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Phospholipid Fatty Acid Profiling for Soil Microbial Community Analysis in Soil Conservation Farming, Missouri

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ABSTRACT

Cover crops and crop rotations are utilized for improving degraded soils by creating a continuous ground cover and adding organic matter. They improve soil microbial biodiversity which is also an indicator of soil health. The objective of this study was to evaluate the impact of cover crops and crop rotation on soil microbial properties using phospholipid fatty acid (PLFA) profiles. Corn (*Zea mays* L.)/soybean (*Glycine max.* L), (CS) and corn/soybean/wheat (*Triticum aestivum* L.) (CSW) rotation with various cover crop mixtures was tested. The control treatment had no cover crops. The PLFA profile results indicated that total bacteria (3331-1487 ng g^{-1} soil), fungi (980-355 ng g^{-1} soil), protozoa (111-25 ng g^{-1} soil), actinomycetes (613-263 ng g^{-1} soil) were significantly higher in CSW plots compared to CS rotation plots and control, indicating the impact on soil microbial population. The short period of cover crop incorporation did not influence soil microbial population significantly.

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INTRODUCTION

Historically, farmers have been using cultural practices including crop rotation, green manuring and cover crops to replenish organic matter and nutrients back into the soil (Paine and Harison, 1993). However, advancements in conventional agriculture have led to the widespread use of inorganic fertilizers, which has contributed to the reduction in various conservation cultural practices (Ritter *et al*., 1998; Franzluebbers, 2007).

In the recent past, there has been a renewed interest in the benefits of conservation agriculture practices to improve soil health and nutrient status following a period of significant deterioration of land resources and nutrient status of soils (Zalidis *et al.*, 2002). Various soil physical benefits of conservation agriculture include reduced wind and water erosion, reduced non-point source pollution, and improved water infiltration (Hartwig and Ammon, 2002; Malik

et al., 2008; Udawatta *et al.*, 2011). Among conservation agricultural practices, crop rotation and cover crops improves oil structure by providing ground cover, increasing soil organic matter and microbial biomass (Steenwerth and Belina, 2008), improving habitat for soil microorganisms, increasing soil fertility, reducing nutrient leaching, and suppressing weed growth (Reeves, 1994). Cover crops also enrich soils and have positive effects on crop yield (Kremer and Kussman, 2011; Delgado *et al.*, 2007). Cover crops increase carbon sequestration potential of the soil and soil microbial diversity, which, in turn, will enhance soil health (Kremer and Kussman, 2011; Dabney *et al.*, 2001). Since cover crops enhance the availability of soil nutrients (Delgado *et al.*, 2007; Zhu *et al.*, 1989) farmers can save on fertilizer application, production costs while reducing negative water quality effects.

The use of cover crops as an effective conservation practice has been well documented because it had been found to provide multiple benefits to soil health (Kuo and Jellum, 2002). Soil health has a distinct correlation to soil microorganisms and soil microbial processes that in turn, influence soil functions and productivity (Sparling *et al.*, 1997; Niemeyer *et al.*, 2012). Crop rotation, plant species diversity, and associated soil physicochemical properties affect soil microbial population and functions. Plant-microbial interactions drive the recycling of nutrients along with other biogeochemical processes in the soil (Fierer and Jackson, 2006; Schlatter *et al.*, 2015). There is limited information in the published body of knowledge on how microbial diversity is influenced by cover crops and crop rotation. It is important to develop a better understanding of how cover crops and crop rotations influence soil microbial biomass and functions thus helping the development of efficient land management practices for increased land productivity and environmental benefits.

Molecular techniques such as denaturing gradient gel electrophoresis (DGGE) community profiling (Muyzer *et al.*, 1993; Bardhan *et al.*, 2012), DNA cloning, probing and sequencing (Borneman *et al.*, 1996), amplified rDNA restriction analysis (Vaneechoutte *et al.*, 1992) and phospholipid fatty acid profiles (Frostegrad and Baath, 1996; Schutter and Dick, 2000) are used to better identify the microbial community structure in different ecosystems. PLFAs are integral parts of cell membranes and are used as markers to represent active and viable populations of microbes within soil environments (Zelles, 1999). Microbial profiles obtained from PLFA are unique to different groups of microorganisms such as bacteria, fungi, and actinomycetes, and therefore are widely used in microbial ecology research to identify metabolically active microbial communities and compare these communities between various ecosystems. We hypothesize that incorporating cover crops will improve soil microbial abundance and diversity. The specific objectives of this study were to evaluate the impact of crop rotations and cover crop combinations on soil microbial biomass and function and soil microbial community structure using PLFA profiling in a cover crop demonstration farm in Chariton County, north-central Missouri.

