Conservation Cropping Systems Initiative

Report Structure

This report is prepared for an individual farmer cooperator, with data from commercial soil health tests taken in 2015 and 2016. The report is structured as follows:

- Goals of the soil health tests analysis
- Summary of results from this individual cooperator
- Results in detail—this section discusses in detail the individual site results that are summarized in the immediately previous section, for the cooperator and others who may want to study the results in more depth.

A short summary of the results from all cooperators is provided in a separate report. Further synthesis of all data from all sites is ongoing, and will be provided as available.

Soil Health

Soil health has been defined as "the capacity of soil to function as a vital living system to sustain biological productivity, promote environmental quality and maintain plant and animal health."[1] Developing sustainable agronomic practices is directly related to their ability to influence soil health. Any attempt to categorize an agricultural practice as sustainable must first consider the effect on the soil.

Goals of Soil Health Analyses

A key component of the project conducted by the Conservation Cropping Systems Initiative (CCSI) is the evaluation of four different commercial soil health tests—Phospholipid Fatty Acids (PLFA), Earthfort Biological Soil Analysis, Cornell Soil Health Assessment, and Haney-Soil Health Tool. The objectives of this facet of the project are to assess the usefulness and value of the different commercial tests on evaluating the health of Indiana soils as well as the ability of the soil health indicators to distinguish among different cropping practices. Each of the four commercial soil health tests contain upwards of 10 separate soil health measures and most also include a ranking or calculation of overall soil health. While each of these commercial tests includes a large number of different soil properties, they each are supposed to evaluate overall soil health. One of the main goals of this project is to assess the usefulness of these tests on Indiana soils when comparing different cropping systems.

1 Doran et al., 1996; Doran and Zeiss, 2000

Climate	
Mean Annual Temperature:	50.2°F
Mean Annual Precipitation:	42.9 in

Treatments
No-till + Cover Crops + Low N
No-till + Cover Crops + Medium N
No-till + Cover Crops + High N

Individual Site Report:

Cameron Mills

Cassy County

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Summary of Mills Site

At the Mills farmer site, the treatment consisted of no-till cover crop plots with three different N rates on the corn crop. Most of the significant differences among N rates occurred in 2015. Bacterial and fungal biomass as well as total microbial biomass was significantly lower under the high N rate than the two lower N rates. The soil environment with more N applied may be less favorable for the microbial community. Organic matter was greater under the lower N rate than either of the two higher N rates in 2015. This may be related to more rapid decomposition of crop residues with higher N rates resulting in less stable organic matter.

Another important trend in these results is that the majority of these differences were found in 2015, but not in 2016. In fact, many of the averages were higher in 2015 than in 2016, like the Cornell quality score and PLFA diversity index calculations. These are likely related to the very different sampling dates in the two years. In 2015, the soil health samples were collected in June, but in 2016, the recorded date of sampling was much later on August 23. This date much later in the growing season would affect the soil health measures as many of them, especially those that directly measure microbes or microbial activity are very dependent on the soil temperature and moisture. These would be much different near the end of August than in June.

More work is needed to further evaluate the potential usefulness of these commercial tests for characterizing differences in soil health as found in Indiana cropland. The commercial tests as performed in this project, were often unable to distinguish between treatments that appear in the field to show differences. This may reflect a lack of sensitivity of the tests to important characteristics of key field soil

functions. Please refer to the separate overall summary report for further discussion of overall questions, further analyses planned, and questions for future research on soil health assessment methods.

Results

Results are presented in the following tables with a subset of a soil health measures from each of the commercial soil health tests evaluated in 2015 and 2016 at the Mills farmer site. The selected variables were chosen based on preliminary analysis that indicated that these soil parameters had the greatest potential to be sensitive to conservation cropping practices and allow us to distinguish between treatments.

