REGIONAL CHARACTERIZATION OF ALFALFA AND MANURE LEGACY IMPACTS ON SOIL QUALITY IN CROP ROTATIONS

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Abstract:

Managing agricultural soils for improved soil quality has the potential to enhance resilience and yield, reduce environmental impacts, and increase economic profitability in agricultural production systems. We investigated the impacts of a one-time dairy manure application on soil chemical properties, soil microbial communities, and yield in a corn-alfalfa rotation at three Minnesota sites. Treatments included a high manure rate, low manure rate, mineral fertilizer only, and a control with no nutrient inputs. Yields of first-year corn following alfalfa were, on average, 56 bu. ac^{-1} higher than second year corn across all sites. The high manure rate improved yield of first-year corn and first year alfalfa, but no other impacts of manure applications on yield were found. Soil bacterial community diversity was greater following the fall harvest than in spring prior to planting. Fungal diversity also varied significantly by sampling date and seasonal differences were greater in alfalfa than corn. Overall, microbial community structure differed significantly among study site, sampling date, and crop phase. High manure rates significantly impacted soil fungal, but not bacterial, community compositions. Fungal communities were consistently correlated with available soil K across all sites, and correlations with other soil chemical measurements were site specific. Our results suggest that the impacts of a spring manure application on the overall composition and diversity of microbial communities is limited. However, these results represent only one field season so far. On-going data analyses characterize the impacts of manure additions on different taxonomic groups of bacteria and fungi, and it is possible that this will provide more detailed insight into the specific impacts of manure application on agricultural soil biota

Introduction

Managing agricultural soils for improved soil quality has the potential to enhance resilience and yield, reduce environmental impacts, and increase economic profitability in agricultural production systems. Soils provide fundamental functions in agricultural systems related to nutrient availability and cycling, water availability and infiltration, and the control of pests and diseases. A healthy soil is one that has a high capacity to provide these desired functions (Karlen et al., 1997), many of which are mediated by soil microbial communities. Agronomic practices such as fertilization, organic matter additions, and crop rotation alter soil physical and chemical properties with significant implications for soil microbial communities (Fernandez et al., 2016). Understanding the long-term effects of agronomic practices on soil properties and biota is essential for improving management to optimize these microbial communities for agricultural sustainability.

Alfalfa, a perennial forage legume, is the fourth most widely grown crop in the US, covering over 17 million cropland acres in 2017 (USDA NASS, 2018). Alfalfa provides many benefits to cropping systems, including the "rotation effect" in which subsequent crops experience increased yield potential compared with following other crops. Some of this benefit is attributable to reduced weed, insect, and disease pressure, as well as the substantial amount of symbiotically fixed inorganic nitrogen available in the soil as alfalfa residues decompose (UMN Extension, 2018). Alfalfa also improves soil quality by increasing soil organic matter (SOM) and nitrogen, reducing erosion, and improving soil infiltration, and has been shown to increase soil microbial biomass relative to grass forages (Campbell, 1992; Min et al., 2003). However, little research has been conducted to assess the broader impacts of alfalfa on soil microbial community composition and function. Understanding how alfalfa affects soil microbial populations and function will shed light on the mechanisms contributing to the "rotation affect" and will have far-reaching implications for US forage production systems.

Manure application can also greatly improve soil health. However, to date, most research has been devoted to understanding the impacts of manure applications on crop yield and soil physical and chemical properties. However, there is growing recognition that the benefits of manure application extend beyond simple additions of N and P (Robbins et al., 1997; Tarkalson et al., 2018). For example, manure applications can have long-lasting impacts on soil organic carbon (Grandy et al., 2002) and there is evidence to suggest that manure applications have beneficial impacts on soil microbial communities. A meta-analysis of 64 studies showed that when applied at high rates, cattle manure can increase soil microbial biomass by 60% over and above inorganic fertilizer additions (Kallenbach and Grandy, 2011). Further, the impacts of manure on microbial communities can confer soil health benefits for years after application. For example, Zhang et al., (2018) demonstrated that manure still had a legacy effect on soil microbial community composition for up to 13 years after suspending manure application. They noted increased populations of soil N-fixing and ammonia oxidizing bacteria compared to soils that never received manure, which improved soil N-cycling and crop N utilization in the previously manured soils. However, despite the broad recognition of the potential benefits of dairy manure for increasing soil health and forage crop yields, the specific long-term impacts on soil microbiome community composition and function are relatively unknown.

Here, we investigated the impacts of one-time dairy manure applications on soil chemical properties, soil microbial communities, and alfalfa forage and corn grain and biomass yields and explored relationships between microbial community structure, soil characteristics, and crop yield. This ongoing project is being conducted in coordination with other USDA-ARS locations (Kimberly, ID; University Park, PA; Bushland, TX; Madison, WI) as part of the ARS Dairy Agroecosystem Working (DAWG) group common experiment. The objective of the DAWG experiment is to determine the long-term effects (economic, environmental, and soil chemical, biological and physical) of a one-time manure application. The study idea originated at the USDA-ARS Northwest Irrigation and Soils Lab in Kimberly, Idaho where researchers observed improved crop yields from plots that had received manure nearly a decade ago, but now received only mineral fertilizer, compared to plots that have only ever received mineral fertilizer. Their findings suggest that even short-term or single manure applications can influence soil properties, and likely soil microbes, to the benefit of crops for years following application. However, the mechanisms responsible for this benefit are unknown. Quantifying the benefits of manure to long-term soil health and crop production could enhance its value and improve the economic feasibility of longer distance manure hauling. This will expand the acreage available for land-application, reduce the likelihood of overloading soils near livestock operations, and therefore reduce the risk of N and P losses.

