

Metagenomic analysis reveals the impact of sampling methods on intestinal microbiota in donkeys and cows

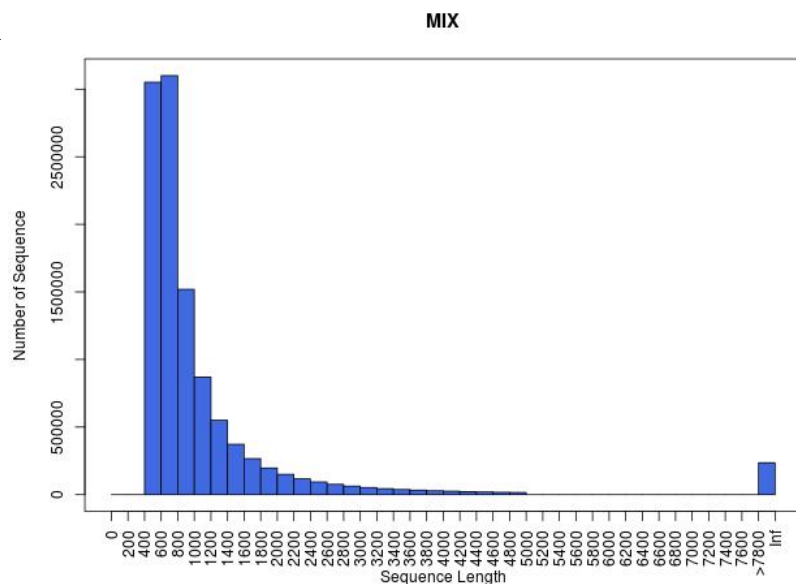
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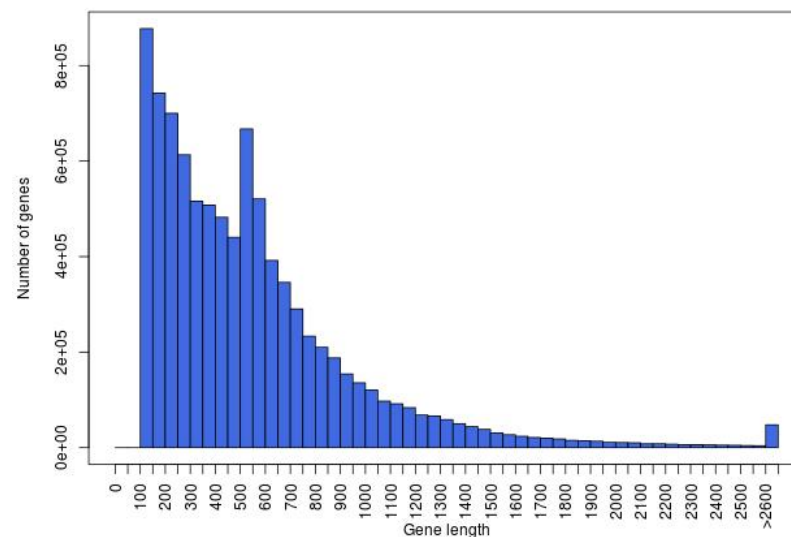
†Lei Su, Jindan Guo, Weixiong Shi and Wei Tong contributed equally to this work.

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a



b



c

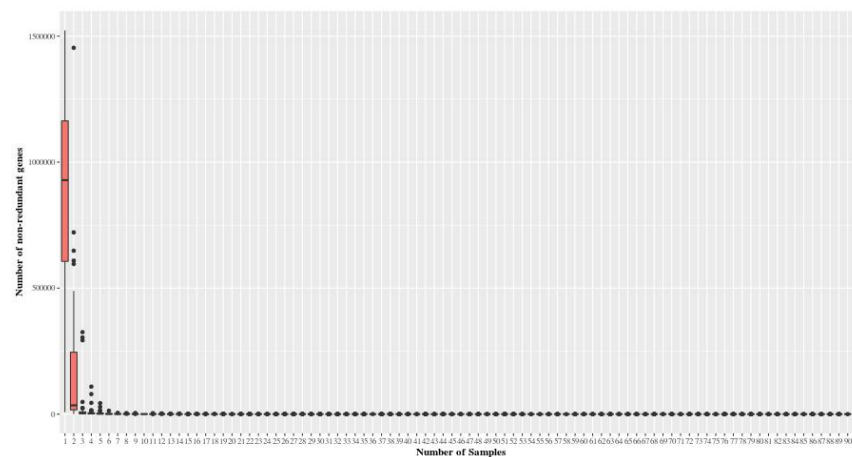


Fig. S1 The results of assembly and gene prediction. (a) Assembly sequence length. (b) Predicted-gene length. (c) Core gene rarefaction curve. The curve nearly plateaus when sufficient sequence data are included.

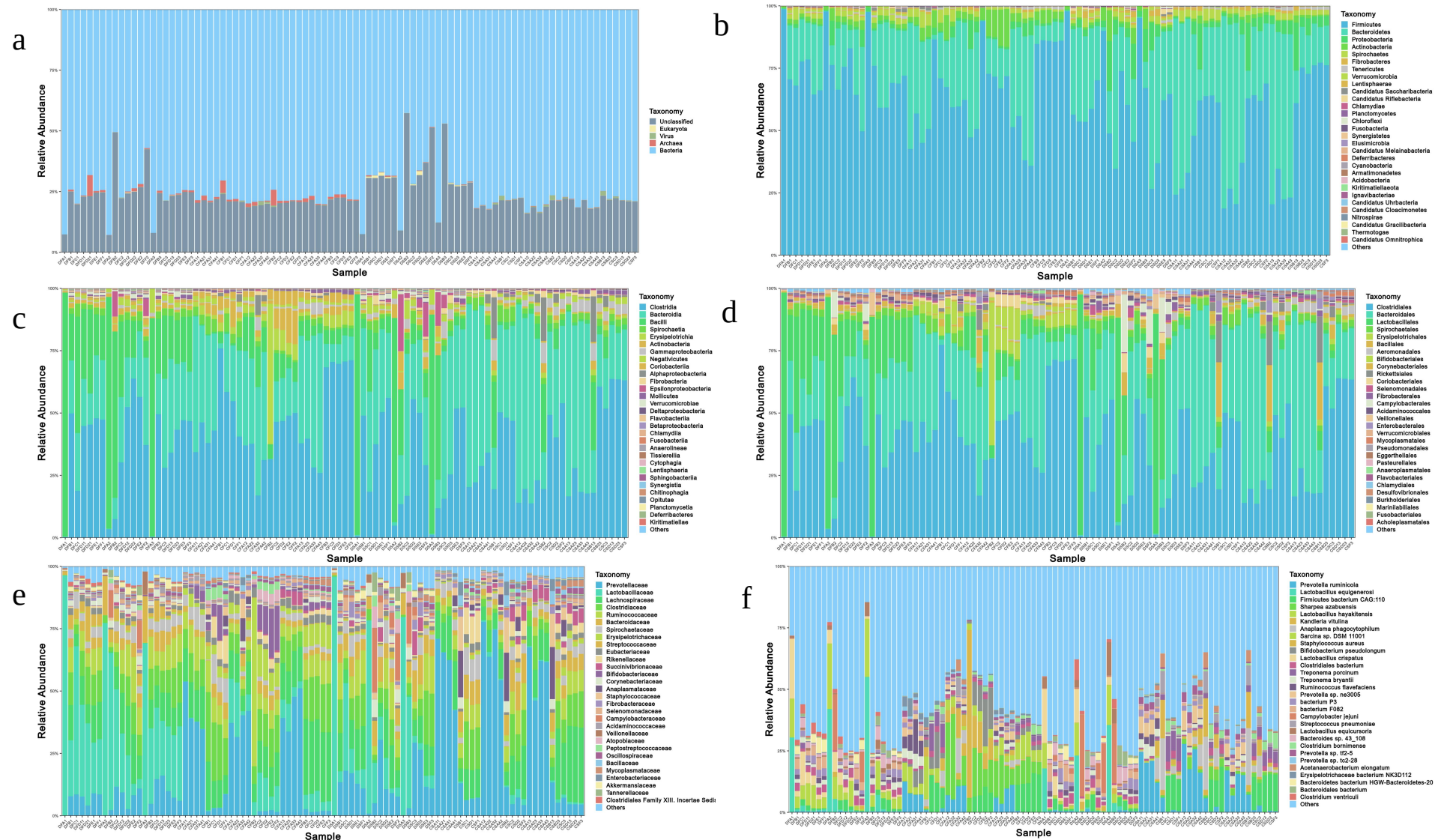


Fig. S2 The top30 microbial composition of all samples at different levels. The microbial composition at the kingdom, phylum, class, order, family and species level respectively. The horizontal axis is the name of 91 samples, and the vertical axis is the relatively abundant of microbes.

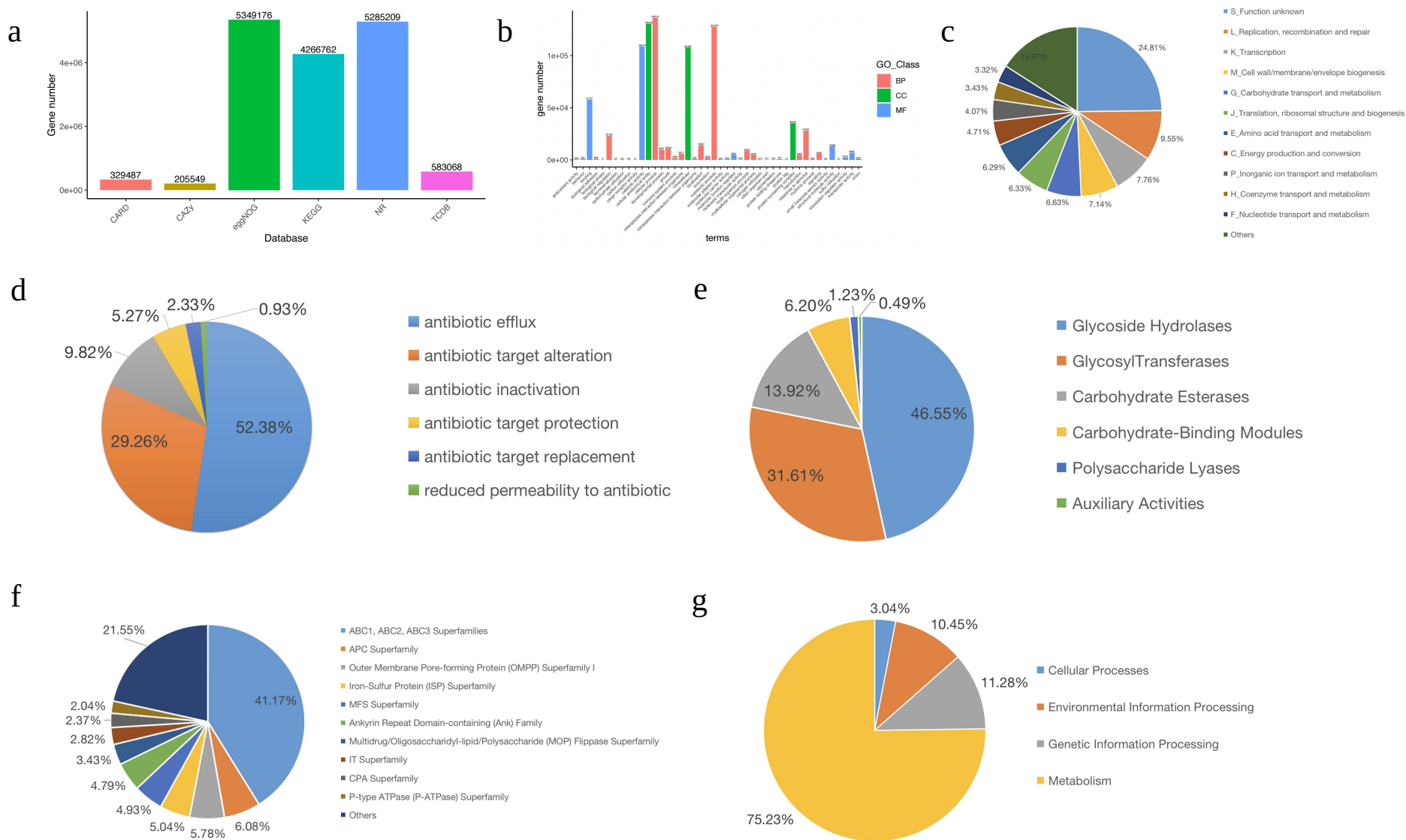


Fig. S3 Function annotation. (a) The distribution of gene number for GO class. (b) The gene number that are mapped to different database (CARD, CAZy, eggNOG, KEGG, NR and TCDB). (c) The annotation of COG database. (d) The resistance mechanism of mapping results in the CARD. (e-g) The annotation of CAZy/TCDB/KEGG database.

