

Microbial DNA methylation links rumen transcriptional activity to methane emission divergence in dairy cows

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Xinyue Zhang, Guirong Xu, Liwei Zhao, Ye Tao, Jiawei Zhang, Cameron EF Clark, Yangyi Hao, et al. 2026. Microbial DNA methylation links rumen transcriptional activity to methane emission divergence in dairy cows. *iMeta* 5: e70153.

<https://doi.org/10.1002/imt2.70153>

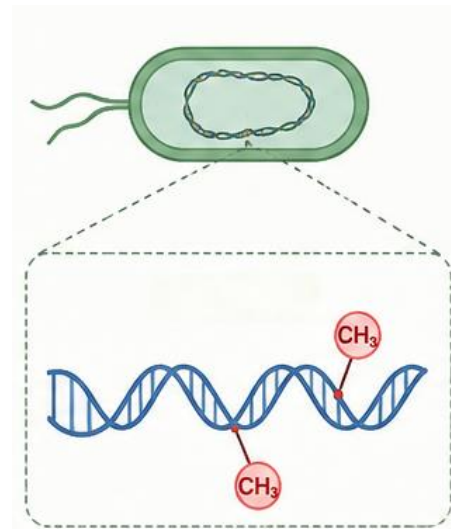
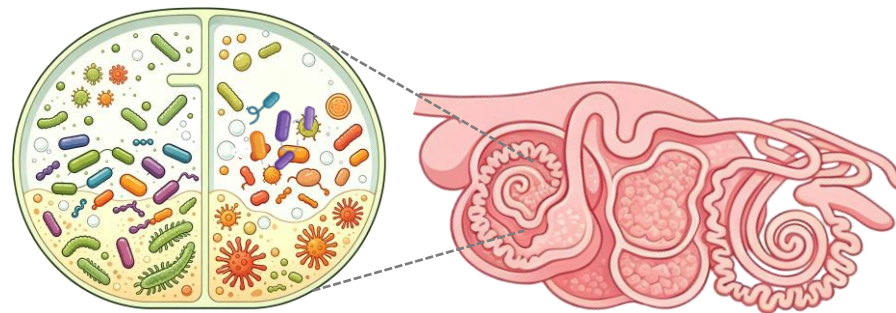


Introduction

- ① Enteric methane is a major dairy greenhouse gas and an energetic loss (2% - 12%) to the host.

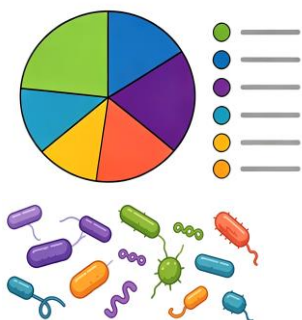


- ② The rumen microbiome plays a central role in fermentation and methanogenesis.

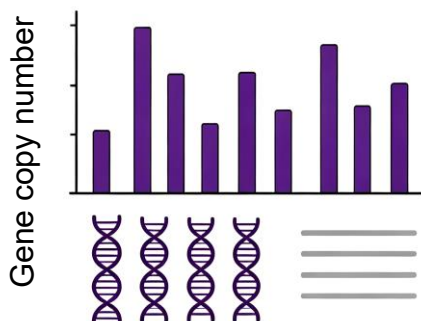


- ③ Previous studies mainly focused on microbial composition and gene abundance.

Microbial composition



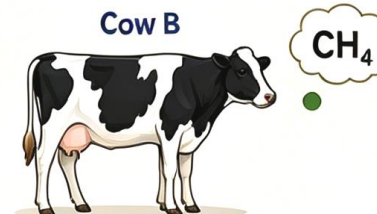
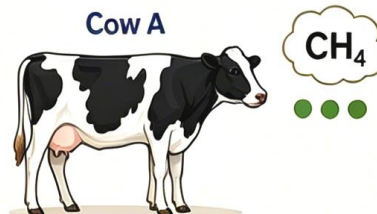
Gene abundance



- ④ Taxonomic differences alone cannot fully explain methane emission divergence.



Similar microbial community but different methane emission.



