

INTRODUCTION

Aphid honeydew, a sugar-rich excretion, influences aphid populations and their natural enemies. Recent studies suggest that the microbial communities within honeydew may affect the behavior of predators and parasitoids. Understanding honeydew microbes' dynamics is key to optimizing aphids' management.

We analyzed honeydew bacterial communities from two aphid species over time using NGS. Cultivable bacteria were isolated on differential media based on dominant genera, identified, and prepared as bacterial solutions for behavioral assays with natural enemies.

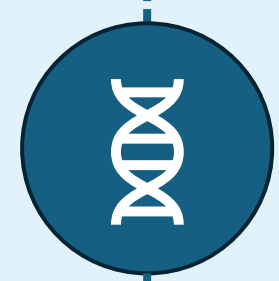
METHODS



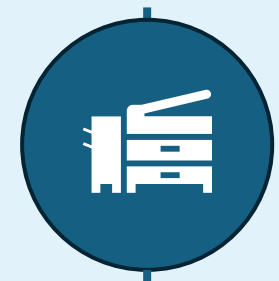
Honeydew collection from *Aphis fabae* and *Acyrtosiphon pisum*



Honeydew was left to age naturally under four conditions: fresh (no aging), 24-hour, 48-hour, and 72-hour aged



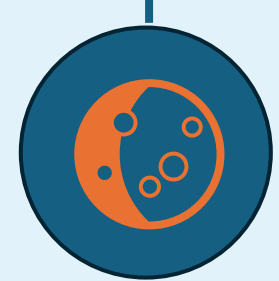
DNA was extracted from honeydew, and the V3-V4 region of the 16S rRNA gene was amplified



