



Spatio-temporal characteristics of the gastrointestinal resistome in a cow-to-calf Model and its environmental dissemination in a dairy production system

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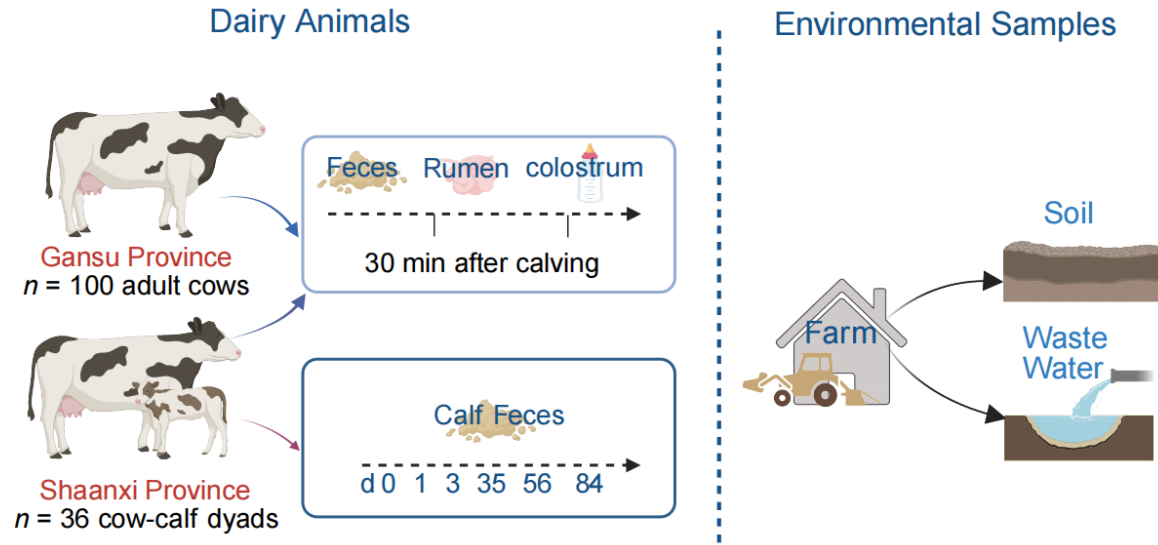
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Introduction



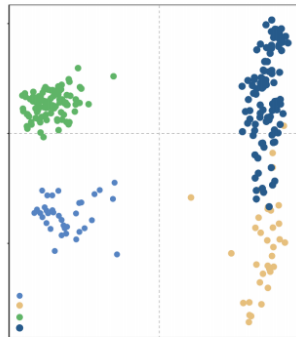
16S rRNA gene sequencing + Metagenome+Metatranscriptome

$n = 529$ sampls

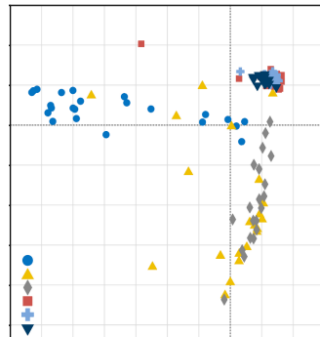
$n = 325$ sampls

$n = 62$ sampls

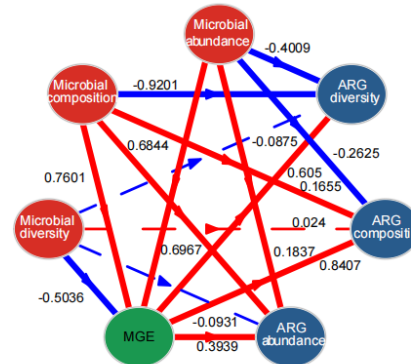
Gut ARG profiles of cows distinguished by regions



