

# Soil Microbial Seasonal Community Dynamics in Response to Cover Crop and Phosphorus Fertilizer Usage in a No-Till Corn-Soybean System in 2018

*C.L. Stewart, L.M. Starr, N.O. Nelson, K.L. Roozeboom, G.J. Kluitenberg, D.R. Presley, and P.J. Tomlinson*

## Summary

This study examined microorganism community composition in plots managed with and without cover crops and three contrasting phosphorus (P) fertilizer management techniques in a no-till corn-soybean system. This work was performed in the spring and fall of 2018 at the Kansas Agricultural Watershed Field Laboratory (KAW), Manhattan, KS. The study design was a 2 × 3 complete block factorial design with three replications, with cover crop presence or absence and three levels of P fertilizer management (control, fall broadcast, and spring injected). To examine microorganism community composition, phospholipid fatty acid (PLFA) analysis was used. Only the main effect of cover crop was found to have a significant impact. Results show greater microbial biomass within plots that had a cover crop as compared to those that did not. The community structure between cover crop plots and non-cover crop plots was similar; however, their abundance was less in non-cover crop plots than in those that had a cover crop.

## Introduction

There are numerous indicators for soil health, soil microorganisms are one component of soil health. A deeper understanding of how soil microbial dynamics respond to management practices can aid in providing more efficient and effective indicators of soil health to benefit producers. A PLFA analysis quantifies phospholipid fatty acids present in a soil sample. Phospholipid fatty acids are found in all cellular membranes and vary in different organisms. For this reason, quantifying phospholipid fatty acids in a soil from contrasting management scenarios can detect differences in the microbial community. Microorganisms tested for in this PLFA analysis include bacteria (prokaryotes) and eukaryotes (Thies, 2008).

Bacteria are prolific within agricultural soils; it is predicted there could be 300,000 different kinds of bacteria within one gram of soil (Gans et al., 2005). There is still much that remains unknown about soil microorganisms; however, some soil bacteria are known to have agriculturally beneficial roles. Many bacteria contribute to agriculture in making nutrients accessible to crops. Some specific kinds of bacteria are known

to be helpful to plants. Actinomycetes are fibrous bacteria that look similar to fine roots. Actinomycetes can form associations with crops to allow crops to have greater access to water and nutrients (Bhatti et al., 2017). A PLFA analysis separates gram positive and gram-negative bacteria, which refers to structural characteristics of the bacterial cell wall. The PLFA analysis performed in this study separates bacteria into the following categories: actinomycetes, gram-negative, gram-positive, and anaerobic. All bacteria are either gram-negative or gram-positive, and this classification relates to the structural characteristics of the bacterial cell wall. Anaerobic bacteria thrive in low oxygen conditions such as wet soils. Bacteria that fall into multiple categories within the PLFA are not counted in multiple categories, for example actinomycetes are a kind of gram-negative bacteria, however, they are quantified only in the actinomycetes category.

Every living organism that is not a prokaryote is a eukaryote. Organisms are classified as eukaryotes based on their cell structure. An PLFA analysis provides the following eukaryotic categories for soil microorganisms: arbuscular mycorrhizal fungi (AMF), fungi, and eukaryotes. Arbuscular mycorrhizal fungi form beneficial associations with crops similar to actinomycetes. Arbuscular mycorrhizal fungi allow crops to have greater access to water and nutrients and are also known to aid in soil structure by producing glomalin. Glomalin can protect plant roots and also binds soil particles together aiding in soil aggregate stability (Chen et al., 2018). Fungi can break down complex organic material that allows greater nutrient availability for crops. There are many different kinds of soil eukaryotes that are not AMF nor another type of fungi, one kind of eukaryote common in agricultural soils are protists. Protists largely consume bacteria and also increase nutrient availability (Bonkowski and Clarholm, 2012). Arbuscular mycorrhizal fungi are a kind of fungi, and all fungi are eukaryotes. However, the PLFA analysis does not list members in more than one group.

This study aims to better understand the dynamics of soil microorganisms in relation to cover cropping and fertilizer treatments. The KAW is managed as a corn, soybean rotation with cover crops planted after harvest each year. Results discussed in this report were from samples taken in the spring and fall of 2018 before termination of a cover crop of triticale and rapeseed (spring) and after harvesting of soybean (fall).

