Limited impact of one-time dairy manure applications on soil microbial communities in corn-alfalfa rotations

Dan C. Schlatter[1], Sarah C. Castle[2], Melissa L. Wilson[3], Joshua D. Gamble[1]

[1]USDA-ARS Plant Science Research Unit, Saint Paul, MN.

[2]Sustainable Conservation, San Francisco, CA.

[3]University of Minnesota, Department of Soil, Water, and Climate, Saint Paul, MN.

email: Daniel.Schlatter@usda.gov

Introduction

Animal manure is a valuable soil amendment in agroecosystems to dispose of waste, recycle nutrients, and promote sustainability. Manure applications can meet some or all of the nutrient requirements for optimal crop growth while also providing soil health benefits, many of which are mediated via their impacts on soil microbial communities. Recently, single, high-rate applications of manure have been hypothesized to 'prime' soil systems by stimulating organic matter mineralization and nutrient cycling processes, thus having long-lasting impacts on nutrient provisioning to subsequent crops. This 'priming' effect of large manure applications suggests that lasting soil health benefits may offset transportation costs of hauling manure. However, it remains unclear how single, high-rate applications of manure drive soil microbial dynamics across space and time. **The goal of this work is to determine how high and standard rates of dairy manure amendments modify soil microbial communities in corn-alfalfa forage cropping systems across distinct locations that vary in climate and soil characteristics.**

Methods

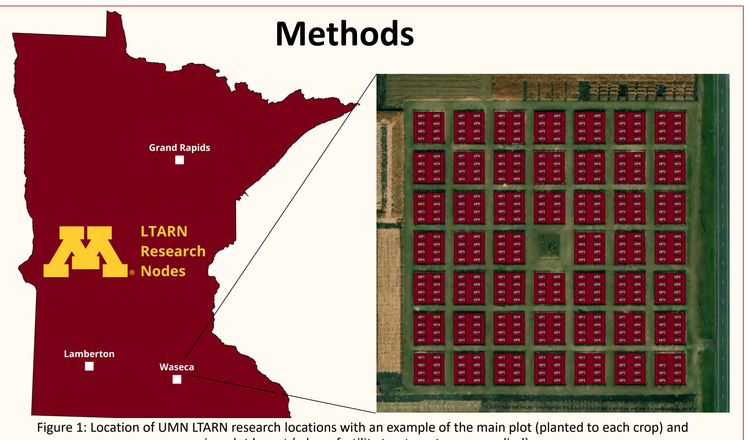

Figure 1: Location of UMN LTARN research locations with an example of the main plot (planted to each crop) and microplot layout (where fertility treatments were applied).

- Main plots (24 x 24 m) were in a 5 year corn-alfalfa rotation (3 years alfalfa, 2 years corn) with 3-4 replicates at three UMN long-term agricultural research sites. Each crop occurs in every year.
- Within main plots, 6 x 6 m microplots received one of four fertility treatments. These included raw dairy manure slurry applied at 1) agronomic recommended rates based on soil tests; 2) high rates of manure for yields 10-20% higher than expected; 3) mineral fertilizer at recommended rates; and 4) no fertility.
- At each site, soil samples (0-15 cm depth) were taken in spring 2019, prior to fertility treatments, and in fall 2019, spring 2020, and fall 2020. Corn and alfalfa rotations were present at each site in each year. Spring sampling occurred before planting and fall samplings after the last harvest.
- Soil DNA was extracted and prokaryotic 16S rRNA (V4 region) and fungal (ITS2) targets were PCR amplified and sequenced on an Illumina MiSeq (2x300bp) flow cell at the University of Minnesota Genomics Center.
- After data processing and denoising, microbial community composition and diversity was evaluated with ordination (NMDS), PERMANOVA , and differential abundance tests (DESeq2) to identify significant shifts in microbial communities and populations among treatments and time points.

Results

- Overall, location (r^2=0.12, p=0.001); time (r^2=0.08, p=0.001), crop phase (r^2=0.02, p=0.001), and fertility treatment (r^2=0.01, p=0.017) all explained significant variation in fungal communities (Figure 2).
- Similarly, for bacteria location (r^2=0.24, p=0.001); time (r^2=0.03, p=0.001), and crop phase (r^2=0.01, p=0.001) explained significant variation, while fertility treatment was not significant (r^2=0.01, p=0.108; Figure 3).

