Tillage, Cover Crop and Nitrogen Fertilization Effects on Soil Microbial Carbon Dynamics under Long-term Cotton Production

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Abstract

Effective soil management requires a deeper understanding of how internal soil carbon (C) works. This is vital as cycling is tightly linked with nutrient cycling processes. This study evaluated the effect of agronomic practices including tillage (no-till and tilled), cover crops (no cover, hairy vetch - *Vicia villosa L*, and winter wheat - *Triticum aestivum. L*) and different N fertilizer rates (0, 34 and 101 kg N per hectare) on soil microbial C dynamics as measured by soil microbial biomass C (SMBC) along with soil microbial respiration over the 2013 cotton growing season at Jackson, West Tennessee. The study was set as split -split plot with N fertilizer rates as the whole plot, cover crops as the split plot and tillage as the split-split plot. Sampling was carried out four times; in June, July, September and October. Both SMBC and soil respiration responded similarly to the treatment factors. Early in the season, June and July, N fertilizer rate affected SMBC with 101N fertilizer rate having greater SMBC. In July, in addition to N fertilizer rate, tillage affected SMBC with tilled-vetch treatment at 34N and 101N fertilizer rate having a significantly greater levels of SMBC while soil respiration was greater under the no-till no cover treatment across all N fertilizer rates. However in September, tillage and cover crop affected SMBC and soil microbial respiration. The no-till no cover and no-till wheat treatments had greater SMBC and soil microbial respiration compared with no-till vetch at 0N and 34N fertilizer rate. At the end of the growing season prior to harvest, the no-till no cover treatment still had effect on soil microbial respiration while none of the treatment factors affected SMBC. Early in the season N fertilizer influences microbial activities while later in the season tillage and

cover crop become the dominating factors. Overall the effects of N fertilizer, tillage and cover crops on these soil properties were season dependent.

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CHAPTER I
Introduction and General Information

Nitrogen (N) is often the limiting nutrient in many cropping systems. For resource-poor farmers in developing countries too little or no N fertilizer application results in lower yields. The lower N fertilizer application rates in these systems and corresponding low yields results in the expansion of agriculture into marginal lands (extensive agriculture), the consequences of which is massive soil loss through soil erosion and overall land degradation. However, in developed countries, excessive N application results in environmental degradation including stream eutrophication and subsequent fish death due to dissolved oxygen depletion and a decrease in aquatic biodiversity (Breitburg et al., 2009).

Resource-poor farmers are challenged by financial constraints to obtain adequate quantities of fertilizer to achieve optimum yields. There is also a paucity of information on the use of fertilizers and the appropriate fertilizer rates for optimum yields. These challenges lead to food insecurity. Many Sub-Saharan countries like Lesotho are able to produce only 30% of their food (Turner, 2009) and the remaining food deficit must be imported. Since most of the families derive their livelihood from agriculture, and the majority of the household members are illiterate, there are few means outside of agriculture through which these households can supplement the shortage of food production (Silici et al., 2011).

For developed countries, the challenge is to reduce the amount of N lost from their agricultural ecosystems. Randall et al. (2010) reported that farming systems in the United States and Europe lose approximately 30 to 35 kg N ha^{-1} $year^{-1}$ while China

loses nearly five times as much (149 kg N ha^{-1} $year^{-1}$) due to high N fertilizer application rates of up to 800 kg N ha^{-1} $year^{-1}$ (Li et al., 2007). While environmental degradation including ground water contamination and eutrophication caused by these N losses are unacceptable, other economic losses are daunting. Baligar et al. (2001) estimated the worldwide economic losses of about 26.0 to 36.4 billion US dollars from N fertilizer loss through either leaching or dinitrification from agricultural ecosystems.

Due to low yields that result from inadequate application of N in resource-poor farming systems and the environmental degradation and economic losses that result from the N losses in the resource-rich farming systems, it is vital to seek more sustainable approaches to N management. Harnessing biologically fixed N would help improve the yields in N-limited farming systems and reduce the fertilizer applied in the systems receiving excessive quantities. As an example of how the biologically fixed N could be beneficial to crop yields and perhaps reduce excessive fertilizer application, Tanzanian farmers were able to see an increase in maize production from 2.5 t to 7 t ha^{-1} from the biologically fixed N by incorporating the leguminous crops in their cropping systems without any additional chemical fertilizers use (Owenya et al., 2011). This increase in yield seen by Tanzanian farmers is an indication that the cropping systems applying excessive N could still maintain high yields if they could reduce the amount of N applied by including legumes into their cropping systems.

The best solution for over and under application of N fertilizer would be that which could bring together different management practices that would add N into the soil, reduce its loss and maintain its cycling and retention within the soil system. Conservation agricultural practices including reduced tillage, cover cropping and crop rotation could

be that best management practices to help achieve N fertilizer use efficiency (Kassam et al., 2009). Cover cropping through the use of leguminous crops could be the soil source of N. Minimum soil disturbance may reduce the rate at which soil organic matter is mineralized and thus reduce N loss while crop rotation could facilitate N cycling and retention in soil. Nitrogen fertilizer additions increase in the above ground crop biomass which tends increase soil organic C (Halverson et al 1999; 2002). According to Delgado (2002), if cropping systems are to benefit from such management practices, they should be adopted in the light of N-cycling; taking into consideration different pathways, flows and mechanisms for gains and losses. Thus it is important to identify the key controlling factors for these processes within the conservation agricultural practices.

Microbial populations are major players in the nutrient cycles and biogeochemical transformation as a whole as they make nutrients available to crops. Thus it is critical to assess soil microbial biomass as a whole unit (soil microbial biomass carbon (C)) along with its activity as measured by soil microbial respiration in order to determine how they are affected by soil management practices. However, their activity is tightly controlled by soil environmental factors. Thus it is necessary to determine soil microbial biomass and its activity especially in how it interacts together with soil environmental factors. Furthermore, it is critical to assess these microbial dynamics of C over the growing season in order to determine how they are affected at different crop growth stages.

Literature review

Nitrogen Cycle

Though N is available in abundance in the atmosphere as di-nitrogen gas (N_2), this form of N is not available to most crops except legumes. Leguminous plant species through associations with N-fixing bacteria (bacteria –rhizobium) and some free living prokaryotic organisms such as *Azotobacter, Clostridium* and *Cyanobacteria* (Delgado, 2002) are able to fix atmospheric N_2. These microorganisms play a crucial role in the transformation of N and hence make it available to other crops and organisms. This transformation of N—the N Cycle— (Figure 1) is one of the most important biogeochemical cycles in agriculture and other ecosystems. The N cycle has the three major steps; N fixation and N mineralization, nitrification and dinitrification. Nitrogen fixation is the process by which N_2 is converted biologically into NH_4^+ by certain groups of microorganisms (N_2- fixers) using a nitrogenase enzyme and N mineralization is the release of inorganic N (NH_4^+) from the decomposition of organic matter. The second step is nitrification which results in the conversion of NH_3 or NH_4^+ into NO_2^- and finally into NO_3^-. The group of organisms called the *Nitrosomonas spp.* is responsible for the first step and the second step is mediated by the group called *Nitrobacter spp.* Though there are intermediate steps in nitrification, the third and last step is referred to as denitrification that results in the reduction of NO_2^- and NO_3^- into gaseous forms of N such as NO, N_2O, N_2. This step is mediated by a wide range of genera of microorganisms including but not limited to *Pseudomonas, Azospirillum,*

Flavobacterium, Paracoccus and Agrobacterium. All of these steps are governed by soil environmental conditions.

Because N-cycling is mediated by different microorganisms, it is crucial for the farmers and land managers to understand the different environmental controls of these organisms and processes they mediate and how different agricultural or land management practices could affect them. Understanding these interactions will help with the identification of the best management practices which will enable food security, sustained use of soils, while at the same time reducing environmental degradation and pollution.

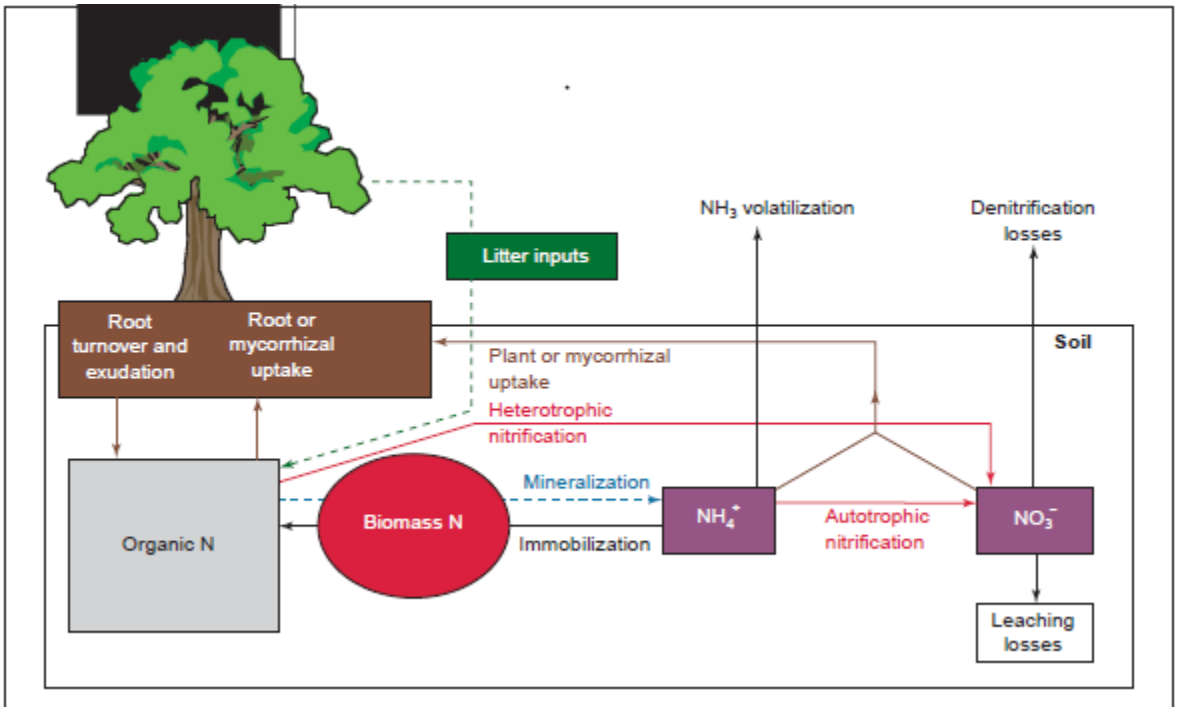


Figure 1. N cycle highlighting the main pools of N. The boxes are main pools while arrows show fluxes. The solid arrows are flux rates while the broken ones are slow rate fluxes which are limiting steps for the cycle. (adapted from Hodges et al., 2000)

The predominant form of N in soils is organic N which, to most crops and a few specific groups of microorganisms, is considered to be unavailable (Schimel and Bennett, 2004). To be available to crops and some microbial groups, N should be in an inorganic form (Hodges et al., 2000) though Chapin et al. (1993) and Jones and Kielland (2002) argue that some forms of organic N such as amino acids are plant available. The availability of soil inorganic N is made possible by the decomposition of organic N by heterotrophic soil microorganisms. This process results with the release of ammonium through mineralization which will afterwards be converted into nitrate through nitrification by ammonium oxidizing bacteria (Myrold et al., 2011). Generally, this process is considered to be the rate-limiting step in the N cycle (Martens-Habbena et al., 2009) and the ammonia oxidizing bacteria are the organisms controlling this process. Both ammonium and nitrate are available to plants but uptake of either depends on the internal plant transport system. Following absorption of nitrate much energy is needed to reduce nitrate to ammonium that is needed for peptides and amino acids formation. Due to this energy cost Jackson et al. (2008) suggest plants prefer to take up ammonium but the higher concentration of nitrate in the soil and because of its higher mobility, nitrate is likely to be taken up by the plants in greater concentrations (Jackson et al., 2008).

Best management practices to increase N use efficiency

Cropping systems that aim at building soil organic matter, reducing tillage intensity and maintaining soil cover may help with soil nutrient retention and cycling while also helping to reduce the amount of fertilizer applied (Fageria et al., 2005). Conservation

agriculture (CA) is such a system. Kassam et al. (2009) define CA as a cropping system based on the three principles: reduced soil disturbance, crop residue retention, and crop rotations. CA increases soil organic matter content in the soil surface layer, improves soil aggregation, and through increased biological activities can stabilize the soil structure (Oades, 1993) and improve soil resilience (Kenny and Smith, 1995; Nannipieri et al., 2003). According to Kassam et al. (2009), the driving force of sustainable soil systems is its biological component that ensures the formation of soil humus, nutrient cycling and improvement of soil structure (Tisdall, 1991).

Though these CA principles can exhibit intertwined benefits, each could have a standalone benefit. In the case of N addition to cropping systems, especially in areas where fertilizers are scarce and cost is a prohibitive factor, leguminous cover crops could be used to supplement N. Seo et al. (2000) in Japan showed that a hairy vetch (*Vicia villosa)* cover crop can supply approximately 50 -150 kg N ha^{-1} to the following crop in the subsequent cropping season. Velvet been (*Mucuna pruriens)* was also shown to contribute 50 to 200 kg N ha^{-1} from biological fixation in West Africa (Carsky et al., 2001). Delgado et al. (2010) with the use of ^{15}N reported higher N use efficiency from the use of cover crop residues (87%) than from the use of inorganic fertilizers (69%) showing that cover crops provide higher N recovery than the inorganic fertilizers. Nitrogen use efficiency is defined as the amount of N inputs that are incorporated into the harvestable dry matter and or recovered from harvestable biomass and incorporated into soil organic matter and in inorganic pools through residues (Delgado et al., 2010) Although cover crops like hairy vetch show potential for recovering N from the soil, other cover crops like ryegrass (*Lolium multitiflorum L.)* tends to immobilize it. Clark et al.

(2007) reported that a ryegrass cover crop will need approximately additional fertilizer of 10 to 50 kg N ha^{-1} for establishment.

In addition to N fixation, cover crops could provide other benefits to the soil as well. They can reduce the amount of N lost through leaching (Rasse et al., 2000). The prevention of nitrate leaching by cover crops from the soil also enhances nutrient cycling by accumulating the organic N and releasing it through decomposition to the next crop (Dinnes et al., 2002). Cover crops also help to conserve soil moisture by reducing evaporation rate through their canopy during their growth and by residues left on the soil surface after their termination. The reduction of evaporation rate from the soil helps to maintain the moisture for the current growing crop and also preserve moisture to the subsequent crop in the following cropping season (Fageria et al., 2005). Through their rooting network and released exudates, cover crops could provide an excellent habitat for the soil microbial population that will improve soil structure (Fageria, 2002). The improved soil structure will increase water infiltration, aeration and reduce soil erosion potential.

Soil microbial biomass, concept and measurement

Microbial biomass is defined as the living portion of the soil organic matter with the exclusion of plant roots and soil organisms greater than 5 x 10^3 μm^3 (Gonzalez-Quinones et al., 2011) and is a primary factor for any soil decomposition process (Wardle, 1992). When referred to as soil microbial biomass carbon (C) (SMBC) and / or soil microbial biomass N, this reflects only that portion of C and or N mass within the microbial cells (Gonzalez-Quinones et al., 2011; Dwivedi and Soni, 2011). The

acquisition of either C or N into microbial cells is through the decomposition of organic materials within the soil and that carbon serves as an energy source for both growth and maintenance of microbial populations while N is used for the synthesis of amino acids and proteins (Smith and Paul, 1990; McGill et al., 1986). Therefore the growth and functioning of the soil microbial biomass is usually limited by the amount of readily available C (Wardle, 1992).

The quantity of SMBC in soil is a function of C inputs into the system and is thus strongly dependent on the soil organic matter (Zhang et al., 2005). Soil management practices that maintain and facilitate soil organic matter build up tend to have higher SMBC compared to those with lower soil organic matter contents. The main source of organic matter additions to soil is crop residues that are left on the soil surface to decompose. Zhang et al. (2005) saw an increase in the SMBC when the soil was amended with the crop residues from the harvest. The amount of C and or N that is assimilated into the microbial biomass during decomposition will thus be reflected in the SMBC soil microbial biomass N (SMBN). Much of the C or N that is assimilated comes from readily decomposable materials such as glucose, hemicellulose and amino acids.

