April 2023

The Use of Biological Soil Health Indicators to Quantify the Benefits of Cover Crops

Alexander Wu
University of Massachusetts Amherst

THE USE OF BIOLOGICAL SOIL HEALTH INDICATORS TO QUANTIFY THE BENEFITS OF COVER CROPS

A Thesis Presented

By

ALEXANDER WU

Submitted to the Graduate School of the

University of Massachusetts Amherst in partial fulfillment

of the requirements for the degree of

MASTER OF SCIENCE

February 2023

Plant & Soil Sciences

Stockbridge School of Agriculture

THE USE OF BIOLOGICAL SOIL HEALTH INDICATORS TO QUANTIFY THE BENEFITS OF COVER CROPS

A Thesis Presented

By

ALEXANDER WU

Approved as to style and content by:

Ashley Keiser, Chair

Rachel Hestrin, Member

Masoud Hashemi, Member

Masoud Hashemi, Director
Plant and Soil Sciences Graduate Program

THE USE OF BIOLOGICAL SOIL HEALTH INDICATORS TO QUANTIFY THE BENEFITS OF COVER CROPS

A Thesis Presented

By

ALEXANDER WU

Approved as to style and content by:

_____
Ashley Keiser, Chair

_____
Rachel Hestrin, Member

_____
Masoud Hashemi, Member

_____
Masoud Hashemi, Director
Plant and Soil Sciences Graduate Program

ABSTRACT

THE USE OF BIOLOGICAL SOIL HEALTH INDICATORS TO QUANTIFY THE BENEFITS OF COVER CROPS

FEBRUARY 2023

ALEXANDER WU, B.S., UNIVERSITY OF MASSACHUSETTS AMHERST

M.S., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Professor Ashley Keiser

Soils provide many essential functions that support the world. With a decline in soil health, these functions also decrease in efficiency, and can threaten the health of billions of people around the world. Typically, soil health tests do not use biological indicators, however microbes drive and perform vital functions to increase soil health. One way to increase soil health is through the use of cover crops to reduce soil erosion during fallow periods, increasing soil organic matter, as well as collecting nutrients from soil into their biomass. These cover crops are then terminated through various methods such as herbicides, disk tillage, or no tillage. The termination method can have an impact on soil health, by chemically affecting soil microbes with herbicides, disturbing soil, microbial communities, and fungi with tillage, or creating residue barriers on the surface of soil such as with using roller crimping (no tillage). Fertilization can also affect soil health, controlling rates of nutrient turnover and decomposition through the needs of microbes for carbon and nitrogen. This study quantifies the effects of four termination methods and four fertilization treatments on soil biological indicators during one growing season of sweet corn. Plots that were not tilled and terminated using roller crimping showed highest rates of decomposition, as well as increased labile carbon pools to feed microbes slowly

throughout the growing season. Microbial activity was also observed to respond to fertilization, as patterns in activity spiked directly after fertilization. This study informs agricultural land management by the usage of biological indicators to further support the use of cover crops to increase soil health along with using no-till termination methods. Root biomass contributions toward soil health was also investigated, and how they may be affected by tillage.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER 1

INTRODUCTION

The NRCS defines soil health as, “the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans.” The world depends on soil, but it is often not thought of often in daily life, despite holding five essential functions. First, soils regulate water, controlling where it flows over the land, and through the soil. They can also filter and buffer pollutants through minerals and microbes in soil. This can help detoxify harsh materials produced by anthropogenic activities. They also help sustain plant and animal life as an ecosystem, as well as providing a physical foundation and medium for plants and buildings. Lastly, they are part of many nutrient cycles, transforming nutrients through decomposition so that they may be used again (USDA NRCS, 2022). About 75% of Earth’s land area has been degraded due to floods, erosion, salinization, human settlement, mining, deforestation, climate change, and more. This threatens the health of 3.2 billion people around the world (Talukder, B. et al., 2021). In degraded soils, these functions decline significantly or are lost. Importantly, food quality and quality are intricately linked to soil quality. As the quality of soil declines, the nutritional value of the food falls as well (Oliver, M. A., & Gregory, P. J., 2015). The food may even contain toxic substances or pathogenic organisms that can make people ill (Brevik, 2009; Brevik & Burgess, 2013). The soil health movement seeks to restore and protect soils so that soils continue to support the essential functions they provide. Healthy soils are essential to sustain our global population, and agricultural management strategies that improve soil health should be the focus of researchers and practitioners.

Decades of research have already demonstrated beneficial land management strategies which improve the health of agricultural soils. One documented management strategy which improves soil health is no-till farming. Tillage is widely used in agricultural land management, mixing soil, and breaking down soil aggregates to create ideal conditions for seedling germination and growth. However, tillage is one of the major agronomic activities that results in a large release in CO_2 from the turnover of carbon held within soil organic matter (Abdalla, M. et al. 2013). Soil organic matter (SOM) is the nonliving organic fraction of soil, composed of plant residues or animal tissues in different stages of decomposition. SOM is very beneficial for soil health, and improves cation exchange capacity, water holding capacity, aggregation, water infiltration, aeration, as well as provides slowly available carbon and nutrients to soil (Talukder, B. et al., 2021). However, tillage increases access to and the turnover of SOM, which contributes to greenhouse gas emissions, and in turn, increased climate change (Abdalla, M. et al. 2013; Mohammed, S. et al. 2022). No-till farming reduces this carbon footprint. As the land is not tilled, soil structure is maintained, and large amounts of CO_2 are not released when SOM can remain protected in soil aggregates. The carbon then stays in the soil. Importantly, SOM is maintained or increases, which brings in co-benefits such as nutrient and water holding capacity, intact soil structure and reduced erosion (Lal, R. 2006).

Another documented practice that improves soil health is the use of cover crops. Cover crops provide many benefits to soil such as increasing soil organic carbon, reducing soil erosion, improving soil structure and weed suppression (USDA NRCS,

n.d.). Some cover crops such as legumes, have the additional function to affect soil nutrient dynamics. Legume cover crops like peas and clover symbiotically fix N_2 with bacteria in the genus *Rhizobium* and supply significant amounts of nitrogen in low-fertility soils (Smil 1997). This provides nitrogen for the next crop to be grown and reduces the amount of nitrogen fertilizer needed during the growing season (Blanco-Canqui et al. 2015). When cover crops are terminated, the residues are incorporated into agricultural soils as coarse fragments, which then get broken down by soil microbes. With the introduction of crop residues, microbial activity is stimulated, which can result in increases in the microbial biomass nitrogen pool (St. Luce et al. 2014). The amount could change however, depending on the chemical quality of the cover crop incorporated into the soil. For example, rye plants can contain C:N ratios of 26:1 all the way up to 82:1 depending on their growth stage. Pea plants have a lower ratio at 29:1 (USDA NRCS, n.d.).

The C:N ratio of the cover crop litter influences the amount of cover crop nitrogen that ends up in soil pools that are plant accessible. It can also be influenced by the nitrogen demands of the soil microbial community balanced with the amount of available nutrients already in the soil (Gillespie et al. 2014). The soil microorganisms are the ones which drive the chemical decomposition of the terminated cover crops, but they also require nitrogen to meet their own nutrient demands. If there is not enough nitrogen available in the decomposing cover crop biomass, the microbes will immobilize the nitrogen in their own biomass making it unavailable for plant uptake. The initial C:N ratio of cover crop as well as the initial nitrogen content determines the rate of

decomposition, or how fast the biomass becomes mineralized and released into soil pools, as well as whether the nitrogen will be immobilized by soil microbes or mineralized (released). Generally, litter with high C:N ratios slow decomposition, whereas lower ratios could be slow or fast, depending on existing soil resources (Prescott 2010). For example, if microbial communities are in a soil with excess soil nitrogen, there would be no need to decompose the litter quickly for nutrients, as they are already available. Evidence also suggests that decomposition may proceed quickly because the microorganisms are not limited by nitrogen and instead demand carbon from the cover crop residues (Hobbie, S. E., 2008; Knorr, M. et al., 2005). Therefore, cover crop type combined with existing soil resources and microbial nutrient demand can control the rate of cover crop litter decomposition as well as nitrogen made available for crop uptake across the growing season.

Different species of cover crops are chosen across farms in the U.S. depending on location as well as soil needs. One common cover crop is winter rye. This cover crop is winter hardy, grows early in the Spring, and reaches an optimum growth stage sooner than other small grains. It can also scavenge for excess soil NO_3^-N, and reduce NO3-N leaching from the soil. Additionally, winter rye provides ground cover to reduce soil erosion, and its biomass can help maintain organic matter in the soil (Krueger et al. 2011). It is a low nitrogen cover crop, which induces immobilization of nitrogen in the soil microbial community from existing nitrogen pools when decomposed as compared to high nitrogen cover crops. Legume cover crops such as pea can be used to fix nitrogen into the soil, increasing available N for cash crops. As legume cover crops decompose,

the nitrogen in the biomass undergoes mineralization into inorganic forms of nitrogen such as ammonium and nitrate. By using both leguminous and non-leguminous cover crops as a mixture, the soil can have an increase in benefits. For example, cover crops of rye-pea mixtures suppress more weeds compared to monocultures of pea cover crops (Akemo et al. 2000). More research is needed to increase our knowledge of what impact mixtures of high and low nitrogen cover crops such as pea and rye have on immobilization and mineralization of soil nutrients. Theoretically, when cover crops are terminated, all of the nitrogen in the biomass enters the soil and should be available to be taken up by the cash crop grown above, however that is not the case. Research has indicated that cover crop nitrogen ends up in plant accessible and inaccessible soil pools as well as microbial biomass (St. Luce et al. 2014). These pools can be impacted by the termination method of the cover crops, as well as timing of termination.

Termination methods can impact the size and diversity of soil microbial communities. The impact could then influence the rate of litter decomposition as well as nutrient availability for cash crops. Mechanical fragmentation of cover crops such as with tillage can increase cover crop residue surface areas, increasing space for microbes to colonize and increasing decomposition of the litter. However, tillage also disturbs soil, and in turn the soil ecology, by redistributing and mixing all soil layers, increasing carbon turnover with the loss of SOM (Abdalla, M. et al. 2013). Chemical termination using herbicides may successfully terminate the cover crop but can have negative effects on the microbial community. Past studies have shown that herbicides can decrease soil denitrification as well as temporarily changing microbial respiration and biomass (Kim et

al. 2020). Roller crimping is another termination method, killing cover crops and weeds while creating a residue barrier on the soil surface. Unlike tillage methods, this does not disturb soil or microbial and fungal communities, leaving the soil structure intact (Bloszies, S. A. et al., 2022). The timing of the termination method can have direct impacts on cash crop yield. For example, rye termination at the time of corn planting can result in decreased corn yield, but when terminated a week or more before, the yield was similar or slightly greater. Cover crops may also be terminated earlier to limit resource depletion, preventing cover crop from using all the nutrients in the soil before growing the corn (Krueger et al. 2011). While using cover crops is a large soil-health promoting practice, more research is needed to connect how the management of cover crops can be used to improve soil health beyond reduced erosion and nutrient retention, especially with their impacts on improving the soil ecosystem, specifically soil microbial communities and biogeochemical cycling.

Microbial communities are often overlooked when talking about soil health, but perform very important functions in soil, including their role in SOM turnover and formation or nutrient release noted above. While these microbial communities are of great importance, their overall function is not represented in standard soil health tests. Therefore, there is increasing concern that current soil metrics lack sufficient weight for differences in soil management practices (Roper et al., 2017; Stewart, R. D. 2018). The Soil Health Institute uses 19 “Tier 1” indicators for soil health such as soil carbon, pH, texture, cation exchange capacity etc., and only recently included biological indicators

(Stewart, R. D. 2018). The question lies as to why biological indicators are not widely used if they perform such vital functions to increase soil health.

Many factors contribute to the effectiveness of microbial communities as drivers of a healthy soil ecosystem. These communities may thrive depending on soil conditions such as the moisture content in soil, disturbance, soil composition, and nutrient availability (Van der Heijden, M. G. A. et al. 2008). As mentioned before, bacteria such as *Rhizobium* can supply significant amounts of nitrogen in low-fertility soils (Smil 1997). These bacteria live in a mutualistic relationship within the root nodules of legumes. Mycorrhizal fungi also form symbiotic relationships with plants, delivering limiting nutrients across large distances where the plant roots may not reach, as a sort of nutrient highway in exchange for carbon. Fungal networks in the soil are disrupted with the soil is physically disturbed (i.e., through tillage), but maintained with continuous live roots (i.e. cover crops) (Bowles, T. M. et al. 2017). A combination of microbial players helps decompose organic materials, breaking them down into soil organic matter, and releasing those nutrients into the soil for plant use. This is a critically significant role with regards to nutrient cycling and release. Therefore, a diverse and fully functioning soil microbial community provides the engine for improved soil health.