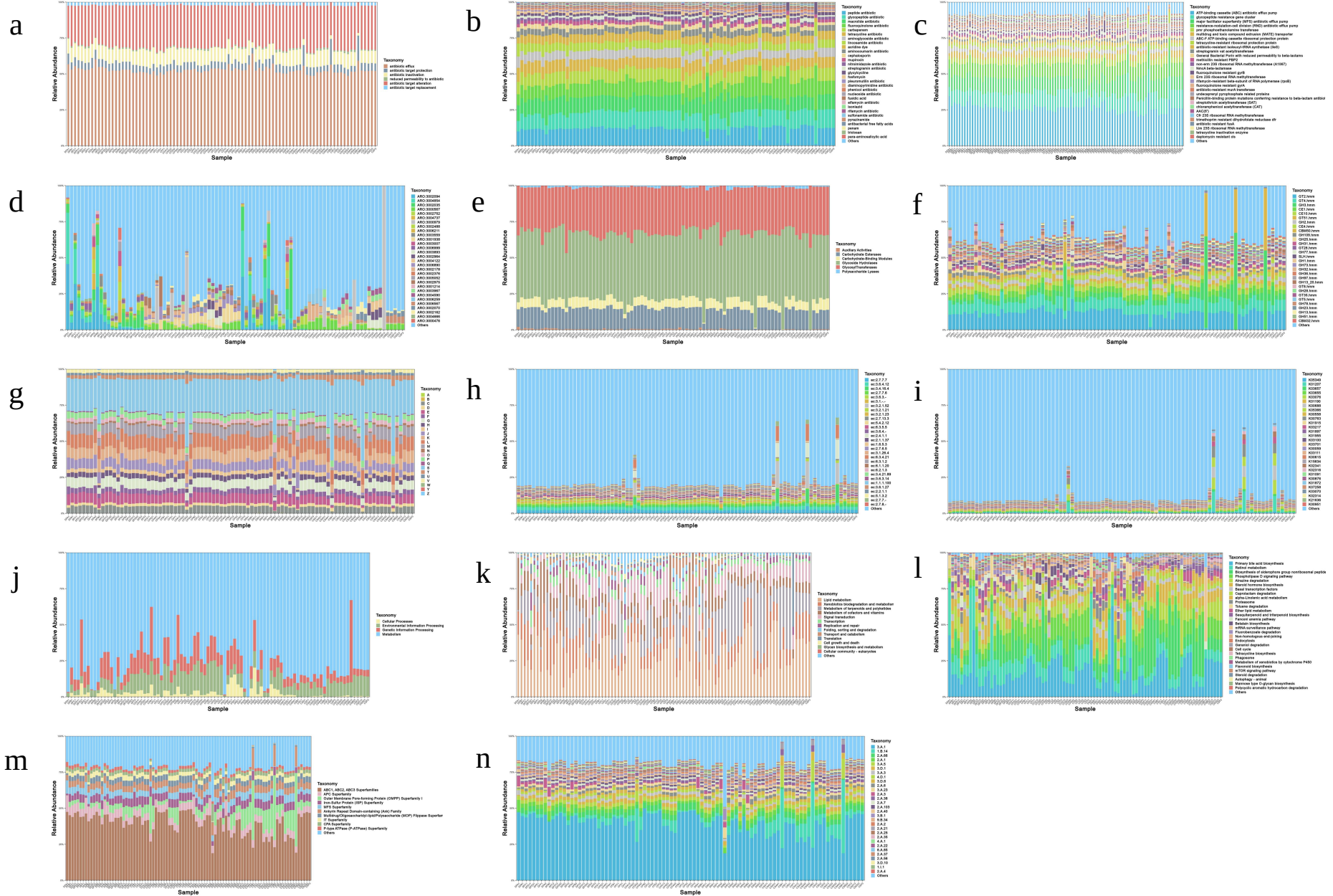


Fig. S4 Functional composition of different samples at different database and level (CARD_I1, CARD_I2, CARD_I3, CARD_I4, CAZy_I1, CAZy_I2, COG_I2, Pathway.EC, Pathway.KO, Pathway.I1, Pathway.I2, Pathway.I3, TCDB.I1, TCDB.I2). The horizontal axis is the name of 91 samples, and the vertical axis is the relatively abundant of functional annotation.

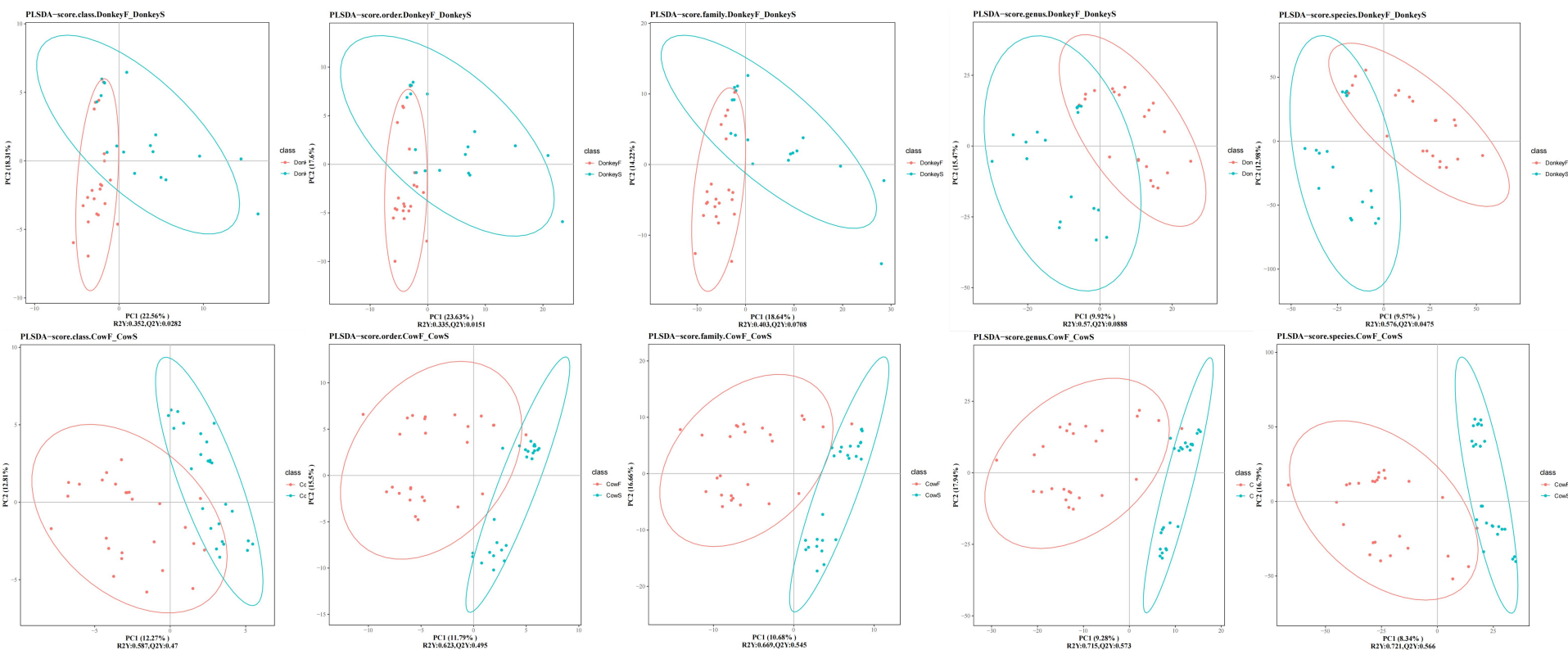


Fig. S5 PL-SDA results for different sampling methods. They were calculated based on donkey (top) and cow (bottom) samples at the different levels (class, order, family, genus and species) in order.

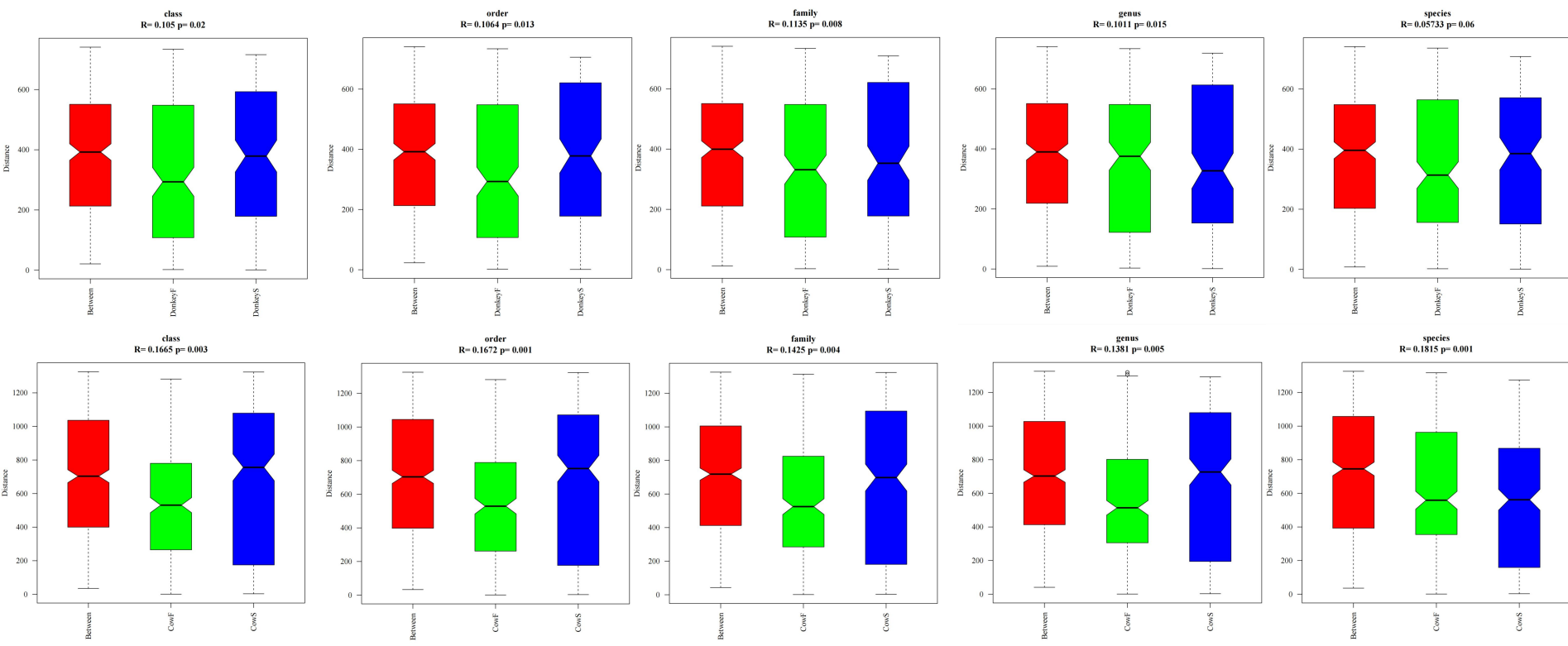


Fig. S6 ANOSIM for different sampling method. They were calculated based on donkey (top) and cow (bottom) samples at the different levels (class, order, family, genus and species) in order.

