EVALUATION OF THE MICROBIOMETER® MOBILE SOIL TEST AS AN INDICATOR OF SOIL MICROBIAL BIOMASS AND SOIL HEALTH

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

in Partial Fulfillment of the Requirements for the Degree of

Master of Professional Studies in Agriculture and Life Sciences

Field of International Agriculture and Rural Development

by

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December 2021

ABSTRACT

Increased interest in holistic soil management is driving a demand for analytical approaches to assessing soil health that integrate biological and physical assessment of soils with chemical analyses. In the Global North, there is an expanding interest among farmers to engage in measuring and evaluating soil nutrient status and soil health. There is also a need for on-site soil testing in developing regions of the Global South, where access to quality soil testing services is often limited. Here, I report research results on measuring soil biological activity, specifically, assessing soil microbial biomass carbon (SMB-C) using a recently developed, rapid, on-site testing tool called microBIOMETER® (Prolific Earth Sciences, Inc., Montgomery, NY, USA).

Soil samples were taken from cover crop trials established at two sites in New York, the Hudson Valley Farm Hub (Farm Hub) and Cornell Musgrave Research Farm (Musgrave). Results of SMB-C from use of the microBIOMETER® were compared to the following soil health assays: chloroform fumigation-extraction (CFE), chloroform fumigation-incubation (CFI), soil respiration, permanganate oxidizable carbon (active carbon), and autoclaved citrate extractable soil protein (soil protein). For each location, a Pearson's correlation analysis was used to explore linear relationships between the laboratory soil health assays and the microBIOMETER®. Multivariate linear regression was used to compare the microBIOMETER® results to the other soil health assays to account for effects of cover crop treatments and location.

Active carbon and soil respiration were significantly correlated at both the Farm Hub, $r=0.73$ ($p<0.0001$) and at Musgrave, $r=0.55$ ($p=0.0055$). At the Farm Hub, active carbon was also significantly correlated with soil protein ($r=0.64$, $p=0.0008$) and the microBIOMETER®

(r=0.43, p=0.0343). As anticipated, there was a significant correlation between CFE and CFI at the Farm Hub (r=0.65, p=0.0048).

In the multivariate modeling, location was significant, but treatment (cover crops planted) and the treatment-location interaction were not significant in all models. Multivariate models with microBIOMETER® as the response variable and location with one soil health assay as explanatory variables were fit. Of the five soil health assays tested, soil protein was significantly related to microBIOMETER® (p=0.0453) and active carbon was significant at p=0.0829. There was no evidence of a significant relationship between microBIOMETER® and the other three soil biological assays.

The results of this study indicate that the microBIOMETER® tool is reflecting some aspects of soil biological health, but that further research is needed to understand more precisely how useful this tool will be as a soil health indicator. While the microBIOMETER® is easy to use and affordable, its use as a soil health test for agroecosystem management is unclear.

BIOGRAPHICAL SKETCH

Eric Benjamin Gordon, known to many as “Ben,” grew up in Larchmont, New York, a New York City suburb. Since attending Bard College in Upstate New York for his Bachelor of Arts in Theater & Performance has called the mid-Hudson Valley region of New York his home. During his time at Bard, he helped start the campus farm growing vegetables organically, and after graduating knew that he wanted to start a career path related to agriculture and growing food. After working on vegetable and livestock farms in New York and Vermont for three years, he spent two years in Paraguay as an Agriculture Extension Volunteer for the Peace Corps. While serving in the Peace Corps, he began to focus on agricultural science with the goal of working in extension to assist small-holder farmers with soil management, both in the United States and internationally. While finishing this thesis for the Master of Professional Studies program at Cornell, he began a Master of Science in Soil Science degree at North Carolina State University in August 2021.

ACKNOWLEDGEMENTS

The first acknowledgement and thank you is to my advisor, Dr. Janice Thies, who helped foster me as a scientist at Cornell and continues to make time to guide me as a scientist after Cornell.

Additionally, the following people and groups have my thanks and appreciation:

Dr. May Boggess of the Cornell Statistical Consulting Unit, for her invaluable help with drafting this paper and the statistical analysis,

Dr. Judith Fitzpatrick and Prolific Earth Sciences, Inc., for funding parts of this study and supplying the microBIOMETER® test,

Dr. Alan J. Franzluebbers of North Carolina State University, for his contribution and assistance in soil testing and his continued guidance for this study,

the Cornell Sustainable Cropping Systems Laboratory, especially Dr. Matt Ryan, and Ph.D. candidate Uriel Menalled, for the use of the soil samples from their experimental plots,

and Alexandria Casteñeda & Whitman Barrett of my Cornell master's degree cohort, for their assistance in the laboratory.

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Introduction

Soil testing is a fundamental component of agroecosystem management. Often used as an agronomic indicator for yield, standard soil nutrient testing allows farmers to evaluate the soil's ability to promote plant growth and health in horticultural, row crop, and pasture-livestock systems. Moreover, soil testing allows land managers to implement site-specific nutrient management (SSNM) to reduce costs, pollution, and environmental degradation associated with excessive fertilizer application beyond plant requirements for growth (Dimkpa et al., 2017).

Soil testing and SSNM have focused historically on providing farmers a fertilizer recommendation based on analysis of soil chemical-nutrient contents, generally the macronutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S), and eight micronutrients. Farmers collect soil samples representative of their fields and send them off-site for analysis of the chemical-nutrient constituents in a laboratory. Recently, however, there has been an increasing interest among farmers in assessing soil health (Carlisle, 2016; Lobry de Bruyn & Andrews, 2016) in a manner that integrates biological and physical assessment of a soil with the chemical components for a more holistic management approach (USDA-NRCS, 2020). Among other benefits, managing soils to promote biological activity may reduce reliance on and increase efficiency of agrochemical inputs and fertilizers (Franzluebbers, 2016; Zhao et al., 2016). Considering the importance of soil biological activity and managing agroecosystems to promote soil health, I examined and compared laboratory measurements of soil microbial biomass carbon (SMB-C) with use of the microBIOMETER®, a relatively new, rapid, and mobile test designed to be used in the field by farmers and members of the general public.

The research reported here contributes to the literature in two ways. First, I compared the microBIOMETER® with established, laboratory measurements of SMB-C and other well-established assays of soil biological health. Five assays in total were used in comparison with microBIOMETER®, including two that measure SMB-C, chloroform fumigation-extraction (CFE) and chloroform fumigation-incubation (CFI). The other assays performed were soil respiration (burst of CO_2), active carbon (permanganate oxidizable carbon), and autoclaved citrate extractable protein (soil protein) (Moebius-Clune et al., 2016). Currently, to my knowledge, there are no studies published in peer-reviewed scientific journals that benchmark the microBIOMETER® test in this way.

Second, I discuss expanding the use and access to soil nutrient and biological testing for the development of agriculture in both the highly industrialized Global North and for resource-poor farmers of the Global South. Specifically, I consider the implications of improving SSNM and agroecosystem management with mobile, non-laboratory based soil analysis such as the microBIOMETER®.

Soil Testing and International Agriculture Development

In contrast to farmers in high-income and industrialized nations of the Global North (the North), farmers of the Global South (the South) – most of them smallholder and resource-poor – do not typically evaluate the nutrient levels of their soils (Chambers & Jiggins, 1987b; Dimkpa et al., 2017). Although research has indicated that soil testing in the North is also relatively low on average (Lobry de Bruyn & Andrews, 2016), biophysical and socioeconomic factors are more limiting to soil testing in the South (Dimkpa et al., 2017; Kokoye et al., 2018; Stewart et al., 2020). Affordable and accessible mobile soil testing that is scientifically verified and comparable

to laboratory standards can help increase widespread soil analysis based SSNM in both the North and South.

Here, I identify four main obstacles to soil analysis based SSNM, biophysical and socioeconomic in nature, that include, but are not limited to: (1) direct financial costs, (2) heterogeneity and variation of soils; (3) location and systemic inaccessibility; and (4) farmer awareness, perception, and knowledge. Increasing farmer access to and use of mobile soil testing kits that are user-friendly and affordable can help overcome these obstacles. Mobile soil testing should also include soil health parameters that incorporate management recommendations beyond chemical fertilizer recommendations typically given by standard chemical analysis of nutrients.

The upfront, direct cost of laboratory soil analysis is the most apparent obstacle that falls under the socioeconomic category. In sub-Saharan Africa (SSA), though the prices of various soil test packages are ostensibly lower than in U.S. laboratories (see pricing tables in Dimkpa et al., 2017), the costs are generally not affordable for most smallholder farmers in SSA compared to wages and income for the average farmer (Dimkpa et al., 2017; Kenya National Bureau of Statistics, 2017). Outside of SSA, Kokoye et al. (2018) also found that farmers most often cited the high financial burden of soil analysis in Haiti as an obstacle to its use. The four factors listed above each have their own influence, but are also interconnected in their effect on a farmer's ability or likelihood to utilize soil analysis services for SSNM. For example, hyper-variation within a farmer's field of soil type or topography (obstacle 2) could require a higher sampling density and incur greater financial burden for the analysis of multiple samples (obstacle 1). Thus, there is an inherent link between these obstacles that adds to the complexity of trying to overcome them.

Spatial heterogeneity of soils – specifically, the spatial variation of soil characteristics and quality – is a primary influence on agricultural yield. Indigenous communities (Pawluk et al., 1992; Barrios & Trejo, 2003) and pre-industrial peasant-feudal societies (Baveye & Laba, 2015) have historically confronted the realities of farming in highly varied landscapes. A major shift occurred with the industrial revolution of the late 19th and early 20th century that prompted high agricultural mechanization, altering the agricultural landscape and the perception of the spatial variation of soils due to increases in tillage intensity. The biotechnological engineering and increased agrochemical use of the Green Revolution later in the mid-20th century also enabled farmers in the North to overcome and, to a limited extent, homogenize heterogeneous soil-landscapes; meanwhile, much of the South, particularly SSA, did not see these advancements (Pingali, 2012). A study of nutrient heterogeneity in southern U.S. Appalachian soils found that past land use homogenized the spatial density of soil C, K, and P, and retained its uniformity 60 years after abandonment, indicating that land use has persistent, multi-decadal effects on the spatial heterogeneity of soil resources (Fraterrigo et al., 2005).

While modern practices may have allowed some homogenization to occur, farmers were encouraged to consolidate small fields into drastically larger units, where landscapes were treated as expansive, uniform units whose treatment as a unit was more economical and time-efficient (Baveye & Laba, 2015). The result has been a low modern sampling density, with the average in the U.S. of one sample per 67 ha, ranging from 1 in 9 ha in Georgia and 1 in 1214 ha in Wyoming (Fixen, 2002). Such a low sampling density does not allow for practical accuracy in SSNM, so fields are often over-fertilized in the U.S. regardless of nutrient levels (Lobry de Bruyn & Andrews, 2016).

In contrast, fields of resource-poor farmers in the South often do not receive enough fertilizer. Many studies have recently documented the hyper-local heterogeneity of soil fertility and the obstacles farmers of SSA face in SSNM (Vanlauwe & Giller, 2006; Tittonell et al., 2008; Vanlauwe et al., 2015). The higher variation observed in the South most often relates to the presence of highly weathered soils with varying contents of soil organic matter typical of tropical climates, as organic matter affects most nutrient cycles (Vanlauwe et al., 2015). Spatial variation in soil makes SSNM difficult at the local level because variation of soil type and quality can occur within less than one hectare of land, either within one farmer's field or between these and the farm fields of their neighbors.

Studies of nutrient response trials in Zimbabwe (Zingore et al., 2007), Kenya (Tittonell et al., 2008), and Togo (Wopereis et al., 2006), for Southern, East, and West Africa respectively, have confirmed such field-to-field variation. Harou et al. (2018) analyzed 1,007 different soil samples from farmers' primary maize fields from 50 different villages in Tanzania, East Africa. They found that only 55% of farmers shared similar soil test results and recommendations with those of their closest neighbor. Their study also suggested that the government's blanket fertilization recommendation did not correlate with recommendations from their hyper-local soil testing regime. As mentioned previously, the hyper-local heterogeneity of tropical soils requires a higher sampling density, incurring greater cost for laboratory soil testing, even within a small farm of 2-5 ha or less.

While soil testing laboratories are available across the globe where crops are produced, the access to laboratories is limited more in the South compared to the North. In a survey of various agriculture stakeholder groups across SSA, access to quality soil testing was determined the top-

ranked limiting biophysical factor for soil management (Stewart et al., 2020). The number of public and private soil testing service providers in SSA is low due to limited infrastructure development. This is in stark contrast to the plethora of both private and public soil and plant testing facilities across the United States, where state and federally funded agencies or Land-Grant universities often provide soil testing services in each state (Dimkpa et al., 2017).

High farmer-to-extensionist ratio is an increasingly common trend in the South (Fisher et al., 2018), and the result is a decrease in assistance to farmers with tasks including soil testing and SSNM (Stewart et al., 2020). The access to trained extension providers as part of the knowledge-transfer value chain (KTVC) is critical in driving the process of implementing soil analysis and a SSNM plan (Stewart et al., 2020). Soil sampling protocols, for instance, are not straightforward for an untrained practitioner due to zoning delineation, topographical change, and soil type identification within a heterogeneous soil-landscape. As such, the lack of direct involvement in sampling by an extensionist will often hinder the implementation of SSNM from inception (Middendorf et al., 2017).

Mobile soil testing can improve the KTVC by bridging the gap between scientific or technical indicators of soil quality (TISQ) and local indicators of soil quality (LISQ) in the South (Barrios & Trejo, 2003; Barrios et al., 2006). Farmer knowledge of soils in the North is more closely aligned with Western scientific principles and TISQ largely due to greater literacy rates, formal education levels, and trust of and reliance on crop advisors and the scientific community (Barrios et al., 2006; Lobry de Bruyn & Andrews, 2016). Using observable features of the soil and surrounding agroecosystem, farmers in the South largely rely on LISQ and often have little understanding and greater mistrust of TISQ (Barrios et al., 2006; Dawoe et al., 2012). The

potential to incorporate mobile soil testing into participatory research and extension activities may therefore help to integrate LISQ and TISQ for farmers.

