ABSTRACT

EPTING, CARLEE GRACE. Impacts of Tillage Practices and Cover Crop Adaptation on Soil Health and Soil Organic Matter Partitioning in Different Ecosystems (Under the Direction of Dr. Ekrem Ozlu).

Intensive management and land use changes can globally cause loss of fertile topsoil, soil degradation, and loss of soil carbon stocks over time. This study examines the impacts of tillage, land use and cropping systems diversification on soil health and organic matter partitioning in the Coastal Plain and Tidewater regions of North Carolina. We performed two individual studies to investigate this question. First, a long-term study investigates how land use, tillage, erosion, and duration of management practices impact the interactions between carbon and nitrogen stocks of soil organic matter fractions and other soil properties. This study contains data from eight different sites in the Coastal Plain of Pender County including no-till less than two years, no-till twenty-five years with slope, no-till twenty-five years without slope, no-till longer than forty years, vertical till longer than forty years, strip till longer than forty years, a pasture longer than forty years, and forest longer than forty years. Soil samples were collected up to the depth of 55 cm and analyzed for soil texture, pH, electrical conductivity, bulk density, and particulate organic matter and mineral associated organic matter. Results showed variation in bulk density and soil texture across sites. pH and electrical conductivity were found to be influenced by organic matter fractions and texture. Organic matter inputs impacted soil carbon and nitrogen stocks. A second short-term study was focused on evaluating effects of tillage (no-till, conventional tillage (disking)) and cover cropping (cereal rye, crimson clover, no cover crop, and cereal rye and crimson clover mix) on soil health indices under corn-soybean rotation in Eastern North Carolina. Soil samples were collected to the depth of 20 cm and analyzed for soil bulk density, texture, pH, electrical conductivity, water extractable carbon and nitrogen, soil microbial respiration, and soil enzymes (β-glucosidase and urease). Results from this study indicated lower bulk density values in no tillage sites when compared to conventional disking. Soil microbial activity and soil respiration were highest in no-till treatments with one or more cover crops, especially in Tidewater soils. Water extractable carbon and nitrogen had over all higher values in no tillage treatments. These studies highlight the influence of cover cropping and tillage systems on soil health, as well as the influence of soil properties on soil organic matter fractionations.

Impacts of Tillage Practices and Cover Crop Adaptation on Soil Health and Soil Organic Matter Partitioning in Different Ecosystems

by

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DEDICATION

To my mother, Stacey, and grandmother, Maura, who have always encouraged my pursuit of education, and supported me along the way. To my older sister Bailey, who followed me to Raleigh to help support my dreams and kept me on track. To my friend Jessica, who always supported me even 300 miles away. To my partner Connor and his family, who have showed their support throughout my entire academic journey. And finally, to all my close family, who I carry on my mind as I pursue my next adventure.

BIOGRAPHY

Originally from West Columbia, South Carolina, Carlee Epting spent her childhood visiting and spending time on her grandfather's generational farm. These experiences sparked her original interest in agriculture. Carlee graduated from Brookland-Cayce High school in 2018. After graduation she attended The University of North Carolina at Pembroke where she completed her bachelors of science in environmental science in 2022.

After completion of her bachelor's degree, she decided to pursue her masters of soil science degree at North Carolina State University. During her journey at NCSU, she worked directly with farmers for her research. These encounters sparked her passion for outreach and farmer connections. After graduating she hopes to pursue a job where she can develop her passions.

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Abstract

Intensive management and land use change can cause loss of fertile topsoil and lead to soil degradation over time. This study provides insight into potential indicators that improve carbon stocks for each particulate and mineral associated organic matter fractions. Study sites, in Pender county North Carolina, include no-till less than two years, no-till twenty-five years with slope, no-till twenty-five years without slope, no-till longer than forty years, vertical till longer than forty years, strip till longer than forty years, a pasture longer than forty years, and forest longer than forty years. Intact and disturbed soil samples were taken to a depth of 55 cm with 5 cm increments. These were then analyzed for texture, bulk density, pH, electrical conductivity, particulate organic matter carbon, mineral associated organic matter carbon, particulate organic matter carbon stocks, and particulate organic matter nitrogen stocks. Biplot principal component analysis results showed clustering along two components. Soil pH, opposite to particulate organic matter carbon and nitrogen and bulk density, opposite to mineral associated organic matter carbon and nitrogen, suggest a negative correlation between organic matter fractionations and these soil properties. Organic matter fractions clustered together indicating a common variance, offering a possible link to soil carbon dynamics. Multiple linear regression analyzing possible predictors for particulate organic matter carbon, mineral associated organic matter carbon, particulate organic matter carbon stocks, and particulate organic matter nitrogen stocks, showed soil properties such as particulate organic matter carbon and nitrogen stocks, and mineral associated organic matter carbon and nitrogen being correlated with each other. This study focused on an important gap of knowledge

on soil health indices which factors are improving soil structure and soil carbon fractions in North Carolina Coastal Plain soils.

1.2. Introduction

Soil health indicates the capacity of soils to function (USDA, 2024) for greater agricultural productivity and sustainable ecosystem services due to soil's critical role in food security, water filtration, and carbon (C) sequestration. However, unsustainable agricultural practices, intensive management, and land use associated changes such as the loss of fertile topsoil via erosion, can lead to soil degradation and loss of organic matter stocks over time.

Soil organic matter (SOM) is among the most studied attributes of soil due to its importance for enhancing soil fertility and productivity while mitigating climate change through C sequestration. Soil organic carbon (SOC) improves soil health, supports soil structure, and provides more sustainable farming (Ozlu and Arriaga, 2021). Soil carbon stocks (SCS) are important to evaluate overall impacts of management practices and land use change on SOM, however, the concept of SOM partitioning into different C fractions such as particulate organic matter (POM) and mineral associated organic matter (MAOM), provides insights about SOC stability and persistence (Lavallee, 2020; Mosier et al., 2024). In this concept, POM indicates physically protected organic matter while MAOM indicates chemically bonded – more stable fractions (Golchin et al., 1997; Kleber et al., 2015; Lavallee et al., 2020).

Conservation practices including but not limited to no-till and reduced tillage aim to provide less aggressive and less frequent disturbance. Conservation management practices can help with improving soil structure and stability of organic matter (OM) in smaller pores (Ozlu, 2020). In contrast, conventional management practices tend to provide more disturbance and require frequent entry into the field by heavy equipment which can cause aggregate disruption, compaction, and hence OM destabilization. Although the goals of tillage, such as preparing seedbeds and managing weeds (Bajwa, 2014) help with farming management, the unintended consequences on soil structure and health could be limiting factors for conventional-aggressive tillage applications (Ozlu, 2020). In North Carolina, recommendations on tillage applications can specifically be challenging due to soil types and climate being quite variable.

Sandy soils in North Carolina are prone to compaction and erosion under intensive tillage applications (Reyes et al., 2024). Conventional management practices can disrupt soil structure, especially in soils containing high clay content. This disruption and soil texture cause soil compaction at various layers within the soil profile where clay is more prevalent (Ozlu et. al.2023). Conventional management practices also cause limitations in water infiltration and increase susceptibility to erosion (Djillo et al., 2024). It is well documented that the gradual breakdown of soil aggregates leads to the loss of protective structure, accelerates OM decomposition by exposing SOC to microbial activity and leads to long-term SOC depletion, and increases degradation of topsoil (Ozlu and Arriaga, 2021). Conservation tillage in contrast reduces soil disturbance, improves soil structure, and potentially preserves SOC by promoting slower decomposition, re-formation of soil aggregates, and enhanced aggregate stability (Ozlu and Arriaga, 2021).

Land use change by human engineering and naturally by erosion increases the complexity of tillage operations and impacts in sandy soils. The long-term impact of erosion on SOC loss, nutrient depletion, reduction in topsoil depth, and agricultural productivity is well documented (Ozlu and Arriaga, 2021). Land use changes, in particular the conversion of forests or grasslands to agricultural fields, alters soil properties. If they have previously been under long-term forest vegetation, soils converted to agricultural management come with great potential to be compacted due to heavy machinery and clay rich subsurface compressing and compacting the soil below leading to rapid SOC loss and erosion (Ozlu et al., 2020). Therefore, the long-term implications of land conversion are critical to study due to impacts on loss of C stocks and stability of OM. Reducing soil disturbance and improving plant root systems, helps protects soil loss from erosion and stabilize SOC. This root system and increased stabilization can be achieved through crop diversification and conservation management (Ozlu and Arriaga, 2021: Lehman et al. 2020).

Soil C fractions and pore diversity are promoted as indicators of a soil's ability to function (Lehman et al., 2020). Disturbance via conventional tillage, land use change, and erosion tend to reduce POM due to break down of aggregates and accessibility of SOC to microbial attacks (Ozlu and Arriaga, 2021), while conservation tillage can promote POM accumulation by minimizing soil disturbance (Yulnafatmawita et al., 2020). The proportion of POM is inversely associated with MAOM where the MAOM is closely associated with mineral particles like clay and stay in soils longer. Previous studies reported that no-till has greater POM and MAOM stocks in soils in comparison to conventional tillage management (Ozlu and Arriaga, 2021). However, some other studies report that tillage can help with protecting MAOM within micro aggregates (Yulnafatmawita et al., 2020). It is known that MAOM is more stable, less sensitive to short-term management changes, but may still be influenced by long-term tillage practices and aboveground vegetation.

Studies focusing on soil health indices that improve soil structure and increasing soil C fractions, have not been expanded upon in North Carolina Coastal Plain soils in detail. This lack of investigation has created a large gap of knowledge. Conducting on farm field observational studies will allow the gap of knowledge to become bridged with baseline information of SCS based on different managements, temporal changes, erosion and land use change. This paper aims to investigate SOC partitioning (POM and MOAM) over extended periods. Specific objectives of this study are:

- To evaluate key soil properties influencing soil C fractions
- To examine the correlations and influences of soil properties on one another
- To examine particle size distribution and bulk density correlations with SOC partitioning.

1.3. Materials and Methods

1.3.1. Study sites description

Soil samples were taken from eight different study sites (Table 1.1) within Pender County, North Carolina, including; (i) no-tillage of more than 40 years (NT40) (ii) strip tillage of more than 40 years (ST40) (iii) vertical Tillage of more than 40 years (VT40) (iv) no-till of 25 years with slope (NT25s) (v) no-till of 25 years without slope (NT25ns) (vi) forest of more than 40 years (F40) (vii) no-till of less than 2 years (NT2) (viii) pasture of more than 40 years (P40). Study sites generally have similar soil types, and all cropped sites are under the same cropping system of corn-soybean rotation. These sites are also under the same Soil Water Conservation Management programs that regulates soil management practices and fertility. The NT40 site was on a Foreston series (coarse-loamy, siliceous, semiactive, thermic Aquic Paleudults) where no-till management was performed for about 40 years.

The ST40 site was on an Aycock series (fine-silty, siliceous, subactive, thermic Typic Paleudults) where strip tillage was performed for about 40 years using an Unverferth Ripper Stripper to a 12-inch depth. VT40 was on a Woodington series (coarse-loamy, siliceous, semiactive, thermic Typic Paleaquults) where vertical tillage was performed for about 40 years using a Case IH vertical tiller. All no-till sites were managed under conventional disking prior to their current management and land use. Both NT25s and NT25ns were on a John's series (fine-loamy over sandy, siliceous, semiactive, thermic Aquic Hapludults) and Norfolk series (fine-loamy, kaolinitic, thermic Typic Kandiudults) under no-till management for about 25 years. NT2 is on Rains series (fine-loamy, siliceous, semiactive, thermic Typic Paleaquults) managed under no-till for only 2 years. Both F40 and P40 sites were on Norfolk series (fine-loamy, kaolinitic, thermic Typic Kandiudults) and Aycock series (fine-silty, siliceous, subactive, thermic Typic Paleudults) which transitioned to current forest and pasture vegetation from agricultural management.

Total precipitation for Pender County annually for 2024 was 120.75 cm. The average air temperature for Pender County was 18°C (North Carolina State Climate Office, 2025).

1.2.3 Soil Sampling and processing

Intact core samples (8.25 x 5.08 cm) were collected from 0-50 cm depths within 5 cm increments using the pit method at three locations within each field in the summer of 2023 for a total of 240 samples. Composite samples were also taken from 0-50 cm depth with 5 cm increments and at three locations. These samples were then placed in a labeled plastic Ziploc bags and transferred to laboratory for further processing and analysis.

1.3.3. Study Analysis

Soil particle size distribution

Soil particle size distribution analysis was performed using the 2-hour hydrometer method (Bouyoucos, G.J. 1962). Briefly, composite samples (50 g of 2 mm sieved soils or 10 mesh) were weighed out and mixed with 100 ml of 5% Sodium Hexametaphosphate solution. These were then stirred and set out for 30 min, then transferred into a dispersion cup to be placed on an electronic mixer for 5 min. The soil suspension was then transferred into a 1 L sedimentation cylinder, mixed thoroughly and then the first hydrometer and temperature reading are taken at 40 seconds. After the first reading, the suspension was left to rest for 2 hours, and another hydrometer and temperature measurement was taken at the end of 2 hours. For temperature correction, we added 0.36 g L^{-1} to the hydrometer reading for every 1°C above 20°C, whereas we subtracted 0.36 g L^{-1} from the hydrometer reading for every 1°C below 20°C. The temperature-corrected hydrometer reading from the soil suspension should be subtracted from the temperature-corrected hydrometer reading taken from the blank solution (without soil) at 40 seconds for sand measurement and 2 hours for clay measurement.

1.3.4. Bulk density

Intact core samples taken directly from the field and weighed to obtain a wet soil weight, before being placed in a drier at 105°C. Dry soil mass were taken after samples were fully dried for about 48 hours (Grossman and Reinsch, 2002).

1.3.5. Soil pH and electrical conductivity

Composite air-dried ground samples were passed through a 2 mm sieve used to conduct soil pH using a 1:1 soil and water ratio (Wang, 2014 and Rhoades, 1996) and EC analysis using 1:2.5 soil and water ratio (Sikora et al., 2014).

1.3.6. Particulate organic matter and mineral associated organic matter fraction analysis

Unground bulk air dried soil was used for POMC, MAOMC, POMN, and MAOMN analysis. After the soils were passed through a 2 mm sieve, 10 g was weighed out and placed in pre-weighted centrifuge tubes and placed in a 60°C oven overnight. Once soils were dried, they were then weighed with the tubes, 12 glass beads were added to each sample, and then 30 ml of water was added. Samples were then placed on an orbital shaker and shaken for 18 hours at 120 oscillations per minute. After samples are done, they are then shaken in the tube to make sure that the soil is in suspension and passed through a 53 µm sieve that is placed in a shallow pan to catch the outwash. Once placed through the sieve, soil is washed through with deionized water until the water passing through became clear. After water ran clear, soil remaining on the top side of the sieve is then backwashed into a pre-weighted metal tin (POM), and outwash passed through the sieve previously is collected in another pre-weighted tins (MAOM). Tins with soil samples were then placed in an oven set at 60°C for 24 hours or until dried, then weighed after drying (Cotrufo et al., 2019, Leuthold et al., 2024; Lavallee, et al., 2020). Samples were then finely ground and placed in tubes and sent to the Department of Biological and Agricultural Engineering, Environmental Analysis Lab (BAE) where they were passed through an Elementar Unicube Organic Elemental Analyzer to determine the C and N content.

1.3.7. Soil carbon and nitrogen POM and MAOM stocks

C-N concentrations received from the BAE lab were used with BD values to calculate the C stocks and N stocks for each fractionation. Calculations for C stocks for each fraction was done using same equation used for total organic carbon (TOC) and total organic nitrogen (TON) carbon stocks calculations provided in below section. Calculations for C and nitrogen (N) stocks were performed using following equation:

$$C\ or\ N\ stocks = \sum_{i=1}^{n}(Ci\ x\ \rho b\ x\ di)$$

where C and N stocks are the TOC and TN stocks (kg m^{-2}), Ci is the TOC and TN contents (kg kg^{-1}), rb is the soil BD (kg m^{-3}), and di is the depth (m).

