Quantitative Microbiome Profiling Reveals the Developmental Trajectory of the Chicken Gut Microbiota and its Connection to Host Metabolism

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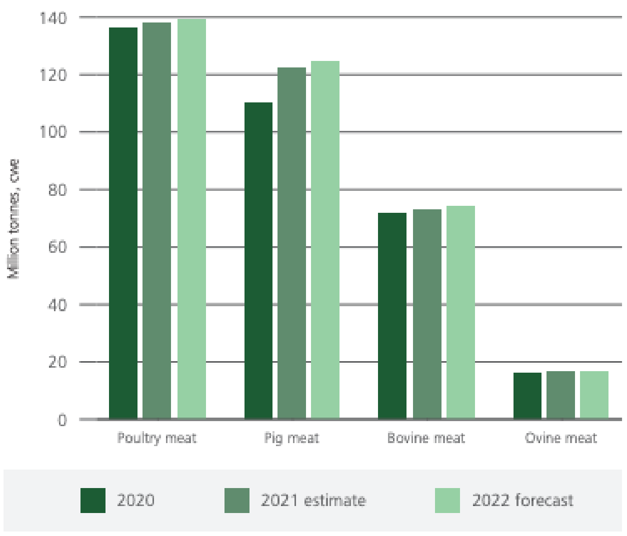
Introduction

Chicken meat has become the most consumed animal meat worldwide.

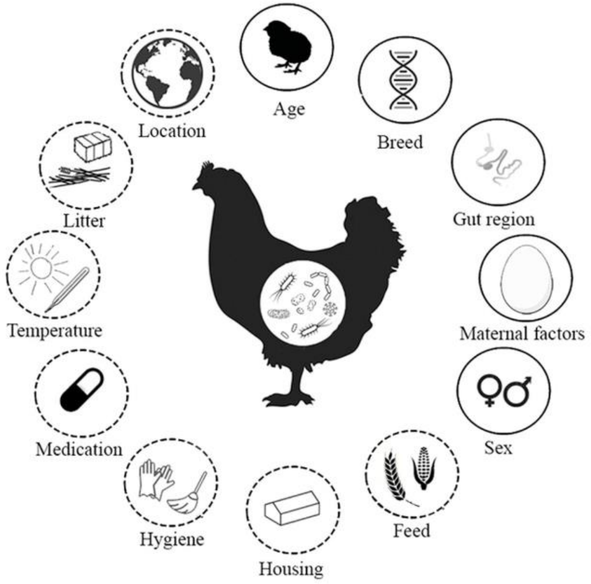
The chicken gut microbiota strongly influences immunity, nutrient digestion, and feed efficiency.

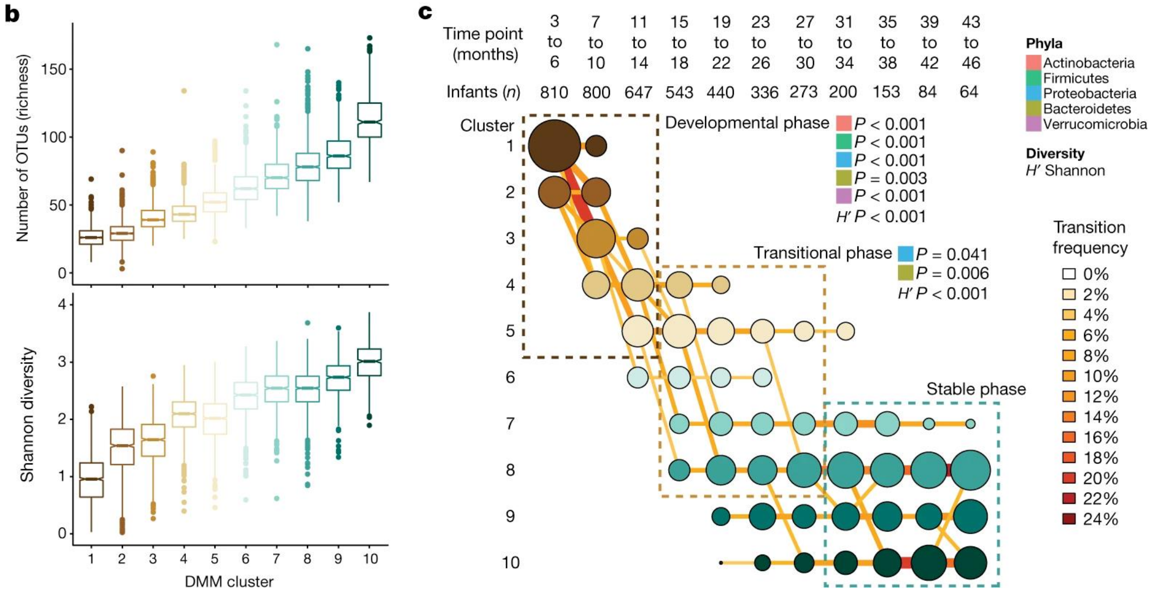
Most studies mainly focused on the characterization of the chicken gut bacteriome, with less effort to reveal the fungal community.

The development of gut microbiota during early life has a far-reaching impact on host health.