Fecal ARG variation in calves driven by age and diet



MGEs are an medium between ecological niches interactions

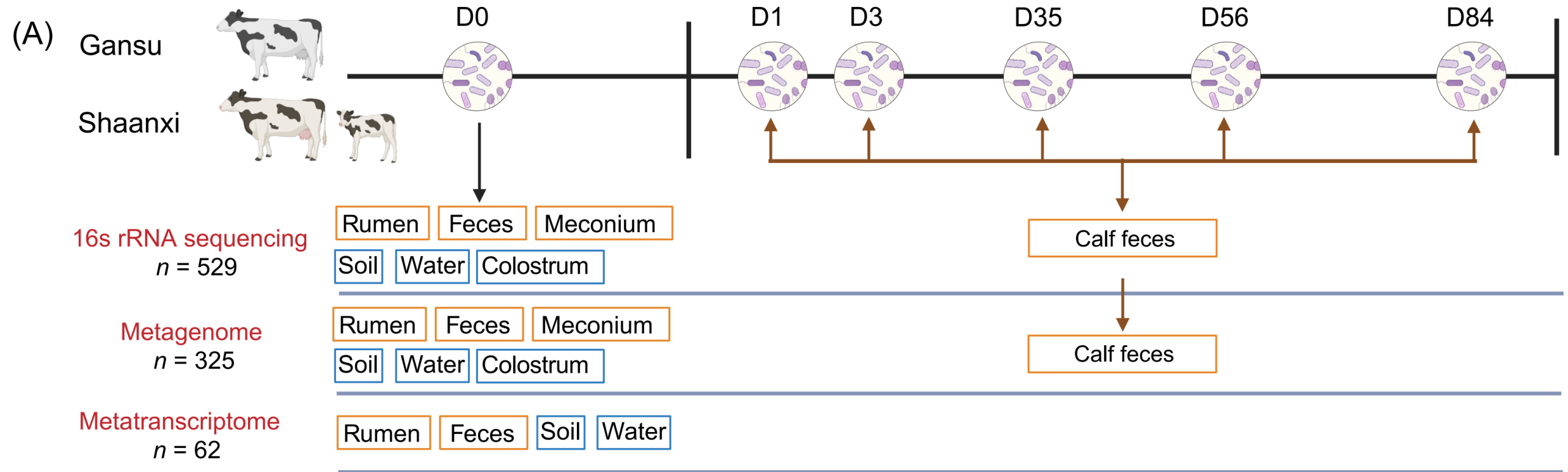


Multidrug resistance holds paramount significance recognized by both metagenome/transcriptome level





Experimental Design



Sample types: (Gansu, Shaanxi) 136 cows, 36 calves, and environmental samples (soil, water).

Omics technologies: 16S rRNA sequencing, metagenomics, and metatranscriptomics.