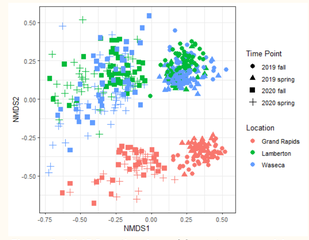

Figure 2: NMDS ordination of fungal communities across locations and time points.

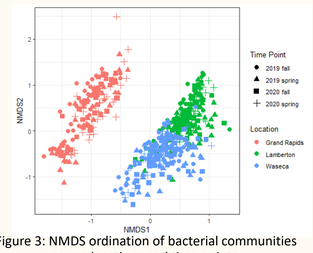

Figure 3: NMDS ordination of bacterial communities across locations and time points.

Table 1A. PERMANOVA r^2 (p-value) of fungal communities after removal of pre-treatment time point (Dissimilarity ~ Time Point + Treatment + Crop Phase + Time Point x Treatment, stratified by main plot).

Factor	Grand Rapids	Waseca	Lamberton
Time	**0.100 (0.001)**	**0.106 (0.001)**	**0.14 (0.001)**
Crop Phase	**0.068 (0.001)**	**0.042 (0.002)**	**0.059 (0.001)**
Treatment	**0.040 (0.001)**	**0.020 (0.001)**	**0.017 (0.001)**
Time : Treatment	0.043 (0.750)	0.032 (0.953)	0.034 (0.574)

Table 1B. PERMANOVA r^2 (p-value) of bacterial communities after removal of pre-treatment time point (Distance ~ Time Point + Treatment + Crop Phase + Time Point x Treatment, stratified by main plot).

Factor	Grand Rapids	Waseca	Lamberton
Time	**0.054 (0.001)**	**0.081 (0.001)**	**0.062 (0.001)**
Crop Phase	**0.061 (0.009)**	0.034 (0.097)	**0.064 (0.005)**
Treatment	**0.022 (0.001)**	**0.031 (0.001)**	**0.015 (0.001)**
Time : Treatment	0.032 (1.000)	0.028 (0.740)	0.025 (0.990)

- Considering post-treatment samples, time point, crop phase, and to a lesser degree fertility treatment significantly impacted microbial community structure (Table 1A, 1B). Crop phase is considered a co-variate in this analysis.
- There were no significant interactions between treatments and time points, indicating similar temporal trajectories of microbial communities across fertility treatments (Table 1A, 1B).

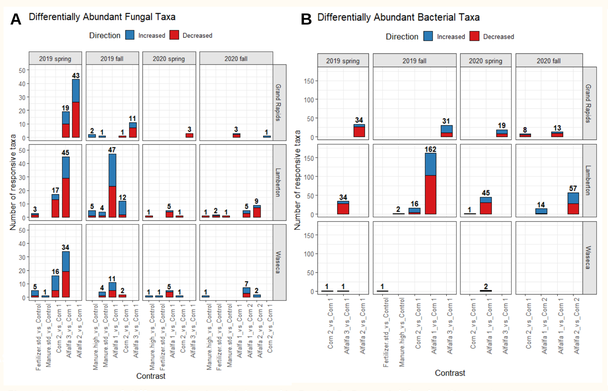

Figure 4: Number of differential microbial taxa (A: Fungi, B: Bacteria) for fertility and cropping phase contrasts among locations and time points. Numbers indicate the total number of differential taxa, where colors indicate whether they were relatively enriched or depleted in a given contrast.

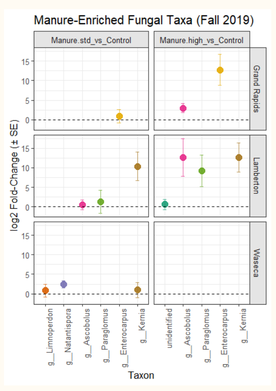

Figure 5: Log-2 fold differences of fungal taxa enriched by manure amendments I the fall of 2019.

- Few individual microbial taxa were responsive to manure applications (Figure 4A, 4B), though some fungal genera were enriched in response to manure in the fall of 2019 (Figure 5). These were often coprophilous taxa (e.g. *Kernia*, *Ascobolus*, *Enterocarpus*).
- Crop phase significantly shifted the relative abundances of many taxa, particularly for fungi in 2019 (Figure 4A). These represented diverse families that were largely location and time specific (data not shown).