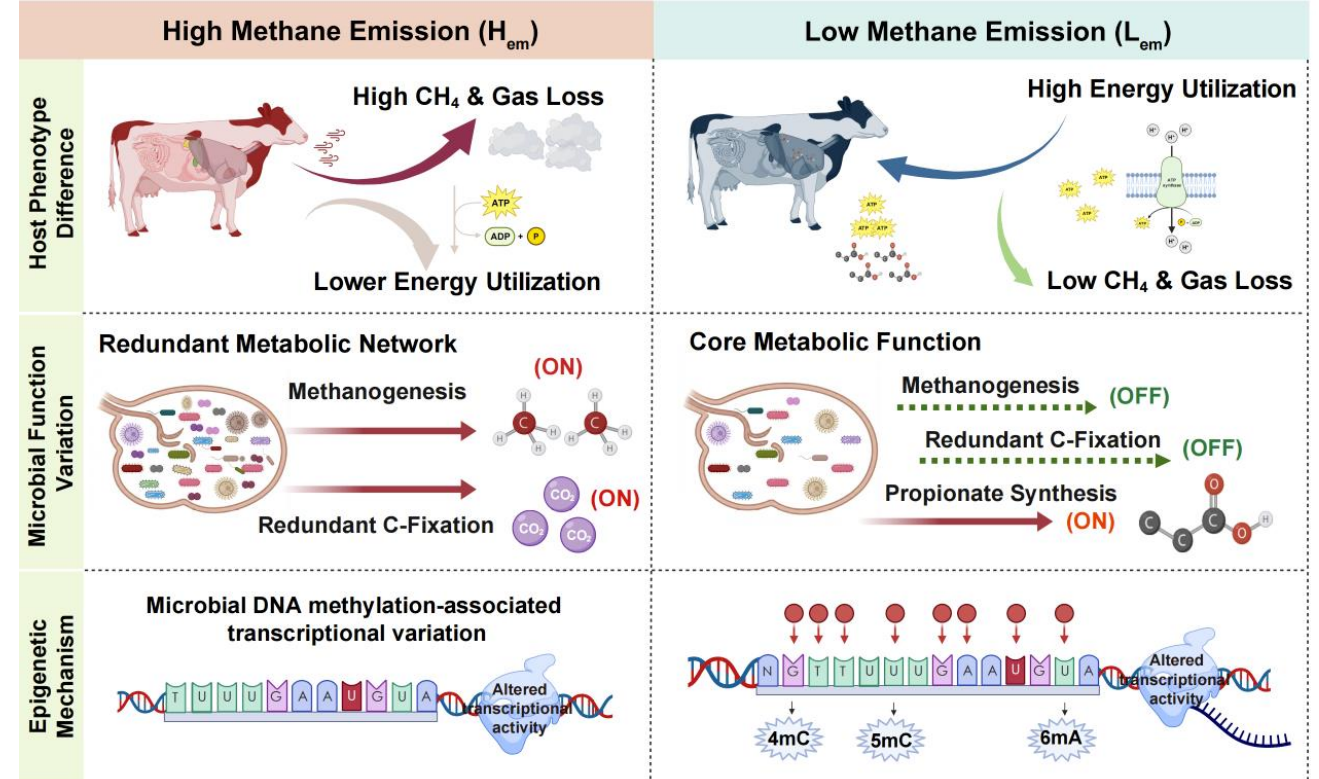
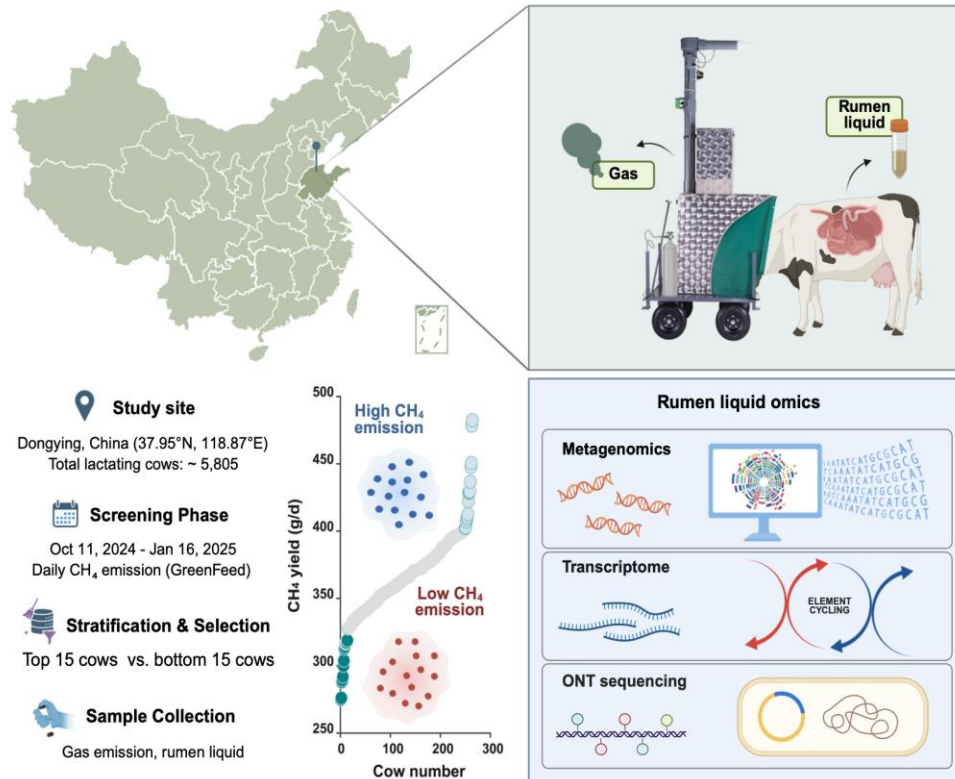
Scientific question:

Does rumen microbial DNA methylation participate in the process of methane production?





Highlights



Resoruce

➤ **First atlas —**
Genome-resolved methylomics uncovers the first DNA methylation atlas of the dairy cow rumen microbiome.

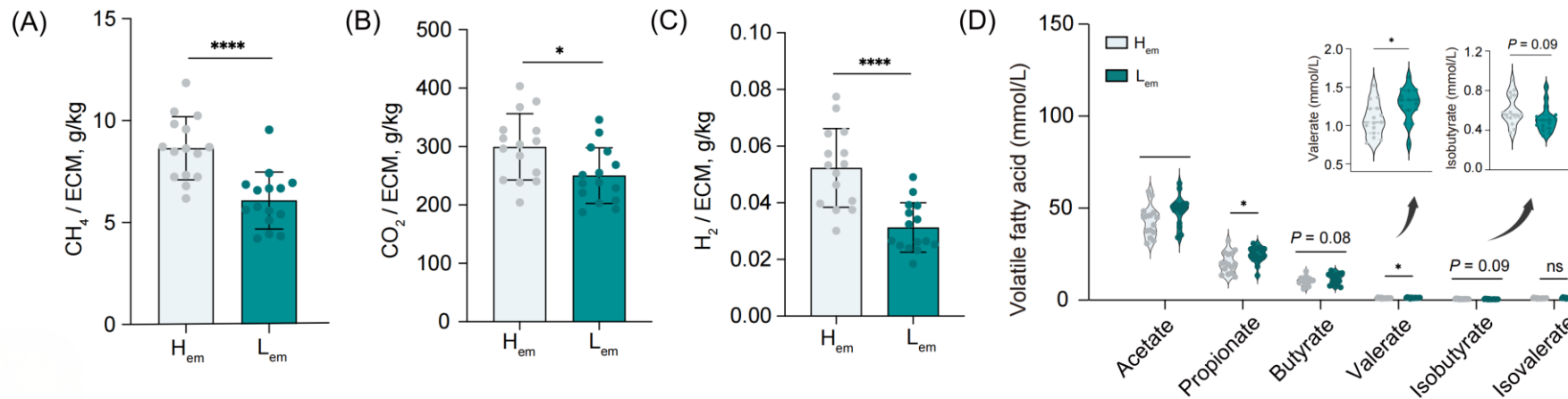
Discover

➤ **Epigenetic divergence —**
Microbial DNA methylation profiles diverge between low- and high-methane-emitting dairy cows despite limited taxonomic differences.