## Procedures

The KAW is located at Kansas State University Ashland Bottoms Research Farm, Manhattan, KS. There are 18 plots at this site that range in size from 1.2 to 1.6 acres. The predominant soil at the site is on Smolan silty clay loam with an average slope of 6 to 8%. Three fertilizer systems were tested: fall broadcast (FB) application of -us, spring injected (SI) application of phosphorus, and no fertilizer application (CN). Each of these fertilizer applications were performed with a cover crop (CC) and with no cover crop (NC). This study utilized a 2 × 3 factorial design with three replicates laid out in a randomized complete block design.

Cover crops were first planted in 2015 and have been planted every year since. Cover crops have included: winter wheat before soybean in 2016, triticale and rapeseed before corn in 2017, and before soybean in 2018. Every year, the same amount of P fertilizer was applied as either a fall broadcast or spring injected applications. The form of P applied in the fall broadcast treatment was diammonium phosphate (DAP) at 120 lb/a

(55 lb P<sub>2</sub>O<sub>5</sub>/a), and the form of P applied in the spring injected treatment was ammonium polyphosphate at 14 gal/a (55 lb P<sub>2</sub>O<sub>5</sub>/a). Nitrogen (N) fertilizer, 28% urea ammonium nitrate, was injected below the surface at a uniform rate of 130 lb N/a for all plots in corn years. In spring and fall of 2018, just before the CC was terminated (spring) and after the cash crop was harvested (fall), soil samples were collected from the 0- to 2-inch depth.

These samples were passed through a 2 mm sieve, frozen, and freeze dried prior to being sent to the Soil Health Assessment Center at the University of Missouri for phospholipid fatty acid (PLFA) analysis. The University of Missouri soil testing lab extracts the samples with an organic solvent and then uses gas chromatography to analyze the samples (Buyer and Sasser, 2012).

## Results

There were no main fertilizer treatment effect and no interaction treatment effects in spring 2018 ( $P > 0.05$  in all categories) (Table 1) nor fall 2018 ( $P > 0.05$  in all categories) (Table 2). The cover crops' main effect was significant in all categories of the PLFA analysis for both spring 2018 ( $P \leq 0.01$  for all categories) (Table 1) and fall 2018 ( $P < 0.05$ ) (Table 2). Total microbial biomass was significantly greater in the cover crop treatment in both spring 2018 ( $P \leq 0.01$ ) (Table 1) and fall 2018 ( $P \leq 0.01$ ) (Table 2) (Figure 1). The microbial community composition as a percentage of the total community in the treatments were managed with cover crops and without cover crops in both the spring and fall 2018 samplings (Table 3).

## Discussion

The findings from the PLFA analysis show a higher abundance of microorganisms present in plots that had a cover crop as compared to plots that did not have a cover crop. This finding was not surprising given that cover crops are known to support microbial populations, which is likely due to their ability to provide nutrients to microbes when cash crops are not present in fields, specifically increasing organic carbon in soil (Finney et al., 2017; Lehman et al., 2015; McDaniel et al., 2014; Nair et al., 2012; Spedding et al., 2004). The community makeup of microorganisms was found to be similar between both the cover crop and the no cover crop plots; however, this is in contrast to other research that demonstrates cover crops impacting the community makeup of microorganisms (Finney et al., 2017). This difference could be due to the sampling depth, as other work (Finney et al., 2017) examined depths deeper than 2 in., and it is possible that at depths beyond 2 in. there may be a different microbial community makeup than what was found in this study. The results discussed here are from a single time point, and as such it will be interesting to see whether findings presented here remain consistent over multiple growing seasons, or if the differences between cover crop and no cover crop plots develop with time.

These results show an increase in the abundance of soil microorganisms with the use of cover crops. Soil microorganisms aid in nutrient cycling processes, allowing nutrients to become available to crops; they also aid in soil structure. Cover crops may offer benefits to soil health in respect to the soil microorganism community, however, their relation to direct yield benefits remains to be determined.