MATERIALS AND METHODS

Study Area and Soil Sampling

The study was initiated in 2012 at the 52 ha Chariton County Cover Crop Soil Health (CCSH) Farm in north-central Missouri (39°50′ N and 92°72′ W; Figure 1). This farm consists of Grundy silt loam, 2-5% slopes (Fine, smectitic, mesic Aquertic Argiudolls) and Armstrong loam, 5-9% slopes (Fine, smectitic, mesic Aquertic Hapludalfs). Claypans between ~25-20 cm depths in these soils with smectitic clays reduce water permeability. Presence of high shrink-swell, smectitic clay in the subsoil results in low saturated hydraulic conductivity, poor infiltration, and high potential runoff. The mean precipitation of the area is 986 mm of which ~63% falls during April through September. The mean temperatures are 30 °C in July and -6 °C in February.

The CCSH farm was planted in corn (*Zea mays* L.) - soybean (*Glycine max*. L) rotation using a no-tillage planter. Crops were cultivated using conventional practices before the establishment of the experiment. Five plots in the northern section of the farm were planted to cover crops mixes (plots A through E;

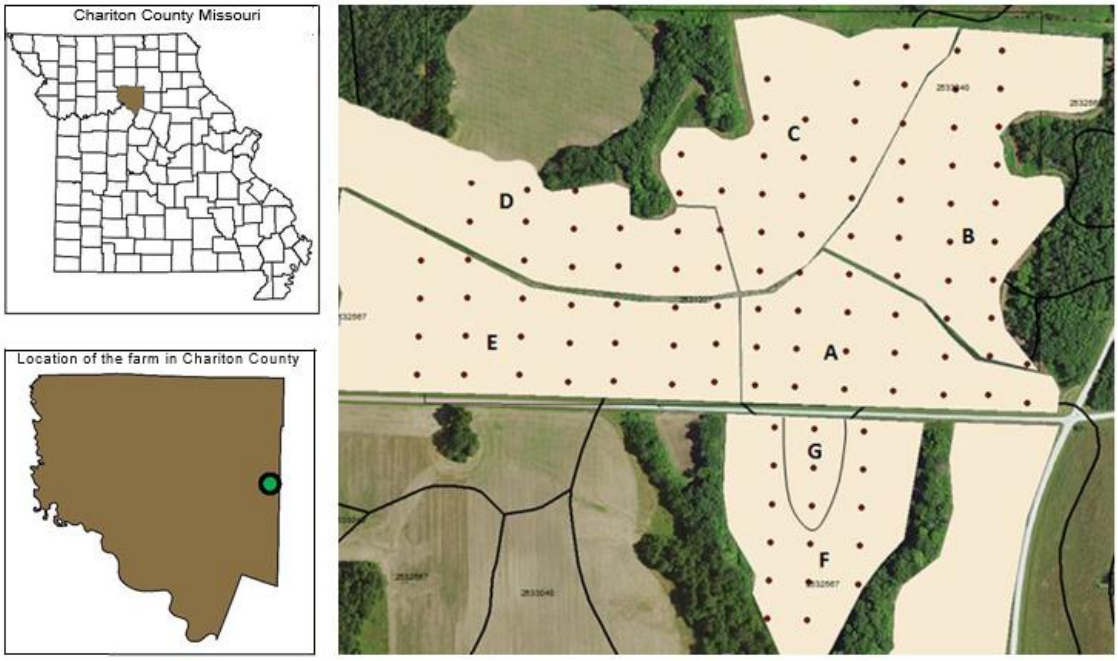

Figure 1: Geographic location and plot demarcation of the Chariton County Soil health farm. The inset map shows the location of Chariton County in Missouri and the farm location in Chariton County. Plots A, B, C had CSW rotation while D, E, F and G had CS rotation. All plots, except, F and G include cover crops

