# Prolific Earth Sciences

# April 2020

# Summary of studies/publications using microBIOMETER®

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## 1. Efficacy of microBIOMETER® in evaluating soil health.

The University of Tennessee maintains 128 experimental plots on which cotton has been continuously grown for 31 years and for which data on soil levels of Total Organic Carbon (TOC) and Carbon Fumigation (CF) estimates of microbial biomass and other indications of soil health were available. These plots have been used to test the effect of five different methods of soil treatment, Till, No-Till, No cover, Winter Wheat Cover and Vetch cover, each at 4 levels of nitrogen supplementation on soil quality and productivity. The team at Tennessee has been evaluating various soil health panels to judge their effectiveness and had not yet found a method that distinguished between the various methods and correlated with their extensive knowledge of the soil health of their 128 plots. But microBIOMETER® was able to do this. Because of this success, Dr. Forbes Walker of University of Tennessee has pulled together a team from the top agricultural schools throughout the nation and submitted grant proposals, to evaluate soil health with microBIOMETER® in many different types of soil.

128 soil samples were sent to Prolific Earth Sciences for measurement of microbial biomass using microBIOMETER® (mB). The samples were blinded by the University of Tennessee as to plot identification and any previous data.

microBIOMETER® results correlated highly with Total Organic Carbon measurement across all soil treatments, with an overall correlation coefficient of $r = 0.9$. In contrast, the correlation of Carbon Fumigation values with Total Organic Carbon was lower, with a negative correlation of $r = -0.74$.

The results confirm the reliability and accuracy of microBIOMETER® measurement of soil microbial biomass, and the superiority of microBIOMETER® to the commonly used Carbon Fumigation technique.



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## Methods

128 soil samples from the top 3-4 inches of 128 different plots were obtained from Professor Forbes Walker, of the U of Tenn on 20th November 2019, blinded as to plot number or plot treatment. Samples were collected over 17-19th November shipped to Prolific Earth Sciences (PRESTO), and assayed within 3 days of arrival. Subsequentially, PRESTO was supplied with the previously obtained data on TOC and CF measurements (Doctoral Dissertation, Mbuthia) and the data were analyzed as given below. Cotton has been continuously grown on the plots for 31 years. During the entire time, one of several agricultural treatments was used on each plot:

1. No till
2. Conventional till
3. No cover crop
4. Winter wheat cover crop
5. Vetch cover crop

Within each treatment category, sub-plots received 0, 30, 60 and 90 kg/N/acre/year.

The data were analyzed using Excel to compute mean values by:

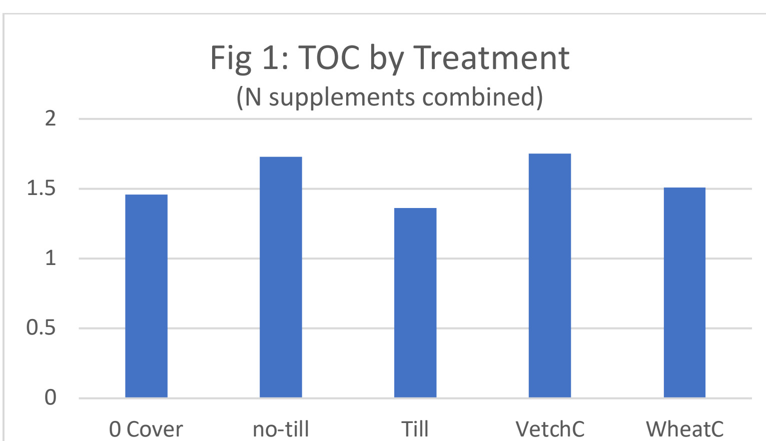
1- Combining the results from all plots subjected to a given treatment (no till, conventional till, etc.) irrespective of the amount of nitrogen supplementation used: presented in bar graphs “by Treatment”.
2- Combining the results for each sub-plot within a given treatment according to the amount of nitrogen supplementation.

## Comments on Methods:

-The microBIOMETER® (mB) analysis of soil microbial biomass carbon was done several years after the measurements of TOC were performed, but we considered this acceptable for the purposes of this study because TOC in soil changes very slowly. Samples for estimation of microbial biomass by Carbon Fumigation (CF) were taken at the same time as TOC.