Though SMBC forms a minute percentage of the soil organic matter (1 to 4 percent) (Brookes, 2001; Wardle, 1992; 1998), it is the most important fraction of soil organic matter when considering soil fertility and nutrition for the production of crops. This organic matter fraction conserves nutrients in the biologically active pools and due to its higher turnover rate those nutrients may become available to crops (Brooks, 2001). Smith, (1990) reported a negative correlation between soil microbial biomass N and N plant uptake and the interpretation he provided for this was the microbial biomass N

was itself the pool being mineralized. Since most of the organic materials pass through the microbial biomass during the decomposition process, the microbial biomass is therefore the agent of nutrient transformation in the soil. The microbial biomass facilitates soil organic matter stabilization which will in turn impart its attributes to the soil including increased soil aeration, enhanced water and nutrient retention and thus the overall soil health and quality (Dwevedi and Soni, 2011). The nutrients that are assimilated into the microbial biomass during the decomposition process, which would have otherwise been lost by being part of the soil solution, become stored in the biological forms (immobilization) and are made available later in the growing season when crop residues would be decomposing. It is from this virtue of mineralization - immobilization cycle that the soil microbial biomass is regarded as both a source and sink for nutrients (Brookes, 2001; Dwevedi and Soni, 2011). This cycle of mineralization –immobilization by the soil microbial biomass becomes even more important in the low input systems where mineralization is the main source of nutrients (Brookes, 2001). Singh et al. (1989) saw an increase in N mineralization to coincide with the decrease in soil microbial biomass N and concluded that the mineralized N was released from the microbial biomass. Brookes (2001) in a Kenyan Ultisol observed increased maize yields under treatments which had higher SMBC. Thus it is crucial to adopt the management practices that enhance the soil microbial biomass.

After the adoption of soil conservation management systems or after the restoration of degraded lands, it becomes prudent to assess the performance of the management practice. The performance of such management systems is measured through certain indicators such as soil organic C which is important in many soil processes (Gonzalez-

Quinone et al., 2011). However, it takes time before there could be any noticeable changes in the concentrations of soil organic C after the adoption of certain management practice or during the restoration process (Brookes, 2001; Dwevedi and Soni, 2011). As a result is suggested that SMBC could be used as one of the indicators of changes in soil microorganism population or biotic activity before that change could be measured in soil organic matter and other soil chemical or physical properties (Dalal, 1998; Carter et al., 1999; Gonzalez-Quinone et al., 2011; Dwevedi and Soni, 2011). Powlson et al. (1987) after 18 years of straw usage in a field study saw an increase of about 50 percent in SMBC while soil organic C remained fairly constant without much change. Regardless of its value in nutrient cycling,, the quantities of SMBC or SMBN remain a controversial issue in terms of what the observed changes imply (Gonzalez-Quinone et al., 2011). The concern arises from the fact that the SMBC responds differently under different management practices, different soil types and different climates. Gonzalez-Quinone et al. (2011) see this as an issue because there are no benchmark numbers for SMBC or SMBN that the management practices should aim at achieving through such management practices. However, since in agricultural land use the most important thing is the retention and cycling of nutrients and thus their availability, greater microbial biomass levels are assumed to be beneficial.

Seasonal effect on SMBC

The seasonal effect on SMBC – combined effects of temperature, soil moisture, day length and crop growth stages - could have a direct or indirect effect on SMBC. The direct effects come through the temporal fluctuation on soil environmental properties such as soil temperature and soil moisture while indirect effects come through the

influence of plant or crop growth stages (Speeding et al., 2004). Soil temperature and moisture affect SMBC through increased activity, with greater and optimum conditions (temperature and moisture) resulting in increased microbial activity. Though not universal, increased microbial activity may result in greater microbial population size which could lead to increased SMBC size. Bardgett et al. (1999) reported greater levels of SMBC and SMBN in summer relative to other times in the season and they attributed this to the increased soil temperature which increased soil organic matter decomposition rate. However, Speeding et al. (2004) argued that temperature could have an effect on the microbial activity which changes microbial composition instead of the microbial biomass size. Through the use of the phospholipid fatty acids (PLFA) analysis they found that during the hot summer months, only certain microbial communities were affected but the microbial biomass as measured by SMBC remained constant. According to Paul and Smith (1990), the seasonal effect may not impact SMBC because the biomass may respond to the plant growth over the growing season. Early in the growing season the microbial biomass may utilize the C from the decomposing residues from the winter growing season and when that source becomes depleted the root exudates of the growing plants become the microbial C source. Finally, at the end of the season the decomposition of the old plant roots becomes the main C source (Speeding et al., 2004). This sustained level of C input into the soil could be the result of the lack of variability of the SMBC over the season. According to Smith and Paul, (1990), the only time that the SMBC may increase could be as a result of flushes of C input into the system. However when the system gets depleted of that C source, the SMBC size will not return to the levels that it was prior to the C input as

SMBC size might have doubled and any further additions of C could sustain those new levels. In order to have a pronounced cumulative effect on SMBC, microbes would require sustained C inputs in contrast to sporadic C source inputs (McGill et al. (1986).

N effect on SMBC

Nitrogen and C are the major controls of microbial activity, composition and size. Though C availability in most cases contributes positively to microbial communities, N can either have a negative or positive contribution. This ambiguous effect of N additions on SMBC, especially in the form of inorganic fertilizer can be through different mechanisms. N fertilizer can increase the net plant productivity (Treseder, 2004; He et al., 2013) which in turn through crop residue when returned to the soil may positively affect the soil microbial biomass. The crop residues could form part of the C source and provide energy to the soil microbial communities hence increase the SMBC size. Zhang et al. (2005) observed increased SMBC in Chinese grassland soils due to N fertilization for over 2 years. According to VitousekA and Howarth, (1991) N availability can strongly influence microbial growth and abundance.

However N fertilization can also reduce the plant's production of fine roots which are critical for nutrient acquisition and as such reduce the plant's soil C addition through rhizodeposits (Treseder, 2008). This corroborates He et al. (2013) who observed the plant's below ground biomass to decline with increasing N fertilization rate. In addition, osmotic potential in soil solution as a result of introducing additional ions through N fertilization could become toxic to microbes (Treseder, 2008). Over time nitrogen fertilization can result in increased H^+ activity (soil acidity) which will lead to leaching of

basic cations (Barack et al., 2007; Schroder et al., 2011). The leached basic cations will result in increased aluminum (Al) and or boron (B) concentrations which could be toxic to soil microbes.

Nitrogen fertilization can also affect microbial populations and functioning indirectly through the lack of C availability. High N availability tends to affect the activity of soil organic matter decomposing enzymes (Fontaine et al., 2004; Kuzyakova, 2010). An inactivation of ligninase by fungi may affect C availability from the organic materials in soils. Lignin can be found interwoven with other C compounds such as cellulose and, as a result the presence of lignin (due to ligninase inhibition), may prevent soil microbes access to other readily available C sources such as cellulose (Treseder, 2008). All these factors resulting from N fertilization act together to either positively or negatively affect soil microbial biomass size and microbial function. Thus within agricultural ecosystems, we could either expect higher levels of soil microbial biomass which will facilitate N cycling and retention or lower soil microbial biomass which means higher turnover of biomass and thus loss of nutrients from the soil system.

Tillage effect on SMBC

Tillage systems that promote the soil organic matter accumulation usually result in greater SMBC size (Kaschuk et al., 2010). Under tillage systems where soil organic matter exists in greater levels, some of the soil biogeochemical processes (C and N mineralization) occur at relatively slow rates thereby allowing soil microbial biomass to grow. No-till maintains crop residues on the soil surface and periodically keeps replenishing soil organic matter through crop residue additions. This environment is

conducive for soil microbial biomass to grow and increase in size. On the contrary, conventional tillage accelerates organic matter decomposition. Granatstein et al. (1987) observed no-till to have greater SMBC than a comparable tilled system in both absolute numbers and as a proportion of total C. This observation was attributed to the higher levels of crop residues under no-till which consists mainly of fresh organic matter which is less humified compared to one under a tilled system which is more humified. Wright et al. (2005) observed no-till to have higher SMBC than tilled systems within 0-2.5 -cm and SMBC had a strong positive correlation with soil C. Thus SMBC is a reflection of soil organic matter.

Tillage affects soil physical properties and thus influences soil microbial biomass size (Wright et al., 2005). No-till promotes soil aggregate development which tends to protect soil microbes from predation by soil fauna and creates a buffer against extreme environmental fluctuations that could negatively affect the soil microbial biomass. These factors result in the accumulation of microbial biomass under no-till as opposed to tilled systems. However, Six et al. (2004) argued that the protection of soil organic matter or soil microbial biomass by soil aggregates depends on the turnover of the aggregates which is a function of how stable the soil organic matter is. They maintained that stabilized organic matter will lead to a slow turnover rate of soil macroaggregates and thus will protect both the soil organic matter and microbial biomass from degradation. Hernandez and Hernandez (2002) observed no-till to have higher SMBC within the soil aggregates than tilled systems. In fact the minimal disturbance of soil by no-till promotes the fungal hyphal network which is the main component of soil aggregate formation (Oades, 1993). The lack of soil disturbance under no-till will allow fungal hyphae to form

macroaggregates from microaggregates. This may result in occluded organic matter within the micro-and macro-aggregates and leading to greater fungal biomass under no-till systems. All these factors pertaining to tillage suggest that microbial biomass size is not only affected by crop residues but it is also a function of soil aggregates. Therefore soil aggregates are not only important in facilitating soil aeration and water movement within the soil but are also important in occluding C from microbial degradation.

Methods for determining soil microbial biomass

There are a number of methods that can be used to determine soil microbial biomass. These methods include chloroform fumigation – incubation (CFI) (Jenkison and Powlson, 1976); chloroform fumigation – extraction (CFE) (Vance et al., 1987); substrate induced respiration (Anderson and Domsch, 1978) and simultaneous chloroform fumigation extraction (sCFE) (Feirrer and Schiemel, 2002). For CFI, the soil is fumigated with chloroform for 24 hours, flushed repeatedly to remove the chloroform, amended with fresh soil and then left to incubate for 10 days. The fresh soil will act as an inoculum and will decompose the chloroform-killed microbes resulting in CO_2 evolution. The released CO_2 is assumed to be coming from the chloroform-lysed cells and as such is taken as a proxy for SMBC size. In CFE, soil is fumigated with the chloroform vapor for about 24 hours and will thus release a flush of C or N from the dying microbes. The released C and or N will then be extracted with a strong salt solution (2M KCl, 0.5M K_2SO_4). The increased extractable C or N from the fumigated soil relative to the control soil will thus be taken as the measure of the SMBC or SMBN. The sCFE method applies the chloroform directly into soil as liquid and allows the soil to shake for a certain period of time (4 hours). During the shaking process the chloroform

will lyse the microbial cells and release C and or N into the solution. The released C and or N will subsequently be extracted with a strong salt solution (2M KCl, 0.5M K_2SO_4). The difference in the extracted C or N between the chloroform exposed soil and the control soil represent the SMBC or SMBN. The substrate-induced respiration method operates on the basis that after incorporation or addition of a labile organic substrates, the amount of CO_2 that will be liberated would be elevated relative to the soil which is not amended with substrates and the difference in CO_2 between the substrate-amended soil and the control represents the microbial biomass pool (Martens, 1995).

All of the chloroform fumigation methods, including both incubation and extraction, are based on the premise that chloroform fumigation of soil samples will kill and lyse the microbial cells and result in a flush of either C or N. The difference in C or N concentration between the fumigated sample and non-fumigated sample is assumed to be due to the lysed microbial cells and thus represent SMBC or SMBN. Assumptions coupled to this process include that: the chloroform completely kills all the microbes in the samples; the flush of either C or N from the fumigated samples is greater than that of the non-fumigated samples; the flush of C and N from the dead organisms in both fumigated and non-fumigated samples will be equal; the portion of microbial cells killed by chloroform is the same across all soils, regardless of sampled geographic region, hence the use of similar extraction efficiencies; and that chloroform fumigation has no effect on soil except for microbial cell killing (Martens, 1995). However, some of these assumptions fail to hold under certain conditions, especially when the soil receives a large input of organic materials. Under these conditions, an assumption that a fumigated

sample should have a greater flush of C or N than the un-fumigated does not hold resulting in soil microbial biomass calculations becoming negative (Martens, 1995).

Although the chloroform fumigation methods are based on the same principle, each method has its own pitfalls. CFI fails to provide accurate results under acidic soils where certain fractions of the organisms (bacteria) are not active and their development as an inoculum within the incubation period is restrained. Also the CFI method is limited within a certain range of soil moisture contents requiring moisture adjustment before fumigation. The other issue is that CFI is not applicable in soils with recent additions of organic materials as the amount of C that is released could be from the decomposition of the amended materials as opposed to C resulting from the dead microbial biomass (Martens, 1995).

Due to the above mentioned problems associated with CFI, CFE is sometimes used but it is not without flaws either. The extraction efficiency that is used to convert the flush of C released from the lysed cells into microbial biomass calculations has a wide range of variation as it is estimated from different soils which can lead to conflicting results. The chloroform vapor could adsorb to the soil colloids and the subsequent extraction with the salt may have higher C levels that are not coming directly from the lysed cells but from the chloroform itself (Alessi et al., 2011).

The sCFE could have the same concerns as the CFE, that the aqueous chloroform that is added to the soil and together with the ethanol that is used to stabilize it may be adsorbed onto clay particles and be extracted with the salt and thus overestimates SMBC. The major advantage that sCFE has over the CFE is that it is less time

consuming and could allow the processing of many samples within a short time period. Moreover, sCFE can be used with saturated soils that would not allow the diffusivity of chloroform when using the CFE.

Microbial biomass C and N are major component of organic matter decomposition, and as such are critical factors in the nutrient transformation processes. Thus SMBC along with other readily available forms of C including extractable forms of organic C and soil microbial respiration that act as energy sources can be studied together in order to determine how this energy facilitate nutrient cycling in agricultural ecosystems.

Extractable Organic C (EOC)

Extractable organic C is a fraction of the soluble organic C (Ros et al., 2009). This organic C fraction consists of dissolved organic C together with other organic compounds that become soluble during the soil extraction process originating from the microbial biomass and particulate organic matter. Since EOC consists of dissolved organic C it is therefore understood to be part of the C that is readily available to microorganisms (Jandl and Sollins, 1997). Though EOC is part of the soluble organic C, it is operationally defined as the extractable C. Xiang et al. (2008) maintains that it should be referred to as EOC since its concentration depends on how the soil was processed (field moist or dry) during extraction and which extractant was used. It can be extracted with several solutions including water (hot or cold), salt (KCl, $CaCl_2$ and K_2SO_4) and other extractants (Murphy et al., 2000). As an implication of how soil processing and extractant affects the EOC or EON concentration, Choudhary et al. (2013) found hot water to extract more C than cold water while Murphy et al. (2000)

found the $CaCl_2$ to have greater extraction efficiency on EON than KCl. These results indicate the importance of quality analysis and control procedures regardless of chosen extractant.

Since EOC consists of several fractions of organic C with a wide degree of lability, there is a lack of consensus on how bioavailable it is to microorganisms. To settle this issue, there have been studies that aimed at determining its bioavailability. Nelson at el. (1994) in soils under soybean production found the proportion of EOC that was bioavailable to be 22% while Gron et al. (1992) found it to be 11% from the groundwater and Ghani et al. (2003) found it to be above 50 %. Extractable organic C content in soils under sugar beet production that was found to be bioavailable was 85% (Zsolany and Steindl, 1991). These studies show that EOC is not entirely bioavailable, but rather a certain fraction of it is bioavailable; which means that indeed it is an energy source for microbes

Because EOC and EON are bioavailable and thus a microbial energy source, these two components could theoretically control N and C cycling. Ghani et al. (2003) found that water-extracted EOC had a positive correlation with both SMBC and SMBN. They also found that EOC had a high degree of association with potentially mineralizable N which is considered an indicator of both microbial- and plant-available N. In addition, Ros et al. (2009) found EON to be positively correlated with total soil N. According to Schulz et al. (2011) EOC can be used as an indicator for decomposable organic C that results in nutrient release during decomposition. Sparling et al. (1998) reported EOC to be positively correlated with microbial soil respiration as further indication of its importance in C cycling. Although there is a high degree of relationship between EOC and some of

the soil properties and processes that are related to nutrient cycling, the variability of EOC values as determined by different extractants merits further attention. This would become even more important if EOC is to be used in nutrient cycling studies (Ros et al., 2009). Furthermore, it is imperative to report explicitly in the literature which EOC method was employed because the resulting values are method-dependent.

Microbial soil respiration

Soil microbial respiration is a measure of CO_2 production that results from the decomposition of soil organic matter by soil microbes. Soil microbial respiration is one of the indices of soil quality as it can be used to infer the level of soil functionality or general microbial activity. The CO_2 production rate from the soil is a function of soil organic matter level content and some soil environmental conditions including soil temperature and moisture (Yuste et al., 2007). There are two types of soil respiration that can be measured experimentally; basal soil respiration and substrate induced respiration. The former is used to measure the level of soil organic matter and its lability while the latter is used to measure the general microbial activity.