With the goal of increasing forage production system resilience through measures that improve the functionality and the quality of soils, we designed a full factorial dairy manure addition experiment. In order to capture the generality of plant and microbial responses to manure applications, this uses a long-term agricultural research platform including three different agricultural sites varying in climate, soil properties, and historical agronomic practices. Importantly, sites are known to harbor distinct bacterial and fungal communities (Castle et al., 2018; Castle et al. (In Review)).

The objectives of this work are to determine: 1) the impacts of dairy manure applications on soil microbial community structure and yield in forage cropping systems, and 2) the relationships between soil microbial community structure, nutrient-cycling functions, and soil edaphic characteristics in the context of forage-cropped agricultural soils.

Figure 1*: University of Minnesota Long-term Agricultural Research Network (LTARN) locations where the study was conducted.*

Materials and Methods:

This study was conducted at University of Minnesota Long-term Agricultural Research Network (LTARN) sites at three Research and Outreach Centers in Waseca, Lamberton, and Grand Rapids, MN (Fig. 1).

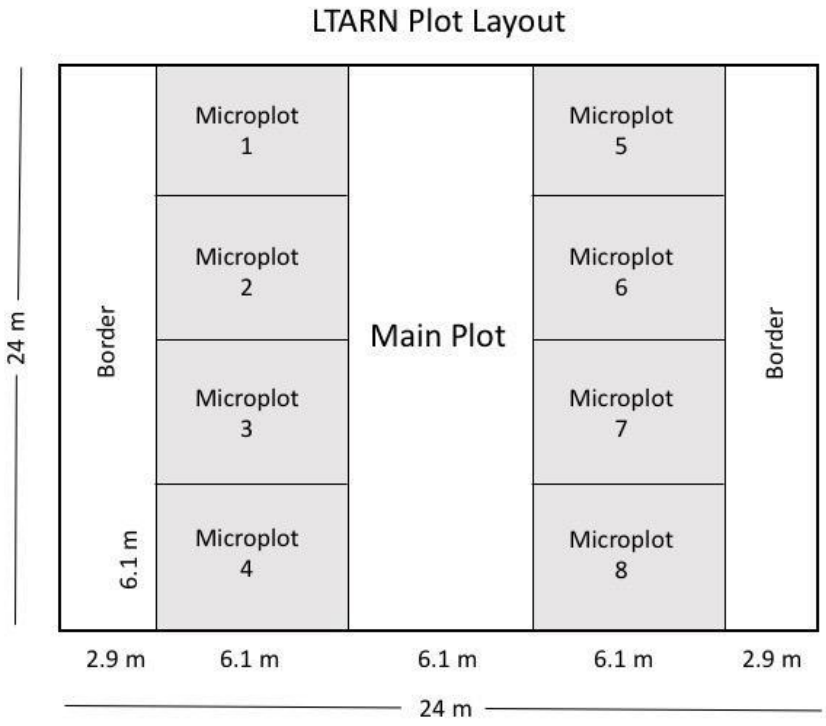

Figure 2: Main plot and microplot layout at LTARN study sites. Manure priming treatments were be randomly assigned to microplots 1 – 4 in each corn-alfalfa rotation main plot.

At each site, experimental plots (24.4 m × 24.4 m) separated by 7.6 m mown grass buffer strips were established on uniform agricultural land representative of regional soil conditions (Fig. 2). Experimental rotations were initiated in four replicates in spring of 2014 at the Waseca and Lamberton locations and in spring of 2015 at Grand Rapids. This research was conducted in one of six main LTARN cropping system treatments, the corn-alfalfa rotation, with both corn and alfalfa present at each site in each year (Table 1).

Table 1: Cropping system sequences at the LTARN locations through 2020

Site	System	Sequence	2018	2019	2020
Waseca	CC/AAA	A	Alf 3	Corn1	Corn 2
	CC/AAA	B	Corn1	Corn 2	Alf1
	CC/AAA	C	Corn 2	Alf 1	Alf 2
Lamberton	CC/AAA	A	Alf 3	Corn1	Corn 2
	CC/AAA	B	Corn1	Corn 2	Alf1
	CC/AAA	C	Corn 2	Alf 1	Alf 2
Grand Rapids	CC/AAA	A	Alf 2	Alf 3	Corn1
	CC/AAA	B	Alf 3	Corn1	Corn2
	CC/AAA	C	Corn1	Corn 2	Alf 1

Within the larger field plots, four microplots (6 m x 6 m) were established and randomly assigned to one of the following treatments:

1. *Agronomic recommended rate of cattle manure + fertilizer (low manure)*. One-time application of manure in spring 2019; from 2020 onward fertilizers applied based on soil tests and established recommendations.
2. *High rate cattle manure + fertilizer (high manure)*. One-time application of manure in spring 2019; from 2020 onward fertilizers applied based on soil tests and established recommendations. Manure and fertilizer for yield goals that are 10-20% higher than expected
3. *Agronomic recommended conventional fertilizer (mineral fertilizer)*: Annual fertilizers applied based on soil tests and established recommendations.
4. *Control*: No nutrient inputs.

