

Evaluation of microBIOMETER® as a tool to estimate soil health in a west Tennessee cotton crop

A Thesis Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

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May 2022

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This thesis is dedicated to my late grandfather, Jim Bell. Without his loving support throughout my life, I would not be who I am today.

Acknowledgments

I want to thank my major advisor, Dr. Forbes Walker, for his guidance and support throughout the pursuit of my degree. I will forever be appreciative of the knowledge and experience that he shared with me. I want to thank my committee members; Dr. Nutifafa Adotey, Dr. Sean Schaeffer, and Dr. Deb Saha, for their support and guidance in the development of this research and culmination of this research into a thesis. I want to thank Mr. Brian Kozlowski for his aid in sampling and sharing his knowledge about the cotton experiment at the West Tennessee Research and Education Center. I want to thank everyone who has been involved with the creation and care of the continuous cotton experiment. Without these people, I would have had nowhere to perform my research. I want to thank the members of Dr. Schaeffer's lab group; Aubrey Fine, Ning Duan, Alexis Gilmore, Cori Martin, and Shannon Newell for allowing me to perform my analyses in their lab and for the extensive and valuable help they provided during my research. I also want to thank the other graduate students under Dr. Walker, Adam Zimmerman and Kara Grosso, who have been the most supportive friends I could have asked for. Finally, I want to thank my parents, my family, and my friends for providing me with all the tools I needed to succeed as a graduate student and for always having my back as I tackled the challenges of graduate student life. Thank you to everyone listed above, I will be forever grateful of the impacts that you have had on my life.

Abstract

Healthy soils are critical for optimized yields and sustainability in agriculture. Soil health testing can provide valuable information on how management practices are affecting soil health. In west Tennessee, existing soil health tests were unable to discern between significant differences in management practices. A new soil health test called microBIOMETER® has been developed by Prolific Earth Sciences® (Montgomery, NY). MicroBIOMETER® utilizes a new methodology for estimating microbial biomass and uses that estimate as a soil health score. This test has not been validated for use in west Tennessee. This research was focused on the validation of microBIOMETER® in a continuous cotton crop on a Lexington silt-loam at the West Tennessee Research and Education Center (WTREC) in Jackson, TN. The continuous cotton experiment at WTREC has undergone the same tillage, cover crop, and nitrogen fertilization treatments since 1981. Tillage treatments are conventional tillage and no-till. Cover crop treatments are no cover crop, hairy vetch (*Vicia villosa*), crimson clover (*Trifolium incarnatum*), and winter wheat (*Triticum aestivum*). Nitrogen application rates are 0, 34, 67, and 101 kg N / ha. Treatments present significant differences in management practices. It is expected that a functional soil health test can distinguish between these treatments. Three sets of samples were taken at WTREC and tested with microBIOMETER®, substrate induced respiration (SIR), and chloroform fumigation extraction (CFE). CFE and SIR are two lab standard methods for estimating microbial biomass and were included as comparisons for microBIOMETER®'s estimate of microbial biomass. Environmental conditions were analyzed to isolate impacts of management treatments. Environmental conditions may have had a role in observed temporal variability of microbial biomass, but did not appear to impact scores within an individual sample. Temporal variability may also have been impacted by cotton growth stages. CFE and SIR were able to consistently distinguish

between management treatments but differences were not significant. MicroBIOMETER® was unable to consistently distinguish between treatments. MicroBIOMETER® estimates of microbial biomass were more variable and less reliable than SIR or CFE estimations. It is not recommended that microBIOMETER® be used as a stand-alone soil health test in the conditions tested in this research.

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Chapter 1

Literature Review

The health of soil, plant, animal, and man is one and indivisible.

– Albert Howard

1.1 Introduction

Since the 1980s, soil health has become an increasingly debated topic among soil scientists. However, this increase in scientific knowledge has not translated into the development of any quantitative soil health tests that are accurate in Tennessee [64].

According to the Natural Resource Conservation Service of the U.S. Department of Agriculture, or NRCS,: "Soil health means assessing and managing so that [soil] functions optimally now and is not degraded for future use" [55]. This definition describes why it is important for producers to understand soil health, as they must be aware of it to optimally manage their soil. Being unaware of the health of one's soil leads to a situation in which the soil cannot be optimally managed.

Without an accurate method of quantifying soil health, Tennessee producers cannot measure soil health or must rely on qualitative evaluations, or "best professional" judgement, from trained soil professionals. In the United States, these evaluations are typically performed by NRCS soil scientists. These professionals can provide quality information to producers; however, these evaluations suffer from two primary issues. It is difficult to compare qualitative sets of data and producers have no way of evaluating soil health on their own.

A reliable soil health test that provided quantitative data would allow producers to measure soil health without professional help, identify areas of concern/low soil health, measure changes in soil health over time, and get recommendations on how to improve soil health scores. This information would allow for more optimally managed land and healthier soils across the state.

In recent years a new commercially available "do-it-yourself" soil health test has been developed. This test, called microBIOMETER®, is a rapid test, relatively cheap, and potentially accurate in Tennessee. The microBIOMETER® test was developed on the assumption that microbial biomass and soil health are directly related. Using this assumption, microBIOMETER® features a new method for estimating microbial biomass and presents that estimate as the health of the tested soil.

1.2 History of Soil Health

The first instance of the term "soil health" in scientific literature was documented in a masters thesis by Henry A. Wallace in 1910. It is likely that Wallace was using "soil health" to refer to soil fertility and the yield from a soil while not directly considering the broader biological or physical components of soil health [73].

Biological components were not considered in soil health until 1931 when Stafford discussed how certain planting regimes in forestry could lead to negative impacts on soil health. [68]. Though Stafford broke the barrier on discussing biological factors when discussing soil health, the concept was still not widely utilized. This was evidenced by the Agricultural Adjustment Administration's 1936 publication "Soil Health and National Wealth", in which biological factors were only briefly discussed [14]. Biological factors are notably absent again in soil health publications until 1956, when Tarrant wrote about the effects of slash burning. Tarrant notes that burning can destroy organic matter and soil microorganisms that are important to soil health but fails to go into any depth on the subject [69].

It was not until 1971 that viewing soil health through the lens of production and fertility was challenged in scientific literature. When answering questions from members of the Commonwealth Section of the Royal Society of Arts after reading his article "High Crop

Yields”, Sir Joseph Hutchinson said “All of us who are farmers have seen farms that have been built up into a very high state of soil health, let us say, rather than soil fertility, and which have been cashed by a single man in his lifetime. This is one of the greatest problems that now needs to be faced” [34]. While the paper does not go into any depth on this distinction, Hutchinson was challenging the idea that a soil should be measured solely by its productivity. Hutchinson was also challenging the idea that immediate crop yields are the most important aspect of farming by noting that a single farmer could ruin a soil’s health and that this problem needed to be addressed.

The idea that soil had benefits beyond production value was expanded upon by Cox in 1975. Cox described one important benefit of a healthy soil was its ability to buffer against the effects of soil-borne diseases [14]. Hutchinson’s argument that declining soil health was a pressing ecological problem was further supported in a 1977 article by Banathy. Banathy argued that short-term economic gains were being prioritized over long-term soil health even though it was known that the long-term effects of poor soil health can be costly [14].

In 1977 George defined soil health as “the inherent replenishment of nutrients in the soil through the process of weathering and soil formation” [14]. The issue with this definition was that it still focused on soil health as a means of discussing yield. Throughout the 1980s, this trend tended to persist, though there was some acknowledgement of other impacts of soil health. In 1987, Hill recognized soil health as an "important piece of natural capital that is essential to production operations" [32]. In 1988, Jain described soil health as being linked to environmental health and discussed soil health’s role in helping feed India’s growing population [37].

It was not until the 1990s that there was an increase in research into soil health [14]. In 1992, Haberern was the first person to describe the need for a soil health index [28]. It is interesting to note that in this same paper, Haberern predicts “an awakening of intense interest, research, and education in the whole area of ... soil quality” [28]. Haberern’s prediction holds true as the 1990s not only contained a debate on how to define soil health, but also evidence that producers began to widely accept the concept of soil health [60], and a debate on whether soil health and quality referred to the same idea [26, 60].

Several definitions of soil health and quality were proposed in scientific papers in the 1990s. In 1994, Larson and Pierce argued that soil quality was defined by a combination of factors that represented the biological, physical, and chemical components of a soil. They listed these functions as: (1) “provide a medium for plant growth and biological activity”, (2) “regulate and partition water flow and storage in the environment”, and (3) “serve as an environmental buffer in the formation and destruction of environmentally hazardous compounds” [44]. Acton and Gregorich proposed that soil health should refer to more than the fertility and productivity of a soil and defined soil health as “the soil’s fitness to support crop growth without resulting in soil degradation or otherwise harming the environment” [2]. Doube and Schmidt suggested a less production-centric definition based on soil’s interactions with all plants. They defined soil health as “healthy soils are those in which the capacity of plants to convert sunlight and CO₂ to structural carbohydrate remains near the upper limits of potential productivity over an extended period” [23]. These definitions have been shadowed by the Doran et al. 1996 definition of soil health. Their definition of soil health was “the continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, maintain the quality of air and water environments, and promote plant, animal, and human health” [21].

Publications in the 2000s on soil health are typically related to the importance of management by producers and other land managers on the health of a soil. Doran states “the ultimate determinant of soil quality and health is the land manager”, which emphasizes the importance that producers have in maintaining healthy soils [20]. Doran also recognized the scientific community’s impact on land management by saying “scientists can make a significant contribution to sustainable land management by translating scientific knowledge and information on soil function into practical tools and approaches by which land managers can assess the sustainability of their management practices” [20]. Doran helped lead the push to translate scientific knowledge into tools for land managers and co-authored a 2000 paper on how to manage soils to enhance or maintain soil health, noting that “anthropogenic reductions in soil health . . . are of pressing ecological concern” [22]. The 2000s also saw a push to identify indicators of soil quality led by van Bruggen and Semenov in 2000 [70].

The 2010s brought about continued interest in soil health and the founding of several institutes to perform research into soil health and promote practices to improve soil health. These included the formation of the Soil Health Institute in 2013 and the creation of the Soil Health Division in the United States Department of Agriculture (USDA) Natural Resource Conservation Service (NRCS) in 2014 [14].

Today, the NRCS offers this definition of soil health: “the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans”. This definition is essentially a pared-down version of Doran’s earlier definition of soil health. The NRCS also lists five functions of a healthy soil: (1) regulate water, (2) sustain plant and animal life, (3) filter and buffer potential pollutants, (4) cycle nutrients, and (5) provide physical stability and support. Again, the NRCS is simplifying earlier work, this time from Larson and Pierce in 1994.

1.3 Soil Health Tests in Tennessee

While there are multiple soil health tests available in the Tennessee, none of these tests have been validated for use in Tennessee. This section will focus on four of those available to Tennessee producers: the Cornell Comprehensive Assessment of Soil Health (CASH), the Alabama Soil Quality Index (SQI), the Haney Soil Health Tool (SHT), and the newly created microBIOMETER® test. Cornell claims the CASH to be the first publicly available soil health assessment [50]. The Haney SHT is focused on biological factors and is likely the most similar to the microBIOMETER® test. The Alabama SQI is one of the newest soil health testing protocols developed and is the first soil health test designed to work in the Ultisols and Alfisols of the Southeast [13].

1.3.1 Cornell Assessment of Soil Health

The Cornell Soil Health Test began as a Program Work Team of interested producers, researchers, Cornell Extension specialists, and private contractors in the early 2000s [50]. Among other accomplishments, this team developed the first soil health assessment protocol for their region, in New York state, known as the Cornell Assessment of Soil Health (CASH).

The CASH evaluates ten parameters to describe soil health: soil texture, available water holding capacity, field penetrometer resistance, wet aggregate stability, organic matter content, soil proteins, soil respiration, active carbon, and macro- and micro-nutrient content. These soil health indicators were selected because of their sensitivity to changes in management practices, their ability to represent important soil processes, their consistency and reproducibility, the ease and cost of sampling, the cost of analysis, and the ease of interpretation by users [50]. These ten parameters can also be augmented with additional, optional indicators like heavy metal content, root pathogens, and salinity.

Once these indicators were selected, the results of these tested parameters were synthesized into a producer-friendly report that gave individual scores for indicators, an overall score for soil health, and management suggestions based on scores [50]. Users can select between three types of soil health analysis: an analysis for (1) field crops, dairy operations, and lawns, (2) organic vegetable crop systems, problem area diagnosis, and home gardens, and (3) urban/suburban gardens and landscaped areas. The primary issue with the CASH is that the indicators are compared to reference values “for assessing the health status of soils in New York and the Northeast region”. This causes issues for users outside of the Northeast as soils are highly variable across the United States. Due to differences in soils and the climate, values for a healthy soil in New York are not the same as in Tennessee. When comparing soils of different expected soil health in west Tennessee, Singh found that the CASH assessment did not give statistically significant results between soils with significantly different management practices [64].

1.3.2 Alabama Soil Quality Index

The Alabama Soil Quality Index (SQI) was developed to address the issue of regional variability. It was recognized that existing soil health tests were not designed to work in the acidic, highly weathered Ultisols of Alabama. Researchers at Auburn University created a soil health assessment that worked in their state [13]. In developing the Alabama SQI, researchers created five criteria for the SQI to meet: (1) make producers aware of soil health, (2) suggest ways of improving soil health, (3) be adaptable to existing soil testing methodologies, (4) be relatively inexpensive, and (5) provide information in an easy-to-understand manner.

