INTRODUCTION

Microbial biomass (MB) is the best single indicator of soil health (Doran, 2000). Microbes feed and protect plants, build soil structure which prevents erosion, increases water holding capacity, and builds soil organic matter (SOC). MB is low in any situation that is harmful to plant growth and vice versa and protects against pathogens reducing the need for pesticides. MB can predict success before plant outcome.

Prolific Earth Sciences spent over four years developing the microBIOMETER®. Results are reproducible in day-to-day testing, as well as under different lighting conditions. The microBIOMETER® correlates well with microscopic and carbon fumigation methods of assessing microbial biomass. In addition, multiple academic studies have shown that MB is highly correlated with other widely accepted soil health indicators, such as Nitrogen, Potassium, Phosphorus and Soil Organic Carbon (SOC) see below.

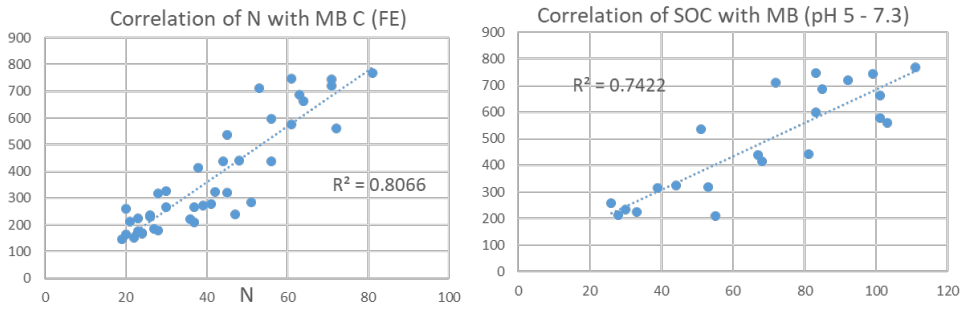

Figure 1. Data taken from Anderson et al 1989 show the correlations between Microbial Biomass (MB) measured by Fumigation Extraction (FE) with Nitrogen (N) and Soil Organic Carbon (SOC).

The microBIOMETER® is a patented, low cost assay for MB that can be performed in-field on living soil. It is simple to use, free of hazardous materials, and produces rapid results. By following four easy steps, the results are available on your smartphone and stored in the cloud:

- Accurately and consistently measure soil by volume
- Extract microbes from soil particles using agitation in a precisely formulated solution
- Allow soil particles to settle, leaving microbes in suspension
- Measure MB by applying the microbial suspension to a test card and analyzing with a smartphone app

The company currently has relationships with several universities and is in the process of obtaining independent validation which will be shared once that data is available.

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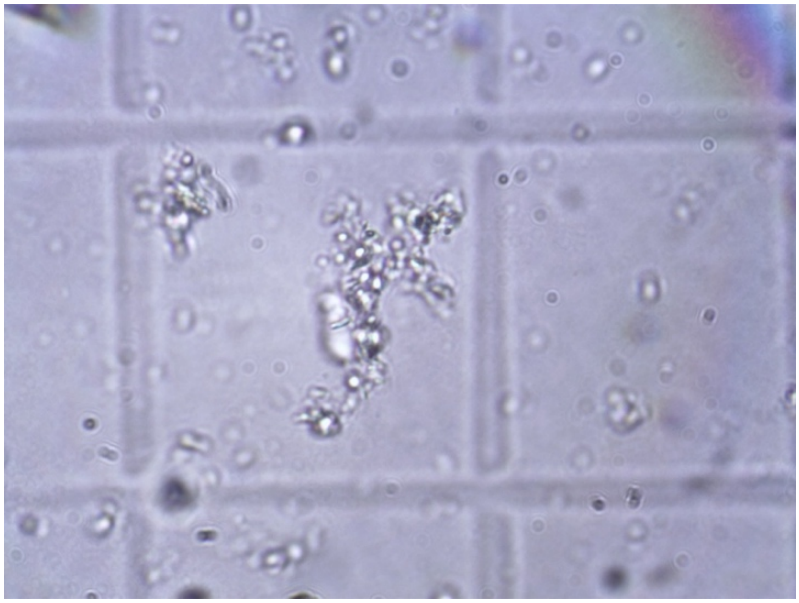
HOW THE TEST WORKS

Healthy soil has good structure due to the sticky substances that bacteria and other microbes produce to bind them to soil particles. The combination of extraction fluid components and mechanical whisking releases the microbes from the nonliving components of soil. The 20-minute settling period allows the nonliving components to settle to the bottom as the microbes remain suspended, for a while.