Raw dairy manure slurry was obtained from a local commercial producer; subsamples of the manure were sent to a commercial lab for nutrient analysis to ensure accurate nutrient application rates. Dairy manure and fertilizer were applied in spring to each appropriate plot in coordination with LTARN/ Research and Outreach Center staff. In both the spring and fall of 2019, two soil cores were collected to a depth of 15 cm in each microplot. Spring cores were collected prior to any fertilizer or manure applications and fall cores were collected following corn harvest. One core was used for soil chemical analysis and the other soil biological analysis. After collection, samples were placed into placed polyethylene bags, transported on ice to the University of Minnesota, and stored frozen (-80 °C) prior to processing. Genomic DNA was be extracted from frozen soil using the DNEasy PowerSoil DNA Isolation Kit (Qiagen, Germantown, MD), and following the manufacturer's instructions. Microbial composition and diversity were evaluated using high-throughput Illumina amplicon sequencing of the V4 hypervariable region of 16S rRNA genes for bacteria and archaea, and the internal transcribed spacer (ITS2) region of the rRNA operon for fungi. All library preparation and sequencing were completed at the University of Minnesota Genomics Center

Samples for chemical analysis were sent to the University of Minnesota Research Analytical Laboratory and analyzed for NO_3-N, Bray P, Exchangeable K, Mg, Ca, Na and organic C. Corn and alfalfa DM yield were determined by hand harvesting a subsample of plants in each plot and collecting wet weights in the field. Raw sequence data were analyzed using the Qiime2 pipeline. Agronomic data were analyzed with standard procedures with crop yield as response variables. All statistical analysis were done using R (R core team, 2018). In total, these data were used to evaluate the impacts of dairy manure additions, cropping system phase (alfalfa vs. corn), and agricultural site on yield, soil chemistry, and microbial communities.

Table 2: 2019 manure or fertilizer rates for all treatments at the three study sites. Note that manure rates were consistent for all crops whereas mineral fertilizer rates were adjusted for each crop according to University of Minnesota guidelines.

Site	**Treatment**	**Manure Rate**	**C**	**Total N**[*]	**NH$_4$ - N**	**P**[‡]	**K**[§]	**S**
		Gal. / Ac.	----------------------*Pounds per acre*-------------------------					
Grand Rapids	High manure	4854	1860	215	107	79	160	26
	Low manure	3236	1247	144	71	53	107	17
	Mineral fert. (corn1)	-	-	60	-	-	100	15
	Mineral fert. (corn 2)	-	-	130[†]	-	-	100	15
	Mineral fert. (alfalfa 3)	-	-	-	-		150	-
	Control	-	-	-	-	-	-	-
Lamberton	High manure	4854	1805	213	104	79	153	25
	Low manure	3236	1204	142	69	53	102	17
	Mineral fert. (corn1)	-	-	60	-	-	100	15
	Mineral fert. (corn 2)	-	-	130[†]	-	52	100	15
	Mineral fert. (alfalfa 1)	-	-	-	-			-
	Control	-	-	-	-	-	-	-
Waseca	High manure	4854	2273	227	85	82	151	27
	Low manure	3236	1508	151	56	55	101	18
	Mineral fert. (corn1)	-	-	60	-	-	100	15
	Mineral fert. (corn 2)	-	-	130[†]	-	-	100	15
	Mineral fert. (alfalfa 1)	-	-	-	-			-
	Control	-	-	-	-	-	-	-

[*] Applied as urea in mineral fertilizer treatments

[†] Split application of 70 lbs. preplant and 60 lbs. at V5 – V6

[‡] Applied as P_2O_5

[§] Applied as K_2O

Project Objectives and Corresponding Results

Project Objectives

1) Determine the impacts of dairy manure applications on forage crop yield
2) Determine the impacts of dairy manure applications on soil microbial community structure in forage cropping systems
3) Evaluate relationships between soil microbial community structure and soil characteristics in the context of forage-cropped agricultural soil

Project Results

1) High manure rates improved yield of first year corn and first year alfalfa in alfalfa-corn rotations. Second year corn yields were lower than first-year corn regardless of fertility treatment.
2) Bacterial community diversity varied with sampling date. Bacterial diversity was greatest following the fall harvest. Fungal diversity also varied significantly by sampling date and seasonal differences were greater in alfalfa than corn.
3) Overall, microbial community structure differed significantly among study site, sampling date, and crop phase.
4) High manure rates significantly impacted soil fungal, but not bacterial, community compositions.
5) Fungal communities were consistently correlated with available soil K across all sites, and correlations with other soil chemical measurements were site specific.

Results and Discussion:

Crop yield

Total dry matter production varied considerably by crop and site (site x crop interaction: F = 16.59, $P < 0.001$). First year corn following alfalfa (corn 1) yielded 47% more than second year corn (corn 2) at Lamberton and 67% more at Waseca (Fig 3). Yields of first- and second-year corn following alfalfa were similar at Grand Rapids. Across sites, corn yielded substantially more biomass than alfalfa. This was not surprising at Lamberton and Waseca where the alfalfa was newly established in 2019. At Grand Rapids, third-year alfalfa yield 33% less than corn, on average.

