpha diversity metrics across four age groups (D 0, D 14, D 28, and D 42): Shannon diversity (A), Chao1 (B), Pielou's evenness (C), and Simpson (D). Based on the results of the Shapiro-Wilk test for normality, either one-way ANOVA or Kruskal-Wallis tests were applied to assess differences across age groups, followed by Tukey's HSD or Dunn's post-hoc tests as appropriate. Pairwise, statistical significance between age groups is indicated by asterisks (**).

demonstrating that both sexes show higher overall diversity and evenness as age progresses.

The cecal microbiome's beta diversity was calculated for Bray-Curtis and Jaccard dissimilarity matrices and PERMANOVA was used to evaluate the difference across age groups and between sexes. The results indicate that age is a major factor that impacts the bacterial diversity in the ceca with both Bray-Curtis and Jaccard dissimilarity matrix in both male and female significantly impacted by age (Fig. 3A, B, D, E). The pairwise comparison between the age (d0, d14, d28, and d42) within male and female indicates that d0 had significantly different microbial composition compared to d 14, d 28, and d 42 (Fig. 3C and F). The results indicate that in case of both male and female d 0 had significantly different composition compared to d14, 28, and 42, additionally d14 had significantly different composition compared to d 28, and d 42. Whereas there was no difference between the microbiome composition between the d 28 and d 42 in female (Fig. 3F). However, interpretation of the Jaccard results for females requires caution. The betadisper analysis indicated significantly different group dispersion among female age groups (permutation $p = 0.003$), meaning that variability within groups differed (S Figure 2).

Additionally, while comparing the microbiome within age between sex (male vs. female), there was no significant difference between the microbiome composition on d 0 and d 28 for both Bray-Curtis and Jaccard, however there was significant difference between male and female for Bray-Curtis on d 14 and d 42 and for and Jaccard on d 14 only (S Figure 3 and 4). Whereas the betadisper analysis showed no significant difference between male and female at any time point (S Figure. 5).

Microbial communities and core microbiome in ceca

The results of the relative abundance of different bacterial species having >1 relative abundance in the male and female cecal samples were summarized in Fig. 4 and S Table 3. On d 0, bacterial species *Enterococcus faecalis,* which belongs to the phylum *Bacillota*, was the most abundant bacterial species in both female (61.35%) and male (58.57%) (Fig. 4). On d 14, *Faecalibacterium hattorii* (31.96%) in females and *Wansuia hejianensis* (13.86%) in males were the most abundant species. Whereas *Negativibacillus massiliensis* was the most abundant bacterial species for both d 28 (7.40%) and 42 (9.68%) in males. On the other hand, *Clostridium perfringens* (11.93%) followed by *Negativibacillus massiliensis* (9.80%) were the most abundant bacterial species at d 28, however, *Romboutsia ilealis* (14.12%) was most abundant on d 42 for the female. Whereas in males at d 42, *Negativibacillus massiliensis* (9.7%) was the most dominant, followed by *Bacteroides fragilis* (4.8%), *Romboutsia timonensis* (4.8%) (Fig. 4).

While analyzing the core microbiome of the species present in the ceca, the shared bacterial species between male and female plateaued at d 14 with 342 common bacterial species, which remained almost constant over the age up to d 42 (Fig. 5A). Whereas, on d 0 female had higher number of unique taxa (136) compared to male (42) and had only 56 shared taxa between male and female. However, from d 14 onward the shared taxa increased drastically and peaked at d 42 (Fig. 5A). Additionally, while comparing the core microbiome between the ages within the sex, in male the common bacterial species between d 14, 28, d 42 were 284 species which was much higher compared to common bacterial species between all-time points (23 species) (Fig. 5B). Whereas in females, 91 bacterial species were common across all time points (d 0, d 14, d 28, and d 42), which was much higher compared to male, and core microbiome between d 14, d 28, and d 42 was 190 bacterial species (Fig. 5C).