Plants require nitrogen to complete photosynthesis (Bojović, B., & Marković, A., 2009) while microbes require nitrogen to produce extracellular enzymes to deconstruct substrates (Mooshammer, M., 2014). Nitrogen demand by growing crops is provided through available soil resources provided through the recycling of nutrients by the soil

microbial community, biological N fixation, conversion of atmospheric N through natural fixation (i.e., lighting) or fertilizer sources. Decomposition could be an important pathway to ensure availability of a limited nutrient, N, within terrestrial systems. As microbes cleave organic compounds within organic matter, such as terminated cover crops, inorganic N is released into the soil where it is available for plant uptake, held within microbial biomass, or held within soil pools. The mineralization of nitrogen from organic to inorganic forms is a two-step process. Ammonification is the first step whereby the tissues which contain organic nitrogen, in the forms of amino acids and DNA, are broken down by microorganisms and released into the soil as inorganic ammonium. Ammonium can be used by plants or undergo nitrification by autotrophic microorganisms to be broken down further. An aerobic process, nitrification converts ammonium to nitrate. Nitrate is highly mobile and can leach easily from soil (Nouri, A. et al. 2022). This depletes the soils of nitrogen, making the nutrient more limited. Soil microorganisms can also immobilize both ammonium and nitrate to meet their own nutrient demands but rendering them unavailable for plants. Soil microbes play an essential role at recycling nitrogen from organic to plant-available inorganic forms. Through decomposition across the growing season, cover crops can provide a consistent source of recycled N to the growing cash crops. We do not yet know, however, how management, such as cover crop termination, impacts the size and activity of the soil microbial community, available soil C pools, and N turnover and availability.

While there have been many studies conducted on cover crop litter decomposition, belowground cover crop inputs such as roots still remain a mystery as to

how big of a contribution they have to the agricultural soil. Roots are primarily in the soil already, and so do not need additional methods to incorporate into soil. Due to their location, they also have close association with mycorrhizal fungal hyphae and microbial communities in the soil. However, roots usually decompose more slowly than the leaf litter of the same species. This may be due to the difference in composition of roots compared to shoots. Roots have more chemical recalcitrance with the recalcitrant compound suberin, physical-chemical protection with lignin, and can have physical protection in soil aggregates (Prescott 2010). In addition to roots, rhizodeposits may be an additional source of soil carbon. About 30-50% of underground soil carbon can be attributed to rhizodeposits, with values up to 40% of total plant input. Additionally, up to 75% of soil carbon inputs to SOM come from roots and rhizodeposits (Austin et al. 2017). Bringing in cover crops, this can bring a large benefit to soil health, as all these belowground sources of carbon can build up SOM in soil. The turnover of cover crop roots and shoots could provide additional soil health biological benefits that have not yet been quantified.

There is a general understanding that cover crops and sustainable management practices improve the physical properties of soil. It is still unknown how these practices directly impact the function of soil microbial communities and implications for C and N turnover and storage. The overall objective of this study was to quantify how four different cover crop termination treatments crossed with four fertilization treatments impact soil microbial community function, carbon and nitrogen turnover and availability, and, thus, soil health. Specifically, I asked:

1. How do different cover crop termination methods impact the soil microbial community size and activity as well as carbon and nitrogen pools?
2. How does the amount of fertilizer added to agricultural soils impact the soil microbial community size and activity as well as carbon and nitrogen pools?

Using these two questions I hypothesized that:

- H1A – Soils with cover crops terminated by roller crimping will have a larger microbial community and readily-available soil carbon pools, compared to methods which apply herbicides or conventional tillage.
- H1B – Soils with cover crops terminated by disk tillage will have higher nitrogen mineralization and nitrification rates compared to methods using herbicides or roller crimper.
- H2A – Soils that are fertilized more will have a larger microbial community and readily-available soil carbon pools compared to soils with less fertilizer.
- H2B - Soils that receive less fertilization will show lower nitrogen mineralization and nitrification in the soil compared to soils with more fertilization.

CHAPTER 2

METHODS

2.1 Experimental Design

This study was conducted at the University of Massachusetts, Amherst Crop and Animal Research and Education Farm in South Deerfield, MA. There were four cover crop termination/weed control treatments chosen, along with four nitrogen fertilization amounts. The termination/weed control methods are as follows: 1) Herbicide Disk Tillage [HDT] - Glyphosate termination (1 lb. a.e./acre) followed by disk tillage to incorporate residues and herbicide application throughout the growing season to control weed growth. 2) Herbicide No Tillage [HNT] - Glyphosate termination with no tillage and no herbicide application during the growing season. 3) Cultivation Disk Tillage [CDT] - Mechanical termination of cover crop using disk tillage along with mechanical weed control using a cultivator. 4) Roller Crimper No Tillage [RCNT] - Termination by roller crimper, with no tillage or herbicides. An early maturing species of sweetcorn (*Zea mays* L.) was chosen as the cash crop for this experiment, with seeds from Johnny's Selected Seeds, in Waterville, ME (Xtra-Tender 20173). The four nitrogen treatments were based upon the recommended fertilizer input for sweet corn (130 lbs./acre). Only nitrogen varied between fertilization treatments. Urea was used for nitrogen (46% nitrogen). There was also evenly applied triple super phosphate (46% phosphorus) and potassium chloride (60% potassium). The four fertilization treatments were no fertilizer (0%), 33% of recommended nitrogen fertilizer, 67%, and 100% the recommended rate. The fertilizer was applied to the fields at two different times. First, one third of the application rate was added as a starter fertilizer. The remaining two thirds of the fertilizer was added when the corn was about 12" tall with six leaves (V6 growth stage).

The experimental plots covered a total of 720 square feet (Figure 1). Within this area, there were 12 rows of plots, each 24 m. long x 2.4m. wide. They were arranged into three blocks with four rows of plots each. Each row of plots was subjected to one of the four termination/weed control methods and was separated into 4 subplots (6m. long) for each of the nitrogen treatments. Each block was randomized for termination and fertilizing treatments, creating three total blocks with the same amount of termination and fertilizer treatments per block. Each plot contained three rows of sweetcorn.

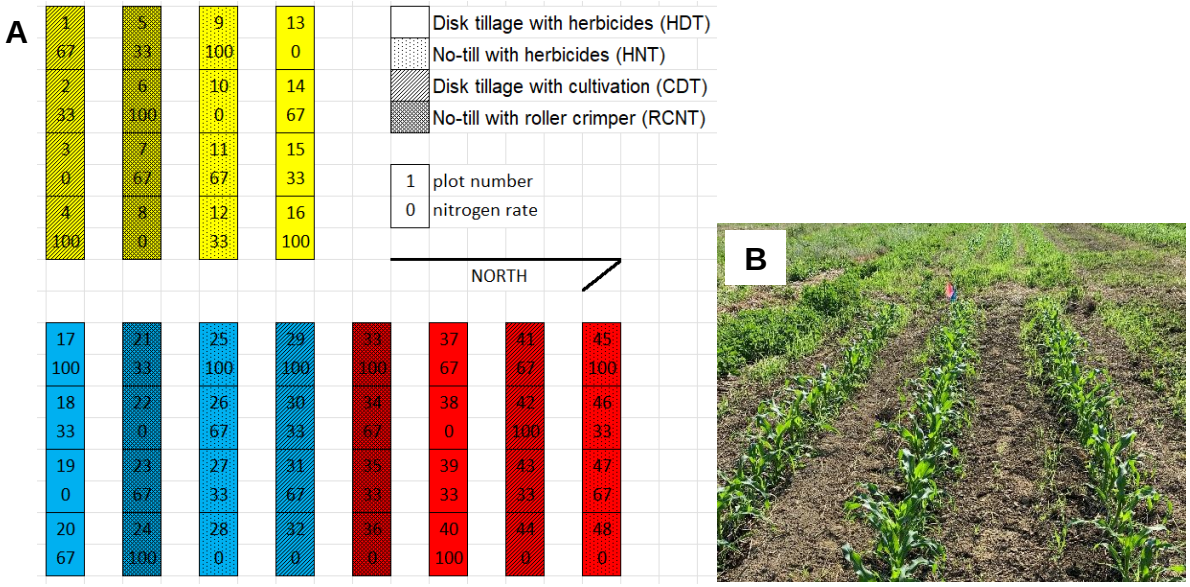

Figure 1: Experimental layout of field experiment (A). There are 4 rows of plots, each subjected to one cover crop termination method. Each row had 4 subplots subjected to fertilization treatment. Plot number appears above nitrogen fertilizer amount, while shading determines termination treatment. Color of plots represent what block they were in. Each row of plots had three rows of sweetcorn planted (B).

A mix of winter rye (*secale cereale*) and field peas (*Pisum sativum*) were the selected cover crops. This resulted in a mixed litter quality, as winter rye plants can contain C:N ratios of 26:1 up to 82:1 depending on their growth stage, while peas have a

ratio of 29:1 (USDA NRCS, n.d.). These cover crops were planted across all plots each fall, following the sweetcorn harvest in September. All termination methods were applied in late May or Early June, when the winter rye reached between 50% flowering and soft milk stage. For this study, termination timing was kept consistent.

The cover crop decomposition study was initiated after two years of the cover crop termination × N fertilization field implementation. While there was cover crop grown in the field, we used cover crop grown in a greenhouse to put in litter/root bags so that they were not influenced by the termination x fertilization treatments. These cover crops were grown in consistent soil conditions in a soil and sand mixture, using topsoil collected from the research farm, but not from the plots used for this study. The cover crops were seeded at an equal mixture of 50% field pea, and 50% winter rye. They were grown until the rye was 50% flowering and soft milk stage, just like in the field. The aboveground biomass was then cut and air dried in the greenhouse. The roots were cleaned of soil by hand and gently rinsed with water before air drying in the greenhouse. Litter bags were 20 cm^2, composed of nylon mesh with 1mm openings. Each litterbag contained about 1g of pea and 1g of rye litter that had been cut into about 4 cm pieces. Root bags were also created but using the roots of winter rye and pea. The root bags were created using 8cm^2 of 53-micron polyester. Each root bag was filled with ~0.05g pea roots, and ~0.15g rye roots. Different mesh sizes were used between litter and root bags to exclude certain micro or macrofauna from decomposing the material. Litter bags used a larger mesh to allow some smaller macrofauna such as worms to assist in

decomposition. However with roots, the 53-micron mesh was used to focus on decomposition dynamics only from microbial activity and soil fungi.

After spring termination, the litter and root bags were placed in the plots between corn seedlings and held in place using a metal stake. Litterbags were placed in each plot with 4 bags per plot (for four collections), with a total of 192 litterbags. Root bags were placed only in CDT and RCNT terminated plots. Root bags in CDT plots had both bags laid out on the surface and secured with a metal stake, as well as buried bags in the soil at a depth of 10cm. This mimicked how disk tillage mixes soils, causing roots to stay buried, or decompose at the surface. In RCNT plots there were only buried bags, as roller crimping does not disturb soil. The aim of these bags was to capture the trajectories of mass loss of the cover crop litter from decomposition, and what influences the litter had on soil and soil microbial communities. Litter/root bags were collected at certain stages of corn growth, to observe the rate of decomposition for the biomass in the bags throughout the growing season. Litterbags were first collected at the V6 stage (20 days after deployment), where the corn plants have 6 true leaves. The second collection was at the V12 stage, with 12 true leaves. In between the two stages should be when the corn has the maximum amount of nitrogen uptake, as it gets ready to produce corn. The third litterbag collection occurred when the corn tasseled, and the last collection was at corn harvest. Root bags were only collected during the last two collections of litterbags, at corn tasseling and harvest anticipating slower root decomposition dynamics than the litter.

At each collection, a random bag from within each plot was chosen to be collected through a random number generator, to pick out 1 of 4 bags in each subplot. The litter and root bags were then located, cleaned of weeds and soil covering the bag, and placed into Ziploc bags for transport back to the lab. Soil samples were then collected from the area under the bag to a 10cm depth. Temperature was also recorded at each subplot using a soil thermometer. Upon returning to the lab, litter and root bags were placed into paper bags to dry. Meanwhile, soil samples were sieved with a 2mm fine mesh sieve and stored in a refrigerator (4°C) until further analysis.

2.2 Soil Analyses

2.2.1 Gravimetric Moisture, Water Holding Capacity, pH

To determine field moisture conditions for each soil sample, about 5g of each sample was added to a pre-weighed foil tray and weighed. Each soil sample was done with duplicates. These were then dried at 105°C for 24 hours and reweighed (Kramer TD. et al., 2012). For water holding capacity, Whatman #1 (150 mm dia.) filters were folded and placed into funnels. About 5g of soil samples were then added to each funnel, and wet with distilled water. After draining for 2 hours, the soil samples were removed, added to pre-weighed foil trays, weighed, and also dried at 105°C for 24 hours and reweighed after (Bradford MA. et al., 2008a). Soil moisture and water holding capacity were then calculated using the dry soil weight, wet weight, and foil tray weights.

Soil pH was also measured using a 1:1 ratio of soil to distilled water (Allen SE., 1989). After 10 minutes, soil pH was measured using a Fischer Scientific pH probe, with calibration standards done every 16 samples.

