Cropping Sequence, Cover Crops, and Poultry Litter Impact Soil Microbial Community Structure

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Introduction

Soil microorganisms play essential roles in soil organic matter dynamics and nutrient cycling in agroecosystems and have been used as soil quality indicators. The response of soil microbial communities to land management is complex and the long-term impacts of cropping systems on soil microbes is largely unknown. Therefore, changes in soil bacterial community composition were assessed in response to12-years of cropping sequences, cover crops, and poultry litter applications under no-tillage.

Materials and Methods

Main effects of four different cropping sequences of corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), and soybean (*Glycine max* L.) were rotated in four year phases for 12-yrs at two Tennessee Research and Education Centers in a randomized complete block design (Table 1) with split-block treatments of four winter bio-covers: hairy vetch (*Vicia villosa* L.), wheat (Triti*cum aestivum* L.), poultry litter, and a fallow control.

At the Research and Education Center at Milan (RECM, Loring B2 series), 4 cropping sequences of corn, cotton, and soybean were repeated in 4-yr cycles (i.e., Phases I, II, III, and IV; Table 1) beginning in 2002. Bio-covers of wheat, vetch, poultry litter (66.7 kg total N ha^{-1} yr^{-1}), and a fallow (winter weeds) control were repeated annually. The same experiment was carried out at Middle Tennessee Research and Education Center (MTREC, Maury silt loam) without cotton. For cotton at RECM, insecticide and crop growth regulation use was extensive (an organophosphate defoliant, growth regulator, and an organophosphate insecticide).

In Spring 2013 and 2014 samples were collected from 0-15 cm. Total soil DNA was extracted using the PowerLyzerTM PowerSoil® DNA Isolation Kit and PowerLyzer homogenization instrument (MOBIO Laboratories, Inc. Carlsbad, CA) according to the manufacturer's directions. DNA extracted from each plot (replicate) was quantified using the Quant-It™ PicoGreen® dsDNA quantitation assay. Bacterial community composition was determined using Illumina sequencing of 16S rRNA gene amplicons. Amplicon libraries were pooled and 250 base paired end sequences were obtained on the Illumina MiSeq platform. Reads were processed using the open source bioinformatic software package Mothur v 1.36.0 following the MiSeq SOP protocol (Schloss et al., 2009).

Simpson's Diversity index and richness were calculated using Mothur on this subsampled dataset, and analyzed for differences by treatment in the statistical package R (R Core Team, 2012). Bacterial community structure was quantified in a matrix of Bray-Curtis similarities, which was then analyzed in a permutational analysis of variance to compare mean diversity and richness by fixed effects (cropping sequence and bio-cover) in PRIMER-E (Clark and Gorley, 2006). Phylogenetic data were used to predict potential functional capacity of the bacterial communities using PICRUSt (Langille et al., 2013), and results were statistically analyzed for significance using STAMP (Parks et al., 2014).

Results

Table 1. Cropping sequences from 2002 (Yr-1)-2017 (Yr-16) at the Middle Tennessee Research and Education Center (MTREC) and Research Education Center at Milan (RECM).

	Year			
	2002†	2003	2004	2005
	2006	2007	2008	2009
	2010	2011	2012	2013
	2014	2015	2016	2017
Middle Tennessee Research and Education Center				
Crop Sequence				
Continuous Corn	corn	corn	**corn**	**corn**
Continuous Soybean	soybean	soybean	**soybean**	**soybean**
Corn-Soybean	corn	soybean	**corn**	**soybean**
Research and Education Center at Milan				
Crop Sequence				
Continuous Cotton	cotton	cotton	**cotton**	**cotton**
Continuous Corn	corn	corn	**corn**	**corn**
Continuous Soybean	soybean	soybean	**soybean**	**soybean**
Corn-Soybean	corn	soybean	**corn**	**soybean**

† 2002-2005=Phase I; 2006-2009=Phase II; 2010-2013=Phase III; Phase IV=2014-2017.
‡ Bolded crops are those grown in the year previous to microbe sampling in Spring 2013 and Spring 2014.

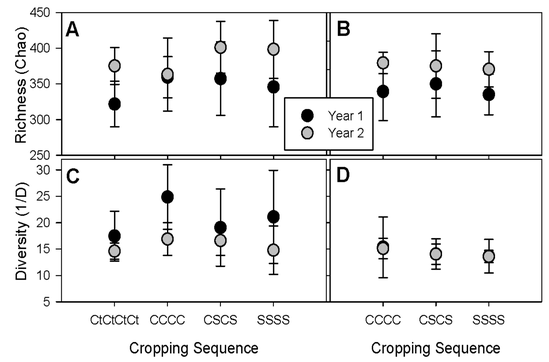

Figure 1. Mean richness (Chao estimate) and diversity (inverse Simpson's Index) in soil bacterial communities by cropping sequence (Ct=Cotton; C=corn; and S=soybean) and year (2013 and 2014) at the Research and Education Center at Milan (RECM) (A and C) and the Middle Tennessee Research and Education Center (MTES) (B and D). Error bars are standard deviation.