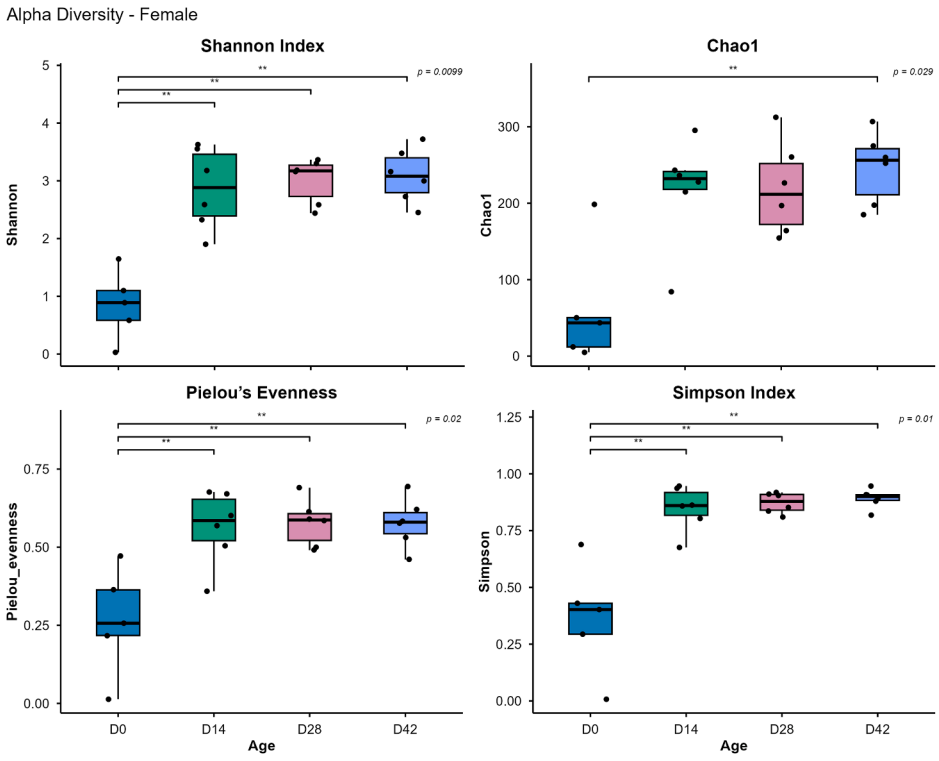

Fig. 2. Alpha diversity metrics for cecal microbiota across age groups of female broilers.
The figure consists of four boxplots presented representing distinct alpha diversity metrics across four age groups (D 0, D 14, D 28, and D 42): Shannon diversity (A), Chao1 (B), Pielou's evenness (C), and Simpson (D). Based on the results of the Shapiro-Wilk test for normality, either one-way ANOVA or Kruskal-Wallis tests were applied to assess differences across age groups, followed by Tukey's HSD or Dunn's post-hoc tests as appropriate. Pairwise, statistical significance between age groups is indicated by asterisks (**).