Table 1: Combination of cover crops by experimental plots for 2013 to 2015

Year	Plots						
	A	B	C	D	E	F	G
2013	Sunn Hemp (100)	Oats (33) Rapeseed (33) Yellow Mustard (33)	Rye (15) Hairy Vetch (85)	Pea (50) Radish (50)	Rye (10) Hairy Vetch (25) Winter Pea (15) Radish (10) Cowpea (15) Turnip (10) Sorghum Sudan (5) Annual Clover (10)	NC[Ŧ]	NC[Ŧ]
2014	Barley (75) Wheat (25)	Triticale (75) Wheat (25)	Wheat crop	Barley (100)	Cereal Rye (50) Triticale (20) Barley (20) Wheat (10)	NC[Ŧ]	NC[Ŧ]
2015	Winter Oats (20) Winter Cereal Rye (40) Winter Triticale (40)	Wheat crop	Winter Oats (10) Canola/Rape (15) Hairy Vetch (50) Crimson Clover (10) Winter Pea (15)	Winter Barley (20) Winter Cereal Rye (40) Winter Triticale (40)	Winter Oats (20) Winter Cereal Rye (40) Winter Triticale (40)	NC[Ŧ]	NC[Ŧ]

[Ŧ]NC: No cover crops; Numbers in parenthesis represent the seeding ratio of the mixes; Wheat crop in bold depicts main crop of the rotation

Figure 1; Table 1). Two control plots (F and G) with no cover crops were located in the southern section of the farm.

Soil cores (0-15 cm) were collected from 115 locations in 17 transects on September 1, 2015. Three soil cores (diam. 2 cm) were sampled from each location in a 50-m grid pattern and a composite sample was prepared for subsequent analysis. The samples were placed in labeled plastic bags, transported on ice in coolers and stored at 4 °C until analysis. Samples were shipped on dry ice to the Ward Laboratories, Kearney, Nebraska for the PLFA analysis. The analysis was performed following the protocol developed from peer-reviewed publications on phospholipid fatty acid research (Table 2).

Table 2: PLFA markers used for taxonomic microbial groups and soil biological stress indicator

Taxonomic group	Specific PLFA markers
Bacteria	10:0 2OH, 10:0 3OH, 11:0 iso 3OH, 12:0 2OH, 14:0 iso, 14:0 2OH, 14:0 iso 3OH, 15:0, 15:0 iso, 15:0 anteiso, 16:0 iso, 16:1 ω7c, 16:1 ω9c, 16:0 2OH, 17:0, 17:0 iso, 17:0 anteiso, 17:0 cyclo, 18:1 ω5c, 18:1 ω7c, 19:0 iso, 19:0 anteiso, 19:0 cycloω8c, 19:0 cycloω9, 19:0 cycloω6
Gram +ve	14:0 iso, 15:0, 15:0 iso, 15:0 anteiso, 16:0 iso, 17:0, 17:0 iso, 17:0 anteiso, 19:0 iso, 19:0 anteiso
Gram -ve	10:0 2OH, 10:0 3OH, 11:0 iso 3OH, 12:0 2OH, 14:0 2OH, 14:0 iso 3OH,16:1 ω7c, 16:1 ω9c, 16:0 2OH,17:0 cyclo,18:1 ω5c, 18:1 ω7c,19:0 cycloω8c, 19:0 cycloω9, 19:0 cycloω6
Fungi	18:1 ω9c, 18:2 ω6c, 18:3 ω3c, 20:5 ω3c
Protozoa	20:2 ω3c, 20:2 ω6c, 20:3 ω3c, 20:4 ω6c
AMF	16:1 ω5c, 20:1 ω9c, 22:1 ω9c
Actinomycetes	16:0 10-methyl, 17:0 10-methyl, 18:0 10-methyl
Microbial Stress Indicators	
S/M ratio	Sat/Mono PLFAs
Pre/Cy	16:1ω7/ cy17:0
	18:1ω7/ cy19:0

Statistical Analysis

Soil parameters were analyzed statistically using JMP (SAS Institute). A one-way ANOVA was used for comparison of variables at a predetermined level of significance (α = 0.05). The assumption of homogeneity of variance was tested using the Levene test. Comparison of means, at a predetermined level of significance ($p< 0.05$), was performed using the least significant difference (LSD) method (SAS Institute) after first confirming that treatment effects were statistically significant ($p< 0.05$).Graphs were generated using Sigma Plot 11.0.

