

Living Soil for a Sustainable Future:
Cover Crop Effects on Soil Health and Productivity

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ABSTRACT

Agricultural land management practices impact the physical, chemical, and biological characteristics of soil, including the structure of the community of microorganisms present in the soil. The community of soil microorganisms, in turn, directly influences processes such as nutrient cycling and water infiltration and retention, which shape the long-term fertility and productivity of an agricultural landscape. The purpose of this study was to examine the effects of cover cropping on the soil biological and chemical features that contribute to soil fertility. The study looked at two summer cover crops – cowpea (*Vigna unguiculata*) and sorghum-sudangrass (*Sorghum xdrummondii*) in comparison with no-cover control – and their effects on soil respiration, soil organic matter and nitrogen availability, and lettuce production. Using soil samples taken from the in-field experiment, a parallel laboratory aerobic incubation study was conducted to examine the effects of the cover crop treatments on the transformation of nitrogen over five weeks. Both cover crops significantly increased soil organic matter, total organic carbon, and extractable potassium and magnesium by over 17% compared to the no-cover crop control. Cowpea significantly increased extractable soil nitrate and respiration rate compared to the no-cover control. Cowpea also resulted in the largest lettuce crop production as measured by both fresh weight and leaf area, but not different from the no-cover control. Sorghum-sudangrass decreased extractable soil nitrate concentration as well as lettuce fresh weight and leaf area compared to the control and cowpea. In conclusion, the study found that sorghum-sudangrass and cowpeas both increased soil organic matter, but only cowpea increased extractable inorganic N and lettuce production for sandy loam soils in the Mid-Atlantic and should be considered for these vegetable cropping systems.

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I. INTRODUCTION

A. Background and Setting

Soil microorganisms are the Earth's original nutrient managers. They have been cycling nutrients through the plant-soil-atmosphere interface for hundreds of millions, if not billions, of years and have evolved highly efficient ways of doing so. The transition to intensive nutrient management and the use of vast amounts of both inorganic and organic fertilizers that occurred as part of the "Green Revolution" overburdened biological nutrient cycling processes and resulted in nutrient overloading and loss of nutrients through runoff and leaching (Novotny et al., 2010). The effects of nutrient runoff are now seen across the world in polluted groundwater systems, eutrophied waters, and aquatic "dead zones." Closer to home, those effects have been seen in the degradation of the Chesapeake Bay watershed due, in part, to agricultural runoff (Pionke et al., 2000). All the while, the decoupling of these biological cycles has left farmers at a loss since they have not been able to capitalize on natural biological processes that may help to decrease input costs and increase yields.

Beyond nutrient cycling, soil microorganisms contribute to disease suppression in soils via competitive and antagonistic interactions in the rhizosphere (Alabouvette et al., 2004). In this sense, a healthy soil with a high diversity of microorganisms would be expected to suppress pathogens more effectively than that of a more homogenous soil due to the competitive interactions taking place between beneficial soil microorganisms and those that are pathogenic to crops. Improving soil health through cover crops that enhance soil microbial activity is expected to reduce chemical inputs such as fertilizers, pesticides, and fungicides and therefore improve environmental sustainability.

This project examines the impacts of two types of cover crops on soil biological and chemical characteristics (soil respiration, fertility, and nitrogen availability) and crop yield and seeks to shed light on practices that have the potential to enhance biological nutrient cycling and other key processes that improve soil health and productivity and increase environmental and economic sustainability. The results of this study could be

used to influence further research into the benefits of cover crops as they relate to soil health, agricultural production, and the structure and functioning of the soil microbiome.

B. Statement of the Problem

In the face of increasingly common environmental issues associated with nutrient runoff and soil erosion from agricultural production, growers across the Southern region are adopting Nutrient Management Plans (NMPs) to decrease nutrient losses and improve water quality in surrounding watersheds (Beegle, 2013). For growers dealing with high leaching potentials in the sandy alluvial plain of the East Coast, the desire to minimize nutrient losses and increase on-farm nutrient cycling and retention is of paramount importance. Much of the research and development into NMPs focused on the impact of physical soil characteristics such as slope, climate, and texture on nutrient dynamics and has essentially ignored the central role that microorganisms play in the cycling and retention of nutrients on farms. In the last decade or so, however, research has focused more on ecological approaches to on-farm nutrient management, utilizing practices such as cover cropping to keep sediments, and, thus, the nutrients bound to them, on the farm and out of watersheds. When combined with a better understanding of how microorganisms regulate the flow of nutrients through a farming system, these practices have the potential to significantly improve the management of nutrients and other resources on agricultural landscapes.

Over the last several years, research demonstrated the vital role that soil microorganisms play in the functioning of agricultural systems (Bhatia, 2008; Brussard, de Ruiter, & Brown, 2007). The living organisms present in the soil, characterized by bacteria, fungi, and other microorganisms, are key regulators of nutrient cycling, water infiltration and retention, and so many other processes in a healthy, living soil. As techniques have become available for assessing the structure and composition of the soil microbial community, research has demonstrated the significance of soil biodiversity for agricultural sustainability (Brussard, de Ruiter, & Brown, 2007). Studies show that agroecosystems high in biodiversity may increase soil fertility and reduce the need for external inputs such as inorganic fertilizers (Mader et al., 2002). Fundamental questions remain, however, around how farming practices influence the soil microbial community and how the microbial community impacts the overall functioning of agricultural soils.

C. Purpose of the Project

The purpose of this project was to examine how the practice of cover cropping, which is utilized in many sustainable farming systems, contributes to soil health and agricultural productivity and, in the long term, associate those impacts with changes in the soil microbial community. The project sought to draw connections between the practice of cover cropping as it relates to soil health, fertility, and agricultural production so that we might be able to better inform farmers on how to fine-tune their management strategies to minimize nutrient losses, improve soil health, and increase crop yields. The experiment looked at two cover crops – cowpea and sorghum-sudangrass. Cowpea was chosen for its ability to form a microbial-plant relationship with high nitrogen-fixing

bacteria. Sorghum-sudangrass was chosen for its expected high biomass input. Our hypothesis was that a cowpea cover crop would increase both soil nitrogen availability and subsequent production of a fall lettuce crop due to its ability to form a symbiotic relationship with nitrogen-fixing rhizobacteria. Conversely, we hypothesized that sorghum-sudangrass would initially decrease soil nitrogen availability and lettuce cash crop growth due to its high biomass contribution which would likely result in nitrogen immobilization in the soil microbial layer.

D. Project Objectives

Objectives are:

- I. To determine how cover crops impact various biological and chemical soil characteristics (i.e. soil respiration, nitrogen transformation, etc.) and fertility (organic matter content, nitrogen availability, etc.)
- II. To determine the effects of these changes in biological and chemical characteristics of the soil on lettuce crop growth and production.

II. REVIEW OF LITERATURE

A. Review of Literature

Part I. Cover Crops, Soil Characteristics, and Crop Production

In their paper, “Summer Cover Crops Fix Nitrogen, Increase Crop Yield, and Improve Soil-Crop Relationships,” Blanco-Canqui et al. (2012) collected data from a 15-year cover crop experiment utilizing a no-till winter wheat and grain sorghum rotation. In the study, hairy vetch was used as a winter cover crop from 1995 to 2000 and, starting in 2002, sunn hemp (SH) and late-maturing soybean (LMS) were used as summer crops between wheat harvest and sorghum plantings. The plots were fertilized with nitrogen at 0, 33, 66, and 100 kg ha⁻¹ to further study the interactions between cover crop treatments and fertilizer rates. The researchers found that summer cover crops produced a large amount of biomass residue in a relatively short period of time (about 12 weeks) (Blanco-Canqui, H. et al., 2012). More specifically, the sunn hemp produced 7.88 Mg ha⁻¹ of residues averaged across N rates and the late-maturing soybean produced 8.76 Mg ha⁻¹ of residues. The study also found that the sunn hemp cover crop tended to increase sorghum yields at 0 kg N ha⁻¹ gradually with time, indicating that the benefits of cover crops for increasing crop yields may be “greater in the long term possibly due to the gradual accumulation in soil nutrients (C and N) and improvement in soil physical properties (Blanco-Canqui, H. et al., 2012). Furthermore, the results from the study indicated that the benefits of cover crops for increasing crop yields were greatest at or below 66 kg ha⁻¹ of N application (Blanco-Canqui, H. et al., 2012). These results led the researchers to conclude that cover crops may be able to provide supplemental nitrogen “especially in cropping systems with limited inorganic N input” (Blanco-Canqui, H. et al., 2012). The results of this study are important to sustainable agriculture, for which one of the goals is to lower inorganic N fertilizer inputs.