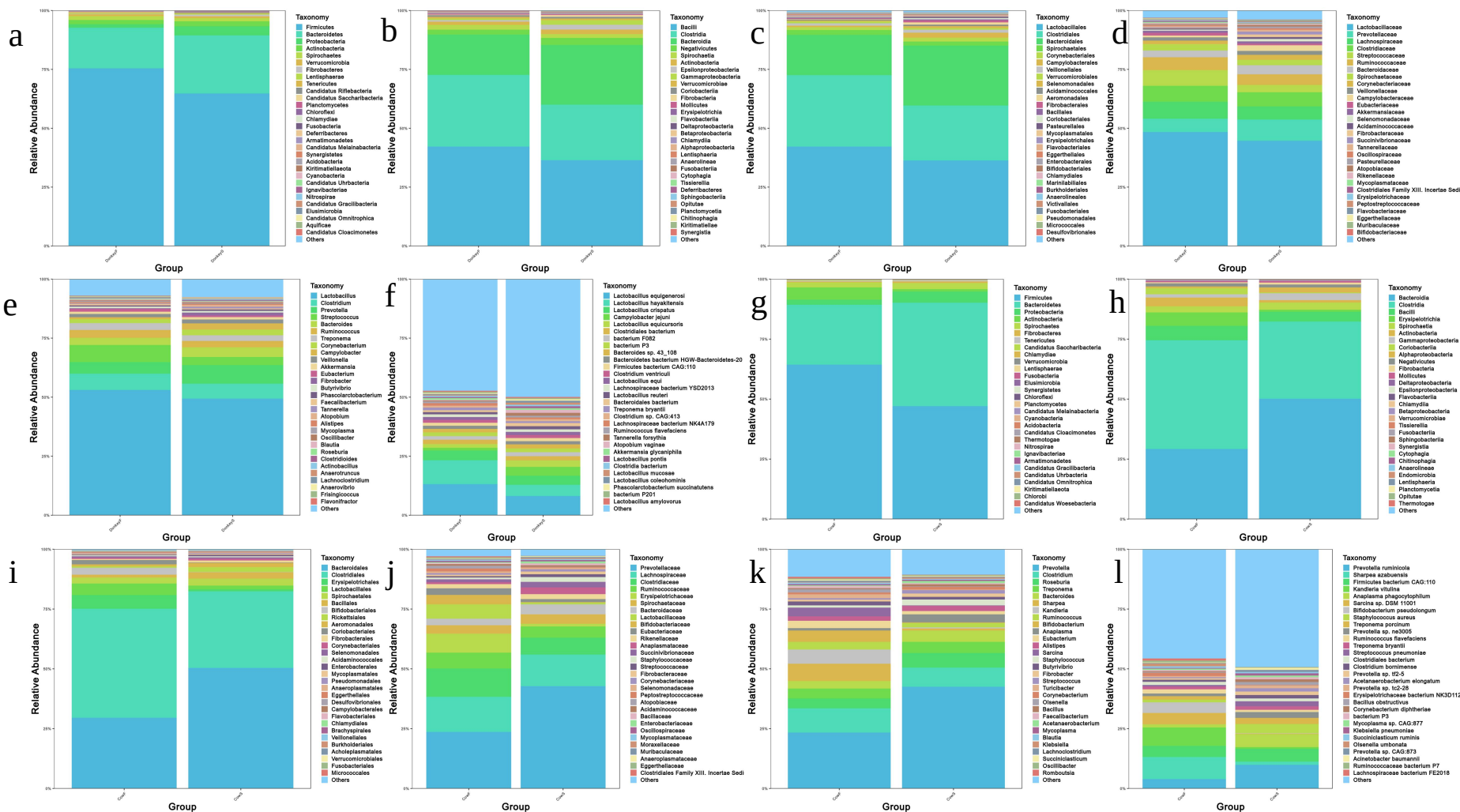


Fig. S7 Bacterial composition for different sampling method. The results were listed at the different levels(class, order, family, genus and species) of donkey (a-f) and cow (g~l) . The horizontal axis is the group name, and the vertical axis is the relatively abundant of microbes.

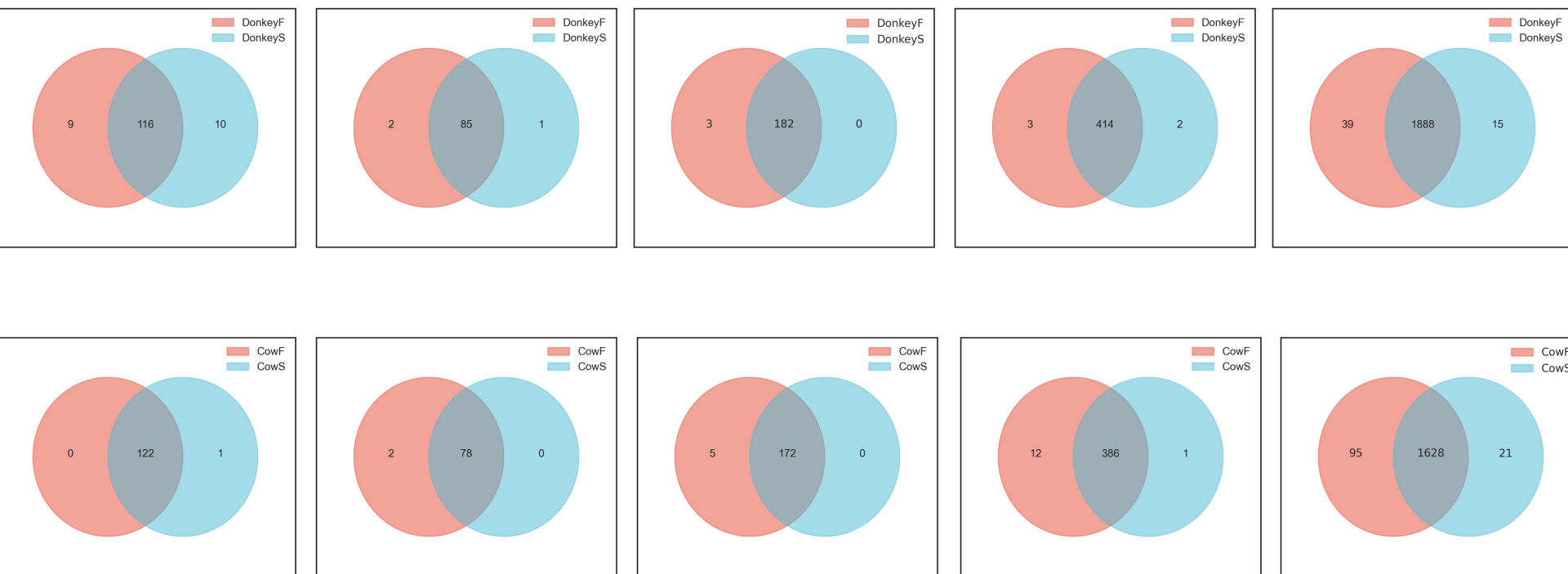


Fig. S8 Venn diagram for different sampling method. The number of microorganisms at each taxonomic level (class, order, family, genus and species) shared by different method for donkey (top) and cow (bottom).

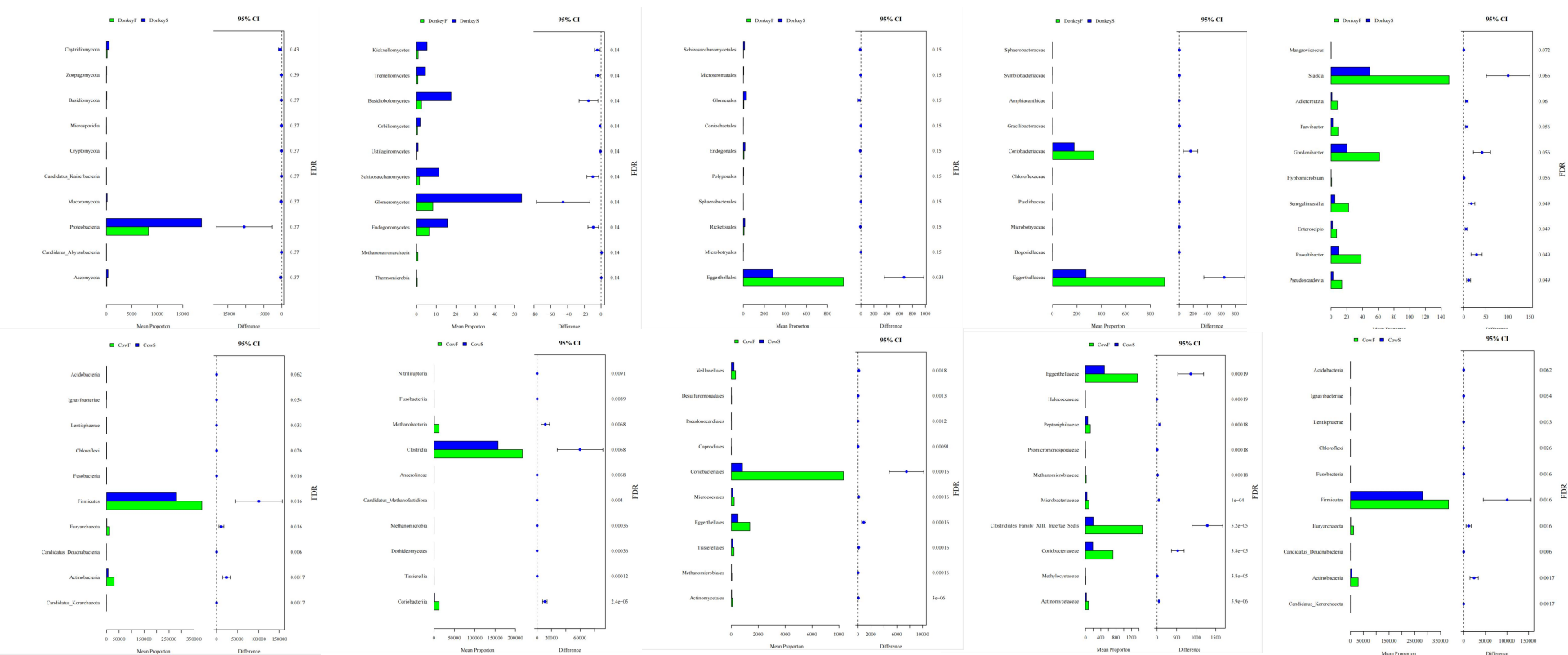


Fig. S9 STAMP analysis for different sampling method. They were listed at the different level (phylum, class, order, family and genus) for donkey (top) and cow (bottom).

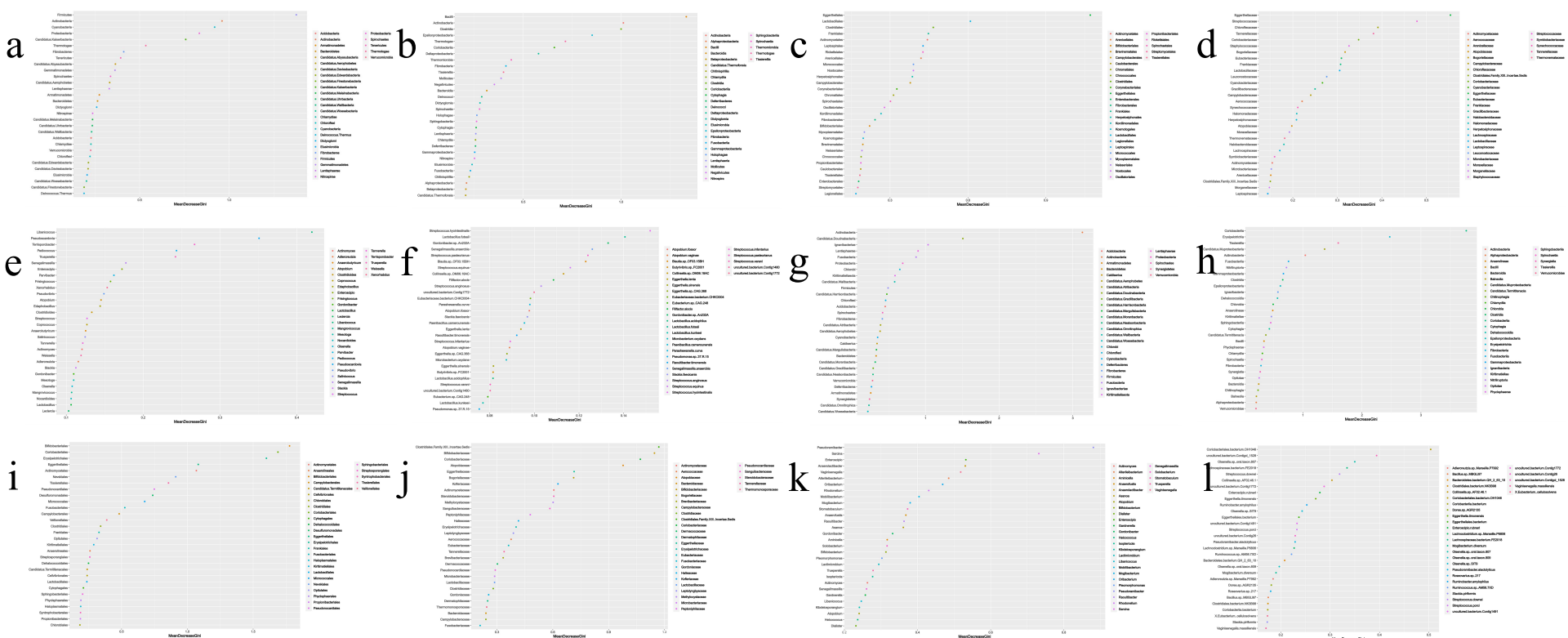
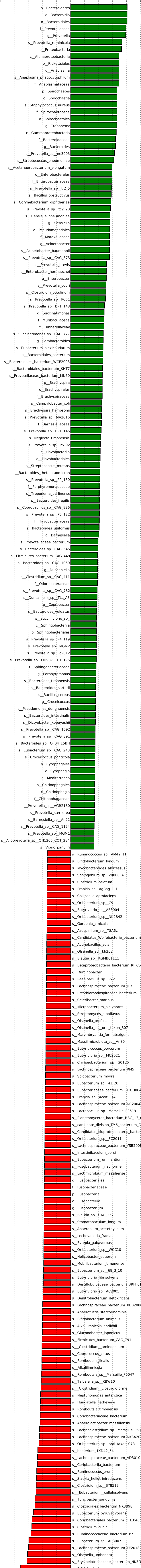


Fig. S10 Microbes importance dot-plot for different sampling method. The result were listed at the different level(phylum, class, order, family, genus and species) for donkey(a~f) and cow(g~l). The horizontal axis is the measure of importance, and the vertical axis is the microbes name in order of importance.