Distribution Characteristics of Microbiota

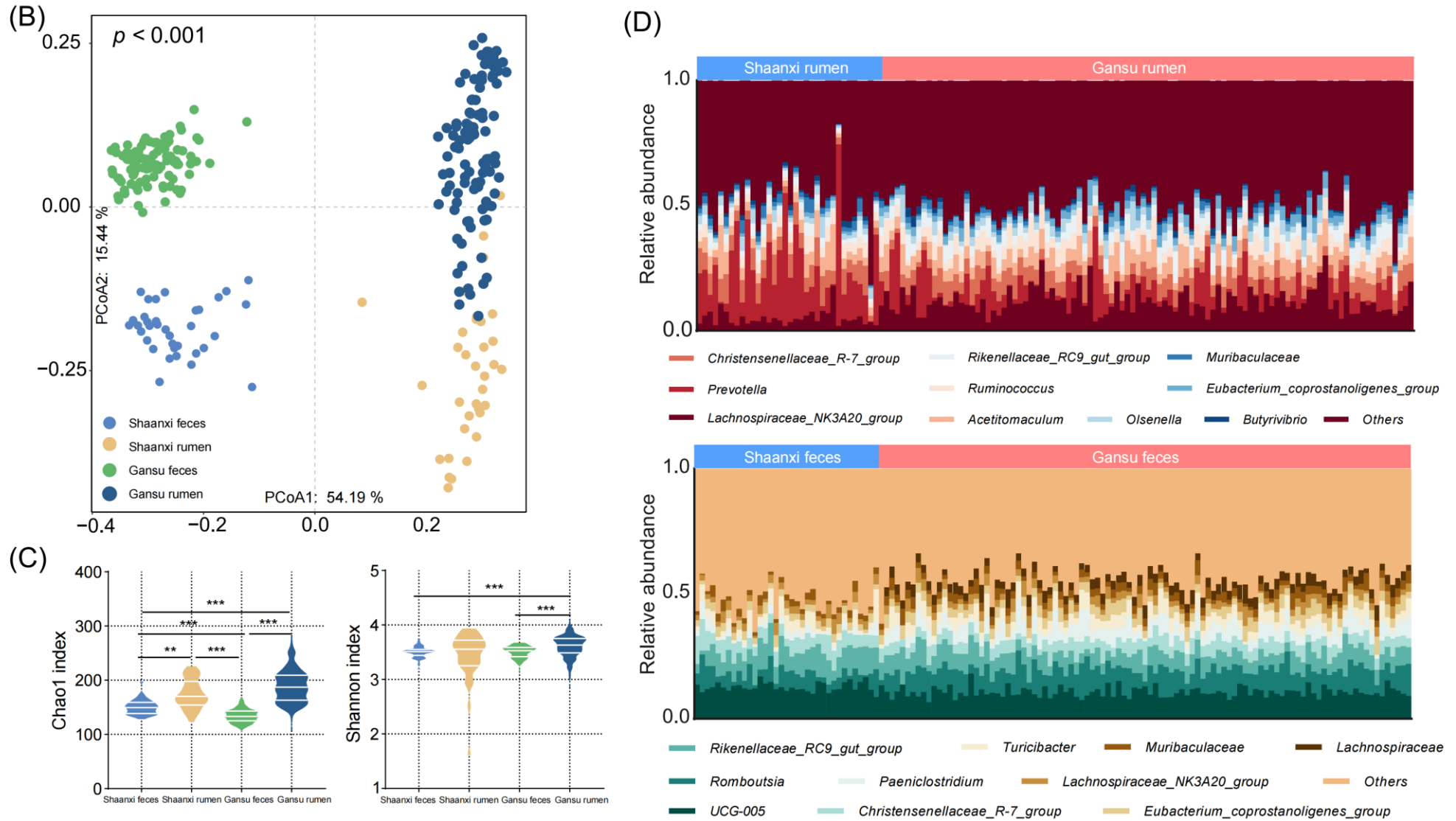


Figure 1 The rumen and hindgut microbiota of cows in Gansu and Shaanxi province.

(B) PCoA of microbiota in the rumen and feces of cows from Gansu and Shaanxi. (C) The α diversity of microbiota in the rumen and feces of cows from Gansu and Shaanxi. (D) The microbial composition of rumen and feces.