## Results

Agricultural treatment (no till, cover crop etc.) was associated with differences in soil TOC, with no till and vetch cover crop giving the highest TOC levels, and till the lowest (Fig 1).



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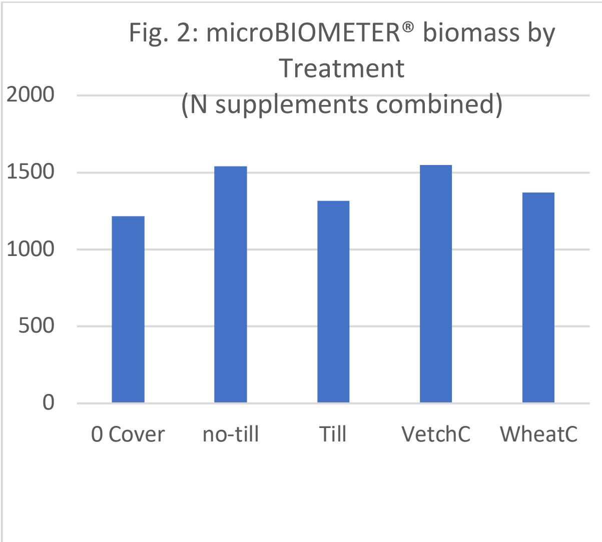
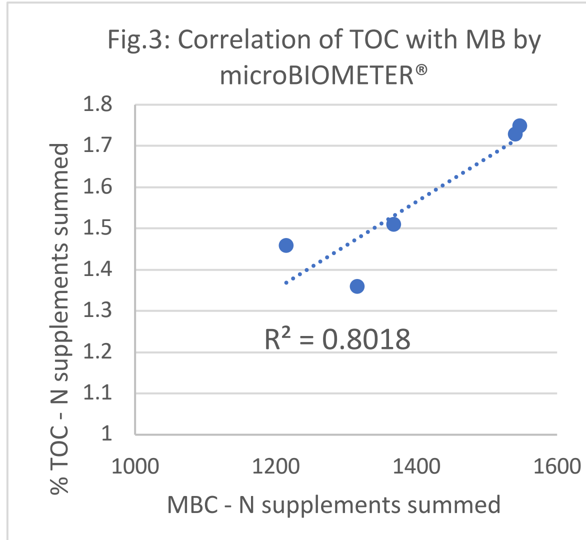
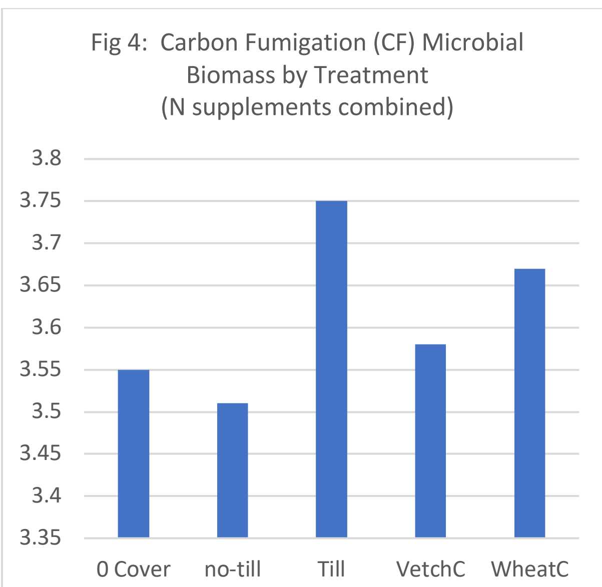
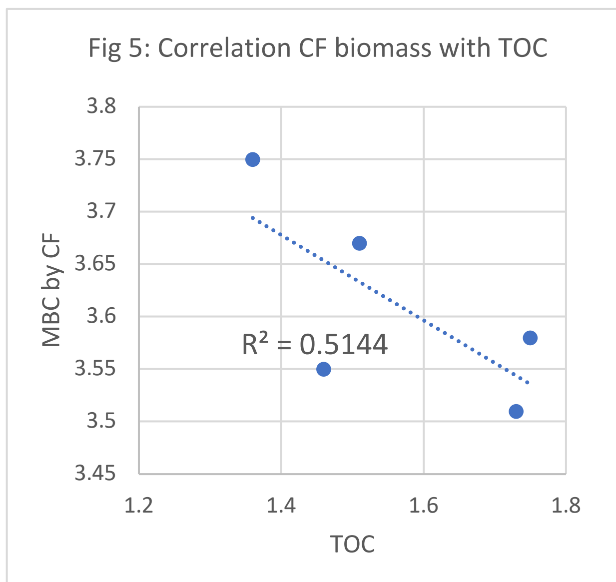
Microbial biomass as estimated by microBIOMETER® was predictive of soil TOC (r = 0.9) and was equally sensitive to the TOC method in detecting the differences in TOC among the treatments (Fig.2 and 3).