Though not without caveats, the advancements in soil testing technologies that make soil analysis more accessible outside of the laboratory have shown promise in overcoming some biophysical and socioeconomic obstacles. Strategies that have increased soil testing access in SSA include the use of a soil testing truck in Uganda (Nakkazi, 2014) and the *SoilDoc* portable kit (see Dimkpa et al., 2017) for use by an extension agent or trained technician in the field. Mobile soil testing, however, should continue to be developed beyond top-down, "transfer-of-technology" (TOT) approaches, which focus less on learning and capacity building for small-holder farmers compared to participatory approaches (Chambers & Jiggins, 1987a; Testen et al., 2018). The soil testing truck and *SoilDoc* kit are examples of TOT because they depend on the technical knowledge of an extensionist to carry out the test and explain the recommendations. Nonetheless, these approaches do increase access for SSNM in highly heterogenous landscapes, are affordable, and improve farmer perceptions of standard soil analysis. Approaches that are developed to directly involve the farmer and their unique socio-cultural environment (i.e., LISQ) may improve the effectiveness of the KTVC for farmer learning and motivation in soil management (Pawluk et al., 1992; Testen et al., 2018).

Farmers' use of soil biota as local and technical indicators of soil quality may be limited in scope and utility and are currently lacking in the farmer-extension KTVC. Surveys in Latin America and SSA showed that many farmers in the South understood, for example, the basic role soil macrofauna (such as earthworms) play in nutrient cycling through litter/residue decomposition and how these processes affect soil physical and fertility properties (Grossman, 2003; Barrios et

al., 2006; Dawoe et al., 2012). However, farmers had limited to no awareness of soil microorganisms or of the roles microorganisms have in decomposing plant and animal residues (Grossman, 2003; Dawoe et al., 2012). Since management practices, such as the use of tillage and pesticides, have a strong effect on the soil microbial community, farmer awareness of critical, microbial-mediated processes (e.g., mycorrhizal colonization) is of great importance.

Due to the focus often given to wet-chemistry nutrient analysis as a baseline or bare minimum for soil testing, the importance of soil biological testing is more likely to be overlooked by farmers in both the North and South. Increasing awareness of integrated soil testing with a soil health approach, that includes biological and chemical testing, is vital to the KTVC. The use of an in-field testing tool, such as the microBIOMETER®, could act as a biological metric and, along with standard soil testing, could allow more integration of soil health into the KTVC. Thus, it is important that the scientific community evaluates the effectiveness of microBIOMETER® as a soil health test.

Soil Health and Measurements of Soil Biological Activity

The concept of soil health, derived from its predecessor, soil quality, is defined as "the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans," (USDA-NRCS, 2020). It is distinguished from "soil fertility," which focuses on the management of soil to produce harvestable crops. Soil health emphasizes a greater sustainability of soils to provide ecosystem services beyond crop needs, such as water regulation, habitat for plant and animal wildlife, atmospheric regulation, and maintenance of biodiversity (Kihara et al., 2020; USDA-NRCS, 2020). For a more concise and applied understanding, soil health is often seen as the integration of biological, physical, and chemical aspects of soil functioning, with chemical

aspects generally referring to standard laboratory testing of nutrient contents. The biological component is the lynchpin of the soil health paradigm, which emphasizes the living organisms – bacteria, fungi, earthworms, arthropods, etc. – as the term "health" can be only used to describe something with life (Franzluebbers, 2016).

Laboratory soil testing services have focused historically on soil chemical indicators of inorganic nutrients and soil acidity (pH), to assess whether the soil has the nutritive capacity to support a certain level of crop growth and yield. While critically important, inorganic nutrient availability alone does not offer a complete assessment of soil properties and processes that determine crop yield and influence environmental quality. Soil testing could achieve more holistic outcomes with the adoption of tests for soil biological activity.

For instance, Franzluebbers (2016) suggested that the flush of carbon dioxide test (soil respiration), which measures the level of microbial respiratory activity, could be used to evaluate basic agronomic performance indicators such as nutrient cycling and the decomposition of organic residues. Haney et al. (2001) and Franzluebbers & Stuedemann (2003) showed that the flush of CO_2 measured in the soil respiration test correlated well with nitrogen mineralization, the process in which organic N in the forms of residues and microbial biomass is converted to plant-available, inorganic N. Soil respiration is generally the least labor and equipment intensive (Moebius-Clune et al., 2016) of the laboratory soil health tests, making its availability more widespread, including in the South (Dimkpa et al., 2017).

The active carbon (C) assay measures the soil organic C (SOC) of organic matter in the soil that correlates to the carbon fraction readily available as a substrate for soil microbial consumption/decomposition and biomass incorporation. The distinction of this pool of SOC as

"active" is to differentiate it from the bulk of SOC that is more passive, recalcitrant, and more slowly affected by microbes (Weil et al., 2003). The active C of a soil is closely related to the particulate organic matter (POM) of soils (Moebius-Clune et al., 2016), which is the a fraction of partially decomposed organic matter from plant and animal residues (Witzgall et al., 2021). Measuring POM, however, is a more labor-intensive assay compared to active C. Total soil organic matter often changes very slowly in soils, whereas, active C represents the more readily decomposable fraction of organic matter that responds to management much faster and acts as a "leading indicator" of management influence for farmers who seek to build organic matter (Moebius-Clune et al., 2016).

The ACE protein (soil protein) assay has replaced potentially mineralizable N in some soil health frameworks as an indicator of how much organic nitrogen may be present in soil. The soil protein test can indicate the capacity of organic matter to provide N, with higher quality organic matter having a low carbon-to-nitrogen ratio. Moebius-Clune et al. (2016) suggested that soil protein is a better indicator of overall soil health due to the importance of N in controlling microbial population growth (biological) and as the most limiting nutrient for plants (chemical). Unfortunately, like the microbial biomass assays discussed below, measuring soil protein is a relatively long and labor-intensive process that precludes it from being an affordable test.

Finally, both historical and contemporary studies of soil health have cited soil microbial biomass (SMB) as a strong indicator of soil health (Pankhurst et al., 1995; Toor et al., 2021). The soil microbial population undergoes various changes over a cropping season, such fluctuations in overall population numbers and community composition (Lauber et al., 2013). Moisture, temperature, and nutrient availability are some factors that can affect soil microbial population

dynamics (Castro et al., 2010), along with soil management practices. Tracking changes in SMB over time can reflect changes in soil health that may occur when new farm management practices are implemented, such as before or after fertilizer additions or in response to the use of cover crop rotations (Zimmerman, 2021). Apart from its use as an agronomic indicator, Dynarski et al. (2020) suggested SMB as an important biological indicator to consider for assessing carbon flows and for understanding the permanence of soil C sequestration, one of the more popular and important endeavors of payment for ecosystem service (PES) projects.

Measurement of SMB-C is accomplished with a variety of methods such as chloroform fumigation-extraction (CFE) or fumigation-incubation (CFI), substrate-induced respiration (SIR), and direct counts of microbial cells and biomass under a microscope after vital staining. The CFE and CFI methods are currently the most common and both were used in this study to compare with the SMB results from the microBIOMETER®. Like the soil protein assay, the CFE and CFI methods require specialized equipment and chemicals and can take a longer time to complete, making them impractical and expensive for farmers.

Materials and Methods

Soil Sampling & Experimental Design

Soils were sampled in 2020 (Time 1, T1) and in 2021 (Time 2, T2) from cover crop trials in two locations in New York State, USA. The cover crop trials were part of an ongoing study being undertaken by the Sustainable Cropping Systems Laboratory of Cornell University. The trials were located at:

- the Hudson Valley Farm Hub (Farm Hub), Hurley, NY (41°55’24.9” N, 74°04’46.0” W).

- and the Cornell University Musgrave Research Farm (Musgrave), Aurora, NY (42°44’10.1” N, 76°39’08.1” W).

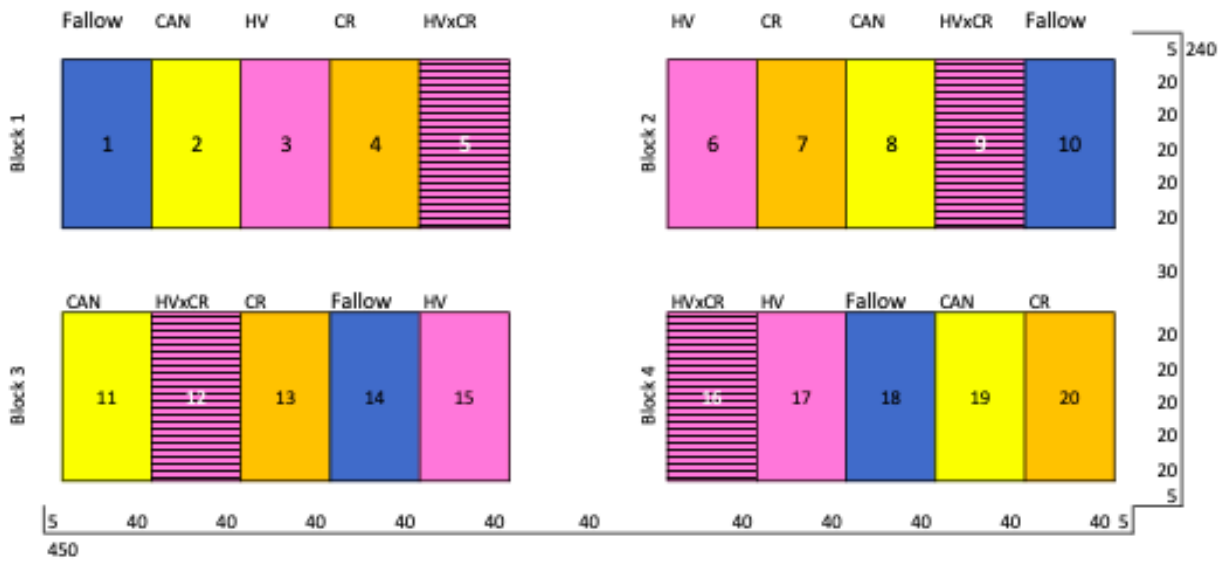

Figure 1. Layout of blocks and treatments at the Musgrave location (courtesy of the Cornell Sustainable Cropping Systems Laboratory).

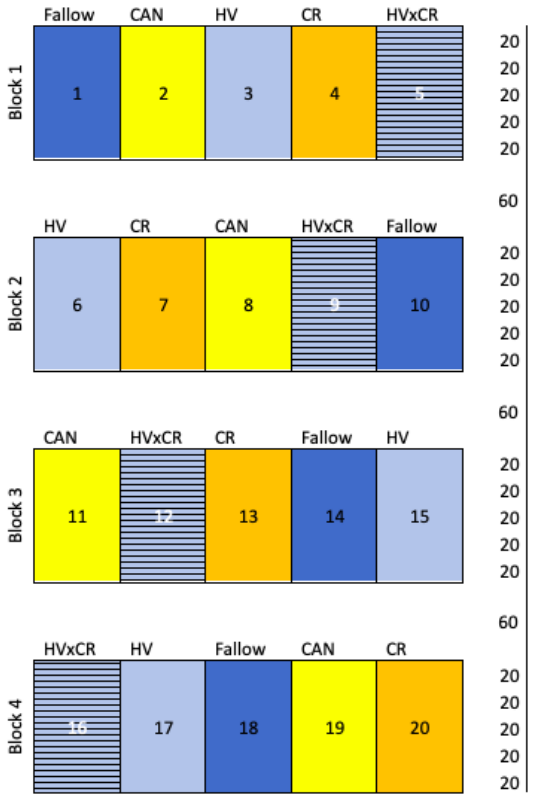

Figure 2. Layout of blocks and treatments at the Farm Hub location (Courtesy of the Cornell Sustainable Cropping Systems Laboratory).

Both experimental locations consisted of four blocks measuring 30.48 m (100 ft) by 60.96 m (200 ft) before cover crops were planted. At the Musgrave farm, blocks were placed to account for spatial variation across the entire field (i.e., no slope or significantly uneven ground, Figure 1). Blocks were placed to account for an elevational gradient at the Farm Hub, where block 1 was at the highest elevation and block 4 at the lowest (Figure 2). Cover crops were planted in September 2020 coinciding with soil sampling for this study. Before seedbed preparation, the field at Musgrave was an unfertilized oat monoculture. Prior to the termination of the oats, the experimental area was fertilized with 1120 kg ha^{-1} of 5-4-3 (N:P:K). Oats were harvested and terminated with a moldboard plow in Mid-July 2020. At the Farm Hub, the field had been a spring wheat monoculture before the experiment began and was fertilized with 1120 kg ha^{-1} of 5-

4-3 (N:P:K) at planting. The wheat was harvested and terminated with a high-speed disc in mid-July 2020. The soil from the Farm Hub was classified as an Unadilla series silt loam, and at Musgrave was a Lima series loam.

Forty-eight samples (4 for T1 and 20 for T2 at both locations) were composited from 25 soil cores taken from the entire block for T1 and each treatment plot (1-20) for T2 (Figures 1 and 2) using a standard 2.3 cm (0.9 inch) diameter soil probe to a depth of 15.2 cm (6.0 inches). The samples were kept on ice until stored in a cooler at 4°C between use for testing and analysis. Soils were homogenized and sieved to 2 mm before all assays.

Baseline Soil Samples (T1)

The cover crop experiments were installed at the two locations in a randomized complete block design with four blocks (Figures 1 and 2). One composite soil sample was taken in each block, after tillage, but before the cover crops were planted. These baseline samples enabled examining any changes in soil biological health after one year of cover cropping. One composite sample for each of the four blocks at both the Farm Hub and Musgrave provided four samples per location and an n=8 sample size for T1.

Cover Crop Treatment Soil Samples (T2)

After tillage and the T1 soil sampling, the blocks were subdivided into five treatment plots and planted with cover crops as shown in Figures 1 and 2. The list of treatments and controls between the T1 baseline (0) and T2 (1 - 5) treatments are as follows:

0. Tilled – baseline control after tillage
1. Fallow – a fallow control after initial tillage eventually growing weedy biomass
2. CAN - canola (*Brassica napus*, L.),

3. CR - cereal rye, (*Secale cereale*, L.),
4. HV - hairy vetch (*Vicia villosa*, Roth)
5. and HV x CR - hairy vetch with cereal rye

T2 samples were taken 11 months after T1 samples in July 2021 after cover crops were terminated using a roller-crimper. Composite samples were taken in each treatment plot yielding five samples in each of the four blocks, thus providing 20 samples for each location and an n=40 sample size for T2.