Distribution Characteristics of Resistome

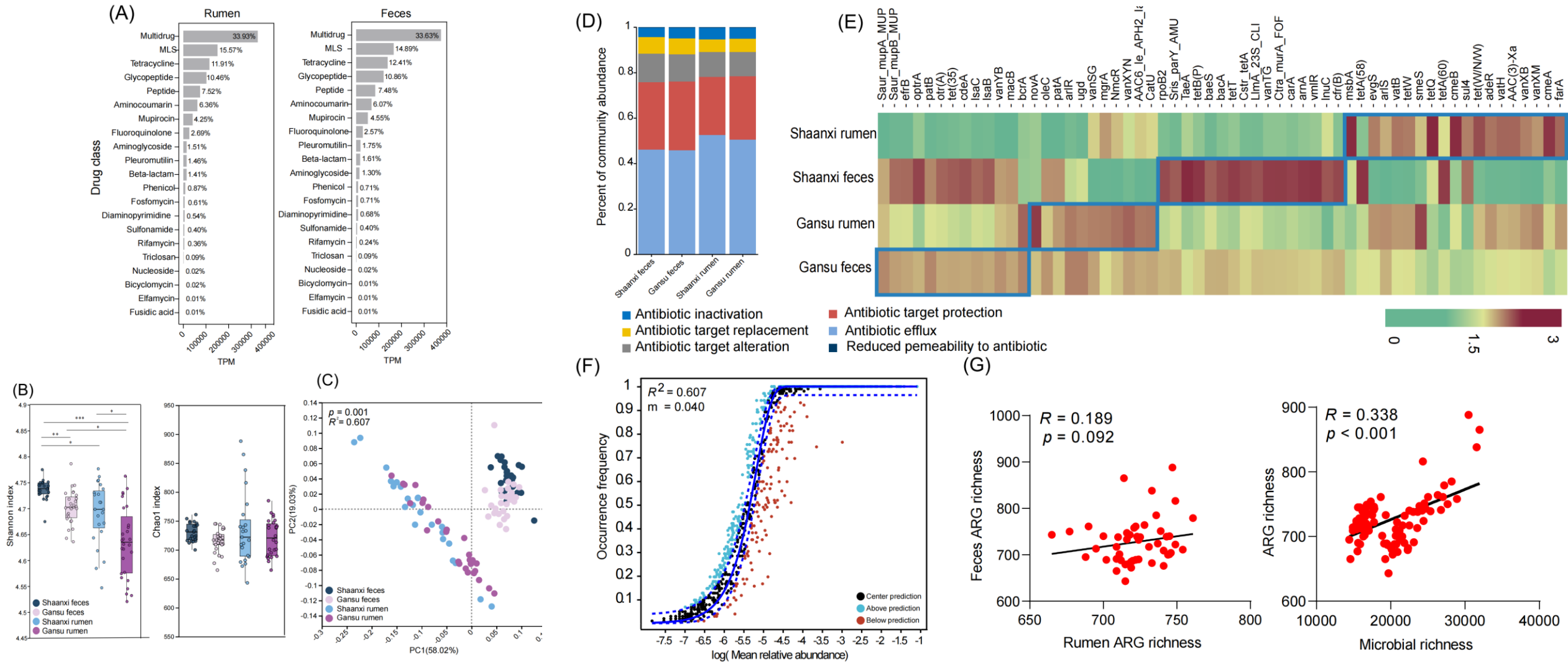


Figure 2 The gut resistome profile of cows in Gansu and Shaanxi.

(A) The total abundance of antibiotics resistance genes (ARG) resistance. (B) The α diversity of resistome. (C) PCoA. (D) The composition of resistance mechanisms. (E) The identification of signature ARGs in the four groups. (F) Fit of neutral model determined the contribution of deterministic and stochastic processes on ARGs. (G) The correlation analysis.