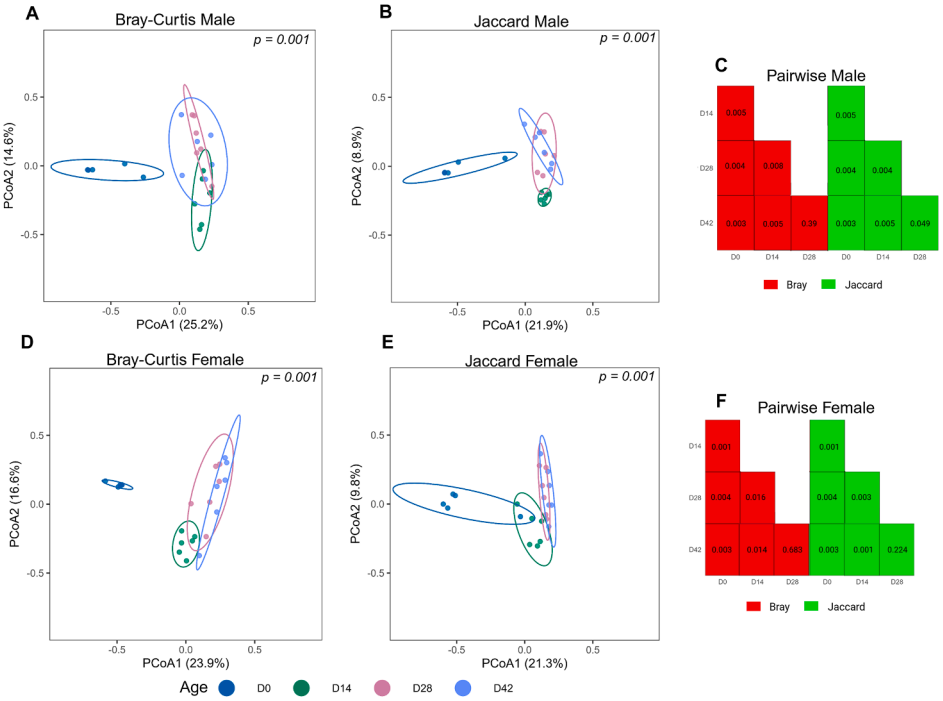

Fig. 3. Temporal changes in cecal microbial composition of broilers in male and female.
Principal Coordinates Analysis (PCoA) plots display beta diversity of the cecal microbiome in male and female birds across four time points (Day 0, 14, 28, 42) based on Bray-Curtis and Jaccard dissimilarity indices figures A, B, D, and E. Each point represents an individual sample, colored by time point. Clear shifts in community structure are observed over time within each sex. Statistical significance of differences in microbial composition was evaluated using PERMANOVA, with $P = 0.001$ indicated for both sexes in both distance metrics. The heatmaps (C and F) show pairwise PERMANOVA p-values, confirming significant differences in microbial communities between time points.

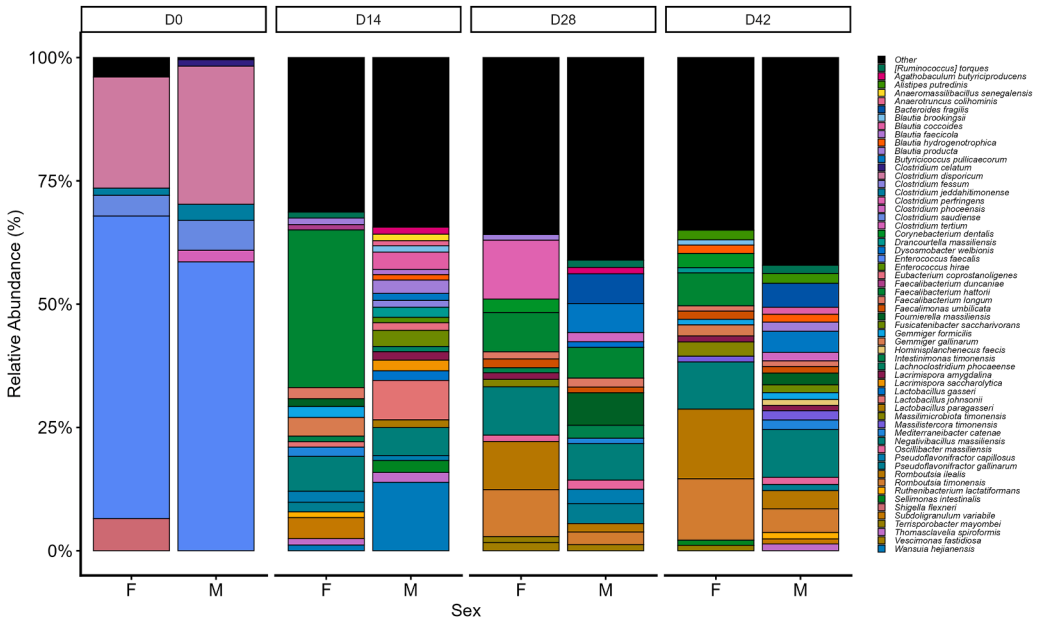

Fig. 4. Species-level relative abundance of cecal microbiota in male and female broilers.
The figure displays the relative abundance of bacterial species identified in the cecal microbiome of male and female broilers across different time points. The stacked bar graph plots represent bacterial species with relative abundance > 1 and bacterial species having relative abundance < 1 grouped as Other. The taxonomic classification was performed at species level using NCBI 16S rRNA database using wf-16S workflow of EPI2ME platform. Additionally, the exact relative abundance values for each bacterial species were listed as the S Table 3.