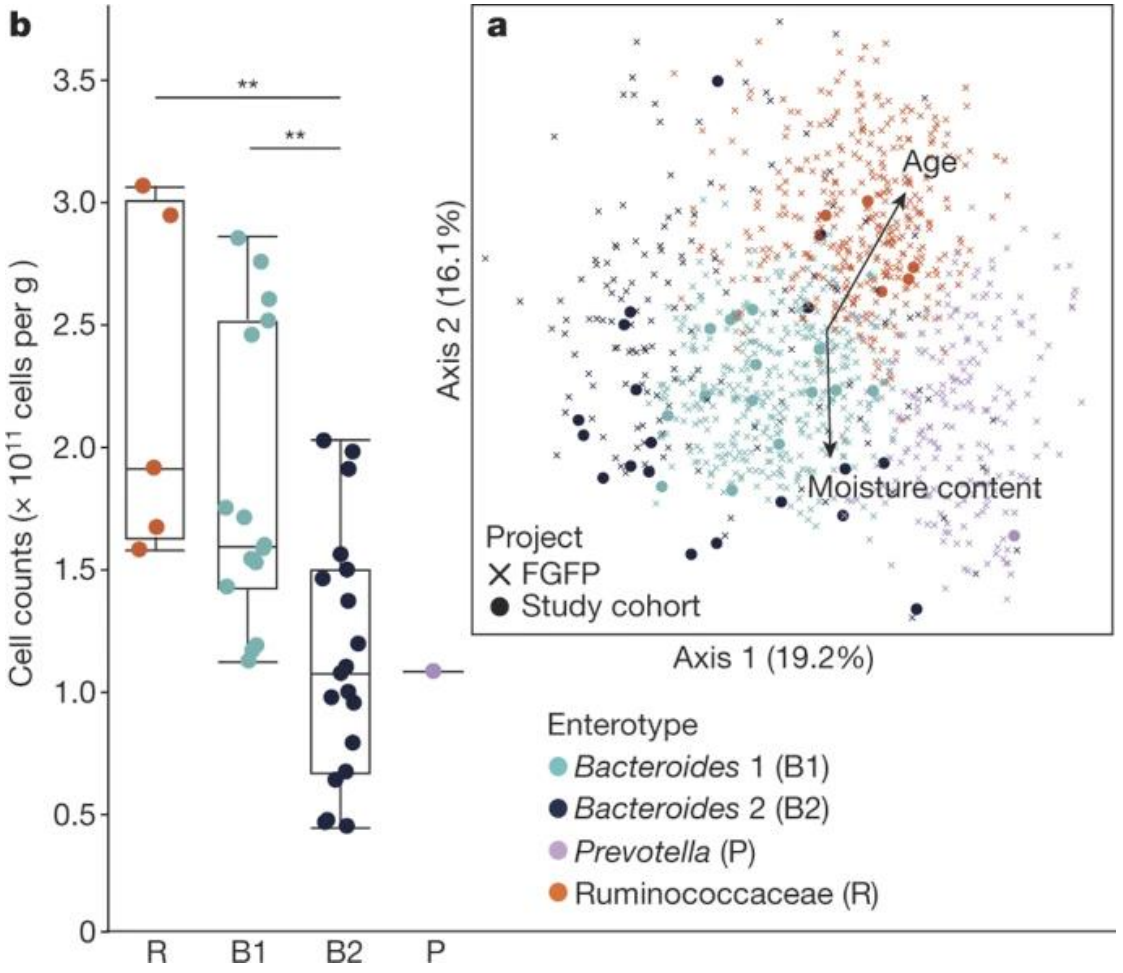
Source: FAO.

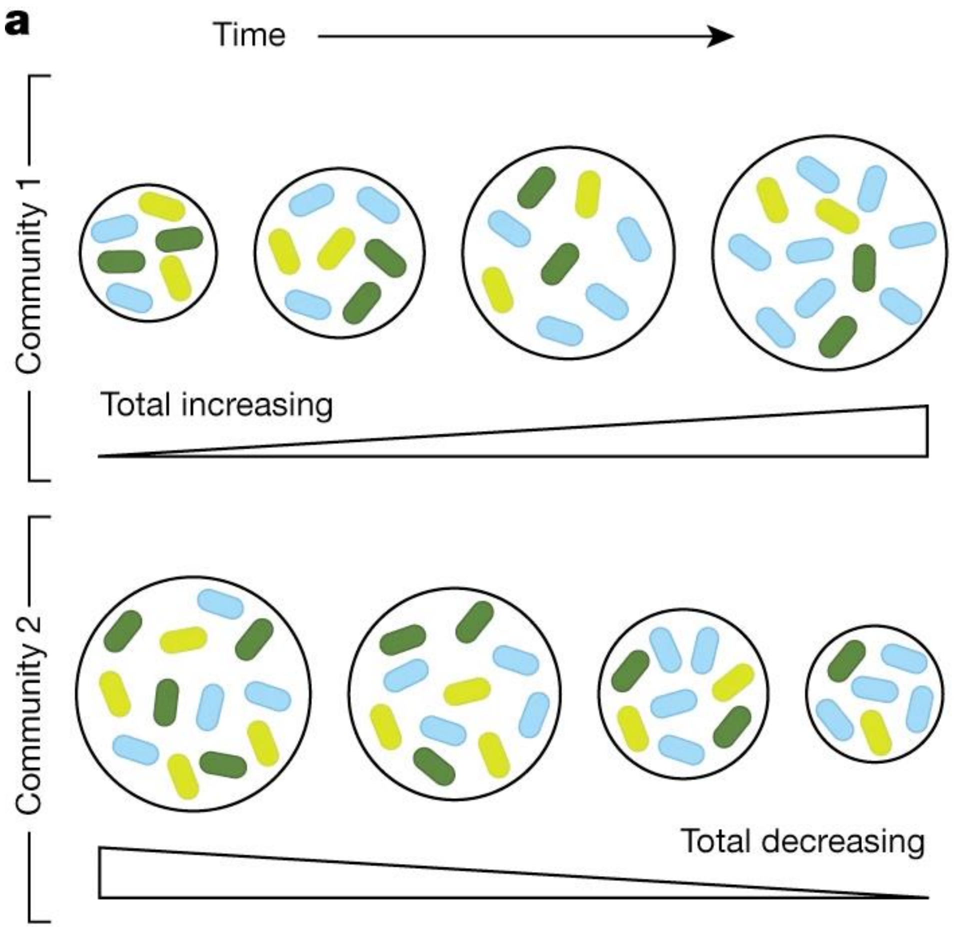




(FAO, 2022; Kers et al, 2018, Front Microbiol; Stewart et al, 2018, Nature)