Microbial biomass as estimated by the carbon fumigation (CF) method correlated less strongly and, negatively with TOC ($R^2$ = -0.5514). This could be an intrinsic shortcoming of the method, and/or it could be due to the time of year of sampling, (June (CF) vs. November (microBIO)) or crop and post-harvest differences.




**The study group at University of Tennessee has demonstrated that neither the Haney or Cornell Soil Heath Panels could distinguish between different treatments or soil quality.**



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## 3. University and Foundation Studies

- Dr. Pochron, Stonybrook University has published results of studies on effect of glyphosate and roundup on worms and soil quality using microBIOMETER®

- Pochron, S., Choudhury, M., Gomez, R., Hussaini, S., Illuzzi, K., Mann, M., Mezic, M., Nikakis, J. and Tucker, C., 2019. Temperature and body mass drive earthworm (Eisenia fetida) sensitivity to a popular glyphosate-based herbicide. *Applied Soil Ecology*, *139*, pp.32-39.

- Pochron, S., Simon, L., Mirza, A., Littleton, A., Sahebzada, F. and Yudell, M., 2020. Glyphosate but not Roundup® harms earthworms (Eisenia fetida). *Chemosphere*, *241*, p.125017.

- Cornell University granted PES, a Jump Start Grant in 2020. Dr. Quirine Ketterings (USDA) laboratory will be evaluating the variation in plant quality in a field with the microbial biomass and the fungal to bacterial ratio.  Dr. Ketterings has also included microBIOMETER® in her upcoming grant applications.

- Dr. Forbes University of Tennessee, USDA, has assigned a graduate student to validating the use of microBIOMETER® as a soil health tool and has submitted a grant for validating the test in four different areas of the U.S. to FFAR.

- Dr. Jill Clapperton has included the test in the Farm Smart grant.



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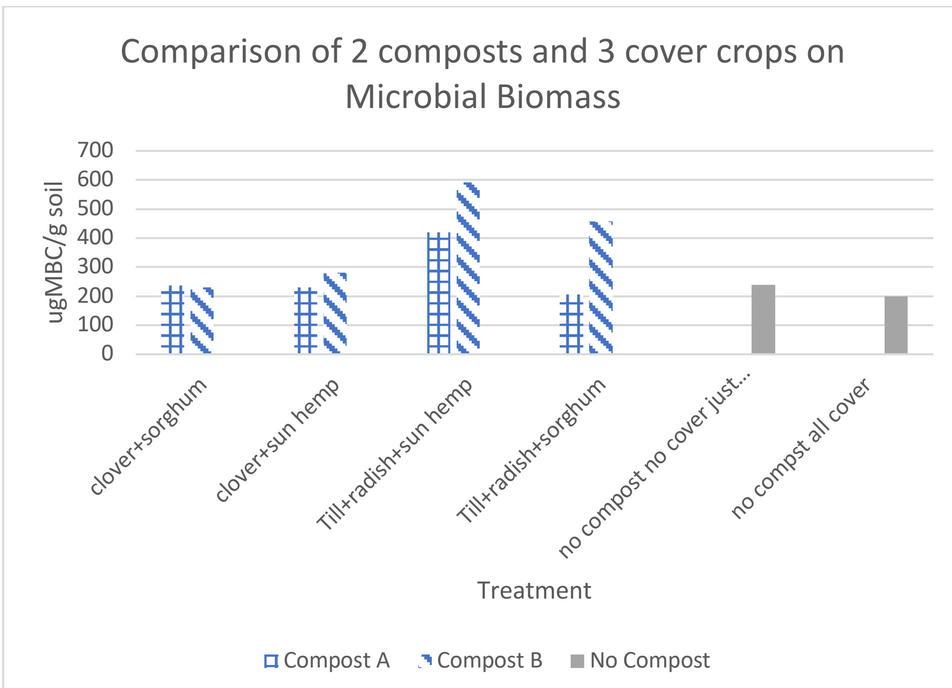
**4. Evaluation of the synergistic effects of multiple sustainable practices.**