In the context of agriculture, soil respiration can be used as a proxy for nutrient levels within the soil organic matter and can also be used as a measure of soil's capacity to cycle nutrients and its ability to sustain plant growth. Central to this process are the soil microbes that mediate the process in an attempt to gain energy. The energy is gained from C molecules, and in the process of degrading the soil organic matter nutrients (like NH_4^+, PO_4^{2-} , and SO_4^{2-}) are released into the surrounding environment or assimilated

into microbial cells. The nutrient of importance to this study- N- is assimilated into microbial cells until the cells' N requirements are satisfied. Once the microbial N demand is satisfied for the generation of energy and biomass formation, nutrients will be released into soil to become part of the plant available nutrients (mineralization). Throughout the whole process of decomposition and nutrient assimilation, there will be a continuous release of C as CO_2 (Coleman et al., 2004). This release or evolution of CO_2 is regarded as C mineralization. Thus the C and N cycle are tightly linked and are therefore studied together in nutrient cycling studies. According to Zak et al. (1993), N mineralization and soil microbial respiration are often correlated.

Soil microbial respiration can be measured in the field or in the laboratory. Measuring soil respiration in the field can be challenging to interpret because it includes microbial respiration, soil animal respiration and root respiration. The other confounding issue with field measured respiration is that it has a high degree of spatial and temporal variability (Martikainen et al., 1996).Laboratory measured respiration, which excludes plant roots and soil animals during the incubation, assumes respiration to be that of microbial origin and activity. However, when the soil is incubated in the laboratory there is a degree of disturbance that destroys the soil structure, and exposes occluded organic matter. Moreover, the homogenizing and sieving of the soil before incubation accelerates the rate at which soil organic matter will be degraded. This process will over estimate soil respiration and is not representative of field conditions. Due to this challenge, sometimes the soil is core sampled in order to avoid the soil disturbance and maintain the soil structure as it was in the field. This way, soil respiration measured within the soil core could mimic that which is occurring in the field. However, using the

soil core method becomes hard to separate the contribution of plant's roots and animal fauna's to soil respiration (Martikainen et al., 1996).

Since N mineralization occurs as a byproduct of organic matter decomposition, the N cycle is always tied to C cycling and thus the two processes would need to be studied together in order to determine how one affect the other.

Rationale for the study

As farmers are encouraged to move from more destructive farm and environmental practices to more sustainable and environmentally friendly management practices, there is a need for information on how to synchronize sustainable biological management practices into their daily management. No-till experiments together with cover crops and different N fertilizer rates were established in 1981 at the West Tennessee Experimental and Education Center, Jackson, Tennessee. However, few studies exist that have quantitatively analyzed how these management practices affect the below ground C dynamics. Therefore it is critical to assess how long-term interaction of these practices affects nutrient cycling and soil C dynamics as determined by soil microbial biomass C and soil microbial respiration. Moreover, there is a need to understand how soil biochemical and soil environmental factors interact together to affect the internal soil C cycling.

The purpose of this study was to determine the impact of tillage regimes, different cover crops and N fertilization on soil microbial C dynamics under cotton (*Gossypium hirsutum .L)* production. This was achieved through the determination of microbial biomass C and

soil microbial respiration and extractable organic C as they represent the most active source of energy to microbes and thus have potential to influence the nutrient fluxes.

Objectives

To determine the long-term interacting effects of nitrogen fertilization, contrasting tillage regimes and different cover crops on soil microbial C dynamics as measured by microbial biomass C and soil microbial respiration and salt -extractable organic carbon under cotton production in West Tennessee.

The specific objectives were:

1. To assess soil microbial biomass C and soil microbial respiration during the cotton growing season as affected by N fertilizer rates ,cover cropping and tillage.
2. To determine the seasonal effects on C dynamics as measured by soil microbial biomass C and soil microbial respiration.

The hypotheses were:

1.0. H_0: Microbial biomass C and soil microbial respiration would not differ between tillage, cover crops and N fertilizer treatments.

1.1. $H_{A:}$ Microbial biomass C and soil microbial respiration would differ between tillage, cover crops and N fertilizer treatments with no-till, leguminous cover crop and higher rate of N fertilizer rate having greater concentration of these properties.

2.0 H_0: Soil microbial biomass C and soil microbial respiration would not differ during the cotton growing season.

2.1 H_A: Soil microbial biomass C and soil microbial respiration would differ during the cotton growing season with these properties being greater during the active cotton growing stages (cotton flowering and boll development) .

CHAPTER II
Materials and Methods

Site description and experimental design

The study was conducted from June to October 2013, on the long-term continuous cotton plots under no-till and conventional tillage at West Tennessee Research and Educational Center, Jackson. Soils at the site are classified as a Lexington silt loam *(fine-silty, mixed, thermic, Ultic Hapludalf)*, well drained with a 0-2 percent slope. The soils are derived from red marine deposits overlain by loess deposits. The experimental plots were established in 1981. The original objective of the experiment was to assess the effect of tillage (no-till and tilled) on cotton production under different cover crop treatments including no cover, winter wheat (*Triticum aestivum. L)*, hairy vetch (*Vicia villosa)*, and crimson clover (*Trifolium incarnatum)* and different N fertilizer rates (0, 34, 67, 101 kg N ha^{-1}).

The objective of this current study was to determine the effect of different rates of N fertilizer, different cover crops and tillage on net N transformation processes and particular this project focused on 0, 34 and 101 kg N ha^{-1} and no cover, wheat and vetch treatments. The treatment and experimental design was set as a Randomized Complete Block Design (RCBD) with a split-split plot. N fertilizer rate was the whole plot treatment with 3 levels (0 Kg N ha^{-1}(0N); 34 kg N ha^{-1} (34N); and 101 Kg N ha^{-1}(101N)), the split plot was a cover crop treatment with three levels (hairy vetch, winter wheat, and no cover crop) and the split-split plot treatment was tillage practices with two levels (no-till and conventional tillage – tilled). Each treatment factor had four replications which

resulted in 72 experimental units. Each experimental unit was 12 m by 8 m in size, with eight rows of cotton.

Soil Sampling

Field operations on the sampled plots were completed in May 2013. On May 8, 2013 cover crops under all the plots were terminated using Gramoxene® SL. The plots under conventional tillage were plowed on May 16, 2013 and on May 17, 2013 all the plots, including those under no – till, were planted; the cotton variety that was planted was *Phytogen 375 WRE.* Fertilizer was applied on May 17, both phosphorus (P) and potassium (K) fertilizers were applied at a uniform rate across all the treatments. Phosphorus fertilizer was applied as triple super phosphate at the rate of 101 kg P_2O_5 ha^{-1} and K fertilizer was applied as potassium chloride at the rate of 134 kg K ha^{-1}. Nitrogen fertilizer was applied as ammonium nitrate and was applied four days after planting (May 21, 2013) at 0N, 34N and 101N. Insecticides (Orthene®) and herbicides (Cornerstone® – glyphosate) were applied on June 4, 2013.

Soil was sampled four times during the 2013 cotton growing season at different developmental and growth stages of cotton. The growth stages were: after planting (June), flowering (July), boll development (September) and at maturity just prior to harvest (October). These time points were selected on the premise that different crop growth stages would affect microbial functioning and population dynamics. The intention was to assess how the seasonal variation during the growing season and crop phenology would affect the soil microbial C dynamics.

The first soil sampling occurred on June 12, 2013, 25 days after planting and 21 days after N fertilizer application. Sampling was delayed so as to avoid the carry-over effect of fertilization (priming effect) on the microbial populations but more importantly on the net N mineralization and nitrification potential determinations. The second sampling was completed at the flowering stage of the cotton crop on July 26, 2013, 70 days after planting. The third sampling was at cotton boll development on September 5, 2013, 110 days after planting. The forth sampling point was prior to harvest, on October 17, 2013, 152 days after planting.

For all of these sampling time points, soil was sampled with a 2.5 cm diameter probe to a 0-7.5-cm depth as this layer of soil is characterized with higher microbial activity (Feng et al., 2003). Sampling was done randomly within the plot and the sample units were taken from the interrow area, 10 -15-cm away from the crop row. The experimental units (sampled plots) had four rows and sampling was concentrated within the 2 middle rows in order to avoid the tractor compaction effects and also to be consistent with other samples that were taken (bulk density). To move from one plot to the other, the samplers were disinfected with 70% ethanol to avoid cross contamination between different plots. Soil from different sampling units within the plot was mixed together into one composite sample (72 samples total). Samples were kept in a cooler using dry ice; the samples remained in the cooler while transported back to the laboratory. Upon arrival at the laboratory, samples were stored at 4^0C until processed.

Measurements

Soil Physico-chemical analysis

Mehlich 1 (Mehlich, 1953) extraction was used to assess soil nutrient availability. Ten grams of air-dried soil was mixed with 20-mL of Mehlich 1 extracting solution and put on an oscillating shaker. The samples were covered with plastic and foam to seal the tops and shaken for five minutes after which they were filtered through a Whatman N0.1 filter paper. After filtering the samples settled for 15 minutes and were analyzed with the inductive couple plasma (ICP), Perkin-Elmer 5300 DV ICP. The extract was analyzed for P, K, Mg and Ca.

Soil pH was determined through a 1:1 (g of soil: mL of water) soil-water paste and the buffering capacity was determined with Moore-Sikora buffer solution (Sikora and Moore, 2008). Total soil N and C were determined through dry combustion using Thermo Flash EA 1112 NC combustion analyzer.

Soil bulk density was determined through the soil core method (Hartge et al., 1986). A 7.5-cm long and 7.5-cm diameter metal cylinder was used. The cylinder was driven into the soil using a hammer. Three soil cores were taken per plot. The samples were taken on the edge of the row, about 15cm away from the row in order to avoid the compaction caused by the tractor in the middle row. The wet sample weight was measured before drying and the samples were oven dried at 105°C for 24 hours. After drying the dry weight of the samples was measured. Bulk density was estimated using equation 1.

$$\text{Bulk density (g cm}^{-3}\text{)} = \text{Mass of dry soil / volume of soil} \quad \text{(Equation 1)}$$

Where:

Mass of soil = Mass of dry weight (g)

Volume of soil (cm^{-3}) = Volume of cylinder = $\pi * r^2 * h$

r = radius of the cylinder (cm)

h = height of the cylinder (cm)

Gravimetric soil moisture determination

Soil samples were obtained from random locations within a plot in the field from a depth of 0 to 7.5- cm using a 2.5-cm diameter soil probe. The soil was composited into one sample, transferred to plastic bags and then sealed to prevent evaporative water loss and placed in the cooler and transported back to the laboratory. Samples were transferred to aluminum weighing tins, weighed and then transferred to a 105° C oven. Samples were removed, placed in a desiccator to cool and weighed to determine dry weight. The soil moisture was determined on weight basis using equation 2 (Topp and Ferre, 1986).

Soil moisture (g of water/ g soil) = wet weight – dry weight / dry weight Equation (2)

Where:

- wet weight is weight of soil before drying
- dry weight is the weight of the soil after drying

Determination of Soil Microbial Biomass C (SMBC) and Extractable Organic C (EOC)

Soil microbial biomass C and EOC were determined using the slurry fumigation extraction method (Fierer and Schimel, 2002) using a 10-g field moist sample mixed with 40-mL of 2M KCl. Two subsamples were prepared from one sample. One sample was mixed with only KCl (control sample) while the other was mixed with KCl and 0.5-mL of stabilized chloroform. Samples were shaken at 150 rpm for four hours. After shaking the samples settled for 30 minutes and the supernatant was filtered through a Whatman N0.1 filter paper. After filtration, both the fumigated samples and control samples were aerated to remove the chloroform using an air spurge. The tubes from the pump were inserted into the filtrate and allowed to bubble for 30 minutes. After bubbling the extracts were kept at -20°C until analyzed. The extracts were analyzed for both extractable organic C using O.I Analytical Aurora, model 1030. Equation 3 through 5 was used to estimate the microbial biomass while equation 6 was used to estimate EOC (Eldor et al., 1999).

Mass of extractable C from the chloroform exposed samples and control samples:

$$C_F = EC_F * V / DW \qquad \text{(Equation 3)}$$

$$C_C = EC_C * V / DW \qquad \text{(Equation 4)}$$

Where:

C_F = extractable C in the chloroform exposed sample in $mgkg^{-1}$ soil

EC_F = extractable C in the chloroform exposed sample in mgL^{-1} extractant

C_C = extractable C in the control sample in $mgkg^{-1}$ soil

EC_C = extractable C in the control sample in mgL^{-1} extractant

V = volume of solution in the extracted soil (mL)

DW = weight of a dry soil (kg)

Soil Microbial biomass C (SMBC) in soil:

$SMBC = (C_F - C_C)/ K_{EC}$ (Equation 5)

Where:

SMBC = Soil microbial biomass C (mg C kg^{-1} soil)

K_{EC} (0.38) = extraction coefficient for extractable C

$EOC = EC_C * V / DW$ (Equation 6)

Note: EOC was determined as the mass of C from the non-chloroform exposed samples.

Determination of microbial soil respiration

Soil microbial respiration was determined every other day through static incubation for a period of two weeks. Soils were visually assessed to remove macrofauna or roots prior to incubation. A 50 g field moist soil sample was placed in air tight 0.5 L glass jar and incubated at an ambient temperature. A 0.5-mL headspace sample was taken with a 1-mL glass syringe and measured for CO_2 concentration with infrared gas analyzer (LI-COR, 820, LI-COR Biosciences, NE, USA). The actual headspace CO_2 concentration was calculated from a standard curve. After about one week or when the CO_2 exceeded 2%, the jars were vented to remove the CO_2 build up as it may interfere with the microbial activity and because it was above the detection limit (Fierer and Schimel, 2002). Respiration was calculated using the ideal gas law (equation 7).

$C_m = (C_v * M * P) / (R * T)$ (Equation 7)

Where: C_m = mg CO2 –C/ L headspace

C_v = μL CO2/ L headspace

M = molecular weight CO2 –C (12 mg/ mg mol)

P= barometric pressure (N m^{-2})

R= universal gas constant (8.3145 j / mol K)

T= incubation temperature in Kelvin (K)

Data analysis

All data was analyzed using the analysis of variance (ANOVA) to test for significant differences for the main treatments effects as well their interactions. The general linear mixed model was used for the analysis and Fisher's protected least significant difference (LSD) was used for mean separation using SAS (9.3) software. The analyses were performed at alpha (α) 5 percent (for soil physical and chemical properties) and at 10 percent (for soil biochemical properties). Simple Pearson moment correlation analysis (at α 5 percent) was performed to determine relationships among the variables measured. Transformations were done to achieve normality and equal variance.

CHAPTER III
Results and Discussions

Results

Soil physical and chemical properties

Soil total carbon and soil total N

Among the three treatment factors, N fertilizer rate and tillage had a significant effect ($p < 0.05$) on both soil total carbon (TC) and soil total N (TN) (Table 1). TC and TN tended to increase with increasing N fertilizer rate. No-till had greater TC and TN compared to the tilled treatments (Table 1). There was also a significant cover crop and N fertilizer rate interaction effect on TC and TN ($p < 0.05$) (Table 1). The no cover and wheat treatments had a positive correlation with N fertilizer rate (Figure 3 and 4). At 0 N fertilizer rate, vetch had significantly greater TC and TN ($p < 0.05$) than both no cover and wheat while at the 101 N fertilizer rate all three cover crops had similar TC and TN contents (Figures 3 and 4).

Mehlich 1 extractable ions (K, Mg, Ca and P), soil pH and Soil bulk density

Nitrogen fertilizer rate was negatively correlated with extractable Ca, K and P, with 0N having significantly greater ($p < 0.05$) concentrations of these ions than the 34N and 101N (Table 1). Both no cover and wheat had significantly greater extractable K and P concentrations than vetch (Table 1). Tillage had a significant effect on extractable Ca, Mg and P ($p < 0.05$) with the no-till treatment having greater concentrations of these ions (Table 1). Soil pH was negatively correlated with N fertilizer rate. Tillage also significantly affected soil bulk density ($p < 0.05$) (Table 1) with the tilled treatment having lower bulk density than the no-till treatment.

Table 1. Cover crop, N fertilizer rate (kg N ha^{-1}) and tillage effects on selected soil physical and chemical properties. Sampling was completed on June 12, 2013 except for bulk density which was sampled in July, 2013. P, K, Mg and Ca are extractable soil ions.