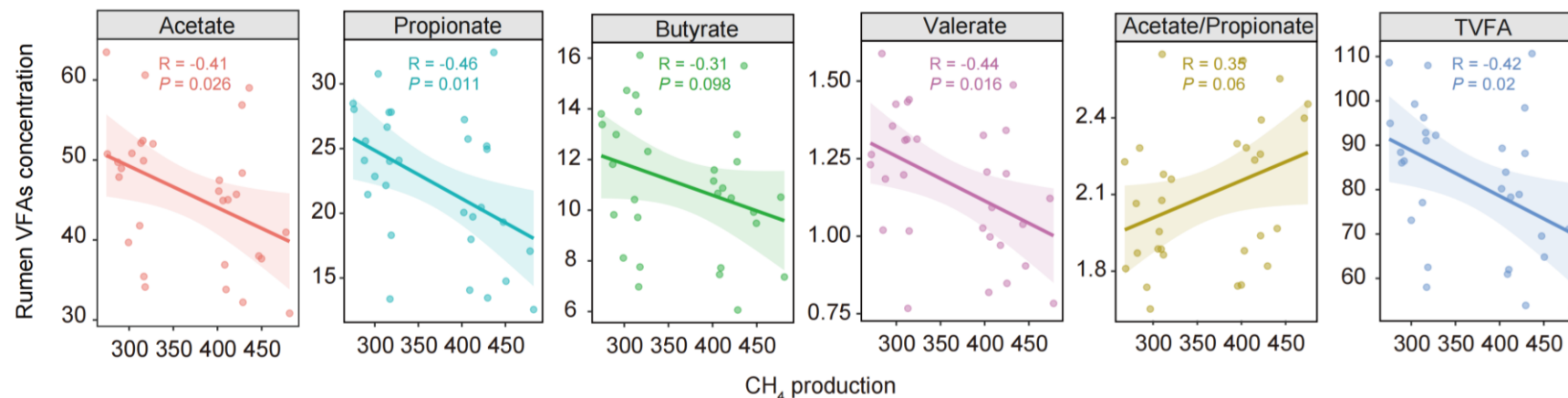
Framework

➤ **Mechanistic link —**
Differential methylation is linked to transcriptional variation in genes involved in carbon metabolism, hydrogen utilization, and methanogenesis.

Metabolic alterations in low-methane emission cows

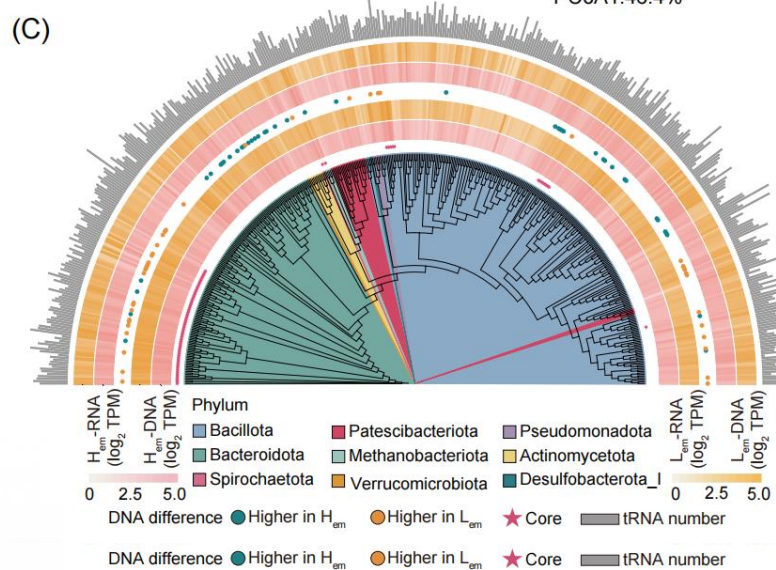
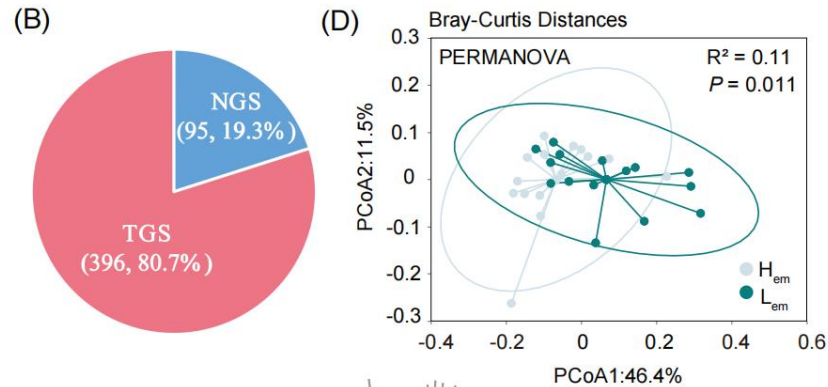