Paired-end sequencing was performed on the Illumina MiSeq system



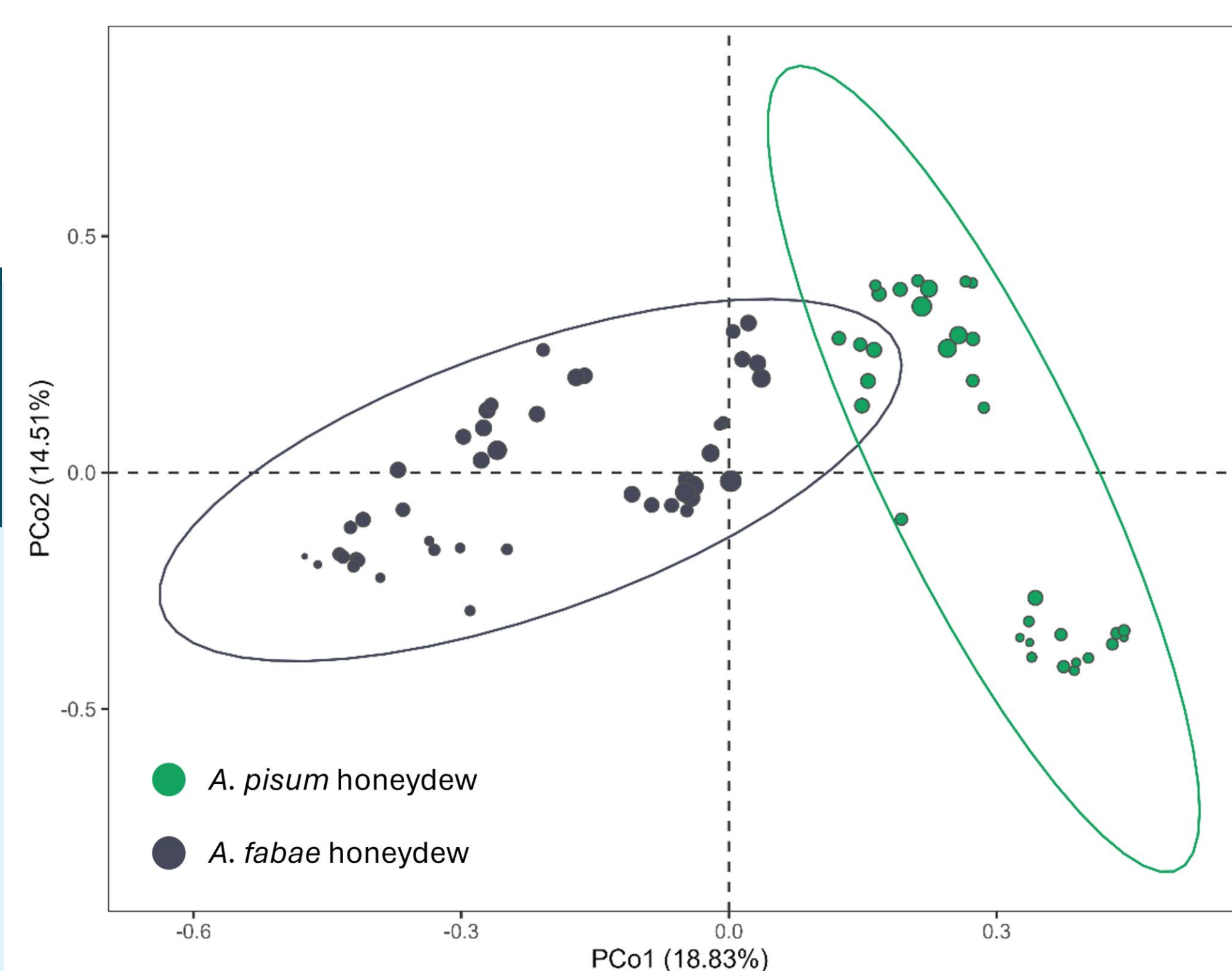
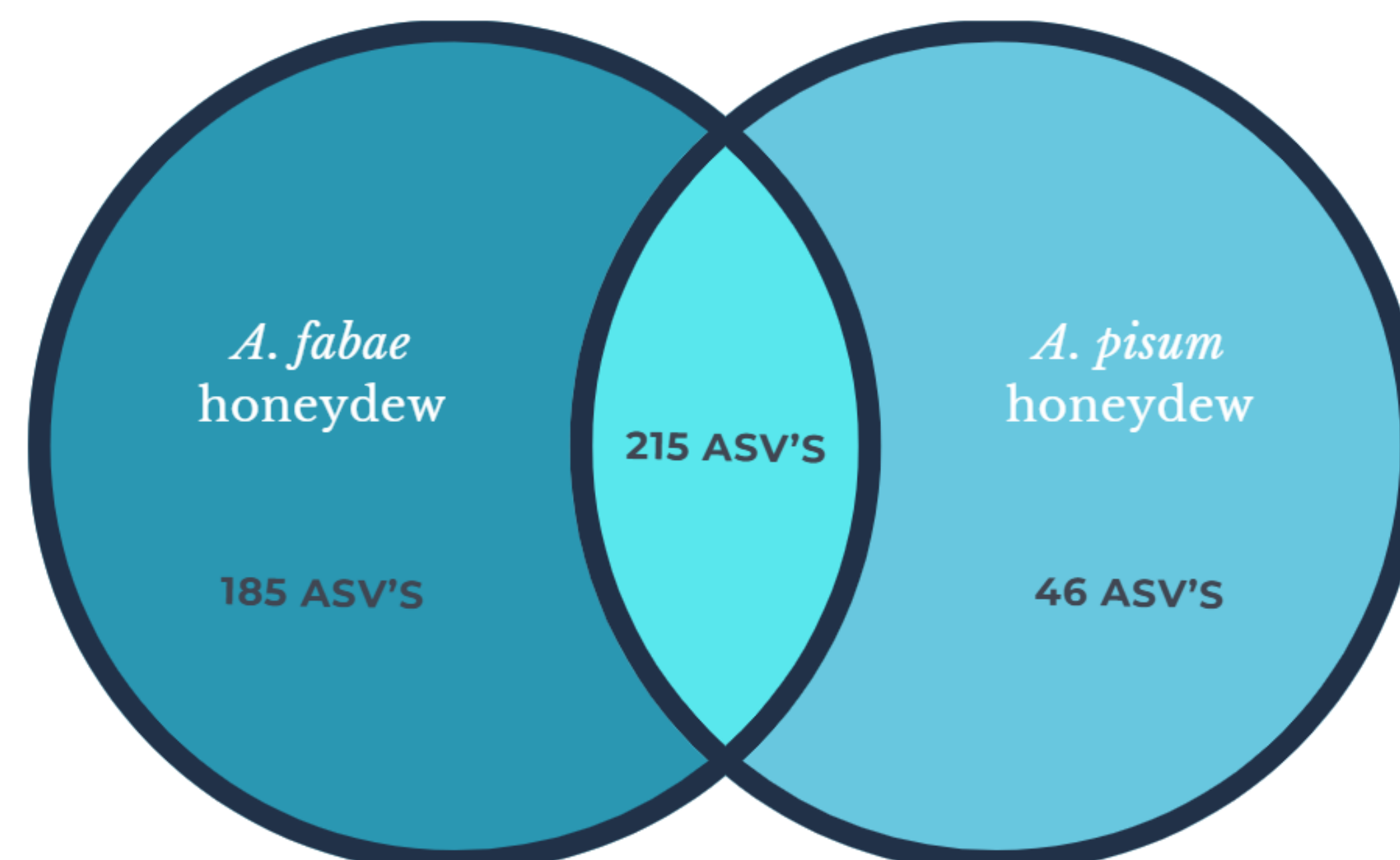
Raw sequencing data was processed using QIIME2, assigned taxonomy against the SILVA 138 database, and filtered to remove contaminants and low-quality samples, resulting in a dataset of 77 honeydew samples for bacterial community analysis



