

Metabolomic profiling of forages to identify anti-methanogenic compounds targeting enteric methane for sustainable livestock systems

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Introduction

- Ruminant enteric methane (CH₄) is a primary agricultural source of anthropogenic GHGs, making practical mitigation strategies a priority for sustainable livestock systems [1].
- Plant secondary metabolites, especially tannins, saponins, and flavonoids, suppress methanogenesis by modulating rumen microbes; tropical tannin-rich legumes consistently lower CH₄ in vitro and in vivo assays [2]. Flavonoids, as flavonoid-3-O-glycosides, further reduce CH₄ emissions by shifting microbial populations and fermentation [3].
- Untargeted metabolomics provides a systems approach to discover and prioritize anti-methanogenic compounds (AMCs) by linking chemical features to CH₄ forage phenotypes [4].

Objectives

To identify anti-methanogenic compounds (AMCs) in forages from CIAT, ICARDA and ILRI Genebanks' using an integrative untargeted metabolomics approach combined with *in vitro* CH₄ production assays.

Methodology

- To pinpoint relevant AMCs, we apply statistical analyses, unsupervised classification, and differential analysis of metabolomic profiles from contrasting high- and low-CH₄-producing samples.
- 26 contrasting CH₄ production samples of 13 forage genus (Table 1) employing untargeted metabolomics SB-Aq method using LC/MS-based technique.
- After acquiring the MS/MS spectra, each spectrum follows two parallel routes to obtain the sample metabolomic profile (Figure 1).
- Preliminary results allowed us to identify a set of candidate under-expressed or over-expressed metabolites associated with methanogenic activity.

Table 1. Forage species from the Alliance, ICARDA and ILRI Genebanks' and their *in vitro* CH₄ (ml/g) concentration production.

Forage specie from CGIAR Genebanks'	
1. <i>Trifolium burchellianum</i> (ILRI – 9.3)	14. <i>Lathyrus sylvestris</i> (ICARDA – 41.1)
2. <i>Cajanus cajan</i> (CIAT – 18.9)	15. <i>Stylosanthes fruticosa</i> (ILRI – 41.8)
3. <i>Leucaena pallida</i> (ILRI – 19.9)	16. <i>Chloris gayana</i> (ILRI – 43.2)
4. <i>Flemingia macrophylla</i> (ILRI – 20.7)	17. <i>Leucaena leucocephala</i> (CIAT – 44.3)
5. <i>Desmodium heterocarpon</i> (CIAT – 22.3)	18. <i>Erythrina velutina</i> (CIAT – 48.6)
6. <i>Erythrina abyssinica</i> (ILRI – 25.0)	19. <i>Desmodium scorpiurus</i> (CIAT – 49.0)
7. <i>Cajanus cajan</i> (ILRI – 26.8)	20. <i>Cenchrus ciliaris</i> (CIAT – 50.1)
8. <i>Hedysarum carnosum</i> Desf (ICARDA – 29.7)	21. <i>Chloris gayana</i> (CIAT – 51.9)
9. <i>Flemingia macrophylla</i> (CIAT – 31.1)	22. <i>Trifolium repens</i> (ICARDA – 53.7)
10. <i>Panicum coloratum</i> (ILRI – 33.8)	23. <i>Panicum maximum</i> (CIAT – 53.8)
11. <i>Hedysarum coronarium</i> L. (ICARDA – 38.2)	24. <i>Vicia tenuifolia</i> (ICARDA – 56.4)
12. <i>Cenchrus purpureus</i> (ILRI – 39.5)	25. <i>Stylosanthes guianensis</i> (CIAT – 57.1)
13. <i>Vicia narbonensis</i> L. (ICARDA – 40,0)	26. <i>Lathyrus sativus</i> (ICARDA – 65.6)

References

- [1]Morgavi, DP, et al. (2023). Review: Reducing enteric methane emissions improves energy metabolism in livestock: is the tenet, right?. *Animal*, 17:3. [10.1016/j.animal.2023.100830](https://doi.org/10.1016/j.animal.2023.100830)
- [2]Ku-Vera JC, et al. (2020). Role of Secondary Plant Metabolites on Enteric Methane Mitigation in Ruminants. *Front Vet Sci*. 7:584. [10.3389/fvets.2020.00584](https://doi.org/10.3389/fvets.2020.00584)
- [3]Oskoueian E, Abdullah N, Oskoueian A. Effects of flavonoids on rumen fermentation activity, methane production, and microbial population. *Biomed Res Int*. 2013;2013:349129. [10.1155/2013/349129](https://doi.org/10.1155/2013/349129)
- [4]Jensen RH, et al. (2022) Untargeted Metabolomics Combined with Solid Phase Fractionation for Systematic Characterization of Bioactive Compounds in Hemp with Methane Mitigation Potential. *Metabolites*. 12(1):77. [10.3390/metabo12010077](https://doi.org/10.3390/metabo12010077)