## References

- Bhatti, A.A., Haq, S., Bhat, R.A. Actinomycetes benefaction role in soil and plant health. *Microbial Pathogenesis*. 111:458-467. 2017.
- Bonkowski, M., & Clarholm, M. Stimulation of plant growth through interactions of bacteria and protozoa: Testing the auxiliary microbial loop hypothesis. *Acta Protozoologica*, Vol. 51, pp. 237–247. <https://doi.org/10.4467/16890027AP.12.019.0765>. 2012.
- Buyer, J.S., Sasser, M. High throughput phospholipid fatty acid analysis of soils. *Appl Soil Ecol* 61, 127-130. 2012.
- Chen, M., Arato, M., Borghi, L., Nouri, E., Reinhardt, D. Beneficial Services of Arbuscular Mycorrhizal Fungi – From Ecology to Application. *Front. Plant Sci.* 2018.
- Finney, D.M, Buyer, J.S., Kaye, J.P. Living cover crops have immediate impacts on soil microbial community structure and function. *Journal of Soil and Water Conservation*. 72 (4) 361-373. 2017.
- Gans J., Wolinsky M., Dunbar J. (2005) Computational improvements reveal great bacterial diversity and high metal toxicity in soil. *Science* 309:1387–1390.
- Lehman, R.M., Cambardella, C.A., Drott, D.E., Acosta-Martinez, V., Manter, D.K., Buyer, J.S., Maul, J.E., Smith, J.L., Collins, H.P., Halvorson, J.J., Kremer, R.J., Lundgren, J.G., Ducey, T.F., Jin, V.L., Karlen, D.L. Understanding and Enhancing Soil Biological Health: The Solution for Reversing Soil Degradation. *Sustainability*. 7:988-1027. 2015.
- McDaniel, M. D., Tiemann, L. K., & Grandy, A. S. (2014). Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. In *Ecological Applications* (Vol. 24). Retrieved from <http://www.metawinsoft.com/>.
- Nair, A., & Ngouajio, M. (2012). Soil microbial biomass, functional microbial diversity, and nematode community structure as affected by cover crops and compost in an organic vegetable production system. *Applied Soil Ecology*, 58, 45–55. <https://doi.org/10.1016/j.apsoil.2012.03.008>.
- Spedding, T. A., Hamel, C., Mehuys, G. R., & Madramootoo, C. A. (2004). Soil microbial dynamics in maize-growing soil under different tillage and residue management systems. *Soil Biology and Biochemistry*, 36(3), 499–512. <https://doi.org/10.1016/j.soilbio.2003.10.026>.
- Thies, J.E. Molecular Methods for Studying Microbial Ecology in the Soil and Rhizosphere. In: *Molecular Mechanisms of Plant and Microbe Coexistence*.

**Table 1. Spring 2018 *P*-values and least significant differences (LSD) of treatments in (pmol/g)**

Spring 2018	Treatment groups	Total biomass	AM fungi	Gram negative	Gram positive	Fungi	Anaerobe	Actinomycetes	Eukaryotes
<i>P</i> -value	Fertilizer × CC	0.42	0.75	0.48	0.44	0.69	0.24	0.37	0.07
	Fertilizer	0.43	0.52	0.40	0.36	0.46	0.36	0.69	0.13
	CC	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*
LSD	CC	11260.69	563.20	3627.8	2704.02	868.17	156.62	1342.78	173.72

\* Indicates statistically significant *P*-values. *P* < 0.05.  
 CC = cover crop. AM = arbuscular mycorrhizal.