1.3.8. Statistical Analysis

A series of generalized regressions was performed using the fit model function in JMP Pro 18 with the general regression personality and the Lasso estimation method where; (i) POMC is the role variable (Y), soil properties soil properties as the construct model effects; (ii) POMN, as Y, and soil properties as the construct model effects; (iii) MAOMC as (Y), and soil properties as the construct model effects; (iv) MAOMN as (Y), and soil properties as the construct model effects. A Principal components analysis (PCA) was conducted using the Multivariate Methods analysis tool in JMP Pro 18, on all soil properties to analyze the similarities and correlation between groups of samples from the data set. Correlation analysis was performed in Microsoft Excel analyzing all of the soil properties from the data set.

1.4. Results

1.4.1. Principal Component Analysis

A principal component analysis was applied in order to reduce the size of data comparisons and visualize the data more cohesively. Clay content had significant positive correlation with bulk density. However, the proportion of clay and silt content had negative correlation with EC, sand, MAOMC, MAOMN, CMAOM, and NMAOM. Further soil BD showed significantly negative correlations with CPOM, NPOM, POMC, and POMN. Besides physical properties, chemical properties like EC had significantly positive correlation with CPOM and NPOM. However, MAOMN had significantly positive correlation with EC but negative correlation with pH.

Thirteen principal components were captured from this data, with components 1 (33.5%) and 2 (28.1%) accounting for the highest variance (Figure 1), Components 3 – 13 contribute 20%. Principal Component 1 is heavily driven by NMAOM, CPOM, NPOM, POMC, POMN, MAOMN, and CMAOM. On the other hand, principal component 2 exemplifies another major source of variance due to strong loadings of MAOMC and CMAOM. The positive correlations between variables like POMC and CPOM advocate that POMC and CPOM increase together, whereas sand and clay are negatively correlated, reflecting their contrasting textural roles.

1.4.2. Relationships between soil health indicators and C-N stocks of SOM fractions

1.4.2.1. Particulate organic matter carbon

A LASSO regression analysis was performed to examine potential predictors for POMC. Soil properties including sand, silt, clay, BD, EC, pH, POMN, MAOMC, MAOMN, NMAOM, CMAOM, NPOM, CPOM, POMC were used for the X variables and POMC was placed as the Y variable.

The Overall model signifies a strong relationship between the X and Y variables (R^2= 0.99). Model p-value is significant ($p<0.05$). The results indicated that POMN was a significant predictor of POMC ($P=0.01$). NPOM also presented to be a predictor of POMC ($P=0.04$). CPOM was also a predictor for POMC (P=<0.0001). All other soil properties are not significant on POMC (P>0.05).

1.4.2.2. Particulate organic matter nitrogen

This LASSO regression analysis was performed to examine the impact of soil properties (X) (sand, silt, clay, BD, EC, pH, POMC, MAOMC, MAOMN, NMAOM, CMAOM, NPOM, CPOM on POMN (Y).

Overall regression model indicated a good fit for this data set (R^2=0.99). The P-value for overall model is not significant (P=0,08) However, BD presented as a significant predictor for POMN in this regression with a P-value of 0.04. NPOM also showed to be a significant predictor for POMN, P<0.0001. Other soil properties did not present as significant predictors for POMN (P>0.05).

1.4.2.3. Mineral associated organic matter carbon

This LASSO regression analysis was performed to examine the impact of soil properties (X) (sand, silt, clay, BD, EC, pH, POMN, MAOMN, MAOMN, NMAOM, CMAOM, NPOM, CPOM on MAOMC (Y).

This model produces a R^2 value of 0.99 indicating this model creates a good fit for this data. The overall p-values indicates significance (P<0.05). Specifically, the proportion of sand content (P=0.001), BD (P<0.05), MAOMN (P=0.03), NMAOM (P=0.045), and CMAOM (P<0.05) all show to be significant predictor for MOAMC content.

1.4.2.4. Mineral associated organic matter nitrogen

This LASSO regression analysis was performed to examine the impact of soil properties (X) (sand, silt, clay, BD, EC, pH, POMC, MAOMC, MAOMN, NMAOM, CMAOM, NPOM, CPOM on POMN (Y).

Model fit presents an R^2 value of 0.99, indicating a good fit for this model and the data. Overall model p-value is significant ($P=0.0001$). Exact significance lies within; EC ($P=0.042$), MAOMC ($P=0.04$), NMAOM ($P<0.05$), CMAOM ($P=0.046$). Specifically, this significance indicates that these soil properties are predictors for MAOMN. Other soil properties do not indicate significance, and not strong predictors for MAOMN.

1.4.3. Correlation Analysis

Throughout the correlation analysis, positive values indicate a positive relationship with other properties, while negative values indicate an opposite correlation, as one increases the other decreases. POMN and NPOM have a correlation analysis of 0.98 indicating a high linear correlation between the two soil properties. POMC has a high correlation with CPOM (0.98), POMN (0.93), and N (0.93). MAOMC, MAOMN, CMAOM, and NMAOM are all highly positively correlated together, with correlation values ranging from 0.73 to 0.96. Several soil properties had strong inverse relationships between each other. The strongest negative correlation is between silt and sand soil properties (-0.96).

1.5. Discussion

This study focused on a large gap of knowledge on soil health indices which factors improving soil structure and SOM fractions in North Carolina Coastal Plain soils. This on farm field observational study provides an investigation of a real-life condition scenario which allows the gap of knowledge to become bridged with baseline information of SOM fractions and stocks.

1.5.1. Principle Component Analysis

Soil texture appeared to be a key factor impacting BD, EC, and SOM fractions. The proportion of sand was positively correlated with MAOMC, while the proportion of clay showed negative correlation with EC, CMAOM, MAOMN and NMAOM fractions. Thus, coarser textured soil particles support SOM stabilization via mineral associations, but finer particles may delay nutrient retention due to soil compaction (Hussain et. al, 2001). The negative correlation between bulk density and POMC or POMN highlights how critical soil physical structure is for SOM dynamics. Moreover, soil chemical composition including pH and EC also impacted CPOM and CMAOM (Zhou et. al. 2019). For example, MAOMN showed a positive correlation with EC but had a negative correlation with pH. This denotes significant impacts of ionic interactions and soil acidity on N stabilization in mineral complexes (Ozlu and Kumar, 2018). PCA further disclosed clear patterns in soil health indices including particle size distribution as the dominant source of variance which also impacts interactions of other soil properties and SOM pools.

1.5.2. Relationships between soil health indicators and C-N stocks of SOM fractions

1.5.2.1. Particulate organic matter carbon

POMN and NPOM are predictors of POMC. Sun et. al. (2023) studied the responses of soil organic C pools to the addition of N. This study suggests that an increase in N even in stock proportions increases the content of POMC. The increase of POMC can be attributed to the increase of N, by changing the main C:N ratio and source for microbial biomass and overall reducing the composition of POMC (Sun et. al., 2023).

1.5.2.2. Particulate organic matter nitrogen

While a predictor for POMN, BD and POMN are negatively correlated to each other. Compaction of the soil (a higher bulk density value) could indicate that there are less pores in spaces where N pools and sources accumulate, decreasing microbial breakdown. This decrease in microbial breakdown can have a negative effect on the POMN present in the soil (Longepierre et. al., 2022). CPOM are more sensitive to level changes than those of NPOM. Due to this an accumulation of stocks can be a predictor for the values of POM in the soil (Manzoni et. al., 2024).

1.5.2.3. Mineral associated organic matter carbon

Much like that of POMN, BD can be a predictor for MAOMC values. Greater pore availability in lower BD soils can cause an increase in overall C availability to microbes and increase the breakdown and formation of MAOMC fractionations in the soil (Longepierre et. al., 2022). Sand content as shown through the PCA analysis is positively correlated with MAOMC, meaning that these two soil properties increase linearly together. Hussin et. al. (2021) suggests that coarser textures sols particles support SOM stabilization through mineral associations.

1.5.2.4. *Mineral associated organic matter nitrogen*

Soil C:N ratio affects MAOMC by effecting the potential predictors. Having a greater increase of N stocks lowers the C:N ratio in the system which leads to slower decomposition of

SOM stocks of different soil fractions. The opposite can be said about an increase in C stocks (Longepierre et. al., 2022). Existing studies show that MAOMN is potentially the largest source of mineralized N (NRCS, 2014; Villarino et. al.) and EC is a strong predictor for N retention. This can explain the changes in soil MAOMN fractions and impact of EC. EC is also documented as reflecting a soil's capacity to retain nutrients such as N (Mirzakhani et. al., 2017).

1.6. Conclusions

This study provides baseline information regarding soil C fractionations and stocks on soils, specifically in the Coastal Plain region of North Carolina. Through evaluation of key soil properties that contribute to soil C fractions, impacts of BD, soil texture, and EC were highlighted through PCA as well as correlation analysis to influence these fractions. Multiple linear regression analysis further highlights the influence and possible predictors of POMN, POMC, MAOMN, and MAOMC. These findings can be used to analyze these predictors influence on POMN, POMC, MAOMN, and MAOMC in differing land use factors, giving way to the implications of soil health management effects on SOC partitioning.

REFERENCES

Bajwa, A. A. (2014). Sustainable weed management in conservation agriculture. *Crop Protection*. Volume 65, pages 105-113.

Bouyoucos, G.J. (1962). Hydrometer method improved for making particle size analyses of soils. *Agron*. J. 54:464-465.

Cotrufo, M. F., Ranalli, M. G., Haddix, M. L. (2019). Soil carbon storage informed by particulate and mineral-associated organic matter. (pp. 989–994) *Nat. Geosci*. 12.

Djillo, S. C., Wolka, K., and Tofu, D. A. (2024). Assessing soil erosion and farmers' decision of reducing erosion for sustainable soil and water conservation in Burji woreda, southern Ethiopia. *Scientific Reports*, 14(1), 8638.

Golchin, A., Baldock, J. A., and Oades, J. M. (1997). A model linking organic matter decomposition, chemistry, and aggregate dynamics. In R. Lal, J. M. Kimble, R. F. Follett, and B. A. Stewart (Eds.), Soil processes and the carbon cycle (pp. 21–32). *Lewis Publishers*.

Hussain S., Hussain S., Guo R., Sarwar M., Ren X., Krstic D., Aslam Z., Zulifqar U., Rauf A., Hano C., El-Esawi M. A. (2001). Carbon Sequestration to Avoid Soil Degradation: A Review on the Role of Conservation Tillage. *Plants (Basel)*. 10 (10).

Lavallee, J. M., Soong, J. L., and Cotrufo, M. F. (2020). Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. *Global change biology,* 26(1), 261-273.

Manzoni S., Cotrufo M. F. (2024). Mechanisms of soil organic carbon and nitrogen stabilization in mineral-associated organic matter - insights from modeling in phase space. *Biogeosciences*. Vol. 21. 4077-4098.

Mirzakhani, H., Mani I., Mirzakhani A.N. (2017). Study on Soil Nitrogen and Electrical Conductivity Relationship for Site-Specific Nitrogen Application. *Sensors.* Vol.22.

Mosier, S., Kelly, L., Ozlu, E., and Robertson, G. P. (2024). Switchgrass (Panicum virgatum L.) cultivars have similar impacts on soil carbon and nitrogen stocks and microbial function. GCB Bioenergy, 16(3), e13125.

North Carolina State Climate Office. (2025). https://products.climate.ncsu.edu/data/

Ozlu, E. (2020). Dynamics of soil aggregate formation in different ecosystems. The University of Wisconsin-Madison.

Ozlu, E., and Arriaga, F. J. (2021). The role of carbon stabilization and minerals on soil aggregation in different ecosystems. *Catena*, 202, 105303.

Ozlu, E., Heitman J., Reyes, A. R. (2023). Managing Agricultural Machinery to Limit Soil Compaction in North Carolina. North Carolina State Extension. AG-439-72

Ozlu, E., and Sandeep K. (2018). Response of soil organic carbon, pH, electrical conductivity, and water stable aggregates to long-term annual manure and inorganic fertilizer. *Soil Science Society of America Journal* 82, no. 5: 1243-1251.

Reyes, A. R., Heitman, J., Vepraskas, M., and Ozlu, E. (2024). Soil Management Practices to Reduce Hardpans and Compaction in Sandy Soils of North Carolina, USA. In Sandy Soils (pp. 201-210). S*pringer Nature Switzerland*.

Leuthold, S., Haddix, M., Lavallee, J., Cotrufo, M. F. (2024). Physical fractionation techniques, Editor(s): Michael J. Goss, Margaret Oliver, *Encyclopedia of Soils in the Environment* (Second Edition), *Academic Press*, pg. 68-80, ISBN 9780323951333,

Sikora, F.J., and Kissel, D.E. (2014). Soil pH. Southern Cooperative Series Bulletin, 419, 48-53.

Sun T., Mao X, Han K., Wang X., Cheng Q., Liu X., Zhou J. , Ma Q., Ni Z., Wu L. (2023). Nitrogen addition increased soil particulate organic carbon via plant carbon input whereas reduced mineral−associated organic carbon through attenuating mineral protection in agroecosystem. Science of the Total Environment. Volume 899. 165705. ISSN 0048-9697.

USDA. (2024). Soil Health. https://www.nrcs.usda.gov/wps/portal/nrcs/main/soils/health/

USDA, NRCS. (2014). Soil Electrical Conductivity. www.nrcs.usda.gov/sites/default/files/2022-10/Soil%20Electrical%20Conductivity%20Educators.pdf.

Villarino, S. H., Talab, E, Contisciani, L., Videla, C., Di G., Paula, M., Matias E., Jackson K., Robert B., and Piñeiro, G. (2023). A large nitrogen supply from the stable mineral-associated soil organic matter fraction. *Biology and Fertility of Soils.* Vol. 59. 833-841.

Wang, J.J., Provin, T. and Zhang,H. (2014). Measurement of Soil Salinity and Sodicity. *Southern Cooperative Series Bulletin*, 419, 185-193.

Yulnafatmawita, Y., Saidi, A., Pratiwi, Z. H., and Haris, Z. A. (2020). Organic matter stock at suboptimal soils under forest and corn cultivation in wet tropical region. In IOP Conference Series: *Earth and Environmental Science* (Vol. 583, No. 1, p. 012010). IOP Publishing.

Zhou, W., Han, G., Liu, M., and Li, X. (2019) Effects of soil pH and texture on soil carbon and nitrogen in soil profiles under different land uses in Mun River Basin, Northeast Thailand. *PeerJ* 7: e788

Table 1.1. Observational site descriptions and soil types.