This experiment conducted by James Sottilo, illustrates the multifactorial problem agriculturists face when deciding a strategy for implementing sustainable practices and how preparation paired with microbial biomass testing can remove some of the guess work. And highlighted how microBIOMETER® measurement of MB allows rapid evaluation of efficacy of a regenerative effect. MB is an excellent indicator of soil health because the microbes contain all the nutrients a plant needs in the ratios that are needed, i.e. if N is low, MB will be low. A cover crop that supplements the missing nutrients in a soil will most effectively restore soil health.

Sottilo compared 2 compost formulations, A and B, and 4 cover crop mixtures, clover/sorghum, clover/sun hemp, radish/sorghum and radish/sun hemp on microbial biomass. The controls, shown in solid bars had no cover, no compost or no compost with all 4 cover crops. As can be seen from the controls adding a cover crop without compost did not raise microbial biomass above control level. Clover cover crop mixtures did not significantly raise microbial biomass above the control. Radish/sun hemp doubled microbial biomass with compost A, and tripled it with compost B. Radish/sorghum did not raise microbial mass with compost A, but it doubled with compost B.




Based on this experiment it was decided to us Compost B with till and radish sum hemp as the cover crop.

**5. Microbial Biomass variations during the growing season on 5 Tennessee Farms**

In 2017 Prolific Earth Sciences was awarded a grant from the State of Tennessee evaluate changes in microbial biomass over the growing season to evaluate: 1). the fluctuations in MB over the course of the season to gain insight into the proper time to sample; 2). To estimate how many samples/acre were necessary for testing; and 3). to evaluate the correlation of MB with yield – the project required farmers to provide yield data. Five Tennessee farms elected to participate. The farms are designated, W, T, S, O and Y were distributed between Memphis and Nashville. The T farm grows various vegetables in small plots. The other 4 are large agricultural farms that identified a 1-acre area for testing. Ten soil samples were collected from the top 5 inches on these one-acre areas. Location was determined by GPS and samples were collected at 4 times during the growing season Mid May, late June, Late August and late September. Field moist soil samples from the top 5 inches were collected over a 2-day period, refrigerated until testing, and were tested within a week of collection. Samples were analyzed using the microBIOMETER® assay for microbial carbon biomass. At the 4$^{th}$ soil collection all the farms with exception of S had been harvested and S was ready for harvest. A sample from the O farm was not available in September.

Samples prior to planting were not available due to the enrollment process but were taken shortly after planting. Initial and final readings of the five farms are shown in Fig 1, below. (T farm had 10 different crops, started at various times and there is no record of the crop maturity at the times of sampling. For the final microbial count, 2 soil samples that were of newly planted crop were not included. The 8 dormant plots were included.)