Introduction

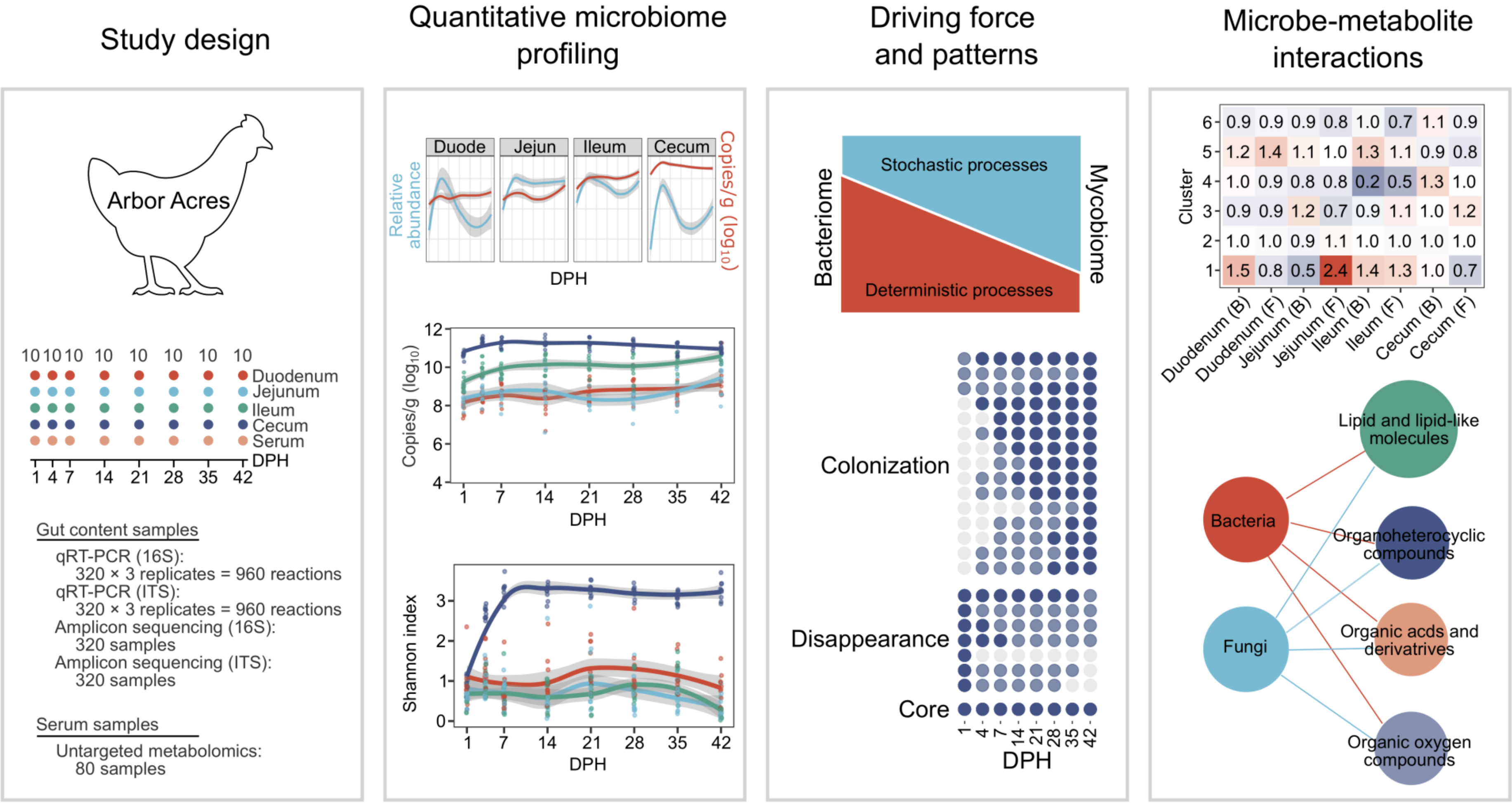




(Vandeputte, 2017, Nature; Rao, et al., 2021, Nature)

Results

Study design



Study design

Quantitative microbiome profiling

Driving force and patterns

Microbe-metabolite interactions

Gut content samples

qRT-PCR (16S):
320 × 3 replicates = 960 reactions
qRT-PCR (ITS):
320 × 3 replicates = 960 reactions
Amplicon sequencing (16S):
320 samples
Amplicon sequencing (ITS):
320 samples