	Soil Type	Crop	Management
No-till > 40 years	100% Aycock Loam	Corn/Soybean Rotation	No-tillage > 40 years
Strip Tillage > 40 years	100 % Aycock Loam	Corn/Soybean Rotation	Unviferth Ripper Stripper at 12" depth
Vertical Tillage > 40 years	88% Woodington + 12% Rains fine sandy loam	Corn/Soybean Rotation	Case IH vertical tiller
No-till > 25 years with slope	96.8% Aycock loam, fine sand	Corn/Soybean Rotation	Previously Disking
No-till > 25 years without slope	96.8% Aycock loam, fine sand	Corn/Soybean Rotation	Previously Disking
Forest > 40 years	90.2% Aycock loam, fine sand	Natural Forest, Previous Disking	Previously agriculture production
No-tillage < 2 years	100% Rains Fine Sandy Loam	Corn/Soybean Rotation	Previously Disking
Pasture > 40 years	66.3% Aycock loam, loamy fine sand	Pasture, Previous Disking	Previously agriculture production

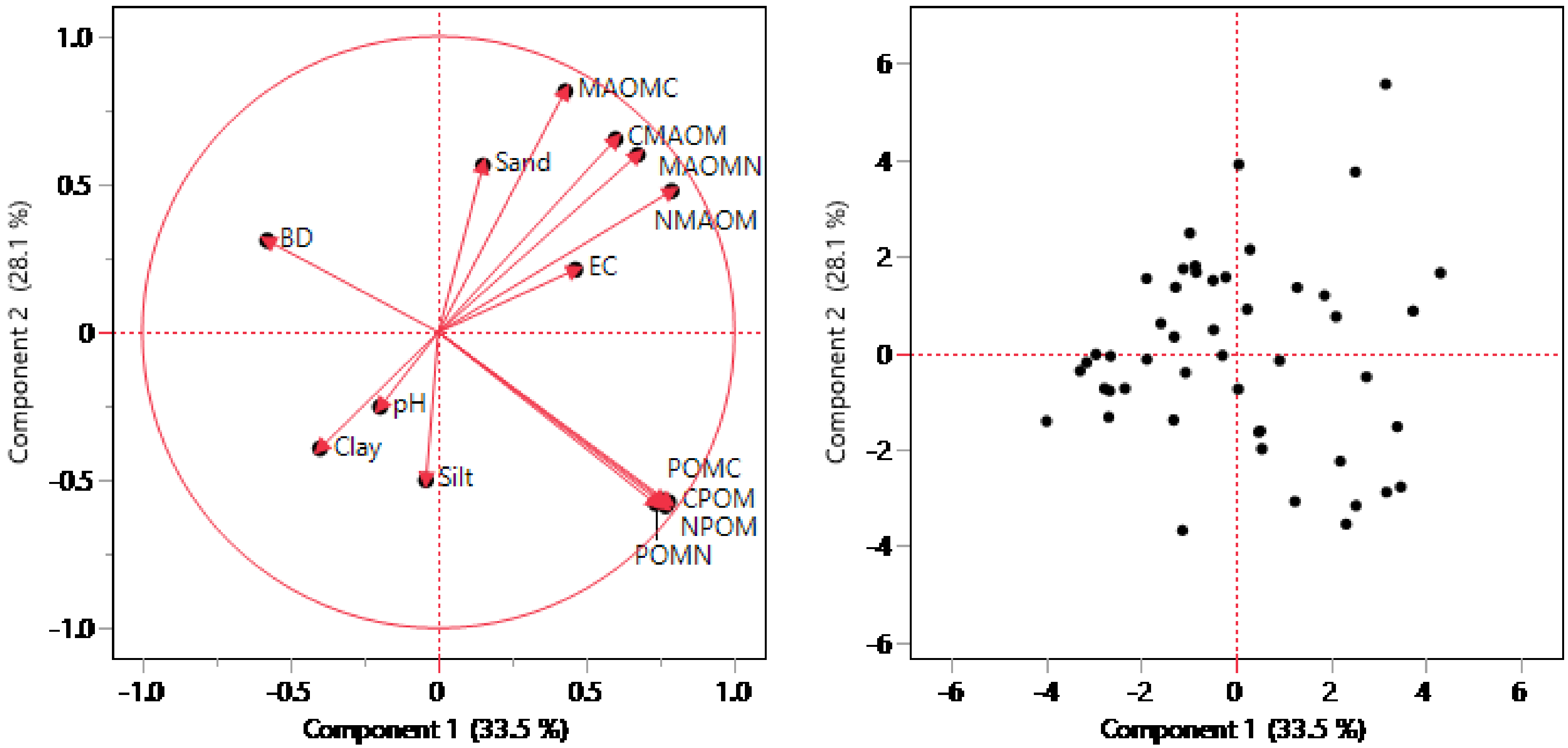

Figure 1.1. Principle Component Biplot Analysis of all soil property† data points. †Indicates all Soil Properties (Sand, proportion of sand in soil's texture; Silt, proportion of silt in the soil's texture; Clay, proportion of clay in the soil's texture; BD, bulk density; EC, electrical conductivity; pH, potential of hydrogen; POMC, particulate organic matter carbon stocks; POMN, particulate organic matter nitrogen stocks; MAOMC, mineral associated organic matter carbon stocks; MAOMN, mineral associated organic matter nitrogen stocks; NMAOM, concentration of nitrogen in mineral associated organic matter; CMAOM, concentration of carbon in mineral associated organic matter; NPOM, concentration of nitrogen in particulate organic matter; CPOM, concentration of carbon in particulate organic matter).

Table 1.2. Multiple Linear Regression analysis, using the Lasso estimation method. Showing estimate, standard error, ChiSquare, Lower 95% and Upper 95%. All Soil properties as the X values and Particulate Organic Matter Carbon Stocks as the Y value.

Parameter Estimates						
	Estimate	Std Error	Wald ChiSquare	Prob > ChiSquare	Lower 95%	Upper 95%
Intercept	-2.9	2.0	2.2	0.1	-6.8	0.9
Sand	0	0	0	1	0	0
Silt	0	0	0	1.00	0	0
Clay	-0.01	0.01	0.6	0.5	-0.03	0.01
BD	1.6	1.2	1.6	0.2	-0.9	4.02
EC	3.78E-05	3.62E-05	1.09	0.3	-3.32E-05	1.09E-04
pH	0.14	0.07	4	0.05	-0.0009	0.3
POMN	8.3	3.08	7.2	0.01	2.2	14
MAOMC	0.09	0.10	0.8	0.4	-0.1	0.3
MAOMN	0	0	0	1	0	0
NMAOM	-0.2	0.71	0.1	0.7	-1.6	1.1
CMAOM	0	0	0	1	0	0
NPOM	-4.7	2.3	4	0.04	-9.2	-0.3
CPOM	0.60	0.03	352	<.0001	0.5	0.7
Normal Distribution Parameters						
	Estimate	Std Error	Wald ChiSquare	Prob > ChiSquare	Lower 95%	Upper 95%
Response	0.15	0.059	7	0.009	0.038	0.27

†Indicates all Soil Properties (Sand, proportion of sand in soil's texture; Silt, proportion of silt in the soil's texture; Clay, proportion of clay in the soil's texture; BD, bulk density; EC, electrical conductivity; pH, potential of hydrogen; POMC, particulate organic matter carbon stocks; POMN, particulate organic matter nitrogen stocks; MAOMC, mineral associated organic matter carbon stocks; MAOMN, mineral associated organic matter nitrogen stocks; NMAOM, concentration of nitrogen in mineral associated organic matter; CMAOM, concentration of carbon in mineral associated organic matter; NPOM, concentration of nitrogen in particulate organic matter; CPOM, concentration of carbon in particulate organic matter).

Table 1.3. Multiple Linear Regression analysis, using the Lasso estimation method. Showing estimate, standard error, ChiSquare, Lower 95% and Upper 95%. All Soil properties as the X values and Particulate Organic Matter Nitrogen Stocks as the Y value.

	Parameter Estimates					
	Estimate	Std Error	Wald ChiSquare	Prob > ChiSquare	Lower 95%	Upper 95%
Intercept	-0.15	0.09	2.7	0.1	-0.3	0.03
Sand	0	0	0	1	0	0
Silt	-0.0001	0.0004	0.1	0.7	-0.0009	0.0006
Clay	0.001	0.001	2.1	0.1	-0.0005	0.003
BD	0.1	0.06	4.3	0.04	0.007	0.3
EC	1.28E-06	2.35E-06	0.3	0.6	-3.33E-06	5.89E-06
pH	-0.004	0.004	1.1	0.3	-0.01	0.003
POMC	0.05	0.04	2.2	0.1	-0.02	0.1
MAOMC	-1.84E-05	0.2	1.09E-08	1	-0.3	0.3
MAOMN	0.06	0.10	0.44	0.5	-0.1	0.3
NMAOM	0	0	0	1	0	0
CMAOM	-0.005	0.1	0.002	0.97	-0.2	0.2
NPOM	0.6	0.02	1179	<.0001	0.6	0.7
CPOM	-0.03	0.02	2	0.2	-0.08	0.01
	Normal Distribution Parameters					
	Estimate	Std Error	Wald ChiSquare	Prob > ChiSquare	Lower 95%	Upper 95%
Response	0.011	0.007	3.00	0.083	-0.0015	0.024

†Indicates all Soil Properties (Sand, proportion of sand in soil's texture; Silt, proportion of silt in the soil's texture; Clay, proportion of clay in the soil's texture; BD, bulk density; EC, electrical conductivity; pH, potential of hydrogen; POMC, particulate organic matter carbon stocks; POMN, particulate organic matter nitrogen stocks; MAOMC, mineral associated organic matter carbon stocks; MAOMN, mineral associated organic matter nitrogen stocks; NMAOM, concentration of nitrogen in mineral associated organic matter; CMAOM, concentration of carbon in mineral associated organic matter; NPOM, concentration of nitrogen in particulate organic matter; CPOM, concentration of carbon in particulate organic matter).

Table 1.4. Multiple Linear Regression analysis, using the Lasso estimation method. Showing estimate, standard error, ChiSquare, Lower 95% and Upper 95%. All Soil properties as the X values and Mineral Associated Organic Matter Carbon Stocks as the Y value.

Parameter Estimates						
	Estimate	Std Error	Wald ChiSquare	Prob > ChiSquare	Lower 95%	Upper 95%
Intercept	-1.08	0.33	10.57	0.0012	-1.73	-0.43
Sand	0.0018	0.00056	10.86	0.001	0.00075	0.0029
Silt	0	0	0	1	0	0
Clay	0	0	0	1	0	0
BD	0.69	0.21	11.16	0.0008	0.28	1.09
EC	-1.25E-06	5.37E-06	0.05	0.8	-1.18E-05	9.26E-06
pH	0.0093	0.012	0.59	0.4	-0.014	0.033
POMC	0	0	0	1	0	0
POMN	0.020	0.059	0.11	0.7	-0.097	0.14
MAOMN	4.04	1.91	4.49	0.03	0.30	7.79
NMAOM	-2.74	1.37	4.01	0.045	-5.41	-0.058
CMAOM	0.67	0.016	1880	<.0001	0.64	0.70
NPOM	0	0	0	1	0	0
CPOM	-0.0026	0.0036	0.51	0.476	-0.0097	0.0045
Normal Distribution Parameters						
	Estimate	Std Error	Wald ChiSquare	Prob > ChiSquare	Lower 95%	Upper 95%
Response	0.026	0.0044	33.27	<.0001	0.017	0.034

†Indicates all Soil Properties (Sand, proportion of sand in soil's texture; Silt, proportion of silt in the soil's texture; Clay, proportion of clay in the soil's texture; BD, bulk density; EC, electrical conductivity; pH, potential of hydrogen; POMC, particulate organic matter carbon stocks; POMN, particulate organic matter nitrogen stocks; MAOMC, mineral associated organic matter carbon stocks; MAOMN, mineral associated organic matter nitrogen stocks; NMAOM, concentration of nitrogen in mineral associated organic matter; CMAOM, concentration of carbon in mineral associated organic matter; NPOM, concentration of nitrogen in particulate organic matter; CPOM, concentration of carbon in particulate organic matter).

Table 1.5. Multiple Linear Regression analysis, using the Lasso estimation method. Showing estimate, standard error, ChiSquare, Lower 95% and Upper 95%. All Soil properties as the X values and Mineral Associated Organic Matter Nitrogen Stocks as the Y value.

	Parameter Estimates					
	Estimate	Std Error	Wald ChiSquare	Prob > ChiSquare	Lower 95%	Upper 95%
Intercept	-0.065	0.050	1.65	0.20	-0.16	0.034
Sand	0	0	0	1	0	0
Silt	-0.00006	0.00006	1.10	0.29	-0.00017	0.00005
Clay	0.0001	0.0002	0.29	0.59	-0.0003	0.001
BD	4.65E-02	0.03273	2.02	0.16	-0.018	0.11
EC	1.78E-06	8.75E-07	4.14	0.042	6.48E-08	3.49E-06
pH	0.0007	0.0016	0.21	0.65	-0.0024	0.0038
POMC	0.0001	0.0007	0.01	0.91	-0.0012	0.0014
POMN	0	0	0	1	0	0
MAOMC	0.050	0.024	4.39	0.04	0.003	0.098
NMAOM	0.68	0.021	1060.86	<.0001	0.64	0.72
CMAOM	-0.035	0.017	3.98	0.046	-0.069	-0.0006
NPOM	-0.0018	0.0057	0.10	0.76	-0.013	0.0095
CPOM	0	0	0	1	0	0
	Normal Distribution Parameters					
	Estimate	Std Error	Wald ChiSquare	Prob > ChiSquare	Lower 95%	Upper 95%
Response	0.0027	0.00066	17.23	<.0001	0.0014	0.0040

†Indicates all Soil Properties (Sand, proportion of sand in soil's texture; Silt, proportion of silt in the soil's texture; Clay, proportion of clay in the soil's texture; BD, bulk density; EC, electrical conductivity; pH, potential of hydrogen; POMC, particulate organic matter carbon stocks; POMN, particulate organic matter nitrogen stocks; MAOMC, mineral associated organic matter carbon stocks; MAOMN, mineral associated organic matter nitrogen stocks; NMAOM, concentration of nitrogen in mineral associated organic matter; CMAOM, concentration of carbon in mineral associated organic matter; NPOM, concentration of nitrogen in particulate organic matter; CPOM, concentration of carbon in particulate organic matter).

Table 1.6. Correlation Analysis results comparing Soil properties factors. Table shows correlation coefficients, positive values indicate a direct relationship, and negative values indicate a possible inverse relationship.

	Sand	*Silt*	*Clay*	*BD*	*EC*	*pH*	*POMC*	*POMN*	*MAOMC*	*MAOMN*	*NMAOM*	*CMAOM*	*NPOM*
Sand													
Silt	-0.96												
Clay	-0.46	0.2											
BD	0.22	-0.34	0.29										
EC	0.003	0.09	-0.29	-0.06									
pH	-0.24	0.25	0.05	-0.06	-0.12								
POMC	-0.05	0.08	-0.09	-0.45	0.18	0.05							
POMN	-0.04	0.04	0.01	-0.42	0.18	-0.12	0.93						
MAOMC	0.41	-0.33	-0.4	-0.01	0.22	-0.12	-0.11	-0.2					
MAOMN	0.22	-0.16	-0.29	-0.08	0.57	-0.32	0.13	0.16	0.77				
NMAOM	0.14	-0.05	-0.34	-0.33	0.54	-0.29	0.24	0.26	0.73	0.96			
CMAOM	0.28	-0.17	-0.45	-0.33	0.22	-0.1	0.06	-0.04	0.94	0.76	0.8		
NPOM	-0.07	0.09	-0.04	-0.55	0.16	-0.1	0.93	0.98	-0.18	0.15	0.29	0.02	
CPOM	-0.09	0.14	-0.13	-0.58	0.15	0.06	0.98	0.91	-0.1	0.11	0.26	0.11	0.94

[†]Indicates all Soil Properties (Sand, proportion of sand in soil's texture; Silt, proportion of silt in the soil's texture; Clay, proportion of clay in the soil's texture; BD, bulk density; EC, electrical conductivity; pH, potential of hydrogen; POMC, particulate organic matter carbon stocks; POMN, particulate organic matter nitrogen stocks; MAOMC, mineral associated organic matter carbon stocks; MAOMN, mineral associated organic matter nitrogen stocks; NMAOM, concentration of nitrogen in mineral associated organic matter; CMAOM, concentration of carbon in mineral associated organic matter; NPOM, concentration of nitrogen in particulate organic matter; CPOM, concentration of carbon in particulate organic matter).