Differentially abundant microbial community

Increased and decreased relative abundance of different bacterial species across different age and sex were analyzed using DESeq2. Within each sex, bacterial species abundance differed significantly across ages, as assessed using DESeq2 likelihood-ratio testing (LTR). Bacterial species such as *Clostridium celatum, Clostridium jeddahitimonense, Clostridium tertium, Clostridium disporicum,* and *Clostridium saudiens* had much higher relative abundance in females at d 0 compared to d 14, 28, and d 42. Whereas bacterial species such as *Bittarella massiliensis, Turicibacter bilis,* and *Turicibacter sanguinis* had higher relative abundance in females at d 28 and d 42 compared to the d 0 and d 14, indicating that the bacterial species abundance increases along with the age (Fig. 6). In case of males, bacterial species such as *Clostridium celatum, Clostridium disporicum, Clostridium jeddahitimonense,* and *Clostridium saudiens* had significantly higher relative abundance in d 0 compared to d 14, 28, and d42. Whereas bacterial species such as *Bittarella massiliensis, Turicibacter sanguinis, Peptococcus niger,* and *Dysosmobacter acutus* had higher relative abundance in females at d 28 and d 42 compared to the d 0 and d 14, indicating that the bacterial species abundance increases along with the age (Fig. 7). Additionally, the pairwise differential abundance analysis using DESeq2 (Wald test, poscounts size-factor estimation) between sex within age group, revealed that a higher number of bacterial species were enriched in females at d 0, d 14, and d 28, whereas a higher number of bacterial species were enriched in males at d 42 (Fig. 8). Moreover, even though there was a higher number of bacterial species enriched at early ages, at d 42 only very few bacterial species were differently enriched between the male and female, indicating gut microbiome modulation due to environmental factors rather than other intrinsic factors.

Discussion

This study provides a comprehensive characterization of cecal microbiome dynamics in broiler chickens from hatch to market age, using full-length 16S rRNA gene sequencing via Oxford Nanopore Technology (ONT). Our results show that age is the primary factor influencing microbial community structure, richness, and evenness, while sex-specific differences, though secondary, become increasingly evident at later developmental stages. To maintain consistent environmental conditions and to minimize potentially confounding factors such as litter and management differences male bird were placed in one pen and female in another pen allowing clear evaluation of age and sex related microbiome dynamics.

In our study, the longitudinal analysis of alpha diversity revealed that d 0 had the lowest diversity, which plateaued at d 14, suggesting rapid microbial maturation within the first two weeks of life. These findings were similar to previous studies showing that poultry cecal microbiota matures early and remains relatively stable thereafter (Montoro-Dasi et al., 2020; Yin et al., 2023, 2024). Similarly, the richness and evenness of the bacterial species of the poultry ceca increased along with age, which was also previously observed by Liu et al. (2023). In addition to the richness and evenness, core microbiome analysis revealed that species richness stabilized around 400 taxa by d 14 in both sexes. This aligns with prior observations that microbial colonization in poultry is obtained rapidly at early life, which ultimately plays a vital role in health maintenance of the host (Sergeant et al., 2014; Oakley et al., 2014; Costa et al., 2017; Crisol-Martínez et al., 2017; Ocejo et al., 2019). Despite sharing most core taxa, males exhibited a slightly higher number of unique species after d 14, suggesting greater ecological heterogeneity, which could translate into differences in pathogen resistance, metabolic function, or growth performance (Jian et al., 2025).

The Bray-Curtis and Jaccard dissimilarity analysis revealed that d 0 microbiome composition was completely different from all later time points, indicating a strong early shift in community structure. This suggests that once the chicks are placed in the production environment, external factors such as feed, water, litter, and overall farm management rapidly and substantially modulate the cecal microbiome more strongly than the intrinsic factors such as sex. Additionally, the pairwise PERMANOVA results indicate that the microbiome comparisons were not impacted by age after d 28 and d 42. This indicates that after the initial establishment period, the gut microbiome tends to stabilize, and age-related changes become less pronounced. This stabilization phase is consistent with previous observations that the chicken microbiome