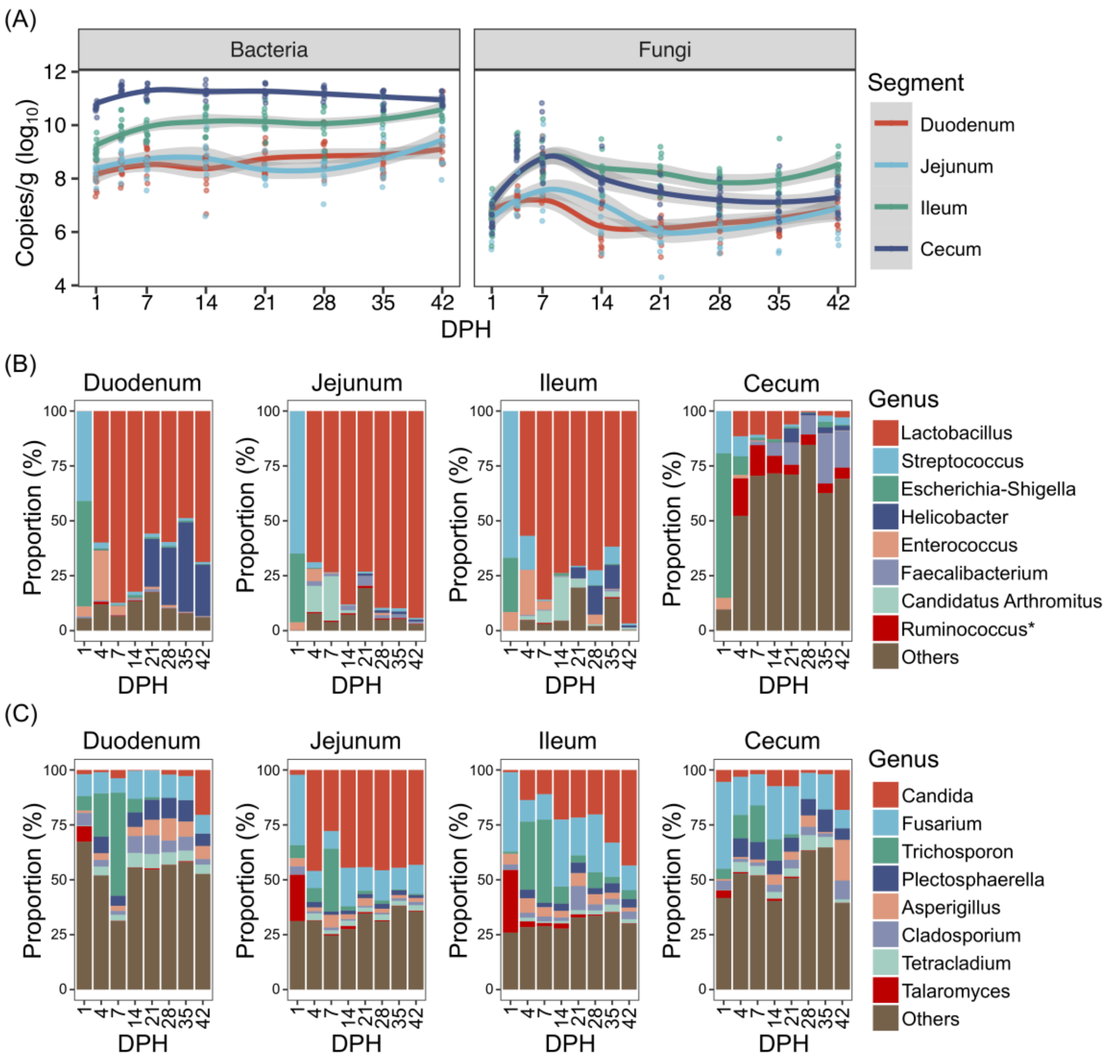
Serum samples

Untargeted metabolomics:
80 samples

Cluster	Duodenum (B)	Duodenum (F)	Jejunum (B)	Jejunum (F)	Ileum (B)	Ileum (F)	Cecum (B)	Cecum (F)
6	0.9	0.9	0.9	0.8	1.0	0.7	1.1	0.9
5	1.2	1.4	1.1	1.0	1.3	1.1	0.9	0.8
4	1.0	0.9	0.8	0.8	0.2	0.5	1.3	1.0
3	0.9	0.9	1.2	0.7	0.9	1.1	1.0	1.2
2	1.0	1.0	0.9	1.1	1.0	1.0	1.0	1.0
1	1.5	0.8	0.5	2.4	1.4	1.3	1.0	0.7

Results

Abundance and diversity of the chicken gut microbiota reach a plateau at 7 days posthatch



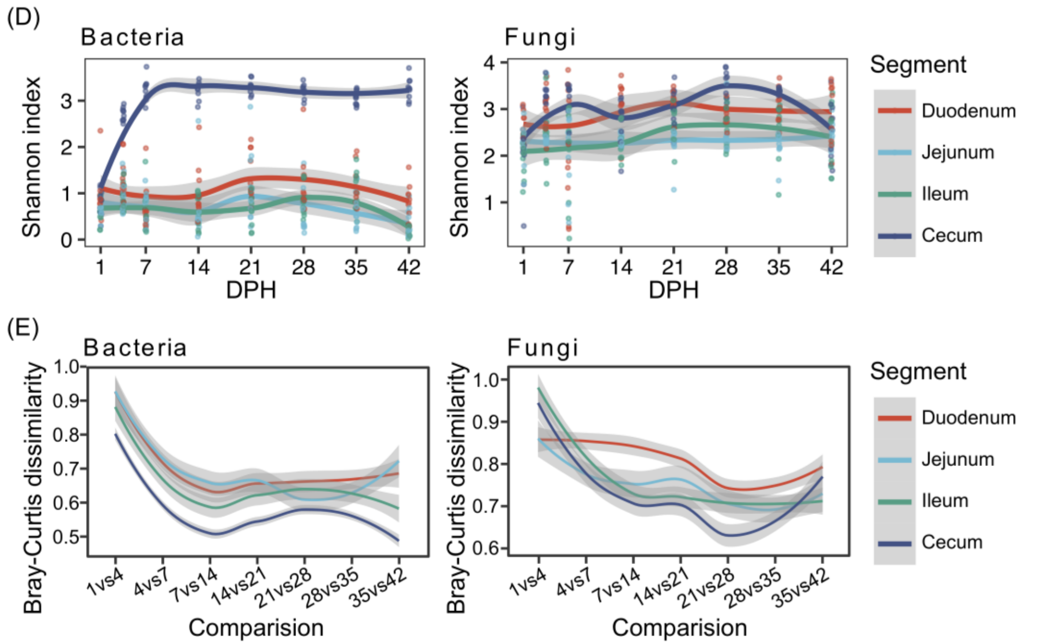
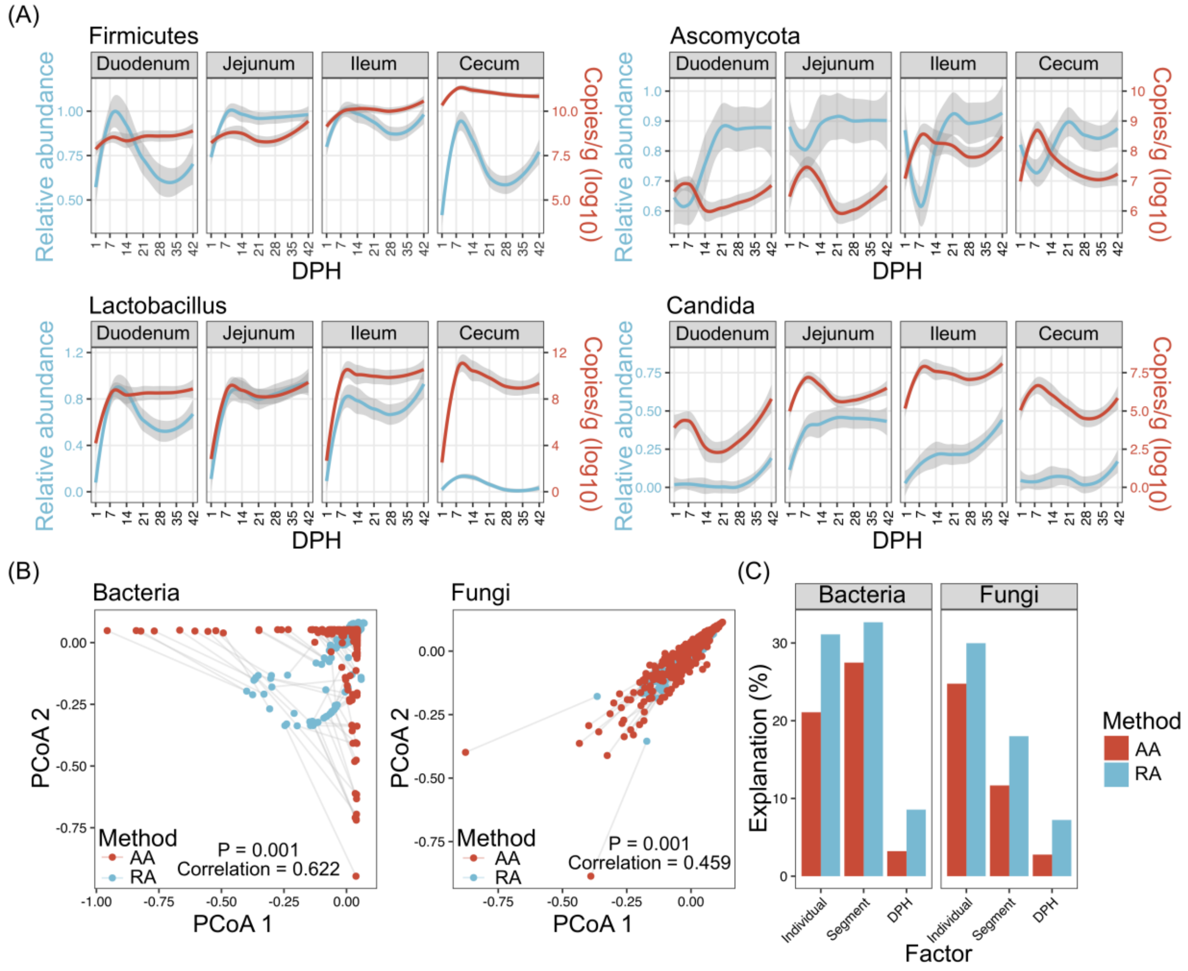
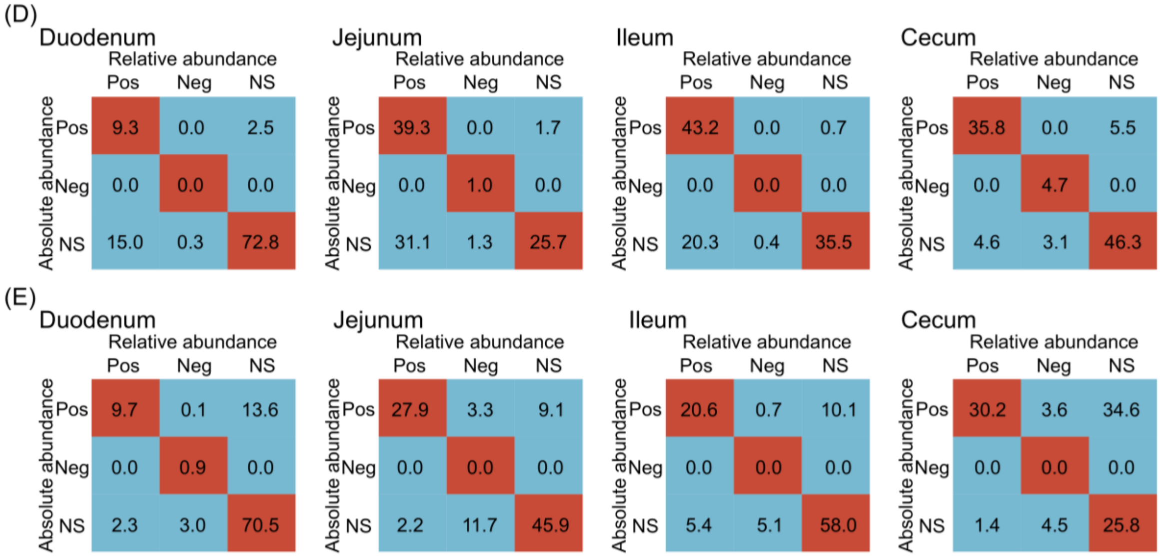