CHAPTER 2: SHORT-TERM IMPACTS OF MIXED COVER CROP AND TILLAGE MANAGEMENTS ON SOIL HEALTH AND SOYBEAN YIELD

Abstract

Soil health, a concept that has been evolving for over a century, indicates the soil's ability to function as a vital and living ecosystem that supports agricultural productivity, environmental quality, and overall ecosystem sustainability. In this study, we studied the impacts of tillage management and crop diversification on soil health including evaluation of all physical, chemical, and microbial aspects under a corn (*Zea mays* L.) soybean (*Glycine max* L.) rotation in Martin (Coastal Plain) and Beaufort (Tidewater) Counties in North Carolina. Study treatments included two tillage practices, conventional disking and no-till, and four cover cropping systems (no-cover crop, cereal rye (*Secale cereale*), crimson clover (*Trifolium incarnatum*), and a mixture of rye and crimson clover (50:50 ratio) using a complete randomized split plot design. Soil samples were collected to the depth of 0-20 cm from both sites in both 2023 and 2024. Study analysis includes particle size distribution, bulk density, pH, electrical conductivity, water extractable carbon and nitrogen, microbial respiration, and enzymes (β-glucosidase and urease). Results showed that soil bulk density was only significantly different ($p<0.02$) in the Tidewater site at the 0-5 cm and 10-20 cm depths. Bulk density was lowest at the Tidewater conventional tillage and crimson clover treatment (0.86 g cm^{-3}) when compared to values under no tillage and crimson clover treatment (1.04 g cm^{-3}). Water extractable carbon for the Coastal Plain site was higher for the depths of 5-10 cm under the no till crimson clover treatment (46.75 mg kg^{-1}) compared to those under no tillage rye (28.93 mg kg^{-1}), conventional tillage and rye and clover mix (28.58 mg kg^{-1}), and conventional tillage and rye treatments (25.58 mg kg^{-1}). A similar trend was observed for the 10-20 cm depths. Soil microbial respiration and enzyme activity were generally higher in the Tidewater site versus

the Coastal Plain sight. Microbial respiration had a higher level of CO_2 (mg 100 g^{-1} day^{-1}) in no-tillage than those under conventional disking at all depths and sites. Similarly, β-glucosidase activity performed the same except for conventional tillage and crimson clover treatment (451.13 μmolp-nitrophenol 100 g^{-1} $hour^{-1}$) versus all other treatments. Urease activity was higher in no till treatments at the Tidewater site, but at the Coastal Plain site urease activity was lower. Generally, no tillage and decreased disturbance treatments had a higher capacity to provide the high levels of soil microbial activity in the soils. Soil microbial respiration and β-glucosidase had higher values in no till treatments with the exception of less than one treatment.

Keywords: disking, no-till, cereal rye, crimson clover, mix-cover crop, soil health, enzyme activities, soil respiration

2.1. Introduction

Soil health is defined as the capacity of soil to function as a living system to support plant growth, regulating water, filtering pollutants, and maintaining ecological balance (Doran and Zeiss, 2000). Historically, soil health assessments include various physical, chemical, and biological soil properties (Lehmann et al., 2020). Some of the key indicators could be named as soil organic matter (SOM), nutrient availability, microbial activity, soil structure, and water infiltration rates. Soil health management may take time (3-5 years) to be statistically visible, however it does have significant direct association with agricultural productivity (NRCS, 2022). Soil health management can also be the reason behind yield limitations, due to poor soil structure, increased compaction, and limited moisture (Mattila and Vihanto, 2024). Hence, healthy soils play a critical role in crop yield maximization depending on soil type, and strategies used in the field that provide sustainable production and resilience against environmental stresses.

Soil is living and finite, and it can face environmental and management related factors causing global and regional soil health concerns like soil degradation, erosion, compaction, nutrient depletion, climate change, population growth. These issues threaten long-term food security. Sustainable management practices like reduced tillage and increased biodiversity in cropping system have the potential to maintain and enhance soil health to ensure long-term productivity (Ozlu and Arriaga, 2024; USDA, 2024).

Soybean production, a large portion of the world's protein consumption, is important for both global and regional scale with its role in human food, animal feed, and biofuel industries. Soybean production is recognized as the most traded agricultural commodity where U.S. and Brazil are leading producers with over 80% of global soybean exports (Valdes et al., 2023). However, soybean cultivation has key challenges like soil fertility issues, pest and disease pressures, and climate variability (Koenning and Wrather, 2010). Thus, there is a demand for effective soil management practices including cover cropping and tillage practices to overcome these challenges and improve soybean yield stability.

One of the sustainable management practices is cover cropping (Roper et al., 2024). A cover crop is a crop grown typically during periods when main crops are not cultivated. Cover cropping provides a multifunctional role in agroecosystems, including preventing soil erosion, improving soil structure, enhancing nutrient cycling, and suppressing weeds (Dabney et al., 2001). Cover cropping may also improve SOM and microbial activities (Dabney et al., 2001), which are critical for long-term soil security. Cover crops can be planted as single species like cereal rye or crimson clover, or in a mixture of species. Cereal rye (*Secale cereale*) is a widely used cover crop, known for its robust root system, ability to scavenge residual nitrogen, and effectiveness in erosion control (Dabney et al., 2010). Crimson clover (*Trifolium incarnatum*) is a leguminous cover crop that can fix atmospheric nitrogen, improve soil fertility, and contribute to biodiversity (Moore et al., 2020).

When a combined rye and crimson clover application takes place in a synergistic cover crop mix, they maximize soil health by balancing nitrogen enrichment with robust ground cover. It is known that increasing biodiversity like using mixed cover crops versus monoculture cropping systems has potential advantages such as; (i) ecological benefits including improved biodiversity, pest and disease suppression and resilience against environmental stresses (Toker et al., 2024), and (ii) complementary effects for biomass and weed suppression and nitrogen fixation and soil fertility. Existing studies also suggest mixed cover crops which can lead to greater improvements in soil structure, nutrient availability, and overall soil health (Roper et al., 2024).

Reducing soil disturbance through tillage management is also one of the principles of soil health management. While conventional tillage like disking disturbs soil more aggressively, conservation tillage including no-till supports improved soil structure and OM stabilization (DeLaune et al., 2019). Conventional tillage, widespread in various cropping systems, may have perceived benefits such as weed control, seedbed preparation and residue management, but it can have negative consequences on soil health including soil structure degradation, loss of OM, increased erosion and disruption of soil microbial communities (Ozlu, 2020). Hence, long-term applications of conventional tillage may not support sustainable soil health and crop productivity. On the other hand, conservation tillage practices including no-till do have benefits and concerns as well. No-till farming minimizes soil disturbance, leaving crop residues on the soil surface and planting directly into the undisturbed soil; thereby, reducing soil erosion, improving water infiltration, enhancing soil structure, and increasing SOM (Ozlu and Arriaga, 2024; Tahat et al., 2020). However, challenges in no-till adoption may appear as increased dependence on herbicides for weed control, potential initial yield reductions, and the need for specialized equipment. In addition, soil type and environmental conditions including variability in climate may create conditions under which no-till practices are most effective, such as in regions with high erosion risk or where maintaining soil moisture is critical.

Although existing studies provide great insights for how these cover crops individually help soil health and crop productivity, application of cover crop mixtures is still not well understood. Similarly, the current state of research on the interaction between cover crops and tillage practices still has limited understanding. Most existing studies have focused either on cover crops or tillage independently, with fewer examining their combined effects. Hence, the demand for research that considers the synergistic or antagonistic effects of different cover crop and tillage combinations on soil health. Moreover, most existing studies focus on long-term outcomes from research experiments while short-term versus long-term impacts of mix cover crops integrated with different tillage practices are not well studied on farm trials which provide insights from real-life conditions and help with immediate management decisions and practices.

This study provides practical recommendations for soybean farmers about tillage and cover crop management and adaptation impacts on soil health, particularly support for sustaining profitability through better soil management practices. The objectives of the study include:

- To evaluate the short-term impacts of four cover crop treatments (no cover crop, single rye, single crimson clover, and a mixture of rye and crimson clover) on soil health indicators.
- To assess the effects of these cover crop treatments under no-till and conventional tillage on microbial activities.
- To explore the interaction between cover crop type and tillage practice in influencing soil organic matter, carbon (C) and nitrogen (N).

The present the study’s hypotheses include:

- Mixed cover crops will lead to greater improvements in soil health compared to single species cover crops or no cover crop.

- No-till practices, when combined with cover crops, will result in better soil health and microbial activities compared to conventional tillage.
- The interaction between mixed cover crops and no-till will produce the most significant positive effects on soil health and soil organic matter.

2.2. Materials and Methods

2.2.1. Site description

Two sites, one in Martin County in the coastal plain region and one in Beaufort County in the Tidewater region of North Carolina were used in this study. Two soil series were present at these sites: Arapahoe series (coarse-loamy, mixed, semiactive, nonacid, thermic Typic Humaquepts) at the Tidewater site and Craven series (fine, mixed, subactive, thermic Aquic Hapludults) at the Coastal Plain site. Previous management at the study sites include; (i) greater than 20 years no-tillage, Coastal Plain; and (ii) greater than 20 years disking, Tidewater. The agricultural fields are under corn (Zea mays) and soybean (Glycine Max) rotation with no cover cropping but residue was left on fields after harvest. Weather station data gathered from North Carolina State Climate Office shows; average precipitation for Martin County (Coastal Plain) in 2024 was 134.85 cm, average air temperature was 16.9°C. Annual precipitation for 2024 in Beaufort County (Tidewater) was 119.99 cm, average air temperature 17.8°C.

2.2.2. Study treatments

Treatments factors were established as follows; two tillage treatments: (i) conventional tillage and (ii) no-tillage; and four cover crop treatments: (i) crimson clover (Trifolium incarnatum) (ii) cereal rye (Secale cereale), (iii) mix of crimson clover and cereal rye, and (iv) no cover crop. The plot design is complete randomized split block design entire plot size is 50 x 20 meters. A total of 8 treatments were implemented in fall of 2023: (i) no-tillage with cereal rye and crimson clover cover crop mix (NTRC), (ii) no-tillage with crimson clover cover crop (NTC), (iii) no-tillage with cereal rye cover crop (NTC), (iv) no-tillage with no cover crop (NTNCC), (v) conventional tillage with cereal rye cover crop (CTR), (vi) conventional tillage with cereal rye and crimson clover cover crop mix (CTRC), (vii) conventional tillage with no cover crop mix (CTNCC), and (viii) conventional tillage with crimson clover cover crop (CTC). Management is the same as used by growers regarding; soybean variety, planting date, seeding rate, row spacing, and weed and pest management.

2.2.3. Soil sampling and processing

Soil samples were taken in spring 2024 after treatments have been placed, terminated, and cash crops have been planted. Ninety–six intact core samples from 0-15 cm and disturbed composite samples from 0-50 cm were collected at each of the two sites in spring 2024, after the treatments and soybean crops have been placed. Intact core samples were collected using a soil core sampler, with 3 x 5 cm liners inserted. Two hundred fifty-six composite samples per site were taken with a soil auger and placed into plastic bags, transferred to the lab and set out to air dry. The soils were then placed into North Carolina Department of Agriculture and Consumer Services (NCDA and CS) boxes and sent for nutrient analysis, further processing includes grinding and passing soil through a 2 mm sieve.

2.3. Study analysis

2.3.1. Texture

Soil particle size distribution analysis was performed using a 2-hour hydrometer method (Bouyoucos, 1962). Briefly, composite samples (50 g of 2 mm sieved soils) were weighed out and mixed with 100 ml of 5% Sodium Hexametaphosphate solution. These were then stirred and set out for 30 min, then transferred into a dispersion cup to be placed on an electronic mixer for 5 min. The soil suspension is then transferred into a 1 L sedimentation cylinder, mixed thoroughly and then the first hydrometer and temperature reading is taken at 40 seconds. After the first reading, the suspension was left to rest for 2 hours and another hydrometer and temperature measurement was taken at the end of 2 hours. For temperature correction, we added 0.36 g L^{-1} to the hydrometer reading for every 1°C above 20°C, whereas we subtracted 0.36 g L^{-1} from the hydrometer reading for every 1°C below 20°C. The temperature-corrected hydrometer reading from the soil suspension should be subtracted from the temperature-corrected hydrometer reading taken from the blank solution (without soil) at 40 seconds and 2 hours.

2.3.2. Bulk density

Intact core samples (5 cm height and 8 cm diameter) were taken directly from the field and weighed to obtain a wet soil weight, before being placed in a drier at 105°C. Dry soil mass was taken after samples were fully dried for about 48 hours (Grossman and Reinsch, 2002).

2.3.3. Soil pH and electrical conductivity (EC)

Composite air-dried ground samples were passed through a 2 mm sieve used to conduct soil pH using a 1:1 soil and water ratio (Wang, 2014; Rhoades, 1996) and electrical conductivity analysis using a 1:2.5 soil and water ratio (Sikora, et al., 2014)

2.3.4. Nutrient analysis

Percent base saturation (BS), cation exchange capacity (CEC), and micro- and macro-nutrient analysis were performed at the NCDA and CS. Macro- and micro- nutrient analysis included phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), copper (Cu), manganese (Mn), zinc (Zn), and sodium (Na). BS is determined based on the portion of the CEC that is occupied by the basic cations Ca, Mg, K (Hardy et al., 2014). CEC is determined by summation of buffer acidity and basic cations (Mehlich et al 1976). Concentrations of P, K, Ca, Mg, S, Cu, Mn, Zn, and Na were determined with Mehlich-3 extraction and followed by a quantification by inductively coupled spectroscopy (Mehlich 1984).

2.3.5. Water extractable carbon and nitrogen

Air dried composite soils were ground and passed through a 5 mm sieve. A total of 3 grams of soil was weighed and placed in 50 ml centrifuge tubes. A total of 30 ml of water was added to each tube then placed on a shaker for 30 minutes before being placed into a centrifuge for 15 minutes at 3500 rpm. Cellulose nitrate membrane filters (0.45 mm) were placed on vacuum filtration system. Samples were filtered until clear solution was present, this solution was then placed into labeled 50 ml centrifuge tubes (Ghani et.al., 2003). These samples were then sent to a laboratory at South Dakota State University for analysis where they were passed through a Shimadzu´s liquid total organic carbon (TOC) analyzer to obtain water extractable carbon (WEC) and water extractable nitrogen (WEN) values.

2.3.6. Soil microbial respiration

Wide mouth canning jars were filled with 20ml of Barium hydroxide ($Ba(OH)_2$) then 100 grams of field wet soils were added to 50 ml beakers and placed on rubber stoppers to prevent the liquid from touching the soil. After this, the jars were tightly sealed and placed in an incubator at 27°C for 24 hours. After incubation was complete, the soil was removed and weighed, and the jar was rotated in order for the Barium hydroxide to capture all of the CO_2. 10 ml of barium hydroxide was then pipetted into 25 ml Erlenmeyer flasks (two replicates per jar) and then titrated using hydrochloric acid (HCL) and phenolphthalein as an indicator. Soils taken from each jar were dried at 105°C and the dry weights were used in calculations (Isermeyer, 1952).

2.3.7. Beta-glucosidase (β-glucosidase)

One gram of field wet composite soils pushed through a 2 mm sieve was used for this analysis. This soil was placed in a 50 ml closable bottle, then 0.2 ml of toluene was added and then left to sit for 15 min, then 4 ml of Modified universal buffer (pH 6.0) and 1 ml p-nitrophenyl-β-d-glucopyranoside (PNG) was added. The soil was then swirled, capped and incubated at 37°C for 1 hour. After incubation, solution was removed and 1 ml of 0.5 m $CaCl_2$ was added swirled and then 4 ml of 0.1 THAM (pH 12) was added. The mixture was then swirled to mix for a few seconds, then solution was filtered through Whatman 2V filters into 25 ml Erlenmeyer flasks (Eivazi and Tabatabai, 1988). After fully filtered, samples were placed into 0.6 mL 96-well plate, then placed into BioTek Epoch 2 Microplate Spectrophotometer to measure p-Nitrophenol (pNP) absorbance.