ArcGIS 10.3 software was used for storage, projection, and analysis of all spatial information and interpolative analyses (ESRI ArcView 10.3). Ordinary kriging was used to analyze the spatial structure and variability of the data. A semi-variogram was fit using a spherical model to minimize error variance and to determine the degree of spatial autocorrelation between pairs of sampled locations (Isaaks and Srivastava, 1989).

RESULTS AND DISCUSSION

Among the different experimental plots, plots A, B, and C were planted under CSW rotation whereas plots D through G were planted under a CS rotation. The cover crop treatments included a diverse mix of species and were highly variable among the plots and are detailed in table 1. As revealed from the analyses, there was a significant effect of crop rotation and cover crop (Figure 2) on the total PLFA concentrations in the various experimental plots. The total PLFA is a measure of active living biomass in the soil and was highest in plot A (6580 ng g^{-1} soil), which was significantly higher than any other experimental plot. Plot B and C had average total biomass of 4208 and 4082 ng g^{-1} soil, respectively. The average total biomass for all other plots ranged from 2688-3469 ng g^{-1} soil, which was lower than the plots with a CSW rotation. Absolute total bacterial and fungal PLFA concentrations followed the same patterns as the total PLFA concentrations (Figure 2). Similar trends were observed for all functional groups of microbial communities (Table 3), which included gram +ve/-ve bacteria, actinomycetes, arbuscular mycorrhizal fungi (AMF), fungi and protozoa.

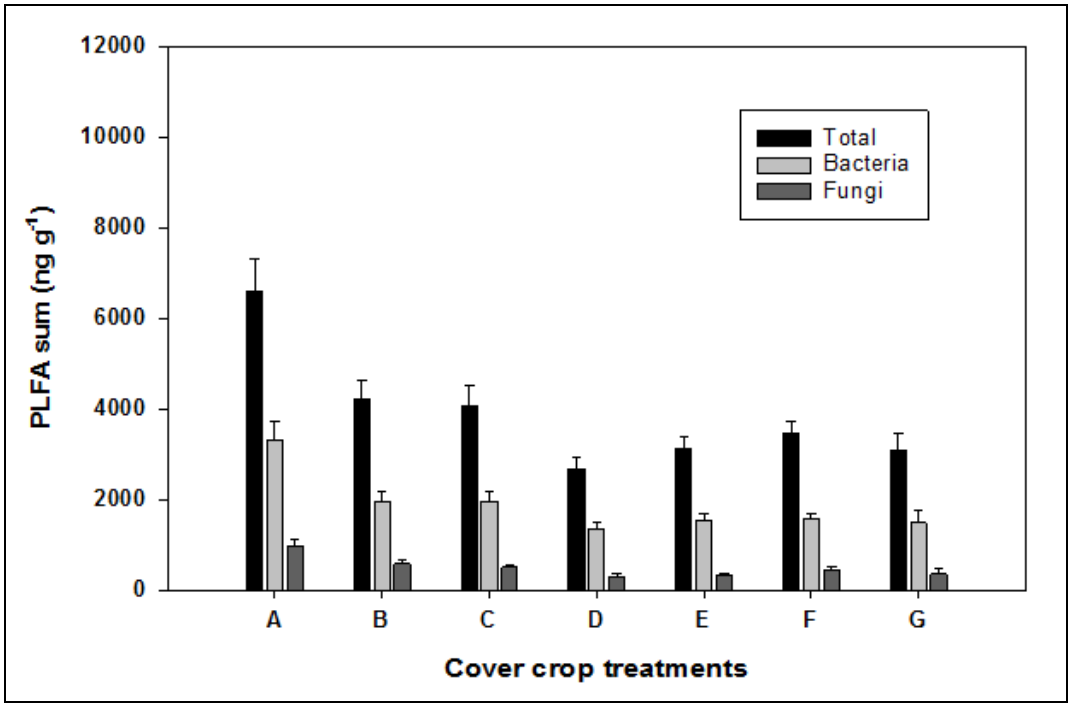

Figure 2: Total PLFA and sum of bacterial and fungal PLFA concentrations for the various experimental plots in the Chariton County Soil Health Farm. Plots A, B, C had CSW rotation while D, E, F, and G had CS rotation. All plots, except, F and G include cover crops