➤ Low-emission group (L_{em}) shows significantly lower CH₄, CO₂ and H₂, but higher volatile fatty acid (VFA), notably propionate and valerate.



➤ Methane emission correlates negatively with the rumen VFA profile.

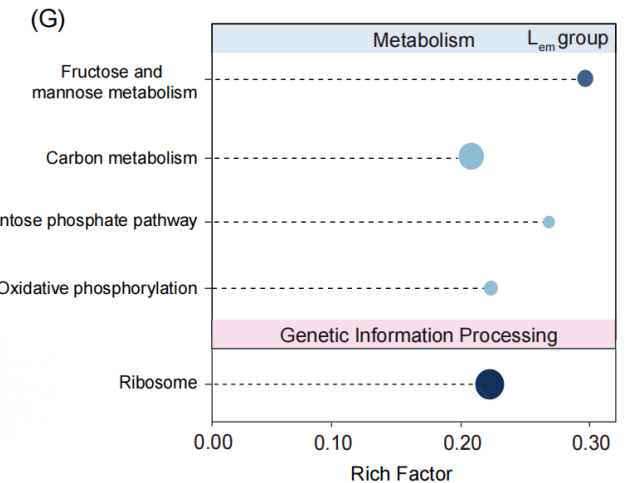
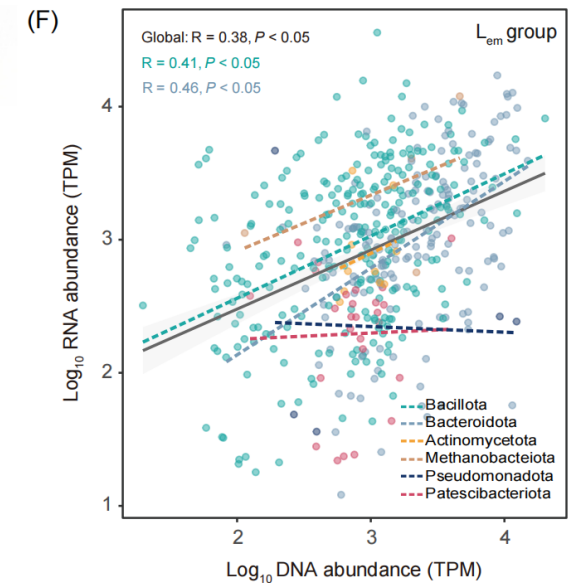
Genome-resolved catalog and transcriptional function