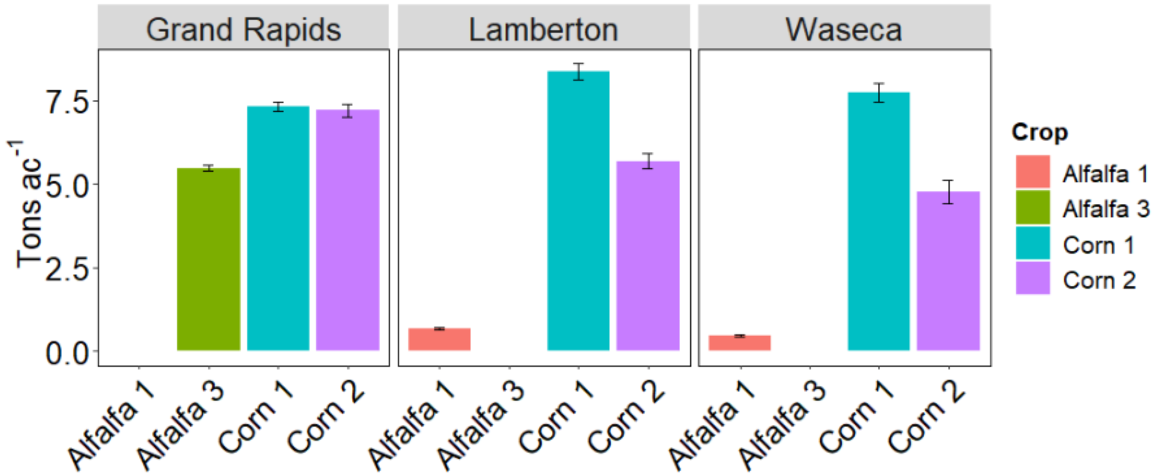

Fig 3: *Crop yield (total dry matter production) by crop and site. This figure shows that first year corn following alfalfa was greater than second year corn at Lamberton and Waseca. No difference in corn yields was observed at Grand Rapids, though third year alfalfa yielded less than corn at this site. Not surprisingly, first year alfalfa yielded less than all corn at Lamberton and Waseca.*

Corn grain yield followed a similar pattern to overall biomass yield, with first year corn yielding 11%, 59%, and 80% more grain than second year corn at Grand Rapids, Lamberton, and Waseca, respectively (Fig 4; crop x site interaction: F = 11.3, $P < 0.001$). This corresponded to an additional 14 bu. ac^{-1} at Grand Rapids, 71 bu. ac^{-1} at Lamberton and 76 bu. ac^{-1} at Waseca.

These findings indicate a substantial yield benefit to corn following alfalfa, and that this benefit diminishes substantially by the second year.

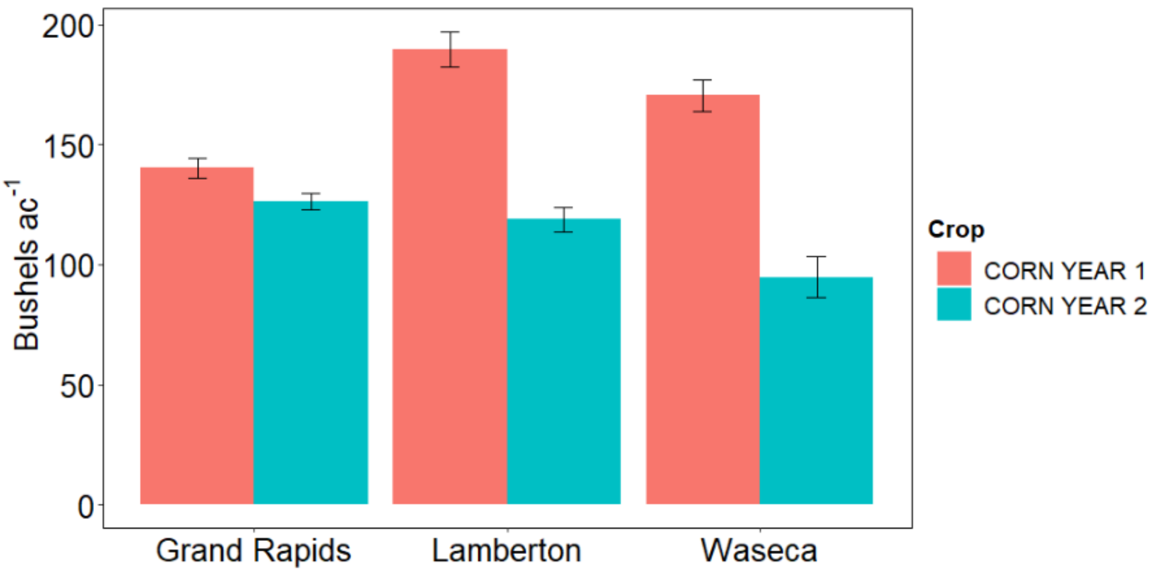

Fig 4: *Corn grain yield by crop (1st vs 2nd year following alfalfa) and site. As with total dry matter, we see here that grain yield was higher for first year than second year corn. This trend was significant across all three sites, though was more pronounced at Lamberton and Waseca.*

Table 3: Corn grain yield by crop and treatment. Values followed by the same letter are not significantly different (P > 0.05) as determined by post-hoc Tukey's HSD

Crop	**Treatment**	**Grain Yield (Bushels ac^{-1})**	**Mean Separation**
Corn 1	Manure (high)	186	A
	Manure (low)	170	A
	Mineral fert.	163	AB
	Control	156	AB
Corn 2	Mineral fert.	132	AB
	Manure (high)	115	BC
	Manure (low)	108	BC
	Control	92	C

Crop dry matter production also varied by fertility treatment for first year corn and first year alfalfa. Across sites, first year corn with the high manure rate yielded more than the first-year corn control (Fig 5; Table 2; treatment x crop interaction: F = 3.97, *P* = 0.012). However, low-rate manure and mineral fertilized first year corn had similar yields to the high manure rate. The high manure rate also resulted in higher first year alfalfa yields compared to the mineral fertilizer and control treatments (main effect of treatment: F = 7.33, *P* = 0.002). No differences in yield were observed based on fertility treatment for third year alfalfa or second year corn (P > 0.05).