For all functional groups, the highest concentration of PLFA was found in plot A followed by B and C, all three of which had CSW rotation management practice. These findings are consistent with the existing body of knowledge regarding soil microbial diversity concerning crop rotations and incorporation of cover crops (Schutter *et al.*, 2004; Lupwayi *et al.*, 1998). Zelles *et al.* (1992) have found that the fallow phase of a crop rotation resulted in a decrease of soil microbial diversity and the addition of winter cover crops enhanced microbial diversity (Schutter *et al.*, 2004). Lupwayi *et al.* (1998) reported increased soil bacterial diversity for crop rotations using red clover (*Trifolium patense*) green manure or field pea (*Pisum sativum*) following wheat. The generally healthy ratio between gram-positive/negative bacteria concentration indicates that there is little difference in ecological stress or limiting resources among the different treatments (Kaur *et al.*, 2005).

Table 3: PLFA concentration for different functional groups in the experimental plots expressed in ng g^{-1} soil

Particulars	A	B	C	D	E	F	G	Avg. All plots
	(ng g^{-1} soil)							
Total Bacteria	3331.6	1966.6	1970.1	1352.4	1541.8	1567.0	1487.2	1945.0
Actinomycetes	613.0	346.4	368.4	263.6	315.6	287.1	309.1	365.7
Gram (-)	1515.9	883.1	820.0	516.2	569.8	665.8	557.0	819.4
Gram (+)	1815.7	1083.5	1150.1	836.2	972.0	901.2	930.2	1125.6
Rhizobia	178.9	104.5	83.9	53.4	32.8	54.4	35.4	82.6
Total Fungi	980.8	590.8	504.3	302.6	339.8	447.8	355.0	520.5
Arbuscular Mycorrhiza	435.9	280.2	176.5	91.0	118.7	156.0	122.4	207.4
Saprophytic	544.8	310.6	327.8	211.6	221.1	291.8	232.6	313.6
Protozoa	111.3	58.5	55.7	25.9	35.9	55.7	41.6	56.2

Plots A, B, C had Corn-Soybean-Wheat rotation while D, E, F, and G had Corn-Soybean rotation. All plots, except, F and G include cover crops

A linear relationship between soil carbon content and total microbial biomass was observed in the experimental plots, *i.e.*, the plots which had the highest total carbon content in the soil also had the highest total biomass (data not shown). It is widely documented that soil organic matter (SOM) is one of the most important components influencing soil microbial population and function (REF).