We calculated microbial biomass as follows. We tested numerous soils with different moisture contents and found that 0.5 cc of compacted soil has mean dry weight (50˚C overnight) of 0.6 g. Therefore the 0.5 cc soil extraction represents 0.6 g of dried soil suspended in 10 ml of extraction fluid. Dilutions of soil extracts were examined microscopically using a slide with precisely spaced grid lines. The area of each square in the grid is known - as is the volume of liquid above each square (using a properly weighted coverslip). Digital image processing combined with visual counting methods were used to calculate the microbial biomass on the slides. The units for biomass of micrograms of microbial carbon per gram of soil (μg C / g) were chosen from the findings from the literature that dried bacteria mass is 50% carbon.



The cell phone app images the sample window and calculates the intensity of the color present. Normally, bacteria are mostly transparent when viewed under a microscope, and stains are often employed to facilitate detection. Since bacteria and other microbes in soil take on the color of the soil due to uptake of humic acid and other dark colored materials, stains are not necessary with the microBIOMETER®. The intensity of the sample window is converted to μg microbial biomass carbon/gram of soil based on the measurements described above.

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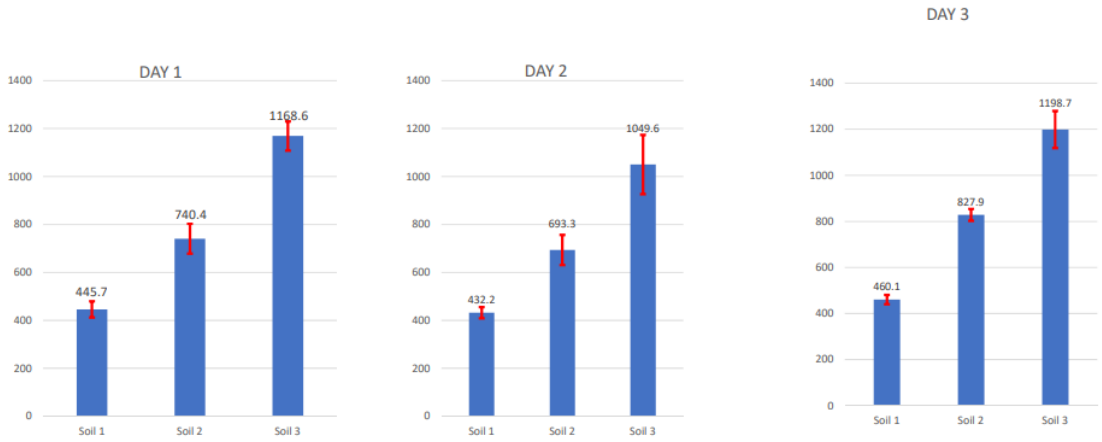
REPRODUCIBILITY AND PRECISION OF THE TEST

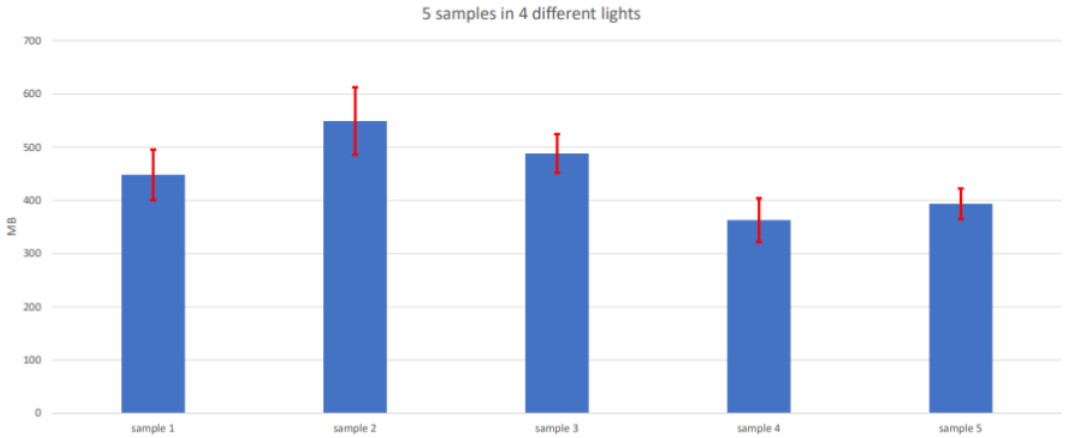
Ten (10) replicates of three (3) different soil samples were analyzed on three (3) different days. The sample measurement variation (CVs) ranged from 3.1% to 11.8%. The error (CVs) across all three days were Soil 1 = 3.1%, Soil 2 = 9.1%, and Soil 3 = 6.9%.