Average values are presented for each of the treatments at the location—three rates of N fertilizer (low, medium, and high). We compared the three N rates to evaluate them for statistically significant differences. The significant differences are found for each year in the column to the right of the averages and degree of significance is indicated by the number of asterisks. Three asterisks (***) indicates a very strong statistical significance while comparisons with fewer asterisks are less statistically significant. Lower significance or lack of significant differences between treatments could be because of a smaller (or no) difference between treatments, but could also be due to greater variability within the measure so we are less confident that the apparent differences between treatments are real. For soil health measures that were significant, average values that are statistically different from each other are labeled with different letters. If two means have the same letter, they are not significantly different; for instance, microbial biomass in 2015 from both low and medium N rate treatments are labeled with the letter 'a'. However, the high N rate is labeled as 'b' for microbial biomass so that treatment is significantly different from both the low and medium N rates.

Brief Statistics Primer—Statistically Significant Differences

Here is an example from one of our farmer cooperators of the highly variable numbers we are analyzing. The average total fungi for four strips of no-till with cover crops was 195 ng/g compared to the neighboring field with an average of 51.5 ng/g of total fungi. These seem like those numbers are very different, but the difference between them is NOT statistically significant.

How in the world can these two numbers not be different? The no-till cover crop is 4x larger than the other, why do the statistics say they aren't different? Statistical analysis tries to determine how confident we can be that this difference is real and would occur again. It's not based just on how large the difference is. We compare how different the two fields are to the amount of variation within each field.

Example

Treatment	Rep #1	Rep #2	Rep #3	Rep #4	Average
No-Till + Cover Crops	98	38	390	254	**195**
Neighbor	32	85	33	56	**51.5**

To make sense of this, we need to look to the numbers that go into the averages. For the no-till, cover crop field, we have numbers that are kind of all over the place with some lower values—38 and 98, but also two very high numbers—254 and 390. For this field, the average is much higher than the average of the neighbor, but there is a high amount of variability in this measure as well. With so much variability in the measure, we can't be confident that this treatment is truly different from the neighbor.

As an example, if you have a field that has a lot of variability in it, you could randomly select a few different spots to check for yield. Depending on what spots you check, you may think you could have record yields or that it's going to turn out to be a disappointing harvest. In this case, eventually you will harvest the whole field and so you know what your true yield is. For the soil health indicators we are looking at, we can only estimate these measures based on the 3 or 4 replicated plots in each field. When there is high amounts of variability, we have no way of knowing what the true average is so we need to be cautious in declaring these differences to be real. If we were to repeat this experiment with four different plots in those fields, we might get a very different average and the difference between the no-till cover crop and the neighbor might end up being much smaller.

The soil health measurements tend to be much more variable than standard soil fertility tests, as the soil biology can be very patchy with microbes clustering near cover and cash crop roots and residues. Wheel tracks can reduce pore space in the soil, affecting water and oxygen availability for microbes. We try to reduce this problem by collecting 20-30 soil cores from each strip to get a more representative sample, but high variability still remains. Soil biology can also change dramatically throughout the summer as moisture and temperature change so these tests only provide a snapshot of these measures at the time of sampling. Ultimately, these issues complicate our ability to detect significant differences even when there are large numerical differences between the treatments.

Site Details—Soils, Treatments

Conservation Cropping System Experimental Plots						
% of Field	Soil Series Name	Soil Texture	Slope	Drainage Class	Native Vegetation	Parent Materials
57%	Cyclone	silt loam	0-2 %	Poorly drained	Transition	Loess over loamy till
43%	Fincastle	silt loam	0-3%	somewhat poorly drained	Forest	

Treatment Details:
All plots have the same cash and cover crop treatments as listed below. All plots were no-tilled with different fertilizer treatments (low, medium, and high N rates) on the corn crop only.

Treatments	Fertilizer Rate (lb N/ac)	Summer 2013	Fall 2013-Summer 2014		Fall 2014-Summer 2015		Fall 2015-Summer 2016		Fall 2016-Summer 2017	
		Cash	Cover	Cash	Cover	Cash	Cover	Cash	Cover	Cash
Low N (CM 1, 4, 7)	**89.6**	CN	AR	SB	AR	CN	AR	SB	AR	CN
Med N (CM 3, 6, 9)	**129.4**									
High N (CM 2, 5, 8)	**179.3**									

Cash and Cover Crop Abbreviations: CN—Corn; SB—Soybean; AR—Annual Ryegrass
Cover crops are color-coded as light green.