Researchers then selected soil health indicators that could easily be analyzed in the Auburn soil lab. The following soil health indicators were selected to use in the Alabama SQI: crop yield, soil pH, extractable macro- and micro-nutrients, carbon content, soil organic matter, soil respiration, electrical conductivity, wet aggregate stability, estimated cation exchange capacity, and base saturation. The values for these indicators in a healthy and unhealthy soil in Alabama were evaluated by analyzing 249 paired samples from across the state and across various soil types. These paired samples came from producers, landowners, and research facilities with the premise that one of the samples came from a low-yield area of the site and the other sample in the pair came from a high-yield area of the site. Developers of the Alabama SQI equated a high-yield soil to a soil with a high level of soil health. As with the CASH, Singh also found that the Alabama SQI did not show significant results between soils with significantly different management practices in west Tennessee [64].

1.3.3 Haney Soil Health Tool

The Soil Health Tool (SHT), developed by Haney and his colleagues in Temple, Texas, aims to provide a better estimation of biological factors in soil health and evaluate soil health on more than potential for productivity [30]. Haney notes that soil health assessments prior to the SHT use inorganic chemicals and treatments to evaluate indicators like extractable nitrogen (N), phosphorus (P), and potassium (K). The SHT is the first test to use organic acids to measure inorganic N, P, and K. SHT uses three indicators to calculate soil health: the one-day CO₂-carbon released by soil respiration, water extractable organic carbon, and water extractable organic nitrogen. The SHT calculates soil health by:

$$\frac{\text{CO}_2 - \text{C released}}{\text{Carbon to nitrogen ratio}} + \frac{\text{water extractable carbon}}{100} + \frac{\text{water extractable nitrogen}}{100} \quad (1.1)$$

While Haney states that his test should be applicable anywhere, Singh again found that the SHT had no significant results between soils with differences in expected soil health [65].

1.3.4 The microBIOMETER® Soil Health Test

Given that the above tests are not calibrated to work in west Tennessee, that leaves an opening in the market for a soil health test that is applicable in west Tennessee. The microBIOMETER® test, a new soil health test developed by Prolific Earth Sciences®, has been designed to measure soil microbial biomass and fungal to bacterial ratio. MicroBIOMETER® uses these factors as a corollary to soil health. Considering that soil microbial biomass is one of the important indicators of soil health, microBIOMETER® may provide reliable and valuable information on the health of a soil. The microBIOMETER® soil test is designed to isolate the microbial fraction of a soil in solution. Once the microbial fraction is isolated, an aliquot can be taken, and microbial biomass can be calculated based on the opaqueness of the sample.

A potential issue with microBIOMETER is that microbial biomass can be a highly variable quantity in soil systems. It is extensively documented that microbial biomass in soils varies temporally [10, 40, 46, 48, 51, 59], with temperature [3, 17, 27, 45, 72], and with soil moisture [1, 7, 18, 75, 41].

Despite this variability, microbial biomass is related to soil health [67]. MicroBIOMETER® does have potential to be of use in two ways: (1) as a relative measurement of soil health when testing across a field and (2) as a less resource intensive method for estimating microbial biomass.

MicroBIOMETER® requires less technical knowledge and is more economically accessible to Tennessee producers than other soil health tests. MicroBIOMETER® costs ten dollars per test while other tests can cost hundreds of dollars to perform. It takes about thirty minutes to perform the microBIOMETER® test, while it might take months to receive results from other tests. There is no technical knowledge required to use or understand microBIOMETER®.

MicroBIOMETER® lacks the independent verification of results that other soil health tests have. Prior to this research, any verification of the microBIOMETER® test has come from within their own labs and with little outside help from independent researchers.

Estimating microbial biomass

Since the microBIOMETER® test utilizes microbial biomass to estimate soil health, part of the research performed for this study is to examine how well microBIOMETER® estimates microbial biomass. To make this determination, two validated lab methods for estimating microbial biomass will be included: chloroform fumigation extraction (CFE) and substrate induced respiration (SIR). These methods for estimating microbial biomass were chosen because it is expected that the values estimated by CFE and SIR will be positively correlated.

This expected trend has been shown in several past studies. Vasquez-Murrieta et al. showed CFE and SIR were significantly correlated at $P = 0.05$ [71]. Kaiser et al. showed that there was correlation between CFE and SIR methods, though this correlation was not as strong as other studies have reported [42]. Wang et al. showed significant correlation between SIR and CFE at $P = 0.01$ [74]. Data collected by Bailey et al. shows a correlation coefficient of 0.6977 when fit to a logarithmic regression [6]. These studies support the expectation that CFE and SIR should be correlated in this study, though there are some previous studies that did not find a significant correlation between CFE and SIR. One example of this is found in the work by Dumontet and Mathur [24].

1.4 Management Treatments and Soil Quality Characteristics

This research utilizes qualitative evaluations of plot health in order to determine if microBIOMETER® and microbial biomass are effective ways to quantify soil health. The qualitative evaluations of soil health are based on the management treatments applied to the plots and the results from past research on the same plots that concern indicators of soil health. The synthesis of this information can be used to make qualitative evaluations of expected soil health in the plots. The effects of each treatment on soil health as well as the information gained from past research about soil health indicators at the research plots are contained in this section.

1.4.1 Effects of Management Practices on Soil Health

The research plots used in this study are part of a long-term cotton experiment started at the West Tennessee Research and Education Center (WTREC) in Jackson, TN, in 1981. The plots have undergone the same tillage, cover crop, and nitrogen fertility practices since the start date. References to "plots" in this section refer to a single split-split level plot in the long-term cotton plots at WTREC that were used in this study.

The long-term cotton experiment is a randomized complete block design using a split-split plot design. Whole plots were treated with four different nitrogen application rates: 0, 34, 67, and 101 kg N / ha. The split plot level contains four cover crop treatments: hairy vetch (*Vicia villosa*), crimson clover (*Trifolium incarnatum*), winter wheat (*Triticum aestivum*), and no cover crop. The split-split level contains two tillage treatments: tilled and no-till (2.1).

Nitrogen Fertilization and Soil Health

Nitrogen fertilizer can have a wide range of effects on soils, including an impact on pH, microbial communities, and amount of soil organic matter present [63]. Depending on the rate of nitrogen application, these changes can be positive or negative in nature [63]. When nitrogen is applied at a rate equal to or lower than the rate at which maximum yields occur, these changes are typically positive. When applied at the yield-optimizing rate, nitrogen can increase the soil organic matter content and cause increases in the population of the microbial community through increased plant growth. However, when applied at a rate higher than the rate at which maximum yields occur, nitrogen fertilizer can become harmful to soil health through mineralization of the soil organic matter and by decreasing the population of microbial communities.

Cover Crops and Soil Health

Cover crops also play an important role in soil health. Using cover crops can increase infiltration, increase the amount of soil organic matter, promote microbial community growth, reduce erosion, and decrease bulk density among other factors [31]. Cover crops have been

attributed to increasing soil health in both tilled and non-tilled agricultural systems [8, 19, 56]. These positive benefits of cover crops have been echoed in other studies [39, 47, 80]. Use of leguminous cover crops is typically more beneficial than use of non-leguminous cover crop [62]. These effects of leguminous cover crops like hairy vetch or crimson clover have been shown in west Tennessee as described in [Section 1.4.2](#).

Tillage and Soil Health

One of the most important management practices for an agricultural soil's health is tillage. The two most common tillage regimes are conventional tillage where the surface horizon of the soil is disturbed, and conservation or no-till systems where the soil surface stays as undisturbed as possible. No-till systems have been recognized as significantly better for soil health than conventionally tilled systems [5, 11, 12, 16, 35, 38].

1.4.2 Past Soil Health Research using Long-term Experiments at WTREC

Past research at WTREC includes studies that quantified different soil health indicators such as soil carbon, yield, microbial communities, and physical properties. The results from these studies can aid in the qualitative description of soil health.

Soil Carbon in response to soil health practices

Increases in soil carbon are correlated with increases in soil health [43]. Keeping carbon levels above certain thresholds, which are dependent on the local conditions, in the root-zone of a soil have been tied to benefits in aggregation, nutrient and water retention, rhizosphere processes, and gaseous emissions.

Jagadamma et al. found that soil organic carbon increased in the upper 2.5 cm of the soil and decreased in the 5-10 cm range of the no-till soil as compared to the conventionally tilled soil [36]. There were no significant differences in soil organic carbon when a single representative sample from 0-15 cm was tested between conventional and no-till. There

were also no significant differences found in soil organic carbon among different cover crop treatments.

Singh et al. found significantly higher levels of soil organic carbon in the upper 15 cm of soils treated with no-till practices as compared to soils treated with moldboard plow tillage [66]. There was no difference in soil organic carbon between no-till soils and soils tilled with a chisel plow or disk plow. Singh et al. found no significant differences in soil organic carbon between different cover crop treatments.

Mbuthia found significantly higher total organic carbon in hairy vetch treated plots compared to other cover crops [49]. Higher total organic carbon was reported in no-till plots as compared to tilled plots. Increased rates of nitrogen application also led to increased levels of total organic carbon [49].

Ceylan found that as nitrogen fertilizer application rate increased, total organic carbon in the system showed less variability between the conventional and no-till plots [15]. This shows that high levels of nitrogen application can cover up the effects that other management practices have on soil health.

Wilson showed that the highest nitrogen application rate led to significantly larger slow soil organic carbon pools than in other nitrogen application rates [79]. No-till practices also led to significantly larger slow SOC pools compared to conventional tillage. Total soil carbon concentrations showed the same trends as the slow SOC pools in regard to response to treatments.

Soil biological properties in response to soil health practices

Mbuthia found no significant differences in microbial biomass between treatments [49]. However, changes in the structure of the microbial community were found. Hairy vetch cover crops led to an increased percentage of Gram⁺ bacteria in samples and no-till led to decreased levels of mycorrhizal fungi [49]. Mbuthia did report significant differences in respiration in soils treated with a hairy vetch cover crop as compared to the other cover crop treatments [49]. Hu et al. determined that the hairy vetch cover crop treatment promoted an increase in the abundance of nitrogen fixing bacteria [33].

Mpheshea found instances of significant differences in microbial biomass between treatments. In June and July, there was significantly higher microbial biomass carbon in the highest nitrogen application rate when compared to the lowest nitrogen application rate [51]. Mpheshea also reported significantly higher microbial biomass in no-till in September.

Crop Yield in response to soil health practices

Nouri et al. found a 12% increase in cotton lint yield in no-till plots as compared to conventionally tilled plots [54]. Nouri et al. also reported higher yields in plots with a cover crop than without, though the differences were non-significant at a 95% confidence interval. Nouri et al. (different co-authors) also published an overview of cotton yield since 1981. Cotton yield decreased significantly in no-till plots as compared to conventionally tilled plots in the first ten years of the experiment [53]. During the following twenty years, no-till plots had significantly higher yields than the conventionally tilled plots. Increased nitrogen application rates led to increases in yield. Hairy vetch cover crop plots had significantly higher yield than plots with a winter wheat cover crop or no cover crop.

Mbuthia also found a 13% increase in no-till yields as compared to yields from conventionally tilled plots [49]. Mbuthia also created a soil quality index for this site and determined a 5% increase in soil quality when hairy vetch cover crops were used compared to other cover crop treatments.

Soil Physical Properties in response to soil health practices

Nouri et al. reported significantly higher volumetric water content and wet aggregate stability in no-till plots as compared to conventionally tilled plots [54]. Significantly larger aggregates were found in the upper 15 cm of the soil in plots with no-till or cover crops as compared to plots that were tilled or did not have cover crops, respectively. Wilson reported similar differences in aggregate distribution where no-till and hairy vetch practices led to higher percentages of large macro-aggregates in the soil [79]. Infiltration rate, cumulative infiltration, and field saturated hydraulic conductivity were all significantly higher in plots treated with cover crops than without [54]. Infiltration rate, cumulative infiltration, and field saturated hydraulic conductivity were all higher in no-till plots than in conventionally tilled plots.

1.4.3 Hypotheses on impacts of soil health practices at WTREC

Based on the synthesis of past research, it is hypothesized that any cover crop treatment should lead to an improvement in soil health over no cover crop. Hairy vetch should provide the most benefits to soil health among the cover crop treatments. It is expected that no-till plots will have greater soil health than plots treated with conventional tillage. It is expected that the 67 kg per hectare nitrogen treatment will have higher microbial biomass than the 0 kg per hectare nitrogen treatment.

Given these results, the combination of no-till treatments with the hairy vetch cover crop will result in the plots with the expected healthiest soil. The combination of conventional tillage with no cover crop will result in the plots with the expected least healthy soils.

1.4.4 Purpose of Research

Other soil health tests have not distinguished between significant differences in contrasting management practices in Tennessee [64]. The microBIOMETER® test has yet to be validated in Tennessee. The purpose of this study will be to validate the uses of microBIOMETER® in a west Tennessee cotton crop. The objectives to be addressed for this validation are as follows:

1. Does microBIOMETER® distinguish between significant differences in management practices?
2. Does microBIOMETER® provide similar estimates of microbial biomass as substrate induced respiration or chloroform fumigation extraction?
3. Do substrate induced respiration or chloroform fumigation extraction distinguish between significant differences in management practices?