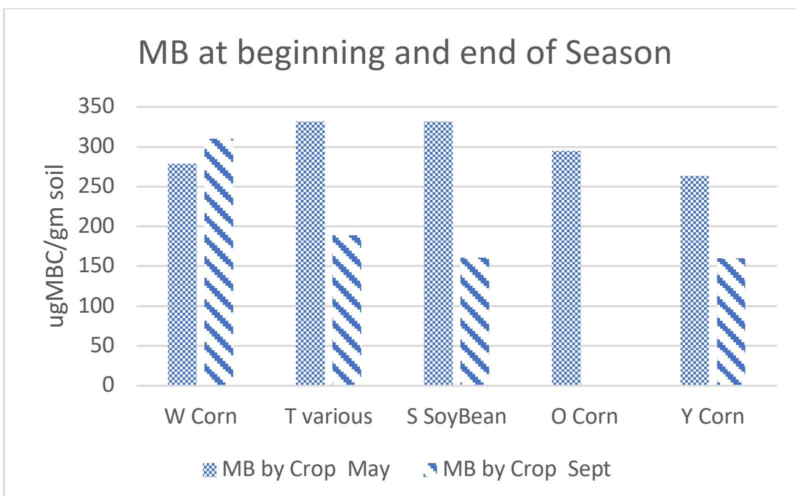
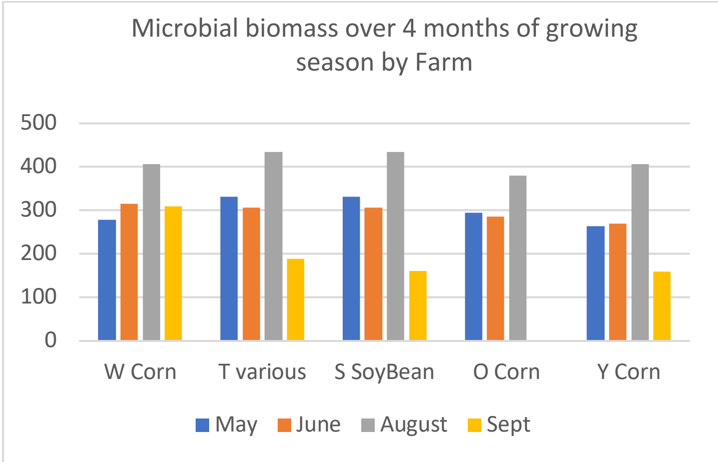
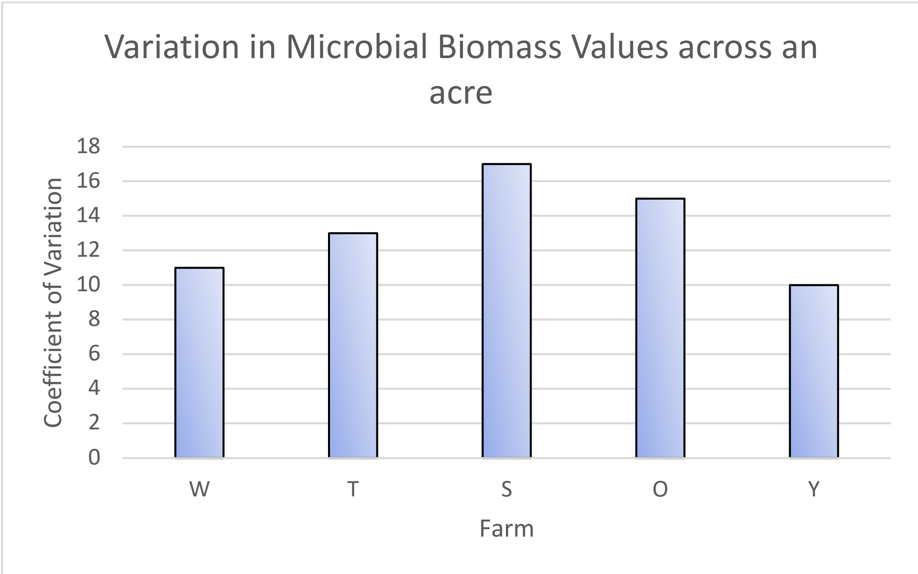


Fig 2 shows microbial biomass over the course of the growing season for all 5 farms and crops. From May through August, microbial biomass increased and decreased in September except for the W farm (Ogle farm was not available for testing in September). The drop in MB in early September is not unexpected and probably reflects the fact that with harvest the plants are no longer providing exudates to the microbes and the stubble and roots are not yet being decomposed.