-6.0 -4.8 -3.6 -2.4 -1.2 0.0 1.2 2.4 3.6 4.8 6.0

LDA SCORE (log 10)

Fig. S11 LEfSe analysis of different sampling method in the cow.

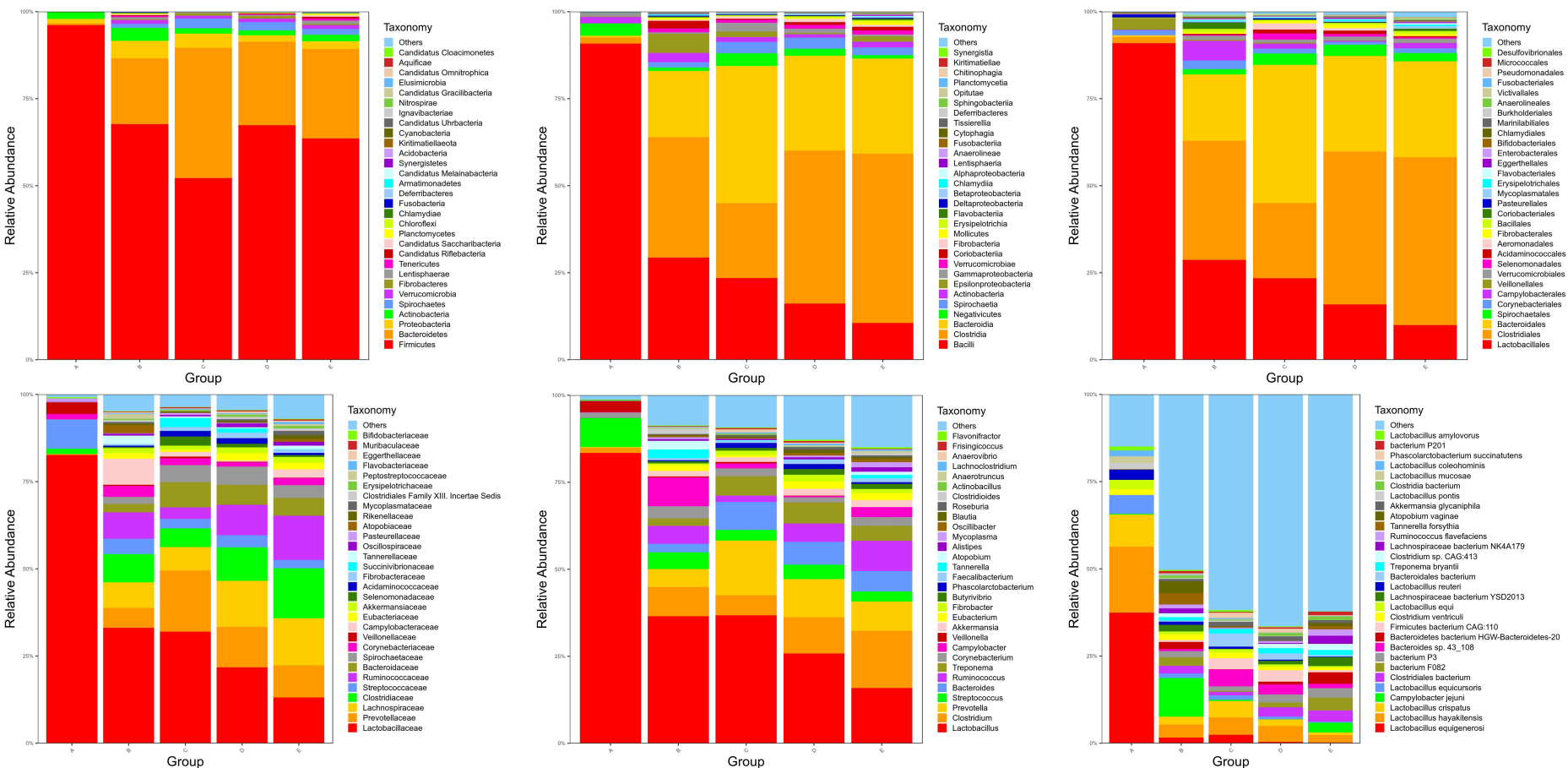


Fig. S12 Bacterial composition of donkey in digestive tract segments at the different levels.
The horizontal axis is the digestive tract segments, and the vertical axis is the relatively abundant of microbes.

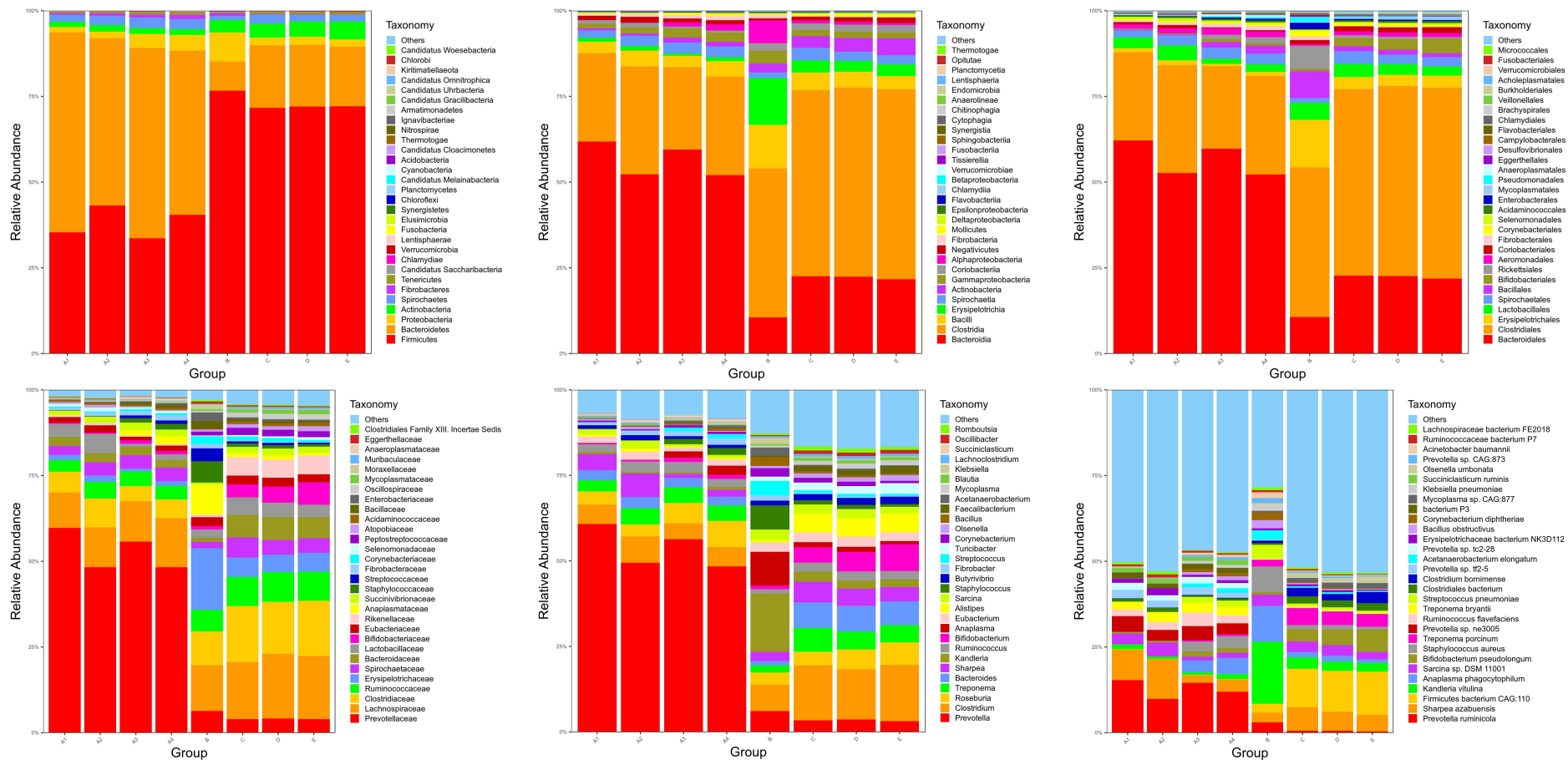
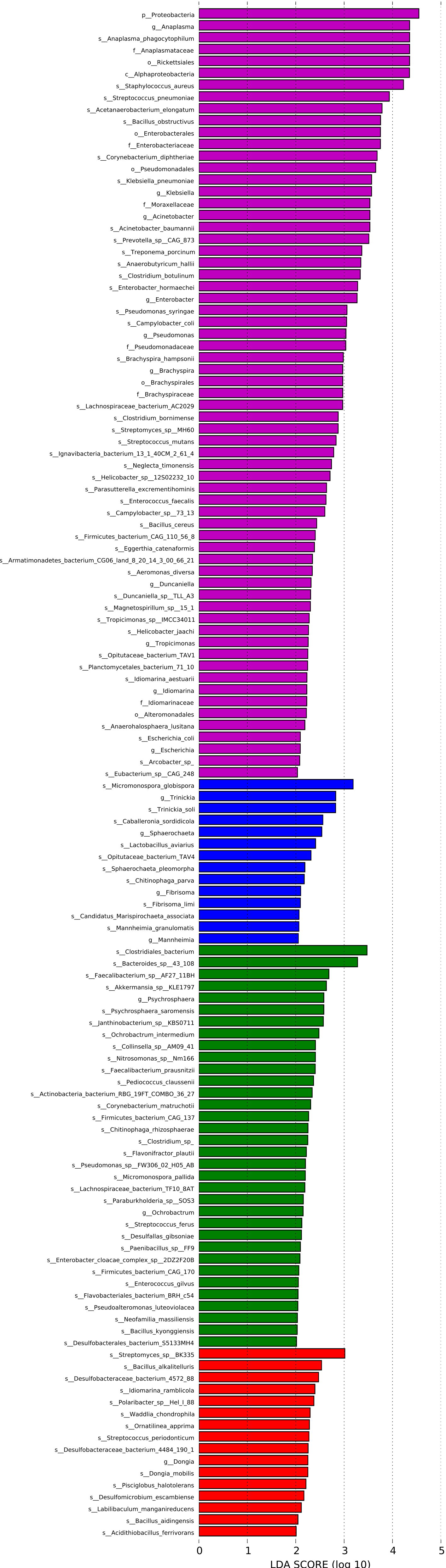


Fig. S13 Bacterial composition of cow in digestive tract segments at the different levels. The horizontal axis is the digestive tract segments, and the vertical axis is the relatively abundant of microbes.

A1 A2 A3 A4



LDA SCORE (log 10)

Fig. S14 LEfSe analysis of different stomachs in the cow.