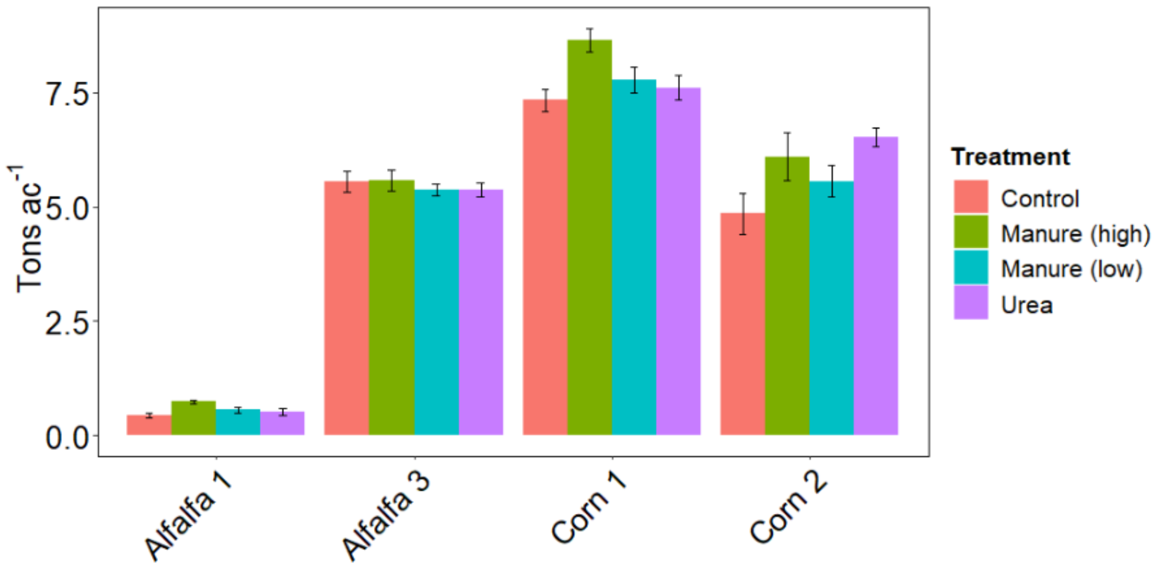

Fig 5: *Crop yield (total dry matter production) by crop and fertility treatment when averaged across sites. We found significant effects of fertility treatment on yield of all crops except Alfalfa 3. Corn and alfalfa were analyzed separately.*

Soil chemical properties

Soil chemical properties to 6-inches depth varied by site. Soil organic carbon, phosphorus, calcium, potassium, and sodium were generally highest at Waseca and lowest at Grand Rapids (Table 3). Soil phosphorus was highest at Grand Rapids. In general, there was little difference in soil chemical properties between the spring and fall sampling. One exception was magnesium, which increased from the spring to the fall sampling as a result of manure application. Spring magnesium levels averaged 11.8 mg kg^{-1} when averaged across sites. By fall, magnesium levels were greater in the high manure treatment (25.8 mg kg^{-1}) and low manure rate (21.7 mg kg^{-1}) than in the mineral fertilized (13.4 mg kg^{-1}) or control plots (12.0 mg kg^{-1}; treatment x season: F = 45.1, $P < 0.001$). No other impacts of manure additions on soil chemical properties were found.

Table 3: Selected soil properties during spring and fall sampling at the three study sites. Means are shown followed by standard deviations in parenthesis.

Site	Season	Total Organic C	NO_3-N	Bray P	K	Ca	Mg	Na
		g kg^{-1}	--mg kg^{-1}--					
Grand Rapids	Spring	13 (1.5)	15.9 (11.1)	43 (14.8)	1559.7 (460.5)	57.9 (23)	7.7 (2.3)	96.3 (32.3)
	Fall	13.4 (1.7)	6.2 (1.9)	40.2 (12.2)	1567.1 (366)	56.9 (21.6)	12.6 (6)	73.8 (24.7)
Lamberton	Spring	16.6 (1.4)	5.7 (3)	17.6 (7.6)	2665.3 (354.1)	706 (102.2)	13.8 (1.7)	164.5 (15)
	Fall	16.9 (1.3)	7.2 (3)	16.4 (5.5)	2772 (372.3)	696.8 (100.7)	21 (8.7)	180 (15.1)
Waseca	Spring	22.1 (4)	9 (7.1)	29.3 (12.6)	3978.5 (800.7)	732.7 (97.8)	13 (1.8)	187.2 (24.3)
	Fall	21.6 (4)	6.7 (3.2)	30.5 (13.3)	4016 (849)	720.6 (97.6)	19.7 (8.2)	210.2 (32.4)

Bacterial Community Metrics

Manure application at either rate (high or standard) did not impact the local diversity of soil bacterial or fungal communities relative to those plots fertilized with urea or those that were not fertilized (Control) ($P > 0.05$ in all cases). Rather, for bacterial communities we observed that diversity varied by sampling date – which was unrelated to manure additions – and this effect depended on site (ANOVA interaction: sampling site x collection date: F = 8.56, $P = 0.0003$; Fig 6). For fungal communities, we observed that diversity also varied by sampling date, but the effect of sampling date depended on whether plots had been planted in corn or alfalfa (ANOVA interaction: sampling site x collection date: F = 4.16, $P = 0.007$; Fig 7).