Soil Biological Health Assays

The five laboratory assays used to measure soil biological health and compare to the microBIOMETER® microbial biomass readings were:

1. Chloroform Fumigation-Extraction (CFE) Microbial Biomass
2. Chloroform Fumigation-Incubation (CFI) Microbial Biomass
3. Soil Respiration (CO_2 burst test)
4. Active Carbon (Permanganate Oxidizable Carbon-POxC)
5. Soil Protein (Autoclaved Citrate Extractable Protein-ACE)

Unless otherwise noted, the author performed the procedures and analyses for the assays.

microBIOMETER®

Soil microbes secrete exopolysaccharides and other metabolites that bind non-living soil particles to each other and to themselves. In the microBIOMETER® assay (Prolific Earth Sciences, Inc., Montgomery, NY, USA), a small soil sample is placed in a test tube with a reagent salt and blended with a whisker. This releases microbes bound to or within soil particles and suspends them in solution, while soil particles settle to the bottom of the tube. The microbes

that remain suspended in the salt solution are sampled by placing drops of the solution on a test card, which is then scanned using a smartphone camera application. The application measures the color intensity of the spot where sampled drops were placed, and the resulting color is compared to a color background surrounding the sample area on the test card. The color generated by the sampled drops is thought to measure the density of microbial cells in the sample by virtue of the chroma taken on by the cells themselves when living in the soil (Prolific Earth Sciences, Inc., 2020).

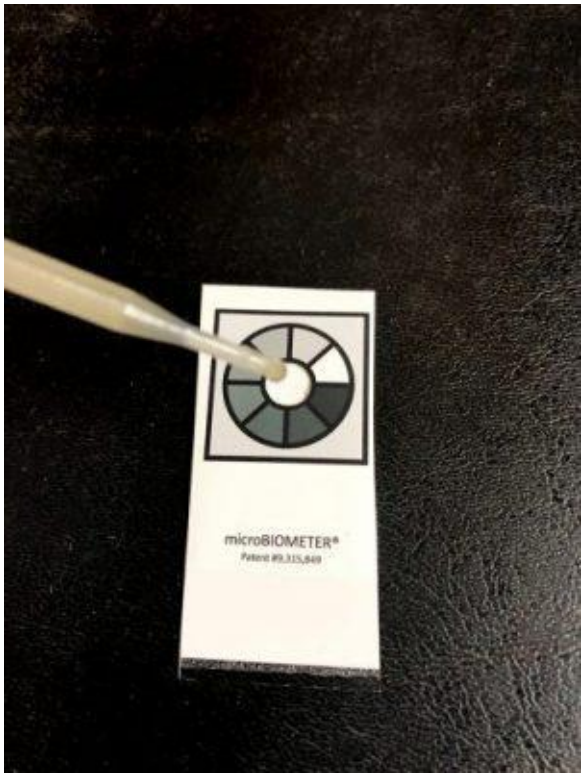

Figure 3. Applying the soil-solution extract to the microBIOMETER® test card before analysis with the app (Prolific Earth Sciences, Inc.).

Figure 4. The microBIOMETER® kit and its components (Prolific Earth Sciences, Inc.).

For each test, approximately 1.4 g of sodium chloride and calcium chloride (NaCl, $CaCl_2$, a proprietary blend provided pre-packaged with the microBIOMETER® soil test kit) was combined with 9.5 mL of water in a small test tube. Approximately 1 mL of sieved, field-moist

soil was packed into a soil sampler syringe and compressed to 0.5 mL. Any excess soil was removed, and the sample placed into the test tube with the salt solution. The soil was broken up manually using a metal spatula and then mixed with the solution for 30 seconds using a battery-operated whisking device (provided with the kit) in the test tube. After mixing, the solution was allowed to settle for 15 minutes before the solution suspension was sampled approximately 1.3 cm below the solution surface with a plastic bulb pipet. Three drops from the pipet were placed onto the sample-area center of the microBIOMETER® test card (Figure 3) and analyzed for SMB-C in µg C g^{-1} soil using the microBIOMETER® mobile app (available for download on iOS and Android devices).

Chloroform Fumigation-Extraction (CFE) Microbial Biomass

The SMB-C can be estimated by measuring the concentration of dissolved organic carbon (DOC) extracted before and after exposure to chloroform in a slurry of soil sample. One set of soil samples is treated with chloroform and a control set is left untreated. Exposure to chloroform in solution solubilizes microbial cell membranes and releases the cell contents into the soil-extract solution, mostly cellular carbon. A potassium sulfate (K_2SO_4) salt-solution is then used to extract the microbial C as DOC from the soil particles. The difference in DOC content of soil extracts with and without chloroform exposure is used to estimate the SMB-C content of the soil. Carbon from the surrounding environment of air, material surfaces, and containers is measured using reagent blanks and factored into the biomass calculation. Included in the final calculation is an efficiency factor (K_{ec}) to account for the proportion of C that is extracted. The direct extraction method used here, suggested by Gregorich et al. (1990) and adapted from the original method by Vance et al. (1987), was used with a lower concentration of K_2SO_4 extractant (0.05 M) than was originally published in both CFE methods (0.5 M) cited. The rationale for reducing

the salt concentration was to allow CFE-C to be measured in a Shimadzu Total Organic Carbon analyzer (TOC-LSH; Shimadzu Corporation, Kyoto, Japan), without endangering the integrity of the column.

Gravimetric moisture content of the soil samples was determined. Then, two, 10 g oven-dry weight equivalent sub-samples of field-moist soil of each sample were placed into individual 50 mL centrifuge tubes. One sample was treated with chloroform and the other was not. Each sub-sample was combined with 40 mL of 0.05 M K_2SO_4, where one set of sub-samples received 0.5 mL of ethanol-free chloroform. Two reagent blanks were also created for each set using the same procedures but without soil added. Both sets of soil extracts were shaken for 1.5 hrs on a platform shaker at 200 rpm and then centrifuged at 4,000 rpm for 5 min. Clarified aliquots were then filtered through Whatman #1 filter paper into clean centrifuge tubes. Samples that did not contain chloroform were frozen. Chloroform-treated samples remained in a fume hood for 16 hours before freezing and storage to allow the chloroform to evaporate. Frozen samples were mailed on ice to the University of New Hampshire Water Quality Analysis Laboratory. There, the samples were thawed and analyzed for DOC concentration by high temperature catalytic oxidation and NDIR detection with a Shimadzu Total Organic Carbon analyzer (TOC-LSH; Shimadzu Corporation, Kyoto, Japan). SMB-C was then calculated from the DOC concentration and reported in µg C g^{-1} soil. A K_{ec} of 0.45 was used in the final calculation (Joergensen, 1996).

Chloroform Fumigation-Incubation (CFI) Microbial Biomass

Like the CFE method, the CFI method employs chloroform fumigation to rupture microbial cells and release SMB-C into the soil solution as mineralizable DOC. Similarly, a non-fumigated sample may be used as a control for subtraction of non-SMB-C organic C, but the CFI assay may

also be conducted without subtraction of the untreated control (Franzluebbers et al., 1999). Instead of calculating SMB-C from analysis of TOC, the CFI method allows for a microbial population to re-colonize the soil under aerobic incubation for ten days. Carbon respired by the recolonizing microbes (CO_2-C, captured by an alkali trap) post-chloroform fumigation represents the SMB-C likely present in the soil prior to chloroform fumigation. Like the CFE method, reagent blanks were also used. Included in the final calculation is an efficiency factor (K_c) to account for the proportion of mineralizable C (Voroney & Paul, 1984).

CFI biomass protocols were adapted from Franzluebbers et al. (2021) based on the original method by Jenkinson & Powlson (1976). Briefly, average gravimetric moisture content of the soil samples was determined, and 20.00 g oven-dry weight equivalent of each soil sample was moistened to achieve 50% water-filled pore space (50% saturation) and incubated for ten days at 25°C in 60 mL glass jars placed within larger 0.9 L canning jars. Ethanol-free chloroform ($CHCl_3$) was placed in a beaker and then placed into a desiccator, along with the glass jars containing the soil samples. A vacuum was applied to the desiccator to vaporize the chloroform and was kept under vacuum for one day. After the chloroform vapors were removed, the samples were replaced in the 0.9 L jars to incubate for another ten days at 25°C with a vial containing 10 mL of an alkali trap of 1 M sodium hydroxide (NaOH) and a vial of water to maintain humidity. The subtraction of a non-fumigated control was not used. SMB-C was determined from the flush of CO_2 captured in the alkali trap, with CO_2-C, as µg C g^{-1} soil, determined by titration with 1 M hydrogen chloride (HCl) with vigorous stirring in the presence of barium chloride ($BaCl_2$, forming barium carbonate [$BaCO_3$] precipitate) to a phenolphthalein endpoint. A K_c of 0.41 was used in the final SMB-C calculation (Anderson & Domsch, 1978).

Soil Respiration

Soil microbial respiration rate can be estimated by measuring the amount of CO_2 released from soil after a defined incubation period. After soils are air-dried and rewetted, the inert microbial population will begin metabolic and respiratory activity, resulting in a burst of CO_2 release. In a sealed container, an alkali solution trap captures the CO_2 respired by the microbial community over a 4-day period. Electrical conductivity (EC) of the alkali trap is measured before and after incubation and the resulting data used to calculate respiration (Moebius-Clune et al., 2016).

Here, 20.00 g of air-dried and sieved soil were placed in a perforated tin container set on top of cellulose filter papers in a pint-sized canning jar. An alkali trap assembly containing 9 mL of 0.5 M KOH was suspended above the soil. Then, 7 mL of deionized water was pipetted to the bottom of the jar so that the soil was moistened via the filter paper by capillary action. The jar was sealed immediately and incubated for 4 days at room temperature. The EC of the KOH solution was measured before and after incubation using an Orion Versa Star Pro meter and probe (Thermo Fisher Scientific, Waltham, MA USA) and used to calculate respiration in mg CO_2 g^{-1} soil d^{-1} by microbes. The Cornell Soil Health Laboratory conducted all procedures and analyses.

Active Carbon

Oxidized soil organic carbon is measured with spectrophotometry after mixing the soil with a potassium permanganate ($KMnO_4$) solution. Active carbon was determined following the protocols of Weil et al. (2003) and Moebius-Clune et al. (2016).

A 2.50 g sample of air-dried soil was shaken in a 0.2 M solution of potassium permanganate ($KMnO_4$) solution for two minutes on a platform shaker at 120 rpm and allowed to settle for 8

minutes. A 202 µL aliquot of the soil-$KMnO_4$ solution was then transferred to 18 mL of deionized water in a centrifuge tube to achieve a 0.02 M $KMnO_4$ concentration, which was then read for absorbance at 550 nm with a Hach Pocket Colorimeter II (Hach Company, Loveland, CO USA). Active carbon, reported in mg C kg^{-1} of soil, was calculated by comparing the absorbance values to a standard curve of known $KMnO_4$ concentrations. Two repetitions were produced from these procedures and the active carbon values were averaged (Moebius-Clune et al., 2016). Procedures and analysis were performed by the Cornell Soil Health Laboratory.

ACE Soil Protein

Soil protein is a measure of the organically bonded amino N readily available for microbial mineralization in soil and serves in place of the potentially mineralizable N assay. The results from this assay indicate the quantity of N present in organic matter that can potentially be turned over through mineralization into inorganic N for plant uptake and growth. Organic N is extracted under high pressure and temperature combined with a citrate buffer and analyzed by colorimetry with a spectrophotometer. Soil protein was determined using the protocols of Moebius-Clune et al. (2016).

A 3.00 g sample of sieved, air-dried soil was weighed into autoclavable glass tubes containing 24 mL of sodium citrate buffer (20 mM, pH 7.0) and sealed. The mixture was shaken to disperse aggregates for five minutes on a rotary shaker set at 180 rpm and subsequently autoclaved for 30 minutes at 121°C and 15 psi. Once cooled, 2 mL of the slurry mixture was centrifuged at 10,000x gravity to precipitate out the soil particles. A 1 mL sub-sample of the centrifuged aliquot was then analyzed for protein concentration by a Pierce BCA Protein Assay kit (Thermo Fisher Scientific) at 60°C. Quantification of protein concentration was determined colorimetrically by

use of a BioTek Synergy HT spectrophotometric microplate reader (BioTek Instruments, Inc., Winooski, VT USA) at 562 nm and compared against a bovine serum albumin (BSA) standard curve using a Pierce Pre-Diluted Protein Assay Standard Set (Thermo Fisher Scientific). Extractable protein content of the soil was calculated by multiplying the protein concentration of the extract by the volume of extractant used and dividing by number of grams of soil used and calculated in mg amino N g^{-1} soil. The Cornell Soil Health Laboratory carried out all soil protein procedures and analyses.

Statistical Methods

Descriptive statistics were means and standard deviations for continuous variables and counts and percentages for categorical variables. Analysis of variance was used to determine significant differences in soil health assays across locations, treatments, and including the location-treatment interaction for T1 and T2 data separately. Pearson correlations were used to evaluate bivariate relationships between soil health assays.

For the microBIOMETER®, multivariate linear regression models were fit to assess the impact of treatment and location in the microBIOMETER® relationship with each of the other assays. For each of these models, the response variable was the microBIOMETER®, and the explanatory variables were one laboratory soil health assay, location, indicators for treatment, and the location-treatment interaction. The interaction was removed first if not significant. Subsequently, the treatment indicators were removed if not significant. Significance was determined at the 5% level. All statistical analysis was done using JMP Pro (Version 16, SAS Institute Inc., Cary, NC, USA).

Results

Soil sampling proceeded as anticipated, and 48 samples were collected. However, three problems were encountered with the soil health assays. First, the initial readings from the microBIOMETER® assay were discarded due to operator error. Subsequently, two more readings were taken, and their average values were used in this analysis. Second, some samples had duplicate CFE biomass readings taken due to questionable values in the initial reading; for those samples, their average was used in this analysis. Three of the CFE biomass readings that were out of range were omitted because the sample was unintentionally destroyed and not available for duplicate measurements, and thus the CFE biomass assay had the analysis sample size of n=45. Finally, the CFI biomass assay was the final assay performed and there was not enough soil left from 22 of 48 samples, leaving a sample size of n=26 from T2 soils only, with n=19 from T2 Farm Hub (1 missing) and n=7 from T2 Musgrave (13 missing).