Based on the dominant bacterial genera identified, differential and selective media were employed to isolate bacteria from honeydew. Hektoen Enteric Agar was used to target *Pseudomonas* spp. and Mannitol Salt Agar was used to target *Staphylococcus* spp.

RESULTS

Bacterial community analysis



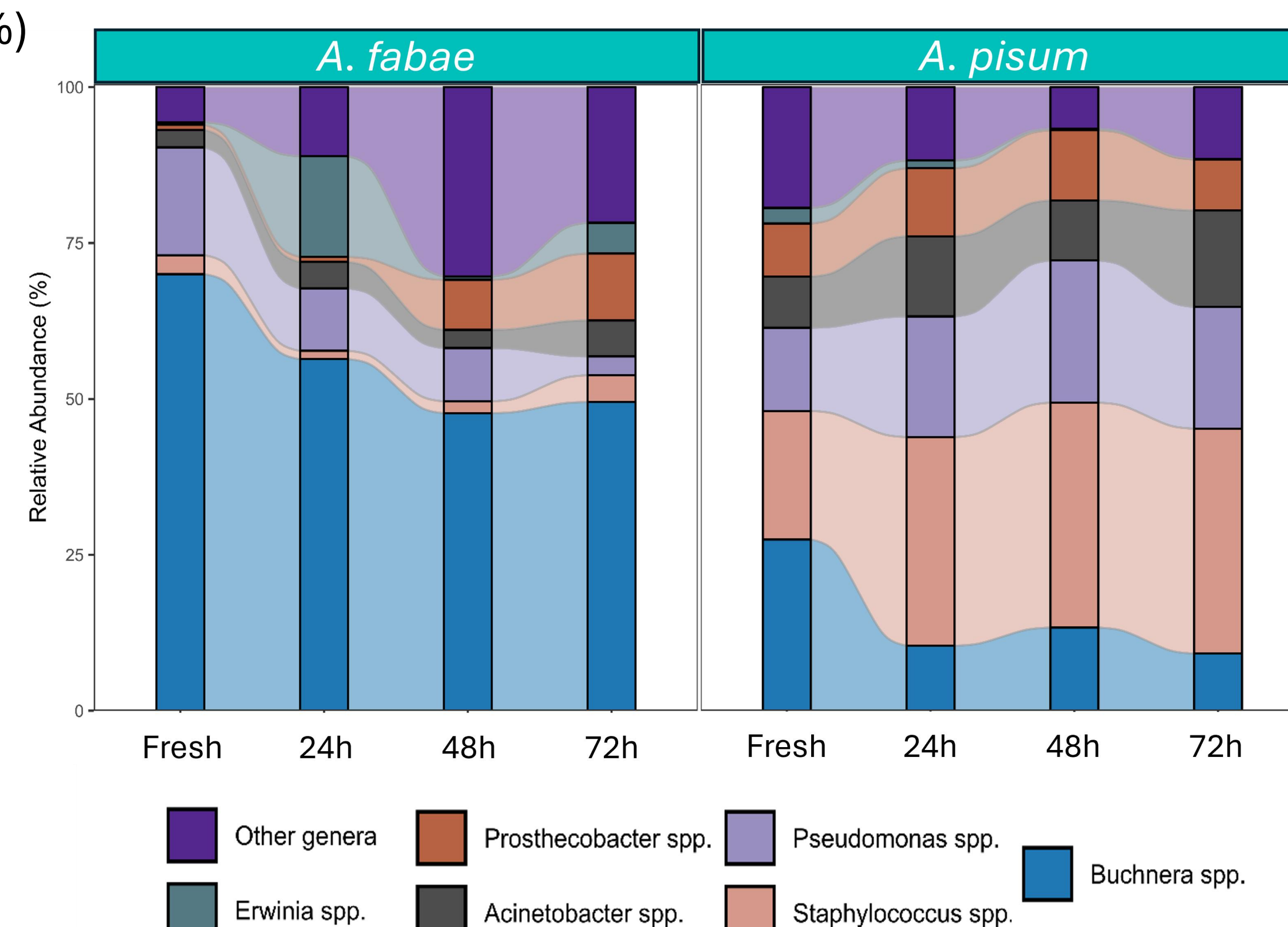
The bacterial community composition of honeydew from both aphid species was significantly different (d.f. = 1; $R^2 = 0.19$; $F = 17.74$; $P < 0.01$).