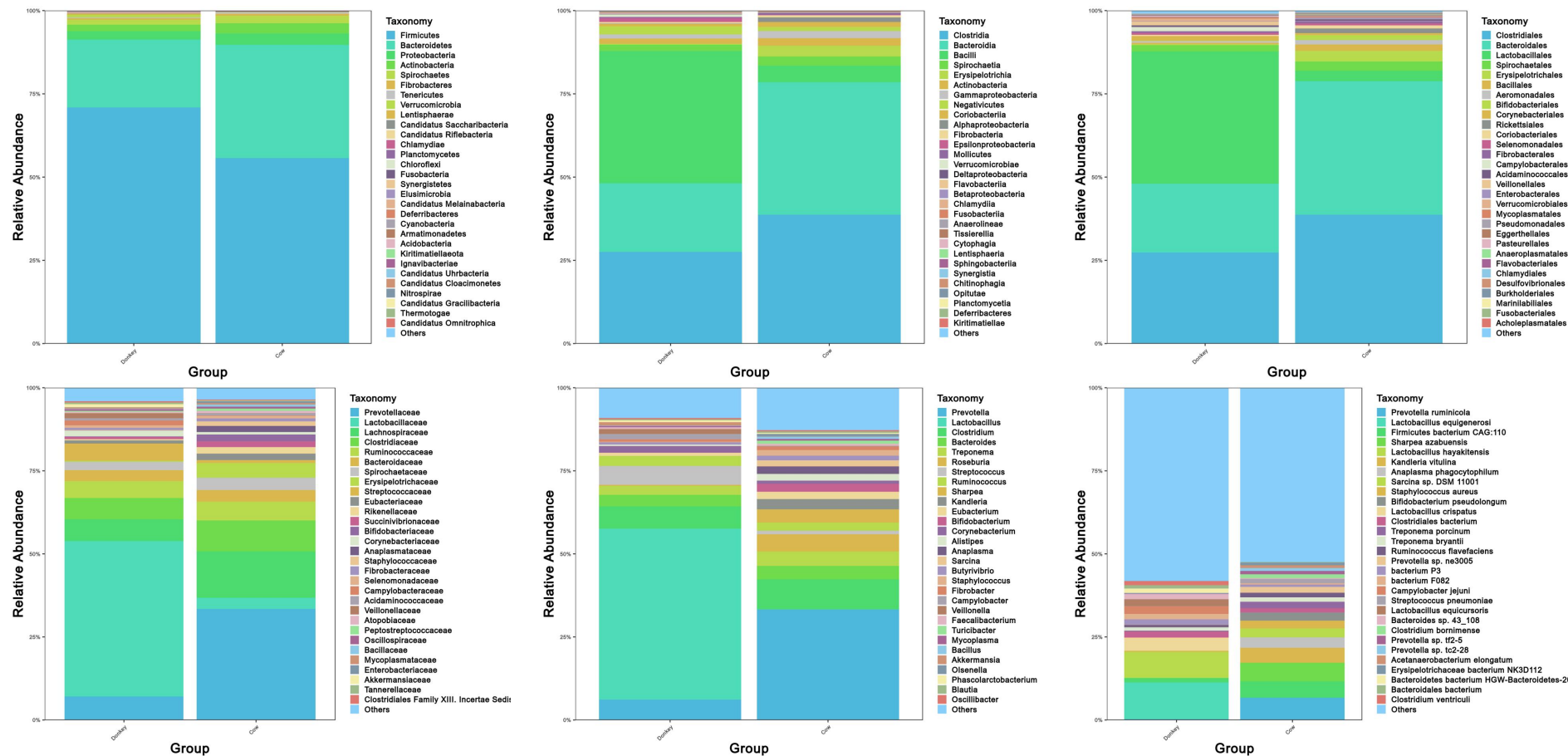


Fig. S15 Bacterial composition of cow and donkey at the different levels. The horizontal axis is the animal name, and the vertical axis is the relatively abundant of microbes.

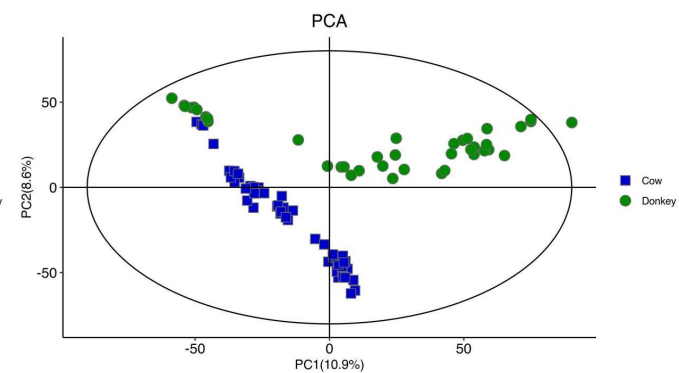
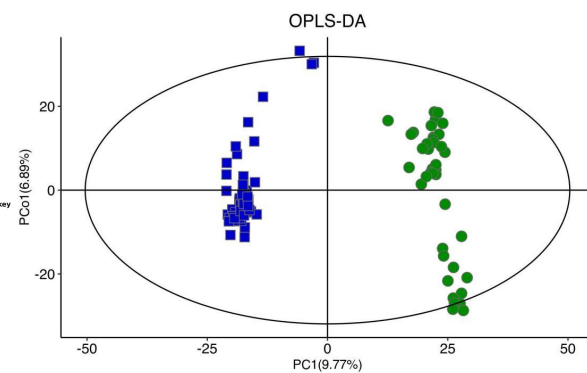
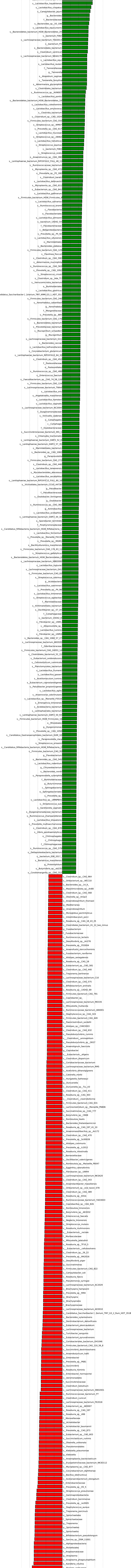


Fig. S16 NMDS, OPLS-DA and PCA analysis



-6.0 -4.8 -3.6 -2.4 -1.2 0.0 1.2 2.4 3.6 4.8 6.0

LDA SCORE (log 10)

Fig. S17 LEfSe analysis of different animals.

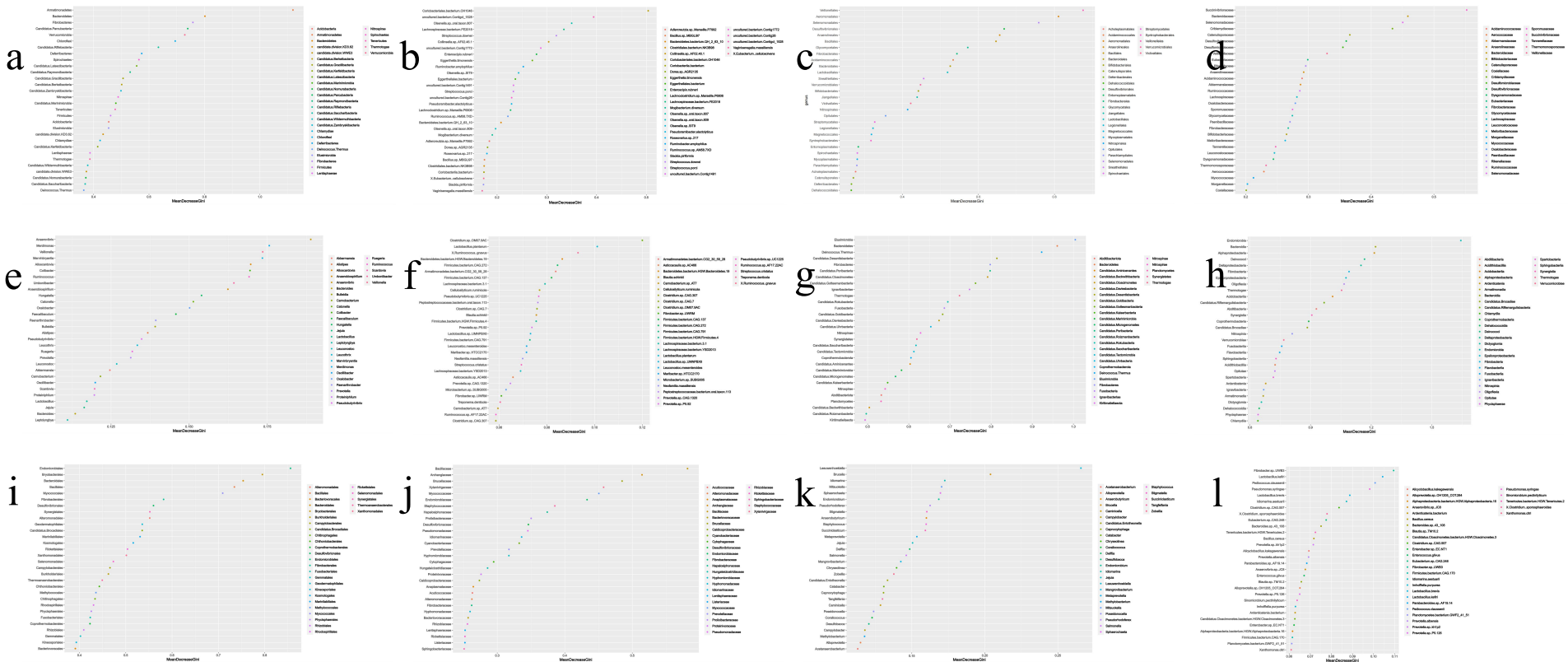


Fig. S18 Microbes importance dot-plot for donkey and cow. The result were listed at the different level(phylum, class, order, family, genus and species) in order. The horizontal axis is the measure of importance, and the vertical axis is the microbes name in order of importance.

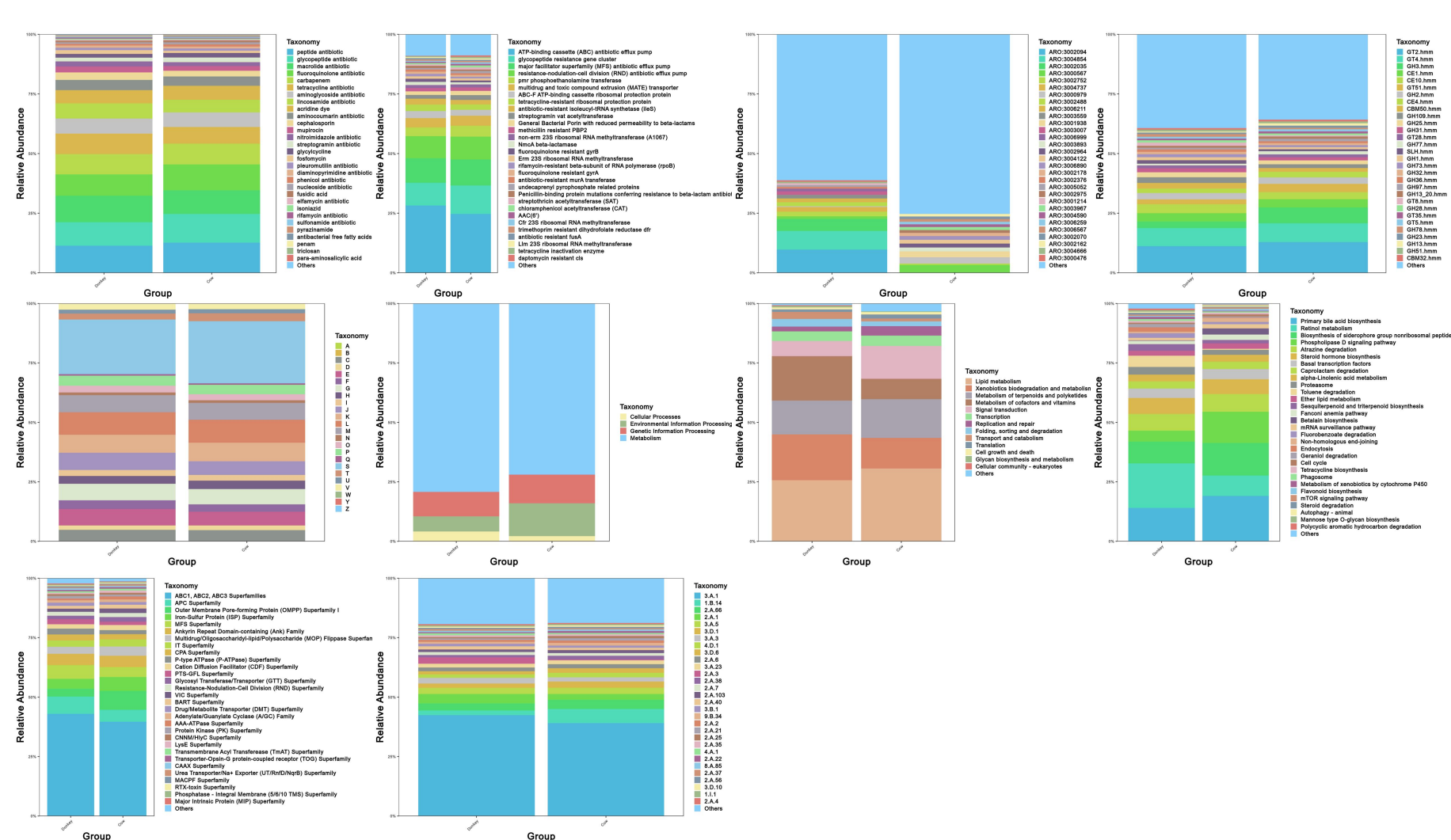


Fig. S19 Functional composition of different animals at different database(CARD_I2, CARD_I3, CARD_I4, CAZy_I2, COG_I2, Pathway.I1, Pathway.I2, Pathway.I3, TCDB.I1, TCDB.I2). The horizontal axis is the digestive tract segments, and the vertical axis is the relatively abundant of functional annotation.