Soil Health Sampling Dates	Soil Moisture (%)		
	Low N	Med N	High N
June 24, 2015	NA	NA	NA
August 23, 2016	20.7	18.9	20.1

Phospholipid Fatty Acids (PLFA)

Phospholipid fatty acids are found in the cell membrane of all cells. Each microbial group also has specific fatty acids only found in the cell membrane of that certain group of microbes—these are called biomarkers. The amount of biomarker fatty acids measured in the soil tell us how large each of these microbial groups are within the soil sample.

- In soils, we look at total microbial biomass as well as several microbial groups—bacteria, fungi, mycorrhizal fungi, and protozoa.
- The PLFA tests in 2015 and 2016 were analyzed by two different commercial laboratories so the units between years are different and make comparisons between 2015 and 2016 difficult.

Table 1. Average values for Phospholipid Fatty Acid (PLFA) for low, medium and high N rates under no-till with cover crops at the Mills farmer site in 2015 and 2016. PLFA tests in 2015 were analyzed by Ward Laboratories and measured in ng/g while in 2016, PLFA tests were analyzed at the Missouri Soil Health Assessment Center and measured in nmol/g. Statistical differences among N rates are indicated as significant at <0.01 by ***, at <0.05 by ** and at <0.10 at *. Average values that are significantly different are indicated by different letters. Measurements in italics are calculations within commercial tests purported to be indicators of overall soil health. **NOTE: Different units and labs between the two years, make direct comparisons between 2015 and 2016 impossible, except for Diversity Index and Fungi:Bacteria Ratio.**

	June 24, 2015			
	Average Values			
PLFA—Ward Laboratories	Low N (CN)	Med N (CN)	High N (CN)	Significant Differences
Total Microbial Biomass (ng/g)	5052 a	4851 a	3221 b	**
Total Bacteria (ng/g)	2610 a	2461 a	1742 b	*
Total Fungi (ng/g)	730 a	703 a	383 b	*
Mycorrhizal Fungi (ng/g)	313	299	148	
Protozoa (ng/g)	69	61	39	
Fungi:Bacteria Ratio	0.28	0.29	0.22	
Diversity Index	1.65	1.61	1.57	
	August 23, 2016			
	Average Values			
PLFA—Missouri	Low N (SB)	Med N (SB)	High N (SB)	Significant Differences
Total Microbial Biomass (nmol/g)	83.2	95.8	83.0	
Total Bacteria (nmol/g)	46.2	53.4	45.9	
Total Fungi (nmol/g)	0.93	1.08	1.04	
Mycorrhizal Fungi (nmol/g)	3.16	3.81	3.22	
Protozoa (nmol/g)	0.50	0.72	0.52	
Fungi:Bacteria Ratio	0.19	0.19	0.19	
Diversity Index	1.32	1.33	1.32	

CN—Corn; SB—Soybean (No fertilizer N applied)

PLFA, cont

Total Microbial Biomass

Represents the overall size of the microbial community within the soil; larger microbial communities indicate a more favorable environment for microbial growth and a healthier soil.

- In 2015, both the low and medium N rates had higher total microbial biomass than the high N rate.
- However, no significant difference between treatments was detected in 2016.
- Ward Laboratories, which analyzed PLFA in 2015, has a rating system for total microbial biomass (see Appendix).
    - According to the rating system, the low and medium N rate plots have microbial biomass that fall in the excellent category and the high N rate plot are very good.

Total Bacteria

Bacteria are decomposers that help break down residues and cycle nutrients and are an important part of the microbial community. However, for optimal soil health, it is important that the microbial community not be dominated by bacteria. Therefore, a high bacteria number does not indicate by itself that the soil has high soil health.

- The same pattern was found for the bacterial portion as the overall microbial biomass.
    - No difference in 2016, but greater bacteria in low and medium rates than in the high N rate in 2015.

Total Fungi

Fungi, like bacteria, are decomposers, but some fungi have fairly specialized enzymes that break down residues that are more complex and difficult to break down. They are also important to soil organic matter formation and soil aggregation. This makes fungi a very valuable part of the microbial community, and high levels of fungi can be a strong indicator of soil health.

- As with bacteria, the same pattern was found in fungi.
    - No significant difference in 2016, but greater fungi in low and medium rates than in the high N rate in 2015.