Descriptive Analysis

Table 1 (T1) and Table 2 (T2) display the means of the soil health assays over the four blocks at each location. Block 4 at the Farm Hub, the block placed at the lowest end of an elevation gradient, demonstrated lower values for all assays except CFE and CFI biomass (Table A1).

Table 1. Means of soil health assays at T1 by location (n=4)

Test		Treatment	Farm Hub	Musgrave
microBIOMETER®	(µg C g^{-1} soil)	Tilled	359.4 **A**	190.8 **B**
CFE Microbial Biomass	(µg C g^{-1} soil)	Tilled	83 **B**	198.5 **A**
CFI Microbial Biomass	(µg C g^{-1} soil)	Tilled	.	.
Soil Respiration	(mg CO_2 g^{-1} soil day^{-1})	Tilled	0.051 **B**	0.162 **A**
Active Carbon	(mg C kg^{-1} soil)	Tilled	355.2 **B**	503.8 **A**
ACE Protein	(mg amino N g^{-1} soil)	Tilled	4.28 **A**	4.67 **A**

Notes. CFI biommas test was not performed for T1 due to insufficient sample. Letters between the Farm Hub and Musgrave columns indicate significant differences between locations, where A indicates that mean is significantly larger than B ($p<0.05$).

Table 2. Means of soil health assays at T2 by location and cover crop treatment (n=4, except where indicated), with grand means of all treatments at each location.

Test	Cover Crop Treatment	Farm Hub	Musgrave
microBIOMETER®		----(µg C g^{-1} soil)----	
	Fallow	339.1	226.6
	CAN	312.4	231.5
	CR	353.7	201.0
	HV	371.0	207.2
	HV x CR	384.3	223.1
	Mean	**352.1 A**	**217.9 B**
CFE Microbial Biomass		----(µg C g^{-1} soil)----	
	Fallow	77.8	122.4
	CAN	170.7[1]	156.8
	CR	141.2[1]	132.0
	HV	84.7	147.3
	HV x CR	103.5	173.6[1]
	Mean	**115.6 B**	**146.4 A**
CFI Microbial Biomass		----(µg C g^{-1} soil)----	
	Fallow	405.0	608.0[1]
	CAN	451.0[1]	650.0[3]
	CR	454.5	708.5[2]
	HV	429.0	686.0[3]
	HV x CR	510.0	.
	Mean	**449.9 B**	**663.1 A**
Soil Respiration		----(mg CO_2 g^{-1} soil day^{-1})----	
	Fallow	0.574	0.741
	CAN	0.551	0.775
	CR	0.541	0.627
	HV	0.564	0.807
	HV x CR	0.565	0.798
	Mean	**0.559 B**	**0.750 A**
Active Carbon		----(mg C kg^{-1} soil)---	
	Fallow	388.4	557.8
	CAN	395.1	582.5
	CR	421.6	564.1
	HV	405.5	574.8
	HV x CR	420.3	578.5
	Mean	**406.2 B**	**571.5 A**
ACE Soil Protein		----(mg amino N g^{-1} soil)---	
	Fallow	3.98	4.43
	CAN	4.03	4.68
	CR	4.65	4.13
	HV	4.63	4.50
	HV x CR	4.40	4.63
	Mean	**4.34 A**	**4.47 A**

Notes. 1. n=3 due to missing data; 2. n=2 due to missing data; 3. n=1 due to missing data. Letters between the Farm Hub and Musgrave columns indicate significant differences between locations, where A indicates that mean is significantly larger than B at $p<0.05$.

microBIOMETER®

Biomass estimated by the microBIOMETER® were dissimilar to the laboratory tests of CFE and CFI biomass. Biomass estimated by the microBIOMETER® was higher compared to use of the CFE method (Tables 1 and 2) and lower compared to use of the CFI method (Table 2). For T1 (Table 1), the mean biomass based on the microBIOMETER® at Farm Hub (359.4 µg C g^{-1} soil) was almost twice that of the Musgrave (190.8 µg C g^{-1} soil) samples. The microBIOMETER® was the only soil health assay that produced higher mean values at the Farm Hub location compared to Musgrave for both T1 and T2 samples. Among the five cover crop treatments at T2 (Table 2), average biomass based on the microBIOMETER® ranged from 312.4 (CAN) to 384.3 µg C g^{-1} soil (HV X CR) at the Farm Hub, and 201.0 (CR) to 231.5 µg C g^{-1} soil (CAN) at Musgrave. The microBIOMETER® biomass estimates were significantly higher at the Farm Hub site, whereas, most other soil health metrics, including CFE biomass, Respiration, and active carbon were significantly higher at the Musgrave site.

CFE Microbial Biomass

CFE biomass resulted in overall lower biomass estimates than those of the microBIOMETER® assay (Tables 1 and 2). As with most of the other soil health assays, average CFE biomass was higher at the Musgrave site. This contrasts with the higher values at the Farm Hub measured using the microBIOMETER®. For the T1 measurements, Musgrave soils (198.5 µg C g^{-1} soil) had higher biomass than Farm Hub soils (83.0 µg C g^{-1} soil). At T2, average CFE biomass ranged from 84.7 (HV) to 170.7 µg C g^{-1} soil (CAN) at the Farm Hub, and 122.4 (Fallow) to 173.6 µg C g^{-1} soil (HV x CR) at Musgrave. All T2 treatments had higher CFE biomass at Musgrave, except for CAN and CR, where the Farm Hub values of those treatments were higher.

CFI Microbial Biomass

Microbial biomass measured by use of the CFI assay was higher overall than either the microBIOMETER® or CFE biomass assays. Although several data points are missing from the Musgrave location, Tables 2, A1, and A2 suggest that biomass was higher at Musgrave compared to the Farm Hub site. For T2, average CFI biomass ranged from 405.0 (Fallow) to 510.0 µg C g^{-1} soil (HV x CR) at the Farm Hub, and 608.0 (Fallow) to 708.5 (CR) µg C g^{-1} soil. There was no CFI biomass data for T1.

Soil Respiration

Soil respiration was lower in the T1 soil samples from the Farm Hub than at the Musgrave site, where respiration was approximately three times higher (0.161 mg CO_2 g^{-1} soil d^{-1}) than at the Farm Hub (0.051 mg CO_2 g^{-1} soil d^{-1}). Like T1 soils, mean respiration at Musgrave was also higher than at the Farm Hub at T2, which was 0.750 and 0.559 mg CO_2 g^{-1} soil d^{-1}, respectively. Respiration at T2 ranged from 0.63 (CR) to 0.81 mg CO_2 g^{-1} soil d^{-1} (HV) at Musgrave and 0.54 (CR) to 0.57 mg CO_2 g^{-1} soil day^{-1} (HV x CR) at the Farm Hub site.

Active Carbon

Musgrave soils had higher active carbon than soils at the Farm Hub in both the T1 and T2 soils which, like the respiration and soil protein assays, differed from the microBIOMETER® that had higher biomass at the Farm Hub. Mean active carbon at T1 for Musgrave (503.8 mg C kg^{-1} soil) was greater than at the Farm Hub site (355.2 mg C kg^{-1} soil). All T2 treatments for both locations had higher average carbon than their respective T1 values. T2 Musgrave ranged from 557.8 (Fallow) to 582.5 mg C kg^{-1} soil (CAN), and T2 Farm Hub ranged from 388.4 (Fallow) to 421.6

mg C kg^{-1} soil (CR). The Fallow treatment had the lowest average values among all T2 treatments at both locations.

ACE Soil Protein

Unlike the results of the other soil health assays and the microBIOMETER®, there was no location nor time that consistently contained higher soil protein. While mean soil protein for T1 Musgrave (4.67 mg amino N g^{-1} soil) was greater than at the Farm Hub (4.28 mg amino N g^{-1} soil), T2 treatments varied in which location had greater soil protein (Tables 1 and 2).T2 treatment average values ranged from 3.98 (Fallow) to 4.65 mg amino N g^{-1} soil (CR) at the Farm Hub, and 4.13 (CR) to 4.68 mg amino N g^{-1} soil (CAN) at Musgrave. Accordingly, not all T2 treatments had greater protein than T1 samples at both locations, which was the case for both the active carbon and soil respiration assays.

Relationships Between microBIOMETER® and Soil Health Metrics

To better understand how biomass data generated by use of the microBIOMETER® was related to other metrics of soil biological health, scatterplots and associated correlation analyses were employed. The strength of the relationship between biomass estimated by the microBIOMETER® and biomass estimated by the CFE is shown in Figure 5. Visual inspection indicates no significant relationship between these two metrics. In fact, a slight negative relationship between these two variables for both locations ($r = -0.05$, $n = 22$ at the Farm Hub and -0.19, $n = 23$ at Musgrave) is evident. An inverse relationship was not anticipated as the microBIOMETER® and CFE biomass assays ostensibly both measure SMB-C in µg C g^{-1} soil. The significant difference in between sites in both metrics is also evident in Figure 5.

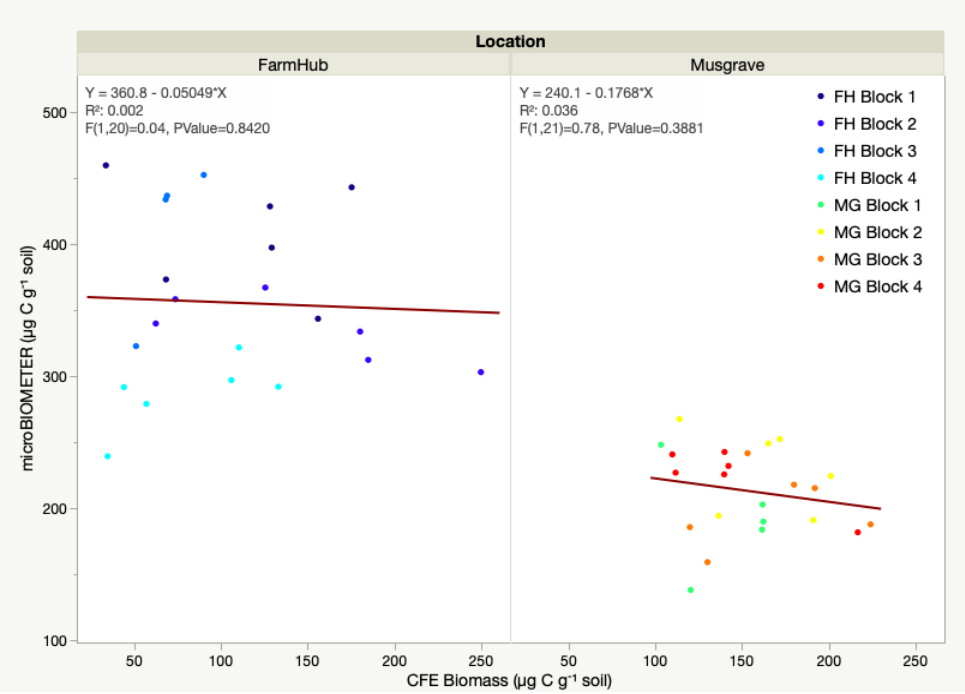

Figure 5. Relationship between biomass estimated by microBIOMETER® and CFE biomass.

The relationship between the microBIOMETER® and biomass estimated by the CFI assay is shown in Figure 6. As previously mentioned, CFI biomass was the last assay undertaken and there was insufficient soil to complete this assay for all samples. Thus, the correlation between these two variables could not be explored fully, particularly at the Musgrave location. Like the results for the CFE biomass, there was no apparent positive relationship between CFI biomass and the microBIOMETER®. The correlation coefficients were r = -0.08 (n =19) at the Farm Hub and r = -0.02 (n=7) at the Musgrave site.

The relationship between biomass measured using the microBIOMETER® and soil respiration at the two sites is shown in Figure 7. The slope of both lines of best fit are positive. The correlation coefficient was r = 0.16 at the Farm Hub but was r = 0.40 at the Musgrave site.

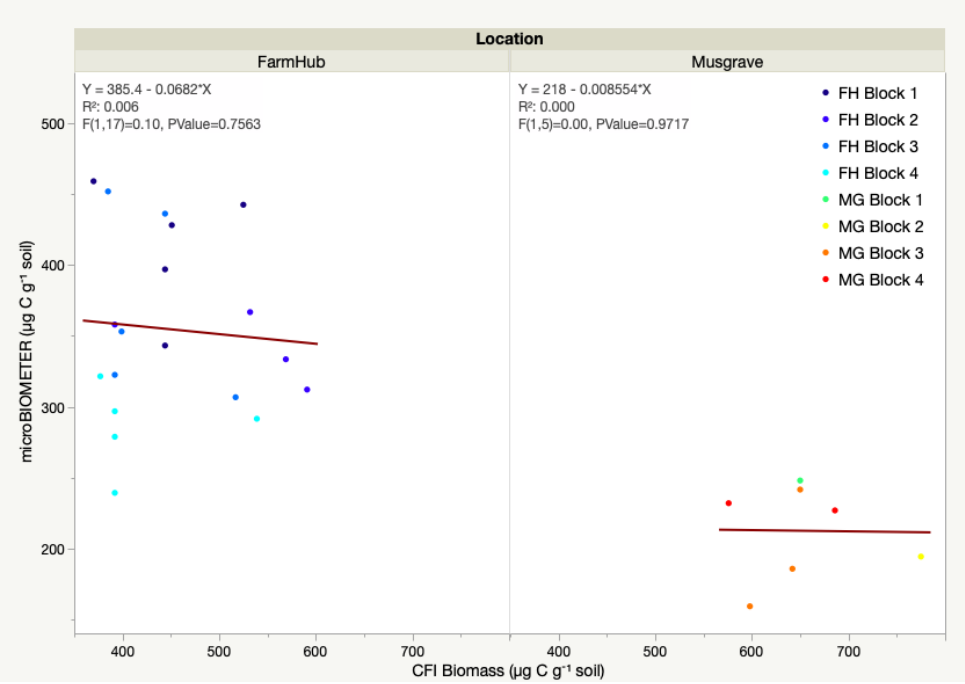

Figure 6. Relationship between biomass estimated by microBIOMETER® and CFI biomass.