The study by Kruse and Nair (2016) entitled “Summer cover crops and lettuce planting time influence weed populations, soil nitrogen concentration, and lettuce yields,” investigated four different short-term summer cover crops and their effects on fall lettuce production in the Midwestern United States. The study included four cover crop treatments: buckwheat, southernpea (a.k.a cowpea), black oats, sorghum-sudangrass, and one no-cover crop (control). In the first year of the study, the researchers found that southernpea (or cowpea) significantly increased soil N and lettuces grown in the southernpea treatment were among the first to be harvested due to their faster rate of maturation. This quick maturation also meant that “lettuce plants in the southernpea treatment spent less time in the field being exposed to weather, insects, or pathogens that could decrease marketable yield” (Kruse and Nair, 2016). The authors theorized that one of the key factors leading to this earlier maturation time was the increased soil nitrate and ammonium concentrations that they observed in the southernpea treatment plots at lettuce planting time. Conversely, the authors observed that lettuce from sorghum-sundangrass plots was consistently lower in yield and smaller in head diameter compared to the other treatments (Kruse and Nair, 2016). Similarly, Wang et al. (2008a) and Finney et al. (2009) observed the same trend in vegetable yield following a sorghum-sundangrass summer cover crop. There are two possible explanations for the observed negative effect of sorghum-sudangrass on lettuce: first, that nitrogen could have been immobilized in the microbial biomass due to the high C:N ratio of sorghum-sudangrass and, second, that sorghum-sudangrass has allelochemical properties that could have limited lettuce growth. The authors concluded that more research was needed to determine the appropriate timing for using a sorghum-sudangrass cover crop in order to benefit rather than hurt lettuce crop production.

The study Wang et al. (2008a) should be highlighted because it brings up another key challenge in determining which cover crops to use in vegetable crop production. The study by Wang et al. (2008a) found that different cover crops can have varying effects on crop yields depending on what type of crop succeeds them. In their study, “summer cover crop and in-season management system affect growth and yield of lettuce and cantaloupe,” Wang et al. (2008a) studied the effects of summer cover crops and management system on subsequent fall romaine lettuce and spring muskmelon growth and yield. They used cowpea and sorghum-sudangrass as their two cover crop treatments. They added one more treatment by either incorporating cowpea into the soil in the fall or using it as mulch in the fall. All sorghum sudangrass was incorporated into the soil in the fall. They found that in both cases, either incorporated or used as mulch, cowpea increased lettuce growth and yield whereas sorghum-sudangrass reduced lettuce growth and yield. Similar studies showed cowpea having a much lower C:N ratio than sorghum-sudangrass (Creamer and Baldwin, 2000). This and the nitrogen-fixing property of cowpea are likely contributors to the increased yields in both fall lettuce and spring muskmelon crops following the incorporation of cowpea cover crop in the fall (Wang et al., 2008a). Conversely, “high C:N ratio and biomass production of sudangrass may immobilize nitrogen and reduce nitrogen availability to the next cash crop” (Wang et al., 2008a; Creamer and Baldwin, 2000). That being said, the study found that muskmelon yield increased by 18.2% the subsequent spring following the fall sorghum-sudangrass cover crop (Wang et al., 2008a). The researchers posed that this could have resulted from

“the long and steady availability of nitrogen in the soil from the decomposition of the sudangrass residue” (Wang et al., 2008a). Thus, the use and timing of a sorghum-sudangrass cover crop must be carefully considered to avoid potential losses in yield (Wang et al., 2008a).

Once it has been established that cover crops, and particularly leguminous cover crops, can be of great benefit to farmers in terms of their potential to increase nitrogen availability and crop yield, it then must be asked how farmers can best manage those cover crops in order to maximize soil nutrient and crop yield gains. Numerous studies have looked into this question including a recent study by Coombs et al. (2017) entitled “Legume cover crop management on nitrogen dynamics and yield in grain corn systems.” In their study, they looked at three different management treatments: 1) cover crop, 2) cover crop seeding rate, and 3) cover crop termination timing in order to draw conclusions on how to best manage leguminous cover crops to increase nitrogen availability and yield in grain corn systems on various soil types (Coombs et al., 2017). Their results were mixed but showed variability in cover crop success in terms of cover crop biomass, nitrogen availability, and corn yield in relation to climatic conditions and soil type more than any of the treatments. However, the study did show that spring-terminated crops resulted in higher PAN during the corn season and correspondingly higher corn grain yield as compared to fall-terminated cover crops (Coombs et al., 2017). The researchers posed several possible explanations for why this occurred including “1) higher N content of spring cover crop biomass, 2) better synchrony of mineralization with corn N demand [and] 3) greater N lost from the system after fall-termination as compared to spring-termination” (Coombs et al., 2017). Overall, their study highlights the need for further research into the effects of management on cover crop success.

Part II. The Soil Microbiome, Biodiversity, and Soil Health

Previous research examined the link between the use of cover crops and their impacts on nitrogen cycling and crop yield, however, in general they did not address one of the key components in that linkage – that is, how cover crops affect the soil microbiome and how those effects, in turn, influence nutrient cycling and crop yield. Up until quite recently, research and development in the field of soil health and agricultural production has focused primarily on the physical and chemical aspects of the soil and has given limited attention to one of the most important facets of soil health – that is, the *living* components of the soil.

One of the most thoroughly studied topics in soil microbiology as it relates to agronomy is the role that soil microbes play in nitrogen fixation (Bhatia, 2008). Research has shown that many plant species form symbiotic relationships with soil microbes to help meet their nutritional demands. Since nitrogen tends to be one of the most limiting factors to plant growth, plants devote a lot of energy to forming these bacterial symbioses. In the chapter, “Role of Microbial Diversity for Soil, Health, and Plant Nutrition,” Bhatia (2008) describes how leguminous plants attract symbiotic nitrogen-fixing bacteria by exuding a variety of substances through their roots. This leads to the “formation of specialized root organ(s) – the nodules.” The presence of and degree to

which these exudates are secreted is controlled by both the host and bacterial genes. Bhatia goes on to discuss how genetic manipulation of both the leguminous host plants as well as the Nitrogen-fixing *Rhizobium* species of bacteria might one day reduce the Nitrogen fertilizer needs of many of the world's cereal crops (Bhatia, 2008).

In addition to the importance of singular species of bacteria, such as *Rhizobium*, on agricultural landscapes, studies have explored the importance of microbial diversity for soil health and agricultural production. A joint research project involving scientists from the Netherlands as well as Brazil explored just that and examined evidence for the “importance of soil biodiversity to sustaining (agro-) ecosystem functioning” (Brussaard et al., 2007). In their paper, “Soil biodiversity for agricultural sustainability,” Brussaard et al. (2007) looked at four aspects involving soil biodiversity and agricultural sustainability. These four aspects were as follows: 1. The contribution of soil biodiversity to resistance and resilience against stress and disturbance, 2. The importance of soil biodiversity for the sustainable use of resources such as water and nutrients, 3. How to manage soil biodiversity, and 4. The case for the value of soil biodiversity.