Mycorrhizal Fungi

Mycorrhizal fungi, also known as arbuscular mycorrhizae fungi (AMF), can be beneficial to many crops as they colonize plant roots and form mutually beneficial relationships. Mycorrhizae are able to scavenge for nutrients in the soil that the plant would not otherwise be able to reach—these can be especially important for P and N.

- No significant difference between treatments was detected in either year.

Protozoa

These microbes are important to nitrogen cycling within soils. Protozoa mainly feed on bacteria and as they eat, they release excess nitrogen that is then available for crop uptake.

- No significant differences in protozoa were found in either year.

Fungi: Bacteria Ratio

As mentioned above, fungi can be a strong indicator of soil health so it is important to have a high ratio of fungi to bacteria.

- There were no significant differences among any of the N rates in either year.
- Ward Laboratories has a rating system for this measurement as well (see Appendix).
    - Based on this, the values for the 2015 measurements for the low and medium N rates are in the good range while the high N rate falls in the slightly above average category.
    - The fungi: bacteria ratios for 2016 were all rated as average based on the Ward Laboratories scale.

Diversity Index

This measurement is calculated using the proportion of the microbial biomass that is in each of the microbial groups listed above and indicates how much diversity is found within the microbial community. High diversity is preferred as a microbial community is better able to deal with environmental stresses and able to decompose a more diverse array of residues.

- Ward Laboratories provided a rating system for this calculation as well (see Appendix).
    - For 2015, the diversity index calculated for all three N rates is in the excellent category. The 2016 diversity indices were lower than 2015 and were rated only as slightly above average.

Earthfort Biological Soil Analysis

Similar to PLFA, this commercial test measures the size of various microbial groups; however, these measurements were made using microscopy, directly counting the size of these microbe groups. This analysis was only completed in 2015.

Table 2. Average values for Earthfort Biological Analysis in 2015 for cover crop no-till (NT+CC) and no cover no-till (NT) plots at DeSutter as well as neighbor (NBR). Statistical differences within pairs of treatments are indicated as signif-

Earthfort	June 24, 2015			
	Average Values			Significant Differences
	Low N (CN)	Med N (CN)	High N (CN)	
Active Bacteria (µg/g)	48	56	57	
Total Bacteria (µg/g)	1921	1493	1019	
Active Fungi (µg/g)	40	32	26	
Total Fungi (µg/g)	568	716	559	
Protozoa--Flagellates (µg/g)	1939	2751	2220	
Protozoa--Amoeba (µg/g)	60741	186234	55273	
Protozoa--Ciliates (µg/g)	190	498	446	
Total Fungi: Total Bacteria Ratio	0.31	0.56	0.75	

CN—Corn

Total and Active Bacteria

As mentioned above, bacteria are decomposers, but are not considered strong indicators of soil health. While some bacteria may be dormant or dead, active bacteria gives an indication of how many bacteria are able to actually cycle nutrients and contribute to decomposition of residues at the time of soil sampling.

- There were no differences between any of the treatments for either total or active bacteria.

Total and Active Fungi

Fungi are also decomposers, but because of their contributions to soil aggregation and soil organic matter, it is preferred to have high fungi levels and have a fungal dominated microbial community. Again, the active fungi gives a better indication of how many fungi are currently able to contribute to nutrient cycling.

- There were no significant differences between treatments for total or active fungi in 2015.

Protozoa

As mentioned above, protozoa eat bacteria and release excess nitrogen, which is now plant available. The Earthfort analysis measures the amounts of three different types of protozoa. Flagellates and amoebae are aerobic protozoa that require oxygen to survive. Ciliates are the largest and least common protozoa, and they are able to survive without oxygen in anaerobic conditions.

- There were no significant differences between any of the treatments for any of the protozoa types.

Total Fungi: Total Bacteria Ratio

Fungal dominated microbial communities are a strong indicator of soil health so higher values of the fungi: bacteria ratio are preferred.

- No significant differences were found between any of the treatments.