Chapter 2

MicroBIOMETER® Validation Study

To be a successful farmer one must first know the nature of the soil.

– Xenophon

2.1 Introduction

An accurate, quantitative test of soil health available for use by producers could help improve soil health across Tennessee. The microBIOMETER® tool is a newly developed soil health test that needs to be validated for use in Tennessee. Previously validated soil health tests did not find significant differences across different treatment combinations where there were differences in the qualitative evaluation of soil health [64]. MicroBIOMETER® was not evaluated during the study by Singh. Therefore, the usefulness of microBIOMETER® to Tennessee producers needs to be evaluated.

This chapter focuses on the ability for microBIOMETER® to distinguish between different agricultural management practices and estimates of microbial biomass. Qualitative assessments of soil health are provided in [Section 1.4.3](#). MicroBIOMETER® scores will be tested for correlation to management treatments and the associated qualitative soil health. Data for environmental factors that can affect microbial biomass was also be collected. Correlation between environmental factors and microBIOMETER® scores will be assessed to confirm that microBIOMETER® scores are not being controlled by environmental factors.

MicroBIOMETER® functions on the claim that microbial biomass correlates directly to soil health. This claim will also be tested. Chloroform fumigation extraction (CFE) and substrate induced respiration (SIR), both widely used methods for estimating microbial biomass, were used to assess microBIOMETER’s® ability to estimate microbial biomass. Microbial biomass estimates from these two lab standards will be used on the split-split level plots with the highest and lowest expected soil health (as discussed in [Section 1.4.3](#)) to test for correlation between health and estimated microbial biomass. Similarities in estimates of microbial biomass between CFE, SIR, and microBIOMETER® will also be analyzed.

2.2 Materials and Methods

Soil samples were collected at the West Tennessee Research and Education Center (WTREC) located in Jackson, Tennessee. Samples were taken from a long-term continuous cotton experiment that has been under the same tillage, cover crop, and nitrogen fertility management practices since 1981. The long-term cotton experiment is a rain-fed (non-irrigated) experiment in a humid subtropical climate as defined by the Köppen classification. Humid subtropical climates experience relatively even rainfall throughout the year with high temperatures. Soils under the long-term cotton experiment are Lexington silt loam (fine-silty, mixed, active thermic Ultic-Hapludalf) with 0-2% slope.

The long-term cotton experiment is a random complete block design using a split-split plot design with four replications at the plot level. Plots were treated with four different nitrogen application rates: 0, 34, 67, and 101 kg N / ha. Split plots were treated with four different cover crop treatments: hairy vetch (*Vicia villosa*), crimson clover (*Trifolium incarnatum*), winter wheat (*Triticum aestivum*), and no cover crop. Split-split plots were treated with two different tillage treatments: tilled and no-till. Tilled plots are double disked at a depth of 10 cm and harrow leveled prior to planting. Split-split level plots are 10 meters² in size. A summary of the plots is in [Figure 2.1](#).

Treatments selected for this study were all split-split level plots within the 0 and 67 kg N / ha plots. This included all four cover crop treatments and both tillage treatments. Subsets of these plots were tested with chloroform fumigation extraction and substrate induced

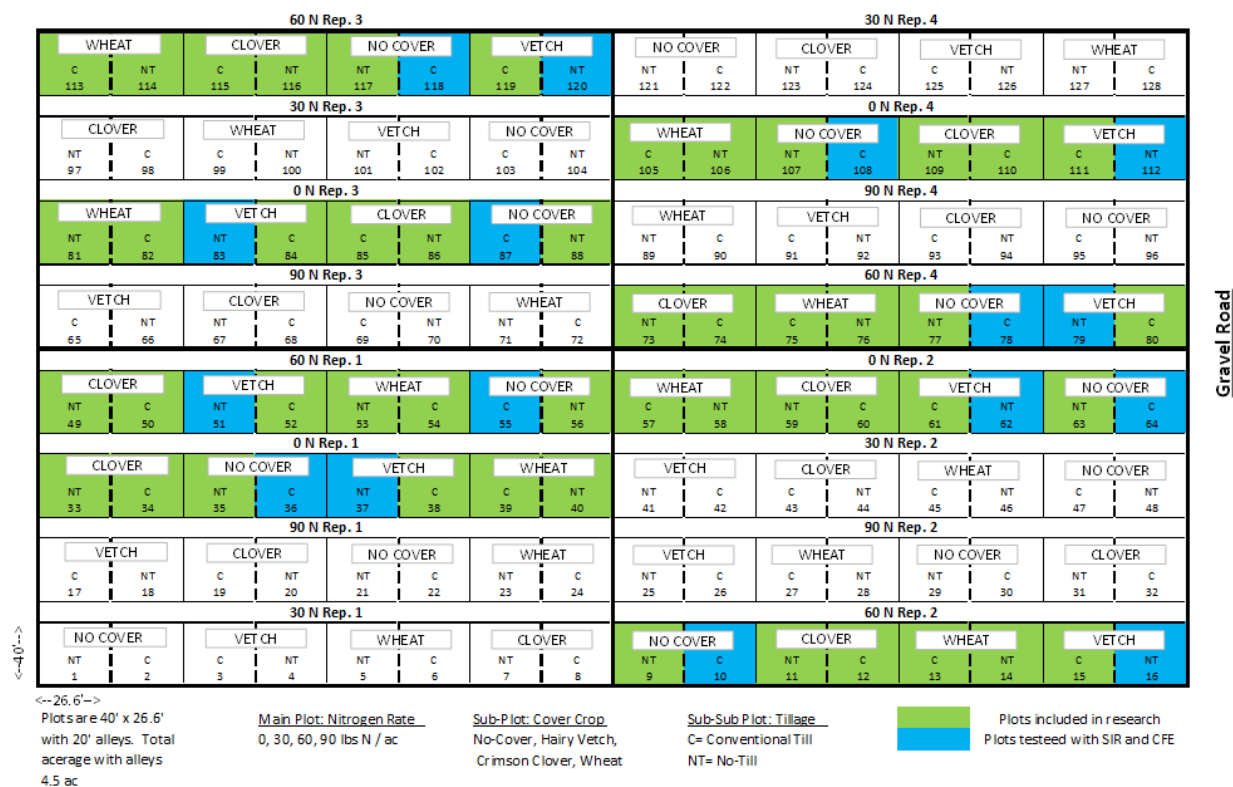


Figure 2.1: Layout of Study Plots at West Tennessee Research and Education Center

respiration. The subset of plots selected for this were split-split level plots with the treatment of hairy vetch with no-till and the split-split level plots with the treatment of no cover crop with conventional tillage. These plots represent the expected highest and lowest soil health, respectively.

For the 2021 season, cover crops were planted on November 6, 2020. The cover crops were terminated with Gramoxone® and Roundup® on April 12, 2021. Cotton was planted on June 14, 2021. Cotton was fertilized on June 16, 2021, using ammonium nitrate. Samples were collected 15 days after planting on June 29, 59 days after planting on August 12, and 126 days after planting on October 18. These sample dates corresponded to sampling during germination, during first flower, and at boll development prior to harvest.

The cotton crop was also periodically treated with herbicides and pesticides. The first application was on April 26, 2021, when Cotoran® and Caporal® were applied. Dual®, RoundUP®, and Acephate® were applied for thrips on May 17, 2021. Transform® and Diamond® were applied for plant bugs on June 15, 2021. Liberty® and Bidrin® were applied for plant bugs on July 13, 2021. Finally, Brigade® was applied for worms on September 17, 2021.

On sampling dates, soil samples were taken to a depth of 8 cm, which is the recommended sampling depth for microBIOMETER®. Cores were collected between rows. Ten cores were collected at random from across the plot and mixed to create a homogenized sample that was representative of the entire plot. Soil temperature was estimated on sampling dates using LusterLeaf® Rapitest® thermometers. Air temperature was collected using the permanent weather station at WTREC.

Prior to processing, soil samples were sieved to 2 mm to remove large aggregates and organic matter. Gravimetric water content was calculated by determining the weight of water lost when oven dried at 105°C for twenty-four hours. Chloroform fumigation extraction (CFE) was performed using the slurry methodology described by Fierer [25]. Step-wise description for methodology performed can be found in [Appendix A](#). Substrate induced respiration (SIR) was performed using methodology from Anderson and Domsch [4] with consideration of the modifications made by West and Sparling [77]. The primary deviations from Anderson and Domsch methodology was the use of yeast extract as the substrate, use of soil water content

to determine amount of extract added, and use of an infrared gas analyzer (IRGA) to analyze carbon content. Step-wise methodology performed for SIR can be found in [Appendix B](#). Step-wise methodology for use of the IRGA can be found in [Appendix C](#). MicroBIOMETER® was performed using methodology adapted from Prolific Earth Sciences® instructions for microBIOMETER® use. Step-wise methodology can be found in [Appendix D](#).

After microbial biomass estimation, values were all converted to micrograms of microbial carbon per gram soil to allow for comparison between different methods of estimating microbial biomass. The collected data was analyzed for statistical significance using One-Way Analyses of Variance (ANOVAs), T-Tests, and Mixed-Factor ANOVAs as appropriate. Tukey’s post-hoc test was performed after ANOVAs to isolate where statistical significance was occurring when ANOVA tests rejected the null hypothesis. Tests were performed at 95% confidence intervals for statistical significance. For statistical tests, the null hypothesis is that there is no significant difference in tested variables and the alternate hypothesis is that there is a significant difference between tested variables.

2.3 Results

MicroBIOMETER® did not consistently return similar values as the other two tests. P-values, correlation coefficients, and covariance data for these relationships are shown in [Table 2.1](#). MicroBIOMETER® also showed higher variance than CFE and SIR in estimating microbial biomass ([Table 2.2](#)). There was only one instance in which two different methods for estimating microbial biomass were not significantly different from each other. This occurred between the CFE and SIR methodologies during the June sampling event. However, there was only a moderate correlation ($R^2 = 0.4146$) between SIR and CFE values during the June sampling event ([Figure 2.2](#)). This was the best correlation between two tests during any of the sampling events.

There was an observed significant interaction between estimated microbial biomass and sampling time (<0.0001) and this occurred regardless of the method of estimating microbial biomass used ([Figure 2.3](#)). Average values from microBIOMETER® were significantly lower in June and October than in August. Average values from CFE were significantly

different between each sampling event, with the highest mean scores in August and the lowest mean scores in October. Average values from SIR were significantly different between each sampling event, with the highest mean scores in August and the lowest mean scores in June. Comparisons between microBIOMETER® and CFE scores over the three sampling events are shown in [Figure 2.4](#). Comparisons between microBIOMETER® and SIR scores over the three sampling events are shown in [Figure 2.5](#). Comparisons between CFE and SIR scores over the three sampling events are shown in [Figure 2.2](#). Comparisons between CFE, SIR, and microBIOMETER® combining data from the three sampling events are shown in [Figure 2.6](#).

MicroBIOMETER® scores were not significantly influenced by soil temperature, air temperature, or water content differences within the same sampling event as shown in [Table 2.3](#). The only instance of environmental conditions leading to significant differences in estimated biomass was in the August sampling event where there was a significant difference in CFE estimates between different gravimetric water contents. MicroBIOMETER® scores were not significantly influenced by the experimental replicate. MicroBIOMETER®, CFE, and SIR scores did vary significantly across different sampling events. This is discussed in further in [Chapter 3](#).

Over the three sampling periods, microBIOMETER® showed no significant difference in mean microbial biomass based on the individual management treatments ([Table 2.4](#)). When testing all interaction effects, microBIOMETER® reported significant differences in microbial biomass during the first sampling event in the cover crop X tillage X nitrogen and tillage X cover crop treatment interactions as shown in [Table 2.5](#). In these interaction effects, winter wheat with no tillage was significantly higher in microbial biomass than all other combinations of tillage and cover crop. Nitrogen application rate did not lead to significant differences in microBIOMETER® scores.

When testing combinations of management practices that lead to the expected highest and lowest soil health, microBIOMETER® reported significantly higher scores in expected low soil health treatments ([Table 2.6](#)). The CFE and SIR estimates of microbial biomass did not show significant differences between soils of different health at $\alpha = 0.05$ but consistently estimated higher microbial biomass in plots with expected higher soil health ([Table 2.6](#)). Qualitative determinations for soil health are found in [Section 1.4.3](#).

2.4 Discussion

2.4.1 Comparisons of Microbial Biomass Estimation

MicroBIOMETER® typically reported different values than CFE and SIR. There was only one instance when microBIOMETER® gave similar estimates for microbial biomass as a different method of estimation. In the August sampling event microBIOMETER® and SIR were not significantly different. However, the values reported by microBIOMETER® and SIR were not well correlated with each other ([Figure 2.5](#)). This indicates that the similar results were reported due to random occurrence.