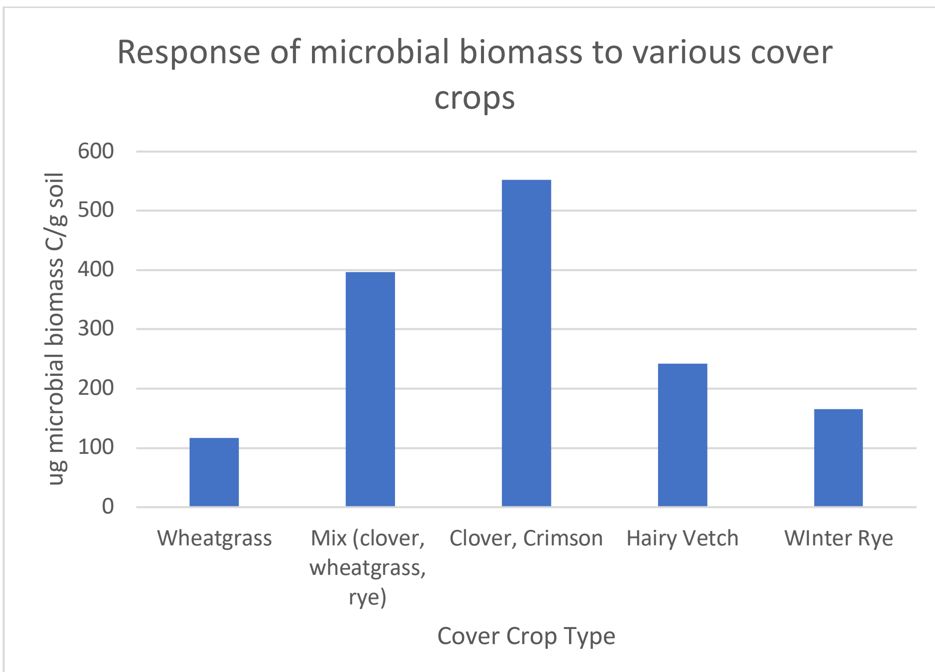

Throughout the season the 10 samples from each acre were remarkably consistent. Fig 3 shows the CV, standard deviation/average, showing a maximum of 17% variation in the values obtained across the acre. This indicates that 1 or 2 samples per acre may be sufficient to evaluate MB.




This study would greatly benefit from the farms providing yields and dates of planting, harvest, fertilization and rainfall. Unfortunately, they did not provide this information.

**6. Evaluation of Cover Crop efficiency using microBIOMETER® to measure microbial biomass increase**

Prolific Earth Science has worked with the Carbon Sponge Project which is funded by a New York State Arts and Science Grants and Patagonia, and City University of New York to educate New Yorkers about how the soil can store carbon. One of their first endeavors was converting an abandoned lot by use of cover crop. Before starting we recommended trying different cover crops in pot experiments. The results of this 1-month experiment, shown below, indicate the superiority of crimson clover in increasing the soil health of their soil. This chart measures microbial carbon and shows that crimson clover increased the microbial carbon by 600%, and so put this carbon back into the soil. An increase like this indicates that crimson clover was replacing a nutrient lacking in the soil. Microbes like humans cannot grow if a critical nutrient is missing. Replacing that nutrient restores their ability to utilize the other nutrients in the soil to grow. Crimson clover provides N to soil, indicating that it was N that was missing.



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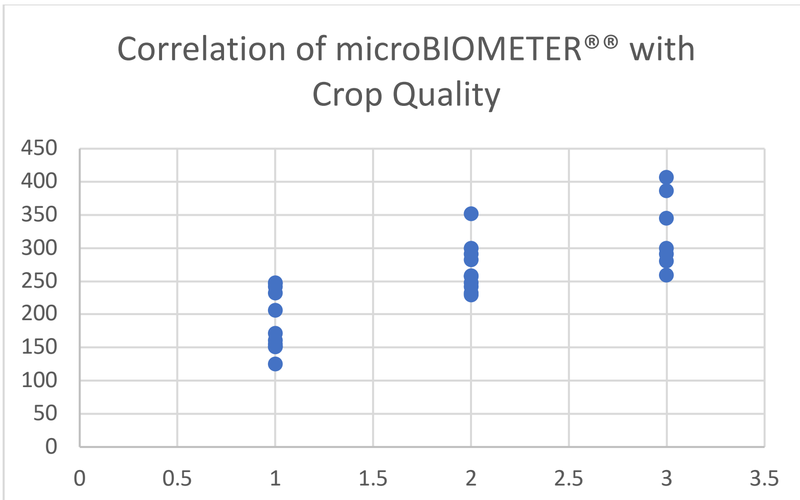
### 7. Correlation of microbial biomass with crop quality

Katharhy Grossman is a graduate student at City University and an agronomist, who went to Ecuador to investigate the relationship between microbial biomass with crop health. He visited 28 different farms growing 15 different crops, (most sites not receiving irrigation), tested the soil with microBIOMETER® and ranked the crop health as poor (1), average (2), good (3), excellent (4). As the graph shows microbial biomass correlated with crop health under all these different conditions. Samples with microbial biomass lower than 225 were all poor (1), samples above 400 were all excellent. The take home lesson is, to improve your plant health and yield, increase your microbial biomass by feeding your microbes with organic amendments. It should be noted, that these farms were not using commercial fertilizers, i.e. they should be considered "organic"; crop yield and soil health do not correlate when comparing "organic" to "conventional" farming.