COMPENSATING FOR DIFFERENT LIGHTING CONDITIONS

One of the most difficult aspects of analyzing with smartphone cameras is differences in color temperature used to image the testcard. The microBIOMETER® app controls for differences in color temperature and allows for consistent readings in all lighting conditions.

Shown are five (5) samples imaged in warm incandescent, fluorescent, direct sun, and shade. The error (CVs) for the five (5) samples across the four (4) different lighting conditions were 10.6%, 11.6%, 7.5%, 11.3%, and 7.3%.



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COMPARISON WITH OTHER METHODS

Overview of current methods run for correlation

- Current methods extract and measure a small component of the MB (e.g. Carbon, phospholipid fatty acids, or DNA) and then multiply the result by 2 factors:
  - An estimate of the fraction of that component in the microbial population.
  - An estimate of the efficiency of the extraction method(s).
- Uncertainties about extraction efficiencies create uncertainties in measurements as does the fact that a single component does not compose the same % in every microbe.
- Current methods are technique dependent and, in many research labs, are not routine. Therefore, run-to-run variation can greatly affect consistency, leading to large study-to-study and lab-to-lab variability.

Method of estimating Microbial Biomass (MB)	% of sample evaluated	Avg Cost	Avg Analysis Time (hours)	Disadvantages	Reproducibility
Carbon fumigation	~40%	$500	336	Cost & Time	Reasonable but not consistent lab to lab
Phospholipid Fatty Acid Analysis (PLFA)	~10%	$80	168	Cost & Time, small % of sample measured	Reasonable but not consistent lab to lab
Microscopy	<10%	$30	168	Dependent on skill of microscopist	Too technique dependent
microBIOMETER®	>50%	$10	0.2		CVs ranged from 3.1% to 11.8%.

Although carbon fumigation is considered by some to be the "gold standard" of measuring soil microbial biomass, we have found widely ranging correlations in the literature between carbon fumigation and other soil microbial biomass tests, such as flow cytometry, ATP, quantitative PCR, and PLFA analysis, with Pearson coefficients ranging from ~-0.01 to ~0.95. While our literature search is by no means exhaustive, it appears that soil type, time of sampling, and geography play a role in these test results and correlations between test types.

Carbon fumigation methods measure the difference in carbon between soil samples that have or have not been exposed to chloroform. The amount of carbon is determined by chemical ***e***xtraction (CFE) or the volume of CO_2 released by respiration during extended ***i***ncubation (CFI). Microbial biomass carbon (MBC) is calculated as E_C/K_C (CFE) or F_C/K_C (CFI) where E_C or F_C is the difference in organic carbon extracted/evolved between fumigated and nonfumigated soil. K_C is a factor used to determine the extent of fumigation and is determined by addition of known amounts bacteria and fungi labeled with ^{14}C and quantification of the extracted ^{14}C or evolved $^{14}CO_2$.

Vance et al 1987 investigated the surprising finding that MBC in acidic soils was underestimated by CFI when compared to microscopic observations. They found that K_C was much lower in acidic soils than for other more neutral soils. With the lack of reliable correlation among the various tests listed above and the trusting of microscopic observations as a basis for determining K_C in carbon fumigation studies, we chose to validate the microBIOMETER® via microscopy.