CFE and SIR also typically reported different values for microbial biomass. This was different than most of the past research in how these two methods compare. In past research, CFE and SIR did not always return similar values but were mostly well correlated. In this research, the two methods returned similar values only once in July and were not significantly correlated across any of the sampling events. The lack of correlation between CFE and SIR does make it possible that microBIOMETER® is similar to CFE and SIR and that there was some error in running the methodologies that led to no correlation between the various methods of estimating microbial biomass. However, microBIOMETER® did produce higher variation in results than the CFE or SIR estimates. As discussed in [Chapter 3](#), impacts from changes in the stage of cotton growth that SIR was potentially more sensitive to than CFE may also account for lack of correlation between CFE and SIR. In either case, it is most likely that microBIOMETER® does not return similar estimates of microbial biomass as CFE or SIR. It is also likely that the microBIOMETER® method of estimating microbial biomass is not as accurate as the CFE and SIR methods.

2.4.2 Microbial Biomass to Estimate Soil Health

MicroBIOMETER® was typically unable to distinguish between any changes in management practices, and consistently reported higher values in the expected low health plots. In one instance where microBIOMETER® reported significantly different results, the conventionally tilled with no cover crop plot scored higher than the no-till with hairy vetch plot. This result

is the opposite of what is expected. It is hypothesized that the differences in wet aggregate stability and aggregate size discussed by Nouri and Wilson for these plots might have led to this situation. MicroBIOMETER® also reported significantly higher microbial biomass in the winter wheat with no-till plots than any other combination of tillage and cover crop during the first sampling event. This result follows the expected trend of increases in soil health and microbial biomass with cover crop usage and no-till practices, though it was expected that crimson clover and hairy vetch would lead to higher microbial biomass than winter wheat.

SIR and CFE both followed the expected trend of higher microbial biomass in the hairy vetch with no-till plots. CFE and SIR did not show significant differences in microbial biomass between high and low health plots (as qualitatively determined in [Section 1.4.3](#)) at $\alpha = 0.05$, though SIR was able to at $0.15 \leq \alpha \leq 0.25$ during different sampling events.

It is possible that environmental conditions were impacting estimates. However, there was only one instance in which microbial biomass varied significantly with changes from environmental factors within a single sampling event. This was during the second sampling event, where CFE had significantly different microbial biomass estimates between different gravimetric water contents. Despite significant differences in means, there seemed to be no correlation between CFE and gravimetric water content ($R^2 = 0.03321$). This shows that the significant differences were random and there was no trend where CFE scores increased or decreased with an increase in water content during that sampling event.

This would indicate that microbial biomass estimated by CFE or SIR can work as a method for evaluating soil health in the conditions sampled. In every sample, CFE and SIR reported higher microbial biomass in the plots that were qualitatively determined to be healthier, even if those differences were not significant at $\alpha = 0.05$. However, microBIOMETER® does not appear to offer an improvement over CFE or SIR in terms of estimating microbial biomass or soil health.

2.4.3 Assessment of the microBIOMETER® tool

There are two possible reasons why microBIOMETER® did not distinguish between management practices in this study: (1) microBIOMETER® is not capable of distinguishing between different management treatments in cotton grown on Lexington silt loam soils or (2)

there were not significant differences in microbial biomass between the various treatments. All methods of determining microbial biomass provide estimates for microbial biomass and are not direct measurements of the soil microbial biomass pool. Therefore, it is not certain that there are significant differences in microbial biomass between treatments at WTREC. However, based on the treatments and past research it is expected that there were differences in microbial biomass. SIR did consistently report differences in microbial biomass to support this, though the differences were only significant at $0.15 \leq \alpha \leq 0.25$. CFE consistently reported differences as well, though they were not as significant as SIR. Additionally, the variance in the data provided by microBIOMETER® indicates that microBIOMETER® is not a reliable method for estimating microbial biomass or soil health under the tested conditions. These all indicate that the microBIOMETER® did not find the differences that do exist in microbial biomass between the tested plots.

Therefore, in this research, microBIOMETER® does not function to estimate microbial biomass or soil health in continuous cotton grown on Lexington silt loam soils. This is likely due to sources of error within the microBIOMETER® methodology. The test assumes total isolation of the microbial fraction of the soil in solution. Any residual mineral, organic, or other fractions of the soil left in solution will impact test results. If the dispersant used by microBIOMETER® is not completely effective, or if the time allowed for settling of the mineral fraction of the soil is not long enough, there will be material other than microbial units left in solution and scores will be impacted. Variations in aggregate size or stability could also impact the microBIOMETER® process and lead to biased estimates of microbial biomass. The idea that an isolated microbial fraction of the soil could be measured photometrically may be valid, but within this research context, it seems that microBIOMETER® does not do an effective enough job of isolating the microbial fraction of the soil for accurate photometric analysis of microbial biomass to occur.

2.5 Conclusion

In the tested conditions, CFE and SIR were able to consistently distinguish between areas of expected high and low health. This would indicate that estimating microbial biomass

can be used as a way to evaluate soil health in cotton grown on Lexington silt loams in west Tennessee. However, microBIOMETER® did not consistently distinguish between the different management practices. MicroBIOMETER® did not provide similar or correlated results as CFE or SIR and was more variable than CFE or SIR. Therefore, while estimating microbial biomass may be a useful tool in evaluating soil health, microBIOMETER® does not provide benefits in estimating microbial biomass or soil health over CFE or SIR.

MicroBIOMETER® may function differently when used to test soil health under other crops or crop rotations. MicroBIOMETER® may also function differently in other soil series. However, it should not be used in isolation to determine soil health or estimate microbial biomass under the conditions tested (continuous cotton grown on Lexington silt loams in west Tennessee) in this research.

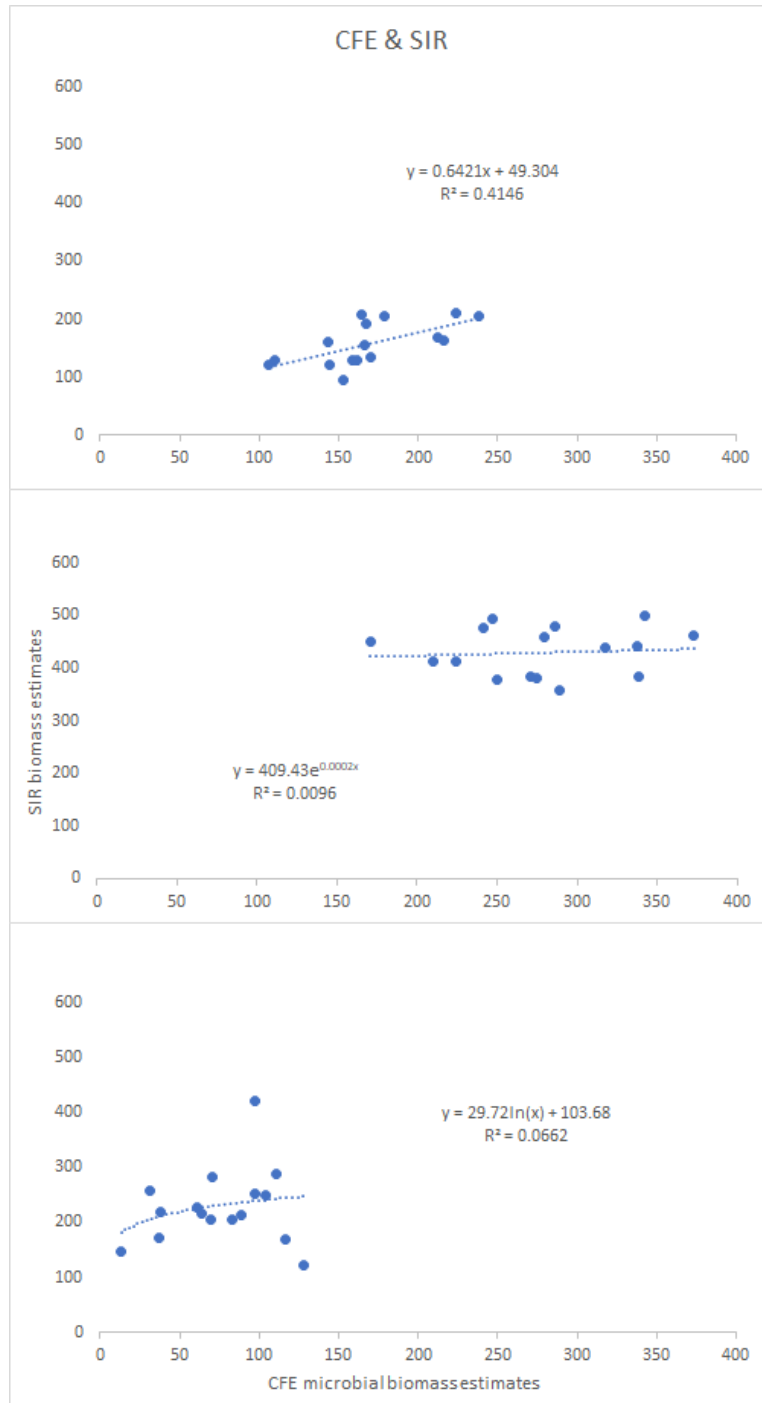


Figure 2.2: Comparison of results between microBIOMETER® and SIR across the three sampling events. The uppermost graph shows June, the middle shows August, and the bottom shows October. Correlations best fit linear, exponential, and logarithmic regressions respectively. Microbial biomass data is presented in μg of microbial biomass carbon / g of soil. $N = 16$ in each graph.

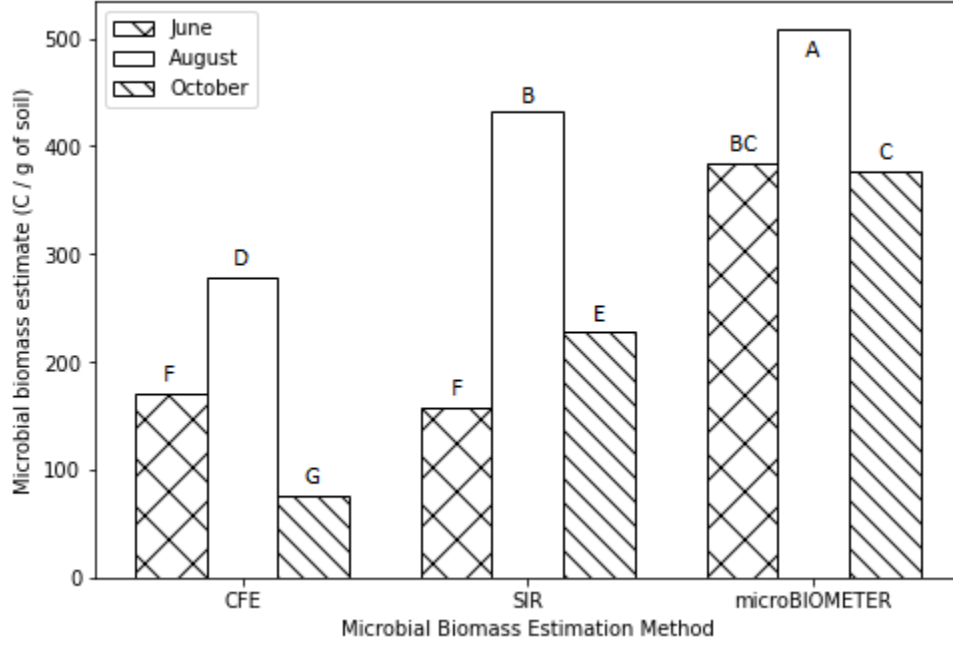


Figure 2.3: Temporal Variation in estimates of microbial biomass. Bars with the same letter are not significantly different.

Table 2.1: Comparisons between estimates of microbial biomass

	June	August	October
microBIOMETER® and SIR			
P-value	0.000001	0.124	0.00022
Correlation Coefficient	0.00008	0.0049	0.0003
Covariance	99.07	1417.44	480.92
microBIOMETER and CFE			
P-value	0.000002	8.65e-8	5.97e-9
Correlation Coefficient	0.0007	0.0825	0.0094
Covariance	34.19	-284.76	114.13
CFE and SIR			
P-value	0.1689	1.63e-7	4.14e-7
Correlation Coefficient	0.4146	0.0227	0.0252
Covariance	842.95	223.32	344.42

P-values, correlation coefficients, and covariance of relationships between different methods of estimating microbial biomass. Correlation coefficients and covariance calculated with Excel.