2.2.2 Net Nitrogen Mineralization and Nitrification

28-day laboratory incubations were performed to determine the nitrogen mineralization and nitrification rate of each soil sample. Published protocols were adapted and followed for this assay (Robertson GP. et al., 1999; Fraterrigo JM. et al., 2005). There were two subsets of samples, a day 0, and a day 28 of the assay. Both used 10 grams of dry weight soil calculated from the soil gravimetric moisture. Each sample was weighed into pre-weighed plastic cups. For the day 0 subset, each cup was then immediately mixed with 50 mL of 2M KCl solution. The samples were then capped and shaken to thoroughly mix the soil and KCl solution. After that, the samples were stored in a refrigerator overnight. The day 28 samples were instead covered in plastic wrap, secured with a rubber band, and set in a room-temperature incubator for 28 days. During those 28 days, moisture of each sample was adjusted to account for any water loss. After that time period, these samples then received the 50 mL of 2M KCl, capped, shaken, and stored in a refrigerator overnight just like the day 0 subset. The day after adding 50 mL of 2M KCl, samples were then filtered using Whatman 1 filter paper, with their extracts collected in 20 mL plastic scint vials. These vials were then frozen until analyzed.

Frozen samples were analyzed using colorimetric nitrification and nitrogen mineralization assays by determining nitrate (NO3) and ammonium (NH4) concentrations per sample. This procedure was adapted from the Hobbie Lab (University of Minnesota) Inorganic Assays in Microplates Protocol (updated 7/4/13 by C. Riggs). Samples were first taken out of the freezer to thaw out. Next, 1 mL of each sample was pipetted into a deep-well 96 well plate, creating a sample plate. A deep-well plate of 0-8

ppm NH4 and NO3 standards was also created. These standards were created using a serial dilution of a 3.608 g potassium nitrate (KNO3) and 2.359 g ammonium sulfate ((NH4)2SO4) stock solution.

The nitrate analysis was adapted from (Doane TA, HorwathWR, 2003) using a vanadium reagent. Using a multi-channel pipette, 100µL of standards were pipetted into specific columns of analytical 96 well plates. In the other remaining columns, 100 µL of samples were pipetted. Each column then had 100 µL of vanadium reagent added. Upon adding the reagent to every column of a plate, time was recorded, and the plate was then placed and stored in the dark for at least 5 hours.

The ammonium analysis used the Salicylate Method, a variation of the Phenate method, with the advantage of being free from mercury salts and phenol. Similar to the nitrate analysis, a multi-channel pipette was used to pipette 80 µL of standards into specific columns of analytical 96 well plates. 80 µL of samples were pipetted into the remaining empty wells. 60 µL of a salicylate solution and 60 µL of a sodium hypochlorite solution was then added to each well. Upon completion, time was recorded, and the plate was stored in the dark for at least 50 minutes.

After the dark incubation period, nitrate and ammonium plates were measured on an Agilent Synergy HTX Multi-Mode Plate Reader at 540 nm and 650 nm respectively. Concentrations of NO3 and NH4 were then calculated using the dry soil weight for the 50

mL KCl extract solution. This determined how concentrations of NO3 and NH4 changed over the 28-day period.

2.2.3 Carbon Mineralization

A 30-day mineralization assay was performed using each soil sample to determine labile carbon, or readily available carbon following methods described in Fierer and others (2005) and Bradford and others (2008b). Five grams dry weight of each soil sample was weighed into pre-weighed 50 mL centrifuge tubes. After weighing out all soil samples, the samples were then placed in an incubator at 20°C for 24 hours. On the next day, the centrifuge tubes were sorted into rows of four, and capped using modified centrifuge caps that could accommodate a septum and O-ring. Outlet needles were then inserted into the tubes through the septa, beginning with the first four samples. Needles connected to a CO2-free air tank were then inserted into those tubes as inlet needles. The samples were then flushed with CO2-free air for three minutes. After three minutes, the outlet needle was removed, swiftly followed by the inlet needle. The outlet and inlet needles were then switched to the next row of four samples to be flushed with CO2-free air for three minutes. This was repeated until all samples had been flushed with CO2-free air. The samples were then placed in the same incubator for 24 more hours. After the 24 hours, samples were removed from the incubator to be measured by a LiCOR Li-7000 infrared gas analyzer for CO2 respiration. A syringe with a hypodermic needle was used to collect air. For each sample, the syringe was flushed with CO2-free air, and then filled with 5 mL of CO2-free air. The needle was then inserted into the sample tube, and the air was mixed into the sample to ensure an even distribution. Then, 5 mL of air from the sample was collected and injected into the LiCor gas analyzer to determine the integral area of

the curve. Standards with a known concentration of CO2 were also measured during this sampling period, at the beginning and end of the sampling period, as well as every 16 soil samples. After finishing the sampling, each tube was uncapped and placed into the incubator until the next flushing and sampling days. The moisture content of the soils was also adjusted weekly to account for water loss. During the 30-day assay, samples were flushed on days 1, 4, 8, 14, 21, and 29. They would then be sampled the following day, on days 2, 5, 9, 15, 22, and 30.

Total labile carbon was determined by calculating headspace CO2 for each tube from the integral area of each sample, as well as the average integral area of the standards that were measured compared to the known CO2 ppm of the standard. The calculated headspace was then used to calculate µg of CO2-C. This was then divided by the dry soil mass for each tube to calculate the contribution of CO2-C from soil. This number was then divided by the incubation time (hours), resulting in CO2-C µg dry weight soil-1 hr-1. These calculations were done for each sampling date, and then integrated across the 30 days, producing carbon mineralization data over the 30 days.

2.2.4 Substrate Induced Respiration

Active microbial biomass was measured using this assay following published methods (West AW., Sparling GP., 1986; Bradford MA., et al., 2008b). This assay is very similar to the 30-day carbon mineralization assay. Each soil sample had 4 g of dry weight soil measured into pre-weighed centrifuge tubes. These tubes then sat in an incubator at 20°C to pre-incubate overnight. The following day, 4 mL of a bacto-yeast solution was pipetted into each centrifuge tube. Then, the samples were incubated at 20°C uncapped on a lab

horizontal shaker for one hour. Directly after, samples were then removed from the shaker and capped using modified centrifuge caps that could accommodate a septum and O-ring. They were then flushed in the same procedure as the carbon mineralization assay, for three minutes each sample. However, the incubation period after flushing was four hours rather than 24 hours. Just like the carbon mineralization assay, CO2-C µg dry weight soil-1 hr-1 was then calculated for active microbial biomass.

2.2.5 Total Microbial Biomass

Direct soil fumigations using chloroform were conducted to determine extractable total microbial biomass. We followed methods described by Fierer and Schimel (2003). Each assay had two subsets. A control/non-fumigated subset, and a fumigated subset. Both subsets used 6 g of dry weight soil, weighed into 70 mL glass tubes. Each soil sample had duplicates in each subset. There were also four blanks run per subset. After weighing out the soil samples, 40 mL of 0.5 M K2SO4 was added to the control/non-fumigated tubes. They were then promptly capped and shaken on a lab horizontal shaker for two hours. After shaking, the samples were taken off the shaker, and each tube was shaken by hand to resuspend soil that may have stuck to the upper parts of the tube. They were then allowed to sit so that soil could settle to the bottom of the solution. Lastly, each solution was then filtered using Whatman No. 42 filter paper into 20 mL plastic scint vials. These vials were then frozen until further analysis.

The fumigated soil subset had one mL of chloroform added to each sample tube. They were then capped and mixed thoroughly using a vortex mixer. The capped tubes were then covered for light exposure for at least 36 hours before being uncapped and

aired out for two additional days. Afterwards, samples were placed into a desiccator and vacuum pumped to ensure that any remaining chloroform had disappeared. The fumigated subset then underwent the same procedure as the non-fumigated subset, starting with the addition of the 40 mL of 0.5 M K_2SO_4.

Frozen samples were thawed and then diluted using distilled water (DI) at a 1:10; sample: DI ratio in disposable glass culture tubes. These samples were then run on a Shimadzu TOC-L CPH analyzer for total organic carbon (TOC) and total nitrogen (TN) analysis. TOC/TN standards were created using 3.822 g NH_4Cl and 2 mL H_3PO_4 combined in a 500 mL volumetric flask. It was then brought to volume using nanopore de-ionized water to create a 1000 ppm carbon and nitrogen standard. The standards were then diluted with K_2SO_4. Microbial biomass C then calculated using the TOC readings by subtracting the average TOC per unit soil (mg C gdw soil-1) of fumigated samples by the non-fumigated samples.

2.2.6 Bulk Soil Measures

A select soil collection was chosen for a loss on ignition assay, to determine the percent soil organic matter content. This procedure was based on a protocol from Cornell University (Moebius-Clune B.N., et al., 2016). First, 5 g of soil was weighed into pre-weighed crucibles. They were then placed into an oven at 105°C to remove all water content. After 24 hours, the samples were removed from the oven and placed in a desiccator. The desiccator was vacuum sealed so that no moisture in the air could be absorbed by the dry soil samples while they cooled down. The cool samples were then reweighed to determine a dry starting mass. After that, the crucibles were placed into a

furnace at 500°C for two hours so that the carbonaceous materials would burn off. Once the two hours were complete, crucibles were removed from the furnace and placed directly into a desiccator as before, and vacuum sealed as they cooled. The cooled crucibles were then reweighed for their change in weight. The percent of mass remaining was calculated using the soil weights before and after exposure to high temperatures.

Soils were chosen to be analyzed for their carbon to nitrogen content. These soils were first ball-milled using a SPEX Sample Prep 8000D Mixer/Mill. The milled samples were then weighed into 9 x 5 mm tin capsules. Around 50-60 mg of milled soil was weighed into each capsule. The capsules were then carefully folded and packaged into an analytical 96-well plate and sent to the Center for Applied Isotope Studies Stable Isotope Ecology Laboratory at the University of Georgia to be analyzed for carbon and nitrogen content.

2.3 Litter/Root bag Analyses

2.3.1 Mass Loss and Ash-Free Dry Mass

Collected litter and root bags stored in paper bags to dry in the lab were used to calculate mass loss over time. Bags were removed from the paper bags and cleaned of excess soil and weeds that had stuck to the outside of the bag. The bags were then opened, and the litter/roots inside were poured into a tin tray. The litter/roots were then slowly picked through and cleaned of soil. The cleaned sample was then relocated to another pre-weighed tin tray and the weight of the sample was recorded. This was repeated for all litter/root samples.

Litter and root samples were then milled in a Spex Sample Prep 8000D Mixer/Mill into a powder. The milled samples were then stored in 20 mL glass scint vials. These samples were then used in an ash-free dry mass assay for mass loss determination. About 0.1 g of milled litter of 0.05 g root samples were added to pre-weighed crucibles. The crucibles were then placed into a muffle furnace at 500°C for four hours. After four hours, the temperature was reduced to 40°C to allow for the crucibles to cool slowly. The crucibles were then placed into a desiccator, vacuum sealed, and left in the lab overnight so that the crucible would cool down completely. Samples were reweighed the next day. Using the crucible weights before and after, the litter percent per sample could be calculated, as it would be the portion of the sample that had burned off. These numbers were then used to adjust the weights recorded for each litter/root bag collected for more accurate mass loss calculations.

2.4 Statistical Analysis

All analyses were performed using R Statistical Software (v4.1.2; R Core Team 2021). Using the lme4 package (v1.1.27; Douglas Bates, Martin Maechler, Ben Bolker, Steve Walker 2015) as well as the nlme R package (v3.1.153; Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team 2021), linear mixed effects models were run to test for differences in litter mass loss across treatments. Mass loss was the response variable, while termination, nitrogen fertilization, and collection date were explanatory variables. Additional linear models were run where the explanatory variables were allowed to interact (2-way interactions only). Block was used as a random effect to account for spatial heterogeneity. The linear mixed effect model structure was also used to test for differences in labile carbon, active microbial biomass, pH, nitrogen mineralization,

nitrification, and C:N ratios according to termination, nitrogen fertilization, and collection date.

After all the models had been made, Analysis of Variance (ANOVA) was run on the lme model to compare variances across the means of each group of variables. The emmeans R package (v1.7.3.; Russell V. Lenth 2022) was also used to look at pairwise comparisons between treatments. The p-values (<0.05) obtained from using both ANOVA and the emmeans function allowed for selection of significant variables as explanations for the data collected. Final graphs and figures were then created based upon these relationships.

CHAPTER 3

RESULTS

3.1 Litterbag Mass-Loss

Looking at the mass loss data in Figures 2 and 3, we observed mass loss of litter over time. All four termination treatments in Figure 2 followed a similar, increasing trend in mass loss throughout the season. Between the first and second litterbag collection, there was a large increase in mass loss. This mass loss slowed down in the later collections to between 80-85% mass loss. Notably, at the first three collections, RCNT has greater mass loss than CDT (Figures 2, 3B) when the corn is in high demand for N resources. At the final collection (harvest), CDT has the greatest mass loss. At the two later dates, both HNT and RCNT had very close data points for mass loss and can be seen to have almost the same values. Eventually, both tillage treatments, CDT and HDT ended with slightly higher mass loss than litterbags in the no-till plots. This shows that termination treatment has an effect on the rate of decomposition for cover crop litter throughout the growing season. A history of N fertilization regime also impacts mass loss. The no fertilizer treatment initially has the faster mass loss. Once fertilizer is applied (between 6/29 and 7/16), the plots with a history of external N inputs, increase in mass loss as compared to the zero-fertilization treatment (Figure 3A). Figure 3 also shows that even though there were four different fertilization amounts and four termination treatments, the main driver behind the mass loss of each litter bag was time (P value <0.0001).