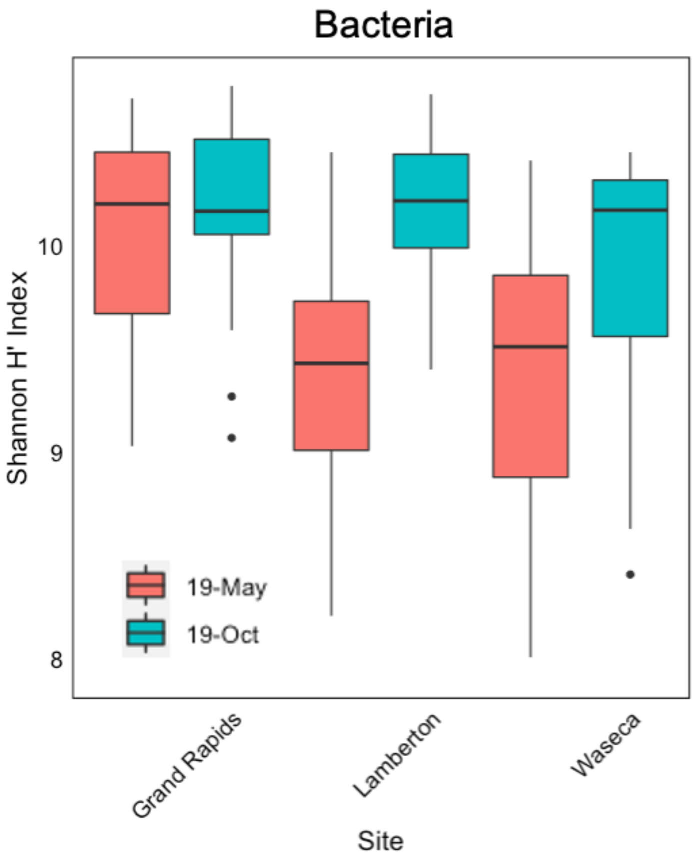

Fig 6. *Bacterial Shannon H' Diversity was significantly greater post-harvest than prior to the spring manure application. The differences by sample collection date occurred regardless of manure application treatment and varied by study site.*

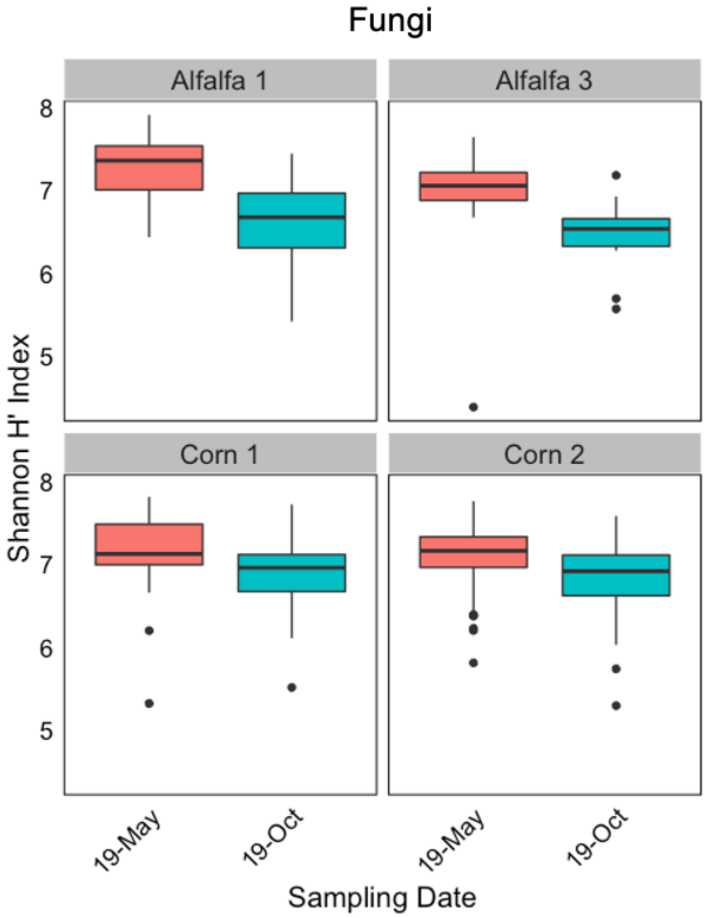

Fig 7. *Soil fungal local diversity (Shannon H' Diversity) varied by sampling date such that diversity was greater at spring than fall sampling, however, seasonal differences were greater for alfalfa than corn plots. Seasonal differences were observed regardless of manure and fertilizer applications.*

Overall and for each site, we observed significant differences in both bacterial and fungal community compositions among different cropping treatments (Fig 8). As has been previously observed in other agricultural systems, we observed that compositional differences were more pronounced in fungal than bacterial communities. This finding is likely due to the close associations that fungal communities are known to have with host plants. In pairwise comparisons, we observed significant impacts of manure application on soil fungal communities. Specifically, we found that plots that had received high rates of manure harbored significantly different fungal communities than those that were treated with urea fertilizer ($r^2 = 0.017$; $P = 0.002$) and those that were not fertilized at all (control, $r^2 = 0.017$; $P = 0.005$).

Additionally, microbial communities were significantly different among sites for both bacteria ($r^2 = 0.16$, $P < 0.001$) and fungi ($r^2 = 0.24$, $P < 0.001$). Within sites, we found that bacterial communities were also significantly different by sample collection date ($r^2 = 0.056$; $P < 0.001$), and the same was observed for fungal communities (($r^2 = 0.041$; $P < 0.001$).