For the first question – that of soil biodiversity as it relates to stress and disturbance resistance and resilience – Brussaard et al. (2007) examined several studies in which a stress event was artificially induced in a plant-microbial ecosystem. In the first study, they examined a two-event stress (disturbance) experiment. The first stress (disturbance) was applied to the agroecosystem in order to assess its effects on biodiversity and the subsequent second stress (disturbance) was applied in order to assess how loss of diversity affected the response of agroecosystem functioning (Griffiths et al., 2000). The first stress event involved the use of a chloroform vapor fumigation at varying time intervals (0 h (unfumigated control), 0.5, 2, or 24 hours). The researchers found that the first stress event reduced the diversity of the soil community progressively as fumigation time increased. In order to apply the second stress event, the researchers either chose a persistent stress by adding a heavy metal (CuSO_4) or a transient stress (brief heating to 40 degrees C). The researchers measured the effects of this second stress event as changes in respiration rates resulting from the decomposition of freshly added organic matter. They found that respiration in the most diverse soils was hardly affected by the Cu addition, whereas respiration in the reduced-diversity soils decreased by up to 70%. However, even with the addition of two other experiments, the team was not able to determine whether the reduction in respiration was due to the reduction in biodiversity or because of the stress event itself (i.e. the initial fumigation). In light of these inconclusive results, more work must be done in order to study the relationship between soil biodiversity and agroecosystem resilience and resistance to stress and disturbance.

For the second question posed by Brussaard et al. (2007) – that of the importance of soil biodiversity for the sustainable use of resources – the authors looked at two resources in particular, nutrients and water, and how efficiently they are used by plants. Studies examining the use of the first resource, the nutrient use efficiency, found that it may not be soil biodiversity *per se*, but the mean functional dissimilarity within a species assemblage that is positively related with nutrient supply, measured as gross nitrate productivity (Brussaard et al., 2007). In terms of water use efficiency, the studies

investigated by Brussard et al. (2007) seemed to point to the fact that “the diversity of soil structural components may be more closely related to water use efficiency than...the diversity of species (groups) themselves.” This points towards a need for further research into these questions as well.

In their third point of analysis of soil biodiversity and its relationship to agricultural sustainability, Brussard et al. (2007) examined how soil biodiversity can be managed. They highlighted a study that proposed several possible “entry points” or agricultural management practices that potentially affect soil biodiversity and biological processes. The study by Swift (1999) proposed management “entry points” that included changes in amounts and/or quality of organic residues entering the soil ecosystem; reducing soil disturbance events such as tillage and the use of pesticides, herbicides, and fertilizers; as well as possible increased soil biological diversity through proactive measures such as inoculation of beneficial soil organisms.

Lastly, the paper by Brussard et al. (2007) looked at the economic valuation of soil biodiversity. The authors pointed to research supporting the “potential immense value of the services provided each year by the soil biota worldwide, possibly exceeding 1.5 trillion US dollars”. They highlighted that one of the key components of the economic potential offered by soil biodiversity lies in the role that these organisms play in the recycling of organic wastes. The recycling of biological material by microorganisms provides an immense opportunity for decreasing the need for synthetic fertilizers and increasing crop growth and productivity. Furthermore, a diversity of microorganisms in the soil has the potential to lessen the prevalence of pathogenic disturbance on agricultural fields by increasing competition and suppression of disease organism. This concept was further explored by Alabouvette et al. (2004).

In their chapter entitled “Microbial Diversity in Soil – Effects on Crop Health,” Alabouvette et al. (2004) discussed the role that microbial diversity has to play in disease suppression on agricultural landscapes. They first examined the current molecular-based techniques available to characterize the diversity of soil microbiota. They highlighted methods that characterize microbial communities by the extraction of nucleic acids from soil samples and sequencing of that DNA. They also pointed to the use of physiological methods that can help provide a representative estimate of microbial diversity as well as community structure. They proposed that these genetic and physiological methods of microbial characterization are critical in order to understand the functional activity of microorganisms in soil and must be combined with assessments of microbial trophic patterns as well as vegetative compatibility in order to gain a full picture of how these microorganisms influence soil health and agroecological functioning (Alabouvette et al., 2004).

Part III. The Impact of Agricultural Practices on Soil Microbial Communities

Once it has been established that the microbial community contributes greatly to soil health and agricultural production, naturally the next question asked should be: how do we foster a healthy and diverse microbiome on our agricultural landscapes? The use of sustainable land management practices such as cover cropping, crop rotation, and

reduced tillage have the potential to foster microbial diversity and improve the health and fertility of farmland. The impacts of several of these sustainable agricultural practices on microbial biomass and microbial community composition as well as on soil fertility and crop production will be examined in the following pages.

In their study, “Crop rotational diversity enhances belowground communities and functions in an agroecosystem,” Tiemann et al. (2015) examined the effects of increased crop diversity on soil aggregation, organic carbon, microbial activity, and the microbial community in an agroecosystem. In the introduction to their journal article, they made the important point that the effects of increased species diversity on belowground microbial community and functioning has, up until this point, primarily been studied only in natural ecosystems. They took the results found in studies based on natural systems and used them to inform their study of increased species diversity on an agricultural landscape. Their study involved three treatments: corn monoculture (Cm), corn and soy rotation (SC), corn with a late summer/winter leguminous cover crop, red clover (Cl) and rotations of corn, soy, wheat with no cover, with red clover (SWC1) and with both red clover and rye cover crops (SWC2). Thus, their experiment aimed to test the effects of various species as well as levels of aboveground species diversity (in the form of commonly used crop rotation systems) on belowground physical, chemical, and biological characteristics. The researchers found that “as crop diversity increased through multiple species rotations...[there were] significant gains in [soil organic carbon] SOC and [total nitrogen] TN that were driven by changes in soil and microbial community structure.” Furthermore, they found that the greatest gains in SOC and TN occurred in micro-aggregates, which tend to be more stable and slower to break down. This is very good news for farmers looking to rebuild degraded and nutrient deficient landscapes so that they provide fertile growing spaces for many years to come.

Another study examining the role of biodiversity and management practices in sustainable agricultural systems took place over 21 years in Therwil, Switzerland and was reported by Mader et al. (2002) The study involved two organic farming systems and two conventional systems, which were compared in order to gain insight about the effects of different farming practices on soil health, ecology, and functioning. Although not specifically stated in the article, the aboveground species diversity was assumed to be higher in the organic systems in light of the avoidance of the use of pesticides and herbicides, however, this assumption was made as a result of the lack of information about the practices used in each system in the text. The study found that the energy used in the organic systems to produce “a crop dry matter unit was 20 to 56% lower than in conventional” and land area used to produce a crop dry matter unit was “correspondingly 36 to 53% lower.” Bringing these results back to the discussion at hand, the study also reported that microbial diversity was highest in the two organic farming systems and lowest in the two conventional systems. Together, these results point to increased crop efficiency on more microbiologically diverse landscapes.

Another practice that has been studied in relation to its effects on the microbial community and soil health is tillage. In their study, “Impact of No-Tillage and Conventional Tillage Systems on Soil Microbial Communities,” Mathew et al. (2012)

examined the impacts of different tillage practices (no-tillage and conventional tillage) on soil microbial community structure, microbial activity, and related physical and chemical soil characteristics. They assessed the structure of the soil microbial community using phospholipid fatty acid (PLFA) analysis, which is a commonly used technique in soil microbiology. Their results showed an increased abundance of PLFA indicators of fungi, bacteria, arbuscular mycorrhizal fungi, and actinobacteria, indicating an increase in species diversity in the no-till soils. Furthermore, the study found that the no-till treatment soils had higher soil carbon and nitrogen contents, which were measured as organic carbon and total nitrogen. Putting these results together, this study supports the theory that increased soil microbial diversity leads to healthier and more fertile soil and goes further to connect tillage practices to their impact on the soil microbial community.

Of course, it must be noted that not all studies examining the impacts of different tillage practices on microbial community structure have had the same results. In their study, “No favorable effect of reduced tillage on microbial community diversity in a silty loam soil,” Degruene et al. (2016) examined the effects of tillage regime (conventional vs. reduced tillage), fate of crop residues (retention or removal) and sample depth on microbial diversity. This six-yearlong study found that “depth emerged as the main factor responsible for variation in microbial diversity, tillage regime ranked second, and finally crop residue fate had no influence on microbial diversity.” These conflicting results point to the need for further research to look at the relationship between agricultural land management practices and soil microbial community characteristics since both of these are so important to sustainable agriculture.