FIGURE 1 Abundance and diversity changes of the quantitative chicken gut microbiota. (A) Absolute abundance dynamics of bacteria and fungi in the four segments of broiler chickens. Bacterial (B) and fungal (C) community composition of the broiler gut microbiota at the genus level. (D) Shannon index of the samples during the 42 DPH. (E) Pairwise comparison of Bray–Curtis dissimilarity of samples from adjacent time points.

Results

RMP biases chicken gut microbiota development and microbial interactions





(D)

Duodenum

	Pos	Neg	NS
Pos	9.3	0.0	2.5
Neg	0.0	0.0	0.0
NS	15.0	0.3	72.8

Jejunum

	Pos	Neg	NS
Pos	39.3	0.0	1.7
Neg	0.0	1.0	0.0
NS	31.1	1.3	25.7

Ileum

	Pos	Neg	NS
Pos	43.2	0.0	0.7
Neg	0.0	0.0	0.0
NS	20.3	0.4	35.5

Cecum

	Pos	Neg	NS
Pos	35.8	0.0	5.5
Neg	0.0	4.7	0.0
NS	4.6	3.1	46.3

(E)

Duodenum

	Pos	Neg	NS
Pos	9.7	0.1	13.6
Neg	0.0	0.9	0.0
NS	2.3	3.0	70.5

Jejunum

	Pos	Neg	NS
Pos	27.9	3.3	9.1
Neg	0.0	0.0	0.0
NS	2.2	11.7	45.9

Ileum

	Pos	Neg	NS
Pos	20.6	0.7	10.1
Neg	0.0	0.0	0.0
NS	5.4	5.1	58.0

Cecum

	Pos	Neg	NS
Pos	30.2	3.6	34.6
Neg	0.0	0.0	0.0
NS	1.4	4.5	25.8

FIGURE 2 Differences in QMP and RMP in profiling the chicken gut microbiota. (A) Changes in the absolute abundance and relative abundance of the main phyla and the main genera in the broiler gut microbiota. (B) Procrustes analyses of the correlation between absolute abundance and relative abundance for bacteria and fungi. (C) The percentage of the variance of the gut microbiota is explained by the factors of individuals, segments, and time. (D) Consistency of the correlations between the taxa based on absolute abundance and relative abundance in bacteria. (E) Consistency of the correlations between the taxa based on absolute abundance and relative abundance in fungi.

Results

Ecological deterministic and stochastic processes shape the segment-specific community types