3.2 Labile Carbon and Active Microbial Biomass

Figure 4 shows labile carbon and active microbial biomass for soils collected from under litterbags. Labile carbon in soil slowly increased in the first three collection periods but

dropped at the last collection. There was a significant interaction ($p = 0.0506$) between termination and date on labile C which seems to be driven by the highest labile C in the RCNT treatment across the first three collection dates, but the lowest at the final collection. This may be linked to increased interactions and in the undisturbed root zones across the active growing season, and a drop in root-microbe interactions at harvest.

Active microbial biomass showed an entirely different perspective though. Active microbial biomass had significant differences due to N fertilization treatments as well as date of collection. Both variables returned a p-value of <0.0001. Figure 4B shows how drastically active microbes were in unfertilized soil plots at the first collection. However, this large difference is not seen in later collections. Plots treated with 100% of the recommended fertilizer amount tended to keep a higher amount of active microbial biomass throughout the season. This was followed closely by the 67% fertilizer treatment as well. 0% and 33% fertilizer treatments appeared to only have a large difference in active microbes in the first collection, and a small difference in the last collection. The middle two collections of soil on 7/16 and 8/04 had around the same active microbial biomass for 0% and 33% N fertilizer.

3.3 Net Nitrogen Mineralization and Nitrification

Figure 5 shows that both nitrogen mineralization and nitrification followed the same pattern throughout each soil collection. An interaction between N fertilization and collection date drove patterns in the data ($p <0.0001$). Nitrogen mineralization and nitrification rates slightly increased or decreased throughout the growing season for fertilization treatments of 0, 33, and 67% fertilizer. For the 100% fertilizer treatment,

nitrogen mineralization and nitrification rates slowly increased through the season. Notably, before fertilization, the 0% treatment has the greatest rates of N mineralization and nitrification. Once fertilized, rates are equal among N fertilization treatments.

In addition, plots terminated by RCNT appeared to have a very broad range of values, as can be seen by the long tail of the boxplot (Figure 6). Given, the large spread in values, we investigated the role of N fertilization on N mineralization within the RCNT plots only (Figure 7). When looking only at RCNT termination, plots with 0% N fertilizer start with high amounts of nitrogen mineralization but decrease between the second and third collection periods indicating an early advantage (prior to the V6 fertilization) of RCNT over other termination methods in promoting N mineralization. N mineralization again increases at the final collection. 33% and 100% N fertilizer, however, increase nitrogen mineralization throughout the growing season, ending with the highest amount in the last collection. 0% and 100% N fertilizer appears to swap places between highest and lowest for nitrogen mineralization over time. 67% N fertilization increased nitrogen mineralization between the first and second collection but decreases afterwards for the rest of the season.

3.4 Microbial Biomass Carbon, TOC, and TN

Figure 8 shows total microbial biomass (as microbial biomass carbon) for the second collection of litterbags, and the final fourth collection. There is a significant drop in microbial biomass between those dates ($p = 1.798e^{-08}$). There was no significant impact of nitrogen fertilization and termination method on microbial biomass.

TOC and TN data showed no significance between fertilization, termination method, as well as date of collection. TN data produced low p-values when date interacted with N fertilization or termination methods, but because of the small, uneven sample size, the data was not conclusive.

3.5 SOM

Figure 9 shows SOM for litter bag soils collection at the last collection on 8/27. There was no statistical significance between termination treatment and SOM. However, HDT terminated plots showed that they had the highest SOM (Avg: 3.97%) followed by RCNT (3.95%), CDT (3.86%), and then HNT termination (3.83%). HDT had the largest spread in data points for SOM compared to the other three termination treatments.

3.6 Root bag Results

Figure 10 shows mass loss data in parts A and C. Mass loss increased over the two collection dates. When separated into the depth at which the root bags were placed, surface root bags appeared to lose mass at a slightly lower rate than buried bags. Within buried bags, those that were placed in RCNT terminated plots had less/slower mass loss than CDT termination methods.

Part B shows labile carbon for soils collected under the root bags. Surface root bags had the highest labile C, followed by buried bags in RCNT terminated plots, and then buried bags in CDT plots. Part D shows active microbial biomass for the root soils. Surface soils had the most active microbial biomass. Soils from buried root bags had around the same amount of active biomass.

Root bag soils also had the carbon to nitrogen ratio of the soils analyzed. Figure 11 shows that soils with higher fertilization resulted in lower C:N ratios. The differences in the ratios were more pronounced between nitrogen fertilization treatments for the soils in the last collection.

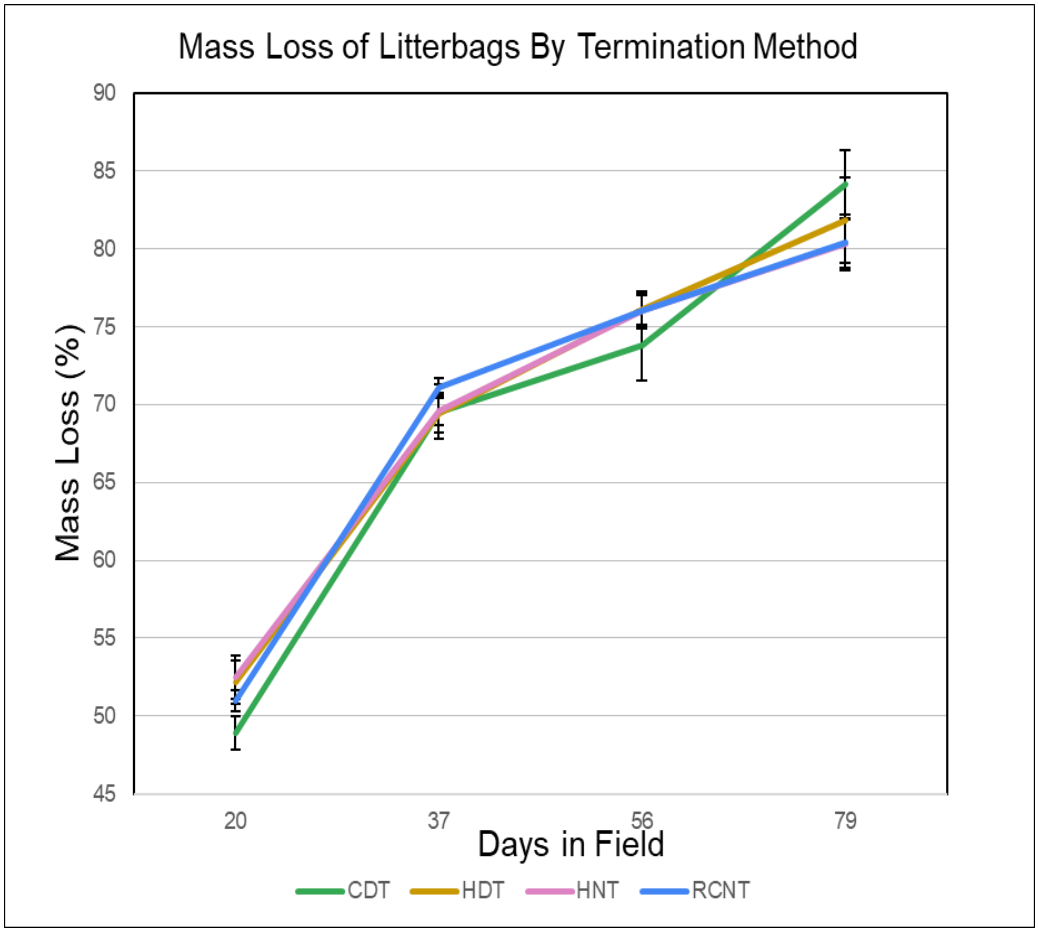

Figure 2: Mass loss of litter bags over time in the field, grouped by the four termination methods, averaged across N fertilization treatment (n=12 ± SE). Color represents each termination treatment. Mass loss is represented as a percentage loss of mass.

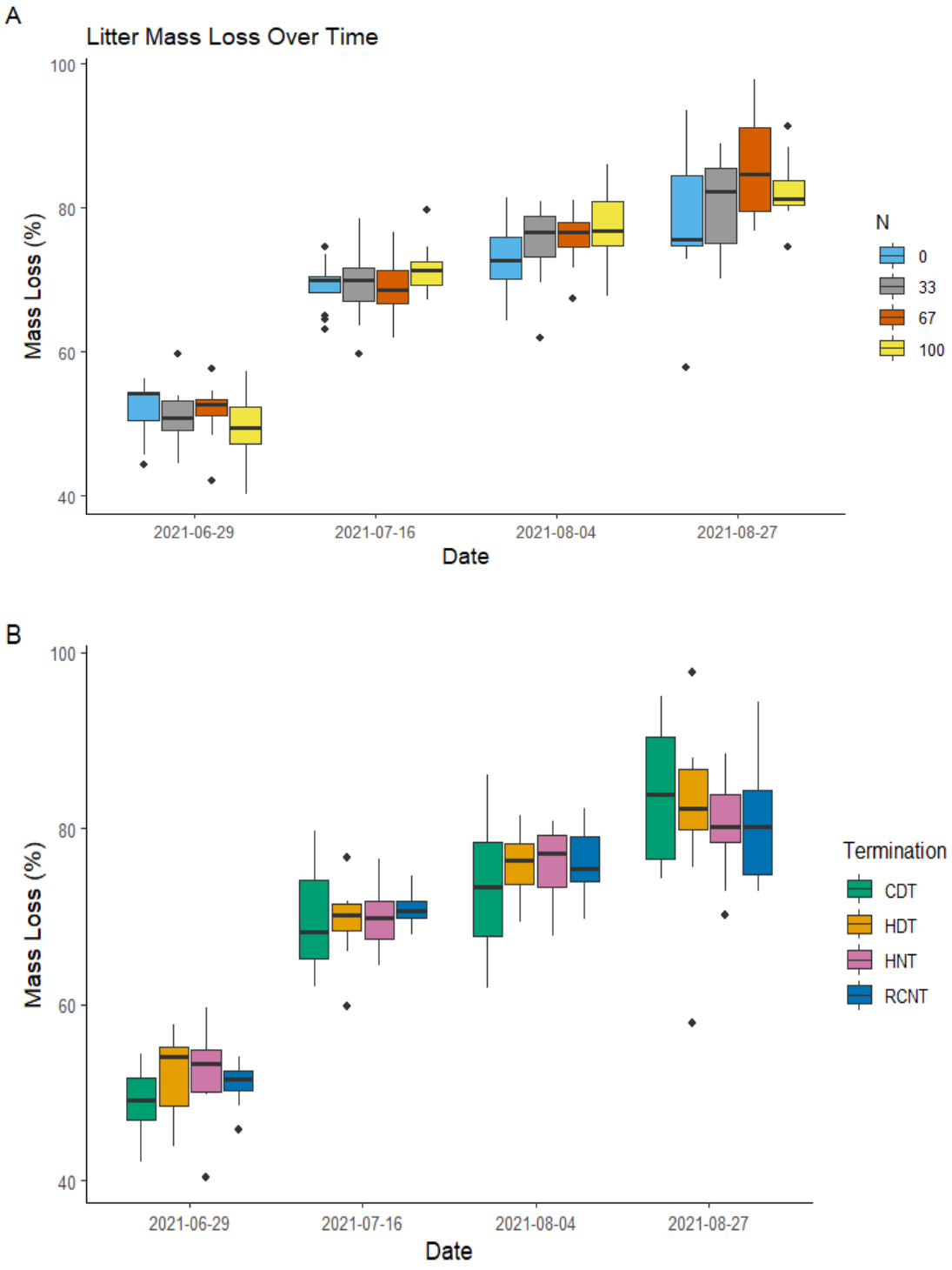

Figure 3: Mass loss of litter bags over time. Part A groups the data by fertilization. Color represents N fertilization treatment. Boxplots represent the average across termination treatment (n = 12). Part B groups mass loss by termination method. Color represents termination treatment. Boxplots represent the average across N fertilization (n = 12).