		Soil physical and chemical properties							
Treatment factors		TC	TN	P	K	Mg	Ca	pH	BD
		(g kg^{-1})				(mg kg^{-1})			(g cm^{-3})
N-rate	**n**								
0N	24	11.06(0.63)b	1.17(0.11)b	113(7.52)a	248(13.84)a	75(8.64)a	988(33.78)a	5.80(0.15)a	1.46(0.08)a
34N	24	12.41(0.90)b	1.23(0.10)b	93 (6.64)b	225(11.74)b	75 (5.96)a	910(32.24)a	5.63(0.12)a	1.44(0.04)a
101N	24	14.24(1.37)a	1.48(0.14)a	85 (8.37)b	206(14.00)c	71(10.53)a	762(61.34)b	5.01(0.13)b	1.38(0.04)a
LSD		**1.63**	**0.14**	**11.79**	**13.79**	**19.32**	**81.25**	**0.18**	**0.08**
Cover crop									
No cover	24	11.98(0.87)a	1.26(0.10)a	104(7.09)a	241(12.60)a	74(7.95)a	906(35.66)a	5.48(0.18)a	1.44(0.06)a
Wheat	24	12.31(0.97)a	1.25(0.10)a	103(6.70)a	231(8.90)a	72(8.88)a	874(19.22)a	5.54(0.09)a	1.45(0.06)a
Vetch	24	13.31(1.24)a	1.31(0.12)a	82 (8.12)b	206(18.08)b	69(8.37)a	880(50.56)a	5.41(0.12)a	1.42(0.05)a
LSD		**1.36**	**0.10**	**9.53**	**13.79**	**6.62**	**60.00**	**0.18**	**0.07**
Tillage									
No-till	36	13.57(1.22)a	1.36(0.11)a	108(8.21)a	231(12.83)a	77(9.91)a	945(54.04)a	5.44(0.14)a	1.47(0.06)a
Tilled	36	11.57(0.90)b	1.22(0.10)b	85 (6.82)b	221(13.55)a	70(7.51)b	830(28.48)b	5.52(0.13)a	1.39(0.05)b
LSD		**1.03**	**0.08**	**7.27**	**11.23**	**4.53**	**31.94**	**0.14**	**0.05**
Sources of Variation					**ANOVA (p values)**				
N-rate		**0.0058**	**0.0077**	**0.0027**	**<0.0001**	0.7474	**0.0014**	**< 0.0001**	*0.0845*
Cover crop		*0.0724*	*0.0877*	**0.0002**	**<0.0001**	0.3617	0.3950	0.3329	0.5942
Tillage		**0.0002**	**0.0050**	**< 0.0001**	*0.0857*	**0.0130**	**< 0.0001**	0.3032	**0.0038**
N-rate*Covercrop		**0.0356**	**0.0123**	0.8586	0.2139	*0.0708*	**0.0315**	0.1714	0.7083
N-rate*Tillage		0.4955	0.6395	0.1067	0.0036	0.6206	0.9222	0.4180	0.7008
Cover crop*Tillage		0.5022	0.6145	0.9579	0.1956	0.8392	**0.0257**	0.7142	0.6987
N-rate*Cover crop*Tillage		0.5822	0.8130	0.9864	0.2936	0.7861	0.6139	0.8122	0.1600

N fertilizer rate (N-rate); Bulk density (BD). Numbers within each column corresponding to N-rate and cover crop treatments are means of twenty four numbers (n=24) while those corresponding to tillage treatment are means of thirty six numbers (n=36).Numbers inside the parentheses are standard error of the mean. Numbers that are followed by the same letters within the same column, corresponding to each of the treatment factor are not significantly different at $p < 0.05$. The bold numbers in the lower part of the table show statistical significance at $p < 0.05$ while the italicized numbers at $p < 0.1$. The degree of freedoms corresponding to each treatment factor and their interactions are described in table 2 to 4.

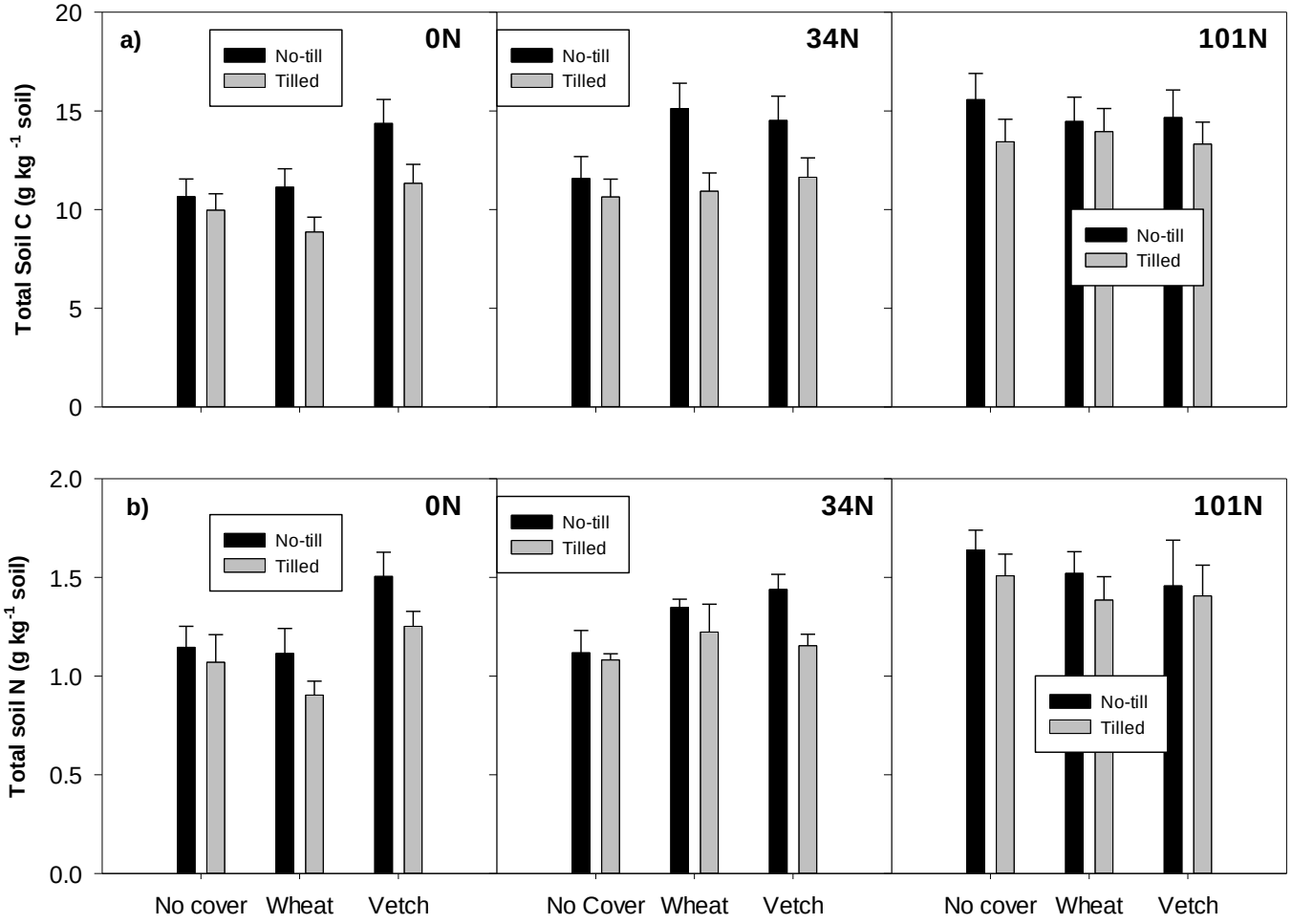


Figure 2. Total soil C and N as affected by tillage, cover crops and N fertilizer rates (kg N ha^{-1}) during 2013 cotton growing season. Sampling was completed in June 2013. Each column is a mean of four numbers (n=4). The error bars are standard error of the mean

Soil biochemical properties

SMBC, soil microbial respiration and EOC

In June, N fertilizer rate had a significant effect on SMBC and EOC ($p < 0.1$) with increasing N fertilizer rate resulting in a corresponding increase in both SMBC (Table 2) and EOC (Table 4). Cover crop and tillage significantly affected EOC ($p < 0.1$) with vetch having greater EOC than wheat and no cover. The no-till treatment had greater EOC (Table 4) compared with the tilled treatment.

In July, N fertilizer rate significantly affected SMBC and EOC ($p < 0.1$) with an increase in N fertilizer rate resulting in increasing SMBC (Table 2) and EOC (Table 4). Tillage significantly affected EOC ($p < 0.1$) with the no-till treatment having greater EOC (Table 4). There was a significant cover crop by tillage interaction effect on SMBC and soil microbial respiration ($p < 0.1$) (Table 3) with the no-till no cover treatments having greater soil microbial respiration across all of the N fertilizer rates (Figure 6 (a)). The cover crop by tillage interaction on SMBC was in tilled vetch treatment, with SMBC being greater at 34N and 101N (Figure 5 (b)).

In September, tillage significantly affected SMBC, EOC and soil microbial respiration ($p < 0.1$) with the no-till treatment having greater SMBC (Table 2), EOC (Table 4) and soil microbial respiration (Table 3). Nitrogen fertilizer rate and cover crop had a significant effect on EOC ($p < 0.1$) and soil microbial respiration ($p<0.1$) with increasing N fertilizer rate correspondingly increasing EOC (Table 4) and soil microbial respiration (Table 3). Vetch cover crop had a significantly greater EOC (Table 4) and soil microbial respiration (Table 3) than both wheat and no cover.

In October, there was a significant tillage and cover crop interaction effect on soil microbial respiration ($p < 0.1$) (Table 3) with no-till, no cover having greater soil microbial respiration (Figure 6 (c)). There was a significant N fertilizer rate and cover crop interaction effect on EOC ($p < 0.1$) (Table 4) with vetch at 34N and 101N having greater EOC than wheat and no cover (Figure 7 (d)). There was a significant tillage and N fertilizer rate interaction effect on EOC ($p < 0.01$) with no-till at 101N having greater EOC than tilled treatment (Figure 7 (d)). For SMBC in October, neither of the treatment factors had a significant effect on SMBC (Table 2).

Table 2. Cover crop, N fertilizer rate (kg N ha^{-1}) and tillage effects on SMBC during the 2013 cotton growing season. Sampling was carried out in June, July, September and October.

Soil microbial biomass C (SMBC) (mg C kg^{-1} dry soil)					
Treatment factors		June	July	September	October
N-rate (kg N ha^{-1})	**n**				
0N	24	72.98(12.94)b	72.51(20.54)b	108.39(16.33)a	107.52 (23.32)a
34N	24	92.93(19.22)ab	97.11(23.34)ab	108.34 (28.96)a	110.96 (23.08)a
101N	24	113.44(18.76)a	127.28 (26.31)a	107.86 (16.76)a	110.63 (30.66)a
LSD		**27.95**	**36.52**	**34.86**	**26.03**
Cover crop					
No Cover	24	94.92 (17.75)a	93.24 (22.70)a	115.98 (25.16)a	100.31 (22.85)a
Wheat	24	84.06 (17.34)a	99.12 (19.99)a	100.96 (16.97)a	106.33 (26.28)a
Vetch	24	100.37 (14.60)a	104.53 (27.50)a	107.66 (19.92)a	122.47 (27.94)a
LSD		**19.49**	**22.51**	**19.13**	**26.03**
Tillage					
No-till	36	87.60 (16.70)a	99.59 (22.20)a	131.34 (26.27)a	104.11 (22.77)a
Tilled	36	98.64 (17.24)a	98.34 (24.60)a	85.06(15.09b	115.30 (28.61)a
LSD		**14.21**	**18.38**	**15.76**	**17.68**

Sources of variation	**Num df**	**Den df**	**ANOVA (p values)**			
N-rate	2	9	**0.0866**	**0.0640**	0.9995	0.9728
Cover crop	2	18	0.3434	0.7047	0.4200	0.3830
Tillage	1	26	0.2075	0.9093	**<0.0001**	0.3152
N-rate*Cover crop	4	18	0.5788	0.1259	0.4534	0.8907
N-rate*Tillage	2	26	0.7152	0.9058	0.4073	0.3242
Cover crop*Tillage	2	26	0.1022	**0.0510**	0.7872	0.1392
N-rate*Cover crop*Tillage	4	26	0.2213	.1983	**0.3481**	0.5921

N fertilizer rate (kg N ha^{-1}). (N-rate); Numerator degree of freedom (Num df); Denominator degree of freedom (Den df). Numbers within columns corresponding to N-rate and cover crop are means of twenty four numbers (n=24) while those corresponding to tillage are means of thirty six numbers (n=36). Numbers inside the parentheses are standard error of the mean. Numbers within each column corresponding to each treatment factor followed by different letters are statistically different at $p < 0.1$.The bold numbers show statistical significance at $p < 0.1$.

Table 3. Cover crop, N fertilizer rate (kg N ha^{-1}) and tillage effects on soil microbial respiration during 2013 cotton growing season. Sampling was carried out in July, September and October.

Cumulative soil microbial respiration (mg C-CO_2 g^{-1} dry soil)				
Treatment factors		July	September	October
N-rate (kg N ha^{-1})	**n**			
0N	24	0.21(0.03)a	0.08(0.02)b	0.14 (0.01)a
34N	24	0.23(0.02)a	0.11(0.02)ab	0.15 (0.02)a
101N	24	0.26(0.14)a	0.13(0.03)a	0.17 (0.02)a
LSD		**0.05**	**0.03**	**0.04**
Cover crop				
No Cover	24	0.22 (0.03)b	0.09 (0.02)b	0.1467(0.02)b
Wheat	24	0.22 (0.03)b	0.10 (0.02)b	0.1378 (0.01)b
Vetch	24	0.25 (0.02)a	0.13 (0.02)a	0.1731 (0.01)a
LSD		**0.02**	**0.02**	**0.0172**
Tillage				
No-till	36	0.24 (0.02)a	0.1155 (0.02)a	0.15 (0.02)a
Tilled	36	0.22 (0.03)b	0.0965 (0.02)b	0.15 (0.01)a
LSD		**0.01**	**0.0150**	**0.01**

Sources of Variation	**Num df**	**Den df**	**ANOVA (p values)**		
N-rate	2	9	0.2374	**0.0537**	0.2905
Cover crop	2	18	**0.0837**	**0.0098**	**0.0014**
Tillage	1	26	**0.0556**	**0.0405**	0.8995
N-rate*Covercrop	4	18	0.1428	0.1914	0.2207
N-rate*Tillage	2	26	0.7592	0.6204	0.6785
Covercrop*Tillage	2	26	**0.0018**	0.8527	**0.0693**
N-rate*Cover crop*Tillage	4	26	0.3651	0.3776	0.6396

N fertilizer rate (kg N ha^{-1}) (N-rate); Numerator degree of freedom (Num df); Denominator degree of freedom (Den df). Numbers within columns corresponding to N-rate and cover crop are means of twenty four numbers (n=24) while those corresponding to tillage are means of thirty six numbers (n=36). Numbers inside the parentheses are standard errors of the mean. Numbers within each column corresponding to each treatment factor followed by different letters are statistically different at $p < 0.1$. The bold numbers show statistical significance at $p < 0.1$.

Table 4. Cover crop, N fertilizer rate (kg N ha^{-1}) and tillage effects on EOC during 2013 cotton growing season. Sampling was carried out on June, July, September and October

KCl Extractable organic C (EOC) (mg C kg^{-1} dry soil)					
Treatment factors		June	July	September	October
N-rate (kg N ha^{-1})	**n**				
0N	24	9.89(1.93)c	18.84(5.79)b	17.65(4.69)b	7.13(1.21)b
34N	24	13.71(3.19)b	18.33(2.66)b	23.42(3.53)a	9.55(1.25)b
101N	24	18.61(3.27)a	34.80(6.90)a	27.63(3.10)a	16.40(1.57)a
LSD		**3.36**	**6.31**	**4.71**	**2.42**
Cover crop					
No Cover	24	13.91(2.98)b	21.80 (5.09)a	20.34(3.79)b	8.73(1.07)b
Wheat	24	12.35(2.36)b	25.14(5.40)a	19.13(3.41)b	10.16(1.30)b
Vetch	24	15.95(3.04)a	25.02(4.87)a	29.23(4.10)a	14.20(1.69)a
LSD		**2.52**	**4.67**	**4.65**	**1.44**
Tillage					
No-till	36	15.61 (3.00)a	26.08(6.02)a	27.23(4.41)a	12.15(1.21)a
Tilled	36	12.53 (2.58)b	21.90(4.22)b	18.57(3.12)b	9.91(1.50)b
LSD		**2.06**	**3.82**	**3.64**	**1.12**

Sources of Variation	**Num df**	**Den df**	**ANOVA (p values)**			
N-rate	2	9	**0.0065**	**0.0036**	**0.0055**	**0.0001**
Cover crop	2	18	**0.0623**	0.3866	**0.0023**	**< 0.0001**
Tillage	1	26	**0.0158**	**0.0715**	**0.0005**	**0.0022**
N-rate*Cover crop	4	18	0.8715	0.3166	0.1640	**0.0096**
N-rate*Tillage	2	26	0.3083	0.1143	0.8342	**0.0083**
Cover crop*Tillage	2	26	0.6350	0.5472	0.5959	0.5130
N-rate*Covercrop*Tillage	4	26	0.8351	0.1021	0.0643	0.1087

N fertilizer rate (kg N ha^{-1}) (N-rate); Numerator degree of freedom (Num df); Denominator degree of freedom (Den df). Numbers within columns corresponding to N-rate and cover crop are means of twenty four numbers (n=24) while those corresponding to tillage are means of thirty six numbers (n=36). Numbers inside the parentheses are standard error of the mean. Numbers within each column corresponding to each treatment factor followed by different letters are statistically different at p <0.1. The bold numbers show statistical significance at (p <0.1).