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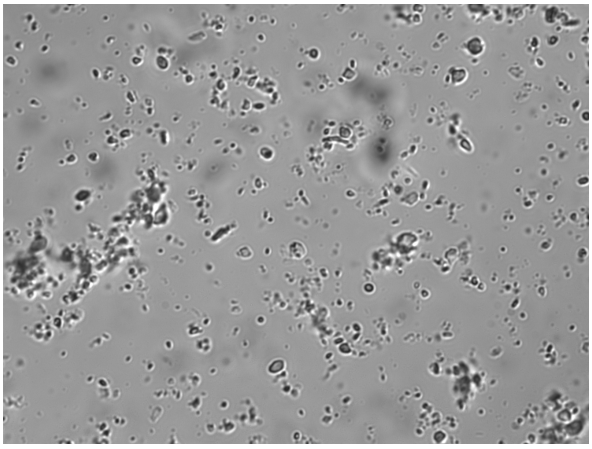
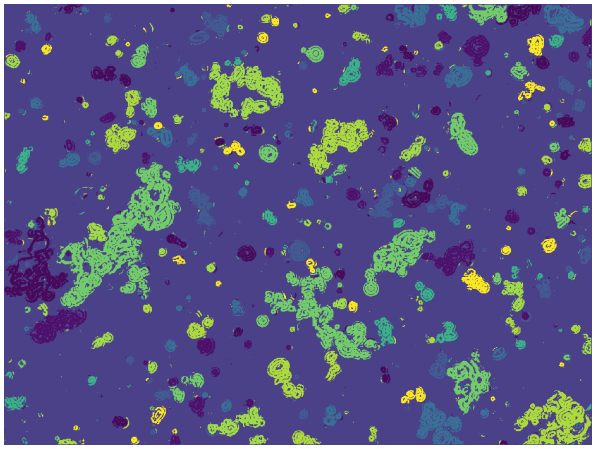
VALIDATION OF microBIOMETER® BY DIGITAL ANALYSIS OF MICROSCOPE IMAGES

Methodology:

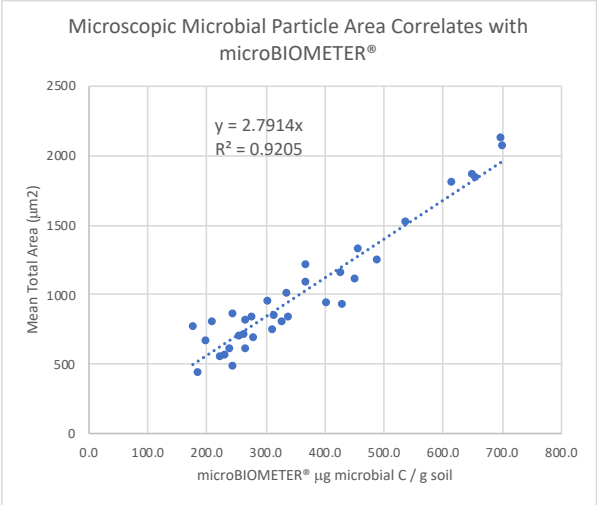
- Extract microbes from non-living soil particles using the same method used in the microBIOMETER® test
  - 3 extracts were used for each soil
- Place 3 or 6 drops of extract onto 3 microBIOMETER® Test cards and analyze with the app.
  - The number of drops depends on the source of the soil samples but is the same for all samples from an individual source.
- Place 14 µl of the same extract on a slide and cover with a slip, 3 samples per slide.
- Acquire 10 images in a spiral pattern around each coverslip.
- Digitally analyze the images to determine the total area of all the particles, using a predetermined scale of 8.1 pixels / µm.

Below is an example of the analysis





On the left is the original image, and on the right is the image after it has been segmented into individual contiguous particles. The area of each particle was determined by the area of the polygon that circumscribed it or more simply by the number of pixels it consumed. Both methods produced similar results. The total area of all particles was calculated for each of the 10 images and averaged. When compared to the average microBIOMETER® reading, the correlation is outstanding.