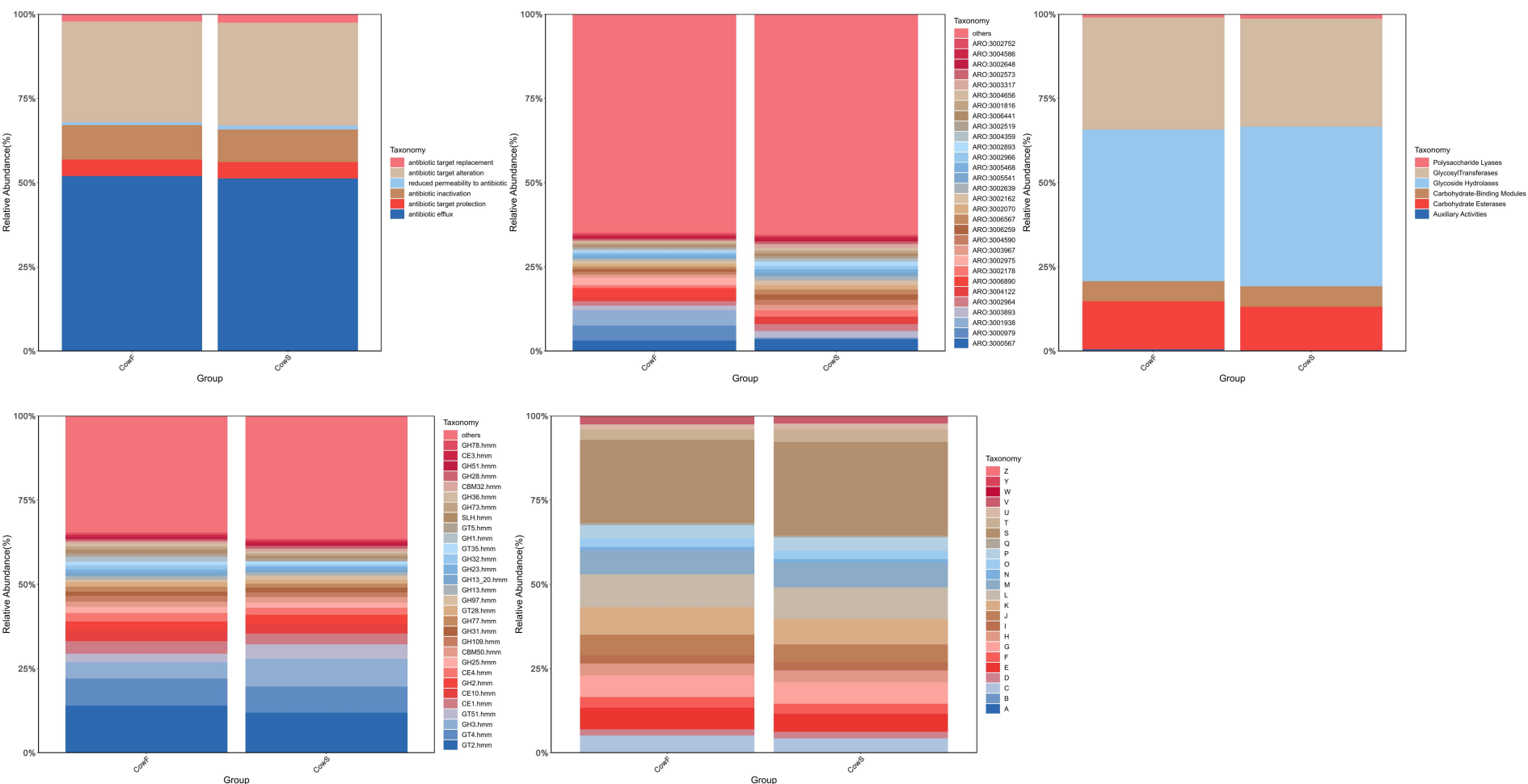


Fig. S20 Functional composition of different method of cow at different database(CARD_I1, CARD_I4, CAZy_I1, CAZy_I2, COG). The horizontal axis is the method, and the vertical axis is the relatively abundant of functional annotation.

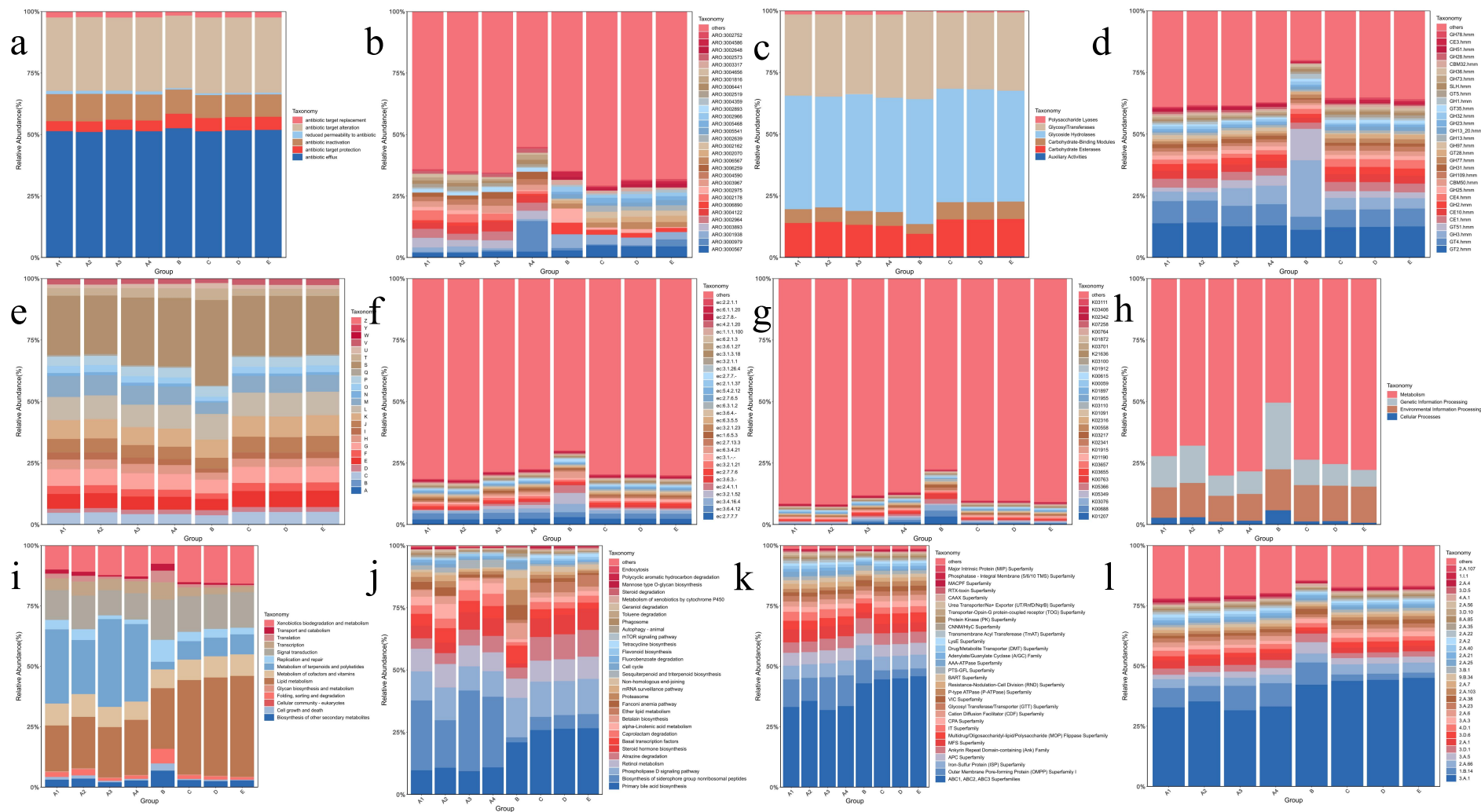


Fig. S21 Functional composition of different digestive tract segments of cow at different database (CARD_I1, CARD_I4, CAZy_I1, CAZy_I2, COG, Pathway.EC, Pathway.KO, Pathway.I1, Pathway.I2, Pathway.I3, TCDB.I1, TCDB.I2). The horizontal axis is the digestive tract segments, and the vertical axis is the relatively abundant of functional annotation.

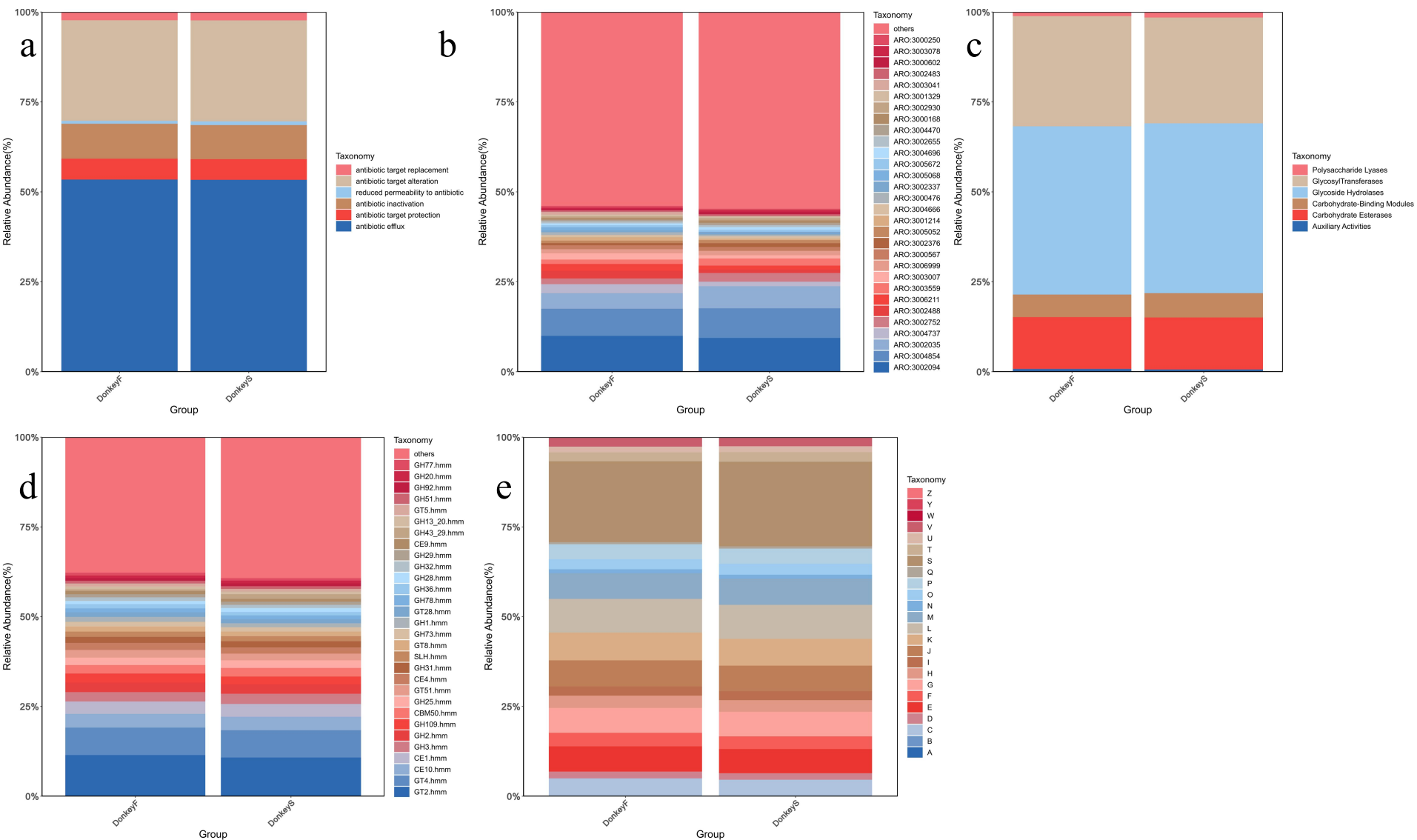


Fig. S22 Functional composition of different method of donkey at different database(CARD_I1, CARD_I4, CAZy_I1, CAZy_I2, COG). The horizontal axis is the method, and the vertical axis is the relatively abundant of functional annotation.