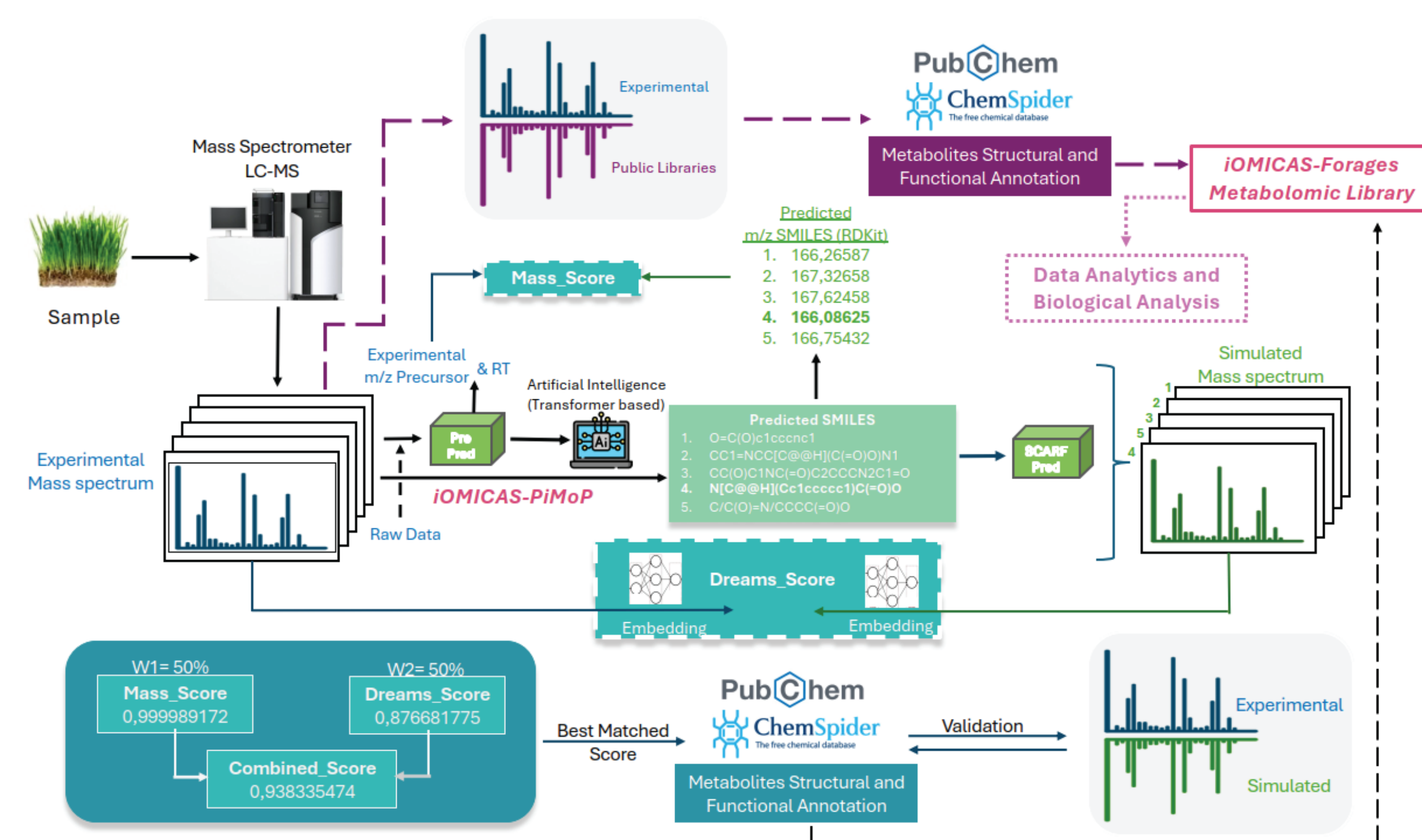


Figure 1. Metabolomics Analysis Workflow. After acquiring the MS/MS spectra (precursor m/z and RT), each spectrum follows two parallel routes. **Route-1:** The spectrum is searched against public spectral libraries; matches above the threshold (90%) proceed directly to structural and functional annotation. **Route 2:** iOMICAS-PiMoP (AI).

Results

- 1784 candidate metabolites were identified with Variable Importance in Projection, VIP>1, that robustly discriminate between high and low CH₄ forages (Fig. 2A).
- Shapley additive explanations (SHAP) further quantified each metabolite's marginal effect on model output (Fig. 2B); metabolites with negative SHAP values for the anti-methanogenic class push predictions toward the "low-methane" state.
- Of the AMC candidates, 110 metabolites were functionally annotated, finding that 50% are flavonoids. Notably, Kaempferol 3- α -L-arabinopyranoside (K3 α LA) and Guaijaverin rank among the top AMCs, and are enriched in *T. burchellianum* and *L. pallida*, respectively.

