

Supplementary Materials for

***Eimeria* infection-related intestinal dynamics and microbiome, growth performance, and nutrient utilization in broiler chickens fed diets supplemented with multienzyme**

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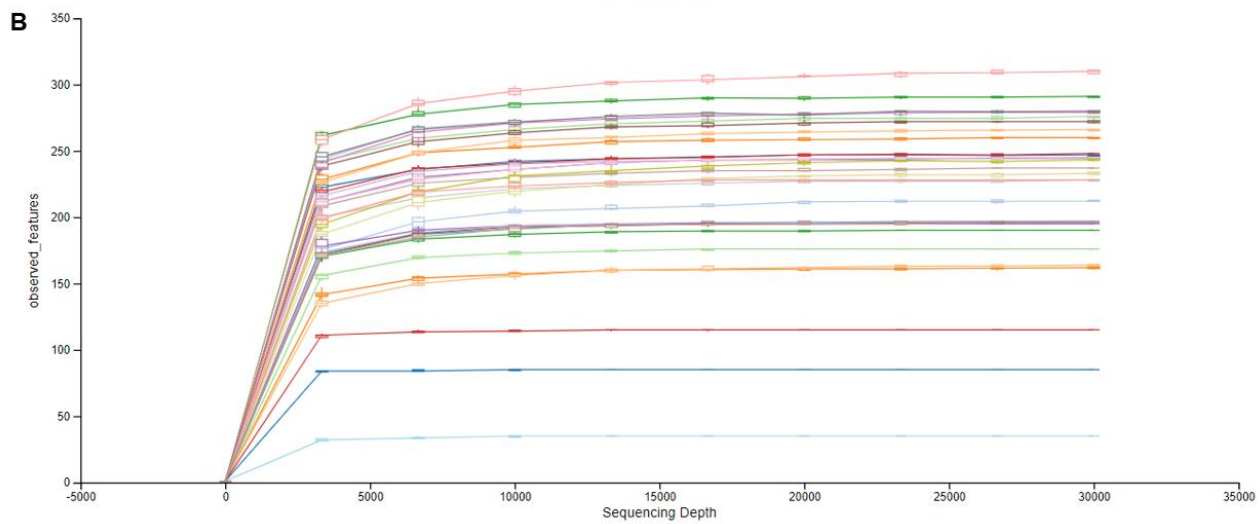
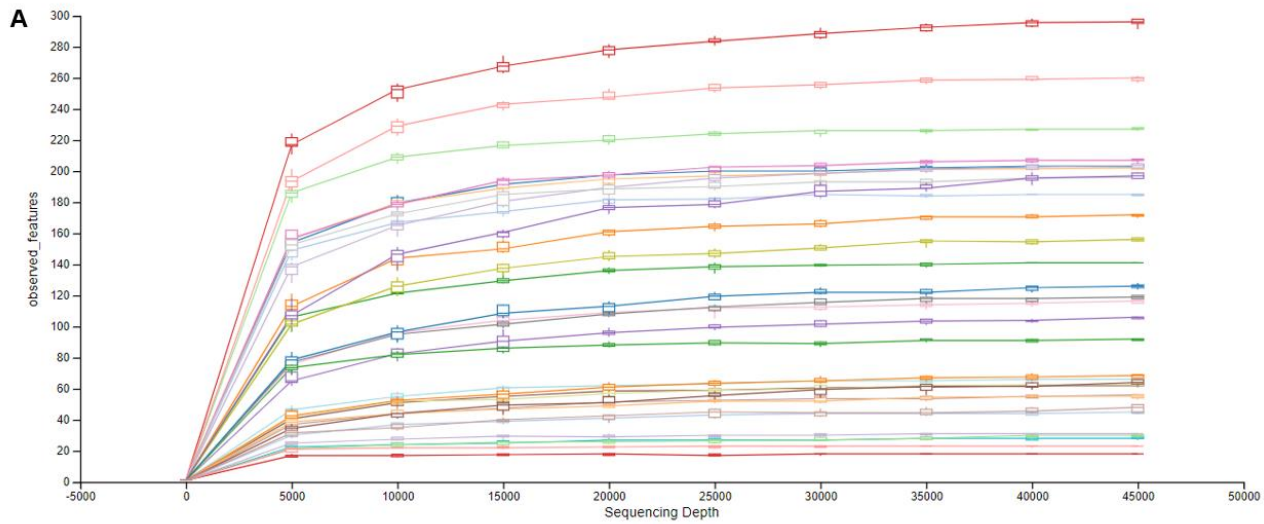
Supplementary Table S1. Primers used in real-time quantitative PCR^a