Linking the study of agricultural practices and their effects on the soil microbiome to measures of microbial activity such as soil respiration and enzyme activity requires the collection of data across the biological and chemical plant-soil continuum. Fernandez et al. (2016) did just that in their paper, “Associations between soil bacterial community structure and nutrient cycling functions in long-term organic farm soils following cover crop and organic fertilizer amendment”. The objectives of their study were to determine the “effects of cover crops and organic fertilizers on soil nutrient cycling activity” as well as the “relationships between soil bacterial community structure, nutrient cycling functions...and soil adaphic characteristics in the context of organically-managed agricultural field soils.” Their experiment included four different cover crop treatments: hairy vetch, winter rye, oilseed radish, and buckwheat. The researchers assessed the effects of their treatments on the soil microbiome by doing assays for nitrogen mineralization, soil respiration, and enzyme activity as well as classifying the bacterial community through 16S rRNA sequencing. Their results showed a positive correlation between enzyme activities and soil respiration as well as soil moisture (Fernandez et al., 2016). Winter rye was their only cover crop treatment to significantly affect soil respiration, which compared to the no-amendment control treatment produced 101% greater soil respiration ($p < 0.001$) during the month of July at their Farmington site. Their results showed a much greater correlation between soil physicochemical differences as well as differences in soil function and bacterial community structure as they related to site than with treatment. Their findings point to the need for further study into the relationships between agricultural practices and their effects on the soil microbial

community and function as well as other biological, chemical, and physical attributes of the soil.

B. Summary of Literature Review

The review of the literature above has surveyed papers related to the effects of cover crops on soil nitrogen availability and crop yield; the soil microbiome as it relates to biodiversity and other indicators of soil health; and the effects of various agricultural production strategies on the soil microbiome and soil respiration. This review was meant to provide background information about the literature already out there around cover crops and their effects on nitrogen availability and crop yield as well as provide a jumping off point for further work resulting from this study as it relates to the microbial soil effects produced by this study. The need for more information linking the use of cover crops to their effects on the soil microbiome and the soil microbiome's contribution to soil health has been thoroughly demonstrated by the literature presented above and will now be discussed as it relates to the project at hand.

III. PROJECT METHODOLOGY

A. Targeted Population and Participating Audience

This project was designed to provide information and resources to farmers along the Eastern Shore of Virginia and around the country who are seeking sustainable methods to improve the health and fertility of their soils. The project was supported by the Virginia Tech Eastern Shore Agricultural Research and Extension Center (ESAREC) as well as, in part, with funding from Southern Sustainable Agriculture Research & Education (SSARE). The project was aimed specifically to help farmers in the southeastern region of the United States.

B. Methodology

Planting Methods

Experimental Design

The summer cover crop experiment involved three treatments including cowpea (*Vigna unguiculata*), sorghum-sudangrass (*Sorghum xdrummondii*), and no-cover crop control. The experiment took place on a 195ft x 72ft (or 59m x 22m) field site at the Eastern Shore Agricultural Research and Extension Center (ESAREC) in Painter, VA on a Bojac sandy loam soil. Each plot was 24ft x 65ft (1,560ft²) or 7m x 20m (140m²). Treatments were arranged in a randomized complete block design with three replications.

The field was previously planted with a mixture of clover and wheat for the year prior to the study. Prior to planting the summer cover crop, the field was mowed and then tilled twice – once three weeks prior to planting and a second time two days prior to planting. The field was prepared with a cultipacker prior to planting in order to make ridges for seeding.

Two summer cover crop treatments were planted on July 13, 2016. The cowpea used was Queen Anne Cowpea from Wetsel Seed (961 N Liberty St., Harrisonburg, VA 22802) and tested in January 2016 with a germination rate of 85%. The sorghum-sudangrass was BMR Sudan Grass F1 OG from Johnny Selected Seeds (955 Benton Ave, Winslow, ME 04901) and tested in March 2016 with a germination rate of 94%.

Inoculant

Cowpea was inoculated at the recommended rate of 5 oz. inoculant/100 lb. seed with Verdesian NDure Premium Peat Inoculant (Verdesian Life Sciences, 1001 Winstead Dr., Cary, NC 27513) for Pea/Vetch/Lentil (*Rhizobium leguminosarum* biovar *viceae*) at a concentration of the Specified *Rhizobium* Species 2×10^8 CFU/g (expiration 12/31/16). The inoculant was applied using the slurry method by dampening the seed with water at a rate of 8.5 oz. (241g) of water per 50 lbs. (22.7 kg) of seed and mixing in the inoculant to evenly coat all of the seeds.

Seeding Density

The cowpea was planted at the recommended seeding rate of 120lbs/acre (Clark, A. (Ed.) 2007). The sorghum-sudangrass was planted at the recommended seeding rate of 40lbs/acre (Clark, A. (Ed.) 2007). Both types of seed were broadcasted by hand into each treatment plot. In order to ensure uniform distribution of the seed, each plot was divided into quadrants and the seed for each plot was divided and spread evenly among the four quadrants. Following planting, a cultipacker was used to cover up the seeds with soil. It should be noted that the field site received a significant rainfall (0.09 in) immediately following planting, which is assumed to have aided in germination.

Maintenance

The entire field area was irrigated using sprinkler irrigation as needed, roughly once a week throughout the summer. Weeds in the no cover crop plots were controlled by tilling those plots every 3 – 4 weeks.

Cover Crop Stand Counts

Cover crop stand counts and weed counts were taken 13 days after planting (DAP) (on July 26, 2017) using a 1ft x 1ft square (5 per plot). The square was thrown five times at random throughout each plot and the number of weeds and cover crops in each square were recorded. Weeds were also identified at the same time.

Aboveground Biomass & Plant Tissue Samples

On October 2, 100 DAP, aboveground biomass samples were taken prior to cover crop incorporation. Aboveground biomass samples were taken using a 1x1ft square (3 per plot). The square was tossed randomly three times per plot and any stems (weeds or cover crop) lying within the square were cut using a serrated scythe. Weeds and cover crop biomass samples were separated into paper bags and each was weighed separately to determine their fresh weights. Samples were dried until a constant weight was obtained at 55°C in a forced air oven and then weighed to determine biomass (dry weight). Nitrogen content (% N) and total nitrogen uptake (kg N ha^{-1}) were determined for both weed and cover crop inputs. Total nitrogen uptake (kg N ha^{-1}) for each plot was determined by

multiplying the dry weight of the weed and the cover crop biomass for each plot by their measured N contents (%) to estimate the total N uptake from the weeds from that plot and the cover crops from that plot and then adding those two numbers together to get the total Nitrogen contribution of plant material in that plot.

Cover Crop Incorporation

Cover crops were incorporated on October 4, 2017 (102 DAP) by first flail mowing the cover crop to break up the plant residue, rototilling, and then incorporating plant residue with a three-row disc bedder. Following incorporation, soil cores from each plot were taken on October 14, 2017 (112 DAP) to a depth of 15cm and 25 per plot using a soil core sampler 2.5cm wide. Soil cores from each plot were thoroughly mixed together, sieved by hand with a 6mm sieve, and separated into plastic bags. One sample was frozen and will be sent out to the GMU Microbiome Analysis Center for microbial community characterization. One sample was dried and sent out to Waypoint Analytical for nutrient analysis. One sample was stored in the refrigerator in order to conduct an aerobic incubation study and determine nitrogen transformation over time using the Lachat 8500 (Lachat Instruments, 6645 W Mill Rd, Milwaukee, WI 53218).

Soil fertility

The soil samples taken on October 14, 2017 (112 DAP) to be sent out to the lab were sent out to Waypoint Analytical to be analyzed for organic matter (OM), total organic carbon (TOC), total nitrogen (TN), and C:N ratio as well as the nutrients: nitrate (NO₃-N), phosphorus (P), potassium (K), magnesium (Mg), and Calcium (Ca). The analytical methods that were used included SMP Buffer pH, Mehlich 3, Loss on Ignition, and Water pH.