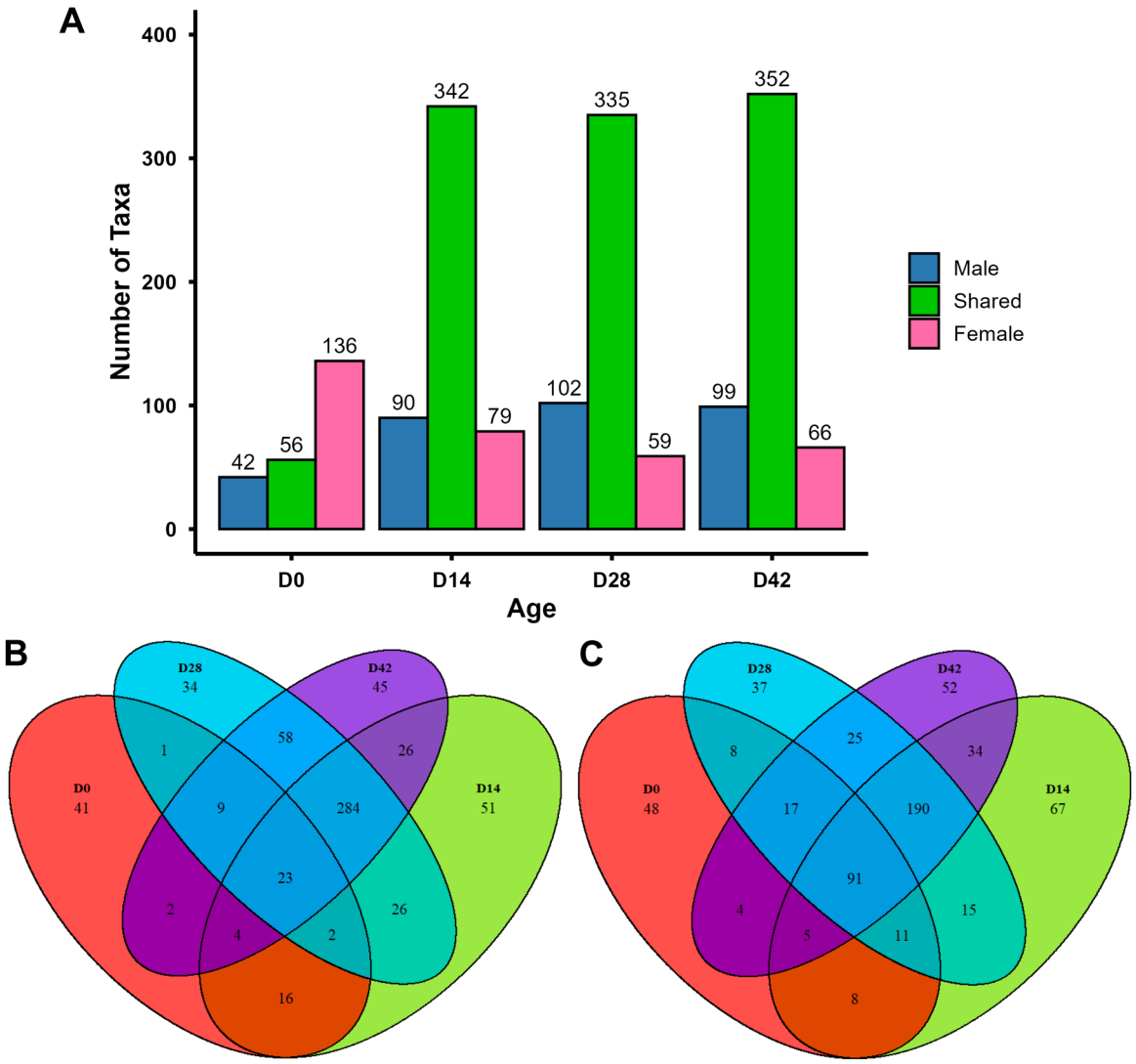

Fig. 5. Unique and core bacterial species detected in male and female broiler chickens across time points.
(A) The bar plot shows the number of bacterial taxa detected in cecal samples of male (blue), female (pink), and shared between male and female (green). The x-axis represents the sampling days, while the y-axis indicates the number of taxa observed. Venn Diagram showing core and unique bacterial taxa for male (B) and female (C) at four time points: D 0, D 14, D 28, and D 42 post-hatch.