Genes	Primer sequence (5'-3')	Accession no.
Housekeeping gene		
β -actin	F: CACAGATCATGTTTGAGACCTT R: CATCACAATACCAGTGGTACG	NM_205518.1
Inflammation-related genes		
IL-6	F: CTGCAGGACGAGATGTGCAA R: AGGTCTGAAAGGCGAACAGG	NM_204628.1
IL-8	F: GGCTTGCTAGGGGAAATGA R: AGCTGACTCTGACTAGGAACTGT	NM_205498.1
IL-10	F: ACTTAACATCCAACCTGCTCAG R: AGGACCTCATCTGTGTAGAAG	
Markers of intestinal integrity		
JAM2	F: AGCCTCAAATGGGATTGGATT R: CATCAACTTGCATTCGCTTCA	NM_0,010,06257.1
occludin	F: GAGCCCAGACTACCAAAGCAA R: GCTTGATGTGGAAGAGCTTGTTG	NM_205,128.1
Nutrient transporters		
b ^{0,+} AT	F: CAGTAGTGAATTCTCTGAGTGTGAAGCT R: GCAATGATTGCCACAACCTACCA	NM_001199133.1
EAAT3	F: TGCTGCTTTGGATTCCAGTGT R: AGCAATGACTGTAGTGCAGAAGTAATATATG	XM_424930.5
Anti-oxidation-related genes		
SOD1	F: ATTACCGGCTTGTCTGATGG R: CCTCCCTTTGCAGTCACATT	NM_205064.1
HMOX1	F: CTGGAGAAGGGTTGGCTTTCT R: GAAGCTCTGCCTTTGGCTGTA	NM_205344

^a Abbreviation: IL, interleukin; JAM2, junctional adhesion molecule; b^{0,+}AT, b^{0,+} amino acid transporter; EAAT3, excitatory amino acid transporter 3; SOD1, superoxide dismutase 1; HMOX1, heme oxygenase 1; F, forward primer; R, reverse primer.

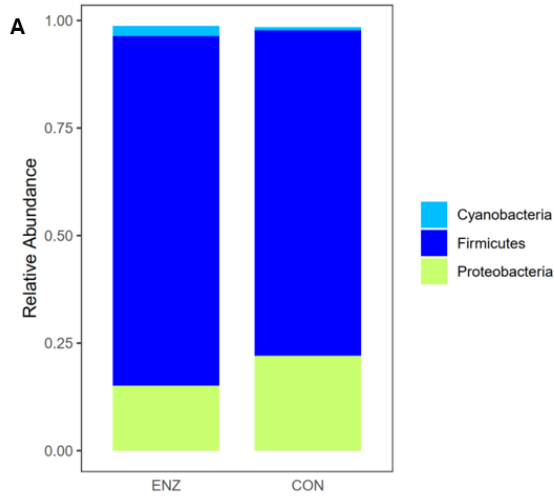
Supplementary Table S2. The average amount of sequencing reads during DADA2 denoising process in ileal and cecal samples

DADA2 denoising process	ileal	cecal
input	122,634.6	60,023.8
filtered	116,401.5	56,768.0
percentage of input passed filter	94.9	94.5
denoised	115,773.4	56,566.3
merged	113,113.8	54,123.5
percentage of input merged	92.2	90.3
non-chimeric	91,038.7	51,834.2
percentage of input non-chimeric	74.2	86.8