2.3.8. Urease activity

Field wet composite soils were passed through a 2 mm sieve and then 5 grams were weighed and placed into closable bottles. This soil was also wetted with 2.4 ml of 0.08 M aqueous urea solution. After this was added the solution was capped, swirled to mix and then incubated at 37°C for 2 hours. After the two hours, 30 ml of 1N KCl was added to each solution and then placed on an orbital shaker for 1 hour. The suspensions were then filtered through Whatman No. 2 filters into 50 ml Erlenmeyer flasks (Kandeler and Gerber, 1988). Samples were then added to into 0.6 mL 96-well plate, then placed into BioTek Epoch 2 Microplate Spectrophotometer to measure the absorbance of ammonium (NH_4^+).

2.3.9. Statistical analysis

Data was analyzed using InfoStat 2020 and JMP Pro 18. Data was analyzed separately within each site (Coastal Plain and Tidewater). Two categories were analyzed; (i) treatment (NTRC, NTC, NTR, etc.) by depth (0-5, 5-10, 10-20 cm), (ii) tillage (conventional tillage and no-tillage) by cover crop (cereal rye, crimson clover, clover and rye mix, and no cover crop) by depth (0-5, 5-10, 10-20 cm). Within each category the interactions were represented through the y-value, whereas the measured properties were placed in the x-value. ANOVA tests were conducted to view significant differences in between each factor in each category. Tukey's honestly significant difference (HSD) was conducted after the ANOVA and was used to determine differences among the means, and within each category, at a=0.05 confidence interval.

2.4. Results

2.4.1. Soil physical health as impacted by tillage and cover crop managements

In general, soil physical properties in these two study sites were not significantly impacted by study treatments. Soil particle size distribution data for 0-5, 5-10, and 10-20 cm depths under all the treatments for Coastal Plain (Martin County) and Tidewater (Beaufort County) sites are shown in Table 2.2. Sand content in the soils of these two fields is proportionally high, ranging from 44.01% to 65.35% at the 0-20 cm depths. This high sand content is not significantly different at either of the two study sites. The proportion of sand has decreased with depth from 0-5 cm to 10-20 cm depths. Similar to sand, treatments did not show difference in the proportion of silt in both fields at any depth, 0-20 cm. The clay content ranged from 9.07% at 0-5 cm to 13.55% at 10-20 cm depths at Tidewater site whereas clay content in Coastal Plain site ranged from 6.67% at 5-10 cm to 18.71% at 10-20-cm depth. This shows that the variability in clay content was greater in Coastal Plain than Tidewater while clay content increased with depth. The proportion of clay did also not differ across all treatments and depths except those of 0-5 cm at Tidewater site and 5-10 cm at Coastal Plain site. For 0-5 cm in Tidewater, the proportion of clay was significantly ($P<0.03$) higher under CTC compared to other treatments. However, clay content was significantly ($P<0.02$) higher for 5-10 cm depth under NTRC than those of other treatments.

Soil bulk density (BD) data showed that soils of the Tidewater region have lower BD, ranging from 0.86 g cm^{-3} at 0-5 cm to 1.18 g cm^{-3} at 10-20 cm depth overall than those in Coastal plain region, ranging from 1.26 g cm^{-3} at 0-5 cm to 1.52 g cm^{-3} at 10-20 cm depth. Soils in both Tidewater and Coastal Plain regions in general showed increasing BD level with depth. Results also showed no significant differences across all treatments for all depths, except those of 0-5 and 5-10 cm depths in the Tidewater site. For 0-5 cm depth at Tidewater, soils had significantly lower BD ($P<0.02$) under CTC (0.86 g cm^{-3}) compared to those under NTC (1.04 g cm^{-3}). Similarly, soils under CTC (0.98 g cm^{-3}) had significantly lower BD than those under NTC (1.10 g cm^{-3}), NTNCC (1.13 g cm^{-3}), NTRC (1.10 g cm^{-3}), and CTRC (1.1 g cm^{-3}) at 5-10 cm depth in Tidewater site. These results indicate that no-till had significantly higher BD at the surface (0-10 cm) depths in Tidewater whereas differences were not significant for the Coastal Plain site. In addition, soils under mixed cover crop, single rye or no cover crop had significantly higher BD than those under crimson clover only in surface depths of Tidewater site whereas soils of Coastal Plain did not show significant differences.

2.4.2. Soil chemical health as impacted by tillage and cover crop managements

Soil pH and EC results for 0-5 cm, 5-10 cm, and 10-20 cm depths for Tidewater and for Coastal Plain under tillage and cover crop treatments are shown in Tables 2.4. and 2.5. Soils in Coastal Plain, ranging from 5.84 to 6.54 across all depths, had slightly higher pH than those in Tidewater, ranging from 4.83 to 5.64. Treatments impacts on the soil pH were not significant at all depths for either region except those for 10-20 cm of the Coastal Plain. Soil pH was significantly different only for NTC versus NTR indicating tillage had no significant effects on soil pH whereas cover crops had significant impacts on pH at 10-20 cm of Coastal Plain only.

Similar to pH, the Coastal Plain site had slightly higher EC than the Tidewater site where EC decreased with depth and treatment impacts showed significant variations across the different depths for either site. In the Tidewater site, data showed that treatments significantly impacted the EC at 5-10 and 10-20 cm depths but not 0-5 cm. The highest EC was observed under NTRC treatment (180 µS cm^{-1}), which was significantly higher than CTRC (105 µS cm^{-1}) by 71%, and CTNCC (87 µS cm^{-1}) by 107% at the 5-10 cm depth for Tidewater ($P<0.007$). However, for 10-20 cm depth, the CTNCC (122 µS cm^{-1}) had significantly higher EC compared to those under CTRC (73 µS cm^{-1}) at Tidewater ($P<0.02$). In contrast to the Tidewater site, differences in EC were significant at 0-5 cm ($P<0.02$) but not at 5-10 cm and 10-20 cm ($P>0.05$). The CTC treatment (278 µS cm^{-1}) had significantly higher EC by 71% compared to CTRC (163 µS cm^{-1}) at the 0-5 cm in Coastal Plain site, respectively. This shows that tillage significantly impacted EC at 5-10 cm depth at Tidewater whereas a mixture of rye with crimson clover significantly lowered EC at 10-20 cm of Tidewater and 0-5 cm of the Coastal Plain.

CEC, BS, and macro- and micro-nutrients showed significant variability across different sites and depths within both the Tidewater and Coastal Plain regions whereas treatment impacts did not show any significant variability, Tables 2.11.1-3 Soils in Tidewater had slightly higher CEC, P, K, S, Ca, Zn and Cu but lower BS and Mn compared to those in Coastal Plain soils. In addition, CEC, P, K, Ca, Mn, and Zn decreased with soil depth for either site. Moreover, BS, Mg, and Cu decreased with soil depth only in Tidewater whereas S decreased with depth only in Coastal Plain. The only significant impact from treatments at either site was observed at 5-10 cm of Coastal Plain where soil P was significantly higher under CTC (71 mg kg^{-1}) compared to those under

CTNCC (33.3 mg kg^{-1}), CTR (27.0 mg kg^{-1}), NTC (36.5 mg kg^{-1}), NTR (33.8 mg kg^{-1}), and NTRC (30.3 mg kg^{-1}), $P<0.004$.

WEC and WEN data for 0-5, 5-10 and 10-20 cm depths under treatments for Tidewater and Coastal Plain sites are shown in Tables 2.6. and 2.7. Both WEC and WEN were slightly higher at Coastal Plain than those at Tidewater. In addition, both WEC and WEN were slightly decreased with soil depth at either site. Moreover, treatments significantly impacted the WEC ($P<0.01$) and WEN ($P<0.0009$) at 0-5 cm depth for Tidewater and Coastal Plain sites. Treatments also significantly impacted the WEC at 5-10 cm ($P<0.01$) and 10-20 cm ($P<0.02$) depths for the Coastal Plain site. The WEC was significantly different at 0-5 cm depth for NTRC (61.4 mg kg^{1}) versus all other treatments for Tidewater. Although a similar trend was observed for 5-10 cm, differences were not significant. The WEC at 0-5 cm depth of the Coastal Plain site was significantly higher under NTR (41.07 mg kg^{1}) and NTRC (41.06 mg kg^{1}) compared to those under CTRC (13.50 mg kg^{1}) and NTC (11.09 mg kg^{1}). However, WEC at 5-10 cm of Coastal Plain site was significantly higher under NTC (46.75 mg kg^{1}) compared to those under NTR (28.93 mg kg^{1}), CTRC (28.58 mg kg^{1}), and CTR (25.58 mg kg^{1}), $P<0.01$. A similar trend was observed for 10-20 cm of Coastal Plain where the highest WEC was observed under NTC (44.02 mg kg^{1}) significantly higher than those under CTRC (22.29 mg kg^{1}), NTRC (21.61 mg kg^{1}), and CTR (20.56 mg kg^{1}), $P<0.02$.

The WEN at 0-5 cm depth of Tidewater was significantly higher under NTNCC (15.95 mg kg^{1}) than that under CTRC (9.05 mg kg^{1}), $P<0.01$. However, the WEN at same depth of the Coastal Plain site was significantly higher under NTRC (25.41 mg kg^{1}) than those under NTC (14.18 mg kg^{1}) and CTRC (11.74 mg kg^{1}) by 79% and 116%, respectively, $P<0.0004$. These show that no-till increases WEC and WEN across the soil depths for either site where the mixture of rye and crimson clover also increases WEC and WEN only at 0-5 cm depth.

2.4.3 Soil biological health as impacted by tillage and cover crop managements

Data for microbial respiration (mg CO_2 100 g^{-1} day^{-1}) and enzyme activities including β-glucosidase (µmolp-nitrophenol 100 g^{-1} $hour^{-1}$) and urease (µmol 100^{-1} $hour^{-1}$) showed significant variability across all treatments, depths, sites (Tables 2.8., 2.9., and 2.10.).

Microbial respiration in general ranged from 6.51 mg CO_2 100g^{-1} day^{-1} at 10-20 cm to 57.56 mg CO_2 100 g^{-1} day^{-1} at 0-5 cm in Tidewater whereas Coastal Plain soils had a range of 3.96 mg CO_2 $soil^{-1}$ day^{-1} at 10-20 cm to 28.21 mg CO_2 100 g^{-1} day^{-1} at 0-5 cm showing double the level of microbial respiration at Tidewater. Microbial reparation results also showed higher CO_2 (mg 100 g^{-1} day^{-1}) levels under no-till treatments than those under conventional disking at all depths and either site. Similarly, soils under a mixture of rye and crimson clover were higher than those with no-cover crop or single cover crop applications at 0-5 cm depth. The only finding for microbial respiration that was in conflict with the above statements was at 10-20 cm depth of Coastal Plain where respiration was very low compared to other depths and sites, ranging from 3.96 mg CO_2 100 g^{-1} day^{-1} to 16.06 mg CO_2 100 g^{-1} day^{-1}.

For β-glucosidase activity, the highest values were present under no-till treatments compared with those under conventional disking except for CTC being highest at 10-20 cm depth in Tidewater (451.13 µmolp-nitrophenol 100 g^{-1} $hour^{-1}$) versus all other treatments. In addition, soils under crimson clover only or a mixture of rye and crimson clover had higher β-glucosidase activity (µmolp-nitrophenol 100 g^{-1} $hour^{-1}$) than other cover crop treatments across different depths at either site.

Data for soil urease activity showed higher urease activity under no-till treatments at Tidewater but this was opposite for Coastal Plain where conventional disking resulted in higher urease activity compared with no-till. Similarly, crimson clover only and a mixture of crimson clover and rye showed higher urease activity in Tidewater versus rye only and a mixture of crimson clover and rye had higher urease activity in Coastal Plain. This could indicate that lower disturbance present in the no-till with higher biodiversity in cropping system could leave the soil structure undisturbed and increasing the potential for microbial habitats in Tidewater whereas residue incorporation with tillage and higher biodiversity in cropping systems support urease activity in Coastal Plain.

2.5. Discussion

This study provides practical recommendations, such as nutrient management, tillage management, cover crop implementation, and organic matter accumulation for growers in Coastal Plain and Tidewater regions. These can directly target soil health management through integration and adaptation strategies of tillage and cover crops, particularly soil's support to sustainable and higher yield and profitability through better soil management practices. The objectives of the study was to evaluate the short-term impacts of two tillage (no-till and conventional disking) and four cover crop (no cover crop, single rye, single crimson clover, and a mixture of rye and crimson clover) treatments on soil health indicators including physical properties, chemical composition, and microbial activities.

2.5.1. Soil physical health as impacted by tillage and cover crop managements

The results of this study suggest that soil physical properties in the Coastal Plain and Tidewater regions did not show significant differences across all treatments. Soil particle size distribution, the key indicator of the texture and associated hydraulic and physical properties also showed minimal differences across treatments and sites (Table 2.2.). It is well documented that particle size distribution does not change under short-term impacts however their variability in a specific area may be another controlling factor affecting other soil properties. In these soils, the sand content dominated the texture with high proportion, ranging from 44.01% to 65.35%. Soils with greater than 50% proportion of sand are considered as sandy soils (Huang and Hartemink, 2020). The consistent decrease in the proportion of sand particles with depth (Gul et al., 2011) indicates a typical stratification pattern. Similar to sand, silt and clay content also did not show significant differences across different treatments for either site but varied significantly with depth. In this study, clay content had greater variability in the Coastal Plain than Tidewater which increases with depth and shows contrasting trends with sand content. The vertical distribution of soil particles is also well known and does cause soil health related issues like compaction.

It was also documented and confirmed with this study's findings that soils of North Carolina are prone to compaction even with no till or cover crop implementation due to the large spaces in between sand particles which allow soils to become compacted easily when heavy machinery passes over (Reyes, et al., 2024; Roper et al., 2024). Sandy soils are more prone to compaction in cases where decreasing sand content and increasing clay content with depth is present. Soils of this study especially for Coastal Plain had greater BD level at subsurface depth (0-10 cm) than those on the surface overlapping with previous studies (Vepraskas, 1987). It is also common in these regions that the sand particles are readily compressed, and compaction level can reach over 1.80 g cm^{-3} bulk density when trafficked and hence can limit the root growth (Reyes et al, 2024). Root growth is one of the significant and economical method to challenge and mitigate re-formation of sub-soil compaction. In this area, crop roots are limited in penetration due to existing hardpan presence, and very low pH levels and saturated conditions at subsurface soil layers. Existing literature also agreed that low pH and saturated soil layers limits root growth (Yan et al., 1992; Souza et al., 2021). However, SOM may behave differently by altering soil compaction level between soils of different regions like Coastal Plain and Tidewater. Soil BD data of this study showed that soils of Tidewater region have lower BD (lower than 1.18 g cm^{-3}) compared to those in Coastal plain region (higher than 1.26 g cm^{-3}).

This study also showed no significant variability in BD under short-term impacts of study treatments except 0-10 cm of Tidewater. Existing literature also reported that soil physical properties including soil particle size distribution and BD does not differ under short-term impacts (Ozlu, 2020; Ozlu and Arriaga, 2024). At the 0-10 cm depth at Tidewater, soils had significantly lower BD ($P<0.02$) under conventional tillage compared to those under no-till (Table 2.3.). Existing studies like Ozlu and Arriaga (2024), Cannell and Hawes (1994), and Dam et al. (2005) reported higher BD level under no-till management compared to conventional tillage in different regions and different soil types. In addition, for soils of 5-10 cm of Tidewater, soils under crimson clover had significantly lower BD than those under a mixture of rye and crimson clover. This might be due to field traffic and heavy equipment impacts are more severe where more moisture is present under higher above ground biomass in mix cover crop application (rye and crimson clover) in comparison to only crimson clover. According to Blanco-Canqui et al. (2015), cover crop effects on BD depend on management duration, showing although some studies does show positive effects of cover cropping on BD, and others reported no changes due to duration of cover cropping.

2.5.2. Soil chemical health as impacted by tillage and cover crop managements

This study also evaluated the impacts of tillage and cover crop management on soil chemical composition across two distinct regions, Tidewater and Coastal Plain. There were no significant differences in chemical composition across all treatments, soil depth, and regional characteristics.