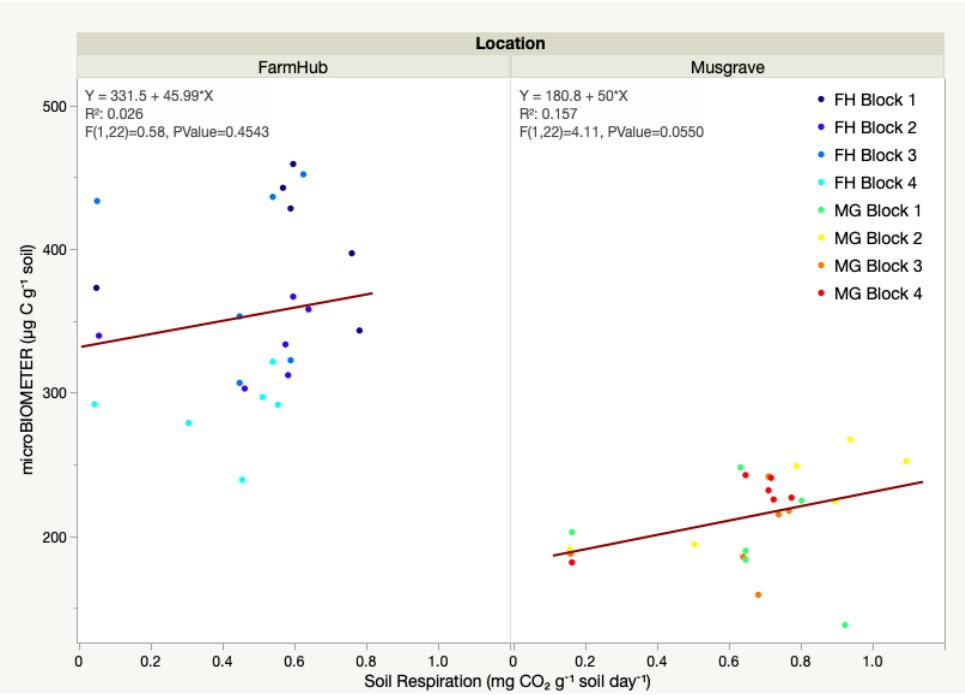

Figure 7. Relationship between biomass estimated by microBIOMETER® and soil respiration.

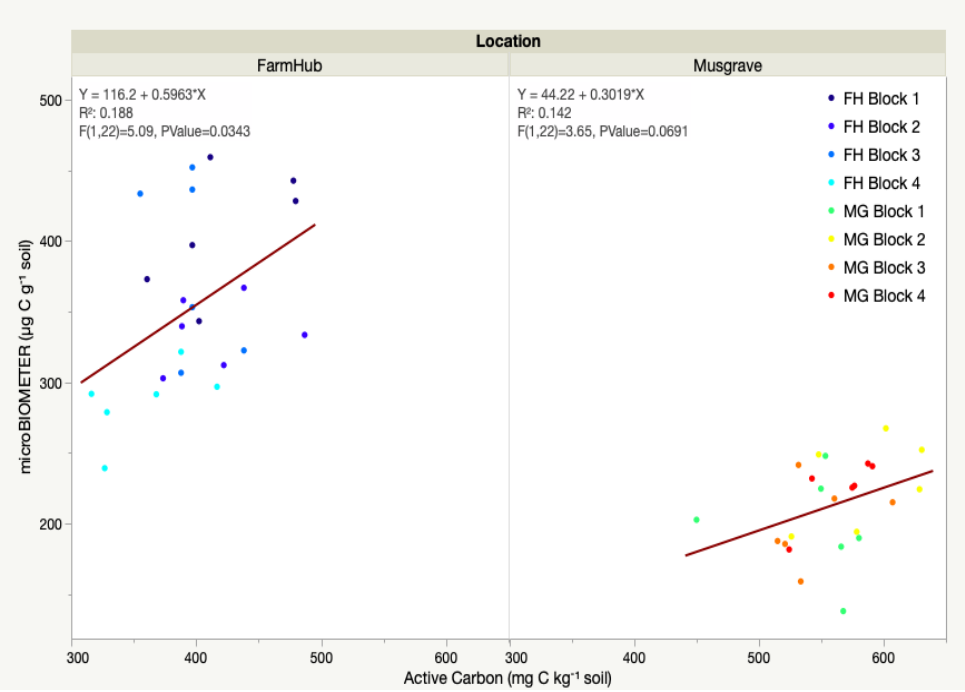

Figure 8. Relationship between biomass estimated by microBIOMETER® and active carbon.

The relationship between biomass measured by the microBIOMETER® and active carbon is shown in Figure 8. Correlation coefficients were positive at both locations, with r = 0.43 at the Farm Hub and r = 0.38 at the Musgrave site.

Figure 9 shows the relationship between biomass measured by the microBIOMETER® and ACE soil protein, where the correlation coefficients were positive at both locations, with r = 0.57 at the Farm Hub, but only r = 0.18 at the Musgrave site.

The strong differences between sites for all metrics can be seen clearly in Figures 5-9.

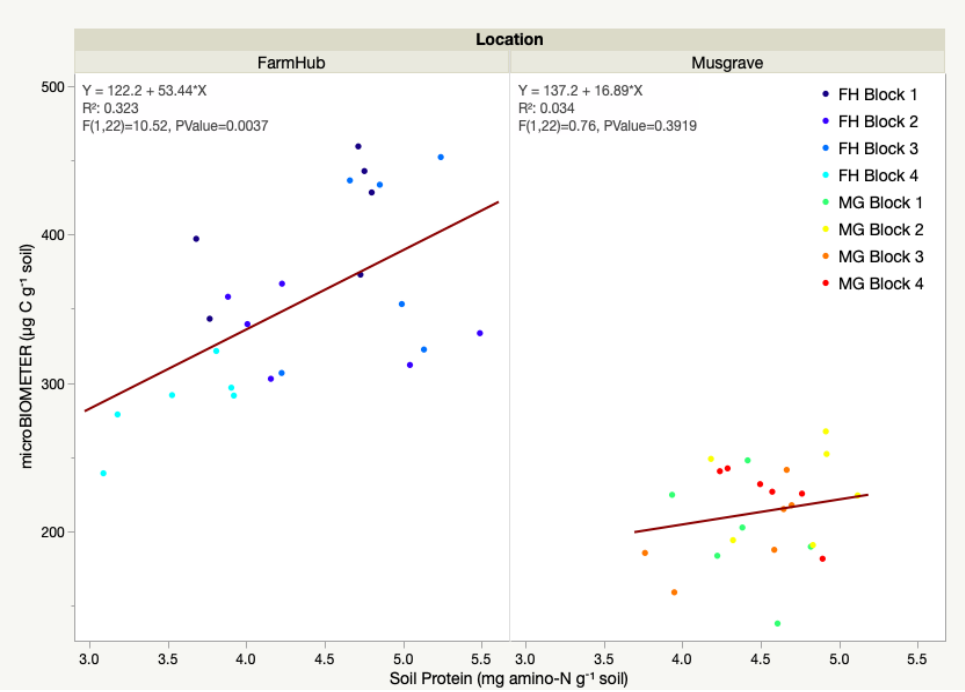

Figure 9. Relationship between biomass estimated by microBIOMETER® and ACE soil protein.

Relationships Between CFE Microbial Biomass and Soil Health Metrics

The relationship between CFE microbial biomass and CFI microbial biomass is shown in Figure 10. The correlation coefficient at the Farm Hub location was r = 0.65 (n = 19) and was r = -0.09 (n = 7) at the Musgrave site. Note that Figure 10 does not include T1 observations for either location, and lacks many observations at the Musgrave location as explained above.

The relationship between CFE microbial biomass and soil respiration is shown in Figure 11. The correlation coefficient was r = 0.24 (n = 22) at the Farm Hub. Whereas, an inverse relationship was observed at the Musgrave site, where the correlation coefficient was r = -0.44 (n = 23).

The relationship between CFE microbial biomass and active carbon is shown in Figure 12. The correlation coefficient was r = 0.39 (n = 22) at the Farm Hub and, as seen for respiration, was inversely related at the Musgrave site, where the correlation coefficient was r = -0.11 (n = 23).

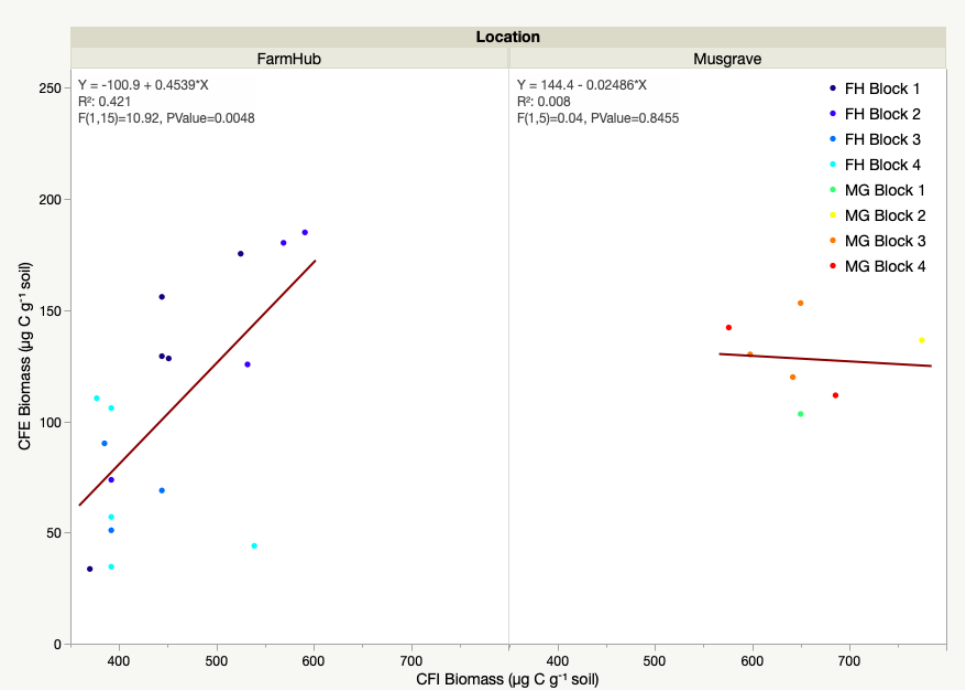

Figure 10. Relationship between biomass estimated by CFE and biomass estimated by CFI.

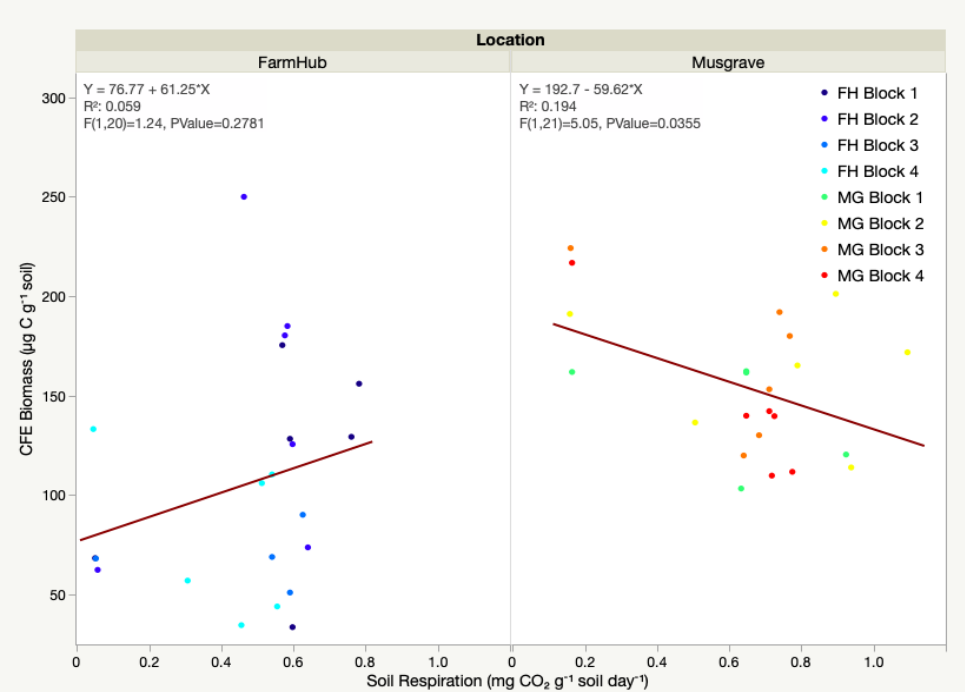

Figure 11. Relationship between biomass estimated by CFE and soil respiration.

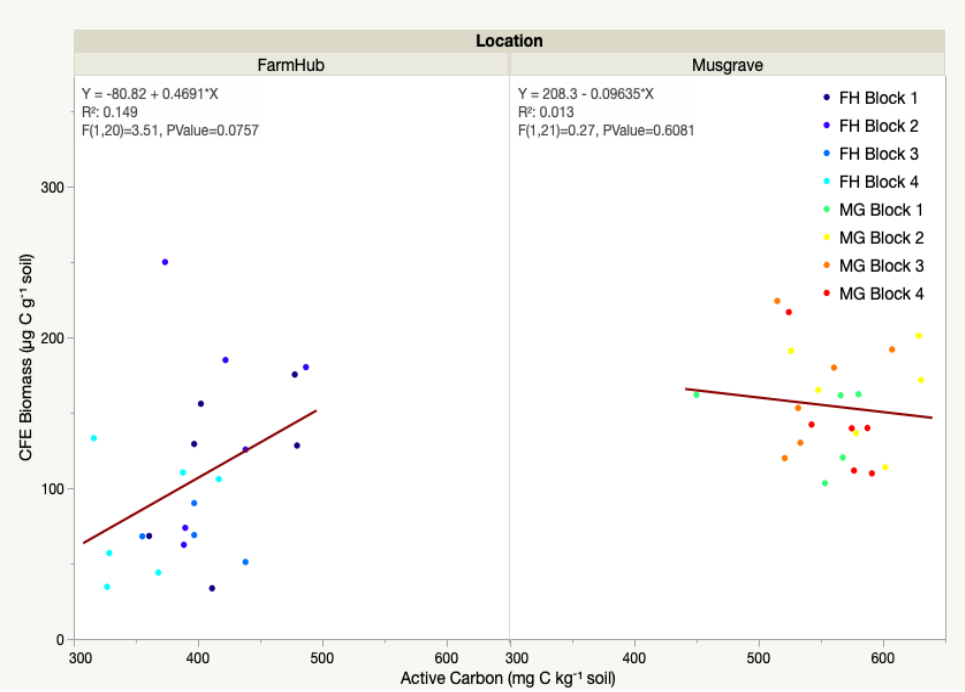

Figure 12. Relationship between biomass estimated by CFE and active carbon.