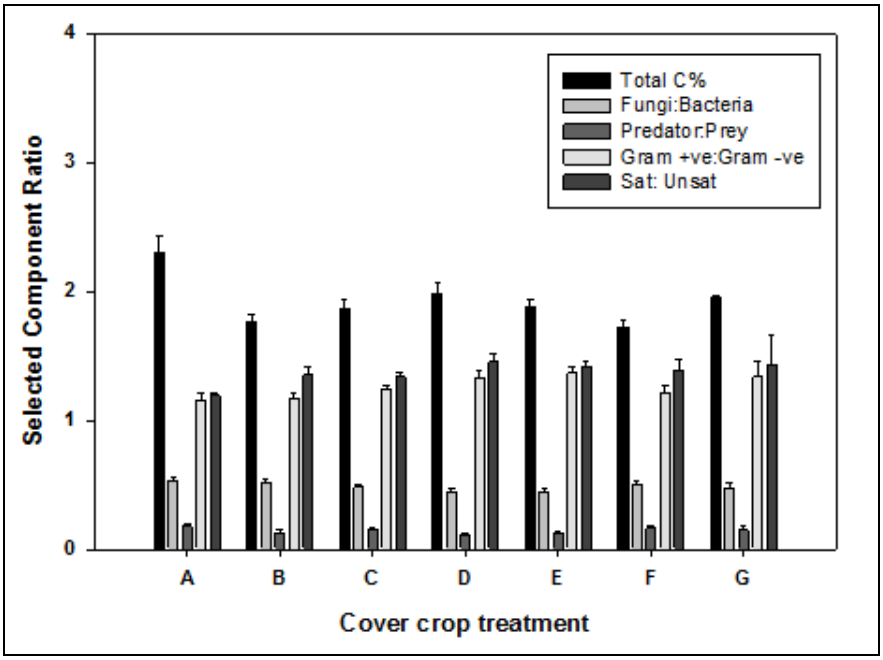

Figure 3: Various microbial parameters revealed by the PLFA profiles generated from the soils in the experimental plots. Plots A, B, C had CSW rotation while D, E, F, and G had CS rotation. All plots, except, F and G include cover crops

The fungal:bacterial ratio has been used to predict C sequestration potential with a higher fungal abundance implying greater C storage in soil (Strickland and Rousk, 2010). The fungal:bacterial ratio (Figure 3) for all the experimental plots ranged between 0.23 to 0.30, with plot A having the highest value of 0.30. A ratio of less than 0.05 indicates very poor soil health while 0.30 and above indicates very good soil health. Usually, bacteria are a dominant biological species in poor soil conditions, as well as

in early spring to late fall due to the seasonal pattern of plant growth function. Although there was variation among several microbial parameters analyzed, the fungal:bacterial ratio for all of the experimental plots indicated a moderate degree of healthy conditions in terms of their ratio throughout the experimental area. These soils are badly eroded with very shallow top soils. Inherent poor soil conditions also may have contributed to lower ratios of fungi:bacteria observed 3 years after the introduction of cover crops.

Several other parameters (Figure 3) such as the ratio of protozoa to bacteria (predator: prey ratio), ratio of gram-positive and gram-negative bacteria, and ratio of saturated and unsaturated fatty acids revealed wide-ranging values which were not easily explained by the cover crop treatments or by the crop rotation practices. The weather conditions of the area since the beginning of the study may have contributed to poor responses to cover crops. The study area has experienced below normal rainfall and extreme temperatures in 2012 and 2013. Cover crops failed in 2012 and some growth was observed in 2013.

Protozoa feed on bacteria resulting in the release of nutrients, especially nitrogen. A higher value indicates better soil health conditions that can support a large number of individuals of a higher trophic level. Although we observed significantly higher protozoa in plot A, the predator: prey ratio was average or better in all of our experimental plots.

We observed a balance between gram-positive and gram-negative bacteria in most of our experimental plots based on the ratio of gram-positive and gram-negative bacteria revealed by the PLFA profiles. Gram-positive bacteria can survive better under stress conditions in soil while gram-negative bacteria are dominant in soils primarily in anaerobic conditions. As expected in the growing season we saw a balanced bacterial community in our experimental plots.

