

Hidden Threats in Organic Fertilizers: A Study of Antibiotic Resistance in Commercial Animal-Based Fertilizers

Background

- Approximately 80% of antibiotics sold in the US are administered to livestock for growth promotion, disease prevention and treatment of illness.
- Manure-based fertilizers can introduce antibiotic-resistant bacteria (ARB) into agricultural soils and native microbial communities.
- Bacteria can acquire antibiotic resistance (AR) through mutation and horizontal gene transfer, allowing resistance to persist and spread.
- Composting and other commercial processing methods do not eliminate ARB completely, creating a need to understand the resistance present in commercially available fertilizers.
- AR poses a threat to soil microbial communities and human health.



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Image by Florida Memory

Research Questions

- Does bacterial richness differ among fertilizer types?
- Does bacterial richness differ between ampicillin amended plates and control plates?
- Does cow manure contain a higher abundance of antibiotic-resistant bacteria (ARB) than other fertilizer types?

Hypothesis

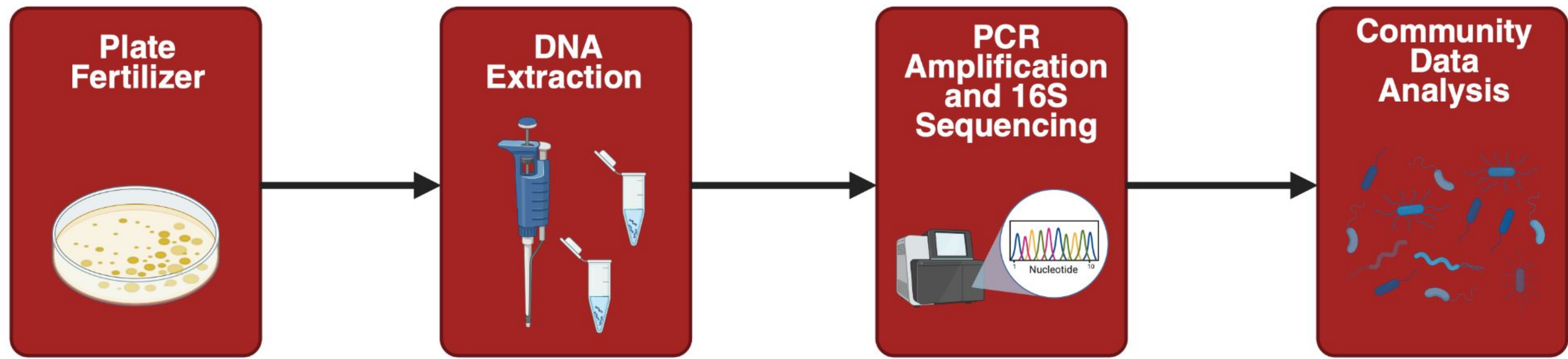
- If cow, chicken, fish, and bat manure are compared, then cow manure will have the highest abundance of antibiotic-resistant bacteria (ARB).
- If bacterial colonies from manure samples are sequenced and analyzed using bioinformatics, then bacterial abundance and community diversity will differ among manure types.



Made with BioRender

Methods

- Fertilizer from each treatment was diluted in sterile water and pipetted onto petri dishes containing ampicillin antibiotic at a concentration of 100µg/mL.
- Plates were incubated at room temperature for two weeks and single bacterial colonies were picked. Bulk DNA was also extracted from whole fertilizer.
- 16s sequencing was performed to identify the colonies and the bulk population.