Soil Respiration Measurements in the Field

On October 15, 2017 soil respiration was measured using the method for field measurement of soil respiration developed by Parkin et al. (1996) and provided in the NRCS Soil Quality Test Kit method (2001). In accordance with this method, soil respiration measurements were taken roughly 24 hours after a rainfall to ensure that the soil was moist but not saturated. First, a 6-in diameter by 4-in wide metal ring was driven into the soil (beveled edge down) using a hand sledge and block of wood to a depth of 3-in so that the height of the headspace was roughly 2-in. For a more accurate measurement of the headspace, four evenly spaced depth measurements were taken inside the chamber in order to calculate the average headspace depth. The metal chamber was covered with a plastic lid with two sealed rubber stoppers. After exactly 30 minutes, a Draeger tube apparatus was used to draw out 100mL of gas from the chamber (RAE Systems, Inc., 3775 North First Street, San Jose, CA 95134). To assemble the Draeger tube apparatus, a needle was connected to one end of a small section of plastic tubing and a Draeger tube was connected on the other end to receive the incoming gas. Modifying the method slightly, the Draeger tube was inserted into a RAE pump in order to more easily take gas samples from the chamber. Before taking the gas samples, a second needle was inserted into the stopper on the plastic chamber lid in order to create a vacuum so that air could be drawn out from the chamber space. The Draeger tube apparatus needle was then inserted into the other stopper on the chamber lid and 100mL of gas was drawn out using the RAE

pump. Following the method, if the respiration reading on the Draeger tube was 0.2% or less, four additional respiration samples were taken for a total of 500mL of gas sampled and the average respiration rate was calculated. Otherwise, if the respiration reading on the Draeger tube was above 0.2%, only two gas samples were taken and the average respiration rate was calculated. The Draeger tube was read according to the method by reading the highest point that the purple color on the Draeger tube could easily be detected. In addition to the respiration measurements, at the time that each respiration measurement was taken, the temperature and volumetric water content (%) were also recorded 2.5cm away from the soil respiration chamber at depths of 2.5cm and 7.5cm (Figure 3). Soil moisture and temperature were taken with a WaterScout SM 100 soil moisture sensor and an external temperature sensor, respectively, connected to WatchDog 1425 data logger (Spectrum Technologies, Inc. Aurora, IL).

Soil respiration was calculated using the methodology laid out in the Soil Quality Test Kit Guide (2001) as follows:

$$\text{Soil Respiration (lb CO}_2\text{-C/acre/day)} = \text{PF} \times \text{TF} \times (\% \text{CO}_2 - 0.035) \times 22.91 \times \text{H}$$

Where:

PF = pressure factor = 1

TF = temperature factor = (soil temperature in Kelvin + 273) / 273

H = inside height of ring = 5.08cm (~ 2 inches)

The soil temperature and moisture at 2.5cm were used to standardize reading according to manufacturer instructions.

Temperature Standardization:

Soil respiration was standardized to 25 degrees C (i.e. 298.15 K) to account for differences in soil temperatures. This method of temperature standardization uses the generally accepted rule that biological activity increases by a factor of 2 with each 10 degree C increase in temperature (Parkin et al. 1996).

The equations are as follows:

For differences in soil temperatures between 15°C and 35°C (i.e. 288.15 – 308.15 K)

$$\text{Standardized soil respiration rate} = \text{soil respiration rate} \times 2^{[(25-T)/10]}$$

For differences in soil temperatures between 0°C and 15°C (i.e. 273.15 – 288.15 K):

$$\text{Standardized soil respiration rate} = \text{soil respiration rate} \times 4^{[(25-T)/10]}$$

After the correct temperature standardization equation was used to calculate the temperature-standardized respiration rate, the result was converted from lbs CO₂-C/acre/d to g CO₂-C/ha/d C·ha⁻¹·d⁻¹ using the equation below:

$$\text{g CO}_2\text{-C}\cdot\text{ha}^{-1}\cdot\text{d}^{-1} = (\text{lbs CO}_2\text{-C}\cdot\text{acre}^{-1}\cdot\text{d}^{-1}) / 11.2$$

Moisture Standardization:

Similar to the rule guiding temperature standardization, it is generally accepted that maximum microbial activity occurs at 60% soil water-filled pore space (Parkin et al., 1996). Water-filled pore space was calculated using a series of equations detailed below.

Determining volumetric water content:

Prior to calculating water-filled pore space, the volumetric water content percentage had to be determined for each plot. This was calculated using the gravimetric water content that was measured at the time the incubation study was started, on October 28, 2016, roughly two weeks after the soil had been collected from the field. To maintain the same moisture content in the soil but prevent the soil from becoming anaerobic, the soil collected for the incubation study at the time of soil sampling was stored in the refrigerator in sealed plastic bags for two weeks prior to the start of the incubation study and the bags were opened once per week to allow carbon dioxide to escape and oxygen to enter into each bag.

The following equations were used to determine gravimetric water content and volumetric water content (Gardner, 1986).

1. Gravimetric -- dry-weight basis

$$w_d = \text{grams water} / \text{grams dry soil}$$

3. Volumetric

$$w_v = \text{volume of water} / \text{volume of soil}$$

Determining water-filled pore space (WFPS):

The following equation was used to calculate water-filled pore space from volumetric water content and soil bulk density (USDA Soil Quality Test Kit Guide, 2001). Soil bulk density was estimated to be 1.6g/cm³ for a sandy soil.

$$\text{Water-filled pore space (\%)} = (\text{volumetric water content} \times 100) / [1 - \text{soil bulk density} / 2.65]$$

Moisture Standardization (60% WFPS):

The following equation was used to standardize respiration measurements to 60% WFPS when WFPS values were between 30 and 60% (USDA Soil Quality Test Kit Guide, 2001):

$$\text{Soil respiration}_{60} = \text{soil respiration rate} \times (60 / \text{measured \%WFPS})$$

The final respiration rate was calculated using both the temperature-standardization and moisture-standardization procedures.

Aerobic Incubation Study

A 5-week aerobic incubation study was started on October 28, 2017, roughly two weeks after the soil samples were taken from the field. The study followed a modified procedure of the methods for an aerobic laboratory incubation laid out in a publication by

Drinkwater et. al. (1996). The purpose of the incubation study was to determine the rate of nitrogen mineralization in an aerobic environment over several weeks by analyzing the nitrate and ammonia content of the soil at weekly intervals over a 5-week period. At the time of each measurement, the soil samples were extracted with a 2M KCl solution and then run on a Lachat 8500 QuickChem 2000 flow injection autoanalyzer (Lachat Instruments, Loveland, CO) to determine their nitrate and ammonia contents.

Five soil samples (20g) from each plot were added to 250mL plastic jars and the plastic jars were covered tightly with parafilm to prevent moisture loss but allow for gas exchange so that the soil environment did not become anaerobic. Distilled water was added to each plastic jar to bring all of the soil in the jars to 15% moisture. The weight of the jars was taken each week in order to ensure minimal moisture loss. Moisture loss over the 5 week incubation study was less than 0.25% in all of the jars, therefore no water was added to the jars after the first week.

Samples were used for nitrate and ammonia determination at 0, 1, 2, 3, and 5 weeks incubation time. Extraction and analysis of nitrate and ammonia were performed following the protocol in the Lachat Instrument's QuickChem Method 12-107-04-1-B. Determination of Nitrate in 2M KCl Soil Extracts by Flow Injection Analysis and QuickChem Method 12-107-06-2-A: Determination of Ammonia (Salicylate) in 2 M KCl Soil Extracts By Flow Injection Analysis.

Following the protocol for nitrate determination offered by Lachat Instrument's QuickChem Method 12-107-04-1-B, soluble, inorganic nitrate was first extracted from the soil samples using a 2M KCl solution prior to being run through the Lachat. While in the Lachat, the extracted solution passed through a copperized cadmium column, thus reducing any nitrate to nitrite. The total nitrite was then determined by "diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl)ethylenediamine dihydrochloride" (Lachat, Krista Knepel, 2003). Following these transformations the resulting solution was a water-soluble dye that had a magenta color, which could then be read at 520 nm by the Lachat's built-in spectrophotometer.