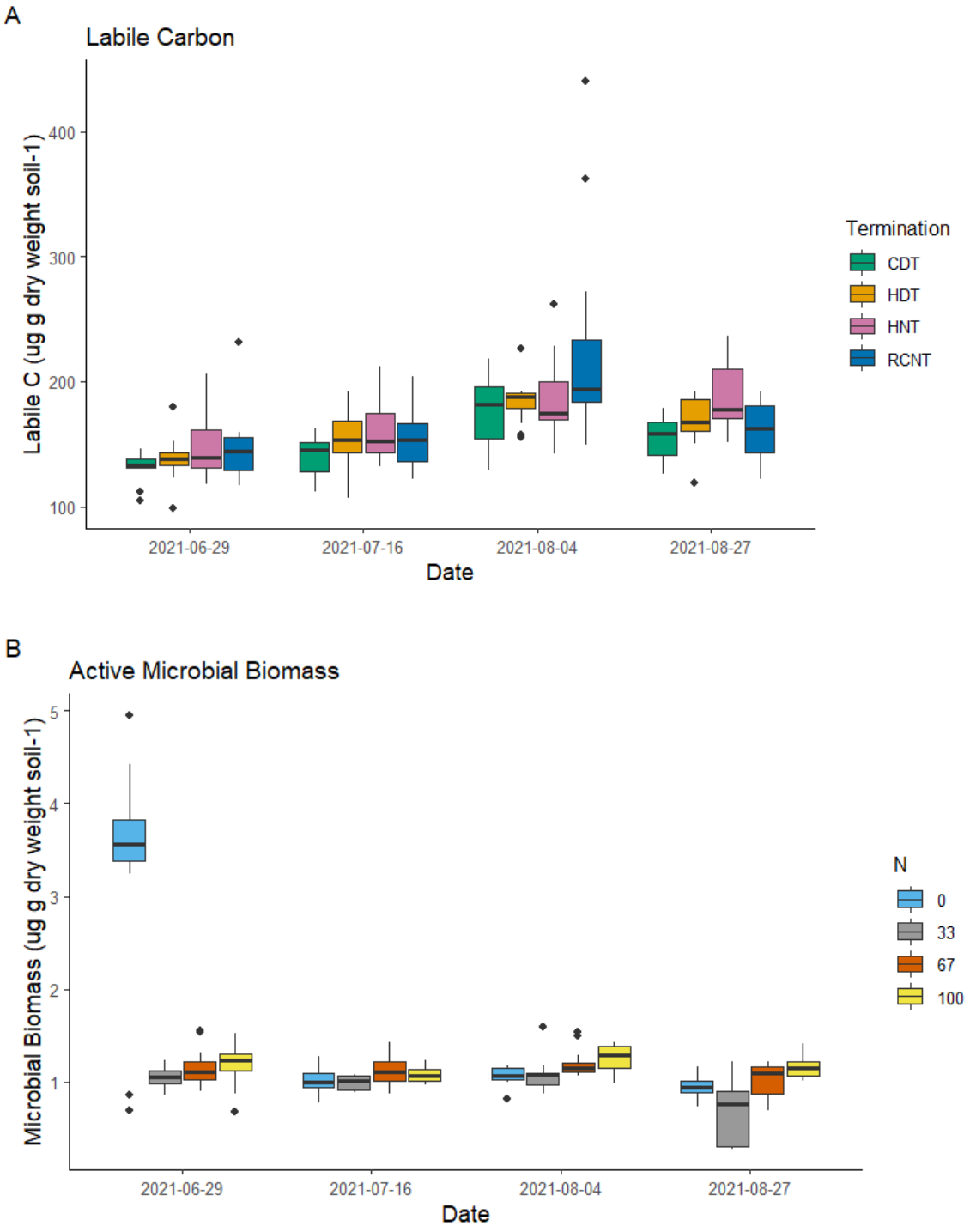

Figure 4: Part A shows total labile C (readily available C) on soils from under litterbags from the carbon mineralization assay. Color represents termination treatment. Box plots represent the average across N fertilization (n = 12) Part B shows active microbial biomass from the litter bag soils based on the substrate induced respiration assay. Color represents N fertilization treatment. Box plots represent the average across termination treatments (n = 12).

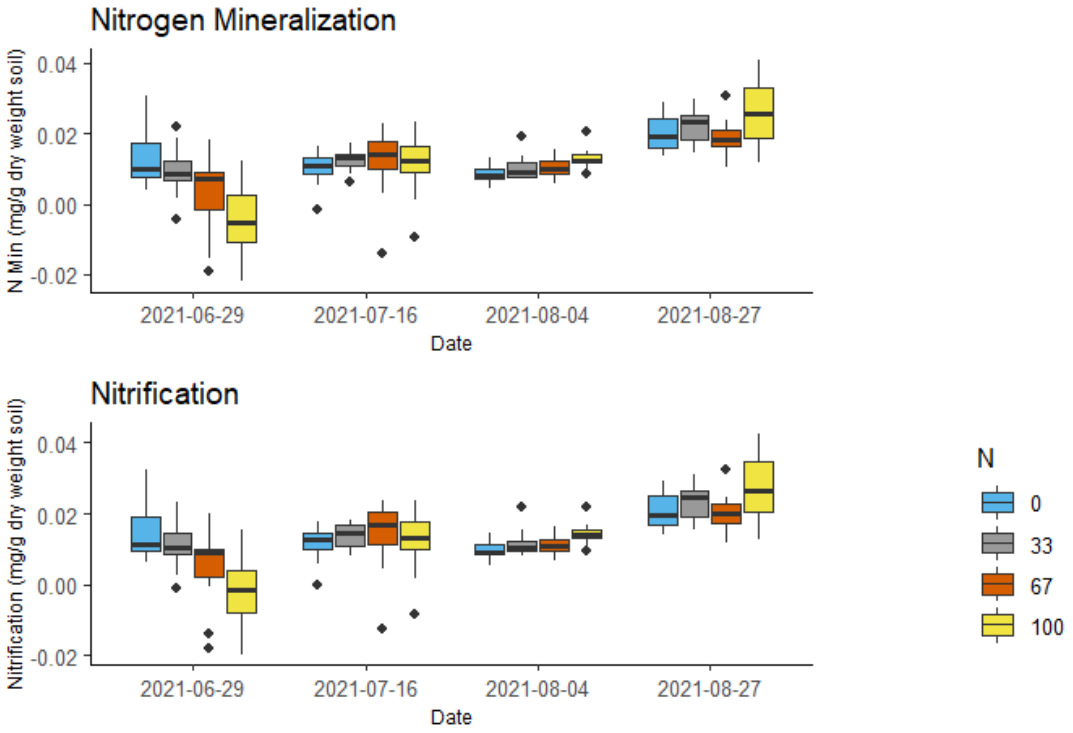

Figure 5: Nitrogen mineralization and nitrification of litter bag soils. Color represents the N fertilization treatment. Box plots represent the average across termination treatments (n = 12).

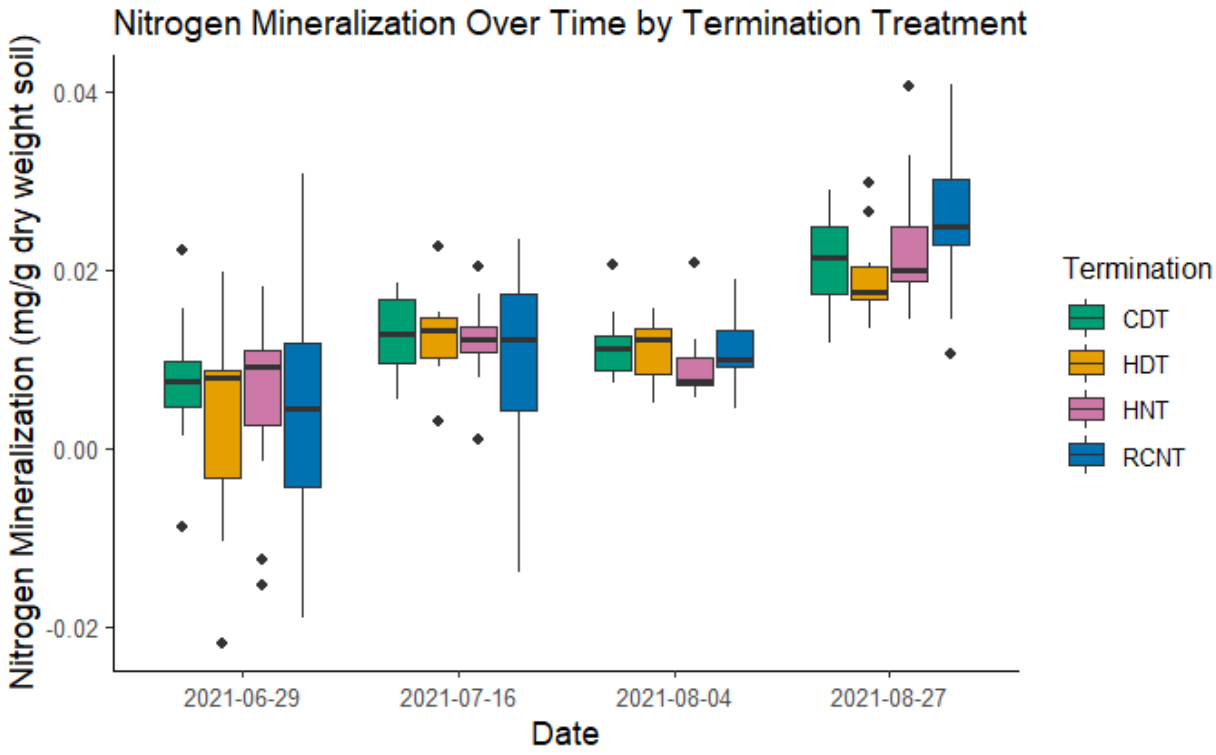

Figure 6: Nitrogen Mineralization over time for litter bag soils. Color represents termination treatment. Box plots represent the average across N fertilization treatment (n = 12).

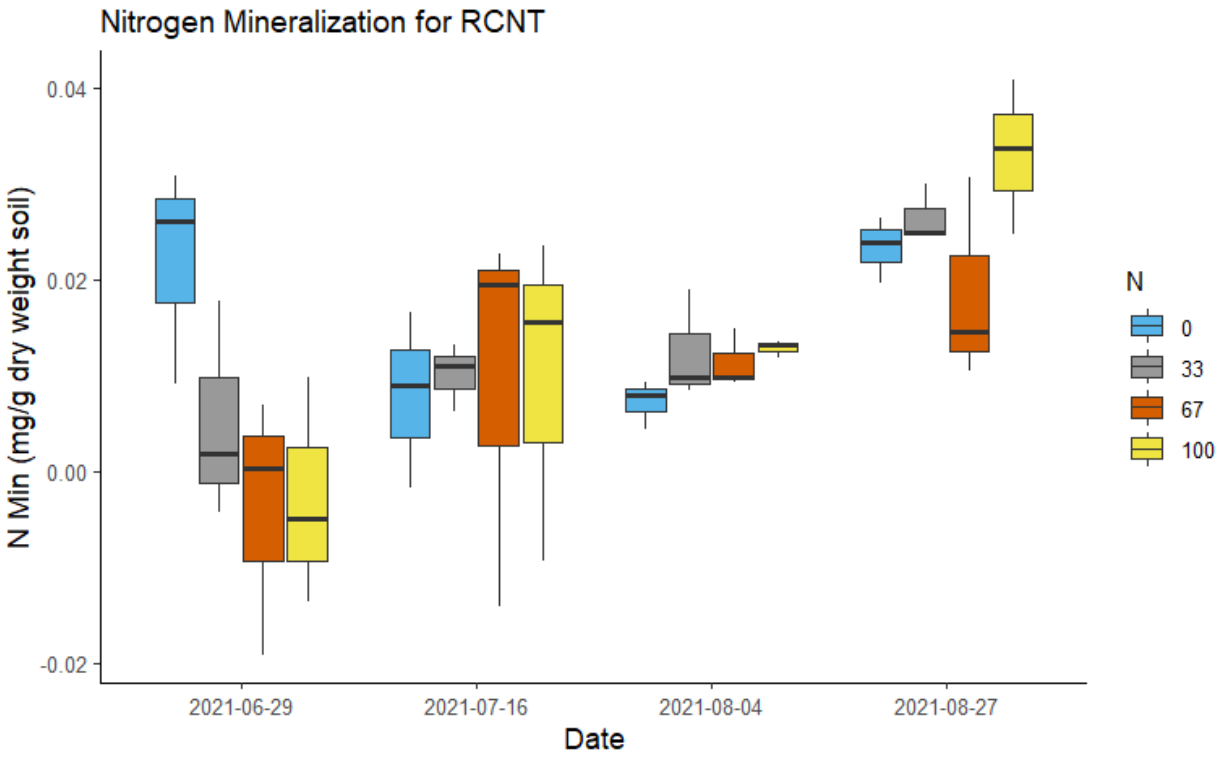

Figure 7: Nitrogen mineralization specifically for the RCNT termination method over time. Color represents N fertilization treatment. Box plots represent the average across termination treatment (n = 12).