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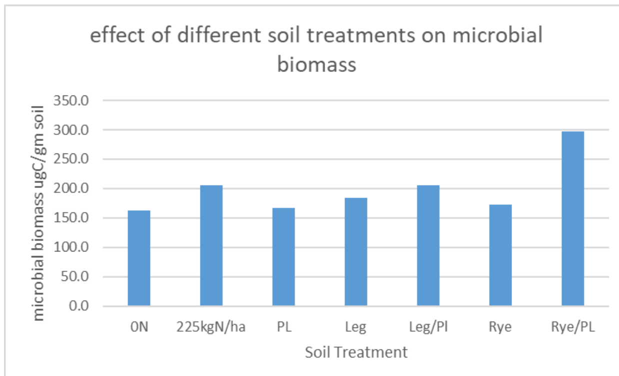
**8. mB effectively identifies the best combination of treatments for building soil health**

Jac Varco, Ph.D. Professor Mississippi University, Mississippi invited Prolific Earth Sciences to provide mB analysis of his experiment testing the effect of 7 different treatments (in triplicate) on corn plots. Testing was performed on top 5 inches of soil from the corn row in July 2017. When mB readings were below 200, 6 drops of extraction fluid were applied and the result divided in half. The treatments were, No nitrogen (0N), 225kgN/ha, poultry litter, (PL), legume cover crop (Leg), Legume + poultry litter (Leg+PL), Rye cover crop, Rye + PL. Microbial biomass is expressed as ug carbon/gram of soil. As shown Fig. 1, the most efficient at increasing MB on a corn crop was Rye cover with poultry litter: it almost doubled as compared to all other treatments that showed little effect over control.




Varco analyzed the effect on soil health on these same samples using the fluorescein di-acetate (FDA) method for soil: briefly this widely used colorimetric test is considered to measure total microbial activity[1]. Correlation of MB with Enzyme activity was r=-0.8 (Fig 2). The negative slope indicates that FDA activity at this time did not reflect microbial biomass carbon (which is the best predictor of soil health).

Yield data was unavailable for further analysis.