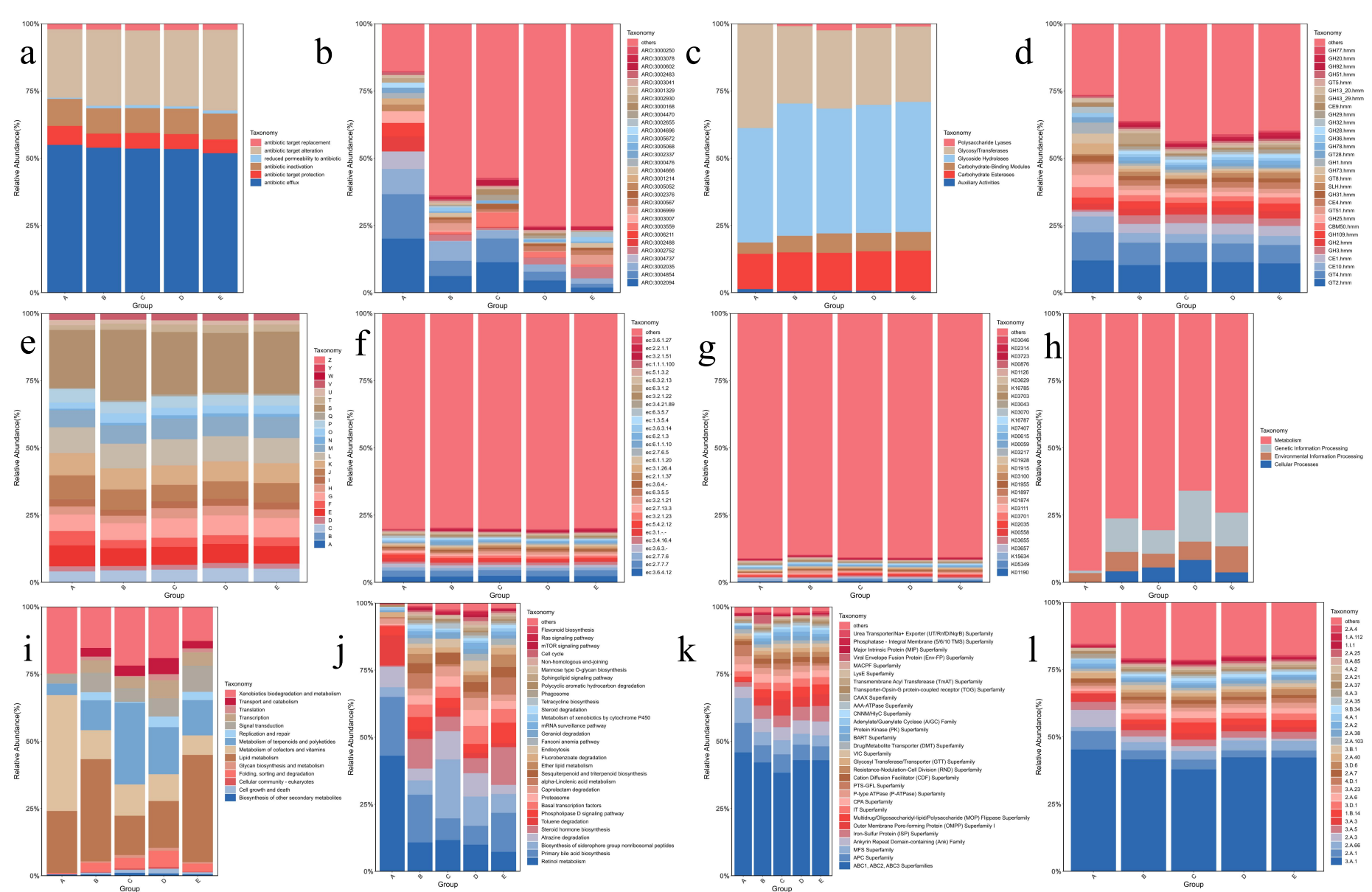


Fig. S23 Functional composition of different digestive tract segments of donkey at different database (CARD_I1, CARD_I4, CAZy_I1, CAZy_I2, COG, Pathway.EC, Pathway.KO, Pathway.I1, Pathway.I2, Pathway.I3, TCDB.I1, TCDB.I2). The horizontal axis is the digestive tract segments, and the vertical axis is the relatively abundant of functional annotation.

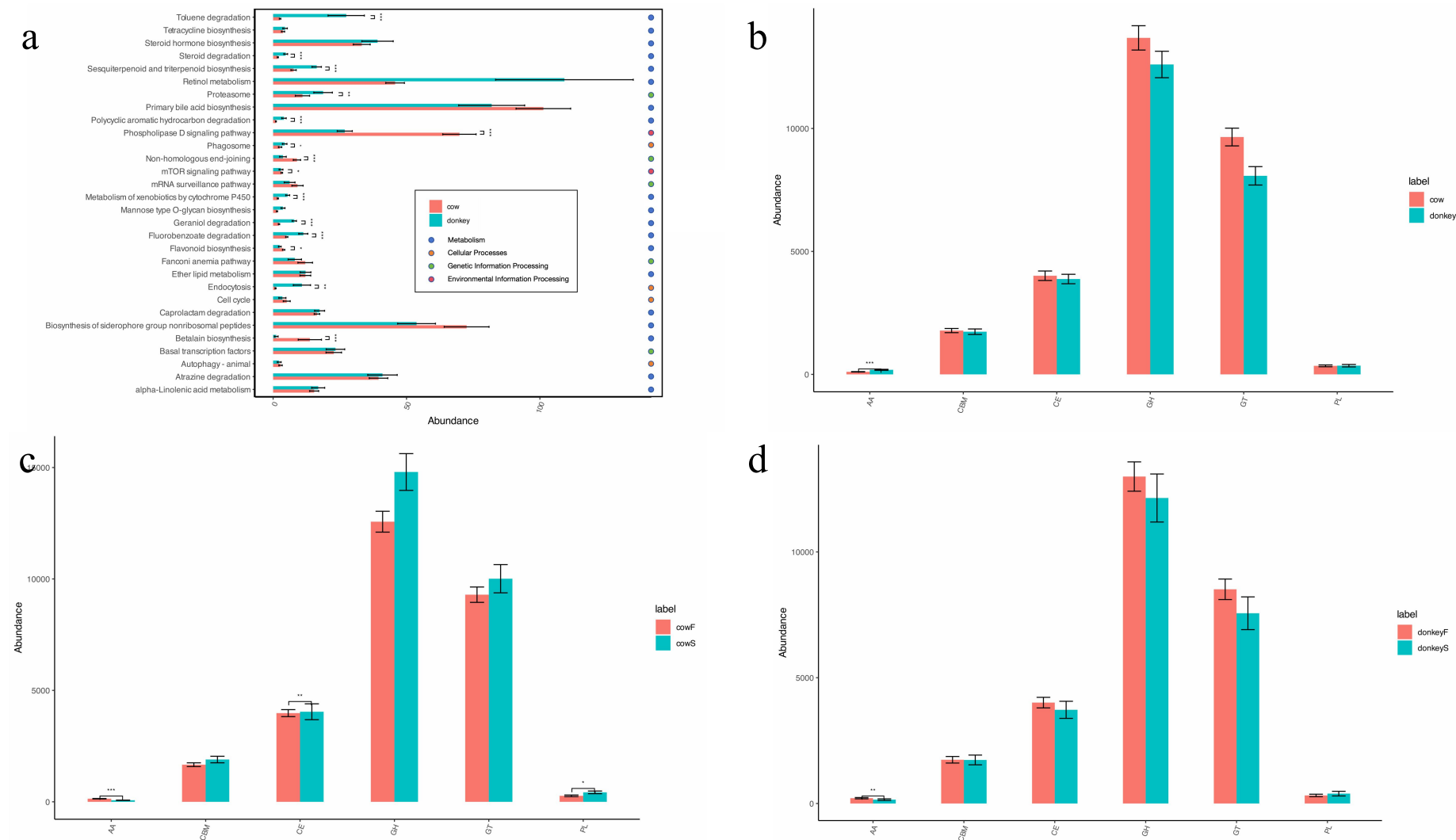


Fig. S24 The comparison of the main annotation function. (a) The comparison of the main KEGG function between cow and donkey. The abundance comparison of GH (glycoside hydrolase), GT (glycosyl transferase), CBM (carbohydrate-binding module), CE (carbohydrate esterases), PL (polysaccharide lyases), and AA (auxiliary activities) of different animals (b) and sampling methods in cow (c) and donkey (d).

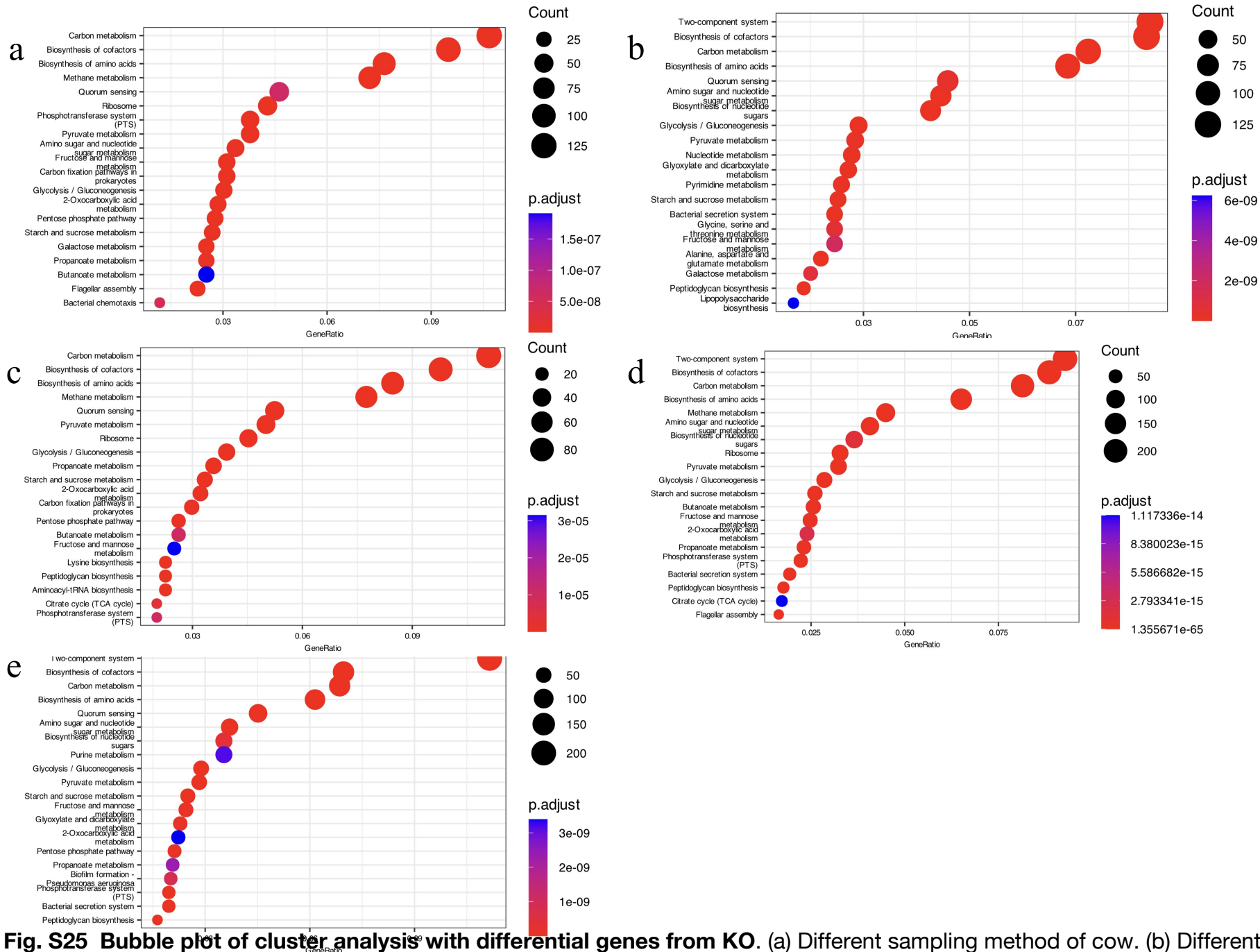


Fig. S25 Bubble plot of cluster analysis with differential genes from KO. (a) Different sampling method of cow. (b) Different digestive tract segments of cow. (c) Different sampling method of donkey. (d) Different digestive tract segments of donkey. (e) Different animals