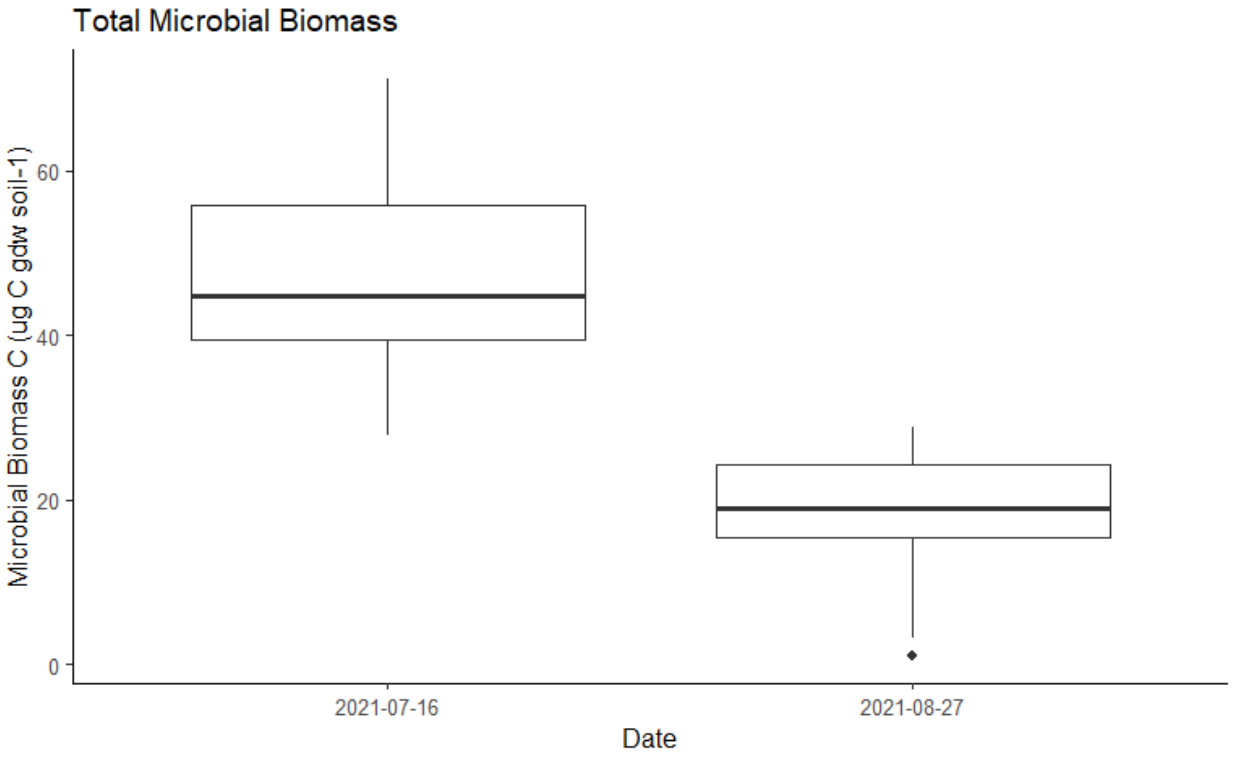

Figure 8: Microbial biomass in litter bag soils for the second and fourth collection dates. Box plots represent an average across both termination and fertilizer treatment (n = 36).

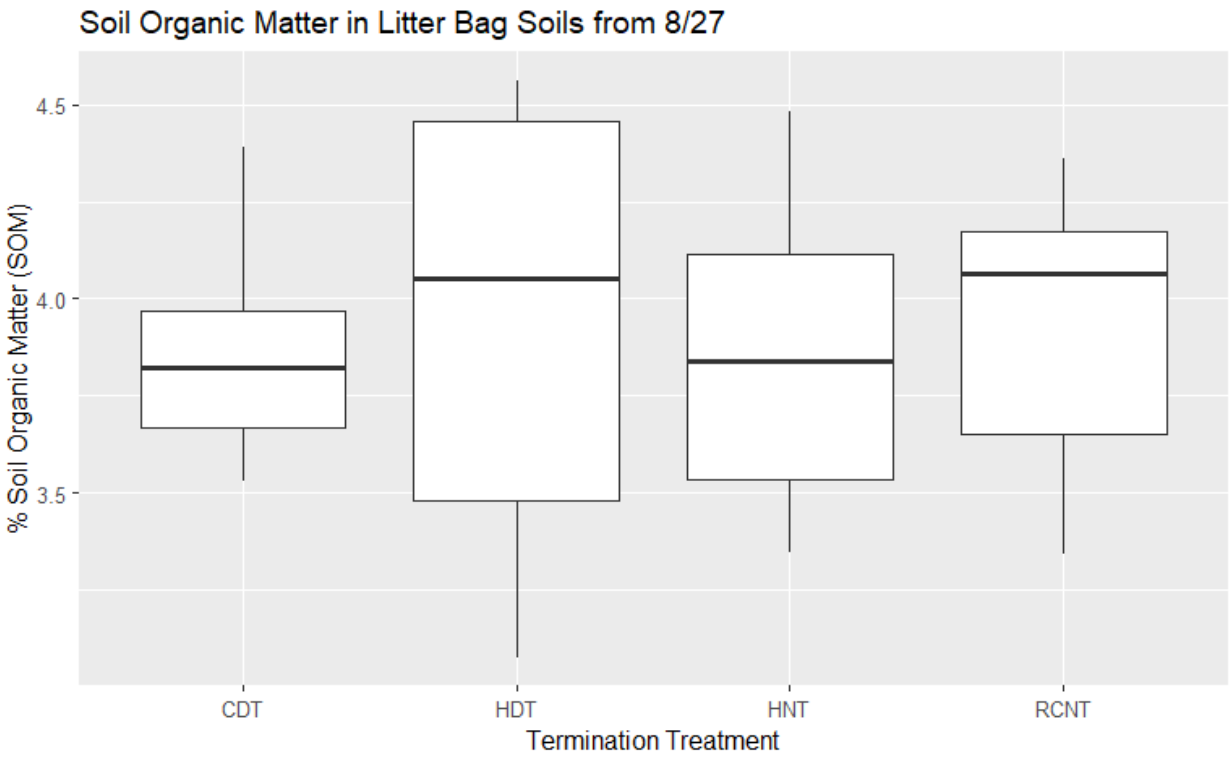

Figure 9: Soil organic matter (SOM) in soils from the last collection of litter bags (8/27). Boxplots represent averages across N fertilization treatments (n = 12).

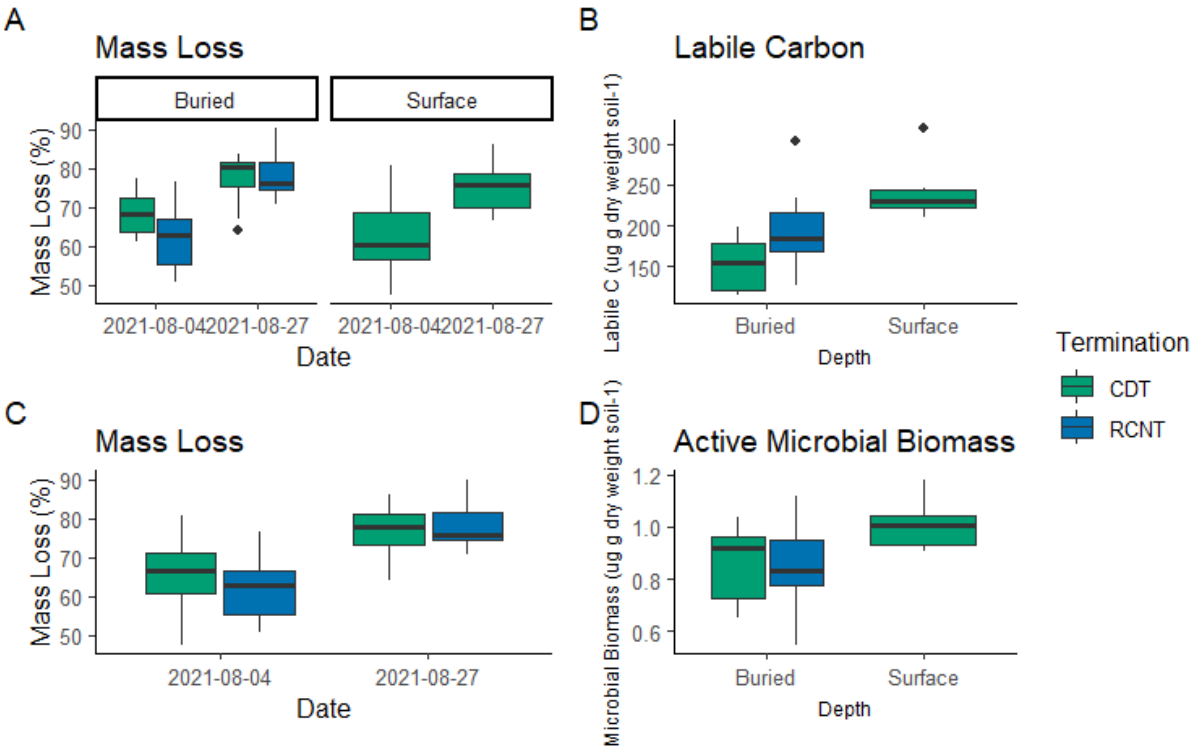

Figure 10: Root bag data sorted into four graphs. Color represents termination treatment. Part A shows mass loss of root bags over time. Box plots represent an average across N fertilization treatment between buried and surface root bags (n = 12). Part C also shows mass loss of root bags over time. Box plots represent an average across N fertilization treatment as well as depth of root bag placement (n = 36). Part B shows carbon mineralization of root bag soils by depth of placement. Box plots represent an average across N fertilization treatment (n = 12). Part D shows active microbial biomass for root bag soils by depth. Box plots represent an average across N fertilization treatment (n = 12).

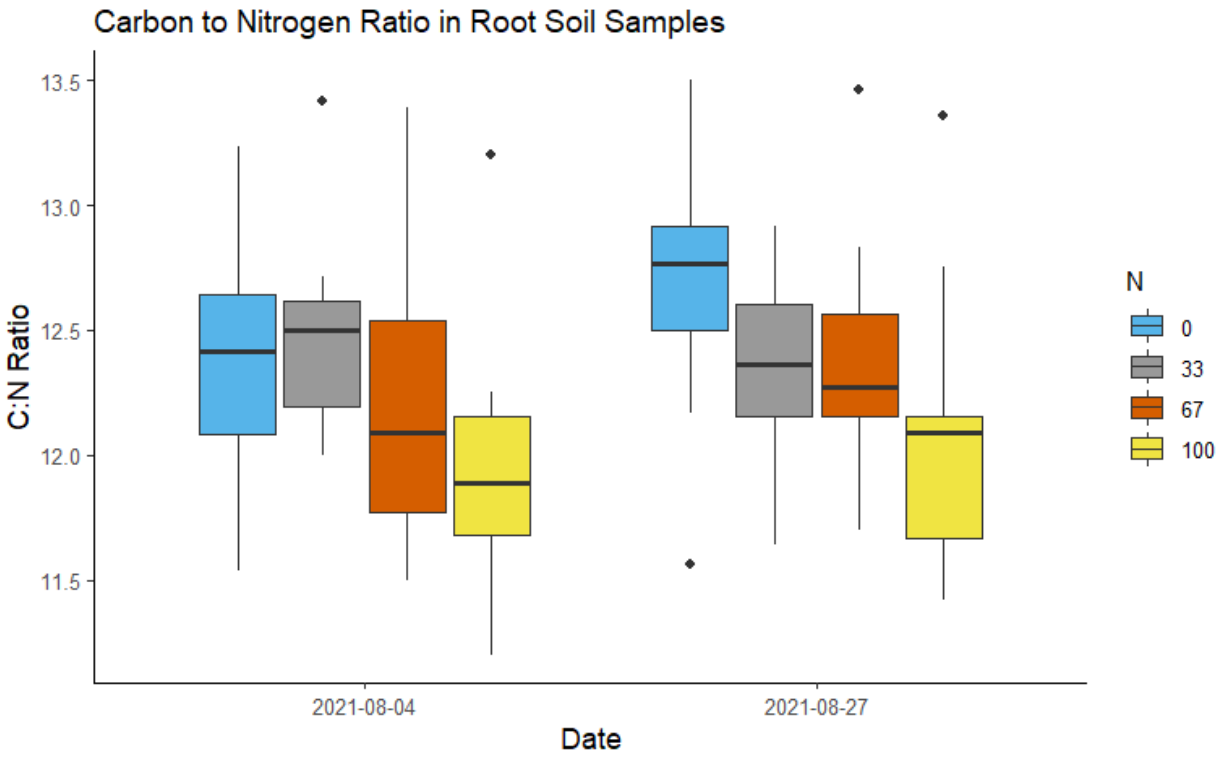

Figure 11: Shows the carbon to nitrogen ratio in root bag soils. Color represents N fertilization treatment. Box plots represent an average across termination treatment (n = 12).

CHAPTER 4

DISCUSSION

This study quantified the impacts of cover crop management – through cover crop termination, weed control methods and N fertilization regime – on soil biological indicators that can be incorporated into soil health tests. These biological soil measures included the decomposition of cover crop above and belowground biomass, soil C and N pools associated with potential microbial activity, such as readily available C and N mineralization, and the size of the microbial community itself. We tested whether termination treatment or fertilizer addition would be positively correlated with soil labile carbon pools (Hyp. 1A & 2A), or nitrogen turnover (Hyp. 1B & 2B). The data gathered supported the H1A hypothesis that roller crimping would result in larger soil labile carbon pools compared to disk tillage or herbicide termination. Termination treatment of cover crops significantly impacted the amount of labile carbon in soil. Significant differences mainly occurred between the earlier two collections of litter bags and the final two collections. In the third collection (8/4/21), RCNT soils had significantly more labile carbon than the tilled terminations, CDT and HDT. Labile carbon trends (Figure 4A), most likely reflect the needs of the sweet corn plants throughout the growing season. Between the first and second collection of litter bags was the V6 and V12 growth stages of corn. Between these stages, corn plants begin to take up a greater amount of nutrients (Hanway, J. J., 1966) Corn plants may regulate microbial communities by utilizing root exudates. Root exudates are a significant carbon cost to the plant and create a carbon rich environment within the rhizosphere so that microbial activity is stimulated. Our data demonstrate that microbial biomass was significantly larger in the second collection,

compared to the last collection of litterbags and soil (Figure 8). This can result in increased nutrient availability for the corn plant as microbes mineralize more nitrogen (Bais, H. P. et al., 2006). The larger microbial biomass also reflects the amount of corn root exudates in soil through the growing season, with less biomass showing a decline in exudate inputs into soil. Using this knowledge, this could explain why the amount of labile carbon increases in the beginning stages of corn growth. However, in the last collection of litterbags and soil (8-27-2021), labile carbon shows decrease throughout all termination treatments. This is because the last collection was also when the corn was ready to be harvested. As the growing season ended, there was no need for the corn plant to excrete more root exudates, and so labile carbon trends decreased. Even though termination was not statistically significant in explaining labile carbon trends, it is important to observe that roller crimping did have an effect on labile carbon (Figure 4A). When the corn plants demanded for more nutrients, plots terminated by roller crimping had the most labile carbon in soil compared to the other three termination methods.