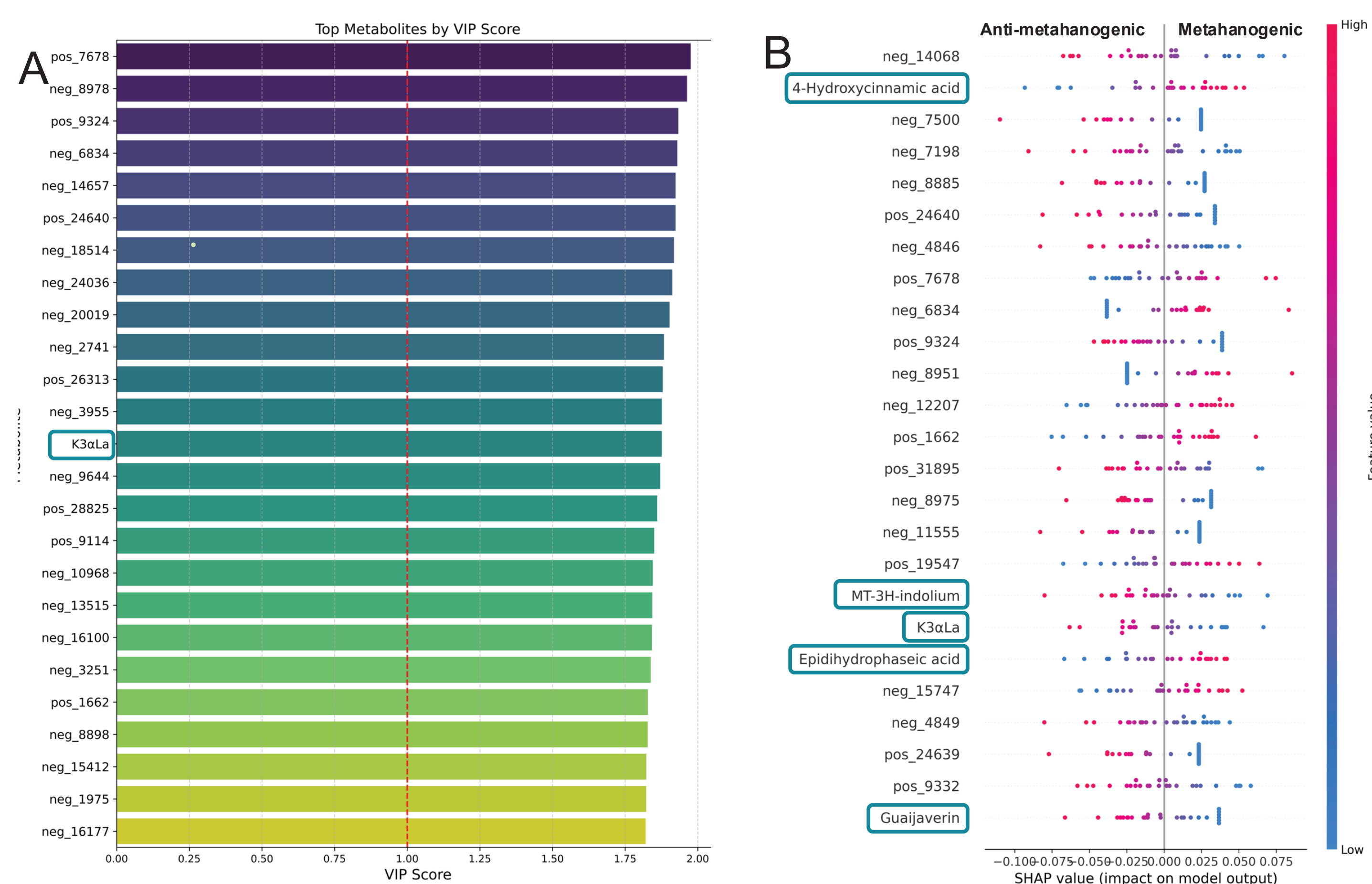


Figure 2. Convergence of SHAP, PLS-DA, and correlation identifies AMCs. **A.** PLS-DA (VIP): The VIP > 1 criterion confirms their strong contribution to separating low- vs. high-CH₄-emitting forages. **B.** SHAP analysis: Metabolites that shift the model's prediction toward the anti-methanogenic state.

Conclusions

- Flavonoids such as Guaijaverin and K3 α LA may inhibit methanogenic archaea directly (by disrupting cell membranes or metabolic enzymes), or alter rumen fermentation pathways, shifting hydrogen utilization away from methanogenesis toward alternative sinks (e.g., propionate production).
- Functional annotation of the remaining candidate metabolites via the iOMICAS pipeline could reveal additional AMCs and strengthen predictive forage selection models (iOMICAS-PiMoP).

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