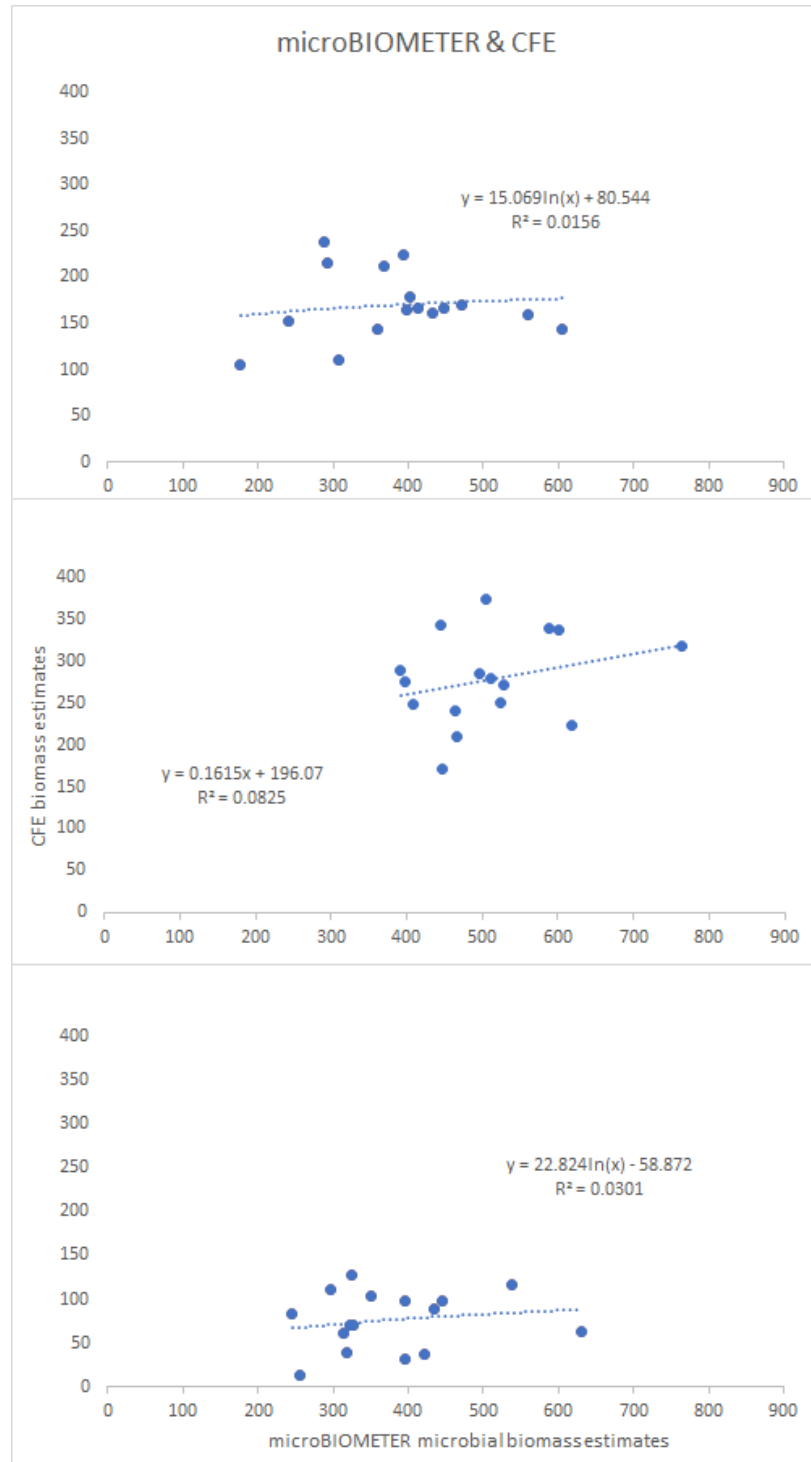


Figure 2.4: Comparison of results between microBIOMETER® and CFE across the three sampling events. The uppermost graph shows June, the middle shows August, and the bottom shows October. Correlations best fit logarithmic, linear, and logarithmic regressions respectively. Microbial biomass data is presented in μg of microbial biomass carbon / g of soil. $N = 16$ in each graph.

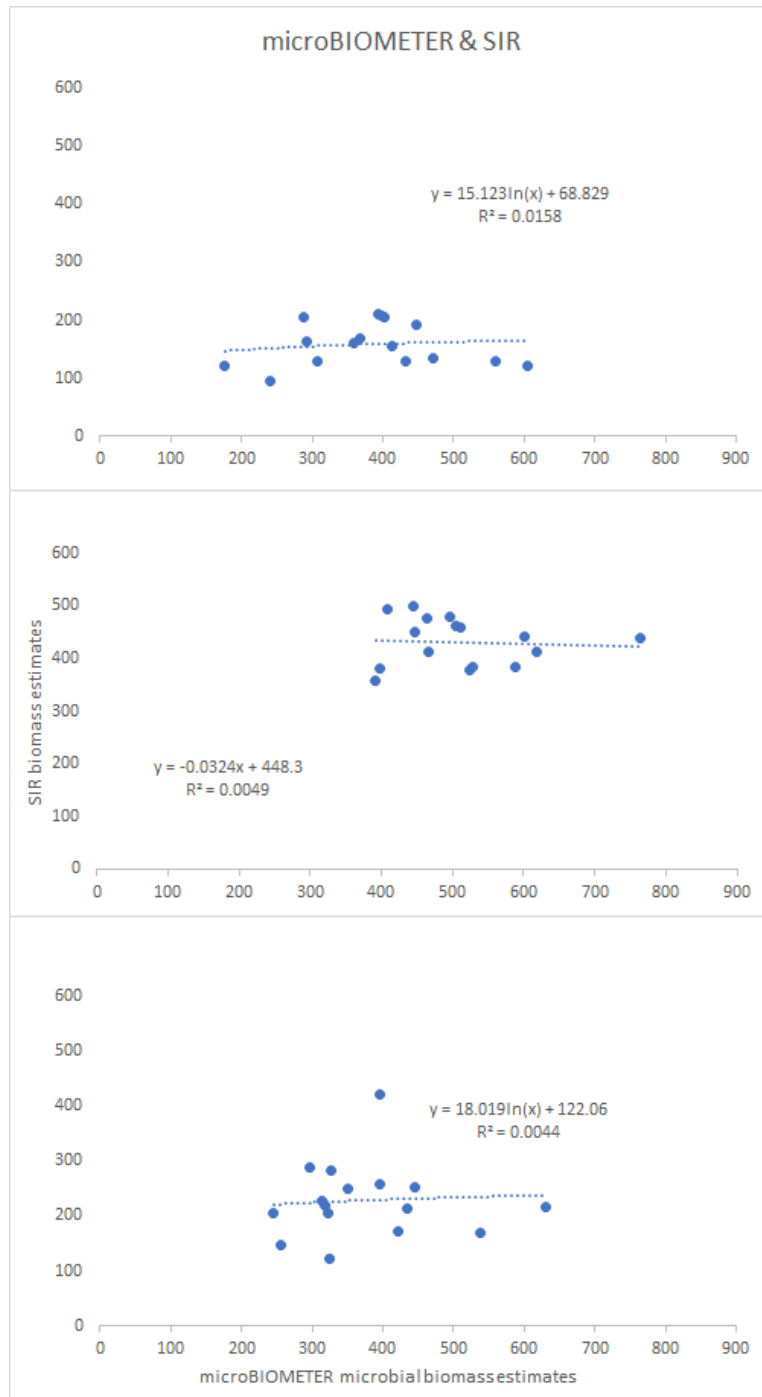


Figure 2.5: Comparison of results between microBIOMETER® and SIR across the three sampling events. The uppermost graph shows June, the middle shows August, and the bottom shows October. Correlations best fit logarithmic, linear, and logarithmic regressions respectively. Microbial biomass data is presented in μg of microbial biomass carbon / g of soil. $N = 16$ in each graph.

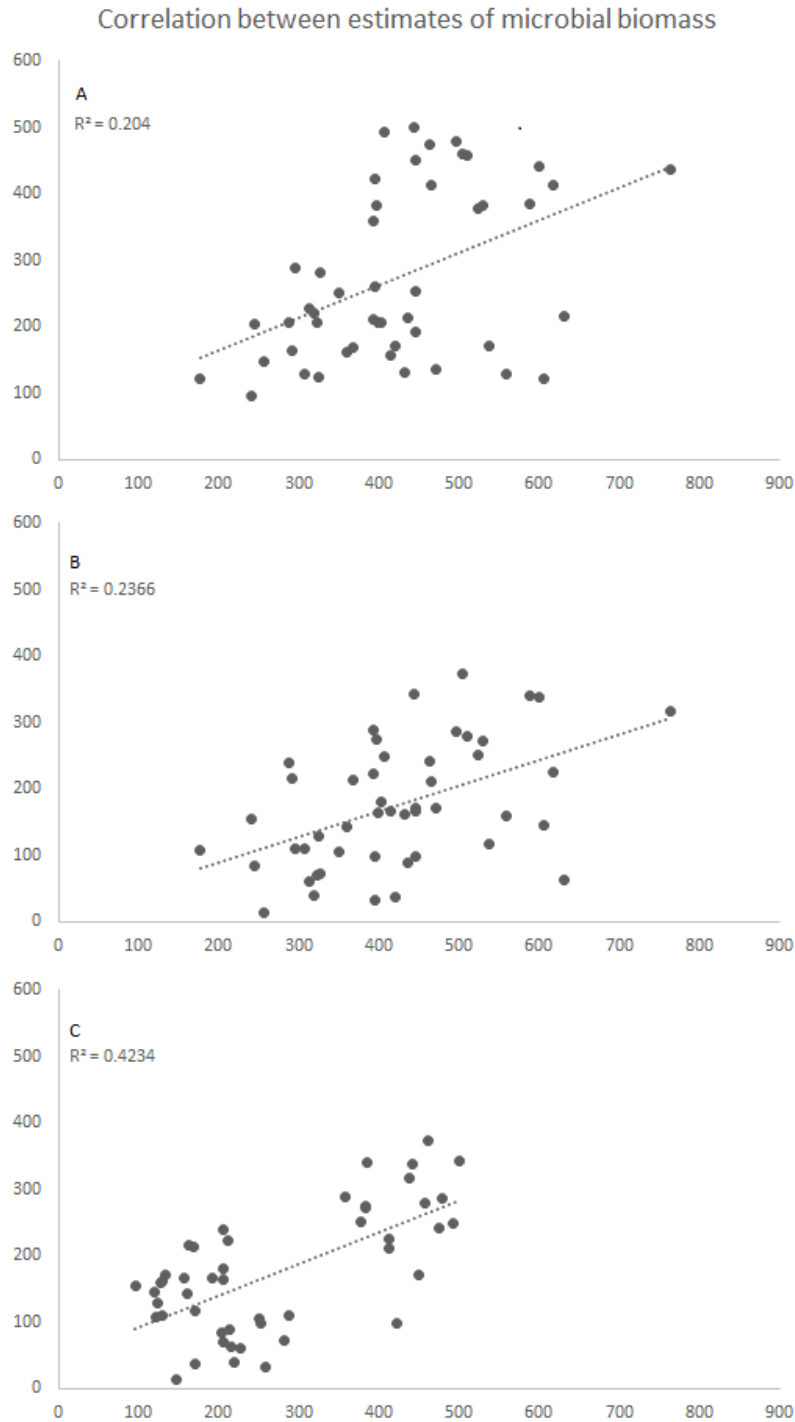


Figure 2.6: Correlation between methods of estimating microbial biomass using data from all three sampling events. Graph A shows microBIOMETER® on the x-axis and SIR on the y-axis. Graph B shows microBIOMETER® on the x-axis and CFE on the y-axis. Graph C shows SIR data on the x-axis and CFE data on the y-axis. All estimates of microbial biomass are in μ g of carbon g of soil. $n = 48$ for each graph.

Table 2.2: Variance in microbial biomass estimates

	June			August			October		
	μ	σ	σ^2	μ	σ	σ^2	μ	σ	σ^2
microBIOMETER®									
All samples	385	111	12231	509	97	9361	376	102	10399
High expected health	328	84	777	508	71	5002	375	89	7949
Low expected health	441	109	11869	511	123	15015	377	120	14333
CFE									
All samples	170	37	1400	278	54	2960	76	33	1116
High expected health	177	45	1996	290	55	3011	87	42	1230
Low expected health	162	29	865	267	55	3032	64	19	348
SIR									
All samples	158	37	1392	432	45	2031	228	69	4793
High expected health	172	44	1932	446	46	2145	239	95	9041
Low expected health	144	25	610	417	42	1742	217	31	957

Table 2.3: Environmental effects on microbial biomass estimates

	June	August	October
microBIOMETER			
Gravimetric Water	0.3973(0.1186)	0.7582(0.0001)	0.429(0.0992)
Soil Temperature	0.2192(0.0067)	0.7915(0.015)	0.6225(9e-5)
Replication	0.5301(0.149)	0.8679(0.0519)	0.6487(0.123)
SIR			
Gravimetric Water	0.0772(0.2915)	0.3839(0.0022)	0.3268(0.1947)
Soil Temperature	0.6261(0.3027)	0.9907(0.0008)	0.0678(0.395)
Replication	0.2941(0.2205)	0.4057(0.0063)	0.859(0.3311)
CFE			
Gravimetric Water	0.4695(0.2886)	0.0056(0.03321)	0.1242(0.1746)
Soil Temperature	0.4906(0.2681)	0.252(0.0139)	0.7344(0.0187)
Replication	0.3016(0.1053)	0.0976(0.2523)	0.438(0.0672)

Correlation coefficients for relationships between methods of estimating microbial biomass and environmental constraints. Parenthetical values are standard error.

Table 2.4: MicroBIOMETER® scores for each treatment group

Treatments	June		August		October	
	μ	σ	μ	σ	μ	σ
Cover Crop						
NCC	391	31	487	38	409	40
WW	464	44	426	41	369	29
CC	415	39	394	38	328	20
HV	390	30	468	28	366	25
Tillage						
CT	401	27	455	30	356	19
NT	448	31	458	21	378	23
Nitrogen Rate						
0 kg/ha	431	34	446	27	363	22
67 kg/ha	418	28	466	24	372	21

NCC: no cover crop, WW: winter wheat, CC: crimson clover, HV: hairy vetch, CT: conventional tillage, NT: no-till. There were no significant differences according to an ANOVA with Tukey's Post-Hoc Test at $P < 0.05$.