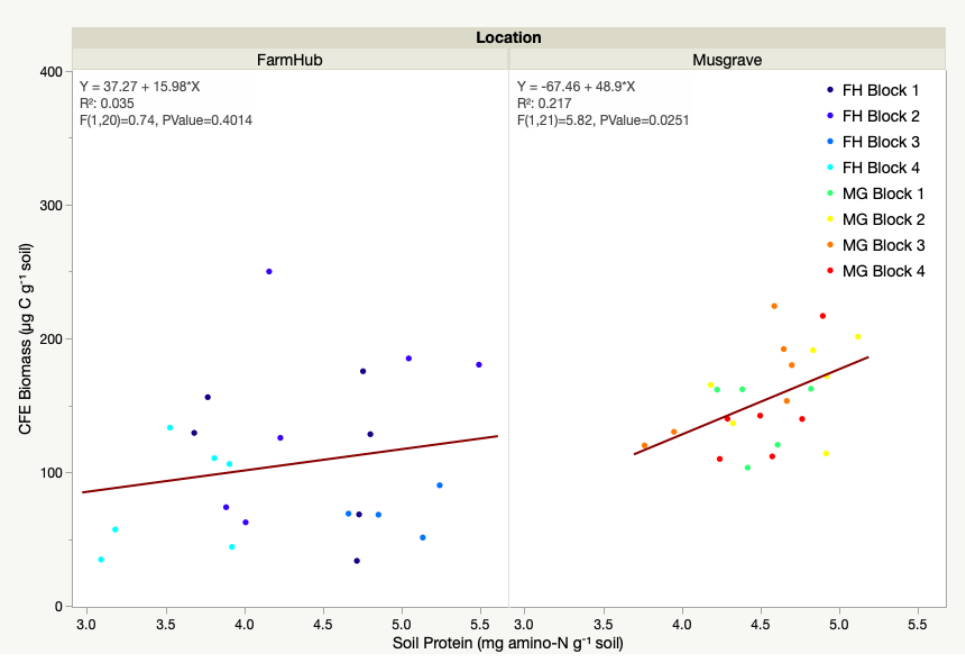

Figure 13. Relationship between biomass estimated by CFE and ACE soil protein.

The relationship between CFE biomass and soil protein is shown in Figure 13. The correlation coefficients were r = 0.19 (n=22) at the Farm Hub and r = 0.47 (n=23) at the Musgrave site.

Excluding the CFI assay which was lacking a substantial number of observations at the Musgrave location, soil protein was the only laboratory soil health assay that showed a positive relationship with CFE biomass at the Musgrave site.

Inferential Statistics

Tests of Correlations

Tables 1 and 2 list the Pearson correlation coefficients mentioned above together with the associated p-values under a null hypothesis that there is no correlation between any of the tested variables.

At the Farm Hub location, two assays were significantly correlated with the microBIOMETER®: active carbon ($r=0.4336$, $p=0.0343$) and soil protein ($r = 0.5687$, $p = 0.0037$). At the same location, a further two variables were significantly correlated with active carbon: soil respiration and soil protein. Note that all correlations that were significant showed a positive relationship between the variables.

At the Musgrave location, no soil health assays were significantly correlated to biomass estimated by the microBIOMETER®. At this site, only three correlations were significant. Active carbon was significantly correlated with respiration ($r = 0.7346$, $p < 0.0001$), CFE biomass was significantly related to soil protein ($r = 0.5080$, $p = 0.0158$), and CFE biomass was inversely related to soil respiration ($r = -0.4404$, $p = 0.0357$) at this location. Visual inspection of Figure 5 reveals four observations with low respiration. These four observations have high leverage and are driving this inverse relationship.

Table 3. Pearson correlations, count, and (p-value) for the Farm Hub location.

	micro BIOMETER	CFE Biomass	CFI Biomass	Respiration	Active Carbon	Soil Protein
CFE Biomass	- 0.0451 n=22 (0.8420)	1				
CFI Biomass	- 0.0763 n=19 (0.0763)	**0.6491** n=17 **(0.0048)**	1			
Respiration	0.1603 n=24 (0.4543)	0.2419 n=22 (0.2781)	0.1112 n=19 (0.6503)	1		
Active Carbon	**0.4336** n=24 **(0.0343)**	0.3864 n=22 (0.0757)	0.4390 n=19 (0.0601)	**0.5487** n=24 **(0.0055)**	1	
ACE Soil Protein	**0.5687** n=24 **(0.0037)**	0.1883 n=22 (0.4014)	0.3080 n=19 (0.1996)	0.1079 n=24 (0.6158)	**0.6400** n=24 **(0.0008)**	1

Notes: p-value is for the test with null hypothesis that the correlation is zero.

Table 4. Pearson correlations, count, and (p-value) for the Musgrave location.

	micro BIOMETER	CFE Biomass	CFI Biomass	Respiration	Active Carbon	Soil Protein
CFE Biomass	- 0.1889 n=23 (0.3881)	1				
CFI Biomass	- 0.0167 n=7 (0.9717)	- 0.0914 n=7 (0.8455)	1			
Respiration	0.3966 n=24 (0.0550)	**- 0.4404** n=23 **(0.0357)**	- 0.6082 n=7 (0.1473)	1		
Active Carbon	0.3773 n=24 (0.0691)	- 0.1129 n=23 (0.6081)	0.7142 n=7 (0.0714)	**0.7346** n=24 **(<0.0001)**	1	
ACE Soil Protein	0.1831 n=24 (0.3919)	**0.4658** n=23 **(0.0251)**	0.1576 n=7 (0.7357)	0.0439 n=24 (0.8387)	0.3822 n=24 (0.0653)	1

Notes: p-value is for the test with null hypothesis that the correlation is zero.

Whilst various correlations are significant at the two sites, the only significant correlation across both sites that between active carbon and respiration. At the Farm hub, the correlation coefficient was r = 0.5487, and at Musgrave it was r = 0.7346. Unfortunately, there were no correlations with the microBIOMETER® that were significant at both sites.

Multivariate Models of microBIOMETER®

The relationships between microBIOMETER® readings and the other soil health assays may have been influenced by location, treatment, and block. Multivariate linear models were used to assess the relationship between microBIOMETER® and each of the other assays while accounting for these effects. Table 4 lists the p-values and indication of significance for the tests of effects of these factors.

*Table 5. P-values for multivariate models of microBIOMETER® and each of the laboratory soil health assays. Explanatory variables are the treatment-location interaction (Trt*Loc), treatment, location, and the assay. Significant p-values at the 5% level are indicated by bold text.*

Model	Trt*Loc	Treatment	Location	Assay
microBIOMETER® by CFE	0.2870	0.7849	**0.0038**	0.3495
microBIOMETER® by CFI	-	0.0924	**0.0288**	0.4795
microBIOMETER® by Respiration	0.1359	0.6009	**0.0016**	0.2181
microBIOMETER® by Active Carbon	0.1940	0.6953	**< 0.0001**	0.0829
microBIOMETER® by Soil Protein	0.2491	0.6856	**0.0011**	**0.0453**

Note. The treatment-location interaction could not be fit in the model with CFI due to a lack in degrees of freedom.

In the model of microBIOMETER® and CFE, the treatment-location interaction was not significant (p = 0.2870). After removing the interaction, the treatment was also not significant (p = 0.7849). After removing treatment from the model, location was significant (p = 0.0038) and CFE was not (p = 0.3495). Figure A1 displays the average microBIOMETER® reading

predicted by this model. The difference between the locations is evident, as is the lack of relationship between the microBIOMETER® and CFE results.

In the model of microBIOMETER® with CFI, it was not possible to include the interaction between treatment and location due to insufficient degrees-of-freedom. This occurred in this model but not others because the CFI was missing 13 samples from the Musgrave location. After removing the interaction, the treatment was not significant ($p = 0.0924$). After removing treatment from the model, location was significant ($p = 0.0288$), but CFI was not ($p = 0.4795$). Figure A2 displays the average microBIOMETER® readings predicted by this model. Like the model with the CFE assay, there is a significant difference between the two locations in the CFI assay, as well as a lack of relationship between the microBIOMETER® and CFI.

In the model of microBIOMETER® with soil respiration, the treatment-location interaction was not significant ($p=0.1359$). After removing the interaction, the treatment was also not significant ($p = 0.6009$). After removing treatment from the model, location was significant ($p = 0.0016$) and respiration was not ($p = 0.2181$). Figure A3 displays the average microBIOMETER® reading predicted by this model with respiration. Again, the difference between the locations is evident, and the model shows a lack of relationship between the microBIOMETER® and soil respiration.

In the model of microBIOMETER® with active carbon, the treatment-location interaction was not significant ($p = 0.1940$). After removing the interaction, the treatment was also not significant ($p = 0.6953$). After removing treatment from the model, location was significant ($p < 0.0001$) and active carbon was not ($p = 0.0829$). Figure A4 displays the average microBIOMETER® reading predicted by this model with active carbon. Again, the difference

between the locations is evident, and the model shows a lack of relationship between the microBIOMETER® and soil respiration.

In the model of microBIOMETER® with soil protein, the treatment-location interaction was as not significant ($p = 0.2491$). After removing the interaction, the treatment was also not significant ($p = 0.6856$). After removing treatment from the model, location was significant ($p = 0.0011$) and, unlike the other soil health assays, the microBIOMETER® relationship with soil protein was significant ($p = 0.0453$). Figure A5 displays the average microBIOMETER® reading predicted by the model with soil protein. The difference between the locations is evident as well as a clear relationship with the soil protein assay.

Discussion

microBIOMETER® as a Soil Health Metric

In this paper, I report results from research on soil biological activity, specifically, the assessment of SMB-C using the microBIOMETER® assay. Using soil samples from two NYS cover crop trials, one at the Farm Hub and the other at the Musgrave site, results of SMB-C from the microBIOMETER® were compared to results from the established laboratory assays CFE and CFI microbial biomass, soil respiration, active carbon, and soil protein.

The intention of this experimental study was to benchmark the microBIOMETER® test as a soil biological health metric and to understand its usefulness to land managers for holistic SSNM and agroecosystem management. Soil health testing facilitates recommendations, such as tillage strategies and organic matter management, that go beyond the typical mineral fertilizer recommendations given by standard nutrient testing (Testen et al., 2018; Kihara et al., 2020). Additionally, easy-to-use soil tests that have simple and inexpensive protocols, such as the microBIOMETER®, can allow for expanded use by farmers in the Global North and South. The microBIOMETER® kit, for instance, is smaller than a laptop, takes about 30 minutes to complete, and does not have expensive, dangerous, or unstable reagents required by many of the laboratory soil biological tests. This allows farmers to better understand and integrate quantitative biological health monitoring with largely qualitative monitoring practices (Grossman, 2003; Barrios et al., 2006; Dawoe et al., 2012), while taking into account the unique biophysical and socioeconomic aspects of their agroecological environment largely ignored by top-down and TOT approaches (Testen et al., 2018; Stewart et al., 2020). There is a greater need for this type of mobile soil health testing for resource-poor farmers of the Global South, who

have less access to soil testing laboratories and extension advisors (Dimkpa et al., 2017; Stewart et al., 2020).

Although the microBIOMETER® does not appear to measure microbial biomass (Tables 3, 4, and 5), the results from this study still indicate that microBIOMETER® does correlate with some aspects of soil health. This was demonstrated by the significant, positive relationships found between the microBIOMETER® and active carbon and with soil protein at the Farm Hub (Table 3). The relationship between the microBIOMETER® and soil protein was additionally supported by the multivariate modeling that isolated its relationship to soil protein as an explanatory variable by accounting for any influences of the cover crop treatments and locations (Table 5 and Figures A1-A5) that affected the microBIOMETER® readings.

Based on my results, the microBIOMETER® should be used for soil health testing by farmers and land managers seeking to monitor their soils and supplement recommendations from standard nutrient testing. The relationship to highly labile C and N, as measured by active carbon and soil protein, respectively, means that farmers can likely use the microBIOMETER® to monitor readily available microbial substrates and potential N released from the organic fractions of their soils. The implications of the significant soil protein relationship with the microBIOMETER® may mean farmers can use it as tool to reduce expensive N inputs that also contribute to greenhouse gas emissions and climate change. Small-holder farmers of the South could use the microBIOMETER® to make such assessments. The portable and widely-used *SoilDoc* kit does include an on-site version of the active carbon assay, but requires a laboratory-trained technician (Dimkpa et al., 2017; Harou et al., 2018), whereas the microBIOMETER® is potentially usable by untrained farmers.

Effects of Cover Crop Treatments and Location on Soil Health Assays

There was a significant correlation between active carbon and soil respiration at both locations (Farm Hub, r = 0.74; Musgrave, r = 0.55). At the Farm Hub, active carbon was also significantly correlated to soil protein (r=0.64) and the microBIOMETER® (r = 0.43). As anticipated, there was a significant correlation and CFE and CFI at the Farm Hub (r = 0.65), which both measure SMB-C.

The average values of the soil health assays indicated statistically significant differences in soil health between the two locations for all assays except for soil protein (Tables 1 and 2) that was further evidenced by the multivariate models that demonstrated location as the main influence on the microBIOMETER® readings (Table 5 and Figures A1-A5).

Differences in soil texture between the two sites may help to explain the higher laboratory soil health values observed for the Musgrave location. Amsili et al. (2021) found that soil protein and active carbon were 84% and 24% higher in coarse-textured compared to fine-textured soils, respectively, and found these two assays were inversely related to clay content (r= -0.49 and r = -0.21, respectively). Although both locations' soils are generally classified as medium-textured, the loam soil at Musgrave is slightly coarser than the silt loam at the Farm Hub site. Similarly, Franzluebbers et al. (1996) found that soil respiration was often higher in coarser-textured soils. While likely to have a strong influence, textural analysis would be needed to confirm the specific proportions of particle sizes, thus differences in soil health due to higher content of colloidal fractions cannot be confirmed.

Cropping system and management, however, are also factors in outcomes of soil health indicators (Nunes et al., 2018; Amsili et al., 2021). The soil at the Farm Hub, while in use as

research plots for organic no-till during this study, has historically been under cultivation for commercial production. Conversely, the Musgrave location has been exclusively a research site for agricultural experimentation for many decades. As such, we can expect a history of more frequent soil disturbance and higher nutrient export at the Farm Hub. Conversely, lower values might have been expected at the Musgrave location due to the use of moldboard plowing for seedbed preparation of the experimental plots. High-speed discing was used at the Farm Hub, which involves less soil inversion and overall disturbance.