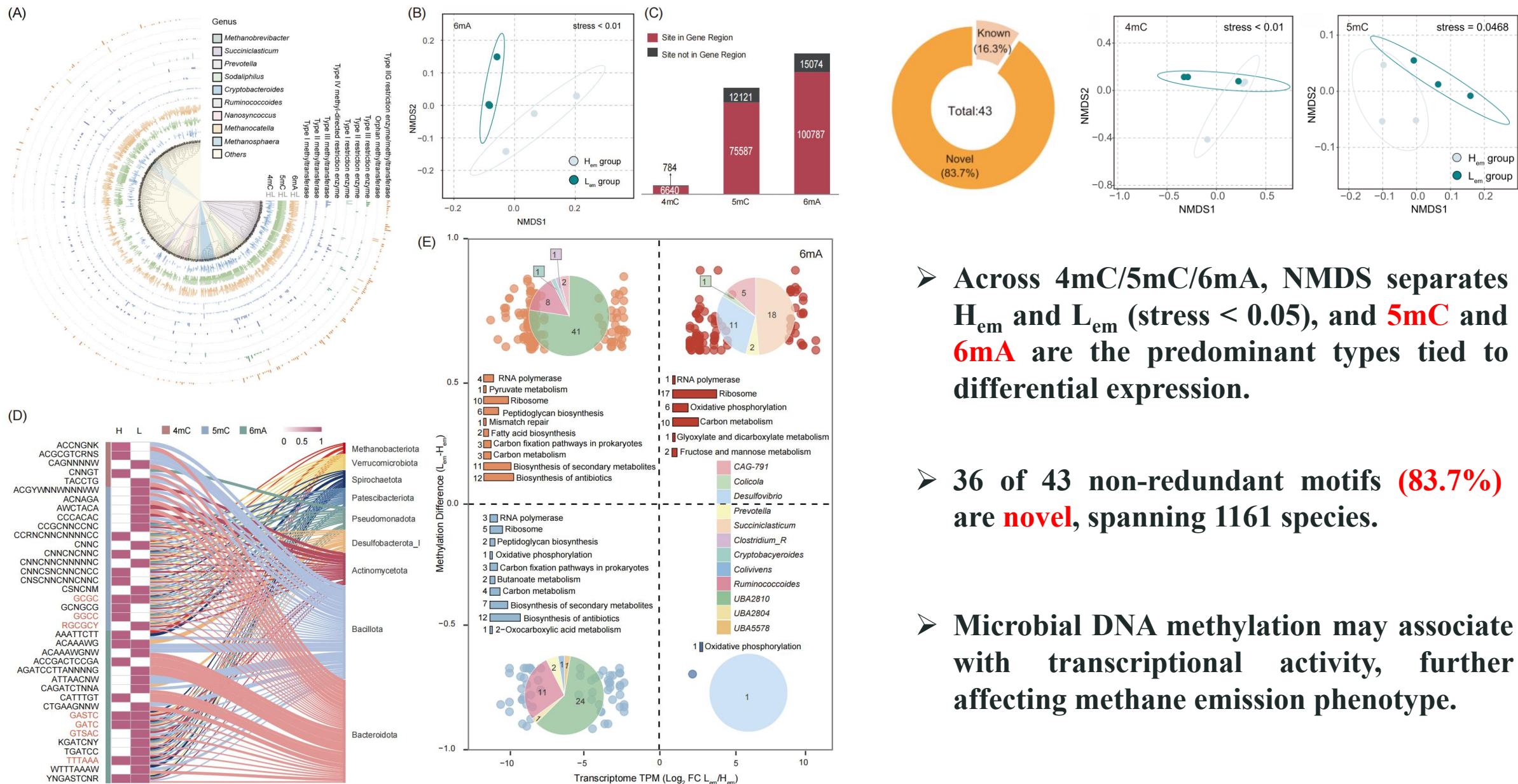
- High-quality catalog from integrated TGS + NGS sequencing.
- Abundance is not consistently correlated with transcription.



- L_{em} group enriched in core metabolic functions without functional redundancy.