Resistome Clustering & Host Bacteria Interaction

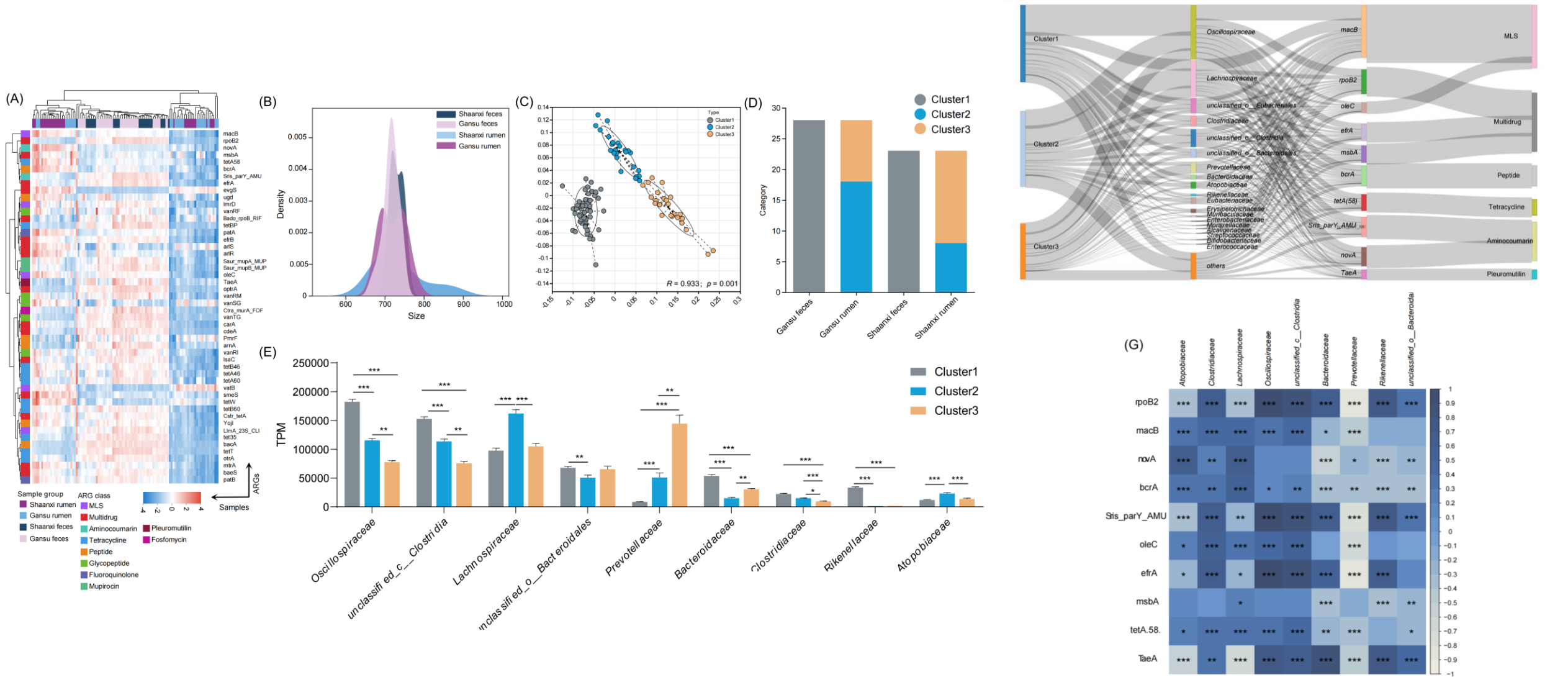


Figure 3 Characteristics of three resistome clusters associated with feces and rumen.

(A) The first 50 abundance values of ARG abundances. (B) Density plot of ARG richness. (C) ARGs were clustered with PAM clustering. (D) The proportion of different clustering samples. (E) The significant difference of top 10 microbial families. (F) Sankey diagram connecting the predicted bacterial hosts.

(G) Spearman correlation analysis between ARGs and bacteria.