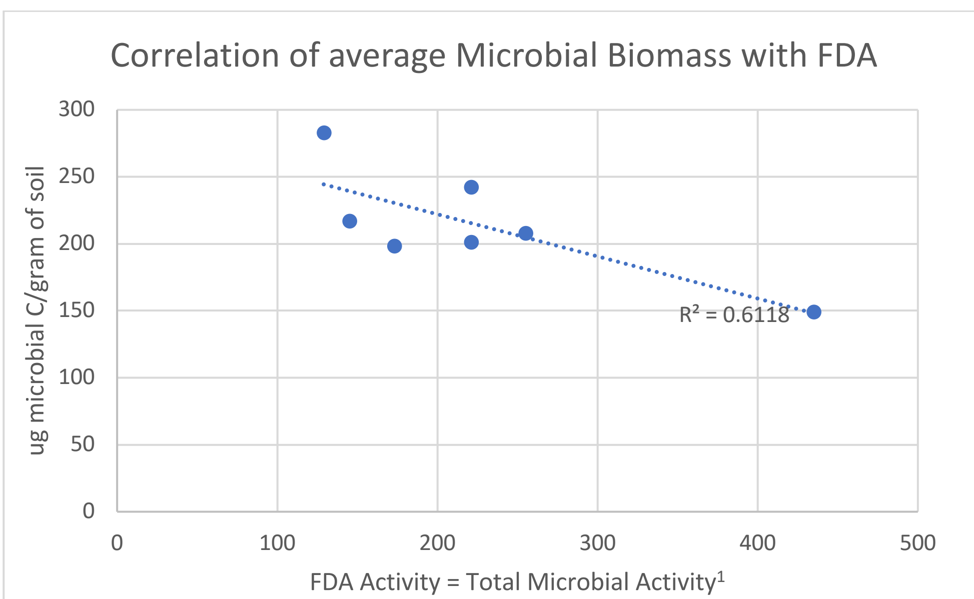





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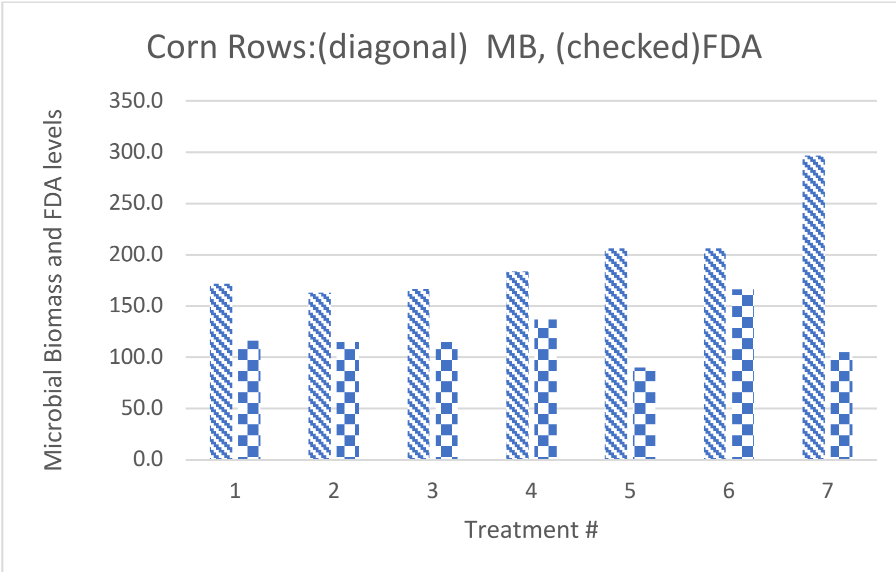
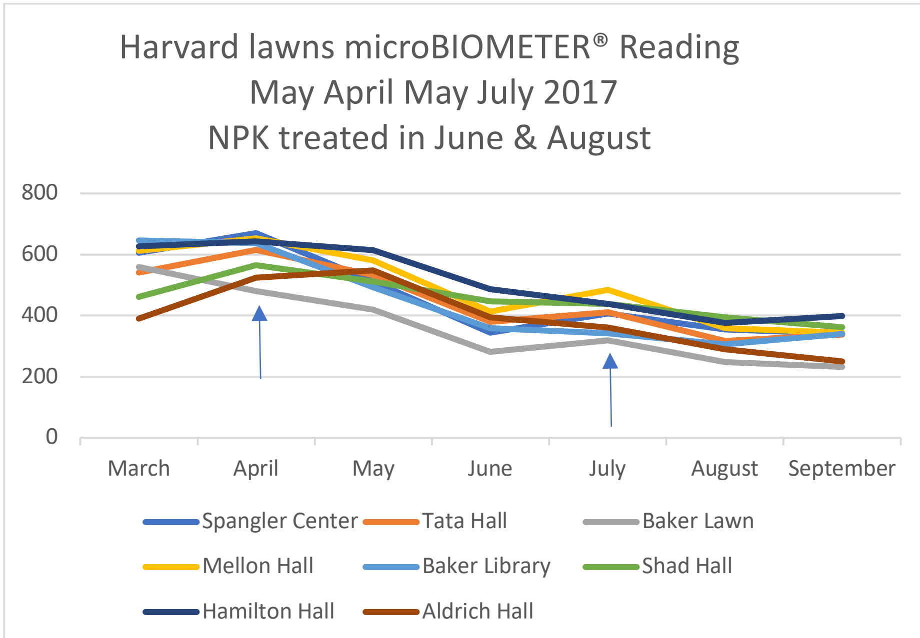
Fig 3 shows the Corn Rye/PL treatment which had the highest MB had the lowest enzymic activity. This is important information because high microbial activity can indicate that microbes are having to work too hard to maintain themselves which means that they are not building soil carbon but putting extra carbon dioxide into the atmosphere.




[1]Adam, Gillian, and Harry Duncan. "Development of a Sensitive and Rapid Method for the Measurement of Total Microbial Activity using Fluorescein Diacetate (FDA) in a Range of Soils." *Soil Biology and Biochemistry*, vol. 33, no. 7-8, 2001, pp. 943-951.

9. Microbial Biomass Seasonal Response on Harvard Lawns.

James Sottilo, PES co-founder, monitored the Harvard lawns in 2017. As can be seen microbial biomass slowly decreased over the course of the growing season. Indicating that monitoring of microbial biomass levels should occur at the same time each year. Also note that microbial biomass decreased after each nitrogen application (arrows). It is well known that chemical fertilizers have a negative effect on microbial biomass.



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