Microbial DNA methylation landscape

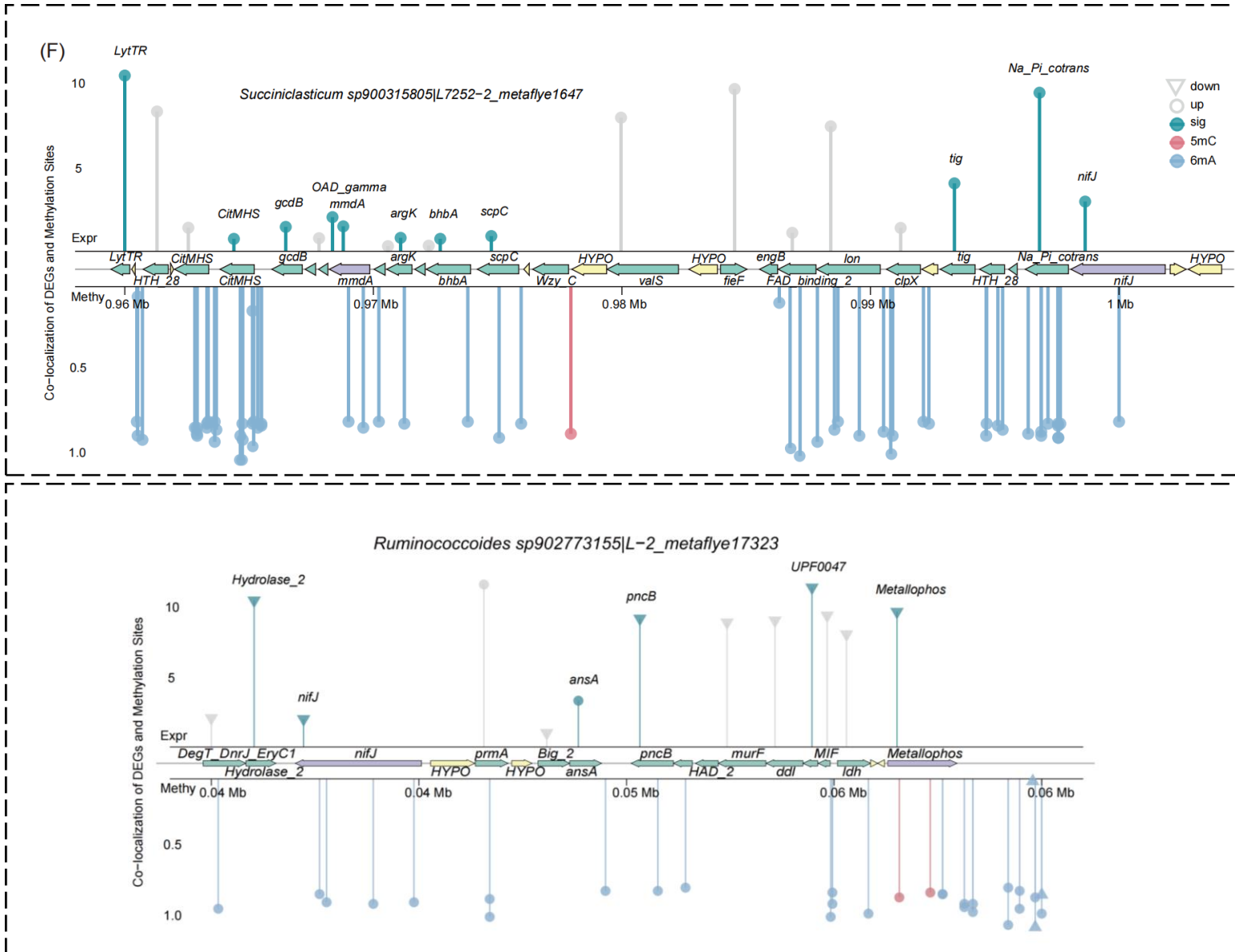


➤ Across 4mC/5mC/6mA, NMDS separates H_{em} and L_{em} (stress < 0.05), and **5mC** and **6mA** are the predominant types tied to differential expression.

➤ 36 of 43 non-redundant motifs (83.7%) are **novel**, spanning 1161 species.

➤ Microbial DNA methylation may associate with transcriptional activity, further affecting methane emission phenotype.