Calf Resistome Dynamics & Maternal Transmission

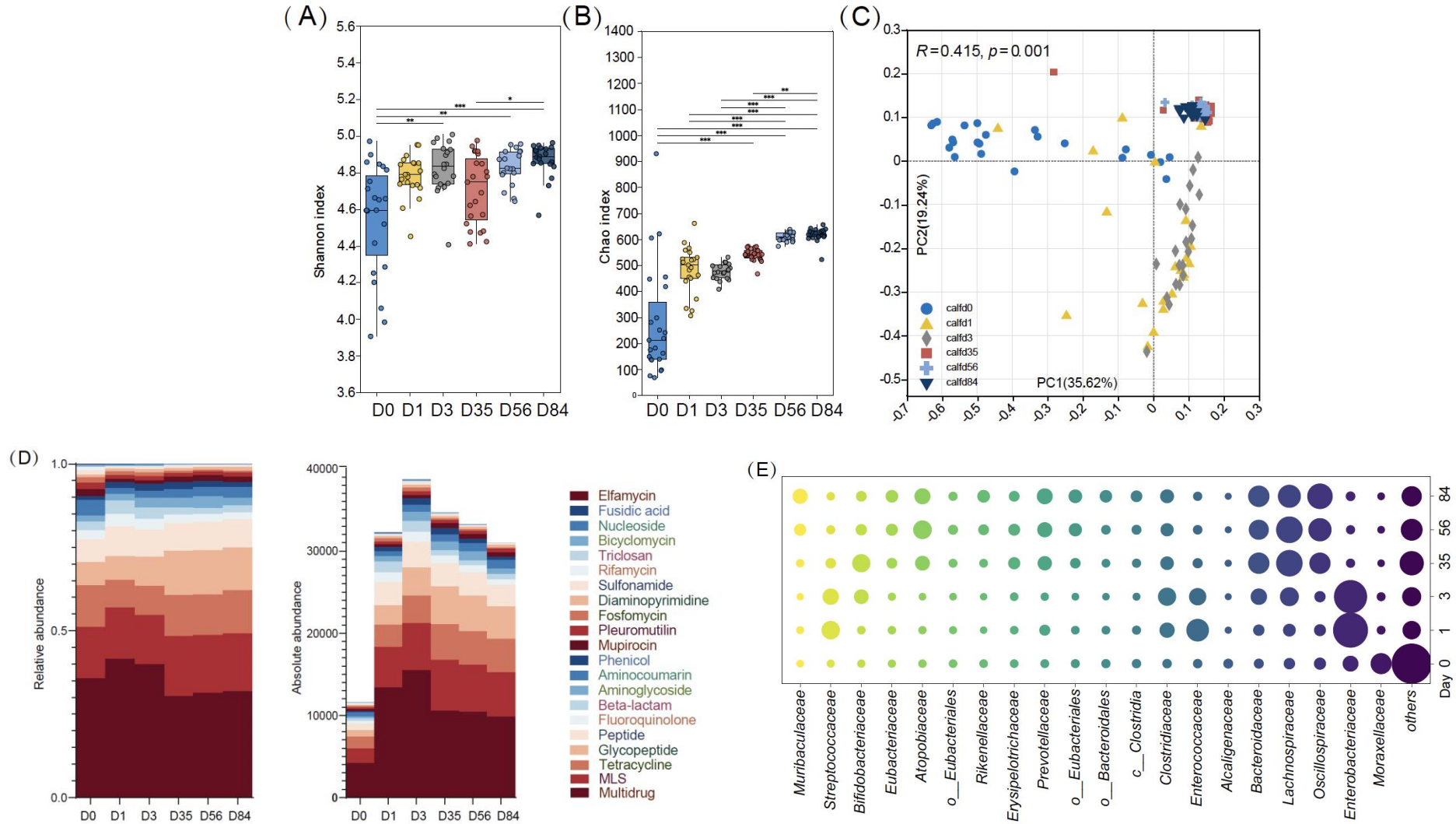


Figure 4 The temporal dynamics of fecal resistome in calves.

(A and B) The Chao1 index and Shannon index of fecal ARG in calves on day 0, 1, 3, 35, 56 and 84. (C) PCoA of resistome.

(D) The relative abundance of fecal ARGs. (E) The predicted bacterial hosts of fecal ARGs.

Calf Microbiome Function - Resistome Association

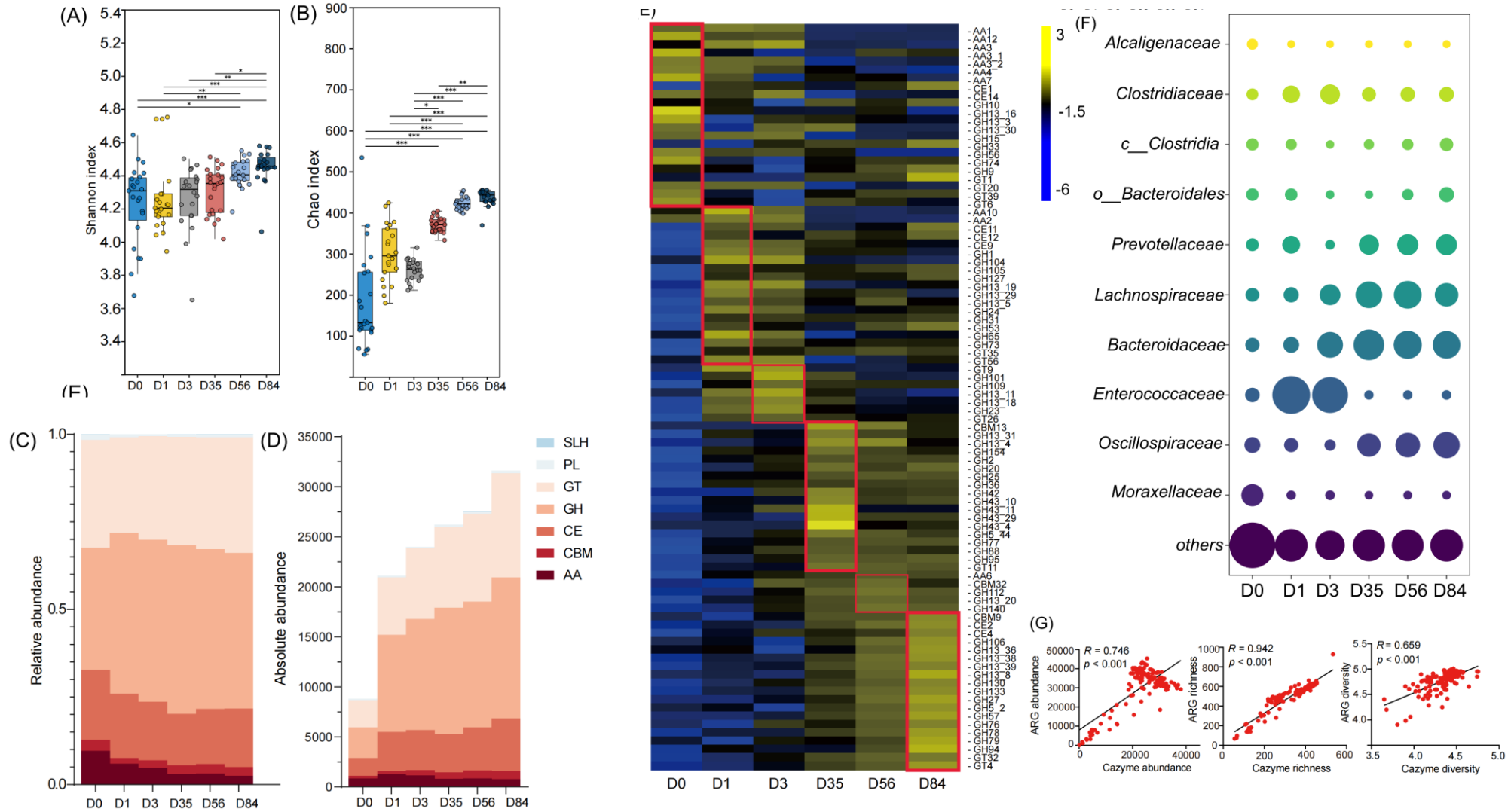


Figure 5 Functional capacity of gut microbiome in the calves.