Cornell Soil Health Assessment

This commercial soil test consists of twelve different measures of different aspects of the soil, which are all rated and then combined together to form an overall quality score (out of 100). The chemical tests of soil pH, P, K and minor elements are not shown in this report as they were not different between treatments, but they are included in the calculated quality score. In general, most of the chemical tests were in the optimal range, reflecting long-term good soil fertility practices.

Note on Rating System:

The ratings in the Cornell Soil Health Assessment are determined by scoring functions for each soil property. The scoring functions used in this report are specific to the Midwest region and some differ based on the soil texture (sandy soils would be rated differently than finer soils). These scoring functions were developed based on a large database of measurements collected from throughout the region. Certain soil measurements rate higher for higher values (Aggregate Stability, Available Water Capacity, Organic Matter, ACE Protein, Soil Respiration, and Active Carbon). Surface and Subsurface hardness are rated higher with lower measured values. Others, such as pH and phosphorus, are rated closer to 100 when within an optimum range; above and below that range are rated lower.

Table 3. Average values for Cornell Soil Health Assessment in 2015 and 2016 for low, medium and high N rates under no-till with cover crops at the Mills farmer site. Statistical differences among N rates are indicated as significant at <0.01 by ***, at <0.05 by ** and at <0.10 at *. Average values that are significantly different are indicated by different letters. Measurements in italics are calculations within commercial tests purported to be indicators of overall soil health.

	June 24, 2015				August 23, 2016			
	Average Values				Average Values			
Cornell	Low N (CN)	Med N (CN)	High N (CN)	Significant Differences	Low N (SB)	Med N (SB)	High N (SB)	Significant Differences
Quality Score	69.1	70.7	67.8		56.8	58.5	60.5	
Aggregate Stability (%)	21.5	16.7	20.0		25.9	32.1	30.3	
Available Water Capacity	0.28	0.26	0.25		0.22	0.20	0.20	
Surface Hardness (psi)	–	–	–		275	283	275	
Subsurface Hardness (psi)	–	–	–		300	300	300	
Organic Matter (%)	3.20a	2.87b	2.93b	*	3.06	2.89	2.97	
Active Carbon (ppm)	518	564	524		469	545	501	
ACE Soil Protein Index	5.43	4.70	4.73		4.85	4.87	4.67	
Soil Respiration-96 hrs (ppm)	640	660	520		460b	530a	470b	*

CN—Corn; SB—Soybean (No fertilizer N applied)

Cornell, cont.

Quality Score
This is calculated based on the rating for each of the 12 different soil measures within this commercial soil health test. It is supposed to indicate overall soil health and values above 60 are considered excellent. Quality scores between 40 and 60 are rated medium and indicate soil health could still be improved. If the values are less than 20, this is considered a constraint and needs to be addressed.

- No significant differences in either year, but values in 2015 were higher than in 2016.
- The 2015 values are all considered excellent indicating high soil health in all three treatments while the 2016 values were rated just below or at excellent.

Aggregate Stability
This measures how well the soil aggregates stay together and can be a strong indicator of how well the soil is able to resist erosion. High aggregate stability can prevent crusting and increase water infiltration

- No significant differences were found between treatments.

Available Water Capacity
This measures how much water the soil holds between field capacity and permanent wilting point, which is the amount of plant-available water the soil can store. Available water capacity is dependent on the soil texture as coarse texture soils are able to store much less water than finer soils. However, for a specific soil texture, more organic matter can increase available water capacity.

- There were no significant differences between any treatment for either year.

Surface and Subsurface Hardness
These are measures of strength of the soil and is an indication of the physical structure of the soil. High levels of surface and subsurface hardness can restrict root growth and influence water infiltration. Surface hardness is measured in the top 6 inches, while subsurface hardness measures 6-18 inches. These measures can also be affected by soil moisture at the time of sampling. These numbers were taken with a cone penetrometer at the time of the field sampling

- No significant differences in surface hardness or subsurface hardness in 2016.
- No surface or subsurface hardness measurements were collected in 2015 at this site.

Organic Matter
Soil organic matter is one of the most important indicators of soil health due to its relationship with many other aspects of the soil, including water infiltration and holding capacity, aggregate stability, and nutrient cycling. However, the limitation of this measure is that it can take several years to significantly alter organic matter.