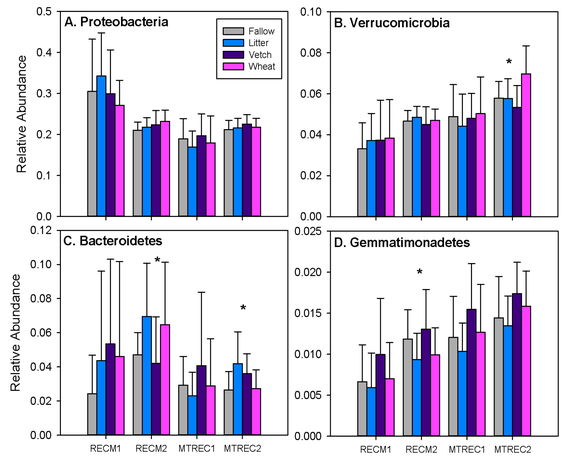

Figure 2. Relative abundance of bacteria phyla (A) Proteobacteria, (B) Verrucomicrobia, (C) Bacteroidetes, and (D) Gemmatimonadetes by bio-cover from 2 sites (RECM, MTREC) in year 1 (2013) and 2 (2014).

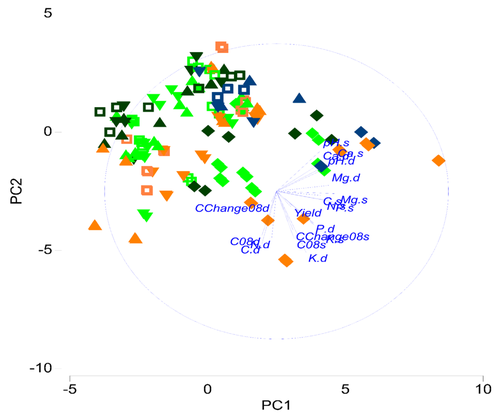

Figure 3. Principle component analysis of soil chemistry and yield for all the plots, coded by treatment. Colors designate cropping sequence: cotton (blue), corn (orange), soybean (dark green) and corn-soybean rotation (light green). Shapes designate bio-covers: fallow (open), poultry litter (diamond), wheat (up triangle), and vetch (down triangle).

Results & Discussion

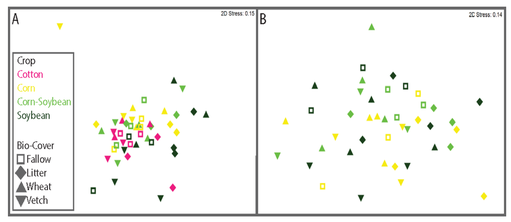

Figure 4. NMDS plots of Bray-Curtis distances of bacterial community structures at RECM (A) and MTREC (B). Samples are coded by cropping sequence (symbol color) or bio-cover (symbol shape).

- Significant differences occurred in both richness (Chao index) ($F = 7.1$, $p = 0.008$) and diversity (inverse Simpson's index) ($F = 28.6$, $p < 0.001$) per locale (Fig. 1).
- Communities under the corn-soybean sequence were not different ($p>0.05$) from either continuous corn, or soybean, but rather fell in between the two continuous cropping systems (Fig. 1, 4).
- After 12-yrs of management implementation, at both sites, poultry litter resulted in the greatest change in bacterial communities (Fig. 2). Poultry litter was significantly different from fallow and hairy vetch treatments (PERMANOVA pairwise test, $t= 1.7, 1.8$; $p<0.05$).
- The change in community composition was driven in part by an increase in Bacteroidetes at both sites (Fig. 2). We also noted that Gemmatimonadetes was sensitive to bio-covers at RECM ($F=3.3$, $p=0.029$), with increased abundances under hairy vetch (Fig. 2).
- Poultry litter amended soils were most linked to soil physical and chemical properties as well as plant yield in PC1, whereas, PC2 had simiar changes at both sites (Fig. 3).
- Decomposer bacterial genera of the phylum Actinobacteria were more abundant under long-term (12 years) hairy vetch cover crops and poultry litter amended plots and under high residue producing, less pesticide-intensive cropping rotations (soybean and corn rotations compared to cotton).

Conclusions

Microbial diversity was greatest under nutrient rich bio-covers (poultry litter) and high residue producing, less pesticide-intensive cropping sequences (soybean and corn compared to cotton), suggesting a more dynamic soil ecology under these no-till cropping systems. This suggests that nutrient management (inorganic fertilizers vs. animal manure) and greater crop rotations (within 4-yr phases) may directly drive phylogenetic community structure and subsequent ecosystem services across agricultural landscapes (Ashworth et al., 2017). Specifically:

i) richness and diversity varied temporally and spatially, coinciding with soil carbon, pH, nutrient levels, and climatic variability;

ii) community composition varied by cropping system, with continuous corn, soybean, and the corn-soybean rotation presenting a hybrid of the continuous corn and soybean communities; however, continuous cotton resulted in the most varied assemblage;

iii) bio-covers asserted the greatest influence on microbial communities; specifically poultry litter treatments differed from cover crops (all of which received inorganic-N).

References

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