Table 2A. PERMANOVA r^2 (p-values) for fungal communities within each location and time point (Dissimilarity ~Treatment x Crop Phase for Treatment and Treatment x Crop Phase; Dissimilarity ~Crop Phase for Crop Phase term, where Dissimilarity is the centroid of microplots within a main plot).

	Time Point	Treatment	Crop Phase	Treatment x Crop Phase
Grand Rapids	Spring 2019 (pre)	0.057 (0.941)	**0.31 (0.028)**	0.124 (0.617)
	Fall 2019	**0.094 (0.001)**	**0.282 (0.0278)**	0.140 (0.281)
	Spring 2020	**0.101 (0.006)**	0.325 (0.056)	0.146 (0.561)
	Fall 2020	0.080 (0.287)	**0.346 (0.028)**	0.133 (0.970)
Waseca	Spring 2019 (pre)	0.062 (0.112)	**0.257 (0.005)**	0.113 (0.455)
	Fall 2019	**0.065 (0.027)**	**0.217 (0.036)**	0.107 (0.561)
	Spring 2020	0.051 (0.771)	**0.283 (0.006)**	0.111 (0.476)
	Fall 2020	0.061 (0.441)	**0.236 (0.049)**	0.116 (0.668)
Lamberton	Spring 2019 (pre)	0.052 (0.625)	**0.30 (0.005)**	0.095 (0.966)
	Fall 2019	**0.056 (0.012)**	**0.284 (0.006)**	0.098 (0.215)
	Spring 2020	0.058 (0.514)	**0.266 (0.010)**	0.128 (0.157)
	Fall 2020	0.064 (0.036)	**0.275 (0.006)**	0.113 (0.257)

Table 2B. PERMANOVA r^2 (p-values) for bacterial communities within each location and time point (Dissimilarity ~Treatment x Crop Phase for Treatment and Treatment x Crop Phase; Dissimilarity ~Crop Phase for Crop Phase term, where Dissimilarity is the centroid of microplots within a main plot).

	Time Point	Treatment	Crop Phase	Treatment x Crop Phase
Grand Rapids	Spring 2019 (pre)	0.064 (0.107)	0.229 (0.139)	0.115 (0.625)
	Fall 2019	0.058 (0.875)	0.231 (0.139)	0.127 (0.481)
	Spring 2020	0.060 (0.257)	0.225 (0.083)	0.120 (0.425)
	Fall 2020	0.054 (0.695)	0.312 (0.083)	0.118 (0.120)
Waseca	Spring 2019 (pre)	0.050 (0.564)	0.198 (0.216)	0.096 (0.731)
	Fall 2019	**0.070 (0.027)**	0.195 (0.265)	0.114 (0.249)
	Spring 2020	0.056 (0.191)	0.199 (0.236)	0.103 (0.353)
	Fall 2020	0.063 (0.099)	0.178 (0.335)	0.095 (0.765)
Lamberton	Spring 2019 (pre)	0.046 (0.640)	0.216 (0.183)	0.087 (0.932)
	Fall 2019	0.041 (0.570)	0.224 (0.134)	0.089 (0.238)
	Spring 2020	0.045 (0.292)	0.194 (0.302)	**0.096 (0.034)**
	Fall 2020	0.043 (0.606)	0.216 (0.192)	0.082 (0.848)

- Within each time point, fungal communities were consistently driven by crop phase, whereas treatment effects were generally limited to the fall 2019 sampling (Table 2A).
- In contrast, soil bacterial communities were rarely significantly associated with fertility treatments or cropping phase (Table 2B).

Conclusions

1. **Spatial and temporal factors were the primary drivers of soil community composition and diversity.**
2. **Cropping phase drove significant shifts in soil community composition, especially for fungi that likely play primary roles in decomposition.**
3. **Fertility treatments, even large doses of manure, had small impacts on soil communities over seasons and crop phases, suggesting that effects of liquid dairy manure were minor and/or short-lived.**
4. **A small number of microbial taxa, especially coprophilous fungi, appeared consistently more abundant after manure treatments. These, however, did not persist over time.**
5. **Priming soil communities for lasting soil health benefits may require higher quantities, different forms, or repeated applications of manure.**

Acknowledgements

This work was supported by funding from USDA-ARS NP215. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture (USDA). USDA is an equal opportunity provider and employer. We greatly appreciate help and guidance from Gregg Johnson and Matt Bickell (UMN LTAR network) in field maintenance and sampling.