Impact of Manure & Nitrogen on the Amount of Antibiotic Resistance Genes in Soil

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Introduction
Antibiotic resistance genes (ARGs) are a concern for human and ecosystem health. Agricultural manure is a source of resistant bacteria with the potential to transfer ARGs by food or contaminate the environment. It is important to study ARGs to have a better understanding of human health, diseases, and the causes of them.

Does animal manure impact the amount of antibiotic resistance genes in soil?

Objective
To evaluate selected Antibiotic resistance genes (ARGs) in manured soil. In this research, the impacts of manure and different amounts of nitrogen on ARGs was studied.

Methods
Collection of Samples, n=48
Replication= 4
Nitrogen rate: N: 125 kg N ha⁻¹, N: 200 kg N ha⁻¹
Precipitation= 100% Irrigation

Culture

DNA Isolation

Quantitative Polymerase Chain Reaction

Results

Quantitative Polymerase Chain Reaction Results

<table>
<thead>
<tr>
<th>Antibiotic Resistance Genes</th>
<th>no manure+N1</th>
<th>no manure+N2</th>
<th>manure+N1</th>
<th>manure+N2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sul1</td>
<td>1.00E+06</td>
<td>1.00E+06</td>
<td>1.00E+06</td>
<td>1.00E+06</td>
</tr>
<tr>
<td>INTI1</td>
<td>1.00E+06</td>
<td>1.00E+06</td>
<td>1.00E+06</td>
<td>1.00E+06</td>
</tr>
<tr>
<td>ermB</td>
<td>1.00E+06</td>
<td>1.00E+06</td>
<td>1.00E+06</td>
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</tr>
<tr>
<td>ctx-m-32</td>
<td>1.00E+06</td>
<td>1.00E+06</td>
<td>1.00E+06</td>
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</tr>
</tbody>
</table>

No difference on amount of gene copies was observed for any of these targets based on manure or nitrogen treatment:
- **sul1**: sulfonamide- important in environment
- **INTI1**: Integrase- helps resistance genes to move
- **ermB**: Macrolide- important in human and animal
- **ctx-m-32**: Beta-lactamase- important in human medicine

Culture Results
Culture based assays indicate no difference on amount of tetracycline resistant bacteria and sensitive antibacterial based on manure or nitrogen treatment.
- **Tet**: Tetracycline- antibiotics

Conclusion
- Based on the results, long term manure application does not increase the ARGs in soil.
- Higher amounts of nitrogen does not impact the amount of ARGs in soil.

Future Work
- 16s microbial community assays are underway.
- Add in 14 assay of tetracycline to collect information on genes.

Acknowledgements
We thank Virginia L. Jin and Marty R. Schmer for maintaining the plots and cooperating in collection of the samples. We also thank all the people in lab: Sue Siragusa, Morgan Meyers, Brandon Nguyen, Alexis Overman, Elizabeth VanWormer, and Vanessa Williams for their support. This program was supported by Grant Program no. 2017-67032-26018 from the USDA National Institute of Food and Agriculture and USDA-ARS NP212, Soil and Air.