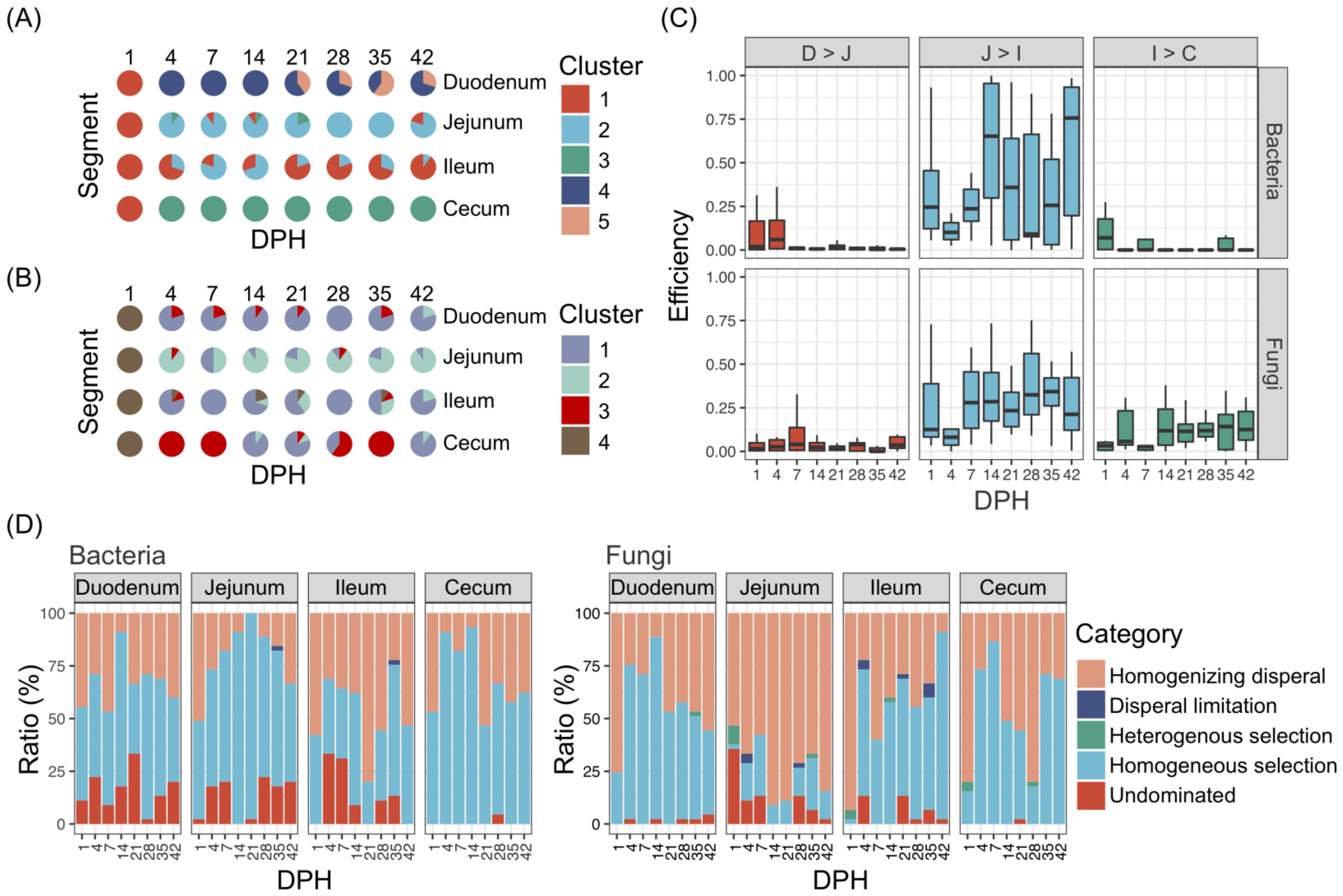


FIGURE 3 Clusters of bacterial and fungal communities associated with the segment and the selection pressure. DMM clustering of bacterial (A) and fungal (B) communities. (C) Relative contributions of upper microbial communities to the adjacent lower microbial communities. (D) Relative importance of different ecological processes.

Results

Chicken gut microbes have different colonization abilities

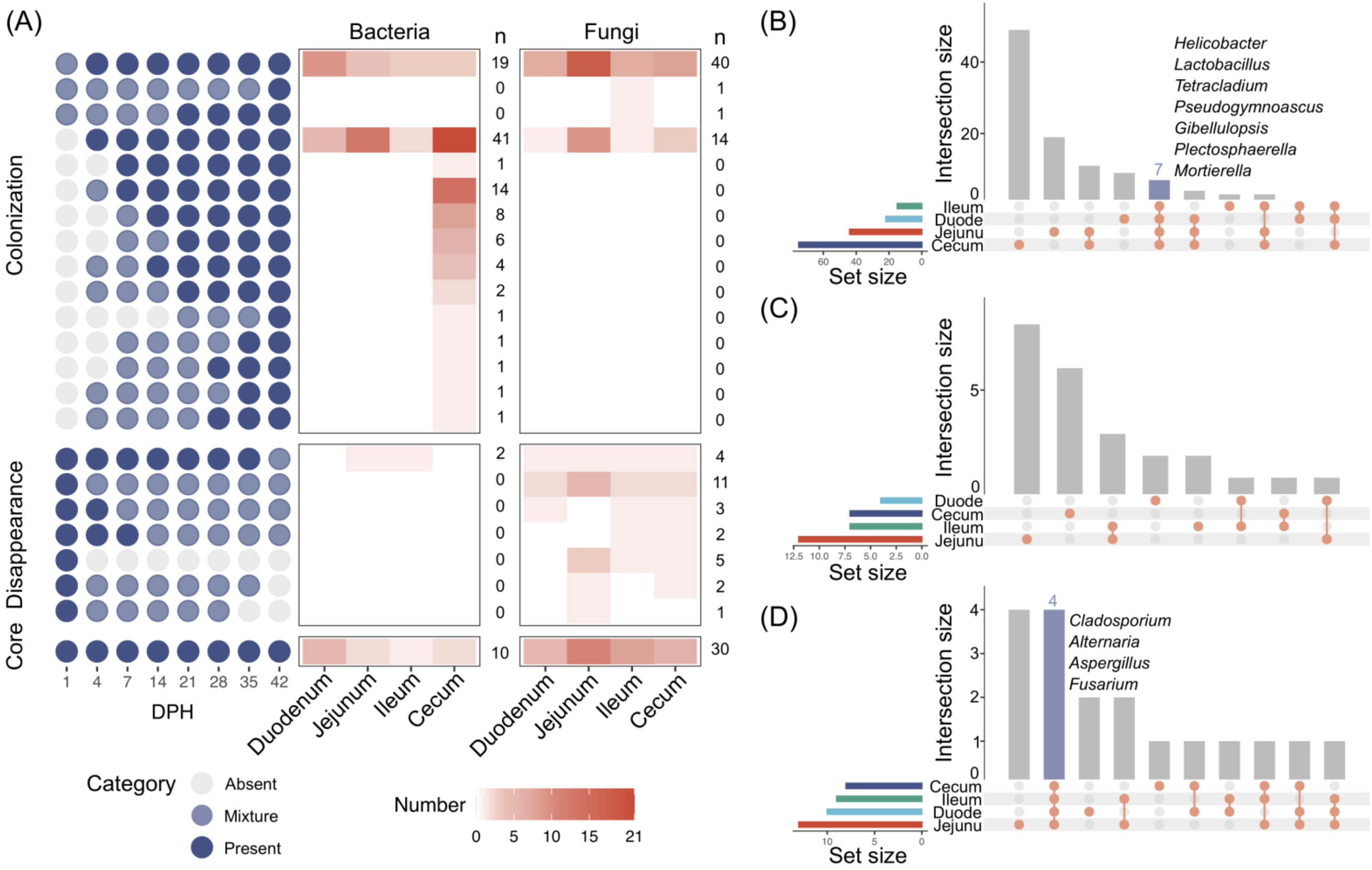


FIGURE 4 Longitudinal occurrence patterns and variants of microbial genera across the four gut segments. (A) Summary of the occurrence patterns matched the three categories (core, disappearance, and colonization) in the four segments. The UpSetR plot highlights the intersection of the genera colonized (B), disappeared (C), and presented (D) in the four segments.