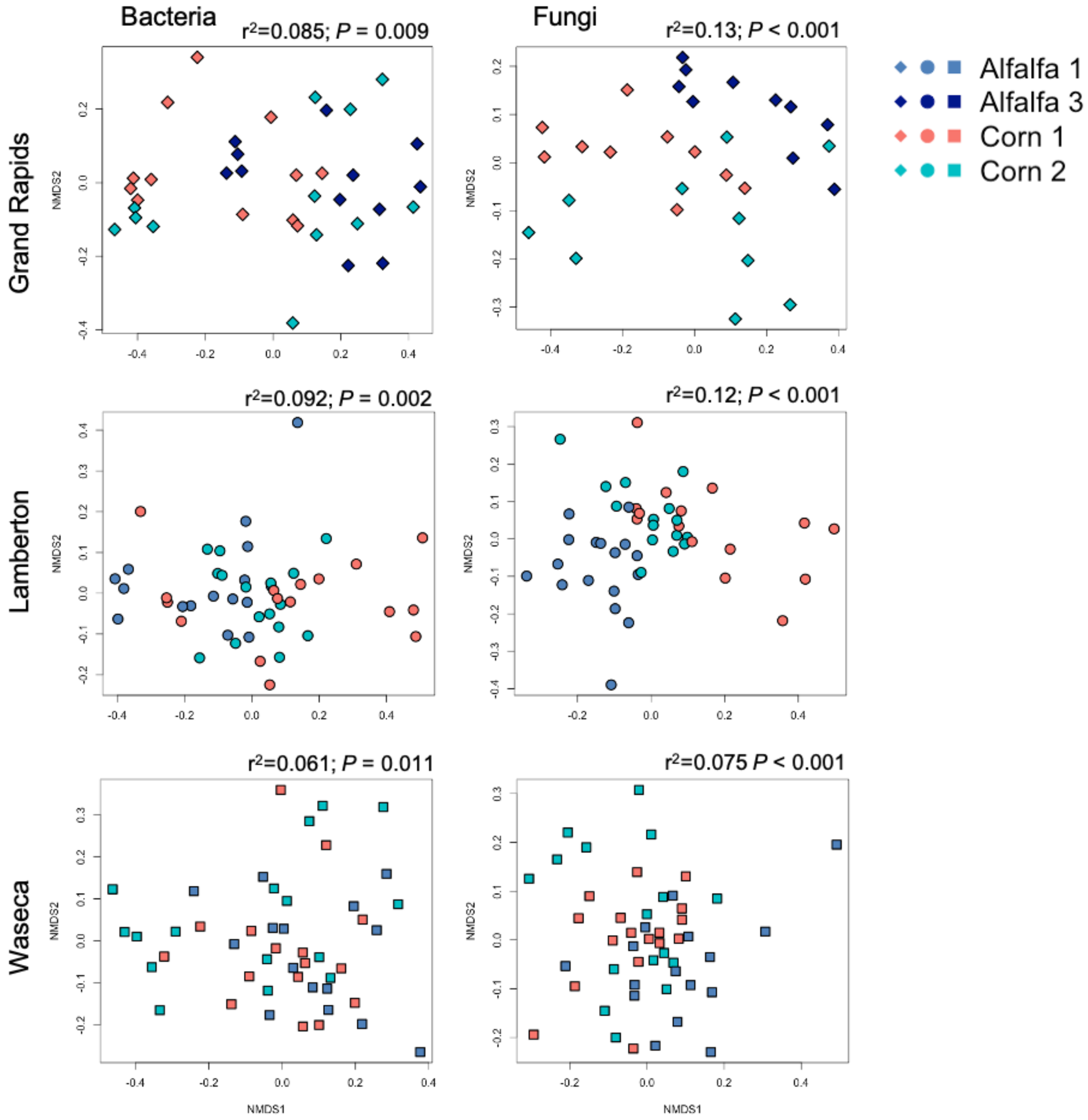

Fig 8. *Microbial compositions were significantly different among crop treatments for all study sites. Data points represent Bray-Curtis dissimilarities of microbial community samples. Statistical significance (shown above each panel) is based on Permanova analyses.*

Bacterial Community and Soil Chemistry Correlates

Spearman's rank correlation analysis of post-harvest sample data revealed that bacterial and fungal community compositions were highly correlated with select soil properties for all sites. Broadly, bacterial community compositions were significantly correlated with soil N and K concentrations for all three sites. Additionally, bacterial and fungal community compositions were uniquely correlated with Bray P, total organic carbon, and available Ca at Grand Rapids, with available Ca at Lamberton, and with N and P concentrations at Waseca. These results, in part, reflect variability in soil chemistry as a result of different crop and manure treatments.

Table 4: Spearman's rank correlations among soil bacterial and fungal community compositions and select soil chemical characteristics for each study site. Analyses included post-harvest (October 2019) data only. Significant correlations are indicated in bold text.

		Bacteria		Fungi	
		rho	*P*	*rho*	*P*
Grand Rapids	NO_3-N	**0.15**	**0.035**	0.10	0.10
	Bray P	**0.31**	**0.0002**	**0.36**	**< 0.0001**
	TOC%	**0.14**	**0.025**	**0.12**	**0.038**
	K	**0.34**	**< 0.0001**	**0.37**	**< 0.0001**
	Ca	**0.44**	**< 0.0001**	**0.46**	**< 0.0001**
	Mg	-0.074	0.90	-0.06	0.79
	Na	0.059	0.19	0.08	0.14
Lamberton	NO_3-N	**0.19**	**0.028**	0.10	0.11
	Bray P	-0.0039	0.51	0.008	0.42
	TOC%	-0.15	0.97	-0.14	0.98
	K	**0.45**	**< 0.0001**	**0.63**	**< 0.0001**
	Ca	**0.21**	**0.0009**	**0.36**	**< 0.0001**
	Mg	0.038	0.26	0.033	0.26
	Na	**0.11**	**0.029**	0.060	0.13
Waseca	NO_3-N	**0.11**	**0.052**	**0.11**	**0.038**
	Bray P	**0.13**	**0.040**	**0.16**	**0.012**
	TOC%	0.087	0.10	0.07	0.12
	K	**0.34**	**< 0.0001**	**0.33**	**< 0.0001**
	Ca	0.041	0.28	**0.12**	**0.053**
	Mg	0.0014	0.47	-0.03	0.67
	Na	-0.061	0.79	-0.02	0.59

Conclusions:

Taken together, our results suggest that the impacts of a spring manure application on the overall composition and diversity of microbial communities is limited. In fact, we observed that only fungal communities were significantly impacted at the high rate of manure application.