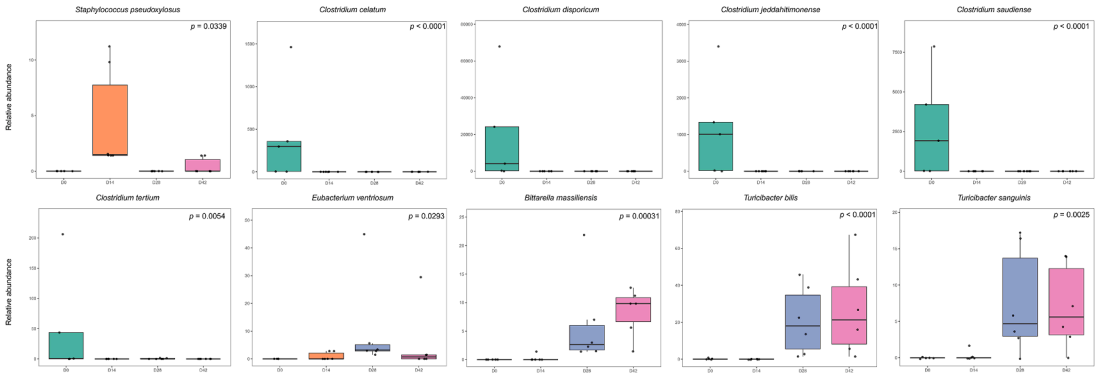

Fig. 6. Age-associated differential abundance of bacterial species in cecal microbiome of female broilers.
Differential abundance was assessed using DESeq2 likelihood ratio testing (LRT). Cecal microbiome count data were normalized using DESeq2 size-factor estimation, and species with Benjamini–Hochberg false discovery rate-adjusted p values < 0.05 were considered significant. Box plots display the relative abundance distributions of significantly age-associated bacterial species in female cecal samples at different ages. The differentially abundant bacterial species were labeled at the top of each box plot.

reaches a relatively stable status (Jurburg et al., 2019; Yin et al., 2023; Glendinning et al., 2022, 2024). Furthermore, the core microbiome

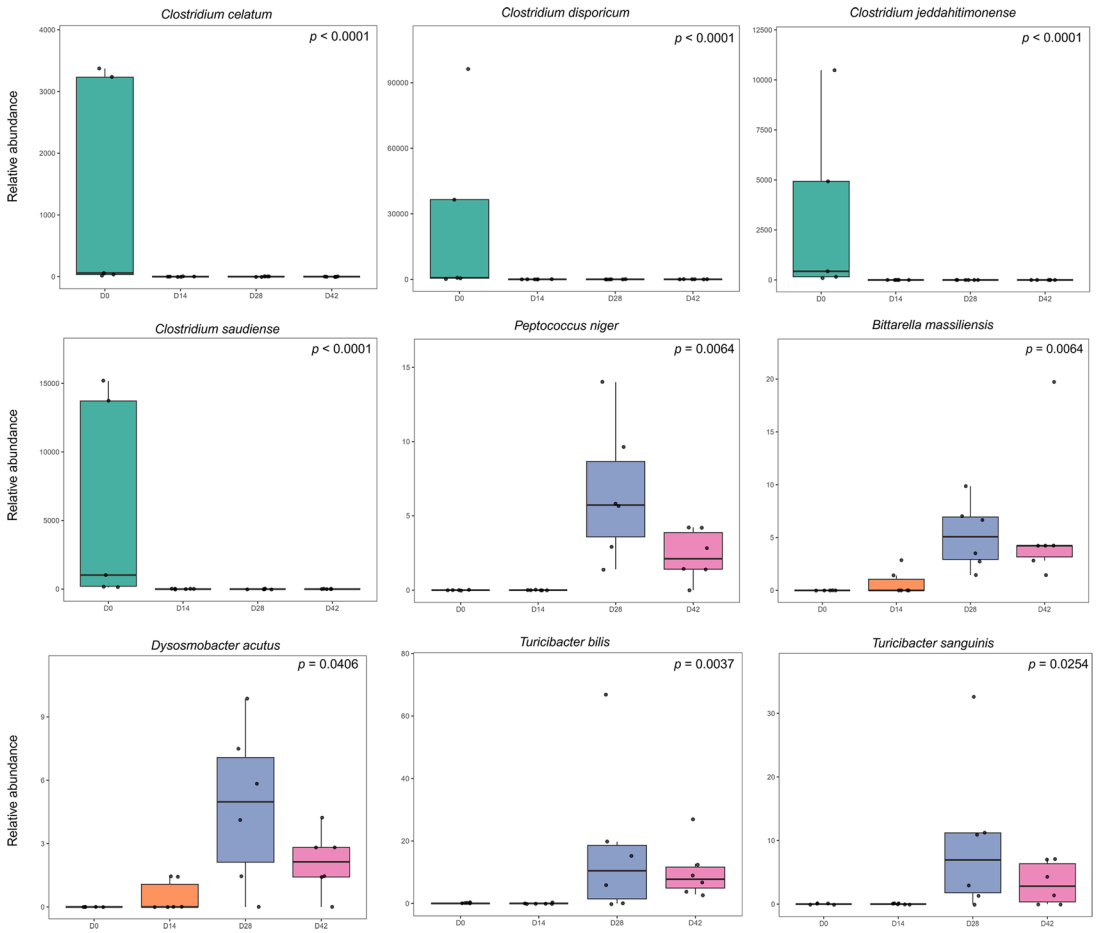

Fig. 7. Age-associated differential abundance of bacterial species in cecal microbiome of male broilers.
Differential abundance was assessed using DESeq2 likelihood ratio testing (LRT). Cecal microbiome count data were normalized using DESeq2 size-factor estimation, and species with Benjamini–Hochberg false discovery rate-adjusted p values < 0.05 were considered significant. Box plots display the relative abundance distributions of significantly age-associated bacterial species in male cecal samples at different ages. The differentially abundant bacterial species were labeled at the top of each box plot.