(A and B) The Chao1 index and Shannon index of fecal carbohydrate-active enzymes (CAZyme) in calves on day 0, 1, 3, 35, 56 and 84.

(C) The relative abundance of fecal CAZymes. (D) The absolute abundance (TPM) of fecal CAZymes.

(E) The identification of signature CAZymes. (F) The predicted bacterial hosts of fecal CAZymes (G) The correlation analysis.

DNA vs. RNA Resistome Comparison

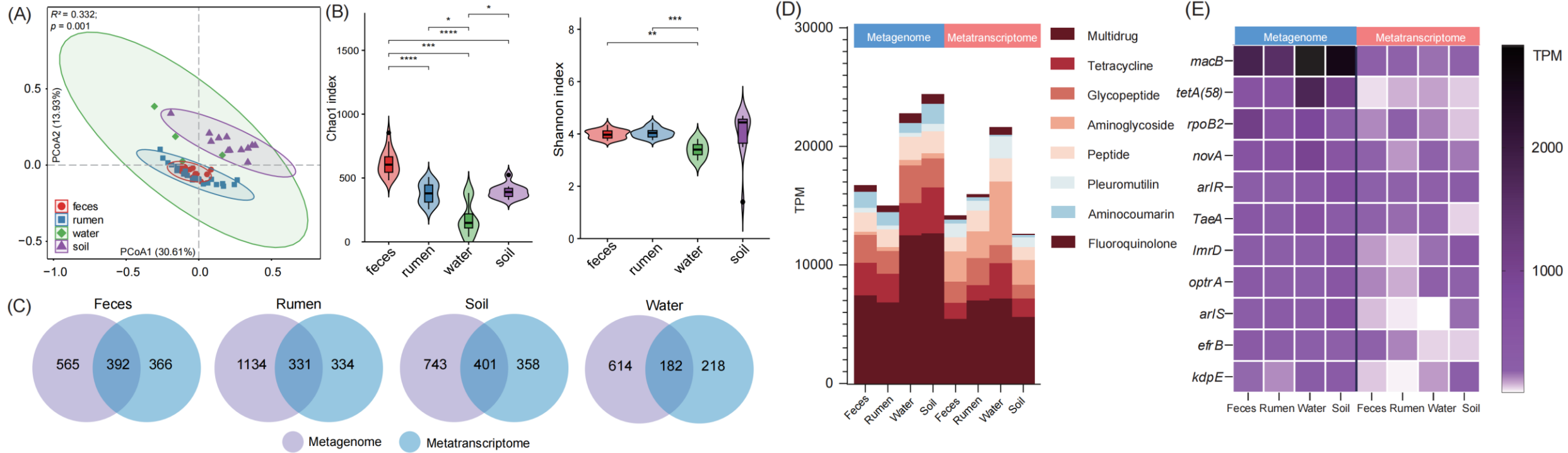


Figure 7 The comparison of ARG at DNA-based and RNA-based level.

- (A) PCoA of ARG transcriptional abundance in the groups of feces, rumen, soil and water.
 (B) The Chao1 index and Shannon index at the metatranscriptome level. (C) ARG identification between the metagenome and metatranscriptome level.
 (D) The comparison of antibiotic class of ARGs. (E) The comparison of ARG abundance.



Summary

1. We demonstrate for the first time that the dairy cow gastrointestinal resistome shows spatiotemporal variations with distinct geographical patterns and gut compartment specificity.
2. Establishes a "cow-calf" model system to clarify the dual pathways of vertical transmission and environmental diffusion of antibiotic resistance genes (ARGs) in the dairy production system.
3. The age and diet are the key factors affecting the early intestinal ARGs, and the water and soil in the farm are the high-risk sources of ARG diffusion.

In the future, the risk of ARG transmission can be reduced by regulating the early diet and optimizing environmental management.

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