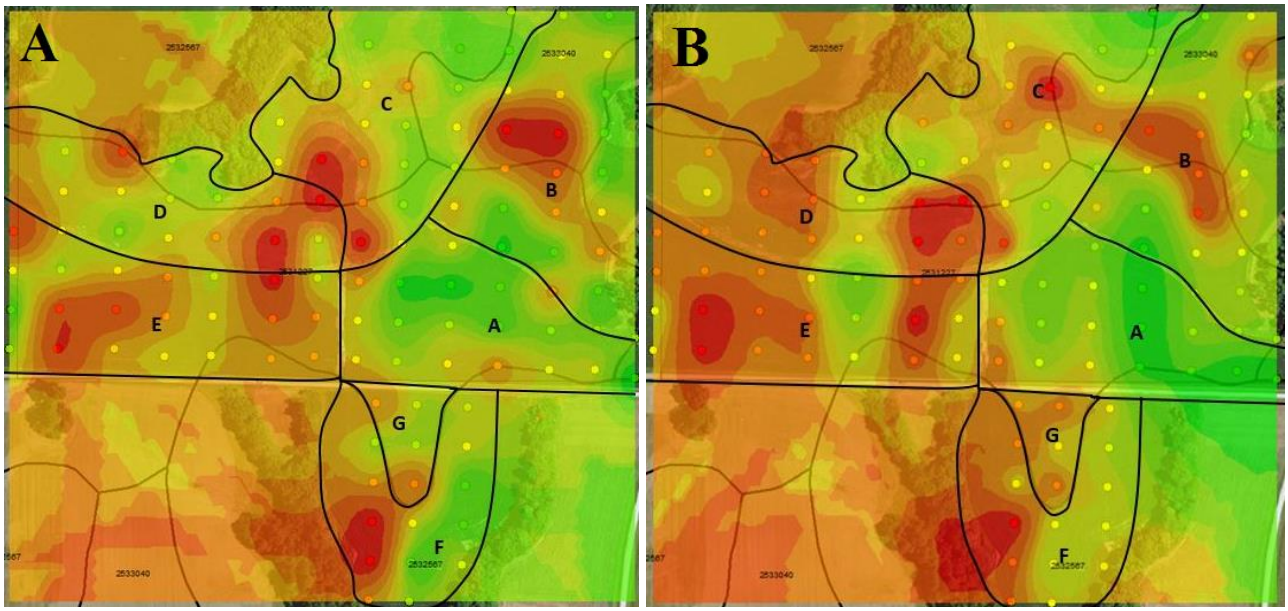

Figure 4: Total biomass (A) and diversity (B) as revealed by the PLFA profiles in the experimental plots modelled using kriging. Plots A, B, C had Corn-Soybean-Wheat rotation while D, E, F, and G had Corn-Soybean rotation. All plots, except, F and G include cover crops

ArcGIS Modelling Interpretation

We also used ArcGIS modeling to visualize the distribution of microbial communities throughout the farm. Ordinary Kriging was used to provide unbiased estimates of total biomass and diversity at un-sampled locations (Figure 4). The kriging models resulted in smoothed prediction surfaces that were then delineated by plot boundaries. This provided information on the structure and spatial dependence

of each variable of interest. Results disclosed slight differences in patterns of spatial dependencies for total biomass and diversity. For total biomass, the distance of discontinuity of spatial autocorrelation was approximately 10 cm. High levels of total biomass were modeled homogeneously within plot A (6580 ng g^{-1} soil); whereas, adjacent plots (B, C, D, and E) revealed several discrete or isolated locations of lower modeled total biomass (up to 80% lower compared to plot A average values). The two control plots also had isolated patches of lower total biomass more similar to the majority of plots except for Plot A. The diversity prediction model exhibited similar spatial patterns to total biomass where plot A was most homogeneous as compared to the adjacent plots. Interestingly, the control plots also exhibited homogeneity in values similar to Plot A; albeit with lower diversity.

The modeled spatial pattern for total biomass and diversity may have been caused by variations in local topographic features and associated hydrological conditions across the study area. The spatial patterns modeled with microbial characteristics may, therefore, exhibit greater variability due to hydrologic activity. This would suggest that the spatial variability in soil properties resulting from the micro-topographic conditions will affect soil microbial activity and productivity. The most observable influence of these patterns was found along with areas where there were visible sheet and rill erosion cuts in the study area. It appeared that there was an interactive effect between local patterns of moving surface water and microbial characteristics.

CONCLUSION

The primary objective of this study was to gain a snapshot of the microbial communities' impact of crop rotation and cover crops on a farm scale. Although we observed some influence of crop rotation on the distribution of soil microbial communities in the experimental plots, it seems there were other confounding influences of soil type, physical and chemical properties, topography, and weather. Soil organic carbon also was a determinant of microbial population and diversity. It is important to note that adding cover crops can aid in increasing natural organic carbon in the soil. Higher cover crop species diversity did not promote higher biomass or diversity of soil microbial communities. Future studies should be designed to eliminate soil type and other physicochemical variables to better evaluate the impact of cover crops and crop rotation on soil microbial functions and diversity.

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Conflict of Interest

The authors declare no conflict of interest.

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