The Lachat 8500 allows both nitrate and ammonia concentrations to be analyzed simultaneously. Following the salicylate method for ammonia determination in the Lachat Instrument's Quick Chem Method 12-107-06-2-A, exchangeable ammonium was first extracted from the soil samples using a 2M KCl solution prior to being run through the Lachat. The solution was then filtered and the filtrate was run through the Lachat. Once inside of the Lachat, the filtrate was heated with salicylate and hypochlorite in a alkaline phosphate buffer. This reaction produced an emerald green color that was proportional to the ammonia concentration. EDTA was used to prevent precipitation of calcium and magnesium and sodium nitroprusside was used in order to increase the emerald green color's intensity so that it could be more easily read by the Lachat's built-in spectrophotometer (Lachat, Scott Hofer, 2003).

For each extraction, 2.5g of soil (wet weight) was weighed out into a 50ml plastic centrifuge tube. 25ml of 2M KCl solution was added to each centrifuge tube using an

electronic eppendorf Xplorer pipet (Matric; 0.5-10ml). The samples were then shaken on an Ebberbach reciprocating shaker for 30 minutes on low oscillation speed. The KCl-soil mixture was then poured through a vacuum filtration system using a vacuum, Buchner funnel, Erlenmeyer flask, and 0.45micrometer, White gridded, 47mm S-Pak Membrane Filters. A filtrated solution of 25ml of 2M KCl without soil was used as a control. The extracted solution was then stored in the refrigerator until samples could be run on the Lachat 8500. If the samples could not be run within 24 hours, they were placed in the freezer at -12°C until they could be run, at which point they were placed in the refrigerator overnight prior to being run on the Lachat.

Time-0 soil was extracted immediately at the start of the incubation study. Week-1 soil was extracted on November 3, 2016. The first soil extracts – Time-0 and Week-1 – were run on the Lachat on November 4, 2016. Week-2 and Week-3 soils were extracted on November 10 and November 16, respectively, and placed in the freezer at 10degrees F until the day before they were run on the Lachat, at which point they were placed in the refrigerator to thaw overnight prior to being run. Week-5 soil was extracted on December 1st. Week 2, 3, and 5 soil extractions were run on the Lachat on December 2, 2016.

Lettuce Production

One row of Red Sail lettuce was planted in each plot on October 27 at the recommended planting density in order to determine the effects of the cover crop treatments on lettuce yield. The lettuce was planted into black plastic, which is a technique commonly used for lettuce and other vegetable crop production on the Eastern Shore of Virginia as well as elsewhere around the United States. In addition, each plot was divided into subplots with and without spun-bonded row cover (1oz/sqyd) to determine differences in growth and nutrient use efficiency. The covered vs. no cover treatments were overlaid onto the experimental study in a split plot design with three replications. The lettuce crop was irrigated as needed via drip irrigation. No fertilizer was used in order to determine the fertility effects of the cover crop treatments.

Lettuces were harvested on December 19, 53 DAP. Four lettuce heads were harvested per subplot and weighed (fresh weight). Out of those four, two representative lettuces were put in plastic bags and stored in cold storage overnight until next day to determine leaf area (LI-COR LI-3100C Area Meter). After the leaf area was recorded, the foliage was dried along with the other harvested lettuce samples to subsequently determine their biomass.

Once dry, a subsample of the dried lettuce leaf material from each treatment subplot was sent out to the lab for nutrient analysis. The data collected from the leaf area meter was also used to determine the leaf area/biomass ratio.

The following equation was used to calculate lettuce yield in Mg*ha⁻¹:

Planting distance: 0.3m (1ft) and 2 rows per bed

Beds: 1.5 m apart (6ft)

$$\text{Mg}\cdot\text{ha}^{-1} = \text{g (lettuce head)} * 2 \text{ (rows per bed)} / 0.3\text{m} / 1.5 \text{ m} * 10000 \text{ m}^2\cdot\text{ha}^{-1} / 1000000 \text{ g}\cdot\text{Mg}^{-1}$$

Data was analyzed by PROC MIXED for ANOVA using SAS 9.4 (SAS Institute Inc., North Carolina)

IV. PROJECT RESULTS, DISCUSSION, AND RECOMMENDATIONS

A. Project Results

Cover Crop Biomass

The average cover crop biomass incorporated into the field provided by each treatment were significantly different between the cowpea, sorghum-sudangrass, and no-cover crop (control) treatments. Sorghum-sudangrass had the highest average biomass (22.1 ton·ha⁻¹), followed by cowpea (8.1 ton·ha⁻¹), which had the second highest biomass. As expected, the no-cover crop treatment provided the least biomass (1.4 ton·ha⁻¹) since those plots were tilled every 3 – 4 weeks to minimize weed growth (Table 1).

Cover Crop Nitrogen Incorporation

The nitrogen content (%) of the cowpea cover crop treatment plant tissue (3.1%) was significantly higher than that of the sorghum-sudangrass treatment (1.3%). The nitrogen contents of the weeds from each treatment were not significantly different. While not significantly different from one another, the total N contributions of the two cover crop treatments were significantly greater than that of the no-cover crop control. Specifically, the cowpea cover crop had a total N uptake of 227 kg N ha⁻¹, the sorghum-sudangrass was 286 kg N ha⁻¹, and the no cover crop was 19 kg N ha⁻¹ (Table 1).

Soil Analysis – Organic Matter and Organic Carbon

The results of the laboratory soil analysis indicated that, while differences in organic matter (OM) as well as total organic carbon (TOC) were not significant between the two cover crops, both of those parameters increased when compared to the no-cover crop control (Table 2).

Soil Fertility – Extractable Nitrate and Other Nutrients

Extractable nitrogen as nitrate (NO₃-N) was significantly different between all three treatments. Cowpea was highest in nitrate (9.3 ppm), followed by the no-cover crop control (4.7 ppm). Sorghum-sudangrass was significantly lower in nitrate than both of the other treatments (1.0 ppm). Both cover crop treatments were significantly higher than the no-cover crop control in both Potassium (K) and Magnesium (Mg), however the differences between the Potassium and Magnesium contents of the two cover crop treatments was not significant. There was no significant difference between the Phosphorous (P) and Calcium (Ca) contents of the three treatments (Table 3).

Soil Respiration

The soil respiration rate of the cowpea treatment was significantly higher than that of the no-cover crop control. However, the soil respiration rate of the sorghum-sudangrass treatment did not differ significantly from either of the other two treatments (Table 4).

Lettuce Production

The yield and leaf area of the lettuce crop was analyzed in two ways: first by comparing the three cover crop treatments (cowpea, sorghum-sudangrass, and no cover crop), and second by comparing a no cover (NC) vs. row cover (RC) treatment on each of the three cover crop treatments (Table 5). The yield and leaf area were both significantly larger for lettuces with or without row cover that followed the cowpea cover crop than those that followed the sorghum-sudangrass cover crop. In contrast, the yield and leaf area of lettuces with or without row cover that followed the cowpea and no-cover crop were not different. The difference between the lettuce yield and leaf area of the no-cover crop treatment compared to the sorghum-sudangrass treatment was not significant under row cover, but significantly different without row cover.

In both the cowpea and no-cover crop treatments, lettuces grown under row cover (RC) had both a significantly higher fresh weight and leaf area than lettuces grown without row cover (NC). The average fresh weight and leaf area did not differ significantly between lettuces grown with or without row cover for the sorghum-sudangrass treatment (Table 5).

Incubation Study Data

The results of the incubation study showed significant differences between the three treatments in the concentration of nitrate at weeks 1, 2, 3, and 5 (Figure 1). Cowpea had a significantly larger nitrate concentration than the other two treatments from weeks 1 through 5. Sorghum-sudangrass nitrate concentration was less than the no-cover control treatments for weeks 1 and 2. The ammonia concentration showed much variation throughout the 5-week study and no significant differences were found between the three treatments (Figure 2). The blank control extraction (2M KCl only) remained below 0.07ppm of nitrate or ammonia for all five weeks of the incubation study.