Methylation links transcription to metabolic flux



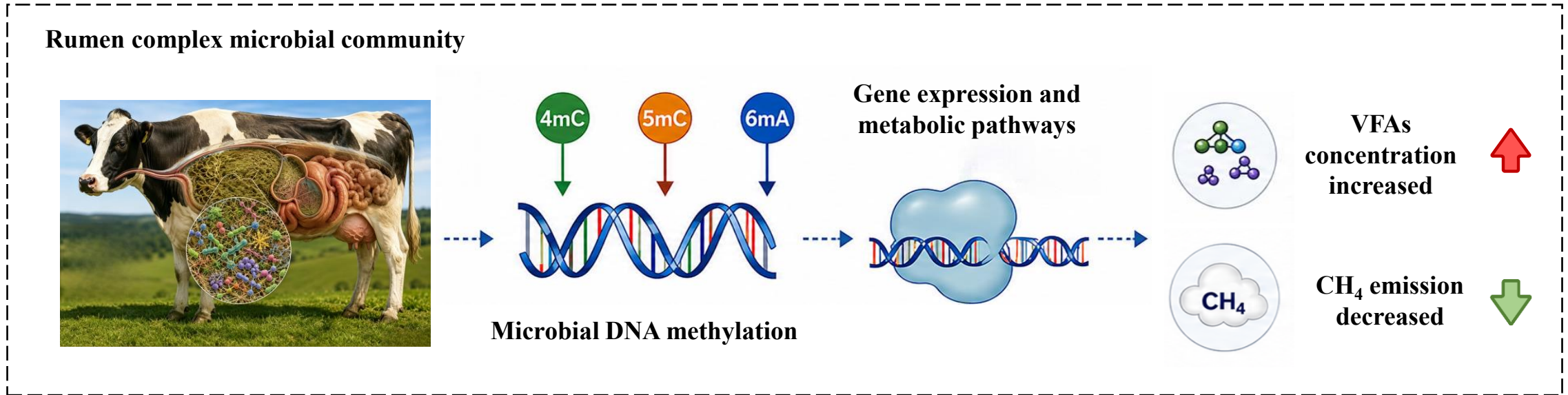
➤ Among differentially expressed genes, 0.12% (4mC), 11.37% (5mC), and 21.65% (6mA) co-localized with microbial DNA methylation sites.

➤ In *Succiniclasicum*, higher 6mA methylation enhanced propionate synthesis gene *mmdA* transcription, promoting propionate production and reducing hydrogen availability for methanogenesis.

➤ In *Ruminococcoides*, elevated 6mA methylation markedly suppressed expression of the hydrogen metabolism gene *nifJ*, reducing free hydrogen supply and inhibiting methanogenic activity.



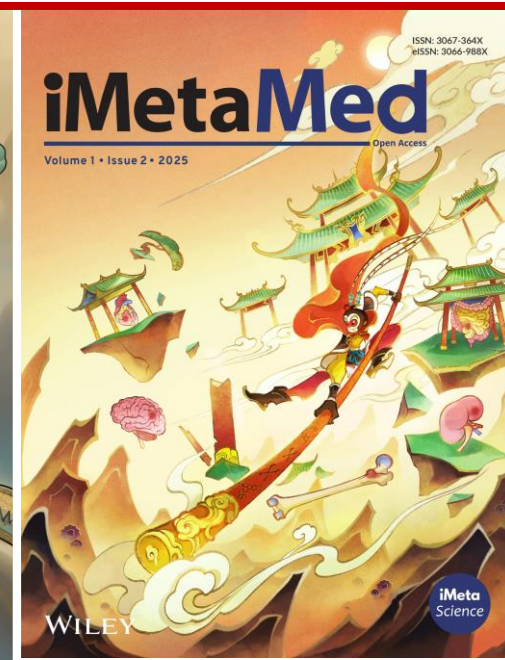
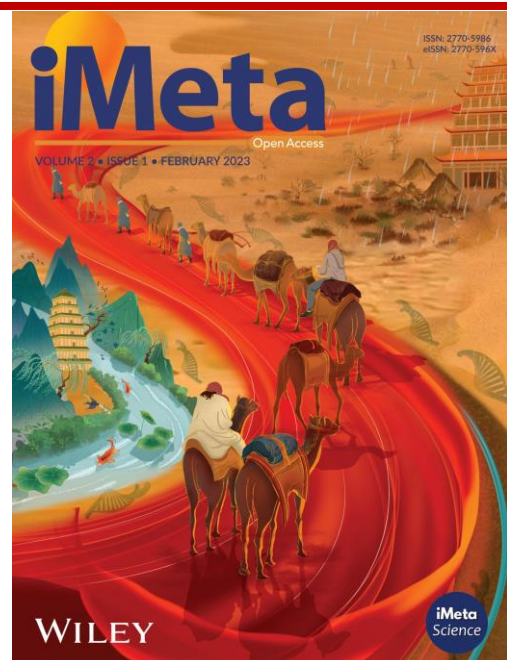
Summary



- Construct the first genome-wide DNA methylation atlas of the rumen microbiome in dairy cows.
- Reveal distinct methylation patterns associated with high and low methane emission phenotypes.
- Advance our understanding from a species abundance-based theory to an epigenetic association-based framework, laying a mechanistic foundation for low-carbon livestock production.

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