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Results

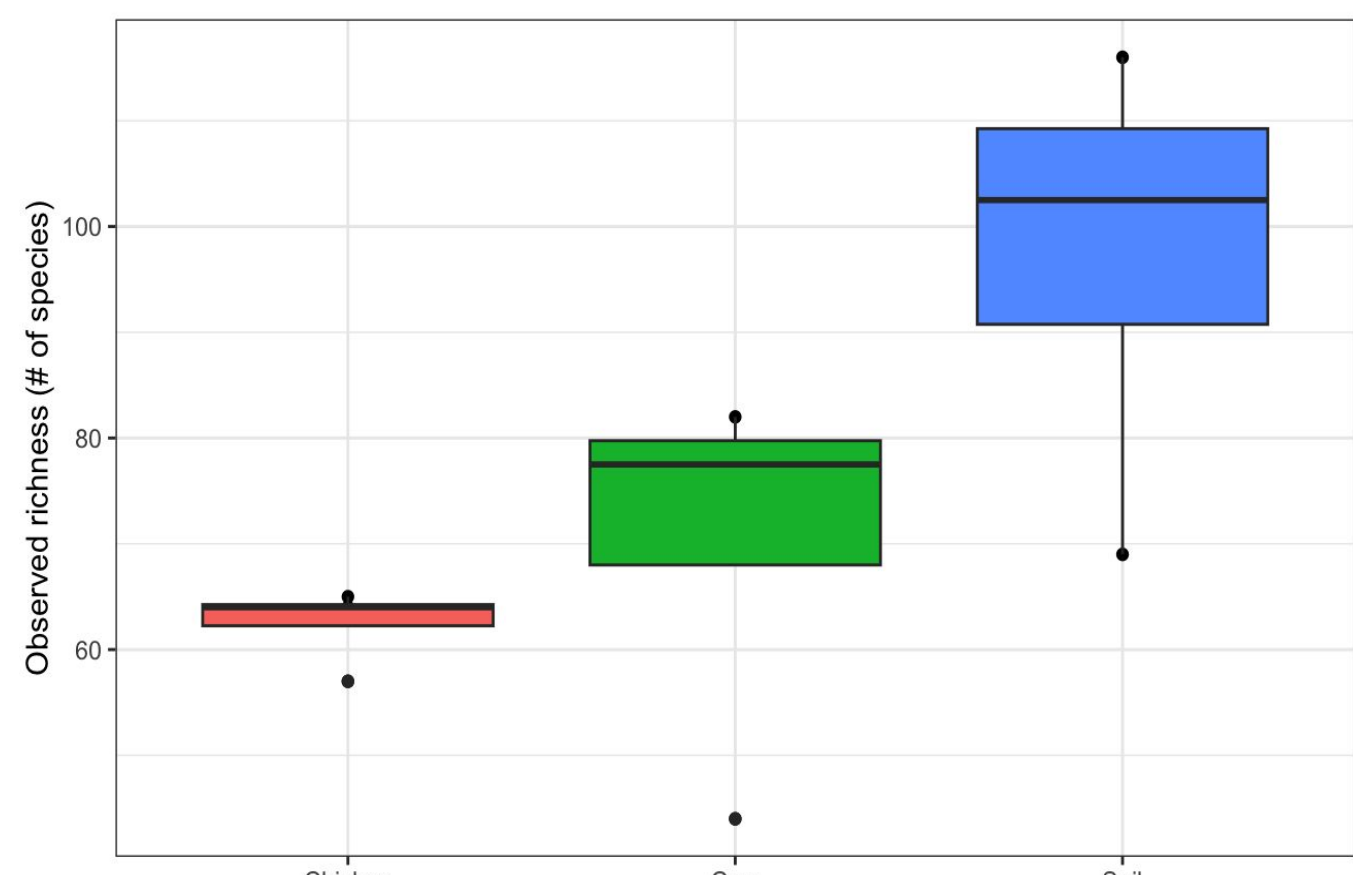


Figure 1: Richness of chicken manure, cow manure and soil

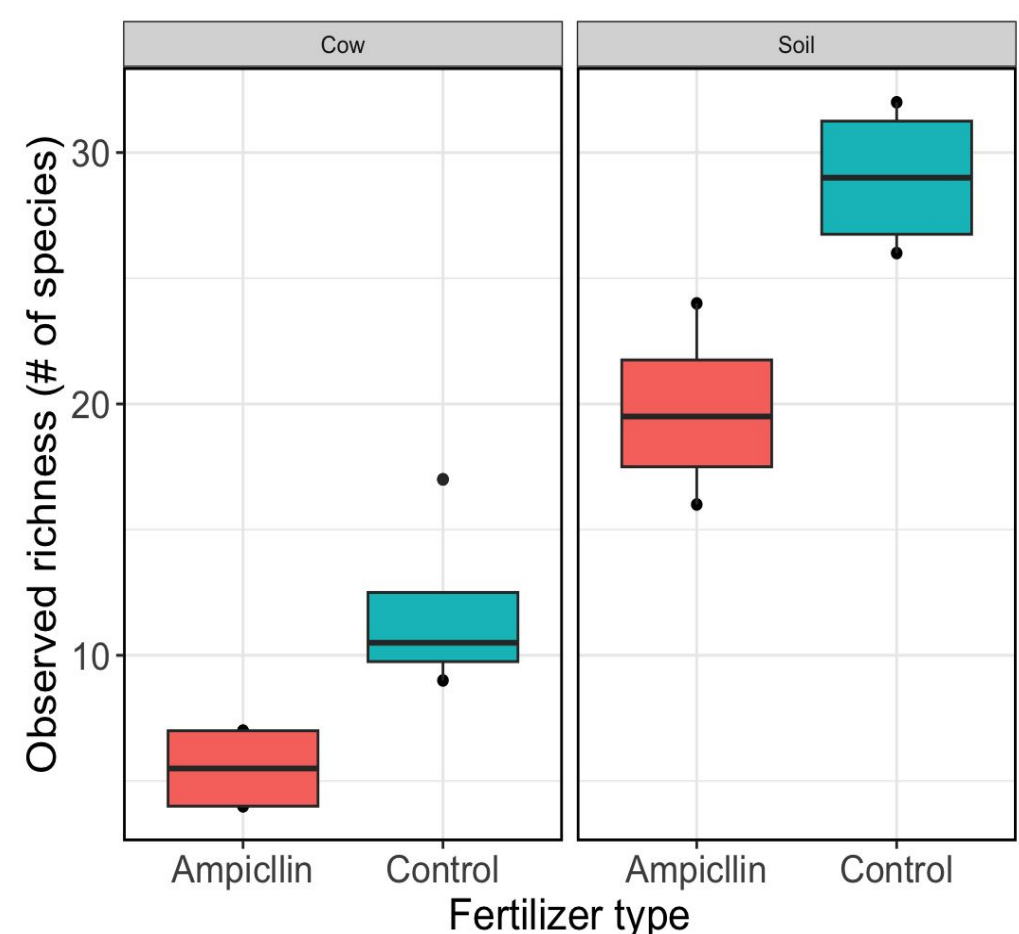


Figure 2: Richness before and after ampicillin selection

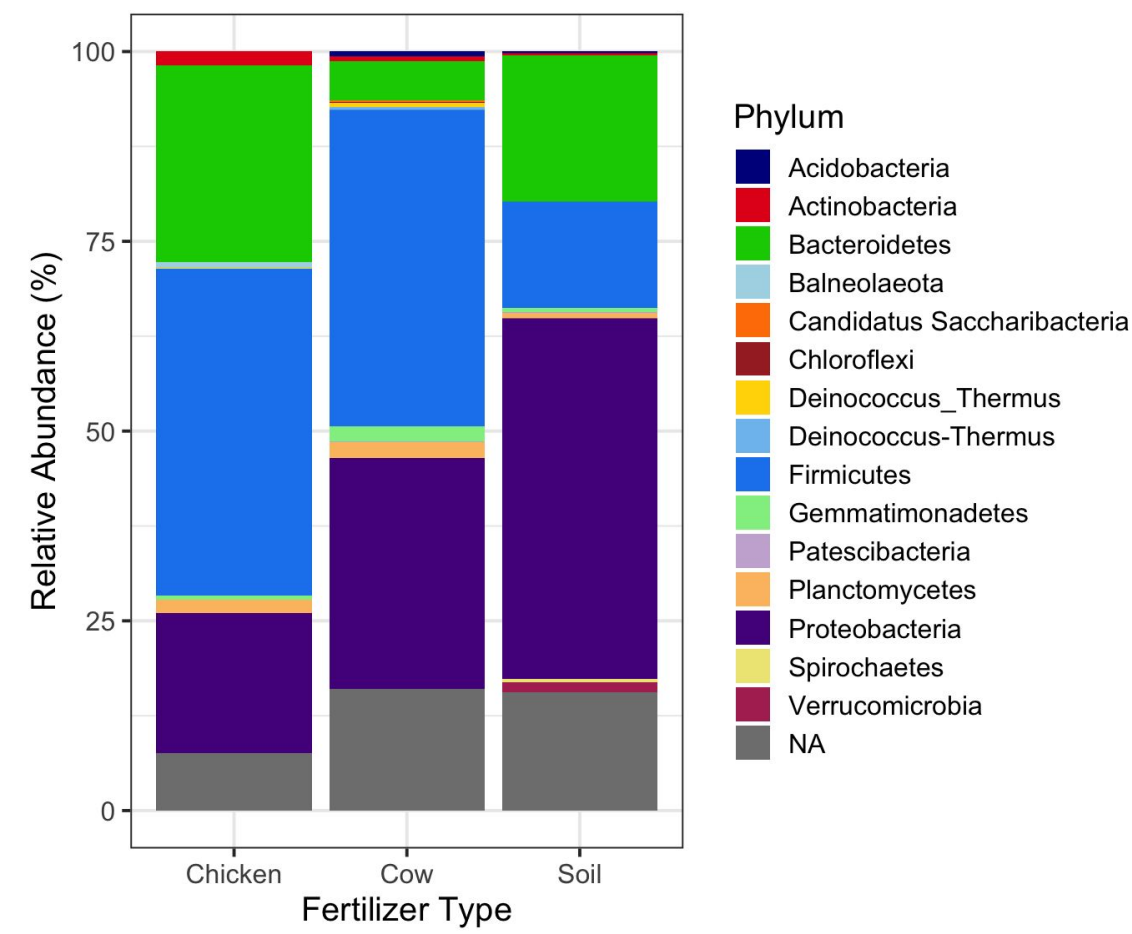


Figure 3: Abundance of phyla across samples

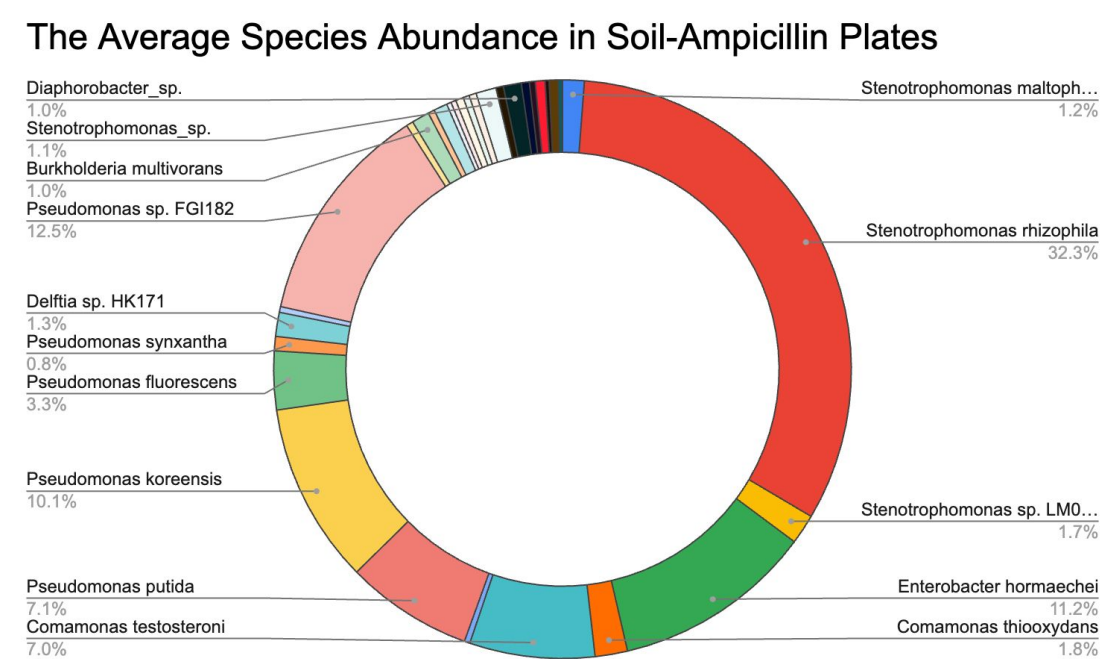


Figure 4: AR species in soil

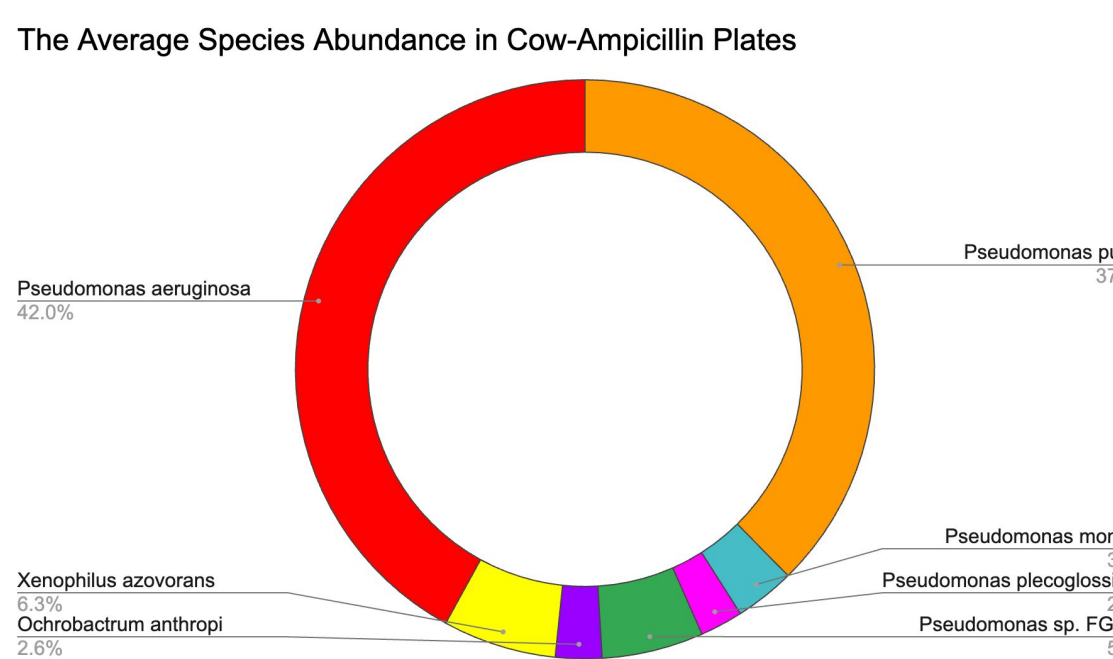


Figure 5: AR species in cow manure