B. Discussion

Both cover crop treatments had some beneficial effects on soil quality as compared to the no-cover crop control, including significant increases in organic matter (OM) and total organic carbon (TOC) as well as potassium and magnesium contents (Tables 2 and 3).

Our finding that lettuce fresh weight and leaf area were both significantly higher for the cowpea treatment than that of the sorghum-sudangrass treatment is consistent with the results reported in other studies (Wang et al., 2008a; Kruse and Nair, 2016). Our results showed a negative impact of sorghum-sudangrass on the lettuce crop fresh weight and leaf area. Similar results were found in a different study involving a sorghum-sudangrass

cover crop with a subsequent fall cabbage crop under both conventional tillage and no tillage management schemes (Finney et al., 2009).

The results from our 5-week incubation study showed a consistently elevated level of nitrate as nitrogen in soil samples taken from the cowpea treatment compared to the other two treatments. The results from our incubation study are supported by the results from the laboratory soil fertility analysis which showed significant differences in the nitrate as nitrogen contents of all three treatments, with cowpea being highest, no-cover control as second, and sorghum-sudangrass having the lowest nitrate as nitrogen content (Table 4). These results align with those of Kruse and Nair (2016), who observed a higher concentration of soil nitrate and ammonia in cowpea treatment plots as compared with other treatments (buckwheat, black oats, and sorghum-sudangrass) from the time of lettuce planting to the end of their study (roughly 3 months). Our soil fertility results are interesting, however, when we look at them in the context of the results from our plant tissue analysis of the three treatments. Those results showed that both cover crops contributed a significantly greater estimated total N (kg ha^{-1}) than that of the no-cover crop control, however the contributions of each cover crop were not significantly different from one another (Table 1). Since the total N contributions from each cover crop treatment were not significantly different, there must be another explanation for the increase in soil nitrate from the cowpea treatment and decrease from the sorghum-sudangrass treatment as compared to the no-cover crop control.

One likely contributing factor that led to increased nitrate in the cowpea treatment compared to the sorghum-sudangrass and control treatments is the ability of cowpeas to fix atmospheric nitrogen through a symbiotic relationship with N-fixing bacteria in the rhizosphere (Creamer and Baldwin, 2000). Another possible explanation for the increased availability of nitrogen in cowpea treatments as compared to sorghum-sudangrass is that, in general, cowpea has a much lower C:N ratio than sorghum-sudangrass and as such, in plots previously under a sorghum-sudangrass cover crop treatment, immobilization of nitrogen in the microbial biomass is likely to occur (Creamer and Baldwin, 2000). Although, it must be noted that, laboratory soil analysis results for our study yielded no significant differences between the C:N ratios of all three treatments. An additional factor that could have also contributed to the lower lettuce fresh weight and leaf area index that we observed in the sorghum-sudangrass plots as compared to the control or cowpea treatments is that sorghum-sudangrass is known to hold certain allelochemical properties, which could have inhibited the growth of the lettuce crop in our study (Finney et al., 2009).

Looking at the results for biomass, soil respiration and nitrate concentration for sorghum-sudangrass together, sorghum-sudangrass consistently had the lowest concentration of nitrate as nitrogen but the second largest soil respiration rate and the largest biomass contribution compared to the other two treatments. These results make sense since soil microbes were likely actively immobilizing soil nitrogen in order to break down the very large amount of biomass contributed by the sorghum-sudangrass cover crop residue. This theory is further supported by the fact that the nitrate concentration of the soil samples taken from the sorghum-sudangrass treatment decreased between time 0 and week 1 and

then gradually started to increase between weeks 1 and 5. Nitrogen was likely actively immobilized by soil microbes during the first week so that they could then begin breaking down the high C:N sorghum-sudangrass plant residue present in the soil. It is also realistic for the cowpea treatment to have the highest soil respiration rate since that treatment appeared to have nitrogen readily available for the living components of the soil to process the left over plant material through mineralization and nitrification as well as other processes.

Despite the negative results observed in this study for using sorghum-sudangrass as a summer cover crop immediately prior to planting a fall lettuce crop, other studies have shown that sorghum-sudangrass might have a beneficial impact on a subsequent spring vegetable crop, likely because of how long it takes to be broken down and its nitrogen to be made available to plants. For instance, Wang et al. (2008a) found that, in contrast to the negative effect sorghum-sudangrass had on the yield of a fall lettuce crop, fall-terminated sorghum-sudangrass increased spring muskmelon yield by 18.2%. Thus, not all cover crops are created equal when it comes to preceding certain cash crops and the timing and nutrient needs of the subsequent cash crop must be carefully considered by farmers when selecting a suitable cover crop.

As noted above, differences in ammonia concentrations across treatments were not significant. Our results yielded much lower values for ammonia concentrations compared to nitrate across all treatments. This is to be expected since ammonium is readily converted to nitrate by soil microorganisms, particularly in warm, moist soil conditions (Agehara & Warncke, 2005). Along those lines, it should be noted that soil samples for the incubation study were first kept refrigerated for roughly two weeks before the incubation study was started. Thus, it is possible that upon warming (transitioning from refrigerator to room temperature), much of the ammonia present in the soil could have been immediately converted to nitrate by soil microorganisms through the process of nitrification. It is also possible that nitrate as nitrogen was much more readily extracted by the 2M KCl extraction since nitrate is much more prone to leaching due to its negative charge. Furthermore, since most KCl extractants were not run through the Lachat immediately following the extraction, it is possible that ammonia was lost through volatilization, although extracted samples were stored in the freezer in order to minimize the potential for volatilization to occur.

C. Conclusions and Implications

Based on the results of this study, we conclude that using cowpea as a summer cover crop has the potential to significantly increase available soil nitrogen and improve the production of a fall lettuce crop. Further studies are needed to confirm this conclusion, however the significance of our findings for year one of this study prove promising in demonstrating the potential benefits of a cowpea summer cover crop in the sandy loam soils of the Mid-Atlantic Region.

Following the results of our study as well as others before it, we conclude that more studies are needed to determine the viability of using sorghum-sudangrass as a summer

cover crop prior to planting a fall vegetable crop. From the literature reviewed in this paper as well as the results from our own study, it appears that when used as a summer cover crop, sorghum-sudangrass has the potential to detrimentally affect a fall lettuce crop due to the likely immobilization of nitrogen in the soil as a result of the high biomass production. It therefore recommended that farmers consider supplementing with nitrogen fertilizer or mixing with another leguminous cover crop if they plan to plant immediately following a sorghum-sudangrass cover crop in order to lessen the probability of nitrogen being tied up in the microbial biomass. Alternatively, as was mentioned above, when using sorghum-sudangrass as a summer cover crop, farmers may want to consider waiting to plant a subsequent cash crop until the following spring in order to allow time for the sorghum-sudangrass plant residue to be broken down by soil microbes and for its plant available nitrogen to be released.

The aim of this study was to connect data on the effects of cover crops on soil nutrients and crop yield with their effects on the soil microbiome in an effort to close the knowledge gap in the connections between management practice and soil biological community and function on agricultural soils. However, the microbiome analysis has not been completed. Therefore, the relationship between microbiome structure and soil biological and chemical characteristics is yet to be determined.

D. Next Steps

A recommendation for future iterations of this study or others is to do an in-field comparison soil incubation study using aerobic soil sample incubation bags left in the field in order to assess how accurately the laboratory incubation study models the nitrogen transformations and nutrient availability out in the field. It would also be beneficial to take soil respiration measurements at the time of each extraction throughout the soil incubation study in order to correlate soil respiration with nitrogen availability.

One further recommendation for future studies would be to include a positive fertilizer control using the recommended nitrogen fertilizer rate for leaf lettuces of 100 – 125 lbs N acre⁻¹. This would help to assess how the lettuce crop grown in the cover crop treatments in this study compares with that of a typically fertilized field (VCE, 2017).

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TABLES

Table 1: Cover crop biomass and nitrogen incorporation from plant tissue analysis.