analysis (Fig. 5C) reinforces these findings, showing that a large proportion of bacterial taxa were shared among d 14, 28, and 42. This high degree of overlap indicates that the microbiome at later stages is largely shaped by stable environmental exposures, with comparatively fewer taxa being uniquely contributed by individual birds, sex, or genetic factors. This pattern supports the interpretation that microbiome acquisition from the environment outweighs host-specific factors during the grow-out period, leading to a consistent core microbiome across later ages. Overall, beyond our expectations, the impact of sex on microbiome diversity was minimal at ceca, whereas the impact of age on microbiome composition and diversity was greatly impacted by environmental factors.

The dominating bacterial community was dynamic and depended on age and sex, as we observed diverse bacterial species dominating different ages and sex as indicated by the mean relative abundance of bacteria. At d 0, *Enterococcus faecalis* was the most dominant bacterial species in both males and females. The dominance of cecal microbiome by *Enterococcus* was different from the previous observations, in which researchers observed early age cecal microbiome dominated by either *Enterobacteriaceae* or *Clostridiaceae* (Glendinning et al., 2019; Ocejo et al., 2019; Ballou et al., 2016; Oakley et al., 2014; Richards et al., 2019). Similar to the previous observation, all other dominating bacterial species at d 0 belong to the genus *Clostridium* and were significantly enriched in both male and female at d 0 compared to later time points d 14, 28, and 42 (Figs. 6 and 7), which has consistently been reported as major early colonizer in newly hatched chicks (Glendinning et al., 2019; Ocejo et al., 2019; Ballou et al., 2016; Oakley et al., 2014; Richards et al., 2019). However, in contrast to our finding of *Enterococcus* dominating the ceca at d 0, Glendinning et al. (2019) reported *Enterococcus* as the dominant genus specifically in the small intestine. This variation in early colonization pattern may be influenced by multiple environmental factors, including hatchery environment, parental microbiota, and immediate post-hatch exposures.

Differential abundance analysis identified some bacterial species differently enriched at certain ages. In both males and females, *C. celatum, C. disporicum, C. jeddahitimonense,* and *C. saudiense*, were significantly enriched at d 0 compared to later time points (d14, 28, and 42). According to the literature, the genus *Clostridium* encompasses a diverse range of species, including beneficial, opportunistic, and potentially pathogenic bacteria (Lopetuso et al., 2013). More importantly, the enriched *C. celatum, C. disporicum,* and *C. jeddahitimonense* at d 0 have not been previously associated with potential pathogenicity in the poultry (Lopetuso et al., 2013; Kieu et al., 2021; Candeliere et al., 2024), whereas *C. saudiense* has been recently reported to cause human bacteraemia (Yoon et al., 2022). However, the bacterial species *C. celatum* has been classified as a gut symbiont in previous human studies, contributing to mucin degradation, carbohydrate fermentation, and the maintenance of gut homeostasis (Lopetuso et al., 2013; Candeliere et al., 2024). Whereas bacterial species such as *Bittarella massiliensis, Turicibacter bilis, Turicibacter sanguinis*, and *Dysosmobacter acutus* were significantly enriched at d 28 and d 42 in male and female. Although the role of *Bittarella massiliensis* in gut remains poorly