Table 2.5: Treatment Interaction Effects on microBIOMETER® scores

	June	August	October
CxTxN	0.0113	0.2286	0.6346
TxN	0.7121	0.1696	0.862
CxN	0.4506	0.13	0.4986
TxC	0.0007	0.1268	0.6346

P-values for treatment interaction effects on microBIOMETER® scores. Bold values indicate significant differences in results. CxTxN refers to unique combinations of cover crop, tillage, and nitrogen application. TxN refers to unique combinations of tillage and nitrogen application. CxN refers to unique combinations of cover crop and nitrogen application. TxC refers to unique combinations of tillage and cover crop. Parenthetical values are the mean variance for microBIOMETER® scores in a given treatment combination.

Table 2.6: CFE, SIR, and microBIOMETER® scores between plots with different levels of soil health

Expected Health		microBIOMETER®			SIR			CFE		
		μ	σ	Grp	μ	σ	Grp	μ	σ	Grp
June										
	Low	441	19	A	144	25	A	162	29	A
	High	328	84	B	172	44	A	177	45	A
August										
	Low	511	123	A	417	42	A	267	55	A
	High	508	71	A	446	46	A	290	55	A
October										
	Low	377	120	A	217	30	A	64	19	A
	High	375	89	A	239	95	A	87	42	A

High and low soil health as described in 1.4.3. Means within a group followed by different letters are significantly different according to the standard T-Test at $P < 0.05$.

Chapter 3

Temporal variability in microbial biomass

We know more about the movement of celestial bodies than about the soil underfoot.

– Leonardo da Vinci

3.1 Introduction

During the research to validate microBIOMETER®, it was found that each method for estimating microbial biomass found significant temporal variation ($\alpha = 0.05$). Mean results from microBIOMETER® were significantly lower in June and October than in August. Chloroform fumigation extraction (CFE) estimations were significantly different between each sampling event, with the August sampling event having the highest estimates and the October sampling event having the lowest estimates. Substrate induced respiration (SIR) was significantly different between each sampling event with the August sampling event having the highest estimates and the June sampling event having the lowest estimates.

Temporal variability in microbial biomass is not a newly recognized phenomenon. In 1998, Wardle published a review of literature on how and why microbial biomass varies temporally. The articles reviewed by Wardle cite changes in moisture content, temperature, or stages of plant growth as reasons that microbial biomass varies temporally [76]. The reviewed articles

also vary in how microbial biomass is correlated to changes in these variables. Changes in soil moisture, temperature, or plant growth have been correlated with both positive and negative changes to microbial biomass. One factor that may have an impact on whether these changes are positively or negatively correlated with microbial biomass is the geographic location in which the study occurred. Wardle does note that in temperature regions, it is most common that microbial biomass increases to a peak in spring or summer and declines to a trough in the winter.

More recent research by Serna-Chavez et al. has identified soil water content as the driving force behind temporal variations in microbial biomass [61]. Changes in soil water content accounted for 34% of the global variance in microbial biomass. This is elevated to accounting for 50% of the global variance in microbial carbon and also in microbial carbon to soil organic carbon ratios. It was determined that soil moisture and soil nutrient availability, not soil temperature, was the driving force behind temporal variation in microbial biomass.

Research by Bardgett et al. has highlighted the importance of plant growth stages on microbial biomass [9]. Microbial communities are known to change throughout growing seasons. In the winter, when plants are typically dormant, the soil microbial pool is often dominated by fungi that feed on complex plant residues. When plants are actively growing, the soil microbial pool is more likely to be dominated by microbes that can feed on the root exudates of growing plants.

Changes in the growth stage of a plant can also lead to changes in how the plant is chemically and physically affecting the surrounding soil. Oftentimes, this occurs through changes in the amount of root exudates released by the plant. Root exudates are often used by plants as a method to try to increase nutrient availability [52]. When plants release root exudates, this is often associated with an increase in microbial biomass around the rhizosphere. This can occur either through an increase in nutrients that microbes then compete for with the plant, or root exudates can be used to signal for an increase in microbial units that are associated with symbiotic methods of nutrient uptake for the plant.

In cotton plants, the most nitrogen is used and the most biomass is added in the growth stages from germination to flowering [78]. As the plant is growing and adding biomass above- and below-ground, the rhizosphere will physically grow as well. As the plant seeks out the

nutrients needed to grow, this likely correlates with an increased number of root exudates produced by the plant. Both of these situations could impact microbial biomass in the cotton plots sampled.

Unfortunately, this research was originally designed to evaluate microBIOMETER® as a soil health tool. Due to this, enough data was not gathered to be able to accurately identify the causes behind the temporal variability that was observed in this research. Data for nutrient cycling, root exudation, and microbial community structure were not gathered and the effects of these variables cannot be quantitatively evaluated. Therefore, this chapter makes hypothesis to explain the temporal variability using quantitative evaluation of data available supplemented with findings from past research on cotton growth, cotton nutrient utilization, and variables that impact microbial biomass.

3.2 Materials and Methods

Data collected for this chapter is the same as the data used in [Chapter 2](#). Data collection methodology is the same as discussed in [Section 2.2](#). Data was collected three times over the course of this research at the West Tennessee Research and Education Center (WTREC) in Jackson, Tennessee. The three sampling dates were June 29, August 12, and October 18. Each sample was during a different stage of cotton growth. The June sample was during the germination of the cotton crop, the August sample was during the first flowering of the cotton crop, and the October sample was during boll development of the cotton crop. These dates are 15, 59, and 126 days after planting of the cotton crop on June 14, respectively. These dates are 13, 57, and 124 days after fertilization of the cotton crop on June 16, respectively. The cotton crop was only fertilized one time on June 16.

The cotton crop was periodically treated with various herbicides and pesticides to aid in cotton growth. The most recent application prior to the first sample collected was on June 15 when Transform® and Diamond® were applied. The most recent application prior to the second sample was on July 13, when Liberty® and Bidrin® were applied. The most recent application prior to the final sample was on September 17 when Brigade® was applied.

One-way Analyses of Variance (ANOVAs) were performed to compare the effect of sampling time on microBIOMETER®, CFE, and SIR estimates of microbial biomass as well as the gravimetric water content and soil temperature. Tukey’s Honest Significant Difference (HSD) was performed as a post-hoc test when significant differences occurred in the ANOVA test. Regression analyses were performed by selecting the regression of best fit in Excel. The growth cycle of the cotton plant was analyzed for possible reasons for changes in microbial biomass between sampling events. Pesticide, herbicide, and fertilizer use at the WTREC were examined for reasons behind why microbial biomass varied between samples.

3.3 Results and Discussion

3.3.1 Changes in Microbial Biomass between Sampling Events

Each of the three methods used for estimating microbial biomass observed significant differences in microbial biomass between sampling events (Figure 2.3). For CFE and SIR, this significant difference was observed between every pair of sampling events. For microBIOMETER®, the August sampling event had a significantly higher mean microbial biomass estimated as compared to June and October. When looking at high and low health plots, this trend persisted and the health of the plot did not appear to impact the temporal variability that was identified (Figure 3.1).

3.3.2 Impacts of Fertilization and Pesticide Application

The cotton plots were only treated with fertilizer once during the growing season. This occurred on June 16, two days after the crop was planted. There was no consistent trend in the data that would suggest that the fertilization application controlled microbial biomass.

The cotton plots were treated with pesticides or herbicides six times during the course of the growing season. These occurred on April 12 (Gramoxone® and RoundUp®), on April 26 (Cotoran® and Caporal®), on May 17 (Dual®, Acephate®, and RoundUp®), on June 15 (Transform® and Diamond®), on July 13 (Liberty® and Bidrin®), and on September 17 (Brigade®). Of those, the June, July, and September treatments were closest to the first,

second, and third sampling event, respectively. The June treatment was 14 days before the first sampling event, the July treatment was 30 days before the second sampling event, and the September treatment was 31 days before the third sampling event.

However, it appears that the application of pesticides did not impact the estimated microbial biomass from any of the three tests. The second and third sampling event were taken 30 and 31 days after pesticide application. There was not a significant difference in time since last pesticide application for those two events, but there were still significant differences in mean estimates of microbial biomass for all three tests. Other studies have found that pesticides applied at field rates is typically not a high enough concentration to damage microbial life ([57], [75]). This appears to be the case in this research as well.

3.3.3 Temporal Variability in Moisture and Temperature

Gravimetric water content (Figure 3.2) and soil temperature (Figure 3.3) both changed significantly between sampling events. To try to further isolate the impacts of moisture and temperature, the correlation between microbial biomass as estimated by each methodology and the two environmental conditions were plotted. Correlations between microbial biomass estimates and soil moisture are plotted in Figure 3.4. Correlations between microbial biomass estimates and soil temperature are plotted in Figure 3.5.

MicroBIOMETER® had the least correlation between estimated microbial biomass and soil temperature ($R^2 = 0.0818$) or soil moisture ($R^2 = 0.2776$). This is likely due to the higher variance in results reported by microBIOMETER®, as discussed in Chapter 2. CFE had the strongest correlations between microbial biomass estimates and the soil temperature ($R^2 = 0.5629$) and moisture ($R^2 = 0.7065$), where lower moisture contents and warmer temperatures led to increased microbial biomass. These correlations make it possible that the increased microbial biomass estimated by CFE during the August sample are due to the soil temperature and moisture. This would also support the findings of Serna-Chavez et al. ([61]) that stated soil water content was the driving force behind global temporal variations in microbial biomass. These correlations are stronger at the seasonal scale than at the individual sample scale for CFE as well. As discussed in Chapter 2, CFE did not appear to be controlled by or correlated to soil moisture or temperature within a single sampling

event. However, the significant differences in soil temperature and moisture that occurred between sampling events correlate better to CFE scores between samples.

The results from SIR correlations are more variable. When looking at the samples taken in June and October, increased moisture was correlated with increased microbial biomass. However, the highest microbial biomass estimated by SIR occurred in August, the driest month sampled, which breaks that trend. This is noteworthy, because other evaluations of SIR have found significant correlations between soil moisture and SIR estimates of microbial biomass [75]. When correlating SIR estimates of microbial biomass to soil temperature, there was little correlation in results. The highest and lowest estimated microbial biomass occurred with mean soil temperatures of 32.8 °C and 32.26 °C, respectively. The median microbial biomass estimated by SIR occurred at an average soil temperature of 12.5 °C. Therefore, it appears that the differences in microbial biomass estimates between samples by SIR were not solely correlated to temporal changes in soil moisture or temperature.

3.4 Temporal Variability in Stages of Cotton Growth

Biological impacts from plant growth may better explain the trends seen in the estimates of microbial biomass from SIR. SIR functions differently than microBIOMETER® or CFE in that it measures the respiration of live microbial units and how those units respond to an added substrate. As discussed in the introduction to this chapter, cotton plants will expand their rhizosphere as they grow, and may increase the amount of root exudates present in the rhizosphere, and it is expected that this trend persisted up to the flowering stage of growth. These effects of cotton growth may lead to increased microbial activity and biomass. SIR estimated the highest microbial biomass during the flowering stage of cotton growth, which is when past research indicates as the most likely time that root exudates and rhizospheric influence from the cotton may peak. As SIR measures microbial activity and respiration, it may be more sensitive to this peak than the other two methods of estimating microbial biomass.

Additionally, the substrate used in the SIR test was yeast extract, which has been shown to be an effective source of nitrogen for bacterial growth [29]. The August sample took

place during the flowering stage of cotton growth. The flowering stage is one of the stages with the highest nitrogen usage and there are associated increases in abundance of bacteria associated with nitrogen uptake ([58], [78]). It is possible that this explains the significantly higher reported microbial biomass by SIR during August. SIR measures respiration of microbial units. If a sample of soil contains a significantly higher abundance of bacteria associated with nitrogen uptake in cotton than other samples, and all samples are treated with a substrate that is high in nitrogen, it is possible that the sample with the highest concentration of bacteria associated with nitrogen uptake in cotton will produce the most respiration. Increased respiration in that sample would then be calculated as higher microbial biomass and could potentially be the reason why increased microbial biomass was reported by SIR in August.

3.5 Conclusions

It is hypothesized that microBIOMETER® estimates of microbial biomass are primarily controlled by soil moisture content. However, the test is currently too variable under the tested conditions to show a significant correlation between soil moisture and estimated microbial biomass.

It is hypothesized that CFE was primarily controlled by soil moisture. Soil temperature was also moderately correlated with CFE, and it is likely that a combination of changes in soil temperature and soil moisture led to the observed temporal variations in estimated microbial biomass. However, past research and the stronger correlation between estimated microbial biomass and soil moisture would indicate that changes in water content were more important than changes to soil temperature for estimates of microbial biomass by CFE.

SIR was correlated with changes in soil moisture, and the trend was that increasing soil moisture led to increased microbial biomass. However, this trend was broken as the driest sample provided the highest estimates of microbial biomass for SIR. Therefore, some other variable had to have had a stronger impact on microbial biomass than the soil water content. It is hypothesized that this variable was impacts from changes in the stage of cotton growth, specifically an increase in nitrogen uptake in cotton plants that increased root exudates and

modified microbial communities to feature more bacteria associated with nitrogen uptake. The August sample likely represented the time with the most nitrogen associated bacteria in the rhizosphere, and treating those bacteria to a substrate with high nitrogen content may have led to higher readings for microbial activity and outweighed the effects of soil water content on estimated microbial biomass.