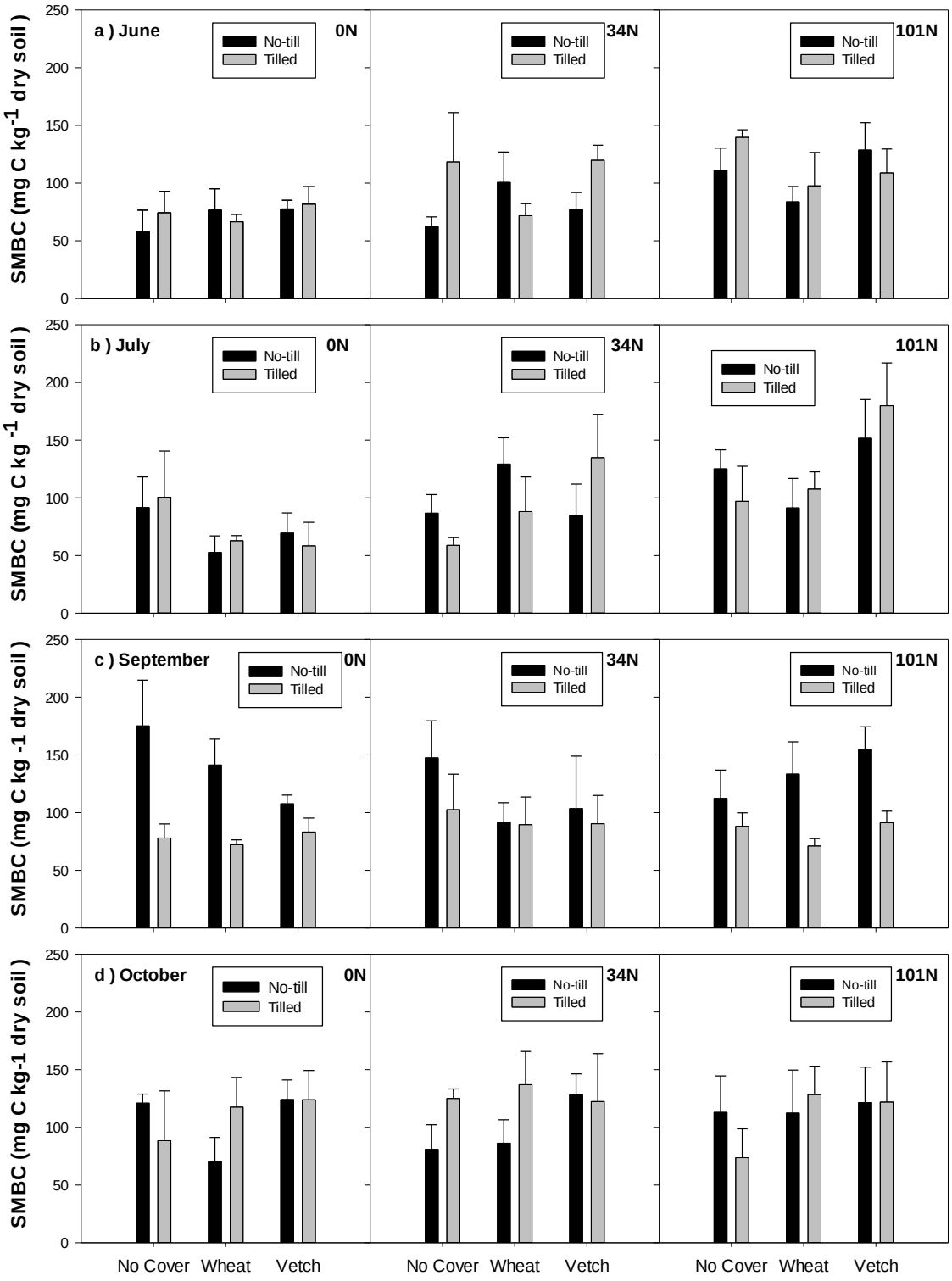


Figure 3. Soil microbial biomass C (SMBC) as affected by N fertilizer rate (kg N ha^{-1}), cover crop and tillage during 2013 cotton growing season. Sampling was done in (a) June, (b) July, (c) September and (d) October. Each column is a mean of four numbers (n=4). Error bars are standard error of the mean

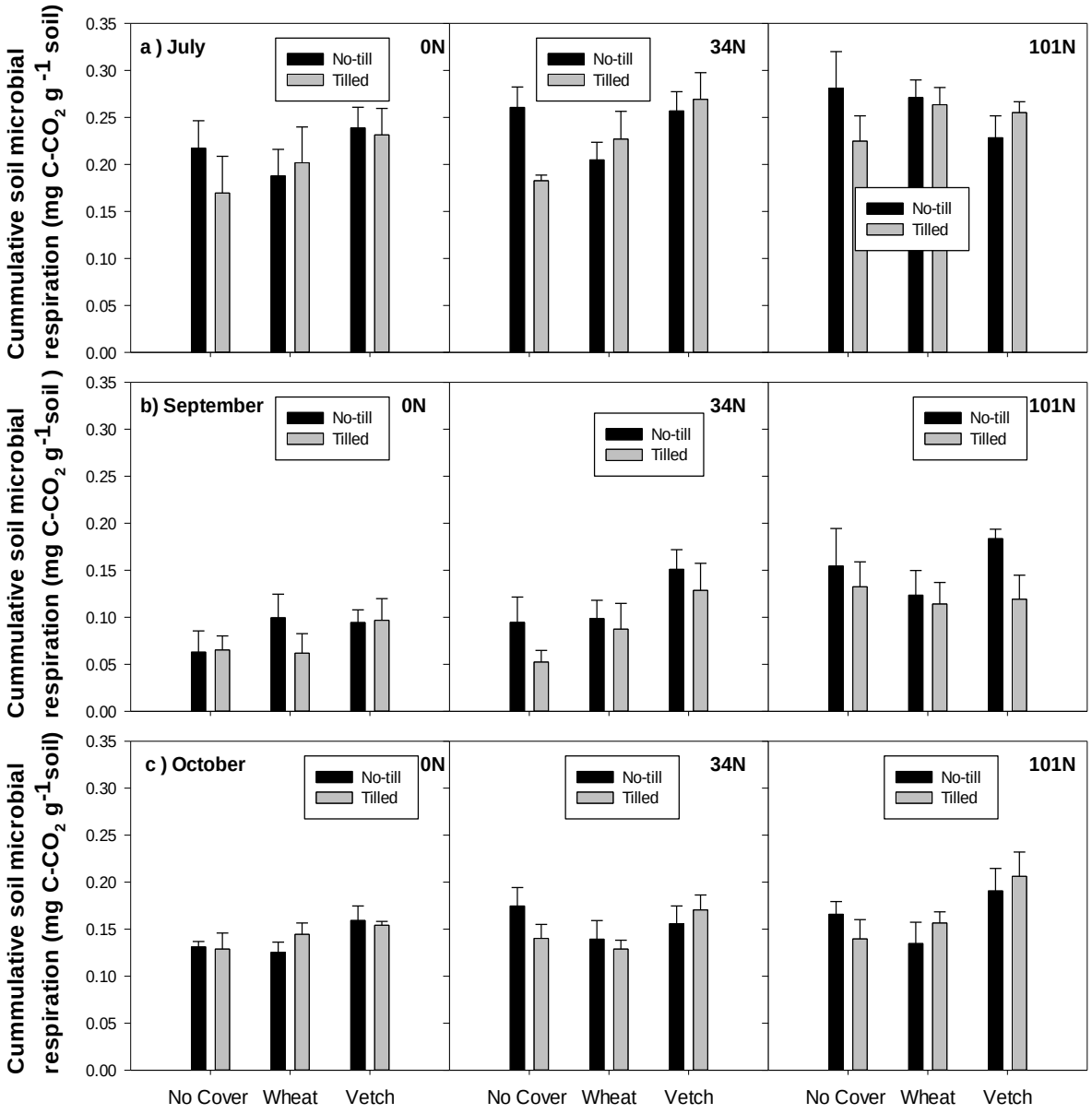


Figure 4. Soil microbial respiration as affected by N fertilizer rate (kg N ha^{-1}), cover crops and tillage during 2013 cotton growing season. Sampling was carried out in (a) July, (b) September and (d) October. Each column is a mean of four numbers (n=4). Error bars are standard error of the mean

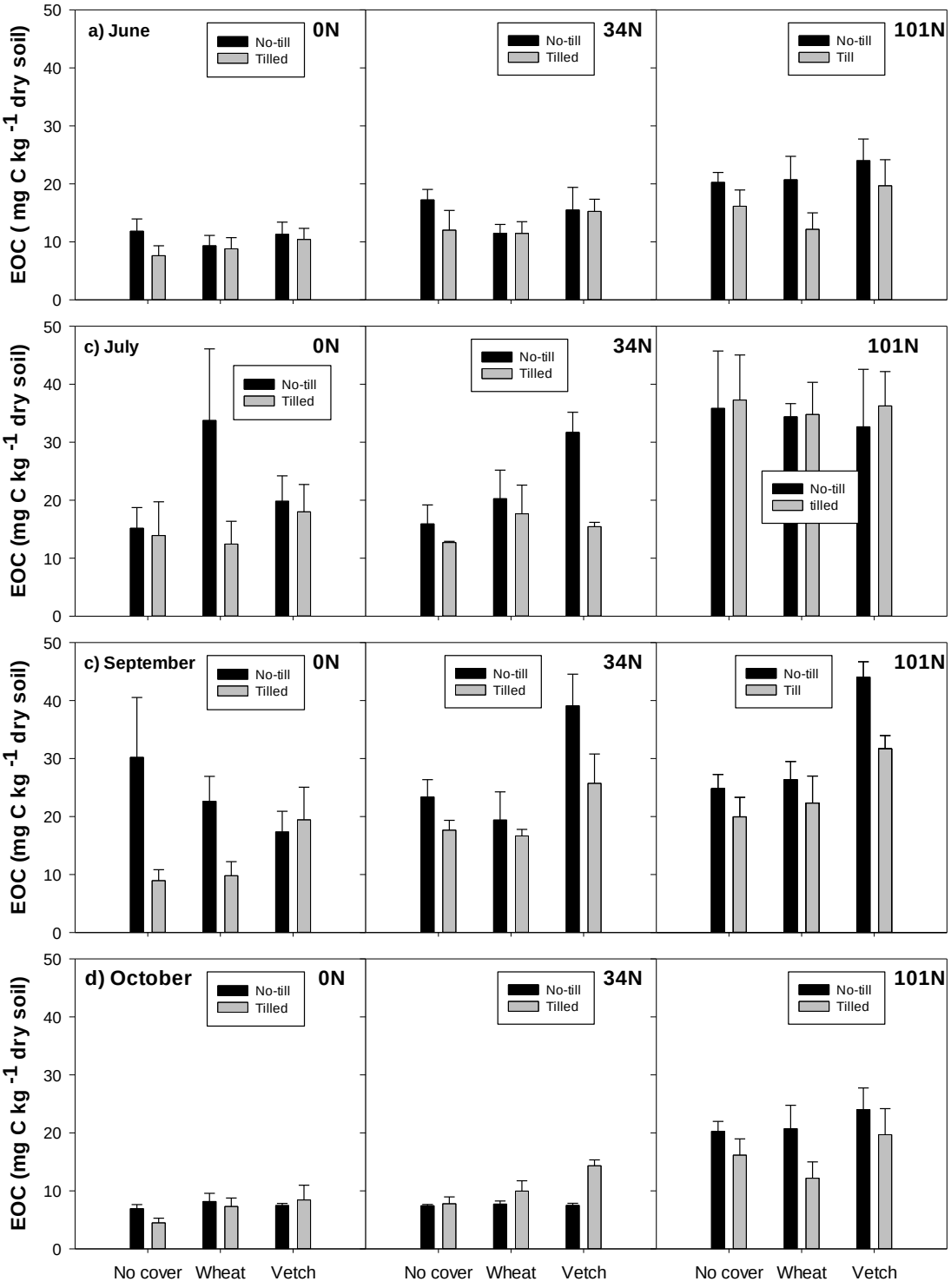


Figure 5. Salt (KCl) Extractable organic C (EOC) as affected by N fertilizer rate (kg N ha^{-1}), cover crop and tillage during 2013 cotton growing season. Sampling was carried out in (a) June, (b) July, (c) September and (d) October. Each column is a mean of four numbers (n=4). Error bars are standard error of the mean.

Discussions

Tillage, cover crop and N-fertilizer effect on TC and TN

The difference in TC and TN between the no-till and tilled treatments (Table 1 and Figure 2 (a) and (b)) could be due to the fact that tilling the soil incorporates the crop residues bringing them into close soil contact and at higher soil moisture than if the residues were left on the soil surface. Tillage can promote favorable conditions for soil microorganisms to decompose the crop residues at a higher rate. Furthermore, tilling the soil allows greater oxygen diffusion into the soil, albeit temporarily, thereby resulting in higher rates of decomposition of soil organic matter hence promoting higher emissions of carbon dioxide (CO_2) which reduce the amount of C stored in the soil. The higher rate of soil organic matter mineralization may result in the release of inorganic N (NO_3^- or NH_4^+) into the soil which is lost through leaching or denitrification and thus reducing c the TN content in soil (Halverson et al., 2002). In contrast to tilling the soil, no-till limits soil contact with crop residues thereby limiting contact with microbes and thus decreasing the decomposition rate. Moreover, soils under no-till management have higher water infiltration rates and more surface residue cover that results in no-till soils having greater soil moisture content. In this current study, no-till had significantly greater soil moisture than tilled (Appendix 3) two out of the four times sampled. The increased soil moisture as facilitated by increased porosity under no-till lowers the rate of oxygen diffusion into the soil which will in turn reduce the oxidative capacity of soil microbes. Well-formed soil aggregates under no-till may physically control soil organic matter decomposition by restricting microbes access to occluded soil organic matter. All of these factors might act together to reduce the C loss through CO_2 emissions and N loss

through leaching and denitrification under no-till and could contribute to no-till having greater TC and TN than the tilled treatment. The difference on both TC and TN between no-till and tilled was mainly under wheat and vetch treatments at 0N and 34N fertilizer rate (Figure 2 (a) and (b)). However, the no-till treatment especially under no cover and wheat had greater concentration of evolved CO_2 (Figure 4). It is therefore surprising to find that treatments that resulted with greater TC and TN also had greater evolution of CO_2. The higher TC under no-till corroborate Al-Kaisi et al. (2005) who found zero tillage to have 15-21 percent more SOC than soils that were chisel plowed (0-10 cm depth) at their study site on a Mollisol in Iowa. This may suggests that even though there was greater CO_2 evolution under no-till, the greater TC and TN is a function of C and N input into the soil.

Figures 2 (a) and 2 (b) show the interaction effects between cover crops and N fertilizer rate on TC and TN. At 0N, vetch had significantly greater TC and TN than both no cover and wheat and that difference tended to decline with increasing N fertilizer rate. At 34N, wheat increased to a similar level of TC and TN compared to vetch (~13.00-g C kg^{-1} soil and 1.3 g N kg^{-1} soil respectively). At the 101N fertilizer rate all the cover crops had statistically similar concentrations of TC and TN (~ 14-g C kg^{-1} soil and 1.4 g N kg^{-1} soil respectively). Overall, the difference in TC and TN contributed by cover crops as influenced by N-fertilizer could be from the plant biomass production. The cotton yield data from this plots show that the yield difference among the cover crops exists only at lower N fertilizer rates while at 101N the is no difference between the cotton yield (Appendix 10). The significantly greater TC and TN at the 0N under vetch treatment could be because of its ability to fix its own N and thus resulting in greater vegetative

growth and plant biomass when compared to wheat and no cover. In fact the data show consistent cotton yields under the vetch treatments regardless of the N fertilizer rate (Appendix 10). However, at the 101N fertilizer rate, all the cover crops had similar TC and TN. Thus the significant difference in TC and TN as a result of cover crops as influenced by N-fertilizer could be a function of their C input from the crop biomass into the soil. Jagadamma et al. (2007) saw a difference of 2.1-Mg ha^{-1} of aboveground biomass yield between corn-soybean and continuous corn cropping system at 0-kg N ha^{-1}N application with corn-soybean having the higher yield and that difference was reversed at higher N-fertilizer rate (280-kg N ha^{-1}) to be -0.94-Mg ha^{-1} with continuous corn system having higher yield of aboveground biomass. Halvorson et al. (2002) found a 13 percent difference in crop residues mass between 101-kg N ha^{-1} and 0 kg N ha^{-1} and the amount of organic N in the residues was also reflected in the N-fertilizer rate. The 101-kg N ha^{-1} rate resulted with organic N residue of 353-kg N ha^{-1} while 0-kg N ha^{-1} rate resulted with 241-kg N ha^{-1} of the organic N residues (Halvorson et al., 2002). Moreover, as an indication that crop residues contribute to both soil C and N, Karlen et al. (1994a) found TN to be more than double in plots that maintained crop residues compared to those with residue removal. These results suggest that high N fertilizer applications result in higher N uptake by the crops and leads to greater production of above ground biomass that will later be returned to the soil through residue decomposition thus leading to higher soil organic C and N.