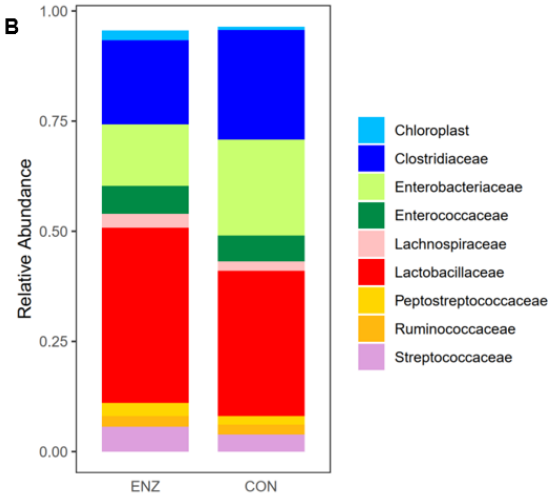


Supplementary Figure S1. Rarefaction curve of ileal (A) and cecal (B) sample sequences. Each line corresponds to an individual chicken. The curves plateau indicating an adequate representative sampling of the community.

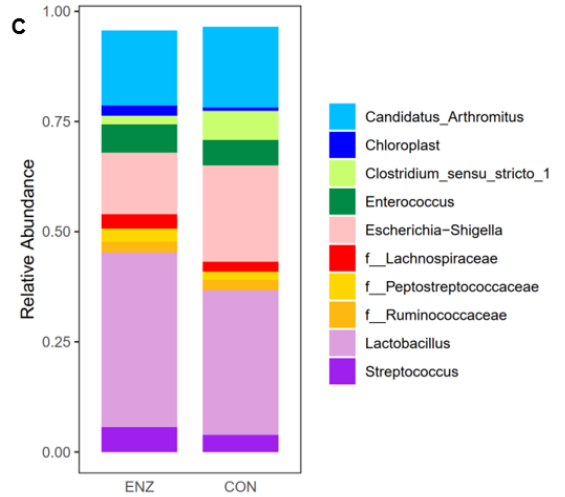
Phylum (> 1%) Composition of Microbiome Samples



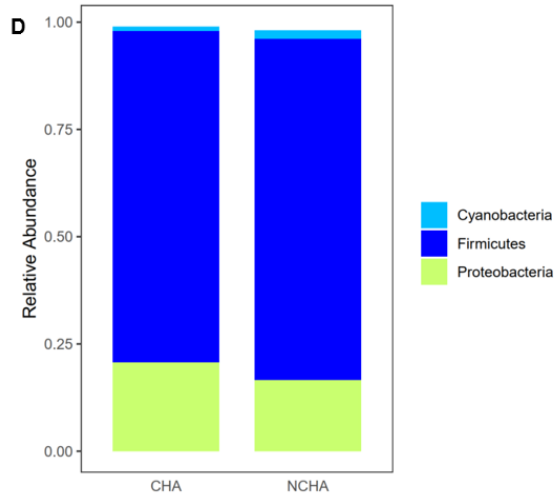
Family (> 1%) Composition of Microbiome Samples



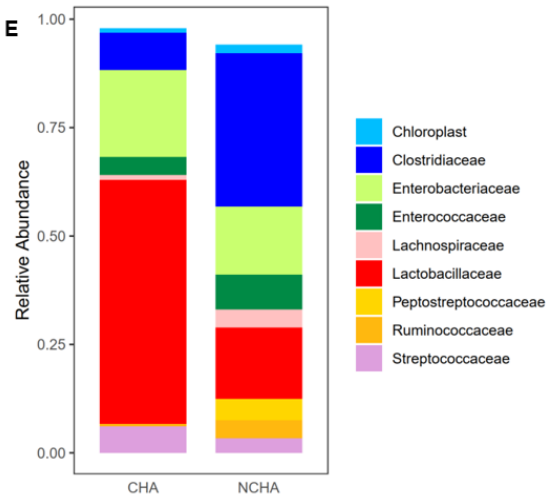
Genus (> 1%) Composition of Microbiome Samples