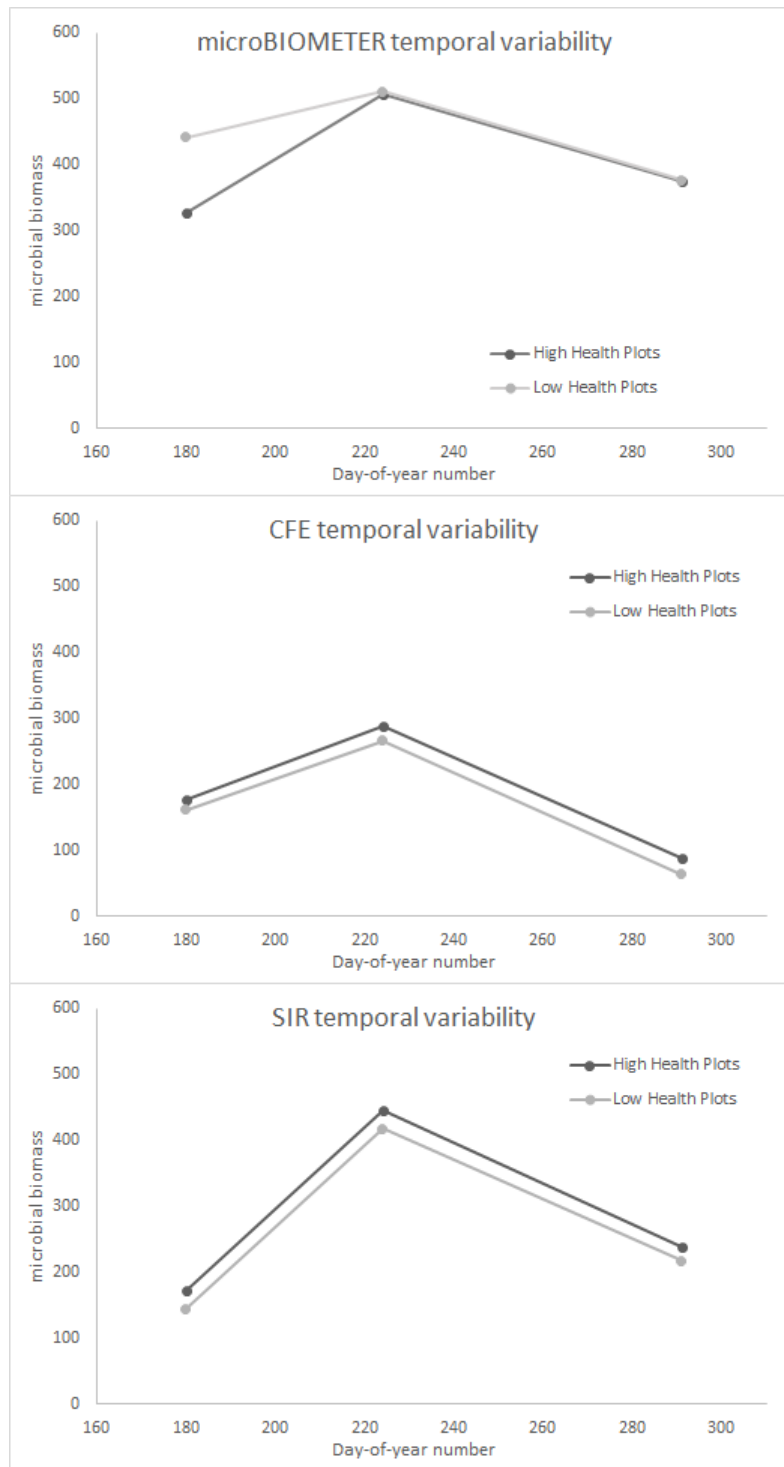
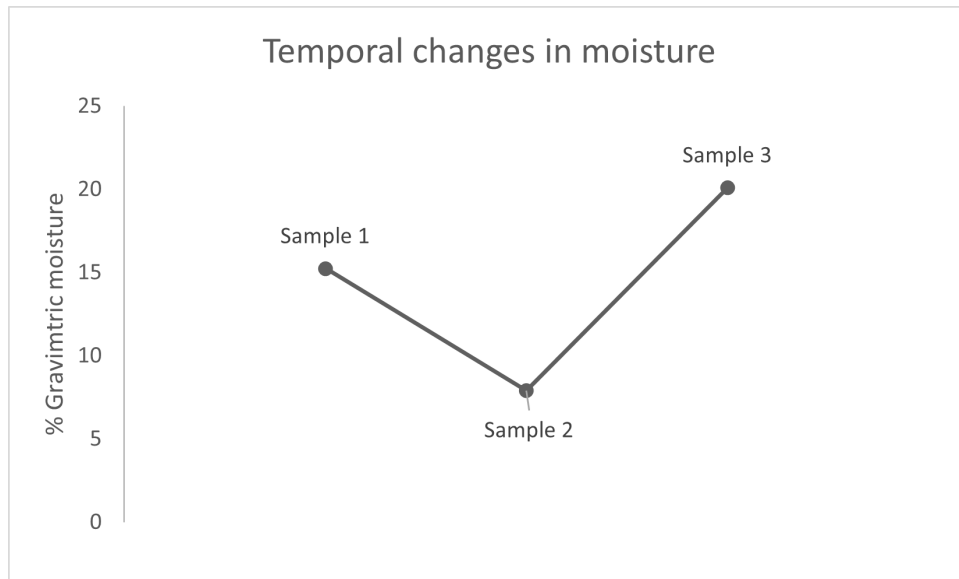


Figure 3.1: Temporal Changes in mean microbial biomass for expected high and low health plots. Microbial biomass is reported in μg of carbon / gram of soil. Time is represented in days since the beginning of the 2021 calendar year.

Table 3.1: Results from Tukey's HSD for microbial biomass between sampling events

Combination (A + B)	Mean(A)	Mean(B)	Standard Error	T Value	p-Tukey
microBIOMETER®					
June + August	385	510	37	-3.42	0.003792
June + October	385	376	37	0.24	0.900000
August + October	510	376	37	3.68	0.001875
CFE					
June + August	170	278	15	-7.20	0.001000
June + October	160	76	15	6.21	0.001000
August + October	278	76	15	13.41	0.001000
SIR					
June + August	158	432	19	-14.78	0.001000
June + October	158	228	19	-3.79	0.001268
August + October	432	228	19	-10.99	0.001000

Mean microbial biomass values reported are in units of μg of microbial biomass carbon per g of soil. Standard error refers to the standard error of the difference of the two means.

**Figure 3.2:** Temporal Changes in gravimetric moisture over the three sampling events.

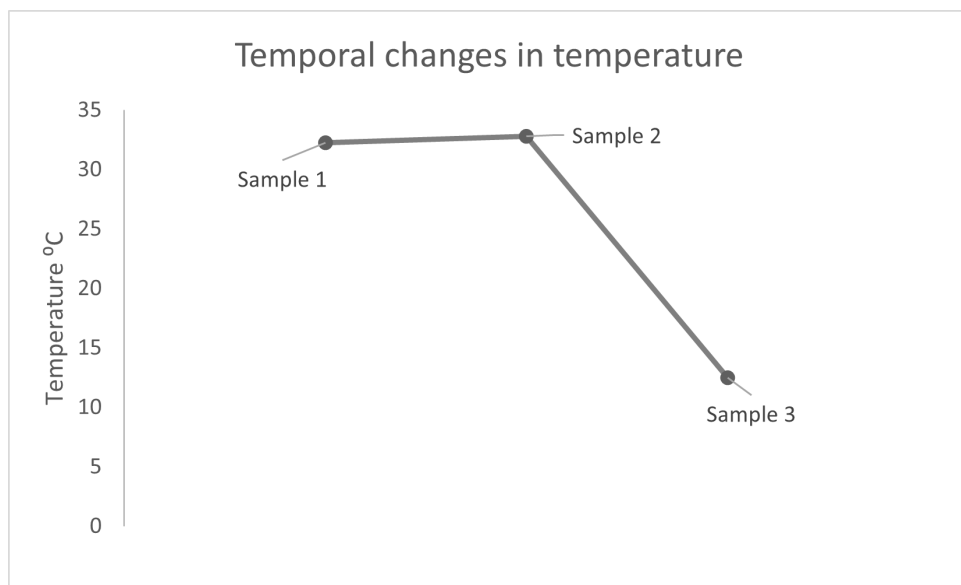


Figure 3.3: Temporal Changes in soil temperature over the three sampling events. Note that while Sample 1 and Sample 2 appear to be the same, they are significantly different at $\alpha = 0.05$

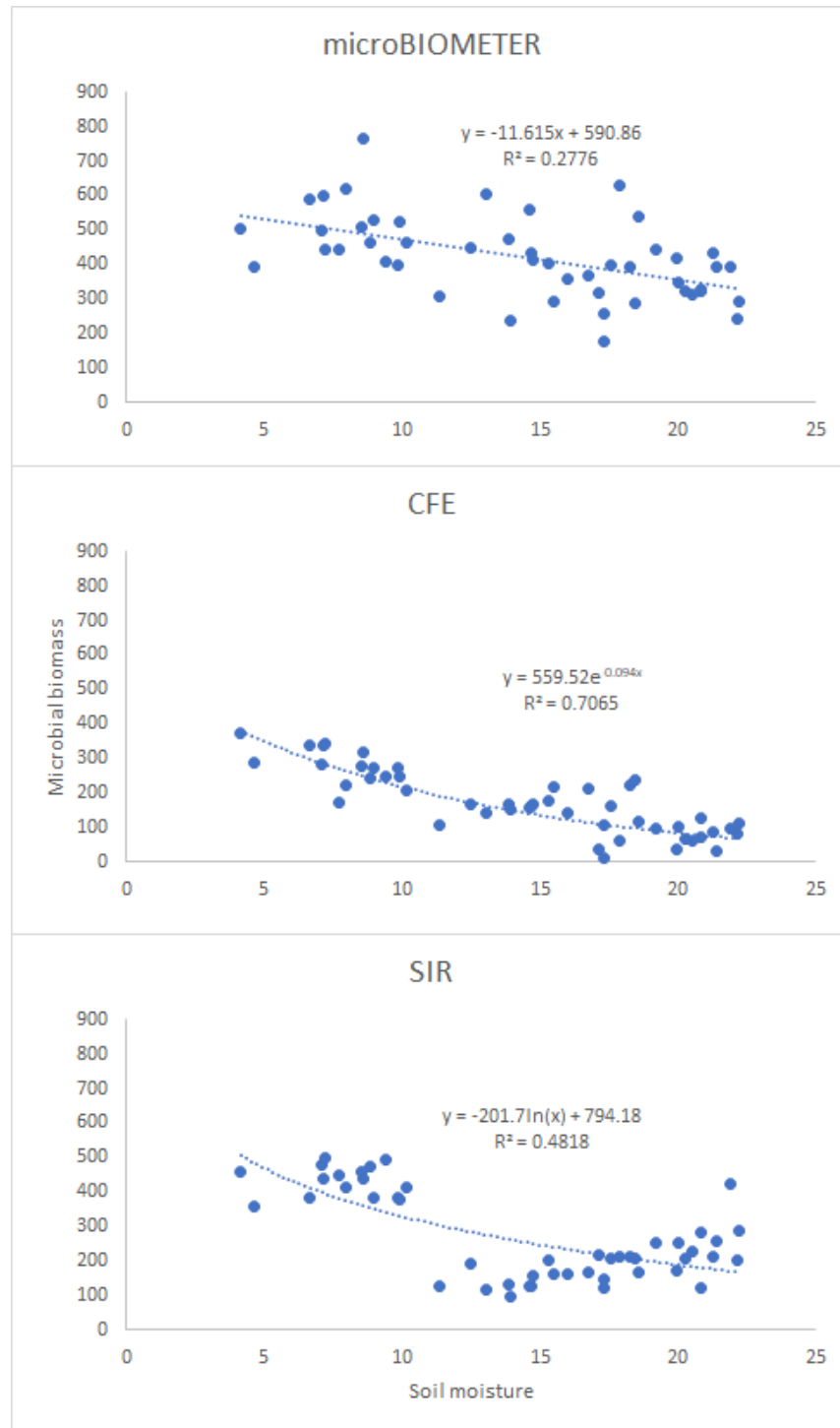


Figure 3.4: Correlation between microbial biomass and temporal variability in soil moisture. Soil moisture is presented in percent gravimetric water content. Microbial biomass data is presented in μg of microbial biomass carbon / g of soil. Correlations best fit linear, exponential, and logarithmic regressions, respectively.