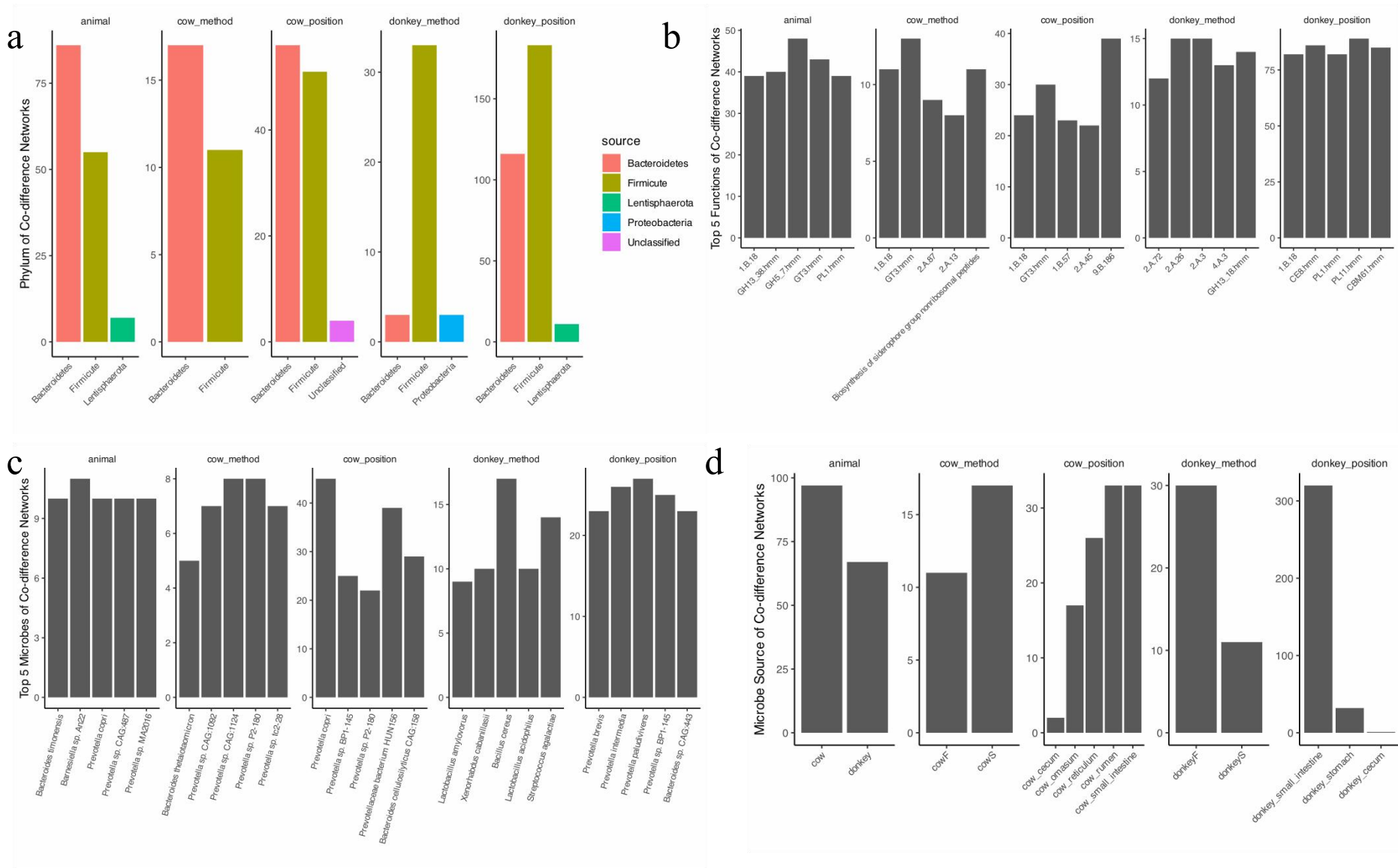


Fig. S26 The information of co-difference networks. (a) The main phylum of different groups. The top 5 abundant functions (b) and speices (c) of different groups. (d) The different microbes' sources of different groups in the co-difference networks.

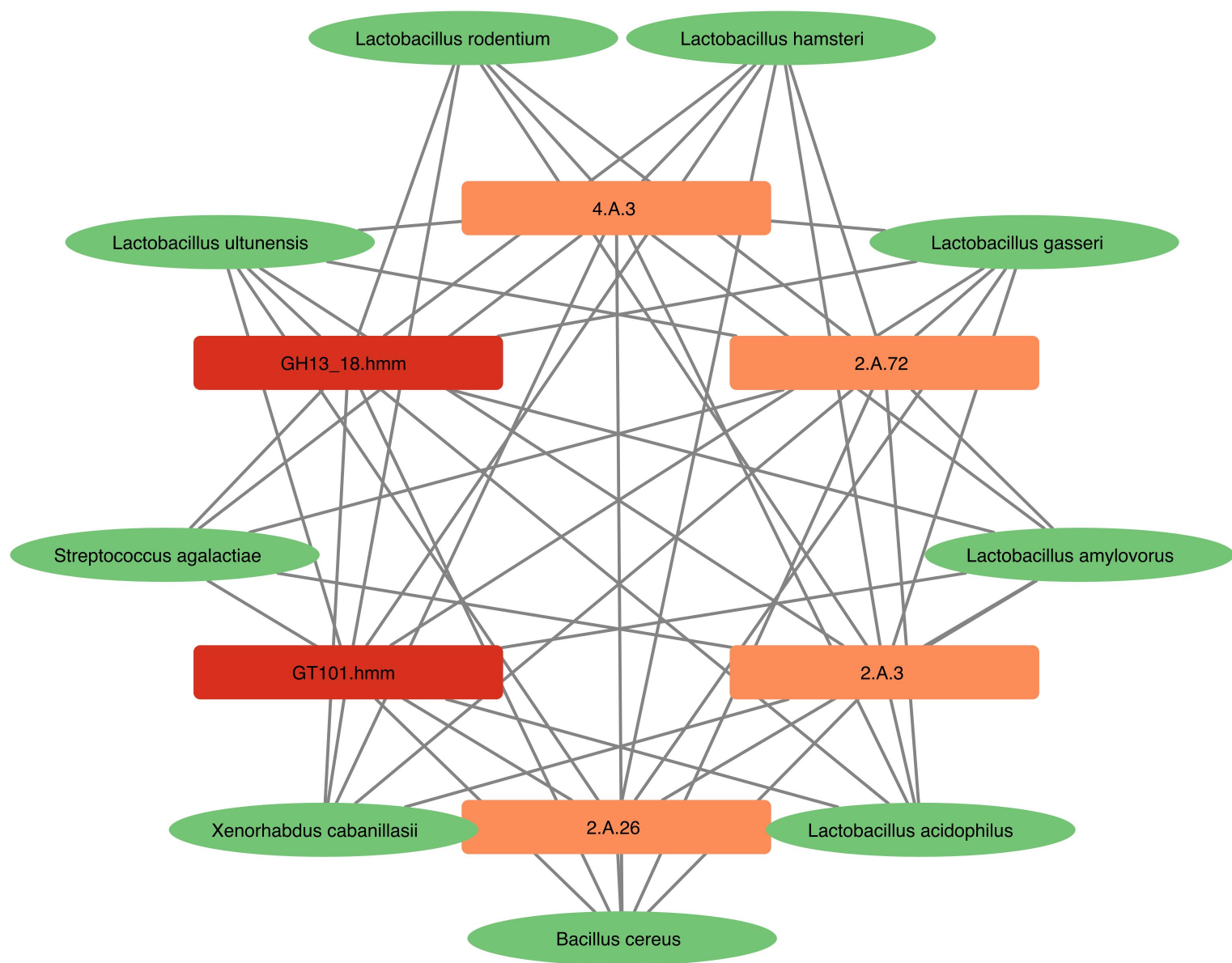


Fig. S29 Network of functions and microbes at the species level grouped by sampling method of donkey.

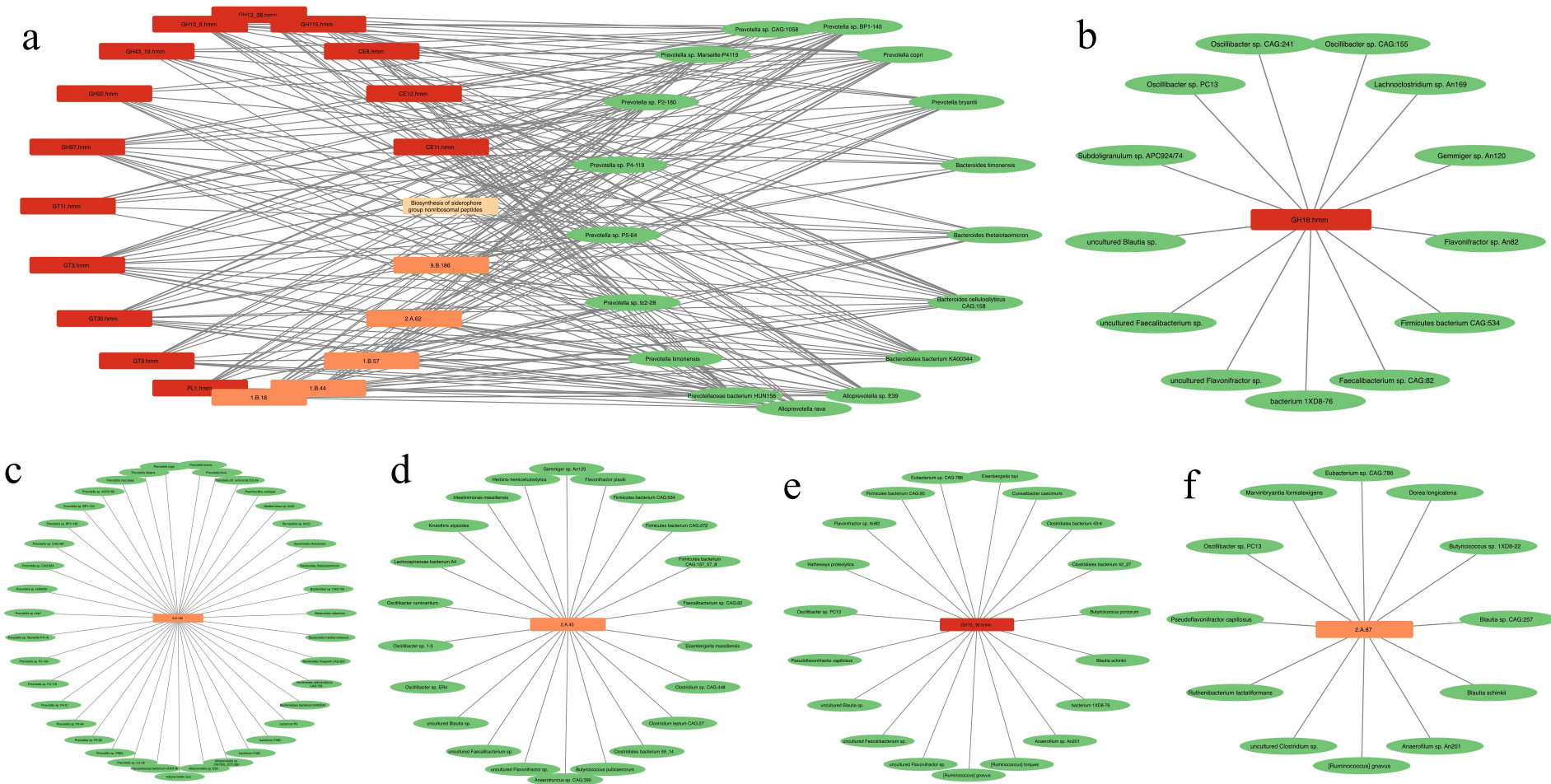


Fig. S30 Network of functions and microbes at the species level grouped by digestive tract segments of cow.