- In 2015, the low N rate had significantly higher organic matter than in the medium or high N rates.
- In 2016, the values were very similar to the 2015 values in the medium and high N rates, but the organic matter was lower in the low N rate than the previous year so there were no significant differences among treatments in 2016.

Active Carbon
This measures the portion of organic matter that is most easily decomposed by soil microbes. High active carbon is an indicator of good soil health and is much more sensitive to management changes than organic matter as a whole.

- In both years, active carbon was not significantly difference.

ACE Soil Protein Index
This is similar to active carbon as it represents the most easily cycled part of organic matter, but measures nitrogen. Proteins are readily broken down by microbes, which mineralizes N into plant-available forms.

- No significant differences were found in 2015 or 2016.

Soil Respiration
Soil respiration measures the amount of carbon dioxide released by soil microbes over a certain period of time. For Cornell, it is measured over 96 hours so the measure is able to stabilize and is more consistent than measures over a short period of time. This measures how active the soil microbes are.

- No significant differences in 2015, but the values were higher than in 2016.
- Soil respiration from the medium N rate plots was significantly higher than the low or high N rate treatments in 2016.

Haney-Soil Health Tool

Like the Cornell commercial soil health test, the Soil Health Tool consists of many different tests that evaluate different aspects of the soil. The tests focus on nutrient availability and microbe activity.

Table 4. Average values for the Haney Soil Health tool for low, medium and high N rates under no-till with cover crops at the Mills farmer site in 2015. Statistical differences among N rates are indicated as significant at <0.01 by ***, at <0.05 by ** and at <0.10 at *. Measurements in italics are calculations within commercial tests purported to be indicators of overall soil health.

	June 24, 2015			
	Average Values			
Haney-Soil Health Tool	Low N (CN)	Med N (CN)	High N (CN)	Significant Differences
Nitrogen (N lb/A)	58	62	53	
Phosphorus (P_2O_5 lb/A)	70	67	70	
Soil Respiration-24 hours (ppm)	53	51	58	
Water Extr. Organic C (ppm)	299	290	275	
Water Extr. Organic N (ppm)	20.7	19.8	20.0	
Carbon: Nitrogen Ratio	14.5	14.6	13.8	
Soil Health Calculation	10.4	9.9	10.6	

Nitrogen and Phosphorus Nutrient Content
These are measures of N and P currently in the soil.

- No significant differences were detected in 2015 for either N or P.

Soil Respiration
As for the Cornell soil respiration, this measures the amount of microbial activity by measuring the amount of carbon dioxide released. For this test, it is measured over 24 hours. Since this is such a short time period, these measures can be highly variable.

- No differences between treatments in this 24 hour measure of soil respiration.

Water Extractable Organic Carbon and Nitrogen
Like active carbon and protein in the Cornell commercial test, water extractable organic C and N are supposed to measure the amount of carbon and nitrogen in organic matter that is readily available to soil microbes.

- No significant differences were found for C or N in 2015.

Soil Health Calculation
This is calculated from the 24 hour soil respiration as well as the water extractable organic carbon and nitrogen. It is supposed to represent the overall soil health and can range from 0 to over 30. While the Soil Health Tool does not provide a rating system, they do suggest that good management practices that improve soil health will cause this calculation to increase over time.

- No differences were detected between treatments in 2015.

Photo Credit: Unknown

Annual ryegrass in Indiana.

Appendix

The rating system provided by Ward Laboratories for Total Biomass, Fungi: Bacteria Ratio and Diversity Index.

Rating	Total Biomass (ng/g)	Fungi: Bacteria Ratio	Diversity Index
Very Poor	< 500	< 0.05	< 1.0
Poor	500+ - 1000	0.05+ - 0.1	1.0+ - 1.1
Slightly Below Average	1000+ - 1500	0.1+ - 0.15	1.1+ - 1.2
Average	1500+ - 2500	0.15+ - 0.2	1.2+ - 1.3
Slightly Above Average	2500+ - 3000	0.2+ - 0.25	1.3+ - 1.4
Good	3000+ - 3500	0.25+ - 0.3	1.4+ - 1.5
Very Good	3500+ - 4000	0.3+ - 0.35	1.5+ - 1.6
Excellent	> 4500	> 0.35	> 1.6