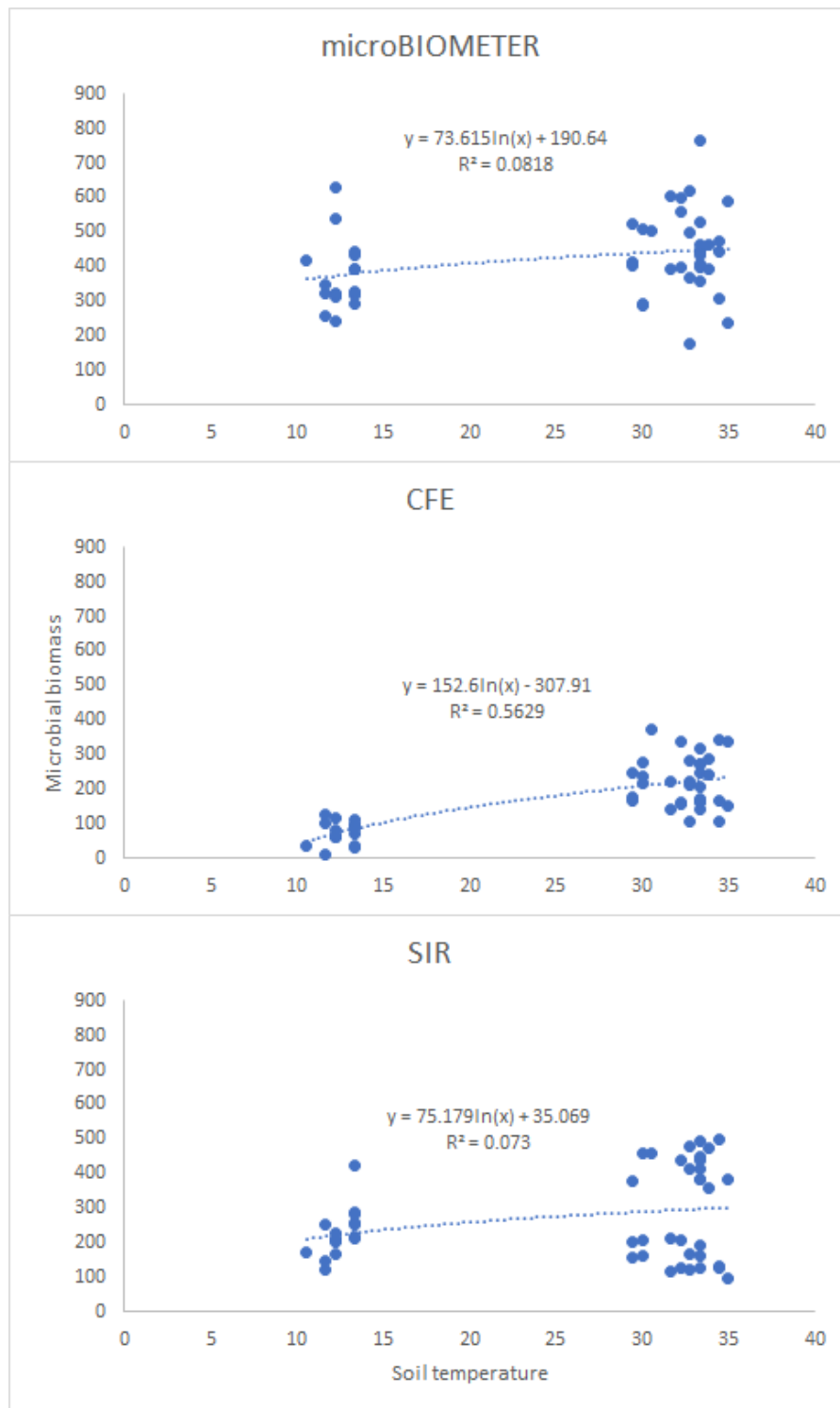


Figure 3.5: Correlation between microbial biomass and temporal variability in soil temperature. Soil temperature is presented in °C. Microbial biomass data is presented in μg of microbial biomass carbon / g of soil. Correlations best fit logarithmic regressions.

Chapter 4

Conclusion

It always seems impossible until it's done.

– Nelson Mandela

4.1 Conclusion

The objectives for this research were as follows:

1. Does microBIOMETER® distinguish between significant differences in management practices?
2. Does microBIOMETER® provide similar estimates of microbial biomass as substrate induced respiration or chloroform fumigation extraction?
3. Do substrate induced respiration or chloroform fumigation extraction distinguish between significant differences in management practices?

For objective one, it was found that microBIOMETER® did not consistently distinguish between significant differences in management practices. In regard to the second objective, microBIOMETER® estimated microbial biomass with significant differences from CFE or SIR estimates, though CFE and SIR estimates of microbial biomass were significantly different from each other, as well. For objective three, neither SIR nor CFE were able to find

significant differences in microbial biomass between significantly different treatments, though both consistently estimated higher microbial biomass where expected.

There were observed differences in microbial biomass estimates over time. Temporal variation in microbial biomass as estimated by microBIOMETER® is unable to be isolated to a specific cause. This is likely due to the high variance in results reported by microBIOMETER®. Temporal variation in microbial biomass as estimated by CFE is correlated to changes in soil moisture and temperature. Temporal variation in microbial biomass as estimated by SIR may be impacted by the nitrogen usage of cotton plants and associated changes in microbial community structure.

The research was only performed under continuous cotton in west Tennessee on Lexington silt loam soils. The results and recommendations from this research may not be applicable to conditions outside of the ones tested.

4.2 Future Work

Based on the results of this study, future work should include continued validation of microBIOMETER®. This study, or a study similar to this one, should be repeated to validate microBIOMETER® under different cropping regimes and on different soil series. While it did not distinguish between management treatments under continuous cotton in west Tennessee, microBIOMETER® still offers improvements over existing soil health tests in regards to accessibility to producers financially and technically. It would be valuable to know if there are places in Tennessee or the United States that this test can distinguish between management practices and differences in soil health.

More research into substrate induced respiration (SIR) as a soil health test should be conducted as well. During this study, SIR was able to consistently distinguish between areas of significantly different management practices. SIR reported significant differences in microbial biomass at a confidence interval of 75% to 85% throughout the study. For field soil health tests, as opposed to laboratory methods, 75-85% confidence in results might be enough for the test to be valuable to producers. SIR is currently a lab-based methodology,

but it might be worthwhile to convert that methodology into a field test if possible. Such a test could possibly function as a valuable method of soil health testing.

Future research into the causes of temporal variability found should be performed. Possible explanations for differences were outlined in [Chapter 3](#). However, there is not enough data in the research performed here to confidently assign reasons for the temporal variability. Hypotheses for why temporal variations were observed with each methodology were put forth, but still need to be tested.

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Appendix

A Chloroform Fumigation Extraction Methodology

Microbial biomass was estimated via chloroform fumigation extraction (CFE). The methodology for CFE is as follows.

1. Sieve the soil sample to remove aggregates and organic matter.
2. The test should be run in triplicate per sample to account for natural variance.
3. Prepare a subsample for chloroform treatment and a subsample for control for each sample.
4. For each subsample, weigh 10g of soil into an appropriate container.
5. To each subsample, add 40mL of 0.5 molar potassium sulfate.
6. To the subsamples to be treated with chloroform, add 0.5mL of EtOH free chloroform.
7. Tightly seal all subsamples and place on a shaker plate for four hours at 150 rpm.
8. After shaking, allow soil to drop out of solution and accumulate at the bottom of the jar for easier solution extraction.
9. Pour the solution through vacuum manifold with filters to remove any soil from the solution. This can be done either by decanting or by using a pipette, but in either case take special care to exclude chloroform bubbles from the extracted solution.
10. Bubble the extracted solution from chloroform treated subsamples for 20 minutes to remove any chloroform that may have gotten into the extracted solution.
11. Once the extracted solution has been collected and bubbled, it can be frozen until ready to be analyzed with the infrared gas analyzer (IRGA).
12. Subsamples cannot be directly measured with the IRGA and must be treated with a permanganate digestion technique before analysis.

Permanganate digestion for extractable soil carbon methodology:

1. Prepare permanganate solution by dissolving, in order, 50g of potassium persulfate, 16.8g of sodium hydroxide, and 30g of boric acid into one liter of Milli-Q water.
2. For this analysis, potassium hydrogen phthalate (KHP), standards are used to curve and correct the measured results and the KHP standards must be created. These are simply a 100 ppm, 50 ppm, 25 ppm, 12.5 ppm, 6.25 ppm, 3.125 ppm, and 0 ppm KHP solution.
3. Measure 2mL of extracted solution for each subsample into an oven safe glass jar.
4. Add 2mL of permanganate solution to each subsample jar and KHP jar.
5. Immediately cap and seal each subsample and KHP standard with a septa and metal ring after adding the permanganate solution.
6. Place sealed jars into an oven at 80°C for at least 8 hours. If the samples are in the oven longer, that is fine as the KHP standards are undergoing the exact same treatment in the oven as the subsamples and will still allow for correction to true values.
7. Once the subsamples and KHP standards have been heated for the allotted period, analysis with IRGA can begin.

B Substrate Induced Respiration Methodology

Microbial biomass was estimated via substrate induced respiration using the following methodology:

1. Sieve soil to remove aggregates and organic matter and homogenize the soil.
2. The test should be run in triplicates per sample to account for natural variance in samples.
3. Determine the amount of soil and yeast extract needed for the test. The test requires a ratio of approximately 20mg of yeast extract for every gram of dry soil. The test is performed with field moist soil, so one must also calculate the amount of field wet soil that is equivalent to a gram of dry soil.
4. Yeast extract should be prepared at a concentration of 12g yeast extract per liter of deionized water.
5. For my tests, I used the equivalent of 6g dry soil weight and added 100mL of 12g/L yeast extract to each sample.
6. Prepare a negative control (blank) that does not receive soil but receives the same amount of yeast extract.
7. Once soil and yeast extract have been added, firmly seal the jar (Mason jars with a septa in the lid were used in this study).
8. Carbon dioxide content inside the jars is determined using a Infrared Gas Analyzer (IRGA operating information can be found in a separate methodology step list) at 0 hours, 2 hours, and 4 hours. The 0 hour measurement should be taken immediately after sealing the jars.
9. After the 0 hour measurement is taken, the jars are placed on a shaker plate and shaken at 120 rpm until it is time to take the 2 hour measurement.
10. After the 2 hour measurement is taken, the jars are returned to the shaker plate and continue to be shaken at 120 rpm until it is time to take the 4 hour measurement.

11. Once all three measurements have been taken, data points must be corrected by accounting for changes in CO₂ in blank samples and corrected for the gas standard curve.
12. Once data points have been corrected, microbial biomass is calculated as the slope of carbon dioxide production over time.

C Infrared Gas Analyzer Methodology

Methodology for operating the infrared gas analyzer (IRGA) for use in substrate induced respiration and chloroform fumigation extraction tests:

1. Turn on IRGA. The machine will take a while to “warm up”, so this should be done thirty minutes to an hour before samples are processed.
2. Open IRGA interface software and connect the IRGA machine with the computer.
3. When ready, begin collecting data on the software.
4. Prior to testing samples, generate a standard curve for the machine. The IRGA machine will read slightly differently each time it is used and so it is important to have a set of known values to calibrate samples to. For this lab, room air, soda lime, 1,000 ppm nitrous oxide, 10,000 ppm, and 50,000 ppm nitrous oxide are used.
5. When testing a sample or standard, use a syringe to pull gas from the sealed container.
6. Expel gas until .5 mL of sample/standard is left in the syringe.
7. Insert syringe into IRGA and smoothly expel the sample/standard gas into the IRGA.
8. Watch the interface software and record the value at the peak after inserting gas.
9. Allow interface software to return to baseline reading before inserting next sample/standard.
10. Each sample/standard should be tested at least twice without getting values more than 10% different than each other to account for natural variance in readings.

D MicroBIOMETER® Methodology

MicroBIOMETER® was processed for this study using the following methodology:

1. Pass soil sample through a sieve to remove aggregates and organic matter.
2. The test was run in triplicates per sample to account for natural variance.
3. Using the provided syringe, loosely fill the syringe with sieved soil to a volume of 1mL.
4. Compact loose soil to a volume of 0.5mL, removing excess soil from the end of the syringe if necessary.
5. Add 9.5mL of deionized water to a test tube.
6. Add the contents of one extraction powder packet to the test tube and water.
7. Mix contents of extraction packet with water for five seconds using a provided electric stirring wand.
8. Add soil sample to extraction solution in the test tube and mix with electric stirring wand for thirty seconds.
9. After stirring, allow the solution to rest for five minutes.
10. After five minutes of rest, gently tap the test tube against a hard surface to get soil that has settled back into solution.
11. Allow the solution to rest for another fifteen minutes.
12. Using a pipette, take an aliquot from the test tube from just under the surface of the solution.
13. Place three drops of the aliquot onto a provided test card.
14. Using the app, name and analyze the sample.
15. Occasionally, the test card will not be dark enough for the app to analyze and will request the addition of three more drops. Do so if this occurs and reanalyze the sample.

16. If a filtered sample is desired, insert the provided test tube filter into the test tube until water is an inch from the end of the filter tube. At this point, steps 11 through 14 can be repeated for the filtered sample.
17. Once analyzed, the app will provide the user with values for micrograms of biomass carbon per gram of soil, fungal to bacterial ratio, percent bacterial biomass, and percent fungal biomass.

Vita

Daniel Thomas Sain was born in Oak Ridge, Tennessee, on September 25th, 1996. Daniel graduated from Clinton High School in 2015, and began attending the University of Tennessee, Knoxville, later that year. Daniel finished his undergraduate studies in 2019 with a degree in Environmental and Soil Science. In 2020, Daniel began his pursuit of a Masters Degree at the University of Tennessee, Knoxville. Daniel graduated with his Masters of Science in 2022.