N-fertilizer, tillage and cover crop effect on Mehlich 1 extractable Soil P

The difference in extractable P due to N fertilizer rate could be due to the fact that N fertilizer application tends to lower the soil pH (Table 1) and impacts soil solution

chemistry. At lower pH, aluminum (Al) and iron (Fe) concentrations increase in the soil solution. These ions will react with P to form sparingly soluble P precipitates that lead to declines in extractable P. Phosphorous and pH had a significant positive correlation (r = 0.31, $p < 0.0093$, Appendix 6). The soil pH at the 101N plots was as low as 5, and at this range the concentration of available Al increases and it's reactivity with P increases as well forming an insoluble precipitate (Haynes, 1982). Moreover, it is at this range of the pH that Al can become toxic plants (Haynes, 1982). Also the difference in extractable P between higher N fertilizer rate and lower N fertilizer rate could be due to the yield differences under different N fertilizer rates over the duration of the experiment. In 2013, the year we sampled for this study, 101N fertilizer rate resulted in significantly greater yields than both 34N and 0N fertilizer rates (Appendix 10). Thus under higher N fertilizer rate (101N) the higher cotton yield could have resulted in more extraction of P but under 0N, lack of N might have affected the amount of P taken up by the plant therefore resulting in less P uptake which could explain the greater extractable P concentration under 0N than the 101N fertilizer rate.

In the case of the cover crops, the no cover crop treatment resulted with higher P concentration than other cover crops (Table 1). Since vetch is a leguminous crop with a lower C:N ratio biomass, organic N is mineralized from the vetch plant biomass more rapidly than from the higher C:N wheat biomass. This N may undergo different transformations (nitrification) that will result in the production of soil acidity. This phenomenon might have had the same effect as the fertilizer N on the extractable P, thus reducing concentration of extractable P under vetch cover crop treatment as compared to both wheat and no cover. The other possible explanation for lower

extractable P under vetch compared to no cover could be related to yield. Under the vetch treatment, the cotton yield was similar across all the levels of N fertilizer rate depicting that the vetch cover crop has the N fertilizer equivalence of 101N fertilizer rate as 0N vetch treatment had similar cotton yield as 101N fertilizer rate (Appendix 10). High N availability under vetch leads to more extraction of P and other nutrients and therefore resulting in lower extractable P.

The difference on extractable P between the no-till and tilled treatments could be due to the fact that when tilling the soil, the ions are brought into contact with soil particles. This may increase the surface reactivity of the particles and in the case P the reaction may be P sorption which will reduce its extractable concentration within soil solution (Lajtha et al., 1999). Also the higher P concentration under no- till could be the effect of biological P cycling resulting from the crop residues mineralization (Dı´az-Zorita and Grove, (2002). According to Dı´az-Zorita and Grove, (2002) organic P cycling under no-till management and the surface residue placement along with surface fertilizer without mixing into soil lead to P stratification. This results in surface soils under no-till having greater extractable P than conventionally tilled soils where soil mixing through lead to distribution of nutrients within the whole plough layer. That is tilling provides a dilution effect of nutrients with shallow depths having greater nutrient concentration than deeper surfaces.

N-fertilizer, tillage and cover crop effect on Mehlich 1 extractable K, Mg and Ca

In general, concentrations of soil extractable K, Mg, and Ca were negatively correlated with N fertilizer rate (Table1). The high N uptake by plants under higher N fertilizer rate

might have resulted in greater uptake of basic cations thereby reducing their concentration in the soil solution.

At higher N fertilizer rates there was a decrease in soil pH. At lower pH, the concentrations of exchangeable Al and Manganese (Mn) become elevated (Schroda et al., 2011; Blevins et al., 1977). The consequence of this would be for these cations (Al and Mn) to replace the basic cations (K, Ca, Mg) from the soil exchange sites and be part of the soil solution. When in the soil solution they become prone to being lost through leaching (Barak et al., 1997).

Soil pH

N fertilizer rate was negatively correlated with soil pH (Table 1). Since the fertilizer form applied to these experimental plots was ammonium nitrate, the transformation of ammonium into nitrate by ammonium oxidizing bacteria (nitrification process) results in the production of acidity thus reducing soil pH, as indicated by the soil pH at higher N fertilizer rate (101N). The last time agricultural lime was applied to these plots was in 1995. The liming rate was in accordance with soil pH and it ranged from 4.5 Mg to 7.8 Mg ha^{-1} for pH of 4.9 – 6.0 to pH of 4.0 respectively (Cochran et al., 2007). In this study the pH ranged from 4.7 at 101N, tilled vetch treatment to 5.9 at 0N, no-till wheat treatment. Thus if lime had not been applied in 1995, pH might have dropped to lower levels than they were by the time of sampling for this study.

Soil bulk density

The tilled treatment had significantly lower soil bulk density than the no-till treatment (Table 1). Tilled the soils are periodically loosened with the plow and other tillage implements while under no-till the soil particles are left to aggregate together without mechanical loosening. Though there was a difference in soil bulk density between these two tillage systems, this may be a difference that exists only within the upper soil surface because sampling was done only at 0- 7.5 cm depths. Tillage operations could sometimes affect bulk density measurements if sampling is carried out shortly after the tillage operation. Such effect, if it occurred, would be more pronounced under the tilled treatments than no-till due to machinery traffic. In our plots there was still lower soil bulk density 7 weeks after the tillage operations on the tilled treatment compared to no-till.

Soil biochemical properties

Nitrogen, cover crops and tillage effect on SMBC and soil microbial respiration and EOC

Both soil microbial respiration and SMBC are the processes of soil organic matter decomposition. Soil microorganisms use organic matter as a substrate in order to gain energy from the C molecules in soil. When microbes degrade soil organic matter, some C will be assimilated into microbial cells and become microbial biomass C and the other C lost due to microbial respiration. Another component of soil organic matter which is vital to the growth of soil microbes is soluble organic matter, which comprises EOC, and

extractable organic N. The relationship between soil microbial respiration, EOC and SMBC is important in consideration of nutrient (N and C) cycles.

Nitrogen availability in soil can have a strong influence on soil microbial activity and thus on microbial growth and abundance (Vitousek and Howarth, 1991). The greater SMBC in June under higher N fertilizer rates (34N and 101N) may be explained by the application of N fertilizer in May, 2013. N-fertilizer application in soil results in increased rates of microbial decomposition of organic matter which is exhibited by the greater levels of SMBC in June especially under higher N fertilizer rates. Regardless of the cover crops and tillage system, an increase in N fertilizer rate from 0N to 101N resulted in an increase in SMBC (Figure 3 (a)). This may be indicative that at 0N fertilizer rate microbes are N-limited. Zhang et al. (2005) in China found an increase of soil microbial biomass after the addition of N fertilizer in grassland soils. They attributed higher SMBC to greater aboveground biomass which was observed under higher N fertilizer rates. However, Ladd et al. (1994) reported that N fertilizer (NH_4NO_3) decreased soil microbial biomass C, with an increase in N fertilizer rate resulting in a decrease in soil microbial biomass C. In their study, plots under high N fertilizer rates had an acidic pH; therefore they attributed the reduction in microbial biomass C indirectly to altered soil environment (soil acidity). According to Treseder (2008), pH may negatively affect microbial biomass due to higher H^+ activity (acidity) which may lead to a leaching of basic cations and mobilization of Al. Under these conditions, microbes may be affected by Al toxicity or become either Ca or Mg-limited (Treseder, 2008).

Nitrogen fertilizer in this current study might have affected SMBC in two ways. First, the higher N fertilizer rate would lead to a decrease in C:N ratio of the substrates therefore

allowing microbes to decompose soil organic matter at a higher rate thus assimilating more C. Second, the higher N fertilizer rate results in greater crop biomass (He et al., 2013), which increases the microbial substrate when the residues are returned to the soil. This corroborates Treseder (2008) whose research found that one of the ways in which N-fertilizer increases soil microbial biomass is by increased net plant productivity. EOC, which is considered an indicator of readily available substrate for microbes (Boyer and Groffman, 1996; Speeding et al., 2004 and Xiang et al., 2008), was greater at the 101N than it was at 0N (Figure 5 (a)). Regardless of cover crop type 101N fertilizer rate had greater TC and TN (Figure 3 (a) and (b)) respectively) which might have contributed to the organic substrates for microbes. In June, EOC had a significantly positive correlation with TC and TN ($r = 0.39$; $p = 0.001$and $r = 0.41$; $p = 0.0005$ respectively). Karlen et al. (1994b) in a long-term no-tillage study in Wisconsin, USA, also found the microbial biomass C concentration to reflect the soil C concentration. He et al., (2013) reported a positive correlation between microbial biomass C and soil organic C. However, in the present study soil SMBC did not have any correlation with either TC ($r = 0.05$; $p = 0.6866$) and EOC ($r = -0.06$; $p = 0.6350$) or TN ($r = 0.08$; $p = 0.5068$) in June. According to Dwivedi and Soni, (2011) soil microbial biomass is a reflection of the total soil organic matter pool even though microbial biomass forms a smaller percentage of this pool.

In July, N fertilizer rate was positively correlated with SMBC (Figure 3 (b)). But in addition to the N fertilizer rate effect on SMBC, in July SMBC was also affected by an interaction of tillage and cover crop, in particular at 34N and 101N fertilizer rate with the tilled vetch treatment having greater SMBC (Figure 3 (b)). Tilling the soil promotes soil

aeration that would be beneficial to soil heterotrophic organisms in soil organic matter decomposition. Greater SMBC under the vetch cover crop in the tilled treatments when compared to no-till (Figure 3 (b)) might be a reflection of how residue incorporation in soil affects the microbial decomposition dynamics. Feng et al. (2003) attributed the tillage effect on SMBC early in the cotton growing season to the incorporated residues from the winter growing season. According to Salinas-Garcia et al. (1997) residue incorporation will result in increased soil microbial biomass by the planting date. Tilling physically buries crop residues and places them into a moist environment conducive to microbial transformation. Higher SMBC in the tilled-vetch treatments suggests that there was more residue decomposition and greater microbial activity. This observation is a reflection of the differences in substrate quality between the cover crop treatments. The lower C:N ratio of vetch combined with the tilling effect facilitates increased microbial activity and microbial decomposition rate.

In July, there was a significant tillage and cover crop interaction effect on soil microbial respiration (Table 3). The no-till, no cover treatment had greater soil microbial respiration than the tilled no cover regardless of N fertilizer rate (Figure 4 (a)). This was in contrast to what was observed for SMBC which was affected by the tilled and vetch treatments (Figure 3 (b)). Carbon dioxide evolution is a result of organic substrate decomposition and is dependent upon the quality (C:N) of the substrate. Lower quality substrates (higher C:N) often result in greater CO_2 evolution than higher quality substrates (lower C:N) (Jenkison and Smith, 1988). This corroborates what was observed in this study in July when the no-till, no cover treatment had higher levels of soil microbial respiration (Figure 4 (a)). The no-till treatment had greater TC and TN

concentration than the tilled (Table 2) and greater EOC (Table 4 and Figure 5 (b)). Cotton, the main source of crop residue under no cover has higher C:N (200:1) compared to any of the cover crop treatments in this study. Thus we can conclude that organic substrates under the no-till no cover treatment are of lower quality and this may help explain the greater soil microbial respiration under the no-till no cover treatment compared to the tilled no cover treatment (Figure 4 (a)). Bossatta and Agren, (1999) define substrate quality as - “the number of enzymatic steps required to release as carbon dioxide a carbon atom from an organic compound”. That is, the lower the substrate quality, many would be the number of enzymatic steps and thus greater CO_2 evolution.

As was observed in July, increasing N fertilizer rate increased SMBC (Table 2) and EOC (Table 4 and Figure 5 (b)) but had no effect on soil microbial respiration (Table 2). Nevertheless, these properties had a significant positive correlation with each other. Soil microbial respiration had a significant positive correlation with both SMBC ($r = 0.37$, $p = 0.002$) and EOC (0.33, $p=0.008$) while SMBC and EOC had no significant correlation ($r = 0.17$, $p = 0.189$) (Appendix 7). Iqbal et al. (2010) also found SMBC to have a positive correlation with soil respiration and Sparling et al. (1998) found a positive correlation between EOC and soil respiration. This corroboration shows that there is a high degree of association between SMBC, EOC and microbial respiration as they are the processes of organic matter decomposition and thus contribute to nutrient cycling.

Tillage can have a strong temporal effect on microbial activity because tillage mechanically exposes soil organic matter to soil microbes. Soil microbial biomass C (Figure 3 (c)), soil microbial respiration (Figure 4 (b)) and EOC (Figure 5 (c)) were

greater under the no-till treatment compared to the tilled treatment towards the end of the growing season in September. These results corroborate those of Karlen et al (1994a) who reported greater soil microbial biomass C and soil microbial respiration under a ten year long-term no-tillage treatment compared to conventional tillage (chisel and plow) Since under no-till systems crop residues are left on the soil surface without being incorporated into the soil, their decomposition will proceed at a slower rate after the termination of cover crops but peak at some later point. As decomposition peaks during the growing season, more crop residue decomposition would be expected to occur under cover crops or crop residues with high C:N ratio as they have a slower decomposition rate. According to Quemada and Cabrebra, (1995) and Sianju et al (2002) residues with high C:N ratio or residues left on the soil surfaces tend to decompose slowly compared to those with lower C:N ratio or incorporated into the soil. Indeed, in September there was higher SMBC under no cover (cotton residues) and wheat regardless of N fertilizer rate (Figure 3(c)). According to Ghidey and Alberts, (1993) cotton roots have a slow decomposition rate due to their wider diameter and high C:N ratio. As residue decomposition progresses, the decomposing residues may contribute to the dissolved organic C which in turn might increase the microbial activity. In fact, EOC, which is a component of dissolved organic C, (Gerard et al., 2011) was highest in September under the no-till, no cover and no-till, wheat treatments than under the tilled treatment irrespective of N fertilizer rate (Figure 5 (c)). This trend held true for soil microbial respiration also (Figure 4 (b)). The greater SMBC and soil microbial respiration in September under no-till no cover and wheat than under tilled treatments may suggest that the organic carbon under no-till is more aliphatic than under tilled

treatment. Extractable organic C which assumed that the labile form of C was also greater under no-till compared to tilled treatment throughout the growing season. Stearman et al. (1989) in his studies from these same plots reported organic C in no-till plots to be more aliphatic than in tilled treatments. This held true only for soils under cotton and corn (*Zea mays*) while soils under soy beans (*Glycine max (L.)* there was no difference between no-till and tilled. This corroborates what was observed in this study under vetch cover crop where no-till and tilled treatment had similar SMBC levels and soil microbial respiration. The residues from the vetch cover crop might be contributing high quality residues that to equalize the C aliphacity between no-till and tilled. Despite the difference in C:N ratios of cover crops, it is interesting to note that the lower C:N cover crop (vetch) at higher N fertilizer rate (101N) under no-till had greater SMBC (Figure 3 (c)), soil microbial respiration (Figure 6 (c)) and EOC (Figure 7 (c)) as well. Overall, September results reflect higher microbial activity and high rate of internal nutrient (C) cycling under no-till.

Soil microbial respiration and EOC in September had a significant positive correlation ($r = 0.61$, $p < 0.0001$); however, SMBC did not have significant correlation with EOC ($r = 0.21$, $p = 0.084$) and soil respiration ($r = 0.18$, $p = 0.164$) (Appendix 8). Other studies also found extractable C to be correlated with soil microbial respiration and SMBC. Ghani et al. (2003) reported a positive correlation between EOC and SMBC and speeding at al. (1998) reported a positive correlation between EOC and soil microbial respiration. The positive correlation between EOC and soil microbial respiration may suggest that EOC as labile form of C might have been the substrate for microbial biomass. Thus it would be expected also to observe a similar relationship between EOC

and SMBC, that some portion of C from EOC will be assimilated into biomass; however, the two properties did not have any significant association.

Soil microbial activity, especially soil microbial respiration depends largely on soil moisture and temperature (Yuste et al., 2007). Soil moisture in September was the lowest compared to other months; however no-till had significantly greater soil moisture content than tilled treatments (moisture content in volumetric percentage, at 10 percent alpha) (Appendix 3). Soil moisture content had a positive correlation with soil microbial respiration ($r = 0.38$; $p = 0.0018$). Higher water content under no-till compared to tilled treatments and its positive correlation with soil microbial activity (soil microbial respiration) might be suggestive of the no-till having greater soil microbial respiration and SMBC than tilled in September. Despite the fact that soil moisture content had a positive correlation with microbial respiration, it had no significant correlation with SMBC ($r = 0.02$; 0.8760). The difference between the no-till and the tilled treatments on soil microbial respiration and SMBC in September suggests that no-till results in a microbial environment that provides a certain kind of a buffer that would promote greater microbial activity at lower soil moisture levels. At similar soil moisture content between conventional plowing and zero tillage, Linn and Doran, (1984) found zero tillage to have lower soil temperature and greater respiration rates than conventional tillage. According to Brady and Weil, (2002) crop residues on zero tillage plots buffer extremes in soil temperature creating a cooler environment and this certainly has implications on soil biological activity.