Overall, soil EC was slightly lower in surface depth of Tidewater than those in the Coastal Plain. Where EC decreased with depth at both sites. This might be due to high freshwater influx through rainfall events and frequent wetting and drying cycles may remove salt from surface depths. Treatment effects were significant for specific depths, for instance, NTRC had significantly higher EC than those under CTRC and CTNCC at the 5-10 cm in Tidewater. This indicates that tillage can influence the movement and concentration of salts. In the previous chapter, results on tillage type (strip till, vertical till and no-till) showed that tillage has significant impacts on soil EC where these impacts can even be seen between different soil layers. In contrast, tillage treatments (e.g., CTC) had significant impacts at the 0-5 cm depth in the Coastal Plain, exhibiting regional differences in the interaction of tillage and soil chemistry. These results align with studies by Dick (1983) and Sharma et al. (2018) where they stated that the impacts of tillage on redistributing solutes and the mitigating effects of cover crops on soil salinity are significant.

CEC was higher in Tidewater whereas BS was higher in the Coastal Plain. This could be related to SOM level being higher in the Tidewater region by 19 times more than soils in the Coastal Plain, providing higher CEC in Tidewater while most CEC in the Coastal Plain is occupied with other elements and hence lower BS. Soil CEC and BS further supported this by reflecting the impacts of SOM through decreasing trends with soil depth in both regions. Soils in Tidewater had higher P, K, and Ca but decreased with depth, showing overlapping results with existing literature that highlights linkage between nutrient stratification, reduced SOM and biological activity deeper in the profile (Singh et al., 2021). The impacts of tillage and cover crop treatments on CEC, BS, and nutrient levels did in most cases not result in significant differences except for soil P at 5-10 cm of the Coastal Plain, where CTC had significantly higher P level than other treatments. The limited enhancement in solubility and downward transport of P under tillage application could cause these which overlaps with what was reported by Nakanishi et al. (2012). In addition, Dick

(1983) also agreed with this statement but also stated that travel distance of P downwards may be depended on clay content and rainfall. Soils of this study showed lower clay content under CTC at 0-10 cm depth in Coastal Plain soils whereas clay content doubled at 10 to 20 cm which further overlaps with Dick (1983). The results shows that the impacts of tillage management on soil chemical health are more visible at particular depths and regions where cover crops continuously support soil chemical properties especially those of pH stability, EC reduction, and nutrient availability at surface depths.

2.5.3. Soil biological health as impacted by tillage and cover crop management

WEC and WEN, the portion of SOM that are soluble in water, are labile-easily accessible and N pools which provide great insights into soil health and biological activity. Soil WEC and WEN are driven by plant residues, microbial biomass, soil structure and soil fertility, and indicates rapidly cycling fractions of dissolved SOM. In this study, soil WEC and WEN were slightly higher in Coastal Plain soils and decreased with depth at both sites. This might be due to lower microbial activity and oxygen availability at lower depths (Nakanishi et al., 2012). In this study, tillage and cover crop treatments had significant impacts on WEC and WEN at 0-5 cm and more limited impacts at lower depths. In Tidewater, NTRC had significantly higher WEC at 0-5 cm, confirming the results of Singh et al. (2021), who disclosed higher surface WEC under no-till with the mixture of rye and crimson clover. As also stated by Singh et al. (2021) who studied impacts of rye, oat and radish, surface WEC and WEN can be increased by aboveground residue, and microbial breakdowns (feed). This also overlaps with our findings that WEN at 0-5 cm was significantly higher under NTNCC in Tidewater and under NTRC in the Coastal Plain, indicating that incorporating legumes such as crimson clover, an N-fixing cover crop, in no-till systems increases the availability of nitrogenous compounds. These findings were further supported by Dick (1983) and Eckert (1991), who highlighted that cover crops and no-till systems together maintain labile organic nitrogen pools and mitigate N losses. This indicates that no-till with mix cover crops which include crimson clover does help with increasing SOM and nutrient levels and offer a sustainable soil management method in Coastal Plain and Tidewater regions.

Similar to WEC, and WEN, data for microbial respiration (mg CO_2 100 g^{-1} day^{-1}) and enzyme activities including β-glucosidase (μmolp-nitrophenol g^{-1} $hour^{-1}$) and urease (μmol g^{-1} $hour^{-1}$) showed significant variability across all treatments, depths, sites (Tables 2.6. and 2.7.). Microbial respiration and enzyme activities were in general higher in Tidewater than in Coastal Plain where these properties also significantly decreased with soil depth. Existing studies also reported significant impacts of soil depth mainly associated with temperature and moisture on microbial respiration (Zhao et al. 2022) indicating Tidewater sites potentially have a higher moisture and better conditions for soil respiration where depth may also impact temperature and the abundance of the microbial groups. The significance of temperature and moisture for microbial community compositions and associated emissions have been well documented (Ozlu et al., 2019; Ozlu and Kumar, 2018). Moreover, soils under no-till had higher microbial respiration than those under conventional disking at all depths at either site. This overlaps with existing literature that no-till systems and biodiverse cropping systems had significant impacts on respiration (Beril et al., 2023), active C, and total C (Aziz et al., 2013). Our study also further resulted that soils under a mixture of rye and crimson clover were higher than those with no-cover crop or single cover crop applications at 0-5 cm depth. The higher respiration with mix cover crop species were also reported by Breil et al. (2023) which further explains that aboveground residue from cover crop can impact soil microbial respiration and having more diverse cropping systems with different decomposition rates elevates the activity of microorganisms.

Aforementioned results are aligned with enzyme activities of these soils. The highest β-glucosidase and urease activities were under no-till treatments than those under conventional disking for all depths except for β-glucosidase under CTC being highest at 10-20 cm depth in Tidewater versus all other treatments. These results overlap with microbial respiration and labile C and N fractions. In addition, Navins and Lacey (2020) reported that soil structure and microbial communities are not disturbed under no-till which provide better conditions to microbes associated with β-glucosidase and urease production while conventional tillage disturbs microhabitats and results lower level of these microbial activity. In addition, soils under crimson clover only or a mixture of rye and crimson clover had higher β-glucosidase and urease activity than other cover crop treatments across different depths at either site. The higher support from biodiversity in cropping systems to microbial activities like β-glucosidase and urease is also well documented (Breil et al., 2023; Navins and Lacey, 2020; Aziz et al., 2013; Dick R. P., 1992).

2.6. Conclusion

The performance of treatments varied across multiple factors including, soil physical properties, chemical composition, and microbial activity. However, no tillage treatments with one or more cover crop implementations have a better predicted outcome for soil health assessment indicators, such as microbial activity. Microbial activity in short term treatments can give insight into the reactions from long term impacts as well as those soil health indicators which take a greater time to change (BD, organic matter).

No tillage and cover crop performance can be highlighted by no till's ability to increase soil respiration and β-glucosidase in soils in both Tidewater and Coastal Plain. This could indicate that cover crop and reduced tillage implements could increase litter biodiversity as well as provide continuous living roots during fallow seasons in long term trials.

Overall, these results support our hypothesis that mixed cover cropping improves soil health, and that no till practices when combined with cover cropping account for greater soil health and microbial activity. While these benefits are short term, existing literature discussing long term impacts follows a similar trend.

REFERENCES

Aziz, I., Mahmood, T., and Islam, K. R. (2013). Effect of long term no-till and conventional tillage practices on soil quality. *Soil and Tillage Research*, 131, 28-35.

Blanco-Canqui, H., Shaver, T.M., Lindquist, J.L., Shapiro, C.A., Elmore, R.W., Francis, C.A. and Hergert, G.W., (2015). Cover Crops and Ecosystem Services: Insights from Studies in Temperate Soils. *Agronomy Journal*, 107: 2449-2474.

Bouyoucos, G.J., (1962). Hydrometer method improved for making particle size analyses of soils. *Agron. Journal*. 54:464-465.

Breil, N. L., Lamaze T., Bustillo V., Marcato, C.R., Coudert, B., Queguiner, S., Jarosz-Pellé, N., (2023). Combined impact of no-tillage and cover crops on soil carbon stocks and fluxes in maize crops, *Soil and Tillage Research*, Volume 233 105782.

Cannell R.Q, Hawes J.D. (1994). Trends in tillage practices in relation to sustainable crop production with special reference to temperate climates. *Soil and Tillage Research*, 30, 2–4, 245-282

Dabney, S. M., Delgado, J. A., and Reeves, D. W. (2001). Using winter cover crops to improve soil and water quality. *Communications in Soil Science and Plant Analysis*, *32*(7–8), 1221–1250.

Dabney, S. M., Delgado, J. A., Meisinger, J. J., Schomberg, H. H., Liebig, M. A., Kaspar, T., Mitchell J., and Reeves, W. (2010). Chapter 9 Using cover crops and cropping systems for nitrogen management. *Soil Water and Conservation Society*. 66. 231-82.

Dam, R. F., Mehdi, B. B., Burgess, M. S. E., Madramootoo, C. A., Mehuys, G. R., and Callum, I. R. (2005). Soil bulk density and crop yield under eleven consecutive years of corn with different tillage and residue practices in a sandy loam soil in central Canada. *Soil and tillage research*, 84. 41-53.

DeLaune, P.B., Mubvumba, P., Lewis, K.L. and Keeling, J.W. (2019). Rye Cover Crop Impacts Soil Properties in a Long-Term Cotton System. Soil Sci. Soc. Am. J., 83: 1451-1458.

Dick R. P. (1992). A review: long-term effects of agricultural systems on soil biochemical and microbial parameters. *Agriculture, Ecosystems and Environment*. 40. 1–4. 25-36.

Dick, W.A. (1983). Organic Carbon, Nitrogen, and Phosphorus Concentrations and pH in Soil Profiles as Affected by Tillage Intensity. *Soil Science Society of America Journal*, 47. 102-107.

Doran, J. W., and Zeiss, M. R. (2000). Soil health and sustainability: managing the biotic component of soil quality. *Applied soil ecology*. *15*. 3-11.

Eckert, D.J. (1991). Chemical Attributes of Soils Subjected to No-Till Cropping with Rye Cover Crops. *Soil Science Society of America Journal*. 55. 405-409

Eivazi, F., and. Tabatabai, M.A. (1988). Glucosidases and galactosidases in soils. *Soil Biology and Biochemistry.* 20. 601–606.

Ghani, A., Dexter, M., and Perrott, K. W. (2003). Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilization, grazing and cultivation. *Soil biology and biochemistry*. *35*. 1231-1243.

Grossman, R., and Reinsch, T. (2002). 2.1 Bulk density and linear extensibility. *Methods of Soil.*

Gul, H., Gul, H., Khattak, R. A., Muhammad, D., and Shah, Z., (2011). Physical properties of soils under sub-surface drainage system. *Sarhad Journal of Agriculture.* 27. 2.

Hardy, D, Tucker R. M., Stokes, C. E., (2014). Crop fertilization based on North Carolina soil tests. NCDA.

Huang, J., and Hartemink, A. E. (2020). Soil and environmental issues in sandy soils. *Earth-Science Reviews.* 208.

Isermeyer, H. (1952). Eine einfache Methode zur Bestimmung der Bodenatmung und der Karbonate im Boden. *Zeitschrift für Pflanzenernährung, Düngung, Bodenkunde*. *56*. 26-38.

Kandeler, E., and Gerber, H., (1988). Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biology and fertility of Soils*. 6. 68-72.

Koenning, S. R., and Wrather, J. A. (2010). Suppression of soybean yield potential in the continental United States by plant diseases from 2006 to 2009. *Plant Health Progress.*

Lehmann, J., Bossio, D. A., Kögel-Knabner, I., and Rillig, M. C. (2020). The concept and future prospects of soil health. *Nature Reviews Earth and Environment*. 1. 544-553.

Mattila T. J., Vihanto N. (2024). Agricultural limitations to soil carbon sequestration: Plant growth, microbial activity, and carbon stabilization, *Agriculture, Ecosystems and Environment*. 367.

Mehlich A, Bowling SS, Hatfield AL. (1976). Buffer pH acidity in relation to nature of soil acidity and expression of lime requirement. *Communications in Soil Science and Plant Analysis.* 7. 3. 253-63.

Mehlich, A. (1984). Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant, Communications in Soil Science and Plant Analysis, 15. 12. 1409-1416.

Moore, V., Davis, B., Poskaitis, M., Maul, J. E., Kissing Kucek, L., and Mirsky, S. (2020). Phenotypic and nodule microbial diversity among crimson clover (Trifolium incarnatum L.) accessions. *Agronomy*. 10. 1434.

Nakanishi, T., Atarashi-Andoh, M., Koarashi, J., Saito-Kokubu, Y. and Hirai, K. (2012), Carbon isotopes of water-extractable organic carbon in a depth profile of forest soil imply a dynamic relationship with soil carbon. *European Journal of Soil Science*. 63. 495-500.

Natural Resources Conservation Service. (2022). Your Soil Health Journey what to look for along the way! https://www.nrcs.usda.gov/sites/default/files/202303/NRCS%20Soil%20Health%20 Journey%20factsheet_FINAL%202.pdf

North Carolina State Climate Office. (2025). https://products.climate.ncsu.edu/data/.

Ozlu, E. (2020). Dynamics of soil aggregate formation in different ecosystems. The University of Wisconsin-Madison.

Ozlu, E., Sandhu, S. S., Kumar, S., and Arriaga, F. J. (2019). Soil health indicators impacted by long-term cattle manure and inorganic fertilizer application in a corn-soybean rotation of South Dakota. *Scientific reports*. 9.

Ozlu, E., and Arriaga, F. J. (2024). Temporal variations in soil aggregate re-formation behaviors after disturbance by tillage. *Soil Science Society of America Journal*. 88. 2. 434-448.

Ozlu, E., and Kumar, S. (2018). Response of surface GHG fluxes to long-term manure and inorganic fertilizer application in corn and soybean rotation. *Science of the Total Environment*. 626. 817-825.

Reyes, A. R., Heitman, J., Vepraskas, M., and Ozlu, E. (2024). Soil Management Practices to Reduce Hardpans and Compaction in Sandy Soils of North Carolina, USA. In Sandy Soils. 201-210. *Springer Nature Switzerland.*

Rhoades, J.D. (1996). Salinity: Electrical conductivity and total dissolved solids. 417-435. In D.L. Sparks (ed.) Methods of soil analysis: Chemical methods, Part 3. ASA and SSSA, Madison, WI.

Roper, W. R., Osmond, D. L., Heitman, J. L. (2024). Wheat cover crop has minimal effect on physical soil properties in the North Carolina Piedmont. *Agronomy Journal*. 116. 5. 2599-2614.

Sharma, V., Irmak, S., and Padhi, J. (2018). Effects of cover crops on soil quality: Part I. Soil chemical properties—organic carbon, total nitrogen, pH, electrical conductivity, organic matter content, nitrate-nitrogen, and phosphorus. *Journal of Soil and Water Conservation*. *73*. 6. 637-651.

Sikora, F.J., and Kissel, D.E. 2014. Soil pH. *Southern Cooperative Series Bulletin.* 419. 48-53.

Singh G, Kaur G, Williard KW, Schoonover JE. Cover crops and tillage effects on carbon–nitrogen pools: A lysimeter study. (2021). *Vadose Zone J*ournal. 20.

Souza, R., Hartzell, S., Ferraz, A. P. F., de Almeida, A. Q., de Sousa Lima, J. R., Antonino, A. C. D., and de Souza, E. S. (2021). Dynamics of soil penetration resistance in water-controlled environments. *Soil and Tillage Research*. 205.

Tahat, M., Alananbeh M., Othman K., A., Leskovar Y., and I., (2020). Soil Health and Sustainable Agriculture. *Sustainability*. 12.

Toker, P., Canci, H., Turhan, I., Isci, A., Scherzinger, M., Kordrostami, M., and Yol, E. (2024). The advantages of intercropping to improve productivity in food and forage production – a review. *Plant Production Science*. 27. 155–169.