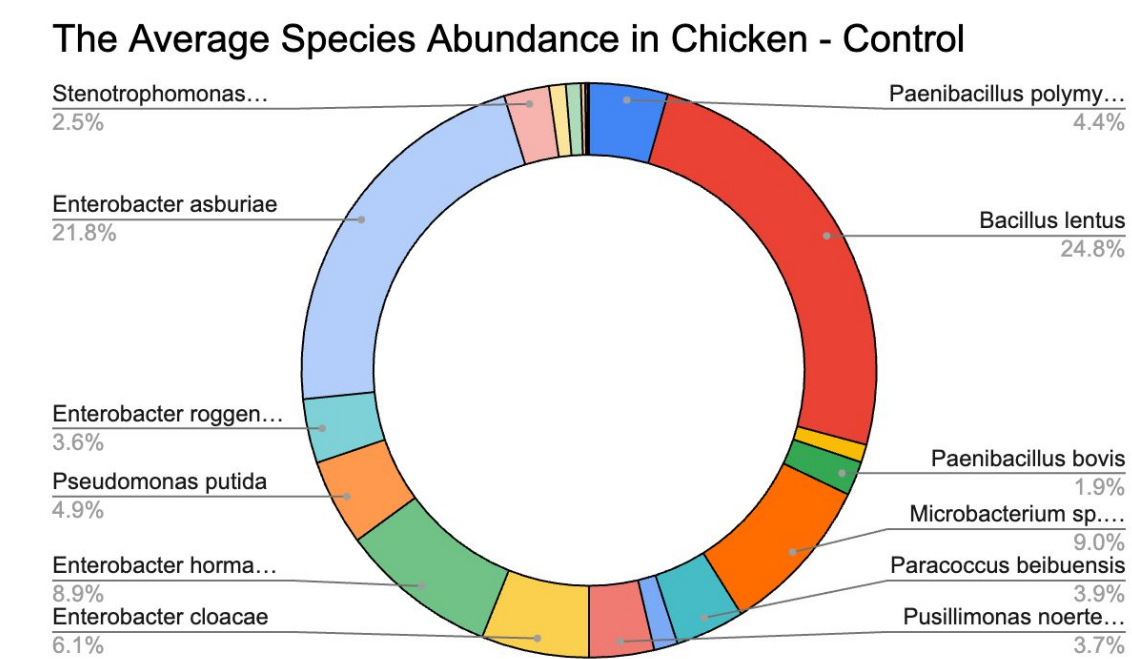


Figure 6: Species in chicken manure

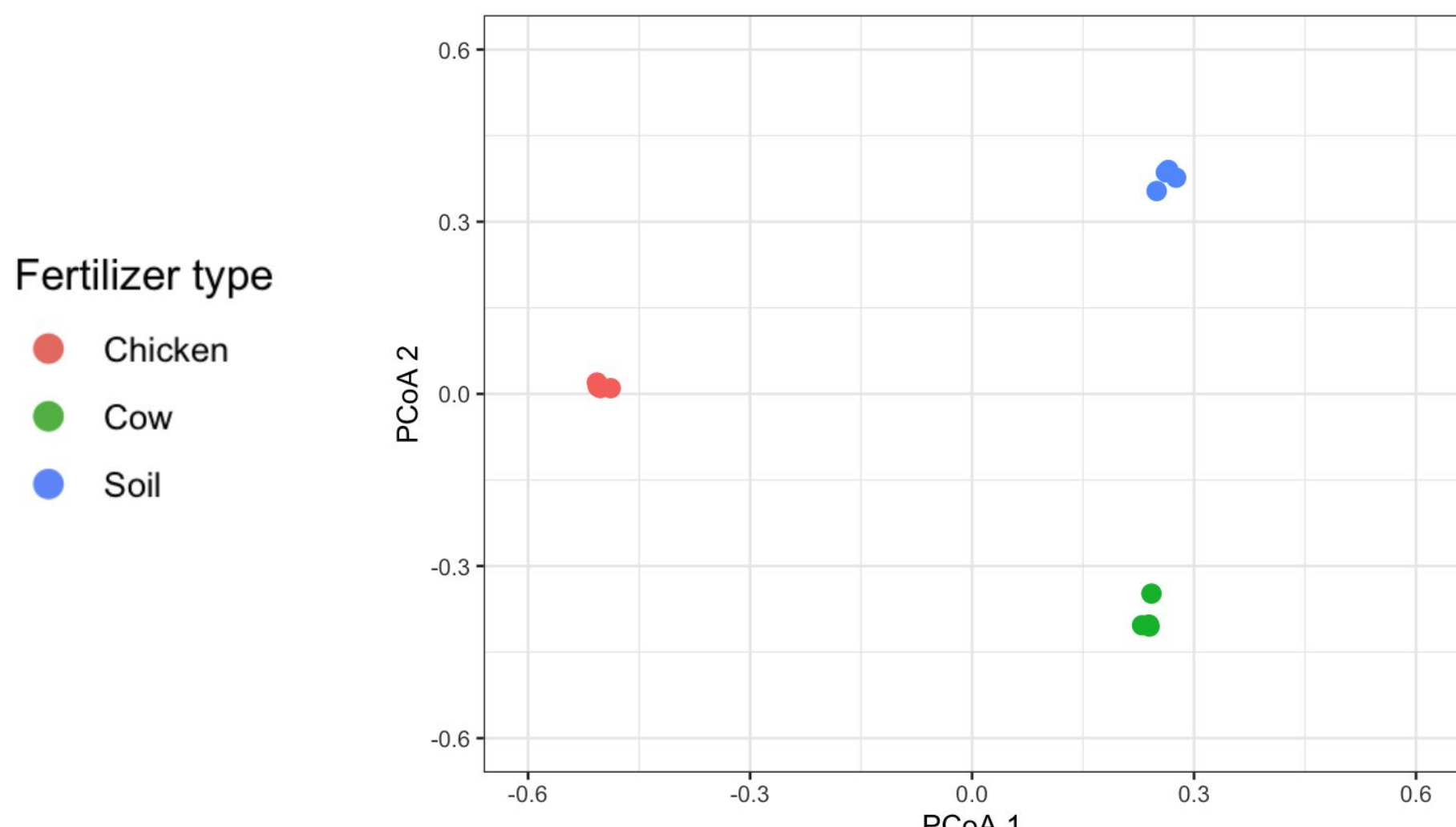


Figure 7: Clustering with PCoA indicates differences in species abundance across samples.

Ampicillin Treated Sample	Antibiotic Resistant Bacteria (ARB)	Percent of Non-Pathogenic Bacteria	Percent of Pathogenic Bacteria
Soil	Present	87.21	13.79
Cow	Present	58	42
Chicken	Present	83.41	17.59
Bat	Not Found	N/A	N/A
Fish	Not Found	N/A	N/A