The decomposition trajectory of the aboveground cover crop biomass varied with time and nitrogen fertilizer treatment (Figure 3A), as expected. There was a large jump in mass loss between the first two collections (6/29 and 7/16). This may be due to the biochemical composition of legume cover crops, and so in this case, our pea cover crop. Legume cover crops have been researched to have a low C:N ratio (USDA NRCS, n.d.). This allows for rapid decomposition of legume litter and fast release of nitrogen into the soil (Buchanan and King, 1993; Jani, A. D. et al. 2016; Sainju et al., 2005). The large jump may also be due to microbial activity responding to fertilizer addition after 6/29. Plots that had been fertilized in the past increase in litter bag mass loss compared to the

0% N fertilization treatment after fertilizer inputs, showing that microbial activity responds to fertilization rates. The rapid decomposition shown in Figures 2 and 3 between the first and second collection dates, followed by a more gradual decomposition rate afterwards may be due to the slower, prolonged decomposition of high C:N rye litter. Termination also shows impacts on decomposition of cover crop litter. RCNT terminated plots had the highest decomposition rate between the first two collections, while CDT had the lowest rate (Figure 2). Both treatments that used herbicides (HDT and HNT) had around the same decomposition rate. This shows that undisturbed soils with no-tillage or herbicide application could have an impact on decomposition rates compared to ones that have been disrupted by tillage.

The other hypothesis that this data supported was the Hyp. 2B, that soils with less fertilization would have lower nitrogen mineralization and nitrification rates compared to those with higher fertilization. Nitrogen mineralization and nitrification rates had almost perfectly identical trends throughout the growing season. The date of collection as well as fertilizer treatment were significant in explaining these responses ($p < 0.0001$). These graphs in Figure 5 can also be explained by corn growth and development. As mentioned before with labile carbon, corn plants excrete root exudates into soil in order to feed microbial communities so they may mineralize more nitrogen for the corn. At the first collection, mineralization and nitrification rates varied between each fertilization treatment. Plots with less fertilizer had higher rates of nitrogen mineralization and nitrification. At this time point, only one third of the fertilizer for the season had been added as a starter fertilizer. Therefore, plots with less fertilization would require more

microbial activity to mineralize nitrogen, as it is not readily available to them in the soil. Right after the first collection, the other two thirds of the fertilizer was added. Corn also would then release increased root exudates to stimulate microbial activity. Figure 5 then shows that plots with higher fertilization rates start to have more nitrogen mineralization and nitrification than those with lower fertilization, observing that microbial activity yet again, responds to fertilization. As corn has a high nitrogen demand, it is not shocking to see these rates increase. However, these rates still went up even at the last collection on 8-27-2021, when the corn was harvested. At this stage, nutrient demand from the corn would have tapered off, as corn stops growing and increasing in grain weight (Hanway, J. J., 1966). The explanation as to why nitrogen mineralization and nitrification rates may have still increased may be due to decomposition of the corn plant underground. More information and research are needed in order to conclude when corn plants start to decompose, and what the chemical composition of the roots are like, to see if they are easily decomposed by microbes.

Termination also appears to play an important role in nitrogen mineralization, and thus the recycling of N from cover crop by available soil pools. The RCNT terminated plots had a large spread of data points in every collection (Figure 6). Because of this spread, the RCNT treatment performs equally or better than CDT across the growing season. With that in mind, RCNT termination methods could be used instead of CDT methods to preserve soil health and increase SOM in soil. In Figure 7, within the first collection, there was the same trend as seen before in Figure 5, where RCNT plots with lower fertilization had higher nitrogen mineralization rates. After fertilizer was added,

RCNT plots with higher amounts of fertilizer also had a higher average mineralization rate, but this time, there was a very large spread in mineralization for plots with 67% and 100% fertilization. Currently, the data is inconclusive as to why these large spreads for RCNT terminated plots happened. The average mineralization then decreases slightly in the third collection which is also peculiar, and then in the last collection, RCNT plots with 67% fertilizer have a lot less average mineralization than the other fertilizer treatments. After running pairwise comparisons on this model, as well as ANOVA, it appeared that fertilization was not significant in determining nitrogen mineralization for RCNT plots. More research is needed to explain this data shown.

The data gathered from our root study demonstrates that termination of cover crop by RCNT could have a positive impact on microbial communities in agricultural soils. Hypothesis 1A, that RCNT plots would have a larger microbial community as well as increased labile carbon pools compared to plots utilizing disk tillage or herbicides to terminate cover crop was supported by this data. Focusing on Figure 10A, root bags that were buried in RCNT plots lost less mass than those in CDT plots. However, 10B shows that those soils gathered from RCNT plots had more labile carbon than CDT plots. The buried bags also had around the same amount of active microbial biomass, in 10D. Due to the slower decomposition of root biomass in RCNT plots, carbon can then be slowly released to feed microbial communities throughout the growing season and increase uptake of this carbon derived from cover crop. Along with this slow decomposition, soil organic matter can then slowly build up in these no-tillage plots, further increasing soil health. There were also surface bags that were placed in CDT terminated plots because disk tillage mixes the soil, and so roots could end up at the surface as well. These surface

bags had the most labile carbon, as well had the most active microbial biomass. This can be attributed to the fact that the surface of soil is more disturbed than underground. At the surface, decomposition of the roots would be influenced by precipitation, UV rays, physical fragmentation, and more. These would also increase the amount of surface area of root litter for microbial activity. Although these bags showed more labile carbon and active microbial biomass than the buried bags, to build upon soil health, a slower release of the carbon from cover crops is better, for the gradual release of carbon throughout crop growing seasons, build soil organic matter, and feed microbial communities. Figure 10 does not touch upon fertilization and its effects on soil and the microbial community. This is because after conducting statistical analyses upon the data gathered, there was no significant effect of fertilization on any of the responses.

CHAPTER 5

CONCLUSTION

The data gathered in this study shows that incorporating biological indicators into soil health metrics emphasizes the overall use of cover crops to improve soil health, and there is variation in soil metrics depending on how cover crops are terminated. By understanding how microbial activity fluctuates in response to termination strategies and fertilization, our knowledge of what happens underground grows further. This study uncovers how seasonal trends in corn growth can have a profound effect in regulating soil microbial communities by looking at the amount of labile carbon in soil, active microbial biomass, and nutrient cycling rates. Hypothesis 1A, that roller crimping would increase labile carbon pools compared to other termination methods was supported by both litterbag and root bag data. The litterbag analyses showed that while termination was not statistically significant, plots that were roller crimped responded to the needs of nutrients by corn plants most positively and resulted in the most labile carbon during corn growth. With root bags, it was shown that using RCNT termination methods, a slower release of carbon throughout the growing season can be beneficial to crop growth, allowing for increased uptake of carbon from the cover crop. Litterbag analyses also supported the hypothesis 2B, that soils with less fertilization would have lower nitrogen mineralization and nitrification rates compared to those with higher fertilization. This is important when studying cash crops that demand so much nitrogen such as corn. Looking at biological indicators also showed that microbial communities do react to fertilization, and that if plots are fertilized regularly, they may rely on it to have increased microbial activity. This data proves that cover crops have a profound impact on increasing soil health, especially

using RCNT termination to not disturb the underground communities. This termination method allows for slow release of labile carbon to feed microbes during growing seasons and allows for faster initial decomposition earlier in the growing season for nitrogen turnover from cover crop to cash crop. In future plans, a similar study that takes place over a couple of growing seasons may provide more solid evidence of effects by termination treatment and fertilizer. But our data of what occurs over one growing season gives us a glimpse of what is happening underground and will allow future research a starting point to start digging at.

APPENDIX

SUPPLEMENTARY TABLES

Carbon Mineralization – Litter Soils			
CO2 ~ Termination*Date, random = ~1|Block			
m.5	df	F-Value	P-Value
Intercept	1	2328.1961	<0.0001
Termination	3	5.2349	0.0018
Date	3	26.2131	<0.0001
Termination*Date	9	1.9297	0.0506

Table S1: ANOVA output for the best model (m.5) to explain patterns in carbon mineralization for litter soils. Model included interactions between termination treatment and the date the sample was collected. Block was added as a random effect for spatial heterogeneity.

Carbon Mineralization – Root Soils			
CO2 ~ Depth, random = ~1|Block			
m.10	df	F-Value	P-Value
Intercept	1	512.1408	<0.0001
Depth	1	10.7291	0.0033

Table S2: ANOVA output for the best model (m.10) to explain patterns in carbon mineralization for root soils. Model included depth that the root bag was buried. Block was added as a random effect for spatial heterogeneity.

SIR – Litter Soils			
CO2 ~ N*Date, random = ~1|Block			
m.18	df	F-Value	P-Value
Intercept	1	893.5461	<0.0001
N	3	26.9385	<0.0001
Date	3	40.1727	<0.0001
N*Date	9	29.7859	<0.0001

Table S3: ANOVA output for the best model (m.18) to explain patterns in active microbial biomass for litter soils. Model included interactions between nitrogen fertilizer treatment and the date the sample was collected. Block was added as a random effect for spatial heterogeneity.

SIR – Root Soils			
CO2 ~ Depth, random = ~1|Block			
m.22	df	F-Value	P-Value
Intercept	1	226.2573	<0.0001
Depth	1	5.0498	0.0345

Table S4: ANOVA output for the best model (m.22) to explain patterns in active microbial biomass for root soils. Model included depth that the root bag was buried. Block was added as a random effect for spatial heterogeneity.

Mass Loss – Litter Bags			
Mass Loss ~ Date, random = ~1|Block			
m.27	df	F-Value	P-Value
Intercept	1	13091.74	<0.0001
Date	3	298.55	<0.0001

Table S5: ANOVA output for the one of the best models (m.27) to explain patterns in mass loss for litter bags. Model included date that the bag was collected. Block was added as a random effect for spatial heterogeneity.

Mass Loss – Litter Bags			
Mass Loss ~ N*Date, random = ~1|Block			
m.30	df	F-Value	P-Value
Intercept	1	12717.12	<0.0001
N	3	2.519	0.0597
Date	3	315.32	<0.0001
N*Date	9	1.638	0.1077

Table S6: ANOVA output for the one of the best models (m.30) to explain patterns in mass loss for litter bags. Model included interactions between nitrogen fertilizer treatment and the date that the bag was collected. Block was added as a random effect for spatial heterogeneity.

Mass Loss – Root Bags			
Mass Loss ~ Date, random = ~1|Block			
m.33	df	F-Value	P-Value
Intercept	1	6328.18	<0.0001
Termination	1	49.17	<0.0001

Table S7: ANOVA output for the best model (m.33) to explain patterns in mass loss for root bags. Model included the date that the bag was collected. Block was added as a random effect for spatial heterogeneity.

Nitrogen Mineralization			
N.Min ~ N*Date, random = ~1|Block			
m.49	df	F-Value	P-Value
Intercept	1	73.51472	<0.0001
N	3	1.59753	0.1918
Date	3	52.55555	<0.0001
N*Date	9	6.29861	<0.0001

Table S8: ANOVA output for the best model (m.49) to explain patterns in nitrogen mineralization for litter soils. Model included interactions between nitrogen fertilizer

treatment and the date that the sample was collected. Block was added as a random effect for spatial heterogeneity.

Nitrification			
Nitrification ~ N*Date, random = ~1|Block			
m.55	df	F-Value	P-Value
Intercept	1	98.99519	<0.0001
N	3	1.43278	0.2349
Date	3	45.76683	<0.0001
N*Date	9	5.9357	<0.0001

Table S9: ANOVA output for the best model (m.55) to explain patterns in nitrification for litter soils. Model included interactions between nitrogen fertilizer treatment and the date that the sample was collected. Block was added as a random effect for spatial heterogeneity.

Microbial Biomass			
Microbial Biomass C ~ Date			
m.58	df	F-Value	P-Value
Date	1	57.713	1.798e^-08
Residuals	30		

Table S10: ANOVA output for the best model (m.58) to explain patterns in microbial biomass for litter soils. Model included the date that the sample was collected.

REFERENCES

Abdalla, M., Osborne, B., Lanigan, G., Forristal, D., Williams, M., Smith, P., & Jones, M. B. (2013). Conservation tillage systems: a review of its consequences for greenhouse gas emissions. *Soil Use and Management*, *29*(2), 199–209. https://doi.org/https://doi.org/10.1111/sum.12030

Akemo, M. C., Regnier, E. E., & Bennett, M. A. (n.d.). Weed Suppression in Spring-Sown Rye (Secale cereale): Pea (Pisum sativum) Cover Crop Mixes. In *Technology* (Vol. 14, Issue 3). https://www.jstor.org/stable/3988907?seq=1&cid=pdf-

Allen SE. 1989. Chemical analysis of ecological materials. Oxford: Blackwell Scientific.