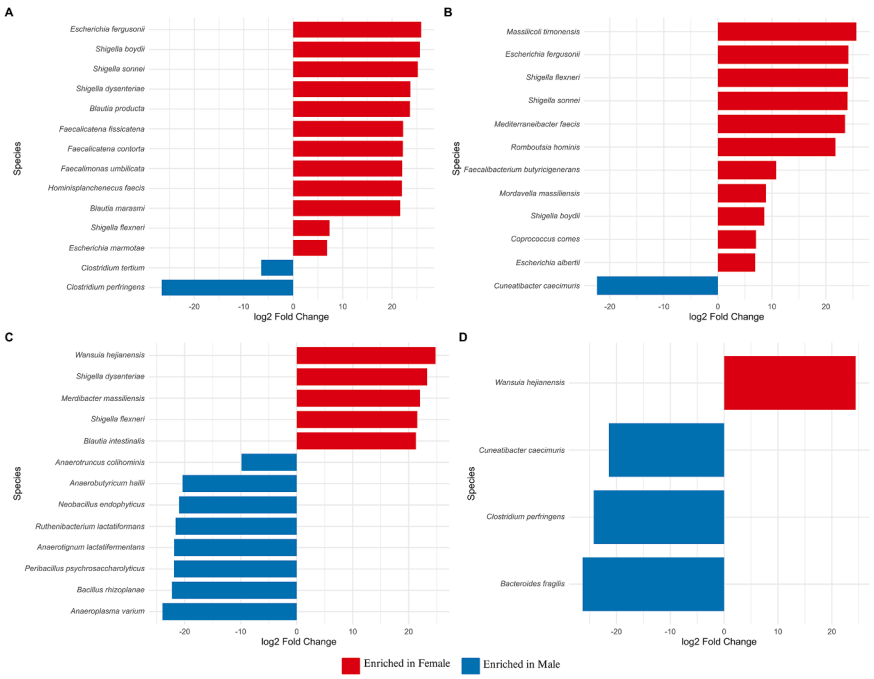

Fig. 8. Sex-associated differential abundance of bacterial species in cecal microbiome across ages.
Differential abundance was assessed using DESeq2 with a Wald test on normalized count data. Size-factor normalization was applied using the *poscounts* method, and bacterial species with Benjamini–Hochberg false discovery rate (FDR)-adjusted p values < 0.05 were considered significantly different. Bar plots display the $\log_2$ fold change in relative abundance of significantly different bacterial species between males and females within each age group: (A) D 0, (B) D 14, (C) D 28, and (D) D 42. Positive $\log_2$ fold-change values indicate species enriched in females (red bars), whereas negative values indicate species enriched in males (blue bars).

characterized and unknown, bacterial species such as *Turicibacter bilis* and *Turicibacter sanguinis* are recognized as gut colonizers with activity towards bile acid and lipid metabolism (Lynch et al., 2023; Maki et al., 2025). Similarly, *Dysosmobacter acutus* enriched in males at d 28 and d 42 might be associated with short-chain fatty acid (SCFA) production, as a closely related species *D. welbionis* was found to produce SCFA in humans (Roy et al., 2020, 2022). These findings reflect the dynamic nature of the chicken cecal microbiome, where early or transient colonizers were gradually replaced by more stable and metabolically active and functionally diverse communities. Additionally, these results provide the much needed species-level resolution of cecal microbiome of broilers offering more precise data for targeted nutritional or managemental interventions, which could not have been achieved only analyzing the genus-level shifts.

Conclusion

In conclusion, age emerged as a primary driver of cecal microbiome composition of broiler in both male and female, probably due to the strong influence of extrinsic environmental and management factors. However, the influence of sex and its potential interaction with developmental stages including hormonal and physiological shifts needs further investigation. Our results indicate that the cecal microbiome was mostly stable after d 28, indicating that any interventions aimed at modulating the gut microbiome, such as probiotics, would be most effective if applied at an early age. Overall, this study enhances our understanding of the cecal microbiome dynamics of broilers. Future research should focus on identifying key extrinsic factors that shape the cecal microbiome, such as litter, feed, and water, which are likely major sources of microbial diversity and key contributors to establishing the gut microbiome. Understanding the key contributors of gut microbiome could lead us to develop targeted interventions, such as early-life probiotics, feed additives, or litter management strategies, to optimize gut health, improve performance, and enhance disease resilience.

Declaration of AI

Codes used for the data analysis were optimized using AI-tools.

Data availability

The raw sequences FASTQ files are available under Bio project no PRJNA1444204.

Supplementary material

CRediT authorship contribution statement

Farazi Abinash Rahman: Writing – original draft, Visualization, Software, Methodology, Data curation. **Erasmo Alexander Flores Granados:** Writing – review & editing, Methodology, Investigation. **Yagya Adhikari:** Data curation. **Muhammad Naeem:** Writing – review & editing, Writing – original draft, Data curation. **Samuel Rochell:** Supervision, Resources, Methodology, Conceptualization. **Dianna Bourassa:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Sabin Poudel:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Disclosures

Authors declare no Conflict of Interest for this article.

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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.2026.106911.

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