Cover crop	Biomass (Mg·ha⁻¹)	N-CC^z (%)	N-Weed (%)	Total N (kg·ha⁻¹)
Cowpea	8.1 b ^y	3.1a	2.6 a	227 a
Sorghum	22.1 a	1.3b	1.6 a	286 a
No Cover	1.4 c		1.6 a	19 b

^z N-CC = cover crop nitrogen content, N-Weed = weed nitrogen content

^y All numbers within a column followed by the same letter are not statistically different at a probability level of 0.05.

Table 2: Soil organic matter and N content.

Cover crop	OM^z (%)	TOC (%)	Total N (ppm)	C/N Ratio
Cowpea	1.23 a ^y	0.72 a	367 a	21.4 a
Sorghum	1.17 a	0.68 a	373 a	18.3 a
No Cover	0.97 b	0.56 b	310 a	18.2 a

^z OM = organic matter, TOC = Total organic carbon, Total N = total nitrogen, C/N= TOC to total N ratio.

^y All numbers within a column followed by the same letter are not statistically different at a probability level of 0.05.

Table 3: Soil Fertility

Cover Crop	NO3-N (ppm)	P (ppm)	K (ppm)	Mg (ppm)	Ca (ppm)
Cowpea	9.3 a ^z	91 a	151 a	54 a	297 a
Sorghum	1.0 c	107 a	147 a	50 a	302 a
No Cover	4.7 b	88 a	116 b	37 b	257 a

^z All numbers within a column followed by the same letter are not statistically different at a probability level of 0.05.

Table 4: Soil Respiration Rate across treatments (measured in-field).

Treatment	Respiration Rate (kg CO₂•ha⁻¹•d⁻¹)
Cowpea	314.29 a ^z
Sorghum	277.45 ab
No CC	100.52 b

^z All numbers within a column followed by the same letter are not statistically different at a probability level of 0.05.

Table 5: Lettuce growth and production.

Fresh weight	NC Yield (Mg*ha⁻¹)	RC Yield (Mg*ha⁻¹)	NC Fresh Weight (g)	RC Fresh Weight (g)	Vertical^y Comparison	Horizontal Comparison
Cowpea	1.73	5.38	39	121	a ^z	***
Sorghum- sudangrass	0.27	0.67	6	15	b	NS
No cover crop	1.73	3.73	39	84	ab	***
Leaf area	NC (cm²)	RC (cm²)				
Cowpea	610	1821			a	***
Sorghum- sudangrass	136	298			b	NS
No cover crop	658	1336			ab	***

^y **Vertical comparison analyzes differences in fresh weight and leaf area between three cover crop treatments; horizontal comparison analyzes differences between row cover (RC) and no cover (NC) for each treatment.**

^z **All numbers within a column followed by the same letter are not statistically different at a probability level of 0.05.**

Table 6: Compiled Data.

	Cowpea	Sorghum Sudan
Soil OM (%)²	+ ^w	+
Soil TOC (%)²	+	+
Soil Total N (ppm)²	0	0
Soil C/N Ratio²	0	0
Cover crop Biomass (g·ha⁻¹)¹	+(b) ^x	+(a)
Cover crop Total N (kg ha⁻¹)¹	+	+
Soil NO₃-N (ppm)³	+(a)	-(c)
Soil P (ppm)³	0	0
Soil K (ppm)³	+	+
Soil Mg (ppm)³	+	+
Soil Ca (ppm)³	0	0
Soil respiration rate (kg CO₂ ha⁻¹ d⁻¹)⁴	+	0
Lettuce fresh weight (g)⁵	0a	0b
Leaf area (cm²)⁵	0a	0b
Soil Nitrate production (mg N/L)^y	+(a)	-(c)
Soil Ammonia production (mg N/L)^z	0	0

^w + signifies values that are significantly greater than that of the no-cover control for that parameter, - signifies values that are significantly less than that of the no-cover control for that parameter; **0** signifies no significant difference between the treatment and the no-cover control for that parameter.

^x All parameter symbols within a column followed by different letters significantly differ from one another at a probability level of 0.05.

¹ Table 1

² Table 2

³ Table 3

⁴ Table 4

⁵ Table 5

^y Figure 1

^z Figure 2

FIGURES

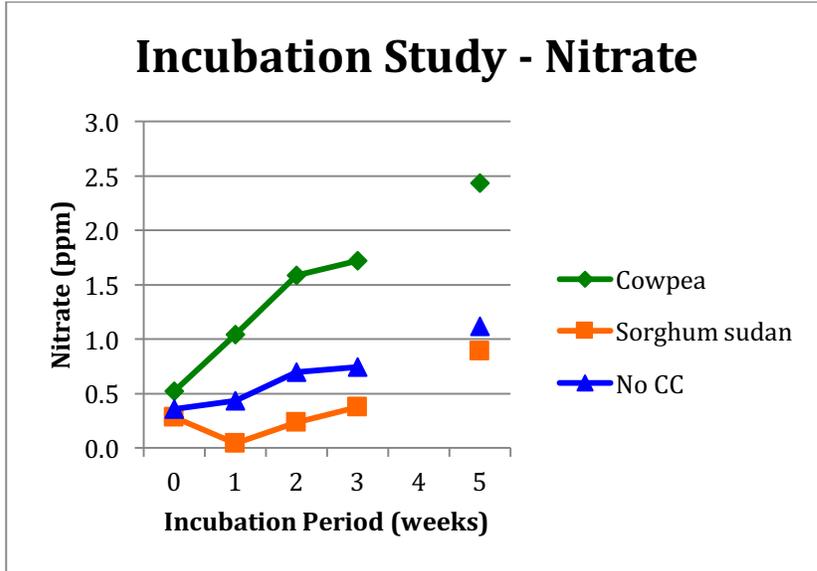


Figure 1: Nitrogen mineralization rate under laboratory conditions.

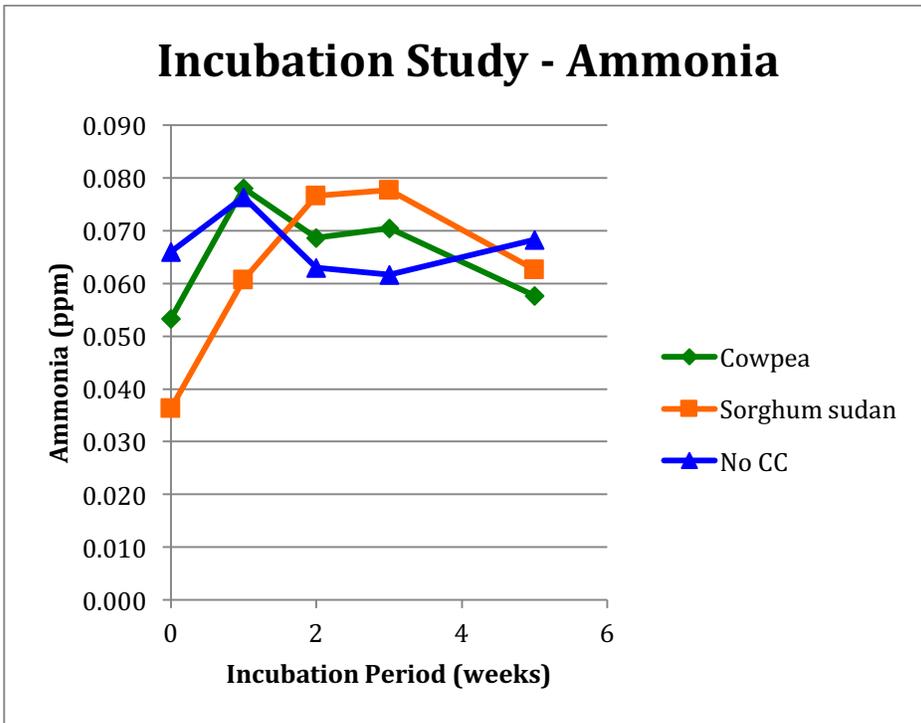


Figure 2: Ammonia production under laboratory conditions.



Figure 3: In-Field Soil Respiration Measurement Set Up.