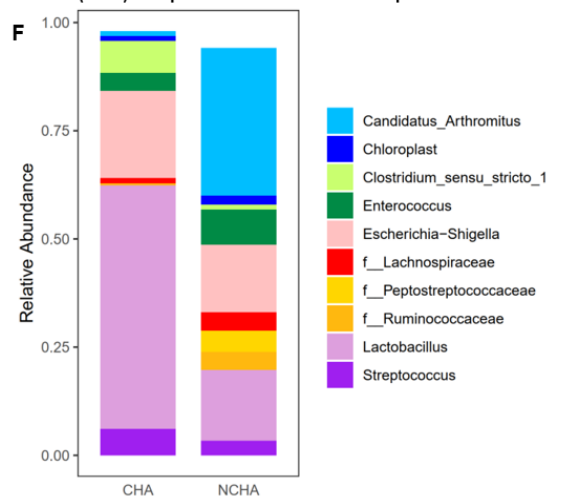
Phylum (> 1%) Composition of Microbiome Samples



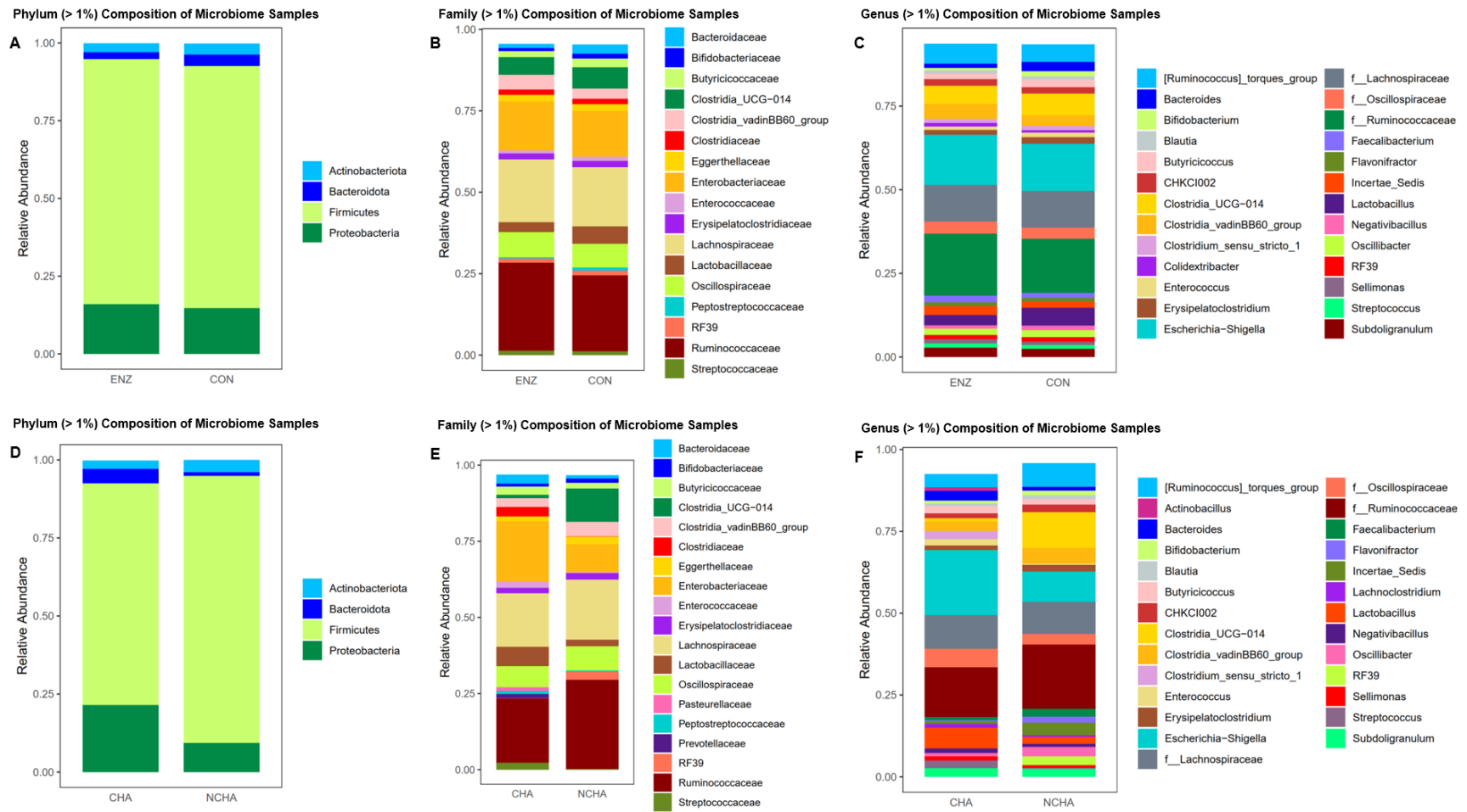
Family (> 1%) Composition of Microbiome Samples