In October, EOC was still greater in the no-till wheat and vetch treatments but only at the higher N fertilizer rate (101N) (Figure 5 (d)) while soil microbial respiration was

greater under no-till no cover at 34N and 101N N fertilizer rate (Table 3 and Figure 4 (d)). Both EOC and soil microbial respiration had a significant positive correlation ($r = 0.35$, $p = 0.004$) (Appendix 9). The positive correlation between EOC and soil microbial respiration shows the importance of EOC as a microbial substrate in the overall soil C-cycle (Sparling et al. 1998). None of the treatment factors, (N fertilizer rate, tillage and cover crop) had effect on SMBC (Figure 3 (d)) in October, just prior to cotton harvest. This reflects the fact that the perturbations that occur within the soil system are dependent on several other factors that include time after their occurrence and to a certain extent upon crop phenology.

Seasonal effect on SMBC, Soil microbial respiration and EOC

Seasonal climates can affect soil microbial processes directly through factors including soil temperature and moisture or indirectly through plant growth. Soil microbial biomass C showed minor variability over the growing season with June having the lowest SMBC when compared to the other months though it had about the same levels as July (Figure 6). SMBC size in June might be reflective of the amount of C that was released from the decomposition of cover crops from the winter growing season and might have had a constant supply of C all throughout June. In July however, there were crops growing in the field and the C that might have resulted from the root exudates and rhizodeposits might have replenished the amount of C that was sourced from the decomposition of the cover crops (previous crop) hence they might have maintained the same level of SMBC size compared to June. Indeed, Speeding et al. (2004) attributed the lack of

variability of SMBC over the maize growing season in their study to the input of C from the growing plant roots which they say it becomes the source of C when the contribution from the previous crop dwindles. According to Smith and Paul, (1990), such a continuous supply of C maintains SMBC at constant level. The higher levels of SMBC in September relative to June are hard to understand as September was drier than June and July (Figure 6) and SMBC had no significant correlation with soil moisture content (r= 0.02; p = 0.8760). This corroborates Debosz et al. (1999) who saw an increase in SMBC during summer (July) when soil moisture was lowest. However, EOC which is assumed to be a proxy for available C (Wang et al., 2003), was greater in September than in all other sampling months (Figure 6). There were higher EOC concentrations in September which resulted in greater SMBC compared to June. It is surprising that in October none of the treatment factors had effect on SMBC but it had greater SMBC than in June. In October, the cotton plants were at physiological maturity and thus not absorbing N or other nutrients. The reduced competition for nutrients between plants and microbes might have led to higher uptake of N therefore allowing them to access more C. Nevertheless, October had lower soil microbial respiration than in September (Figure 7). In October SMBC had a positive correlation with both soil microbial respiration and EOC (Appendix 9). Soil microbial biomass C also had a positive correlation with soil moisture content (r = 0.38; p = 0.0012). The EOC was the lowest in October compared to other months (Figure 6). This might be reflective that the available N due to reduced competition with plants allowed microbes to access more C.

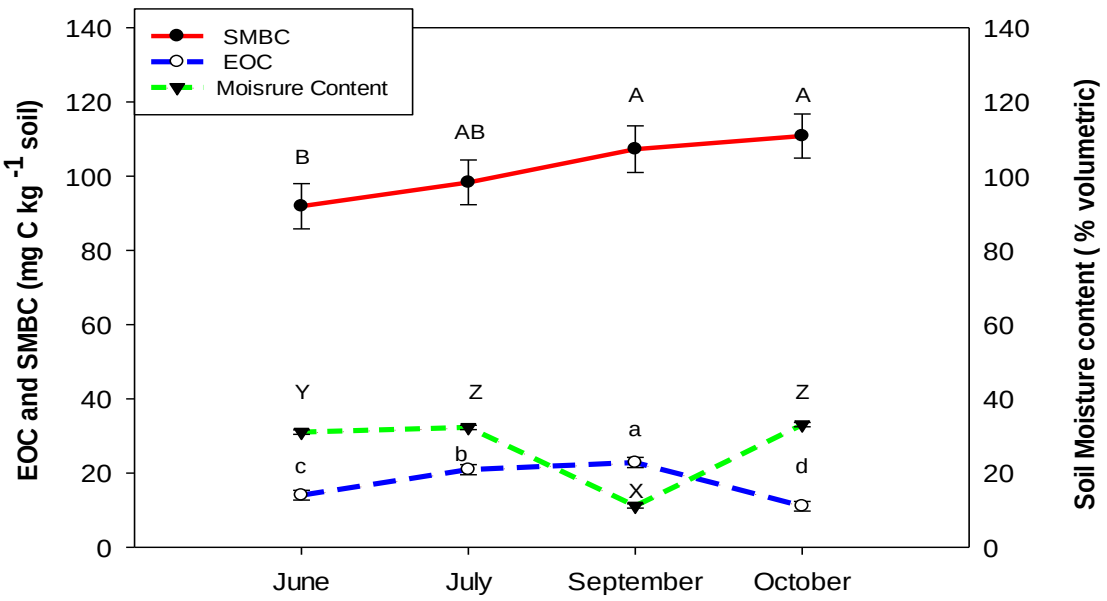


Figure 6. Seasonal effect on soil microbial biomass C (SMBC), KCl extractable oerganic C (EOC) and soil water content during 2013 cotton growing season. Each point is a mean of thirty six number (n=36). The error bars are the standard error of the mean.

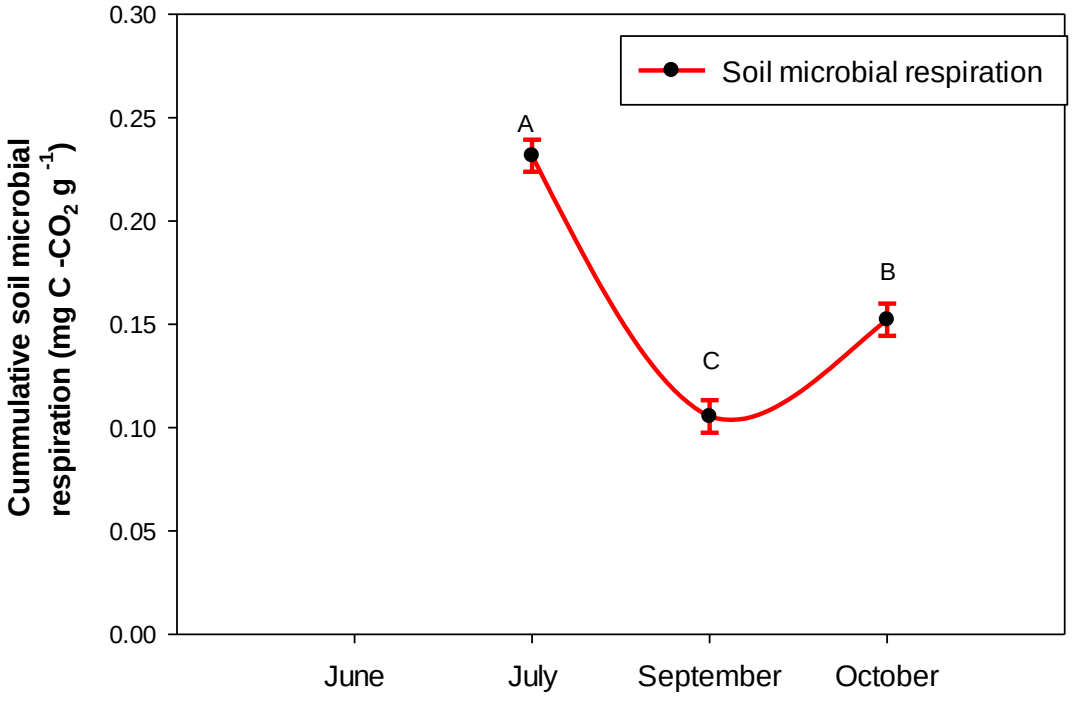


Figure 7. Seasonal effect on soil microbial respiration during 2013 cotton growing season. Each point point is a mean of thirty six numbers (n=36). The error bars are the standard error of the mean.

CHAPTER IV
Conclusions and Recommendation

This study was conducted to investigate the conservation agricultural practices comprising tillage, cover cropping and N fertilization effect on soil microbial C dynamics. In particular, the study aimed at determining the overall effect of these practices on SMBC and soil microbial respiration. The other aim was to determine the seasonality of SMBC and soil microbial respiration during 2013 cotton growing season.

Soil microbial biomass C showed little variation over the cotton growing season. June had the lowest SMBC compared to September and October. Soil microbial respiration was greatest in July and lowest in September.

While we found that there was a difference on both SMBC and soil microbial respiration between treatment factors, the difference was seasonal. What is apparent is that N fertilizer rate, tillage (tilled treatment) and to some extend vetch cover crop treatment were influential on SMBC and soil microbial respiration early in the growing season, shortly after the tillage operations and N fertilizer application. In June the 101N fertilizer rate resulted in greater SMBC and soil microbial respiration while in July the tilled-vetch treatment also resulted in greater SMBC and soil microbial respiration. In September the no-till and cover crop treatments (especially wheat and no cover) were the dominant drivers of both SMBC and soil microbial respiration. In October neither of the treatment factors had effect on SMBC while soil microbial respiration was effected by the no-till no cover treatment. The higher SMBC and soil microbial respiration under no-till especially towards the end of the growing season shows that there is higher carbon cycling under such system which may help to cycle nutrients internally within the biologically active

pools. What this may suggest is that no-till could be effective in nutrient conservation when compared to conventional tillage. What the data suggests is that it is not enough to take one measurement over the growing season and conclude about the behavior of the system. If we did only one time sampling in June, we could have been forced to conclude that only N fertilizer affects these properties whereas the tillage and cover crop effects become apparent later in the growing season. Due to seasonal effect on these soil biological properties (SMBC and soil microbial respiration), to further understand the effect of these treatment factors as influenced by season, future research will need to evaluate them over the winter cover crop growing season. This way we can have an idea of the whole year temporal variability of these properties and thus we can precisely conclude of how these practices affect these soil quality parameters.

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Appendix

Appendix 1. Selected soil physical and chemical properties as affected by N fertilizer rate (kg N ha^{-1}), cover crop and tillage. Sampling for all the properties except bulk density was completed in June 2013 while for bulk density was in July. P, K, Mg and Ca are extractable ions

Treatments			**Soil physical and chemical properties**					
			TN	**TC**	**P**	**K**	**Mg**	**Ca**
N-rate	CC	Tillage	g/ kg soil		mg/kg soil			
0N	NC	NT	1.15 (0.11)	10.66 (1.11)	139.13 (4.95)	297.63 (10.84)	77.00 (7.40)	1025.37 (23.31)
0N	NC	Tilled	1.07 (0.14)	9.97 (1.10)	101.50 (4.95)	247.25 (19.36)	74.13 (6.96)	968.00 (25.28)
0N	W	NT	1.11 (0.13)	11.14 (1.25)	138.63 (6.16)	277.25 (13.21)	73.86 (15.84)	976.62 (43.55)
0N	W	Tilled	0.90 (0.07)	8.87 (0.74)	106.00 (10.09)	228.88 (2.90)	62.25 (6.95)	868.12 (24.94)
0N	V	NT	1.51 (0.12)	14.37 (0.47)	109.88 (10.41)	220.75 (11.35)	87.00 (5.61)	1166.39 (55.26)
0N	V	Tilled	1.25 (0.09)	11.33 (1.25)	80.00 (8.57)	214.25 (25.36)	77.25 (9.06)	926.13 (30.35)
34N	NC	NT	1.08 (0.11)	11.62 (1.89)	108.17 (13.86)	251.19 (12.99)	76.10 (9.61)	911.11 (48.25)
34N	NC	Tilled	1.08 (0.03)	10.64 (0.2)	91.25 (3.91)	227.00 (11.16)	71.38 (7.88)	873.00 (25.33)
34N	W	NT	1.35 (0.04)	15.13 (0.87)	108.38 (5.25)	239.38 (3.79)	77.38 (7.07)	977.50 (25.34)
34N	W	Tilled	1.22 (0.14)	10.93 (0.71)	89.50 (3.81)	223.75 (12.57)	67.75 (1.48)	858.63 (21.46)
34N	V	NT	1.43 (0.08)	14.52 (0.68)	89.25 (10.47)	195.13 (19.44)	83.00 (4.22)	1004.50 (47.35)
34N	V	Tilled	1.15 (0.06)	11.64 (0.93)	69.38 (2.58)	211.88 (10.47)	76.88 (5.51)	834.75 (4.250)
101N	NC	NT	1.64 (0.10)	15.58 (0.67)	101.38 (6.72)	206.75 (10.59)	75.88 (10.24)	885.88 (72.97)
101N	NC	Tilled	1.51 (0.11)	13.44 (0.78)	83.13 (8.18)	218.38 (10.63)	69.63 (5.67)	775.13 (18.79)
101N	W	NT	1.52 (0.11)	14.47 (0.72)	95.38 (8.16)	195.13 (5.02)	73.63 (12.69)	811.37 (63.03)
101N	W	Tilled	1.39 (0.12)	13.95 (1.54)	81.75 (10.51)	221.50 (15.90)	74.25 (9.28)	751.00 (47.09)
101N	V	NT	1.45 (0.23)	14.63 (2.94)	82.17 (7.93)	197.39 (28.22)	70.03 (16.48)	731.80 (107.28)
101N	V	Tilled	1.41 (0.16)	13.32 (1.58)	65.63 (8.76)	199.25 (13.63)	63.13 (8.78)	618.63 (58.853)
LSD			**0.32**	**3.14**	**22.09**	**33.75**	**19.53**	**124.30**

N fertilizer rate (kg N ha^{-1}) (N-rate); cover crops (CC); no cover (NC); wheat (W); vetch (V); no-till (NT); total soil N (TN); total soil C (TC); soil bulk density (BD). Numbers outside the parentheses are means of four numbers (n=4) while number within are the standard errors of the means. The LSDs can be used to compare means between two treatment combinations where only one of the three treatment factors differs and the other two factors are held constant (e.g 0N, NC, NT compared to 0N, NC, Tilled or 101N, NC, NT compared to 34N, NC, NT).

Appendix 1 continued. Nitrogen fertilizer rate (kg N ha^{-1}), cover crop and tillage effects on soil pH and bulk density (BD). Sampling for pH was completed in June 2013 while for BD was completed in July.

			Soil physical and chemical properties	
Treatment Factors			pH	BD (g cm^{-3})
N-rate	CC	Tillage		
0N	NC	NT	5.62 (0.28)	1.54 (0.09)
0N	NC	Tilled	5.66 (0.34)	1.37 (0.09)
0N	W	NT	5.98 (0.03)	1.50 (0.15)
0N	W	Tilled	5.92 (0.02)	1.51 (0.03)
0N	V	NT	5.79 (0.11)	1.51 (0.05)
0N	V	Tilled	5.81 (0.13)	1.35 (0.06)
34N	NC	NT	5.59 (0.13)	1.45 (0.03)
34N	NC	Tilled	5.80 (0.14)	1.47 (0.06)
34N	W	NT	5.48 (0.17)	1.47 (0.04)
34N	W	Tilled	5.71 (0.07)	1.51 (0.03)
34N	V	NT	5.49 (0.07)	1.48 (0.02)
34N	V	Tilled	5.69 (0.11)	1.39 (0.05)
101N	NC	NT	5.05 (0.07)	1.44 (0.06)
101N	NC	Tilled	5.16 (0.15)	1.29 (0.02)
101N	W	NT	5.00 (0.14)	1.45 (0.05)
101N	W	Tilled	5.17 (0.11)	1.38 (0.04)
101N	V	NT	4.95 (0.25)	1.37 (0.05)
101N	V	Tilled	4.73 (0.08)	1.34 (0.05)
LSD			**0.43**	**0.16**

N fertilizer rate (kg N ha^{-1}) (N-rate); cover crops (CC); no cover (NC); wheat (W); vetch (V); no-till (NT); soil bulk density (BD). Numbers outside the parentheses are means of four numbers (n=4) while number within are the standard errors of the means. The LSDs can be used to compare means between two treatment combinations where only one of the three treatment factors differs and the other two factors are held constant (e.g 0N, NC, NT compared to 0N, NC, Tilled or 101N, NC, NT compared to 34N, NC, NT).

Appendix 2. Cover crop, N-rate (kg N ha^{-1}), and tillage effect on extractable organic C (EOC) during 2013 cotton growing season.