In comparing different physical management systems, respiration was found to be lower where moldboard plowing was used compared to discing (Roper et al., 2017), which created less soil inversion and overall disturbance. Similarly, active carbon and soil protein were also higher in chisel and discing systems compared to moldboard plowing (van Es & Karlen, 2019). Amsili et al. (2021), however, found that soil texture had a greater effect than cropping system or management in soil health indicator outcomes, which supports the interpretation that slightly higher values in soil health metrics observed at the Musgrave site were due to a coarser-texture soil.

One confounding issue in the results was that the microBIOMETER® averages were higher at the Farm Hub than at Musgrave, while the CFE, CFI, active carbon, and respiration assays were all higher at the Musgrave site. The results, however, demonstrated relatively low soil health at both locations overall, compared to results reported in other studies (see Roper et al., 2017; Caudle et al., 2020; Amsili et al., 2021). Therefore, a study with similar methods, but using samples of both low and high SMB-C and soil health, may be warranted to examine the capacity of the microBIOMETER® assay to detect such differences. The use of soils from varying

textural classes (i.e., clay loam vs. sandy loam), production systems, and tillage intensities should also be studied for the correlation of soil health indicators with the microBIOMETER®.

Relationships between Laboratory Soil Health Tests

In addition to the comparison with microBIOMETER®, the correlation analysis was used to benchmark the relationship of the soil health assays to each other, essentially to establish that soil health was in fact being measured. There have been more studies in the recent decade that have similarly established the relationship between the soil health assays used here.

Active carbon has been a widely tested and used as a soil health metric due to the simplicity of the protocol, the ability to do the assay directly in the field and demonstrated sensitivity to management changes (Moebius-Clune et al., 2016; Bongiorno et al., 2019). Compared to the Farm Hub correlation ($r = 0.64$, Table 3) , Caudle et al. (2020) found a slightly stronger, but significant and comparable relationship between soil protein and active carbon ($r = 0.74$) in a sandy loam soil, and Bongiorno et al. (2019) found active carbon to be significantly related to respiration ($r = 0.46$). Although I found stronger correlations between other soil health metrics in the literature, I could not find any report of a stronger, positive relationship between active carbon and respiration than what was observed at the Farm Hub site. A possible reason why there are no reports of stronger correlations may be because active carbon differs from soil respiration in that active C measures highly labile and readily available quantities of C, while respiration is a measurement of the microbial activity that results from this available C (Roper et al., 2017).

Although active carbon was significantly correlated with the microBIOMETER® assay at the Farm Hub, it did not meet the 5% significance level when accounting for location and treatment

in the multivariate linear model. Active carbon, however, was the only assay of the five laboratory soil health tests to be positively correlated, with coefficients ranging from $r = 0.37–0.73$, with $p < 0.08$. Meanwhile, the other laboratory soil health assays that were not significant generally had weaker correlations ($r < 0.30$) and considerably higher p-values. Thus, the correlation results here support the idea of active carbon beings a "leading indicator" of soil health and management evaluation (Moebius-Clune et al., 2016).

The significant correlation of soil protein and CFE at the Musgrave and not at the Farm Hub location is surprising, considering the Musgrave location resulted in overall weaker and fewer significant relationships. Decker (2021) found a similar correlation of soil protein and SMB-C ($r= 0.40$), but used the CFI method, which did not correlate with soil protein in the assays performed here. I could not find these assays used together in a correlation analysis in the literature, so the relationship is still unclear. Nonetheless, after available C, microbial biomass accretion is most limited by available soil N, so the potentially available organic N (as measured by soil protein) can influence C fluxes to the SMB-C pool (Blagodatsky & Richter, 1998; Chen et al., 2014).

A few correlation pairs resulted in inverse relationships between the soil health tests, which was unexpected. Aside from marginally negative correlations with microBIOMETER®, which were found at both locations, the only negative correlations with laboratory soil health tests were in the Musgrave soils. Soil respiration, as a burst of CO_2 following re-wetting of dried soil as used in this study, has been well correlated to SMB-C (Franzluebbers, 2018), so the negative correlation found here is surprising. However, for basal respiration assays, in which soils are incubated for longer periods of time (2-3 weeks), weak positive or sometimes negative

relationships with SMB-C have been observed (Wang et al., 2003; Traoré et al., 2007). Respiration, whether as a basal measurement or as a CO_2 burst, depends on substrate availability rather than the size of the SMB pool under favorable conditions of moisture and temperature (Wang et al., 2003). The makeup of the microbial community may have been dominated by certain microbes with a high metabolic quotient (qCO_2), which is the amount of basal respiration per unit of microbial biomass (Insam & Haselwandter, 1989). The moldboard plowing at Musgrave could have influenced the establishment of a microbial community with higher qCO_2, meaning the higher C substrate needs to produce CO_2 in respiration for a given biomass, which has been previously demonstrated (Heinze et al., 2010). Although still unclear, a higher qCO_2 may have affected the soil respiration burst of CO_2 at Musgrave in this study.

CFE and CFI Soil Microbial Biomass Carbon

The CFE and CFI biomass assays, while having a moderately strong and significant correlation, were notably less well-correlated than what has been found in previous reports. Both Vance et al. (1987) and Wu et al. (1990) compared the CFE and CFI methods and both found very strong correlations of $r = 0.99$, much stronger than the correlation in this study, $r = 0.65$.

A few distinctions should be noted in the CFE methods used by Wu et al. (1990) and Vance et al. (1987) compared to the methods employed here which may help explain the discrepancies in our CFE-CFI correlation. First, both published studies used a 0.5 M K_2SO_4 extracting solution for the CFE, a concentration that has traditionally been used and a tenfold greater concentration than the 0.05 M extractant in this study. Using the higher concentration of extractant is a problem for contemporary studies employing the CFE method due to the difficulty the high salt concentration imposes on modern analytical machinery for total organic carbon (see Qian & Mopper, 1996).

Several studies have examined use of various K_2SO_4 concentrations lower than the original 0.5 M concentration used by Vance et al. (1987), finding differences in solubility of organic compounds and microbial carbon based on different soil properties, such as pH (Haney et al., 2001) or content of reactive and stable carbon compounds (Durenkamp et al., 2010). Outside of these specific factors though, other research has suggested there may not be a substantial difference between the extraction efficiency of the 0.05 and 0.5 M concentrations (Makarov et al., 2013). Ultimately, the literature is unclear as to the effect of lower extractant concentrations on SMB-C results, and researchers should continue to examine and resolve this issue.

Apart from the extractant concentration differences, the CFE method I used in this study was the direct chloroform method proposed by Gregorich et al. (1990). In the original method by Vance et al. (1987), soil is exposed to chloroform vapors under vacuum for 24 h to lyse microbial cells, and the K_2SO_4 extractant is added to the samples after the vapors are removed. The direct method combines these two steps by directly adding chloroform as a liquid to the K_2SO_4 with the soil to achieve the same effect after shaking the solutions for at least half an hour. Thus, the direct method is quicker and reduces the complications and equipment needs of fumigating the soils under vacuum. Although Setia et al. (2012) confirmed a strong correlation ($r = 0.87$) between the direct and original CFE methods, few studies have used the direct method (Witt et al., 2000; Fierer & Schimel, 2003). Furthermore, I could not find any publication that has used the direct method in combination with a 0.05 M extractant. Jordan & Beare (1991) compared the direct CFE method with CFI and found strong correlation ($r=0.92$), but only using the original 0.5 M extractant concentration.

Although the microBIOMETER® did not appear to measure SMB-C by correlation to the CFE or CFI assays, the relationship between these three assays should still be investigated further. Particularly, the use of the appropriate CFE methods using the direct or traditional method should be established before comparison with the microBIOMETER® and CFI.

Conclusions

Soil health testing should be considered more frequently for monitoring agronomic and ecosystem services in both the Global North and Global South. Site-specific nutrient and soil health management is a great need, particularly for resource-poor farmers of the Global South where a multitude of biophysical and socioeconomic factors limit soil testing traditionally accomplished in a laboratory. Here, I used the most common laboratory assays of soil biological health to benchmark the microBIOMETER® mobile soil health test, which is marketed as an affordable and easy-to-use test specifically for measuring soil microbial biomass carbon.

The microBIOMETER®, however, did not appear to measure soil microbial biomass carbon as there was no significant relationship with either the CFE or CFI assays of SMB-C. Rather, the microBIOMETER® had a stronger relationship to measures of soil protein and active carbon. Therefore, while there was no evidence that the microBIOMETER® measures SMB-C, the results in this study suggest that the microBIOMETER® does measure some aspect of soil health. Soil protein is a soil health assay that can be used in place of potentially mineralizable nitrogen as a measure of how much nitrogen might be made available from organic matter to support plant growth during a growing season. Active carbon is a widely used soil health test that measures the organic carbon fraction of total organic matter that is readily available for microbial consumption. It is also considered a leading indicator and particularly sensitive to management changes, while soil protein quantifies the soil's ability to provide the most limiting nutrient to plant growth, nitrogen. Based on the relationships established with these two assays, the microBIOMETER® should be used to monitor soil health in both the North and South.

Although positive relationships between the laboratory soil health metrics were observed, some of the correlations between the tests resulted in unexpectedly weak relationships, particularly at the Musgrave location. The cover crop treatments in this study, in place for just a single season, did not influence the soil health results or the overall assessment of soil health at the Musgrave and Farm Hub locations. Additionally, the correlation between the CFE and CFI assays, while moderately strong, was weaker than what has been established by previous studies, possibly due to lower molarity of the extractant used in the CFE assay. Future research should be conducted to benchmark the microBIOMETER® by using samples with a larger variation in soil health and microbial biomass.

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APPENDIX

Table A1. Values of soil health assays and microBIOMETER® at the Farm Hub by treatment and block.

Treatment	Block 1	Block 2	Block 3	Block 4
		microBIOMETER® (µg C g^{-1} soil)		
Tilled (T1)	373	340	433	292
Fallow (T2)	397	358	323	279
CAN (T2)	343	303	307	297
CR (T2)	428	312	353	322
HV (T2)	459	334	452	239
HV x CR (T2)	443	367	436	292
		CFE Microbial Biomass (µg C g^{-1} soil)		
Tilled (T1)	68	62	68	133
Fallow (T2)	129	74	51	57
CAN (T2)	156	250	.	106
CR (T2)	128	185	.	110
HV (T2)	34	180	90	35
HV x CR (T2)	175	126	69	44
		CFI Microbial Biomass (µg C g^{-1} soil)		
Tilled (T1)	.	.	.	.
Fallow (T2)	444	392	392	392
CAN (T2)	444	.	517	392
CR (T2)	451	591	399	377
HV (T2)	370	569	385	392
HV x CR (T2)	525	532	444	539
		Soil Respiration (mg CO_2 g^{-1} soil day^{-1})		
Tilled (T1)	0.050	0.057	0.052	0.045
Fallow (T2)	0.760	0.640	0.590	0.306
CAN (T2)	0.782	0.462	0.448	0.512
CR (T2)	0.590	0.583	0.448	0.541
HV (T2)	0.597	0.576	0.626	0.455
HV x CR (T2)	0.569	0.597	0.541	0.555
		Active Carbon (mg C kg^{-1} soil)		
Tilled (T1)	360.7	388.6	355.2	316.2
Fallow (T2)	396.9	389.7	438.2	328.6
CAN (T2)	402.3	373.5	387.9	416.7
CR (T2)	479.6	422.1	396.9	387.9
HV (T2)	411.3	486.8	396.9	326.8
HV x CR (T2)	477.8	438.2	396.9	368.1
		ACE Soil Protein (mg amino N g^{-1} soil)		
Tilled (T1)	4.73	4.01	4.85	3.53
Fallow (T2)	3.68	3.88	5.13	3.18
CAN (T2)	3.77	4.16	4.23	3.91
CR (T2)	4.80	5.04	4.99	3.81
HV (T2)	4.71	5.49	5.24	3.09
HV x CR (T2)	4.75	4.23	4.66	3.92

Table A2. Values of soil health assays and microBIOMETER® at Musgrave by treatment and block.

Test and Treatment	Block 1	Block 2	Block 3	Block 4
		microBIOMETER® (µg C g^{-1} soil)		
Tilled (T1)	203	191	188	182
Fallow (T2)	248	267	159	232
CAN (T2)	190	252	242	243
CR (T2)	184	194	186	241
HV (T2)	138	249	215	227
HV x CR (T2)	225	224	218	226
		CFE Microbial Biomass (µg C g^{-1} soil)		
Tilled (T1)	162	191	224	217
Fallow (T2)	103	114	130	142
CAN (T2)	162	172	153	140
CR (T2)	162	137	120	110
HV (T2)	120	165	192	112
HV x CR (T2)	.	201	180	140
		CFI Microbial Biomass (µg C g^{-1} soil)		
Tilled (T1)	.	.	.	.
Fallow (T2)	650	.	598	576
CAN (T2)	.	.	650	.
CR (T2)	.	775	642	.
HV (T2)	.	.	.	686
HV x CR (T2)	.	.	.	.
		Soil Respiration (mg CO_2 g^{-1} soil day^{-1})		
Tilled (T1)	0.16	0.16	0.16	0.16
Fallow (T2)	0.63	0.94	0.68	0.71
CAN (T2)	0.65	1.09	0.71	0.65
CR (T2)	0.65	0.51	0.64	0.72
HV (T2)	0.92	0.79	0.74	0.77
HV x CR (T2)	0.80	0.90	0.77	0.72
		Active Carbon (mg C kg^{-1} soil)		
Tilled (T1)	449.9	526.0	514.9	524.2
Fallow (T2)	553.3	601.8	533.5	542.5
CAN (T2)	580.2	630.6	531.7	587.4
CR (T2)	565.9	578.4	520.9	591.0
HV (T2)	567.6	547.9	607.2	576.6
HV x CR (T2)	549.7	628.8	560.5	574.8
		ACE Soil Protein (mg amino N g^{-1} soil)		
Tilled (T1)	4.38	4.83	4.59	4.89
Fallow (T2)	4.42	4.91	3.95	4.50
CAN (T2)	4.82	4.92	4.67	4.29
CR (T2)	4.22	4.32	3.76	4.24
HV (T2)	4.61	4.18	4.65	4.57
HV x CR (T2)	3.94	5.12	4.70	4.76

Table A3. T1 ANOVA tables: test of soil health assay by location and treatment, including the interaction treatment-location. Statistically significant effects are indicated by a bolded p-value.