Results

Co-occurrence networks reveal more positive associations among microbes in the chicken gut

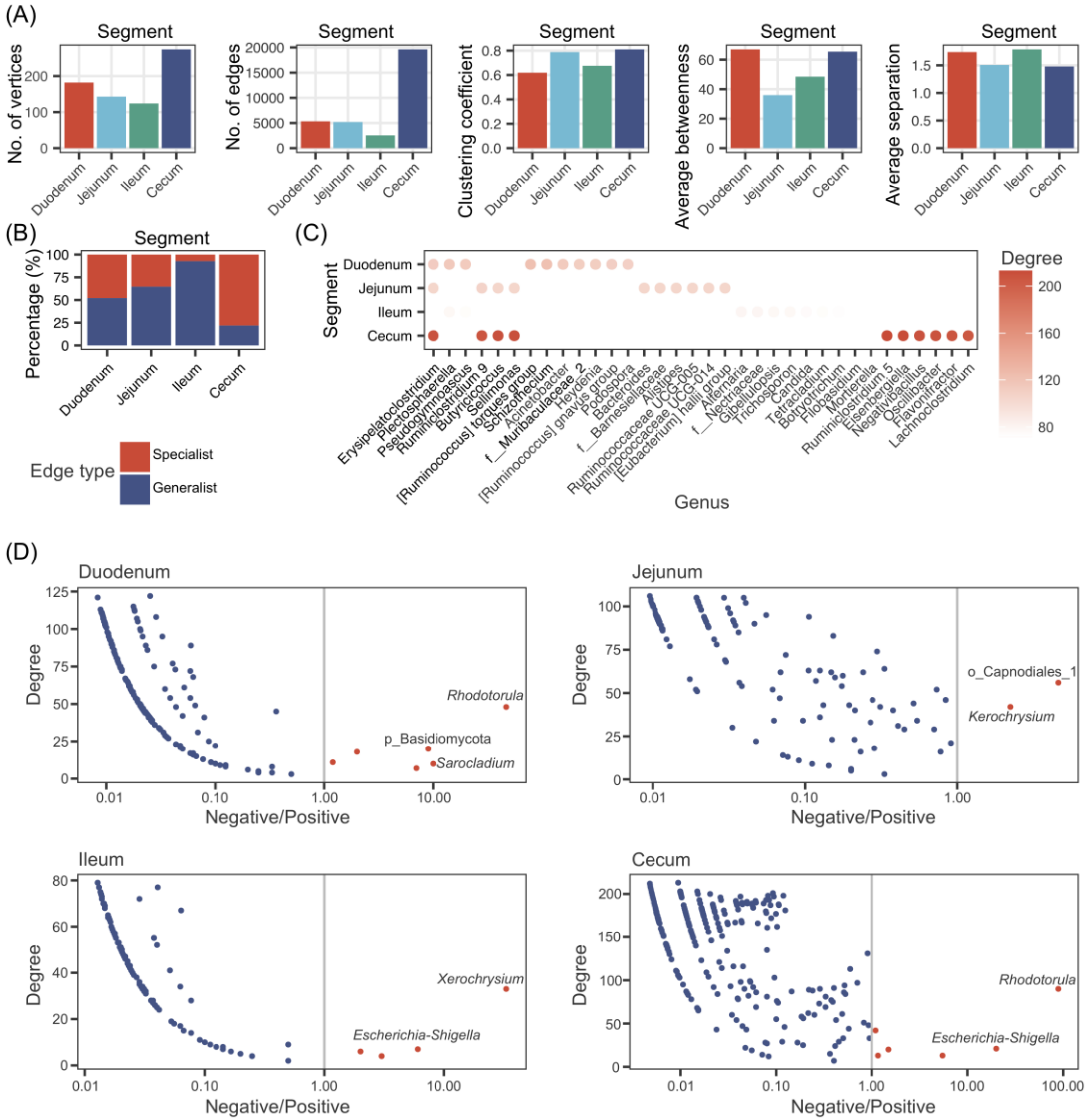


FIGURE 5 Microbial co-occurrence networks in the four gut segments. (A) Network topology of microbial networks inferred from absolute microbiome profiling. (B) Proportions of generalist edges and specialist edges in the four microbial networks. (C) Top 10 key taxa in the four gut segments. (D) Scatter plot of the log-transformed (log10) ratio of negative to positive interactions against degree for each taxon in different segments (duodenum, jejunum, ileum, and cecum).