Treatments			Extractable Organic Carbon (EOC) (mg C kg^{-1})			
N-rate	CC	Tillage	June	July	September	October
0N	NC	NT	11.85 (2.10)	15.16 (3.56)	29.03 (10.32)	6.93 (0.70)
0N	NC	Tilled	7.64 (1.70)	13.90 (5.83)	8.14 (1.90)	4.48 (0.81)
0N	W	NT	9.33 (1.79)	33.73 (12.38)	22.67 (4.30)	8.17 (1.42)
0N	W	Tilled	8.81 (1.94)	12.43 (3.92)	9.80 (2.41)	7.30 (0.54)
0N	V	NT	11.30 (2.10)	19.81 (4.38)	17.34 (3.57)	7.48 (2.56)
0N	V	Tilled	10.43 (1.92)	18.00 (4.71)	19.44 (5.63)	8.43 (0.37)
34N	NC	NT	16.95 (6.18)	15.91 (3.29)	23.35 (3.01)	7.41 (0.22)
34N	NC	Tilled	12.03 (3.42)	12.71 (0.19)	18.16 (1.69)	7.77 (1.18)
34N	W	NT	10.95 (1.51)	18.16 (4.95)	18.35 (4.85)	7.13 (4.85)
34N	W	Tilled	11.46 (3.02)	17.65 (3.32)	16.87 (1.13)	9.96 (1.77)
34N	V	NT	15.50 (3.91)	31.69 (3.48)	39.10 (5.44)	14.31 (2.80)
34N	V	Tilled	15.28 (2.09)	14.72 (0.74)	25.51 (5.06)	10.16 (1.03)
101N	NC	NT	20.28 (1.71)	35.83 (9.91)	32.93 (8.29)	14.24 (0.65)
101N	NC	Tilled	26.77 (10.80)	37.29 (7.77)	32.19 (13.44)	11.54 (2.86)
101N	W	NT	20.73 (4.04)	34.37 (2.30)	35.51 (9.41)	17.28 (2.21)
101N	W	Tilled	12.17 (2.83)	34.77 (5.57)	22.32 (4.66)	10.53 (0.38)
101N	V	NT	23.85 (3.73)	31.56 (3.73)	54.06 (5.69)	25.66 (2.01)
101N	V	Tilled	19.69 (4.50)	36.25 (5.95)	29.46 (2.27)	19.00 (1.37)
LSD			**6.29**	**11.68**	**10.84**	**3.62**

N fertilizer rate (kg N ha-1) (N-rate); cover crops (CC); no cover (NC); wheat (W); Vetch (V); no-till (NT). Numbers outside the parentheses are means of four numbers (n=4) while numbers within are the standard errors of the means. The LSDs can be used to compare means between two treatment combinations where only one of the three treatment factors differs and the other two factors are held constant (e.g 0N, NC, NT compared to 0N, NC, Tilled or 101N, NC, NT compared to 34N, NC, NT).

Appendix 3. Soil moisture content as a affected by N fertilizer rate (kg N ha^{-1}), cover crops and tillage during 2013 cotton growing season. Numbers are means of four numbers (n=4), numbers within the brackets are standard error of the mean.

Treatments factors			**Soil moisture content (Volumetric %)**			
N-rate	CC	Tillage	June	July	September	October
0N	NC	NT	30.25 (1.39)	30.701 (2.32)	10.73 (2.78)	32.73 (1.69)
0N	NC	Tilled	26.11 (2.70)	21.14 (4.96)	9.35 (1.46)	33.84 (1.19)
0N	W	NT	30.76 (1.67)	26.18 (3.18)	8.75 (0.34)	30.68 (0.95)
0N	W	Tilled	28.95 (2.83)	28.97 (2.80)	9.20 (2.4)	33.00 (1.29)
0N	V	NT	36.69 (2.26)	34.88 (1.95)	12.26 (1.00)	35.36 (0.69)
0N	V	Tilled	27.66 (1.92)	31.54 (3.10)	10.39 (2.35)	32.53 (1.45)
34N	NC	NT	29.31 (2.14)	29.82 (1.04)	10.44 (1.33)	31.02 (0.64)
34N	NC	Tilled	28.96 (0.96)	33.27 (2.42)	7.73 (0.97)	35.35 (1.19)
34N	W	NT	32.85 (1.03)	33.40 (1.86)	10.02 (0.97)	31.44 (1.21)
34N	W	Tilled	26.09 (1.32)	30.70 (0.83)	10.64 (1.79)	33.92 (0.78)
34N	V	NT	37.88 (0.87)	35.30 (0.82)	11.99 (1.04)	34.66 (1.21)
34N	V	Tilled	31.25 (1.94)	34. 18 (1.09)	11.42 (0.62))	35.68 (1.02)
101N	NC	NT	34.17 (0.64)	32.85 (2.14)	12.25 (0.82)	33. 17 (1.41)
101N	NC	Tilled	28.82 (0.97	31.47 (3.5)	11.86 (1.85)	31.01 (1.42)
101N	W	NT	35.71 (1.24)	35.96 (1.36)	12.51 (0.33)	32.72 (0.91)
101N	W	Tilled	28.89 (0.58)	34.39 (1.52)	12.76 (1.37)	33.08 (1.78)
101N	V	NT	35.25 (1.73)	34.86 (1.01)	14.83 (1.67)	34.03 (0.32)
101N	V	Tilled	28.97 (1.24)	33.37 (1.17)	10.75 (1.06)	34.66 (1.08)
LSD			**4.40**	**6.37**	**4.12**	**3.33**

N fertilizer rate (kg N ha-1) (N-rate); cover crops (CC); no cover (NC); wheat (W); Vetch (V); no-till (NT). Numbers outside the parentheses are means of four numbers (n=4) while numbers within are the standard errors of the means. The LSDs can be used to compare means between two treatment combinations where only one of the three treatment factors differs and the other two factors are held constant (e.g 0N, NC, NT compared to 0N, NC, Tilled or 101N, NC, NT compared to 34N, NC, NT).

Appendix 4. A three way interaction effect of N fertilizer rate (kg N ha^{-1}), cover crop and tillage on SMBC (SMBC) during 2013 cotton growing season. Sampling was carried out repeatedly in June, July, September and October.

Treatments			Soil Microbial Biomass (SMBC) (mg C kg-1 dry soil)			
N-rate	CC	Tillage	June	July	September	October
0N	NC	NT	57.84 (18.74)	91.47 (26.54)	174.28 (39.62)	120.99 (7.77)
0N	NC	Tilled	74.30 (11.09)	100.50 (39.99)	77.97 (19.56)	88.39 (43.14)
0N	W	NT	76.60 (18.53)	52.55 (14.41)	141.25 (22.31)	70.33 (20.92)
0N	W	Tilled	66.46 (6.59)	62.79 (4.43)	72.03 (4.29)	117.48 (25.72)
0N	V	NT	77.71 (7.56)	69.40 (17.46)	107.59 (12.15)	124.06 (17.01)
0N	V	Tilled	81.77 (15.16)	58.32 (20.43)	83.18 (12.03)	123.85 (25.38)
34N	NC	NT	62.67 (8.07)	86.65 (6.73)	147.38 (32.13)	80.90 (21.33)
34N	NC	Tilled	118.43 (42.70)	58.80 (6.73)	102.51 (30.75)	124.92 (8.32)
34N	W	NT	100.67 (26.30)	129.26 (22.72)	91.56 (16.94)	86.26 (20.24)
34N	W	Tilled	71.80 (10.40)	88.08 (29.95)	89.51 (23.95)	136.95 (28.86)
34N	V	NT	77.01 (14.81)	84.88 (26.98)	103.43 (45.46)	128.09 (18.19)
34N	V	Tilled	119.82 (13.01)	134.79 (37.57)	90.34 (24.55)	122.28 (41.56)
101N	NC	NT	111.04 (19.27)	125.03 (16.56)	112.20 (24.58)	112.98 (31.56)
101N	NC	Tilled	139.66 (6.61)	96.99 (30.30)	88.00 (11.73)	73.69 (25.00)
101N	W	NT	83.83 (13.34)	151.63 (33.54)	133.34 (27.99)	112.31 (37.25)
101N	W	Tilled	97.71 (28.86)	107.59 (14.89)	71.09 (6.31)	128.28 (24.67)
101N	V	NT	128.70 (23.72)	91.26 (25.50)	154.48 (19.85)	121.44 (30.66)
101N	V	Tilled	108.86 (20.75)	179.81 (37.09)	91.14 (10.09)	121.87 (34.85)
LSD			**45.86**	**58.68**	**50.69**	**57.32**

N fertilizer rate (kg N ha-1) (N-rate); cover crops (CC); no cover (NC); wheat (W); Vetch (V); no-till (NT). Numbers outside the parentheses are means of four numbers (n=4) while numbers within are the standard errors of the means. The LSDs were determined at alpha 10 percent. The LSDs can be used to compare means between two treatment combinations where only one of the three treatment factors differs and the other two factors are held constant (e.g 0N, NC, NT compared to 0N, NC, Tilled or 101N, NC, NT compared to 34N, NC, NT).

Appendix 5. Cover crop, N fertilizer rate (kg N ha^{-1}), and tillage effects on basal soil respiration during 2013 cotton growing season. Incubations were carried out for two weeks, in July, September and October.

			Cumulative soil microbial respiration (mg C-CO_2 g^{-1} dry soil)		
N-rate (kg Nha^{-1})	CC	Tillage	July	September	October
0N	NC	NT	0.22 (0.03)	0.06 (0.02)	0.13 (0.01)
0N	NC	Tilled	0.17 (0.04)	0.07 (0.01)	0.13 (0.02)
0N	W	NT	0.19 (0.03)	0.10 (0.03)	0.13 (0.01)
0N	W	Tilled	0.20 (0.04)	0.06 (0.02)	0.14 (0.02)
0N	V	NT	0.24 (0.02)	0.09 (0.01)	0.16 (0.01)
0N	V	Tilled	0.23 (0.03)	0.10 (0.02)	0.15 (0.01)
34N	NC	NT	0.26 (0.02)	0.09 (0.03)	0.17 (0.02)
34N	NC	Tilled	0.18 (0.01)	0.05 (0.01)	0.14 (0.01)
34N	W	NT	0.20 (0.02)	0.10 (0.02)	0.14 (0.03)
34N	W	Tilled	0.23 (0.03)	0.09 (0.03)	0.13 (0.01)
34N	V	NT	0.26 (0.02)	0.15 (0.02)	0.16 (0.02)
34N	V	Tilled	0.27 (0.03)	0.13 (0.02)	0.17 (0.01)
101N	NC	NT	0.28 (0.04)	0.15 (0.04)	0.17 (0.01)
101N	NC	Tilled	0.22 (0.03)	0.13 (0.03)	0.14 (0.02)
101N	W	NT	0.27 (0.02)	0.12 (0.03)	0.13 (0.04)
101N	W	Tilled	0.26 (0.02)	0.11 (0.02)	0.16 (0.01)
101N	V	NT	0.23 (0.02)	0.18 (0.01)	0.19 (0.03)
101N	V	Tilled	0.26 (0.01)	0.12 (0.03)	0.21 (0.03)
LSD			**0.056**	**0.049**	**0.04**

N fertilizer rate (kg N ha-1) (N-rate); cover crops (CC); no cover (NC); wheat (W); Vetch (V); no-till (NT). Numbers outside the parentheses are means of four numbers (n=4) while numbers within are the standard errors of the means. The LSDs can be used to compare means between two treatment combinations where only one of the three treatment factors differs and the other two factors are held constant (e.g 0N, NC, NT compared to 0N, NC, Tilled or 101N, NC, NT compared to 34N, NC, NT).

Appendix 6. Simple Pearson correlation analysis among soil physical and chemical properties. Sampling was completed in June 2913

Properties	TN	TC	P	Ca	Mg	K	pH
TN							
TC	0.85291						
	<.0001						
P	-0.14583	-0.13775					
	0.2284	*0.2555*					
Ca	-0.09805	-0.01534	0.48664				
	0.4194	*0.8997*	***<.0001***				
Mg	-0.10551	0.02196	-0.05239	0.53270			
	0.3847	*0.8568*	*0.6666*	***<.0001***			
K	-0.28516	-0.25497	0.72594	0.34046	-0.12225		
	0.0167	***0.0332***	***<.0001***	***0.0039***	*0.3134*		
pH	-0.36046	-0.34892	0.30893	0.47820	0.00919	0.30978	
	0.0022	***0.0031***	***0.0093***	***<.0001***	*0.9398*	***0.0091***	

Total soil nitrogen (TN); total soil carbon (TC); extractable Phosphorous (P); extractable calcium (Ca); extractable magnesium (Mg); extractable potassium (K). The italicized numbers are p values while the normal numbers are correlation coefficients (r). Probability significance was set at $p < 0.05$.

Appendix 7. Simple Pearson correlation coefficient between soil microbial properties sampled in July

	Soil biochemical properties	
	SMBC	EOC
EOC	0.17	
	0.189	
Soil Resp	0.37	0.33
	0.002	***0.008***

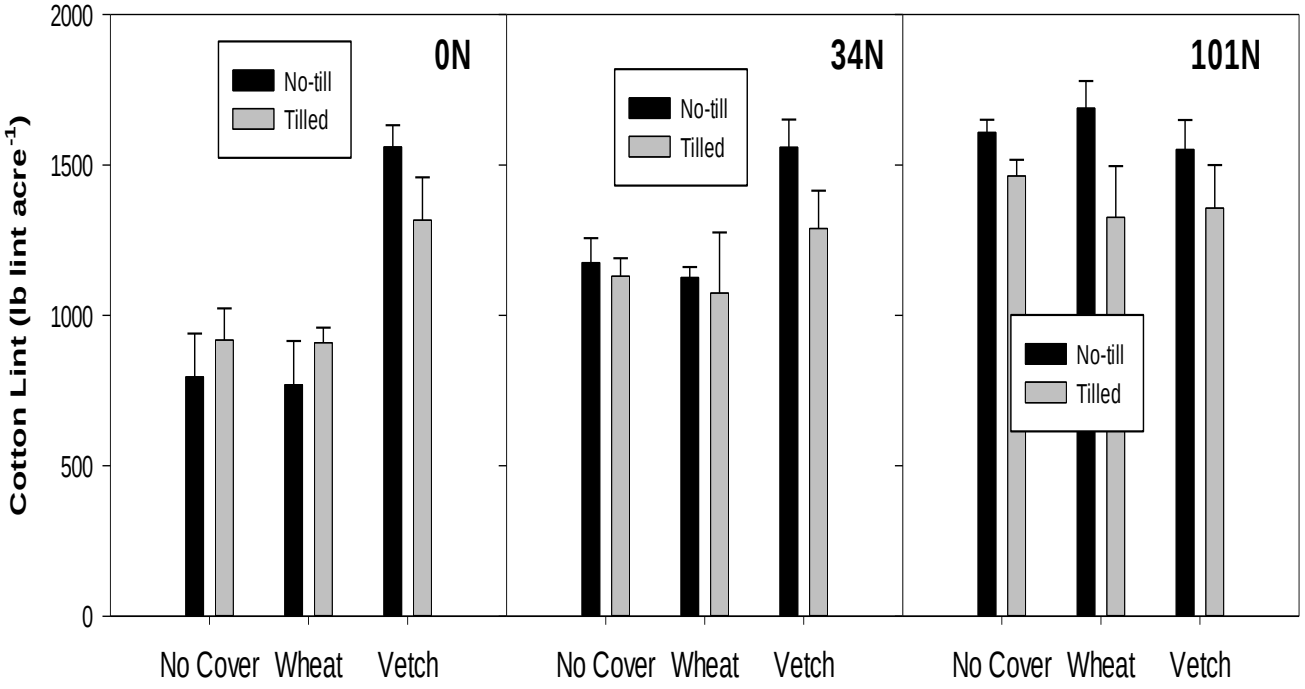
Appendix 8. Simple Pearson correlation coefficient between soil microbial properties sampled in September

	Soil biochemical properties	
	SMBC	EOC
EOC	0.21	
	0.081	
Soil Resp	0.18	0.61
	0.164	***<0.0001***

Appendix 9. Simple Pearson correlation coefficient between soil microbial properties sampled in October

	Soil biochemical properties	
	SMBC	EOC
EOC	0.31	
	0.008	
Soil Resp	0.24	0.35
	0.043	***0.004***

Extractable organic C (EOC); Soil microbial biomass C (SMBC); Soil microbial respiration Soil Resp).The numbers are correlation coefficients (r) while the italicized numbers are p values. Probability significance was set at $p < 0.05$.



Appendix 10. Tillage, cover crop and N fertilizer rate (kg N ha^{-1}) on cotton lint yield over 2013 growing season. Each column is a mean of four numbers (n=4) and error bars are standard error of the mean

Vita

Molefi Mpheshea was born and raised at Khubetsoana, Maseru, Lesotho. He attended his primary school at Mejametalana and finished his high school in 2004 at Khubetsoana high school. Molefi attained his first degree in agriculture with concentration in Soil Science and Resource Conservation at National University of Lesotho in 2010. In 2012 he embarked on a journey to pursue Master of Science in Agriculture majoring in Environmental Soil Science at the University of Tennessee-Knoxville, and earned MSc in 2014.