Response Variable	Explanatory Variable	Sum of Squares	DF	F-Statistic	P-Value
microBIOMETER®	Location	56902.5	1	31.46	**0.0014**
CFE Microbial Biomass	Location	26680.5	1	27.73	**0.0019**
Soil Respiration	Location	0.024	1	1450.72	**<0.0001**
Active Carbon	Location	44149.1	1	40.11	**0.0007**
ACE Soil Protein	Location	0.313	1	1.41	0.2802

Note. CFI was not included because no T1 samples were tested for CFI.

Table A4. T2 ANOVA tables: test of soil health assay by location and treatment, including the interaction treatment-location. Statistically significant effects are indicated by a bolded p-value.

Response Variable	Explanatory Variables	Sum of Squares	DF	F-Statistic	P-Value
microBIOMETER	Location	180136.7	1	64.61	**<0.0001**
	Treatment	4792.5	4	0.43	0.7860
	Trt*Loc	10523.2	4	0.94	0.4524
CFE Microbial Biomass	Location	8658.6	1	4.54	**0.0423**
	Treatment	17336.2	4	2.27	0.0874
	Trt*Loc	11224.2	4	1.47	0.2382
CFI Microbial Biomass*	Location	235938.9	1	55.56	**<0.0001**
	Treatment	34409.5	4	2.03	0.1294
Soil Respiration	Location	0.363	1	23.26	**<0.0001**
	Treatment	0.054	4	0.86	0.4982
	Trt*Loc	0.034	4	0.54	0.7065
Active Carbon	Location	273439.3	1	170.49	**<0.0001**
	Treatment	3016.5	4	0.47	0.7571
	Trt*Loc	2189.2	4	0.34	0.8479
ACE Soil Protein	Location	0.206	1	0.69	0.4118
	Treatment	0.643	4	0.54	0.7064
	Trt*Loc	1.809	4	1.52	0.2208

*Interaction could not be fit due to a lack of degrees of freedom.

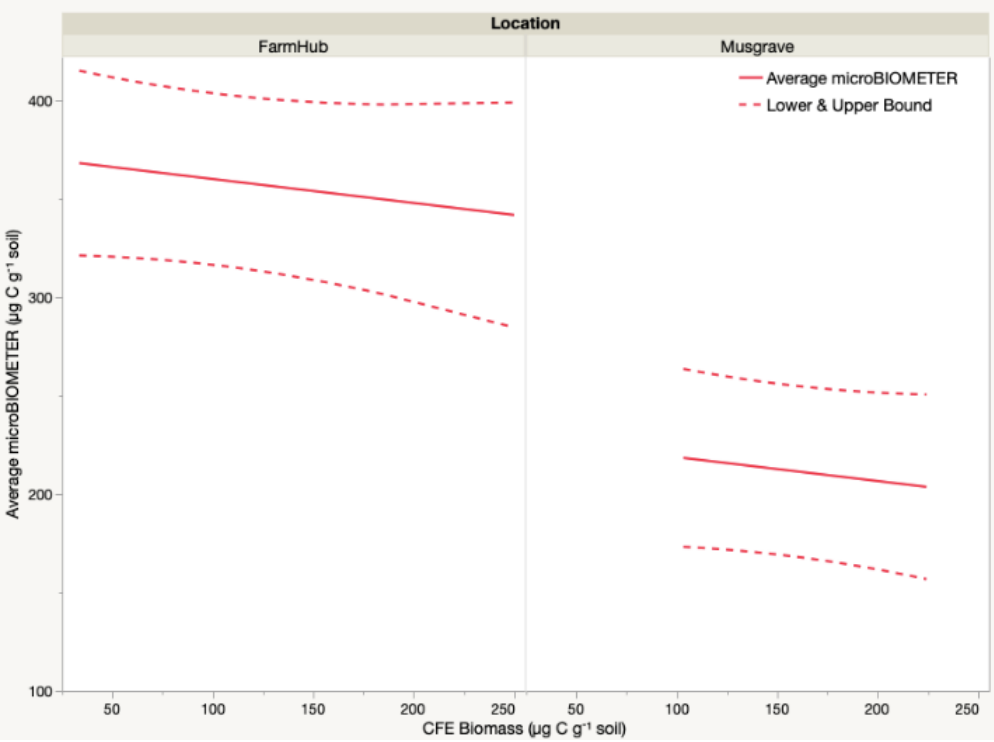

Figure A1. Predicted microBIOMETER® by CFE biomass and location with a 95% confidence interval.

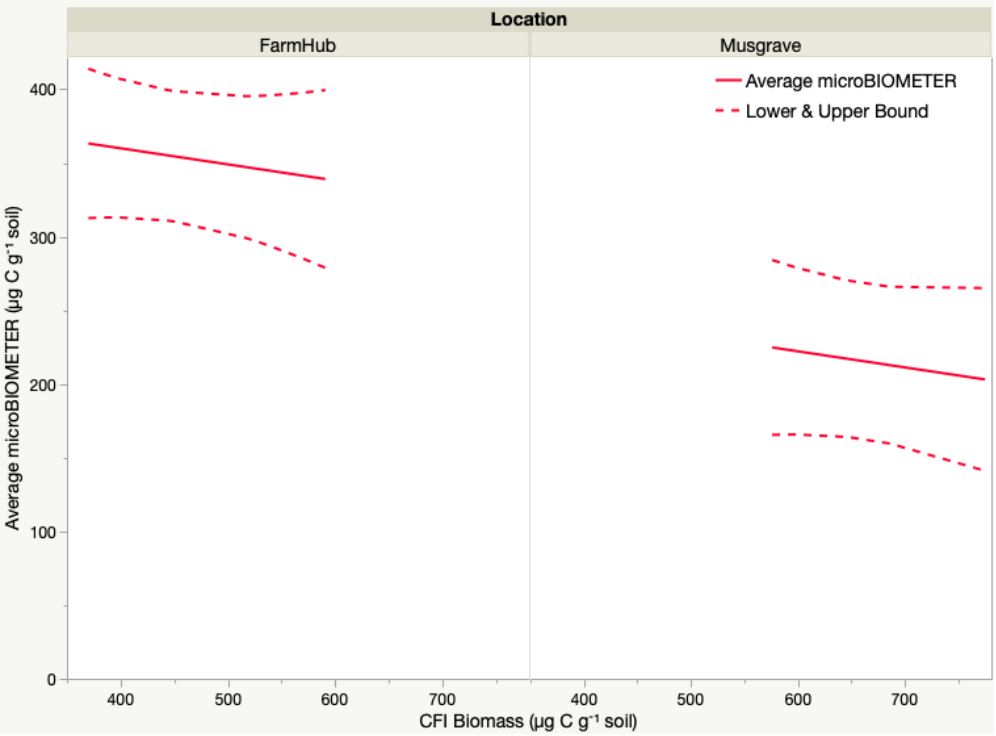

Figure A2. Predicted microBIOMETER® by CFI biomass and location with a 95% confidence interval.

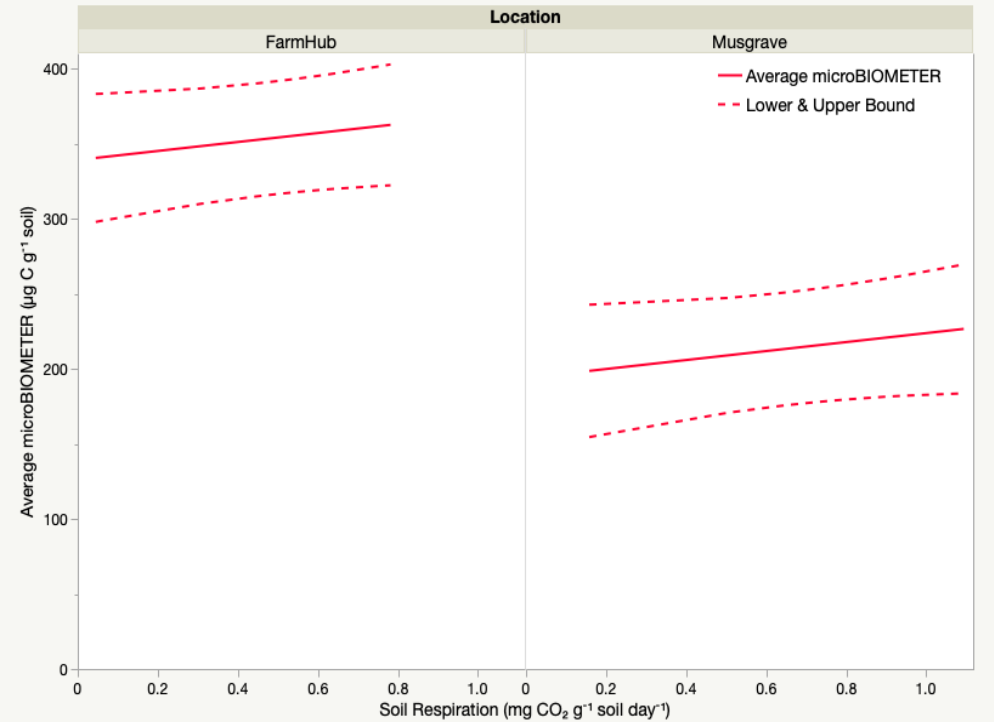

Figure A3.Predicted microBIOMETER® by soil respiration and location with a 95% confidence interval.

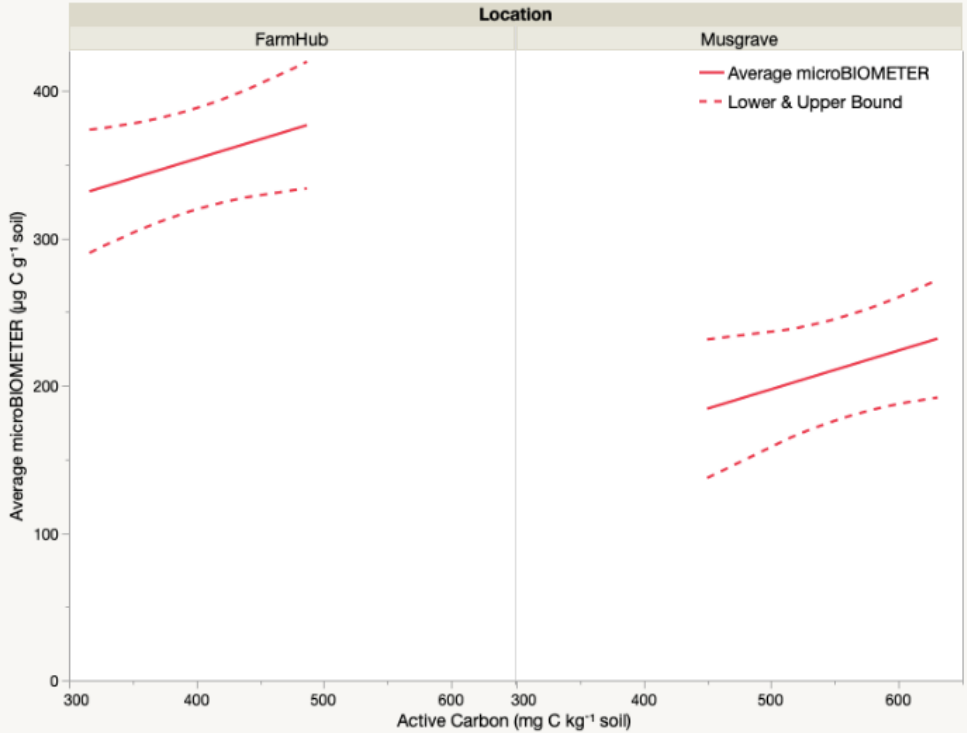

Figure A4. Predicted microBIOMETER® by active carbon and location with a 95% confidence interval.

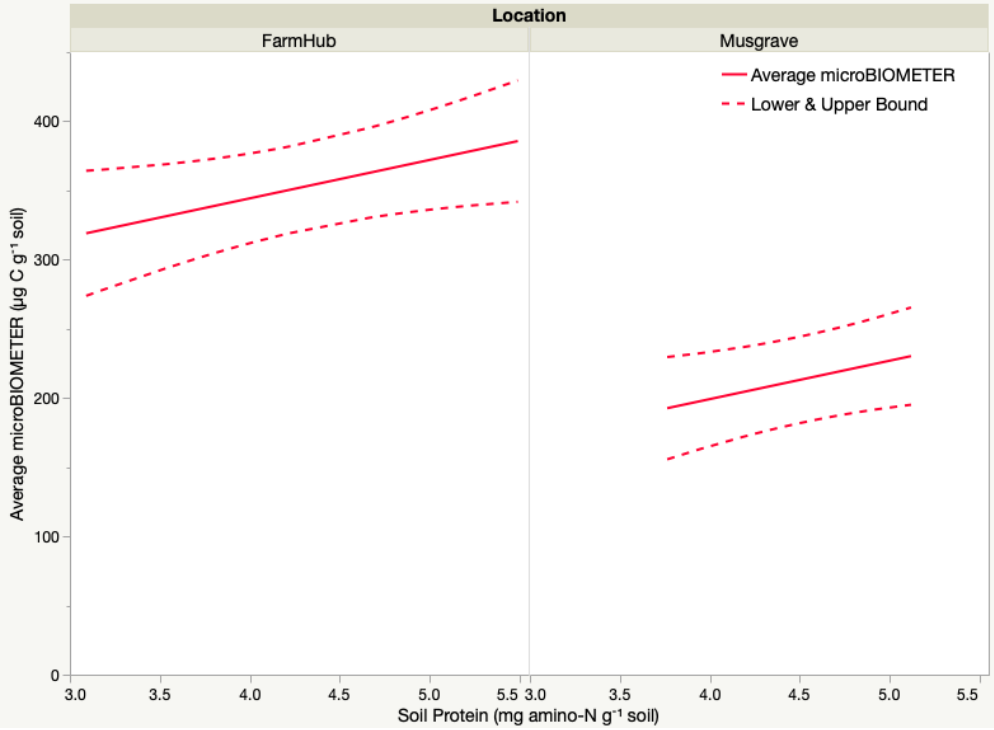

Figure A5. Predicted microBIOMETER® by soil protein and location with a 95% confidence interval.