However, these results represent only one field season so far. Our future data analyses will include characterizing the impacts of manure additions on different taxonomic groups of bacteria and fungi and it is possible that this will provide more detailed insight into the specific impacts of manure application on agricultural soil biota. Further, we will relate changes in soil chemical characteristics to bacterial and fungal taxonomic groups. These analyses will aid in our understanding of how manure applications alter different functional classes of microbiota.

We will also explore the specific impacts of alfalfa on agricultural soil biota. Alfalfa positively influenced subsequent corn yield. Spring soil tests show that N availability was similar for first- and second-year corn, suggesting that the yield benefit to first-year corn was not N-related but could be related to alfalfa's impact on soil biota.

Acknowledgements: Funding for this study was provided by the U.S. Alfalfa Farmer Research Initiative of the National Alfalfa & Forage Alliance. We also thank Dr. Gregg Johnson and Matt Bickell, Ted Jeo, Melinda Dornbusch, Sue Miller, Peter Lenz, Forrest Goodman, and Galen Bergquist for their assistance with planning, field operations, and data collection

Keywords:

alfalfa; manure; soil health; soil microbial community; rotation effect

References:

Campbell, C.A. 1992. Effect of crop rotations and rotation phase on characteristics of soil organic matter in a Dark Brown Chernozemic soil. Can. J. Soil Sci. 416: 1–14. doi: 10.4141/cjss92-034.

Castle, S.C., D.A. Samac, M.J. Sadowsky, C.J. Rosen, J.L. Gutknecht, et al. Impacts of sampling design on estimates of microbial community diversity and composition in agricultural soils. Microb. Ecol. (In Review).

Castle, S.C., Z. Song, D.M. Gohl, J.L.M. Gutknecht, C.J. Rosen, et al. 2018. DNA Template Dilution Impacts Amplicon Sequencing-Based Estimates of Soil Fungal Diversity. Phytobiomes 2(2): 100–107. doi: 10.1094/PBIOMES-09-17-0037-R.

Fernandez, A.L., C.C. Sheaffer, D.L. Wyse, C. Staley, T.J. Gould, et al. 2016. Associations between soil bacterial community structure and nutrient cycling functions in long-term organic farm soils following cover crop and organic fertilizer amendment. Sci. Total Environ. 566–567(June): 949–959. doi: 10.1016/j.scitotenv.2016.05.073.

Grandy, A.S., G.A. Porter, and M.S. Erich. 2002. Organic Amendment and Rotation Crop Effects on the Recovery of Soil Organic Matter and Aggregation in Potato Cropping Systems. Soil Sci. Soc. Am. J. 66(4): 1311. doi: 10.2136/sssaj2002.1311.

Kallenbach, C., and A.S. Grandy. 2011. Controls over soil microbial biomass responses to carbon amendments in agricultural systems: A meta-analysis. Agric. Ecosyst. Environ. 144(1): 241–252. doi: 10.1016/j.agee.2011.08.020.

Karlen, D.L., M. Mausbach, . J., J.W. Doran, R.G. Cline, et al. 1997. Soil Quality: A Concept, Definition, and Framework for Evaluation. Soil Sci. Soc. Am. J. 61(1): 4. doi: 10.2136/sssaj1997.03615995006100010001x.

Min, D.H., K.R. Islam, L.R. Vough, and R.R. Weil. 2003. Dairy Manure Effects on Soil Quality Properties and Carbon Sequestration in Alfalfa–Orchardgrass Systems. Commun. Soil Sci. Plant Anal. 34(5–6): 781–799. doi: 10.1081/CSS-120018975.

Robbins, C.W., L.L. Freeborn, and B.E. Mackey. 1997. Improving Exposed Subsoils with Fertilizers and Crop Rotations. Soil Sci. Soc. Am. J. 61(4): 1221. doi: 10.2136/sssaj1997.03615995006100040030x.

Tarkalson, D.D., D.L. Bjorneberg, and R.D. Lentz. 2018. Effects of Manure History and Nitrogen Fertilizer Rate on Sugarbeet Production in the Northwest U.S. David D.

Tarkalson, David L. Bjorneberg, and Rick D. Lentz USDA-ARS Northwest Soils and Irrigation Research Laboratory, Kimberly, ID. : 1–9. doi: 10.2134/cftm2017.11.0083.

UMN Extension. 2018. Managing the rotation from alfalfa to corn. https://extension.umn.edu/corn-cropping-systems/managing-rotation-alfalfa-corn.

USDA NASS. 2018. Crop Production 2017 Summary. Natl. Agric. Stat. Serv. http://usda.mannlib.cornell.edu/usda/current/CropProdSu/CropProdSu-01-12-2018.pdf.

Zhang, Y., X. Hao, T.W. Alexander, B.W. Thomas, X. Shi, et al. 2018. Long-term and legacy effects of manure application on soil microbial community composition. Biol. Fertil. Soils 54(2): 269–283. doi: 10.1007/s00374-017-1257-2.