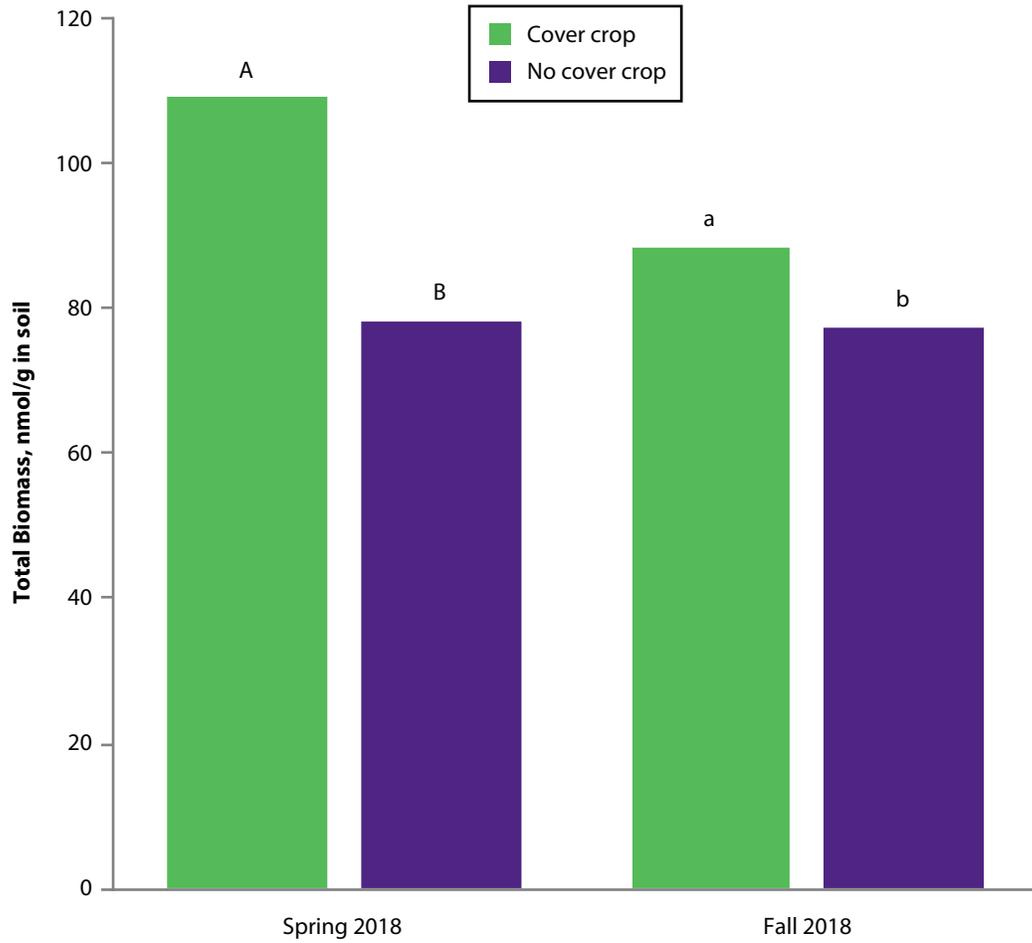
**Table 2. Fall 2018 *P*-values and least significant differences (LSD) of treatments in pmol/g**

Fall 2018	Treatment groups	Total biomass	AM fungi	Gram negative	Gram positive	Fungi	Anaerobe	Actinomycetes	Eukaryotes
<i>P</i> -value	Fertilizer × CC	0.20	0.25	0.31	0.20	0.24	0.33	0.35	0.47
	Fertilizer	0.70	0.73	0.76	0.54	0.76	0.77	0.80	0.23
	CC	<0.01*	<0.01	0.02*	<0.01*	0.04*	<0.01*	<0.01*	0.04*
LSD	CC	6145.54	287.22	2248.92	1564.38	439.14	114.56	883.27	222.25

\* Indicates statistically significant *P*-values. *P* < 0.05.  
 CC = cover crop. AM = arbuscular mycorrhizal.

**Table 3. Phospholipid fatty acid analysis microorganism community category breakdown by percent in spring and fall 2018**

Microorganism category	Spring 2018		Fall 2018	
	Cover crop	No cover crop	Cover crop	No cover crop
	----- % -----			
Fungi	3.8	3.1	3.7	3.5
Arbuscular mycorrhizal fungi	4.6	4.3	5.2	5.1
Actinomycetes	16.3	17.5	15.7	15.8
Anaerobic bacteria	1.8	1.8	1.9	1.9
Eukaryotes	2.0	1.6	2.3	2.3
Gram negative bacteria	39.8	39.0	39.8	40.9
Gram positive bacteria	31.7	32.7	31.3	30.5



**Figure 1. Total microbial biomass measured by phospholipid fatty acid analysis for spring and fall 2018 in plots with cover crop and plots without cover crop (no cover).**