Figure 8: Bacterial composition and ARB across fertilizers

Figures made with Excel and RStudio

Applications

- This study identifies animal-derived fertilizers carrying diverse or resistant bacterial communities.
- Supports public health efforts to monitor & limit the spread of antibiotic resistance.
- Helps manufacturers of fertilizers to minimize ARB introduction into soil.
- Informs safer use of fertilizers still supporting soil health and crop growth.
- Allows regulators to monitor hotspots of resistance and create safer fertilizer-use guidelines

Future Direction

Test additional antibiotics, including tetracycline, kanamycin, and streptogramins to compare resistance patterns.

Conclusions

- The resistant bacteria identified were either environmentally beneficial species or opportunistic pathogens.
- Cow manure had the highest abundance of ARB, with *Pseudomonas aeruginosa* (42%) and *Pseudomonas putida* (37.6%) together making up about 80% of the bacteria found in the ampicillin-treated sample.
- The results supported my hypothesis that cow manure would contain a high abundance of ARB, showing that manure can act as a reservoir and potentially increase the spread of antibiotic resistance into soil and the wider environment.

References

- Zhao, Y.; Li, L.; Huang, Y.; Xu, X.; Liu, Z.; Li, S.; Zhu, L.; Hu, B.; Zhang, T. Global Soil Antibiotic Resistance Genes Are Associated with Increasing Risk and Connectivity to Human Resistome. *Nature Communications* 2025, 16 (1).
- Hacopian, M. T.; Barrón-Sandoval, A.; Romero-Olivares, A. L.; Berlemont, R.; Treseder, K. K. Warming Is Associated with More Encoded Antimicrobial Resistance Genes and Transcriptions within Five Drug Classes in Soil Bacteria: A Case Study and Synthesis. *Environmental Microbiology* 2025, 27 (4).
- Martin MJ, Thottathil SE, Newman TB. Antibiotics Overuse in Animal Agriculture: A Call to Action for Health Care Providers.

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