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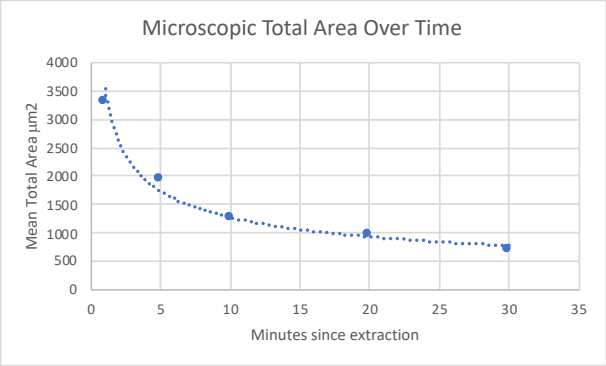
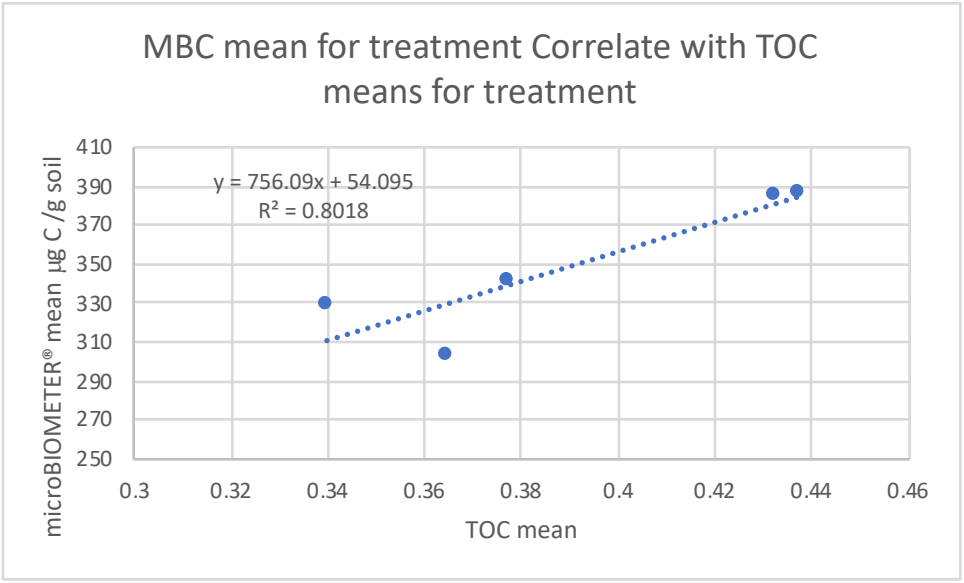
The extraction fluid is formulated to aid in extraction of the microbes and rapid precipitation of the non-living soil particles. Below is a series of total areas derived from microscopic analysis over 30 minutes, showing the stability of the microbial suspension after 10 minutes of settling.



Other Methods in testing for correlation:

- Jill Clapperton with Rhizoterra is currently field testing the Solvita CO_2 Burst field test against the microBIOMETER® and finding good correlation.
- The Carbon Sponge Project at City University of NY (CUNY) is using microBIOMETER® to track carbon sequestration and to evaluate effective sequestration efforts.
- CUNY demonstrated high correlation of microBIOMETER® results with agricultural outcome at 20 agricultural sites in Ecuador.
- Forbes Walker and his associates at the University of Tennessee have found that microBIOMETER® correlates highly with yield from a study of 32 plots, each of which have 30 years or more productivity data. microBIOMETER® is the only soil test they have found with such a correlation.
- In addition, microBIOMETER shows excellent correlation with soil Total Organic Carbon measurements from these fields. See below.



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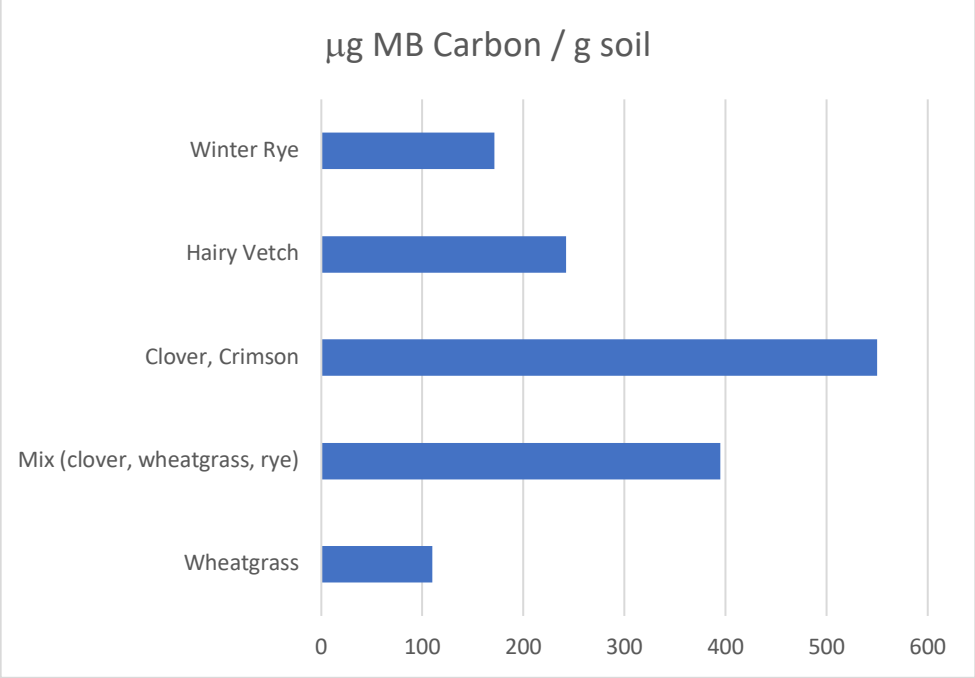
USES FOR THE microBIOMETER®

- Determining the efficacy of a cover crops: microBIOMETER® is excellent for short term (<1 month) potted evaluation of the efficacy of a cover crop. The below graph shows the increase in MB in the soil of a vacant New York City lot with various cover crops.