Bacterial ASV richness was significantly higher in *A. fabae* honeydew compared to *A. pisum* honeydew, as indicated by the rarefaction curves, alpha diversity indices ($P = 0.028$) and Chao1 estimator ($P = 0.028$).

Relative abundance of bacterial communities

At the family level, *Morganellaceae* dominates the bacterial community in *A. fabae* honeydew, accounting for 55.89%, while *Staphylococcaceae* is the most abundant in *A. pisum* honeydew (29.73%). At the genus level, ***Buchnera* spp. is the predominant genus in *A. fabae* honeydew (55.86%)**, while ***Staphylococcus* spp. dominates *A. pisum* honeydew (29.73%)**

Bacterial communities of ***Acinetobacter* spp.** (df = 3, $P < 0.01$), ***Prosthecobacter* spp.** (df = 3, $P < 0.01$), and ***Staphylococcus* spp.** (df = 3, $P < 0.01$) **significantly increased in *Aphis fabae* honeydew across all ageing conditions.**



No significant changes were observed in the overall community of *A. pisum* honeydew. However, *Pseudomonas* spp. ($P = 0.05$) and *Staphylococcus* spp. ($P = 0.05$) showed a marginal increase in abundance at 72h.

Selective isolation of bacteria from honeydew

A total of **38** colonies were isolated and will be characterized using Sanger sequencing

	Hektoen Enteric Agar	Mannitol Salt Agar
<i>A. fabae</i> honeydew	13 colonies (assumed distinct)	4 colonies (assumed distinct)
<i>A. pisum</i> honeydew	18 colonies (assumed distinct)	3 colonies (assumed distinct)

So far, six species or genera have been identified:

- Erwinia* sp. (two strains) and *Acinetobacter** sp. in ***A. fabae* honeydew**
- Pantoea* sp.***, *Klebsiella aerogenes*, and *Enterobacter cloacae* in ***A. pisum* honeydew**

REFERENCES

¹Houtz, J. L., Melo, M., Therrien, J. F., & Cornell, A. (2023). Disentangling relationships between physiology, morphology, diet, and gut microbial diversity in American kestrel nestlings. *Journal of Avian Biology*, 2023(7–8). ²Lopez, J. G., & Wingreen, N. S. (2022). Noisy metabolism can promote microbial cross-feeding. *ELife*, 11. ³Beyer, D., Tang, P. Z., Becker, S., Hoang, T., Bilgin, D., Lim, Y. W., Peterson, T. C., Mayfield, S., Haerizadeh, F., Shurin, J. B., Bafna, V., & McBride, R. (2016). Diversity, productivity, and stability of an industrial microbial ecosystem. *Applied and Environmental Microbiology*, 82(8), 2494–2505.



Bacteria from isolated colonies on Hektoen Enteric agar and Mannitol Salt agar (bottom) from *A. fabae* (left) and *A. pisum* (right) honeydew

DISCUSSION

- Higher ASV richness in *A. fabae* suggests a more complex microbiome, likely influenced by physiological factors rather than diet or environment, as both aphid species were reared under identical conditions on the same plant¹.
- Bacterial growth in *A. fabae* honeydew suggests a dynamic microbial environment, while stability in *A. pisum* may indicate resilience to change².
- Differences in honeydew-associated bacteria between these species may help explain their varying interactions with natural enemies³.
- The higher number of bacterial isolates from *A. pisum* honeydew on Hektoen Enteric Agar suggests a greater presence of *Pseudomonas*-related taxa, which must be confirmed by sequencing.

CONCLUSION

Microbiome complexity differs between *A. fabae* and *A. pisum*, with *A. fabae* showing higher ASV richness and dynamic bacterial growth.

Proteobacteria dominate both species, but species-specific bacterial associations emerge.

Ongoing identification of isolates will help uncover their role in shaping natural enemy behavior.