Genus (> 1%) Composition of Microbiome Samples

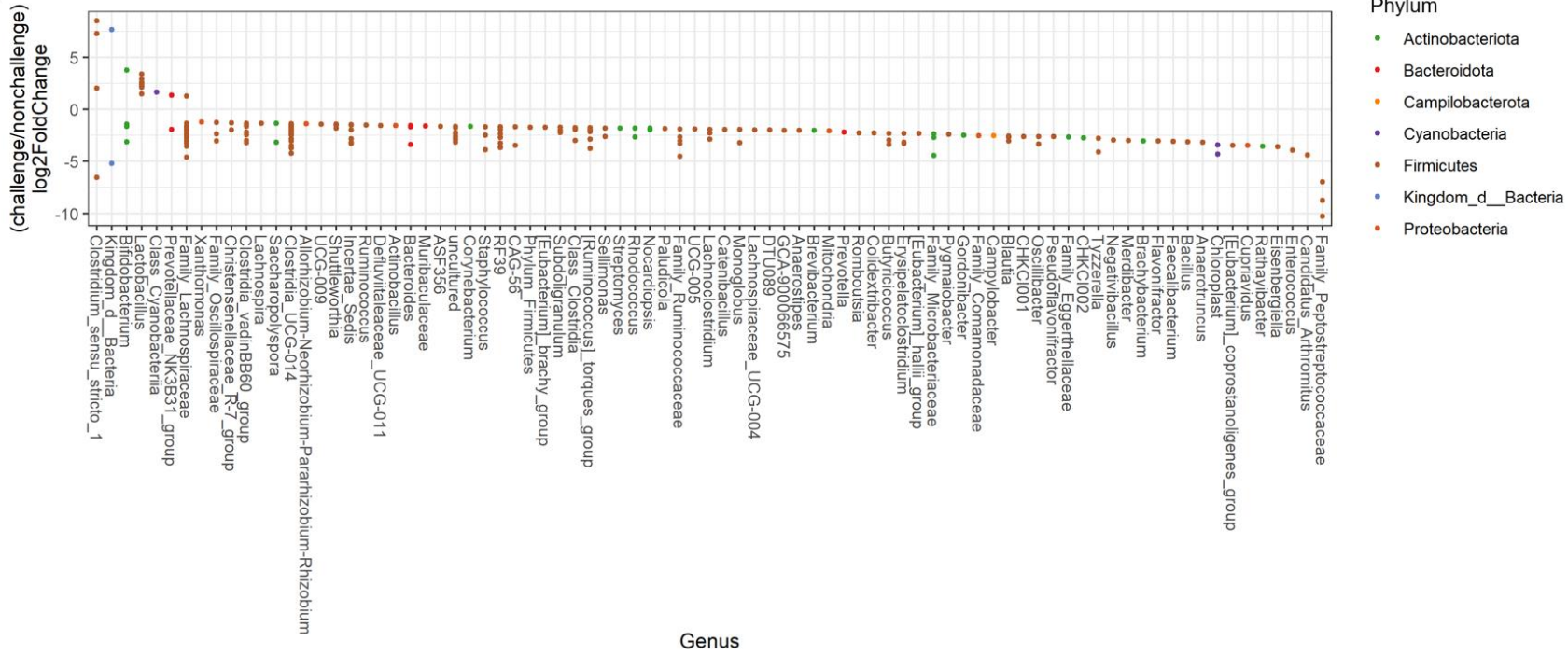


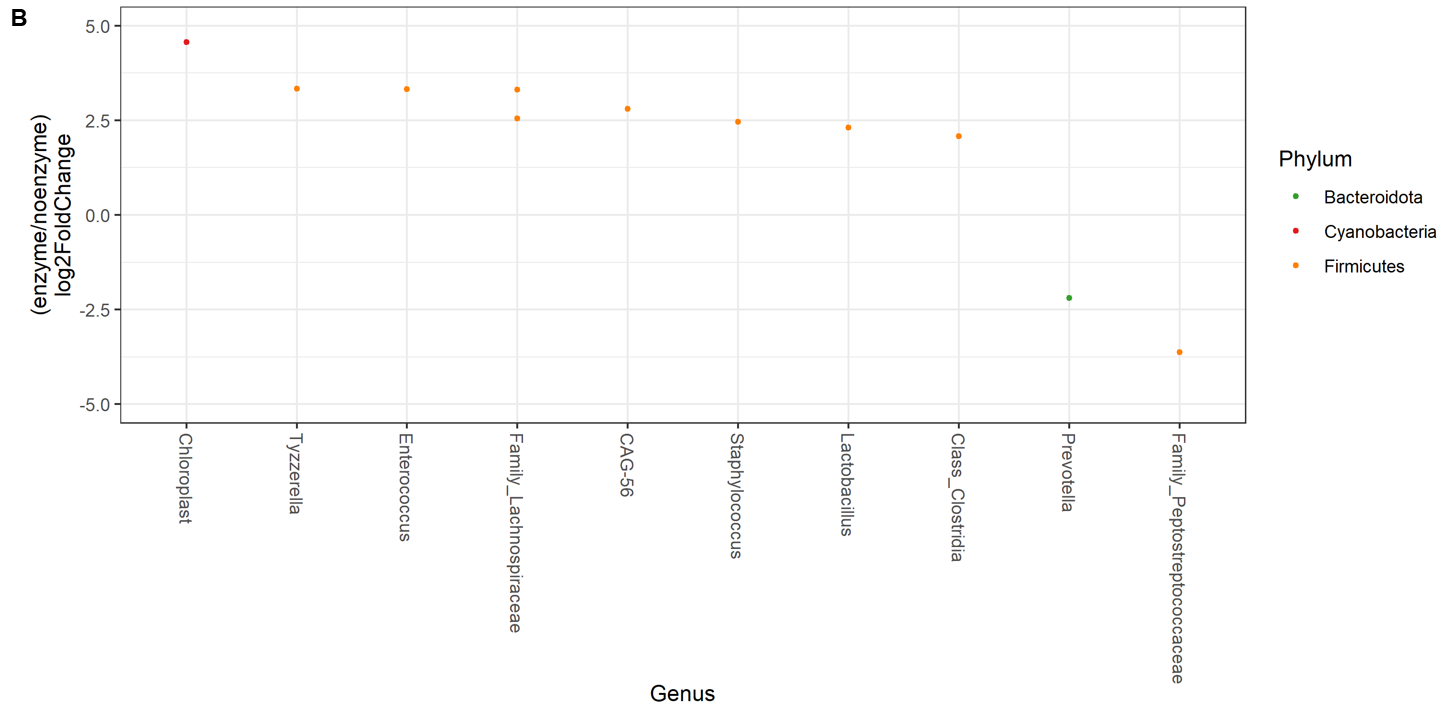
Supplementary Figure S2. Microbial composition of ileal at phylum (A, B), family (C, D), and genus (E, F) level that compose an average of > 1% of the community. Abbreviation: CON, control without multienzyme additives; ENZ, multienzyme additives; NCHA, non-challenge; CHA, challenge.



Supplementary Figure S3. Microbial composition of cecal at phylum (A, B), family (C, D), and genus (E, F) level that compose an average of > 1% of the community. Abbreviation: CON, control without multienzyme additives; ENZ, multienzyme additives; NCHA, non-challenge; CHA, challenge.

A





Supplementary Figure S4. Differentially abundant amplicon sequence variants (ASVs) of ileal microbiota between two groups. Log₂ fold changes calculated by DESeq2 in R for ASVs describe changes in the bacterial community in the ileum with or without corresponding treatment. Each dot represents an ASV with the classified taxonomic level (genus) shown on the x axis, and phylum indicated by color. (A) Ratio of challenge to non-challenge. (B) Ratio of enzyme to no-enzyme. A positive value indicates a significant increase of the specific ASV in challenge (A) (or enzyme, B) treatment relative to that of the non-challenge (A) (or no-enzyme, B) treatment, respectively.