Results

Serum metabolite abundance changes with chicken development

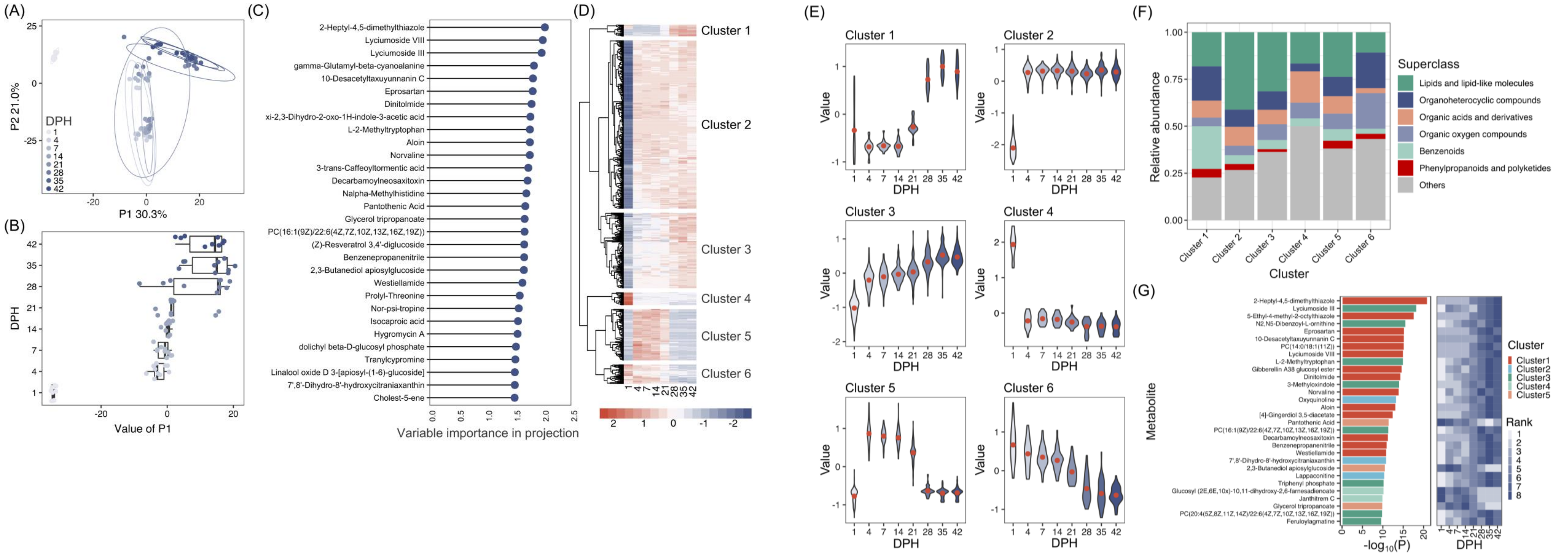
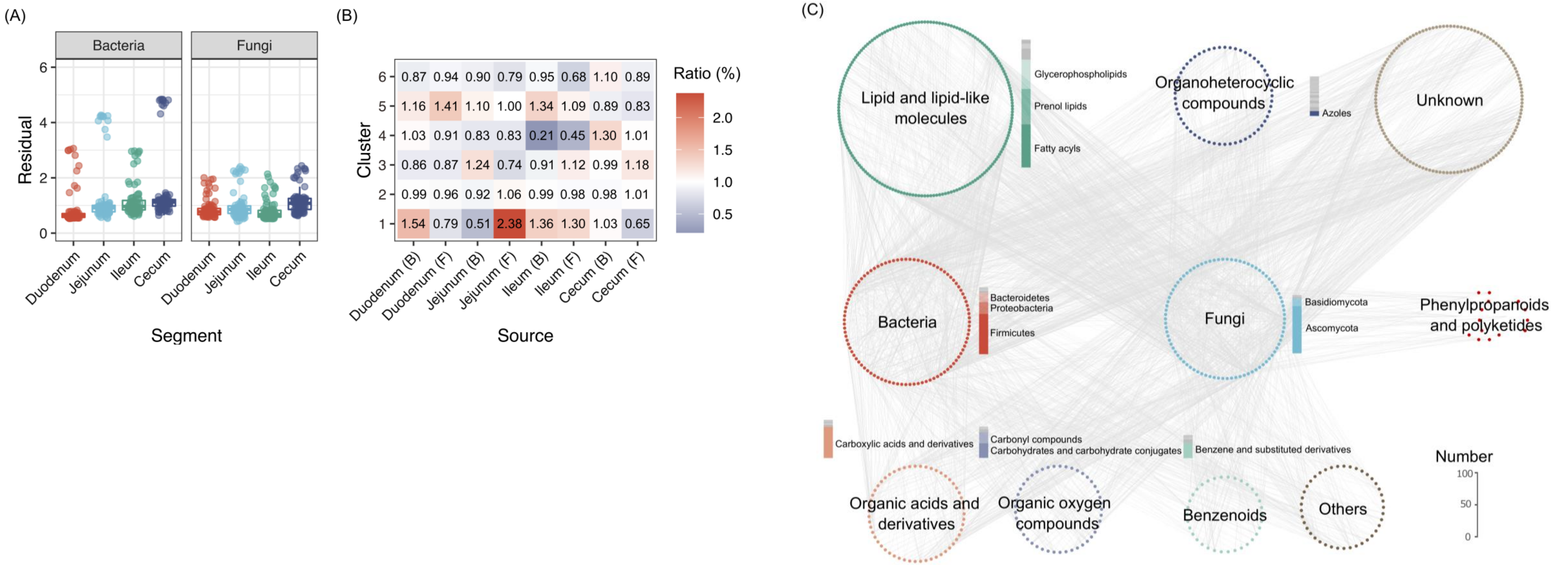


FIGURE 6 Changes in the metabolite profiles during chicken growth. (A) OPLS-DA of metabolite profiles of the chicken serum at different time points. (B) Value of P1 generated from the OPLS-DA at different time points. (C) Importance of metabolites ranked by VIP. (D) Heatmap of metabolites significantly (GLM FDR < 0.05) altered over time. (E) Changes in metabolites of the six clusters over time. (F) Summary of pathway analysis for each cluster. (G) Top 30 most significantly different metabolites over time according to the results of GLM.

Results

QMP of gut microbiota establishes connections between microbes and chicken serum metabolites



Cluster	Duodenum (B)	Duodenum (F)	Jejunum (B)	Jejunum (F)	Ileum (B)	Ileum (F)	Cecum (B)	Cecum (F)
6	0.87	0.94	0.90	0.79	0.95	0.68	1.10	0.89
5	1.16	1.41	1.10	1.00	1.34	1.09	0.89	0.83
4	1.03	0.91	0.83	0.83	0.21	0.45	1.30	1.01
3	0.86	0.87	1.24	0.74	0.91	1.12	0.99	1.18
2	0.99	0.96	0.92	1.06	0.99	0.98	0.98	1.01
1	1.54	0.79	0.51	2.38	1.36	1.30	1.03	0.65

FIGURE 7 Microbe–metabolite interactions. (A) Residuals showing the difference in the microbe–metabolite association from different segments with absolute abundance. (B) Ratio of the top 1% co-occurrence possibilities in all microbe-metabolite interactions. (C) Co-occurrence network of the microbe–metabolite interactions discovered by mmvec from the four segments.

Summary

- The developmental trajectory of the broiler chicken gut microbiota is revealed by a quantitative microbiome profiling approach.
- The chicken gut microbiota displays gut segment-specific community types and growth stage-specific microbes.
- The chicken gut microbes have different colonization abilities, forming different developmental patterns.
- The chicken gut microbiota development is associated with the chicken serum metabolite changes.

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