Austin, E. E., Wickings, K., McDaniel, M. D., Robertson, G. P., & Grandy, A. S. (2017). Cover crop root contributions to soil carbon in a no-till corn bioenergy cropping system. *GCB Bioenergy*, *9*(7), 1252–1263. https://doi.org/10.1111/gcbb.12428

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., & Vivanco, J. M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. Annual review of plant biology, 57(1), 233-266.

Blanco-Canqui, H., Shaver, T. M., Lindquist, J. L., Shapiro, C. A., Elmore, R. W., Francis, C. A., & Hergert, G. W. (2015). Cover crops and ecosystem services: Insights from studies in temperate soils. *Agronomy Journal*, *107*(6), 2449–2474. https://doi.org/10.2134/agronj15.0086

Bloszies, S. A., Reberg-Horton, S. C., Heitman, J. L., Woodley, A. L., Grossman, J. M., & Hu, S. (2022). Legume cover crop type and termination method effects on labile soil carbon and nitrogen and aggregation. Agronomy Journal, 114(3), 1817–1832. https://doi.org/https://doi.org/10.1002/agj2.21022

Bojović, B., & Marković, A. (2009). CORRELATION BETWEEN NITROGEN AND CHLOROPHYLL CONTENT IN WHEAT (Triticum aestivum L.). In *Kragujevac J. Sci* (Vol. 31).

Bowles, T. M., Jackson, L. E., Loeher, M., & Cavagnaro, T. R. (2017). Ecological intensification and arbuscular mycorrhizas: a meta-analysis of tillage and cover crop effects. Journal of Applied Ecology, 54(6), 1785–1793. https://doi.org/https://doi.org/10.1111/13652664.12815

Bradford MA, Davies CA, Frey SD, Maddox TR, Melillo JM, Mohan JE, Reynolds JF, Treseder KK, Wallenstein MD. (2008a). Thermal adaptation of soil microbial respiration to elevated temperature. Ecol Lett 11:1316–27

Bradford MA, Fierer N, Reynolds JF. (2008b). Soil carbon stocks in experimental mesocosms are dependent on the rate of labile carbon, nitrogen and phosphorus inputs to soils. Funct Ecol 22:964–74.

Brevik, E.C. 2009. Soil, food security, and human health. In:Soils,Plant Growth and Crop Production(ed W. Verheye). Encyclopedia ofLife Support Systems (EOLSS), Developed under the Auspices of the UNESCO, EOLSS Publishers, Oxford.

Brevik, E.C. & Burgess, L.C. (eds) 2013.Soils and Human Health. CRCPress, Boca Raton, FL

Buchanan, M. and King, L. 1993. Carbon and phosphorus losses from decomposing crop residues in no-till and conventional till agroecosystems. Agronomy Journal 85(3):631–638.

Doane, T.A. and Horwath, W.R. (2003) Spectrophotometric Determination of Nitrate with a Single Reagent. Analytical Letters, 36, 2713-2722. http://dx.doi.org/10.1081/AL-120024647

Douglas Bates, Martin Maechler, Ben Bolker, Steve Walker (2015). Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67(1), 1-48. R package version 1.1.27.1. doi:10.18637/jss.v067.i01.

Fierer N, Craine JM, McLauchlan K, Schimel JP. (2005). Litter quality and the temperature sensitivity of decomposition. Ecology 86:320–6.

Fierer N, Schimel JP. (2003). A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Sc Soc Am J 67:798–805.

Fraterrigo JM, Turner MG, Pearson SM, Dixon P. (2005). Effects of past land use on spatial heterogeneity of soil nutrients in southern appalachian forests. Ecol Monogr 75:215–30.

Gillespie, A. W., Diochon, A., Ma, B. L., Morrison, M. J., Kellman, L., Walley, F. L., Regier, T. Z., Chevrier, D., Dynes, J. J., & Gregorich, E. G. (2014). *Nitrogen input quality changes the biochemical composition of soil organic matter stabilized in the fine fraction: a long-term study*. *117*, 337–350. https://doi.org/10.1007/sl0533-013-9871-z

H. Wickham. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016. R package version 3.3.5

Hanway, J. J. (1966). How a corn plant develops. http://lib.dr.iastate.edu/specialreports/38

Hobbie. (n.d). *Inorganic Nitrogen Assays in Microplates.* The University of Minnesota

Hobbie, S. E. (2008). NITROGEN EFFECTS ON DECOMPOSITION: A FIVE-YEAR EXPERIMENT IN EIGHT TEMPERATE SITES. Ecology, 89(9), 2633–2644. https://doi.org/https://doi.org/10.1890/07-1119.1

Jani, A. D., Grossman, J., Smyth, T. J., & Hu, S. (2016). Winter legume cover-crop root decomposition and N release dynamics under disking and roller-crimping termination approaches. Renewable Agriculture and Food Systems, 31(3), 214-229.

Kim, N., Zabaloy, M. C., Guan, K., & Villamil, M. B. (2020). Do cover crops benefit soil microbiome? A meta-analysis of current research. *Soil Biology and Biochemistry*, *142*. https://doi.org/10.1016/j.soilbio.2019.107701

Knorr, M., Frey, S. D., & Curtis, P. S. (2005). NITROGEN ADDITIONS AND LITTER DECOMPOSITION: A META-ANALYSIS. Ecology, 86(12), 3252–3257. https://doi.org/https://doi.org/10.1890/05-0150

Kramer TD, Warren RJ, Tang YY, Bradford MA. (2012). Grass invasions across a regional gradient are associated with declines in belowground carbon pools. Ecosystems 15:1271–82.

Krueger, E. S., Ochsner, T. E., Porter, P. M., & Baker, J. M. (2011). Winter rye cover crop management influences on soil water, soil nitrate, and corn development. *Agronomy Journal*, *103*(2), 316–323. https://doi.org/10.2134/agronj2010.0327

Kuznetsova A, Brockhoff PB, Christensen RHB (2017). "lmerTest Package: Tests in Linear Mixed Effects Models." _Journal of Statistical Software_, *82*(13), 1-26. R package version 3.1.3. doi: 10.18637/jss.v082.i13 (URL: https://doi.org/10.18637/jss.v082.i13).

Laghari, M., Naidu, R., Xiao, B., Hu, Z., Mirjat, M. S., Hu, M., Kandhro, M. N., Chen, Z., Guo, D., Jogi, Q., Abudi, Z. N., & Fazal, S. (2016). Recent developments in biochar as an effective tool for agricultural soil management: a review. *Journal of the Science of Food and Agriculture*, *96*(15), 4840–4849. https://doi.org/https://doi.org/10.1002/jsfa.7753

Lal, R. (2006). Enhancing crop yields in the developing countries through restoration of the soil organic carbon pool in agricultural lands. *Land Degradation & Development*, *17*(2), 197–209. https://doi.org/https://doi.org/10.1002/ldr.696

Moebius-Clune, B.N., D.J. Moebius-Clune, B.K. Gugino, O.J. Idowu, R.R. Schindelbeck, A.J. Ristow, H.M. van Es, J.E. Thies, H.A. Shayler, M.B. McBride, K.S.M Kurtz, D.W. Wolfe, and G.S. Abawi, 2016. Comprehensive Assessment of Soil Health – The Cornell Framework, Edition 3.2, Cornell University, Geneva, NY

Mohammed, S., Mirzaei, M., Pappné Törő, Á., Anari, M. G., Moghiseh, E., Asadi, H., Szabó, S., Kakuszi-Széles, A., & Harsányi, E. (2022). Soil carbon dioxide emissions from maize (Zea mays L.) fields as influenced by tillage management and climate*. *Irrigation and Drainage*, *71*(1), 228–240. https://doi.org/https://doi.org/10.1002/ird.2633

Mooshammer, M., Wanek, W., Hämmerle, I., Fuchslueger, L., Hofhansl, F., Knoltsch, A., Schnecker, J., Takriti, M., Watzka, M., Wild, B., Keiblinger, K. M., Zechmeister-Boltenstern, S., & Richter, A. (2014). Adjustment of microbial nitrogen use efficiency to carbon:Nitrogen imbalances regulates soil nitrogen cycling. *Nature Communications*, *5*, 1–7. https://doi.org/10.1038/ncomms4694

Nouri, A., Lukas, S., Singh, S., Singh, S., & Machado, S. (2022). When do cover crops reduce nitrate leaching? A global meta-analysis. Global Change Biology, 28(15), 4736–4749. https://doi.org/https://doi.org/10.1111/gcb.16269

Oliver, M. A., & Gregory, P. J. (2015). Soil, food security and human health: a review. European *Journal of Soil Science*, *66*(2), 257–276. https://doi.org/https://doi.org/10.1111/ejss.12216

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2021). _nlme: Linear and Nonlinear Mixed Effects Models_. R package version 3.1.153, <URL: https://CRAN.R-project.org/package=nlme>.

Prescott, C. E. (2010). Litter decomposition: What controls it and how can we alter it to sequester more carbon in forest soils? *Biogeochemistry*, *101*(1), 133–149. https://doi.org/10.1007/s10533-010-9439-0

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. R version 4.1.2 (2021-11-01) URL: https://www.R-project.org/.

Ritchie, S.W., J.J. Hanway, G.O. Benson. 1993. How a corn plant develops. Spec. Rep. 48. Iowa State University of Science and Technology, Ames.

Robertson GP, Wedin D, Groffman PM, Blair JM, Holland EA, Nadelhoffer KJ, Harris D. (1999). Soil carbon and nitrogen availability: nitrogen mineralization, nitrification, and soil respiration potentials. In: Robertson GP, Coleman DC, Bledsoe CS, Sollins P, Eds. Standard soil methods for long-term ecological research. New York: Oxford University Press. p 258–71.

Roper, W.R., D.L. Osmond, J.L. Heitman, M.G. Wagger, and S.C. Reberg-Horton. 2017. Soil health indicators do not diferentiate among agro-nomic management systems in North Carolina soils. Soil Sci. Soc. Am. J. 81:828–843. doi:10.2136/sssaj2016.12.0400

Russell V. Lenth (2022). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.7.3. https://CRAN.R-project.org/package=emmeans

Sainju, U.M., Whitehead, W.F., and Singh, B.P. (2005). Biculture legume–cereal cover crops for enhanced biomass yield and carbon and nitrogen. Agronomy Journal 97:1403–1412.

Smil, V. (1997). Global Population and the Nitrogen Cycle. 277(1), 76–81. https://doi.org/10.2307/24995834

St. Luce, M., Whalen, J. K., Ziadi, N., Zebarth, B. J., & Chantigny, M. H. (2014). Labile organic nitrogen transformations in clay and sandy-loam soils amended with 15N-labelled faba bean and wheat residues. *Soil Biology and Biochemistry*, *68*, 208–218. https://doi.org/10.1016/j.soilbio.2013.09.033

Stein, L. Y., & Klotz, M. G. (2016). Current Biology The nitrogen cycle. In *R94 Current Biology* (Vol. 26).

Stewart, R. D., Jian, J., Gyawali, A. J., Thomason, W. E., Badgley, B. D., Reiter, M. S., & Strickland, M. S. (2018). What We Talk about When We Talk about Soil Health. *Agricultural & Environmental Letters*, *3*(1), 180033. https://doi.org/10.2134/ael2018.06.0033

Talukder, B., Ganguli, N., Matthew, R., vanLoon, G. W., Hipel, K. W., & Orbinski, J. (2021). Climate change-triggered land degradation and planetary health: A review. Land Degradation & Development, 32(16), 4509–4522. https://doi.org/https://doi.org/10.1002/ldr.4056

Thomas Lin Pedersen (2022). patchwork: The Composer of Plots. R package version 1.1.2. https://CRAN.R-project.org/package=patchwork

U.S. Department of Agriculture Natural Resources Conservation Service. (2022, December 13). *Soil Health*. Natural Resources Conservation Service. Retrieved December 22, 2022, from https://www.nrcs.usda.gov/conservation-basics/natural-resource-concerns/soils/soil-health

U.S. Department of Agriculture Natural Resources Conservation Service. (n.d.). *Cover crops and crop rotation*. Retrieved December 24, 2022, from https://www.usda.gov/peoples-garden/soil-health/cover-crops-crop-rotation

U.S Department of Agriculture Natural Resources Conservation Service. (n.d.). *Guide Sheet: Using a Cover Crop Before Corn.* Retrieved December 29, 2022 from https://www.nrcs.usda.gov/sites/default/files/2022-10/NRCS_Prevent_Planting_CoverCropFACTSHEET2014.pdf

Van der Heijden, M. G. A., Bardgett, R. D., & van Straalen, N. M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, *11*(3), 296–310. https://doi.org/https://doi.org/10.1111/j.1461-0248.2007.01139.x

West AW, Sparling GP. (1986). Modifications to the substrate-induced respiration method to permit measurement of microbial biomass in soils of differing water contents. J Microbiol Methods 5:177–89.