USDA. (2024). https://www.nrcs.usda.gov/wps/portal/nrcs/main/soils/health/

Valdes, C., Gillespie, J., and Dohlman, E. (2023). Soybean production, marketing costs, and export competitiveness in Brazil and the United States (Report No. EIB-262). U.S. Department of Agriculture, Economic Research Service.

Wang, J.J., Provin, T. and Zhang,H. (2014). Measurement of Soil Salinity and Sodicity. *Southern Cooperative Series Bulletin*. 419. 185-193.

Yan, F., Schubert, S., and Mengel, K. (1992). Effect of low root medium pH on net proton release, root respiration, and root growth of corn (Zea mays L.) and broad bean (Vicia faba L.). *Plant Physiology*. 99. 415-421.

Zhao M, Wang M, Zhao Y, Hu N, Qin L, Ren Z, Wang G, Jiang M. (2022). Soil microbial abundance was more affected by soil depth than the altitude in peatlands. *Front Microbial*. 13.

Table 2.1. Tidewater and Coastal Plain general site and background information.

	Tidewater	Coastal Plain
Soil Type	Arapahoe Fine Sandy Loam	Craven Find Sandy Loam
Crop	Corn 2023 Soybean 2024	Corn 2023 Soybean 2024
Management	No-tillage > 20 years	Half conventional tillage and half no-tillage

Table 2.2. Soil particle size distribution (%; sand, silt, and clay) across different treatments in Tidewater and Coastal Plain regions.

	Sand			Silt			Clay		
Depth (cm)	0-5	5-10	10-20	0-5	5-10	10-20	0-5	5-10	10-20
Tidewater									
CTC	56.5^{ns†}	61.4^{ns}	58.6^{ns}	30.5^{ns}	26.6^{ns}	29.3^{ns}	13^{a}	12^{ns}	12.1^{ns}
CTNCC	57	56.5	64.7	32.9	31	21.9	10.1^{ab}	12.5	13.5
CTR	57.2	58.2	54.7	33.2	29.8	34.3	9.6^{ab}	12.1	11.1
CTRC	64.2	63.6	56.9	26.7	25.8	31.6	9.1^{b}	10.6	11.5
NTC	59.3	54.9	58.3	29.5	32	29.6	11.2^{ab}	13.1	12.1
NTNCC	55.8	57.1	58.3	32.7	30.2	28.1	11.6^{ab}	12.8	13.6
NTR	65.4	57.8	59	24.6	32.7	29.5	10^{ab}	9.6	11.5
NTRC	55	56	55	34.8	32.5	31.5	10.3^{ab}	11.5	13.5
Analysis of Variance ($P<0.05$)									
TRT	0.4	0.8	0.8	0.5	0.8	0.4	0.03	0.3	0.9
T x CC	0.2	0.6	0.6	0.2	0.7	0.5	0.2	0.3	0.9
Depth		0.8			0.5			0.006	
TRT x D		0.5			0.4			0.8	
Coastal Plain									
CTC	50.5^{ns†}	50^{ns}	49^{ns}	42^{ns}	43.4^{ns}	38.5^{ns}	7.5^{ns}	6.7^{b}	12.5^{ns}
CTNCC	47.5	51.4	55.5	41.5	36.7	32.5	11	12^{ab}	12
CTR	51.9	52.2	51.9	39.1	37.7	35.4	9	10.1^{ab}	12.7
CTRC	50	50.5	44	41.5	41	42	8.5	8.5^{ab}	14
NTC	49.4	46.7	52	39.3	41.3	35.3	11.3	12^{ab}	12.7
NTNCC	46.9	49.4	50.4	43.6	40.1	38.2	9.5	10.5^{ab}	11.4
NTR	52.1	46.9	44.5	36.3	40.6	36.8	11.6	12.5^{ab}	18.7
NTRC	47.9	46.5	48.9	43.1	40.1	35.1	9	13.4^{a}	16
Analysis of Variance ($P<0.05$)									
TRT	0.8	0.7	0.1	0.1	0.6	0.2	0.6	0.02	0.1
T x CC	0.99	0.9	0.1	0.3	0.6	0.1	0.4	0.06	0.3
Depth		0.97			0.0002			<0.0001	
TRT x D		0.08			0.3			0.2	

† Letters following indicates means are statistically significantly different from each other. CTC – Conventional tillage and crimson clover, CTNCC – Conventional tillage and no cover crop, CTR – Conventional tillage and rye, CTRC – Conventional tillage and rye and crimson clover mixture (50/50), NTC- No tillage and crimson clover, NTNCC – No tillage and no cover crop, NTR – No tillage and rye, NTRC – No tillage and rye + crimson clover mix (50/50). TRT, treatment; D, depth; T, Tillage; CC, Cover Crop.

Table 2.3. Soil bulk density (g cm^{-3}) across different treatments in Tidewater and Coastal Plain regions.

	Tidewater			Coastal Plain		
Depth (cm)	0-5	5-10	10-20	0-5	5-10	10-20
CTC	$0.86^{b†}$	0.98^{b}	1.06^{ns}	1.40^{ns}	1.64^{ns}	1.49^{ns}
CTNCC	0.92^{ab}	1.06^{ab}	1.07	1.40	1.64	1.52
CTR	0.94^{ab}	1.08^{ab}	1.13	1.35	1.61	1.46
CTRC	0.99^{ab}	1.1^{a}	1.13	1.36	1.64	1.49
NTC	1.04^{a}	1.1^{a}	1.1	1.33	1.67	1.43
NTNCC	0.95^{ab}	1.13^{a}	1.18	1.26	1.66	1.45
NTR	0.93^{ab}	1.07^{ab}	1.11	1.32	1.63	1.51
NTRC	0.9^{ab}	1.1^{ab}	1.09	1.37	1.65	1.48
		Analysis of Variance (P<0.05)				
TRT	0.02	0.02	0.11	0.19	0.91	0.91
T x CC	0.003	0.046	0.056	0.22	0.94	0.59
Depth		<0.0001			0.009	
TRT x D		0.3			0.99	

[†] Letters following indicates means are statistically significantly different from each other. CTC – Conventional tillage and crimson clover, CTNCC – Conventional tillage and no cover crop, CTR – Conventional tillage and rye, CTRC – Conventional tillage and rye and crimson clover mixture (50/50), NTC- No tillage and crimson clover, NTNCC – No tillage and no cover crop, NTR – No tillage and rye, NTRC – No tillage and rye + crimson clover mix (50/50). TRT, treatment; D, depth; T, Tillage; CC, Cover Crop.

Table 2.4. Soil pH across different treatments in Tidewater and Coastal Plain regions.

	Tidewater			Coastal Plain		
Depth (cm)	0-5	5-10	10-20	0-5	5-10	10-20
CTC	5.3 ns†	5 ns	4.8 ns	6.04 ns	6.2 ns	6.3 ns
CTNCC	5.2	5.2	5	6	6	6.03
CTR	6	5.6	6	6.2	6.3	7
CTRC	5.2	5.2	5.1	6.02	6.1	6.3
NTC	5.5	5.4	5.2	6.4	7	7
NTNCC	5.3	5.3	5.1	6	6.2	6.4
NTR	5.4	5.3	5.2	6	6.04	6
NTRC	5.2	5.2	5.2	6.1	6.3	7
			Analysis of Variance (P<0.05)			
TRT	0.5	0.7	0.6	0.2	0.3	0.1
T x CC	0.4	0.5	0.5	0.1	0.2	0.04
Depth		0.03			0.001	
TRT x D		0.99			0.9	

† Letters following indicates means are statistically significantly different from each other. CTC – Conventional tillage and crimson clover, CTNCC – Conventional tillage and no cover crop, CTR – Conventional tillage and rye, CTRC – Conventional tillage and rye and crimson clover mixture (50/50), NTC- No tillage and crimson clover, NTNCC – No tillage and no cover crop, NTR – No tillage and rye, NTRC – No tillage and rye + crimson clover mix (50/50). TRT, treatment; D, depth; T, Tillage; CC, Cover Crop.

Table 2.5. Soil electrical conductivity ($\mu S\ cm^{-1}$) across different treatments in Tidewater and Coastal Plain regions.

	Tidewater			Coastal Plain		
Depth (cm)	0-5	5-10	10-20	0-5	5-10	10-20
CTC	$184^{ns\dagger}$	113^{ab}	89^{ab}	278^{ns}	145^{ns}	103^{ns}
CTNCC	187	87^{b}	122^{a}	239	133	95
CTR	177	118^{ab}	97^{ab}	194	126	101
CTRC	197	105^{b}	73^{b}	163	116	71
NTC	128	116^{ab}	98^{ab}	274	108	91
NTNCC	161	132^{ab}	91^{ab}	196	130	113
NTR	120	122^{ab}	90^{ab}	207	101	92
NTRC	184	180^{a}	104^{ab}	270	143	106
			Analysis of Variance (P<0.05)			
TRT	0.3	0.02	0.07	0.02	0.6	0.1
T x CC	0.8	0.08	0.02	0.05	0.3	0.1
Depth		<0.0001			<0.0001	
TRT X D		0.3			0.2	

† Letters following indicates means are statistically significantly different from each other. CTC – Conventional tillage and crimson clover, CTNCC – Conventional tillage and no cover crop, CTR – Conventional tillage and rye, CTRC – Conventional tillage and rye and crimson clover mixture (50/50), NTC- No tillage and crimson clover, NTNCC – No tillage and no cover crop, NTR – No tillage and rye, NTRC – No tillage and rye + crimson clover mix (50/50). TRT, treatment; D, depth; T, Tillage; CC, Cover Crop.

Table 2.6. Water extractable carbon (WEC: mg kg^{-1}) across different treatments in Tidewater and Coastal Plain regions.

	Tidewater			Coastal Plain		
Depth (cm)	0-5	5-10	10-20	0-5	5-10	10-20
CTC	$26^{ab†}$	16^{ns}	19^{ns}	33^{ns}	40.1^{ns}	34.1^{ab}
CTNCC	22^{ab}	15	17	39.4	30	21.19^{b}
CTR	30.4^{ab}	11	6.01	25	26	21^{b}
CTRC	13^{b}	10.3	13.3	14	29	22.3^{ab}
NTC	24^{ab}	11.3	24	11.1	47	44.02^{a}
NTNCC	16.0^{ab}	14	15.2	26	29.4	35.01^{ab}
NTR	20.1^{ab}	18	12	41.1	29	31.2^{ab}
NTRC	61.4^{a}	12	12	41.1	38.1	22^{ab}
		Analysis of Variance (P<0.05)				
TRT	0.02	0.9	0.3	0.04	0.02	0.01
T x CC	0.03	0.6	0.8	0.03	0.7	0.4
Depth		0.09			0.5	
TRT x D		0.06			0.004	

† Letters following indicates means are statistically significantly different from each other. CTC – Conventional tillage and crimson clover, CTNCC – Conventional tillage and no cover crop, CTR – Conventional tillage and rye, CTRC – Conventional tillage and rye and crimson clover mixture (50/50), NTC- No tillage and crimson clover, NTNCC – No tillage and no cover crop, NTR – No tillage and rye, NTRC – No tillage and rye + crimson clover mix (50/50). TRT, treatment; D, depth; T, Tillage; CC, Cover Crop.

Table 2.7. Water extractable nitrogen (WEN, mg kg^{-1}) across different treatments in Tidewater and Coastal Plain regions.

	Tidewater			Coastal Plain		
Depth (cm)	0-5	5-10	10-20	0-5	5-10	10-20
CTC	$14.1^{ns\dagger}$	11^{ns}	14.1^{ns}	18^{ab}	15.2^{ns}	14^{ns}
CTNCC	13.4	12	9	19^{ab}	14	10
CTR	12.4	11.3	11.3	17^{ab}	13.3	10.4
CTRC	9.1	10.1	7	12^{b}	19.03	13.3
NTC	14	14.1	10	14.2^{ab}	21	15
NTNCC	16	16.2	15.1	18^{ab}	21	18
NTR	10.1	14	10.3	21^{ab}	20	14
NTRC	10.2	19.4	9	25.4^{a}	16.1	15
		Analysis of Variance (P<0.05)				
TRT	0.9	0.9	0.3	0.04	0.3	0.2
T x CC	0.9	0.9	0.2	0.3	0.6	0.99
Depth		0.3			0.009	
TRT x D		0.99			0.7	

† Letters following indicates means are statistically significantly different from each other. CTC – Conventional tillage and crimson clover, CTNCC – Conventional tillage and no cover crop, CTR – Conventional tillage and rye, CTRC – Conventional tillage and rye and crimson clover mixture (50/50), NTC- No tillage and crimson clover, NTNCC – No tillage and no cover crop, NTR – No tillage and rye, NTRC – No tillage and rye + crimson clover mix (50/50). TRT, treatment; D, depth; T, Tillage; CC, Cover Crop.

Table 2.8. Soil microbial respiration (mg CO_2 100 g^{-1} day^{-1}) across different treatments in Tidewater and Coastal Plain regions.

	Tidewater			Coastal Plain		
Depth (cm)	0-5	5-10	10-20	0-5	5-10	10-20
CTC	$25.8^{b†}$	14^{a}	8^{ns}	16.4^{ns}	14.04ab	4.2^{b}
CTNCC	25.1^{b}	14.1^{a}	13.3	20	14.1ab	9.71^{ab}
CTR	36.1^{ab}	15^{a}	12.3	15	6b	16.1^{a}
CTRC	37^{ab}	14^{a}	7	16	11ab	11.3^{ab}
NTC	37.3^{ab}	17^{a}	7	28	19a	7^{ab}
NTNCC	37^{ab}	26.1^{a}	13	20.4	13ab	5^{b}
NTR	49.1^{a}	14^{a}	14	16.4	10ab	4^{b}
NTRC	58^{a}	31.3^{b}	11.4	28.2	17.3a	5^{b}
			Analysis of Variance (P<0.05)			
TRT	<0.0001	<0.0001	<0.0001	0.5	<0.0001	<0.0001
T x CC	0.7	0.08	0.4	0.3	0.4	0.03
Depth		<0.0001			<0.0001	
TRT x D		0.01			0.001	

† Letters following indicates means are statistically significantly different from each other. CTC – Conventional tillage and crimson clover, CTNCC – Conventional tillage and no cover crop, CTR – Conventional tillage and rye, CTRC – Conventional tillage and rye and crimson clover mixture (50/50), NTC- No tillage and crimson clover, NTNCC – No tillage and no cover crop, NTR – No tillage and rye, NTRC – No tillage and rye + crimson clover mix (50/50). TRT, treatment; D, depth; T, Tillage; CC, Cover Crop.

Table 2.9. Soil β-glucosidase activity (μmolp-nitrophenol 100 g^{-1} $hour^{-1}$) across different treatments in Tidewater and Coastal Plain regions.

	Tidewater			Coastal Plain		
Depth (cm)	0-5	5-10	10-20	0-5	5-10	10-20
CTC	$1142^{b†}$	351^{d}	451^{a}	480^{bc}	339.3^{bc}	123^{bc}
CTNCC	291^{d}	410^{cd}	414^{ab}	453^{c}	249^{bc}	37.1^{d}
CTR	554^{c}	549^{bc}	296^{d}	424^{c}	173^{c}	149^{b}
CTRC	453^{cd}	253d	199^{e}	408.1^{c}	238.3^{c}	85.2^{cd}
NTC	1585^{a}	614^{b}	369^{bcd}	990^{a}	420^{ab}	445.04^{a}
NTNCC	1196^{b}	582^{b}	330^{cd}	464.3^{c}	195.2^{c}	79.1^{cd}
NTR	1560^{a}	699^{b}	426^{ab}	779.2^{ab}	519^{a}	133.2^{bc}
NTRC	1414^{a}	1297^{a}	389^{abc}	832.1^{a}	549^{a}	108^{bc}
		Analysis of Variance (P<0.05)				
TRT	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
T x CC	<0.0001	<0.0001	<0.0001	0.0045	<0.0001	<0.0001
Depth		<0.0001			<0.0001	
TRT x D		0.0001			0.0003	

† Letters following indicates means are statistically significantly different from each other. CTC – Conventional tillage and crimson clover, CTNCC – Conventional tillage and no cover crop, CTR – Conventional tillage and rye, CTRC – Conventional tillage and rye and crimson clover mixture (50/50), NTC- No tillage and crimson clover, NTNCC – No tillage and no cover crop, NTR – No tillage and rye, NTRC – No tillage and rye + crimson clover mix (50/50). TRT, treatment; D, depth; T, Tillage; CC, Cover Crop.