- Assay health of soil before planting, applying fertilizer, amendment or regenerative regimen. As an example, a plot in Englewood, NJ was planting very expensive trees ($2,000 evergreens) and it was discovered that three (3) had died in a row in the same spot. The microBIOMETER® was used to determine MB of the soils. Thus, the landscaper determined that the spot where they had been planted has <100 µg MBC/g of soil, while trees planted in locations on either side with MB >400 µg MBC/g have thrived.

- Assay progress of remediation or efficacy of treatment. The test quickly, sometimes within days, shows microbial response to treatment providing information before plant outcome.

- The test could be used to discriminate between chemically treated and organically treated soil. Soil that has been treated with chemical fertilizer will generally have MB that is below our level of detection, i.e. < 200 µg MBC/g soil. It is usually around or less than 100. Soil would be considered on a path to health at 200 or above.

- Quality control of compost and compost teas and for evaluating quality of compost and titration of tea.

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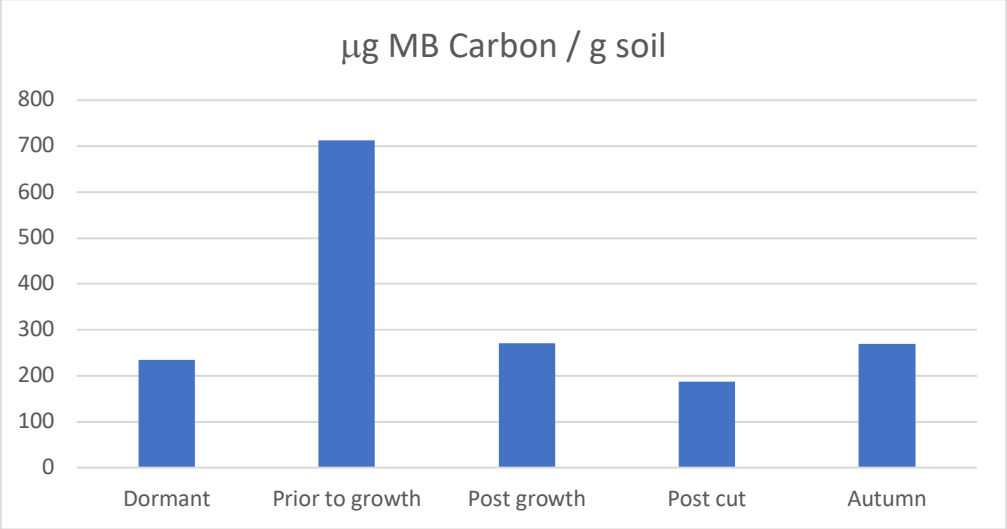
- Research: replaces expensive laboratory test for microbial biomass. The graph below plots the MB in a Texas field over a growing season.



- Microbial biomass has been shown to be a reliable short-term predictor of accumulation of soil organic matter, so should have use in evaluating regenerative methods.
  - Maeder, et al 2002, Science 296 1694.
  - SAFFIGNA et al. 1989. INFLUENCE OF SORGHUM RESIDUES AND TILLAGE ON SOIL ORGANIC MATTER AND SOIL MICROBIAL BIOMASS IN AN AUSTRALIAN VERTISOL Soil Biol. Biochem Vol. 21. No. 6, pp. 759-763.
  - WANG et al. 2012 Fertilization increases paddy soil organic carbon density. Biomed & Biotechnology 13(4):274-282.
  - Valpassos et al. 2001 Effects of soil management systems on soil microbial activity, bulk density and chemical properties. Pesquisa Agropecuária Brasileira.
  - Fontaine et al. 2004. Carbon input to soil may decrease soil carbon content Ecology Letters, 7: 314–320.
  - Parihar et al. 2016. Long term effect of conservation agriculture in maize rotations on total organic carbon, physical and biological properties of a sandy loam soil in north-western Indo-Gangetic Plains. Soil and Tillage Research 161:116-128.
  - Balota et al. 2004. Long-term tillage and crop rotation effects on microbial biomass and C and N mineralization in a Brazilian Oxisol. Soil Tillage Res. 77:137–145.