Table 2.10. Urease (μmol 100 g^{-1} $hour^{-1}$) across different treatments in Tidewater and Coastal Plain regions.

	Tidewater			Coastal Plain		
Depth (cm)	0-5	5-10	10-20	0-5	5-10	10-20
CTC	31.2[bc†]	23[c]	23[c]	27[bc]	16.4[ab]	15[ns]
CTNCC	23.1[c]	14[abc]	14[c]	13[c]	9[b]	14.4
CTR	70.2[abc]	61.3[abc]	61.3[c]	41[ab]	12[ab]	18.3
CTRC	131[ab]	91[ab]	91[bc]	49.4[a]	28[a]	15
NTC	162.1[a]	194.3[c]	194.3[a]	14.3[c]	11[b]	10
NTNCC	27.1[c]	13.2[c]	13.2[c]	16[c]	13[ab]	5
NTR	49[bc]	17[bc]	17[c]	15[c]	7[b]	9.4
NTRC	166[a]	166[a]	166[ab]	46.04[ab]	17.3[ab]	15.3
			Analysis of Variance (P<0.05)			
TRT	0.0001	0.003	<0.0001	<0.0001	<0.0001	0.0003
T x CC	<0.0001	0.12	0.0002	0.02	0.3	0.4
Depth		0.6			<0.0001	
TRT x D		0.7			0.0007	

[†] Letters following indicates means are statistically significantly different from each other. CTC – Conventional tillage and crimson clover, CTNCC – Conventional tillage and no cover crop, CTR – Conventional tillage and rye, CTRC – Conventional tillage and rye and crimson clover mixture (50/50), NTC- No tillage and crimson clover, NTNCC – No tillage and no cover crop, NTR – No tillage and rye, NTRC – No tillage and rye + crimson clover mix (50/50). TRT, treatment; D, depth; T, Tillage; CC, Cover Crop.

Table 2.11.1 Soil nutrients (mg kg^{-1}) across different treatments in Tidewater region.

	P	K	Ca	Mg	S	Mn	Zn	Cu
			----------------------- 0-5 cm -----------------------					
CTC	99 ^{ns†}	255^{ns}	1412^{ns}	254^{ns}	38^{ns}	11^{ns}	12^{ns}	2^{ns}
CTNCC	30	184	1411	285	42	8	8	2
CTR	150	263	2128	423	45	16	19	4
CTRC	81	174	1334	216	35	10	10	3
NTC	46	196	1427	251	35	7	9	3
NTNCC	186	253	1984	375	38	12	28	3
NTR	156	320	1690	359	35	12	17	3
NTRC	175	335	1949	358	53	15	24	4
			Analysis of Variance (P>0.05)					
TRT	0.48	0.57	0.72	0.94	0.87	0.83	0.0349	0.4
T x CC	0.35	0.42	0.5	0.86	0.45	0.58	0.058	0.18
			----------------------- 5-10 cm -----------------------					
CTC	48^{ns}	212^{ns}	1285^{ns}	268^{ns}	52^{ns}	7^{ns}	9^{ns}	2^{ns}
CTNCC	69	147	1263	263	54	5	9	2
CTR	75	160	1528	312	40	8	10	3
CTRC	19	100	902	150	30	5	5	3
NTC	74	184	1308	227	40	6	10	2
NTNCC	148	146	1540	303	39	8	21	3
NTR	124	173	1237	283	36	8	11	2
NTRC	122	205	1468	282	58	12	18	3
			Analysis of Variance (P>0.05)					
TRT	0.46	0.9	0.9	0.98	0.62	0.65	0.08	0.8
T x CC	0.82	0.6	0.64	0.86	0.2	0.4	0.2	0.5
			----------------------- 10-20 cm -----------------------					
CTC	34^{ns}	142^{ns}	776^{ns}	173^{ns}	70^{ns}	5^{ns}	5^{ns}	2^{ns}
CTNCC	58	141	1157	231	61	6	8	2
CTR	82	123	965	204	64	8	8	2
CTRC	71	104	771	127	43	5	5	2
NTC	32	108	775	125	48	5	4	2
NTNCC	89	106	874	173	48	5	9	2
NTR	67	132	831	179	38	5	6	2
NTRC	77	120	843	163	71	9	9	2
			Analysis of Variance (P>0.05)					
TRT	0.84	0.98	0.73	0.95	0.54	0.86	0.34	0.86
T x CC	0.9	0.8	0.71	0.88	0.16	0.46	0.33	0.7
Depth	0.029	<0.0001	<0.0001	0.005	0.007	0.0008	<0.0001	<0.0001
TRT x D	0.84	0.85	0.87	0.99	0.96	0.98	0.49	0.89

† Letters following indicates means are statistically significantly different from each other. CTC – Conventional tillage and crimson clover, CTNCC – Conventional tillage and no cover crop, CTR – Conventional tillage and rye, CTRC – Conventional tillage and rye and crimson clover mixture (50/50), NTC- No tillage and crimson clover, NTNCC – No tillage and no cover crop, NTR – No tillage and rye, NTRC – No tillage and rye + crimson clover mix (50/50). TRT, treatment; D, depth; T, Tillage; CC, Cover Crop.

Table 2.11.2 Soil nutrients (mg kg^{-1}) across different treatments in Coastal Plain region.

	P	K	Ca	Mg	S	Mn	Zn	Cu
			------------------------ 0-5 cm ------------------------					
CTC	$90^{ns†}$	204^{ns}	710^{ns}	189^{ns}	10^{ns}	11^{ns}	4^{ns}	0.4^{ns}
CTNCC	61	212	733	222	10	8	3	0.4
CTR	48	152	735	201	8	9	4	0.4
CTRC	47	118	795	205	8	7	3	0.4
NTC	75	186	814	233	11	9	4	0.5
NTNCC	89	178	705	207	11	7	4	0.4
NTR	91	199	695	218	11	7	4	0.4
NTRC	75	220	832	239	11	8	4	0.5
			Analysis of Variance (P>0.05)					
TRT	0.06	0.42	0.98	0.9	0.27	0.62	0.91	0.86
T x CC	0.17	0.15	0.9	0.88	0.63	0.65	0.8	0.68
			------------------------ 5-10 cm ------------------------					
CTC	63^{a}	127^{ns}	700^{ns}	187^{ns}	9^{ns}	10^{ns}	4^{ns}	0.5^{ns}
CTNCC	30^{b}	134	702	209	7	7	2	0.3
CTR	24^{b}	99	706	195	7	7	3	0.4
CTRC	34^{ab}	106	797	207	7	8	4	0.4
NTC	33^{b}	120	797	224	8	8	3	0.6
NTNCC	39^{ab}	118	719	211	8	5	3	0.4
NTR	31^{b}	123	726	223	9	6	3	0.4
NTRC	27^{b}	131	813	239	8	6	3	0.5
			Analysis of Variance (P>0.05)					
TRT	0.007	0.92	0.98	0.98	0.2	0.5	0.42	0.42
T x CC	0.04	0.63	0.9	0.9	0.09	0.98	0.78	0.87
			------------------------ 10-20 cm ------------------------					
CTC	18^{ns}	82^{ns}	673^{ns}	189^{ns}	7^{ns}	6^{ns}	2^{ns}	0.5^{ns}
CTNCC	17	98	620	191	7	5	2	0.3
CTR	9	78	632	196	7	4	1	0.3
CTRC	14	91	718	216	7	4	2	0.3
NTC	10	90	745	241	7	5	2	0.4
NTNCC	11	87	702	231	17	3	2	0.5
NTR	11	87	659	227	18	2	2	0.4
NTRC	11	95	781	258	13	3	2	0.5
			Analysis of Variance (P>0.05)					
TRT	0.87	0.95	0.94	0.88	0.33	0.53	0.95	0.3
T x CC	0.71	0.81	0.99	0.99	0.64	0.99	0.88	0.15
Depth	<0.0001	<0.0001	0.2	0.8	0.72	<0.0001	<0.0001	0.44
TRT x D	0.73	0.82	0.99	0.99	0.2	0.99	0.99	0.5

† Letters following indicates means are statistically significantly different from each other. CTC – Conventional tillage and crimson clover, CTNCC – Conventional tillage and no cover crop, CTR – Conventional tillage and rye, CTRC – Conventional tillage and rye and crimson clover mixture (50/50), NTC- No tillage and crimson clover, NTNCC – No tillage and no cover crop, NTR – No tillage and rye, NTRC – No tillage and rye + crimson clover mix (50/50). TRT, treatment; D, depth; T, Tillage; CC, Cover Crop

Table 2.11.3 North Carolina Department of Agriculture and Consumer Services CEC (meq 100 cm^{-3}), BS (%) and HM (g 100 cm^{-3}) across different treatments.

	Tidewater			Coastal Plain		
	CEC	BS	HM	CEC	BS	HM
------------------------ 0-5 cm ----------------------						
CTC	11^{ns†}	74^{ns}	4^{ns}	7^{ns}	88^{ns}	0.3^{ns}
CTNCC	11	67	3	7	90	0.2
CTR	14	85	3	7	91	0.3
CTRC	10	72	3	7	90	0.3
NTC	11	75	3	7	93	0.3
NTNCC	13	79	3	7	89	0.2
NTR	12	76	3	7	88	0.2
NTRC	13	82	3	8	92	0.2
Analysis of Variance (P>0.05)						
TRT	0.99	0.99	0.99	0.99	0.95	0.86
T x CC	0.99	0.99	0.99	0.92	0.66	0.85
------------------------ 5-10 cm --------------------						
CTC	10^{ns}	69^{ns}	4^{ns}	7^{ns}	89^{ns}	0.3^{ns}
CTNCC	10	70	4	7	91	0.2
CTR	11	78	4	7	92	0.2
CTRC	8	61	4	7	91	0.3
NTC	11	70	4	7	95	0.2
NTNCC	12	74	3	7	91	0.2
NTR	10	67	4	7	92	0.2
NTRC	11	75	3	8	93	0.2
Analysis of Variance (P>0.05)						
TRT	0.99	0.99	0.99	0.98	0.96	0.7
T x CC	0.99	0.99	0.99	0.99	0.75	0.77
------------------------ 10-20 cm -------------------						
CTC	8^{ns}	55^{ns}	4^{ns}	6^{ns}	91^{ns}	0.3^{ns}
CTNCC	10	69	4	6	90	0.2
CTR	9	67	4	6	91	0.1
CTRC	8	57	4	7	91	0.2
NTC	8	59	5	7	95	0.1
NTNCC	9	61	4	7	87	0.1
NTR	8	61	4	7	84	0.1
NTRC	8	60	4	8	92	0.1
Analysis of Variance (P>0.05)						
TRT	0.99	0.99	0.99	0.87	0.8	0.27
T x CC	0.99	0.99	0.99	0.9	0.62	0.52
Depth	<0.0001	0.0003	0.0013	0.4	0.94	<0.0001
TRT x D	0.9	0.9	0.99	0.99	0.97	0.7

† Letters following indicates means are statistically significantly different from each other. CTC – Conventional tillage and crimson clover, CTNCC – Conventional tillage and no cover crop, CTR – Conventional tillage and rye, CTRC – Conventional tillage and rye and crimson clover mixture (50/50), NTC- No tillage and crimson clover, NTNCC – No tillage and no cover crop,

NTR – No tillage and rye, NTRC – No tillage and rye + crimson clover mix (50/50). TRT, treatment; D, depth; T, Tillage; CC, Cover Crop.

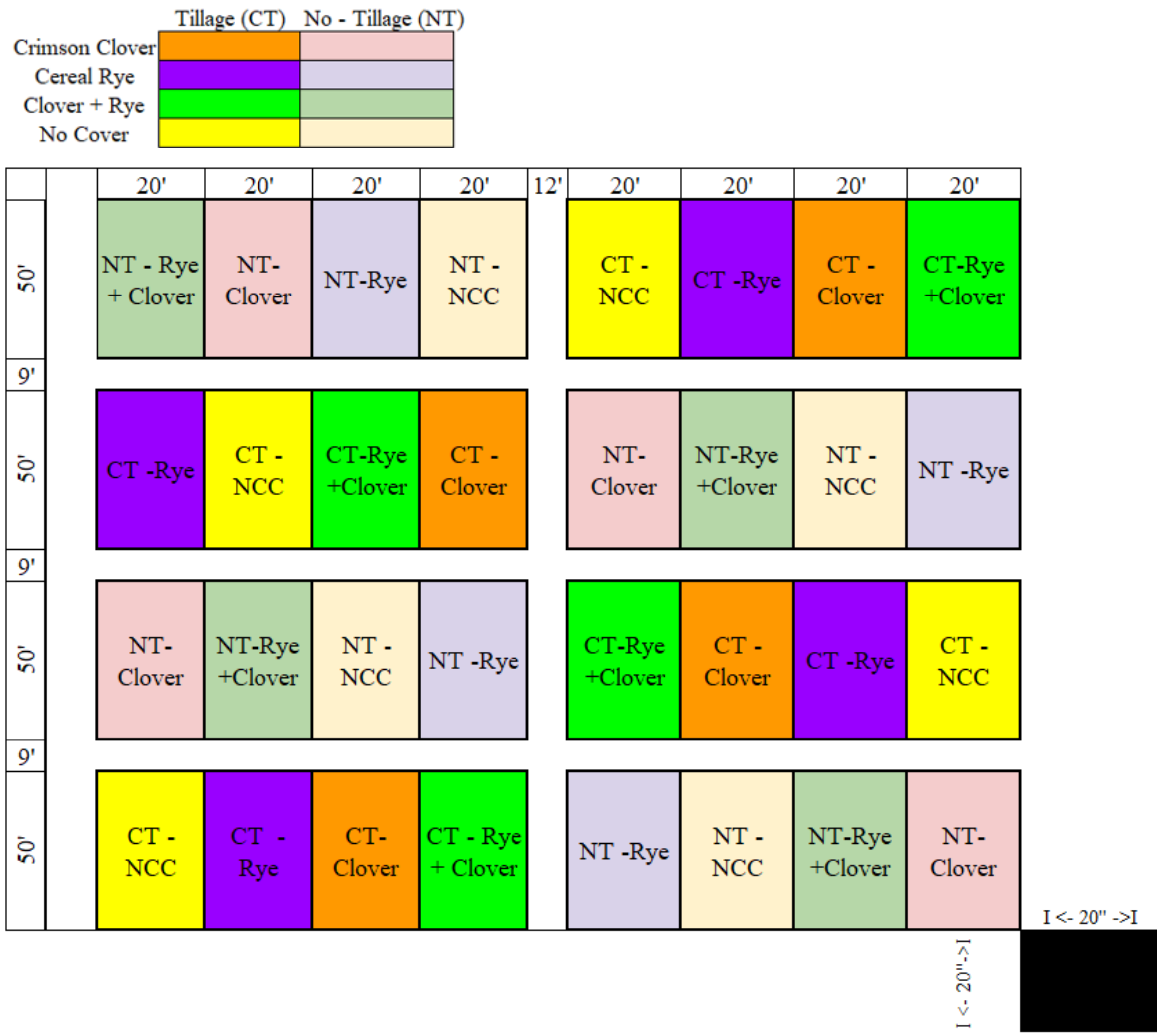

Figure 2.1. Experimental design designating the plot size and treatments.