Distribution Agreement

In presenting this thesis as a partial fulfillment of the requirements for a degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter now, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

Murray Jack Sternberg April 10, 2024

Climate smart farming practices and soil carbon cycle proxies

by

Murray Jack Sternberg

Debjani Sihi, PhD
Adviser

Environmental Sciences

Debjani Sihi, PhD

Adviser

Eric Lonsdorf, PhD

Committee Member

Kristin Phillips, PhD

Committee Member

Biswanath Dari, PhD

Committee Member

2024

Climate smart farming practices and soil carbon cycle proxies

By

Murray Jack Sternberg

Debjani Sihi, PhD

Adviser

An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Science with Honors

Environmental Sciences

2024

Abstract

Climate smart farming practices and soil carbon cycle proxies

By Murray Jack Sternberg

Climate-smart agriculture practices can enhance the ability of small-scale farmers to adapt to issues posed by climate change and meet the growing demand for food, fiber, and fuel. By embracing perennial agriculture, minimizing soil disturbance, and enhancing soil biodiversity, soil microbial activities and the potential for soil carbon (C) sequestration can be increased. While changes in long-term C storage occur over extended timescales, biological parameters provide more immediate insights into these processes.

I evaluated soil C dynamics and associated microbial proxies under different climate-smart agriculture practices (e.g., cover crop, agroforestry, conservation/reduced tillage, and soil amendments such as biochar and compost) within the Southeast USA. Study sites included an annual vegetable farm in Georgia and a perennial agroforestry system in North Carolina. I measured total C and nitrogen (N), potential C mineralization, microbial biomass C and N, microbial necromass, and potential extracellular enzyme activities to improve our understanding of how climate-smart agriculture practices affect soil organic matter (SOM) dynamics and their ability to contribute to long-term C stabilization.

Results indicate that there are significant differences in microbial biomass C between soils using no-till and conservation-till practices, and in microbial biomass C, potential C mineralization, and enzyme activities between agroforestry with pecan vs. pine trees and across alley cover crop types. The main effect of amendment and an interaction effect between amendment and tillage depth on enzyme activities were also observed. This is relevant for comparing the utility of different climate-smart practices for the enhancement of soil biological health and climate change mitigation in a cropping system. This study may provide insights into precursory shifts in soil C dynamics and how they relate to long-term changes in C pools, which is important for agricultural decision-making and land use management.

Climate smart farming practices and soil carbon cycle proxies

By

Murray Jack Sternberg

Debjani Sihi, PhD

Adviser

A thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Science with Honors

Environmental Sciences

2024

Acknowledgements

I would like to thank my research advisor, Dr. Debjani Sihi, for welcoming me so warmly into her lab two years ago as a student only beginning my environmental career, which led to an incredibly transformative and rewarding journey. I appreciate the guidance she supplied at every step of this process, the trust that she has had in me, and her knack for pushing me to do my best work. I would like to thank Dr. Biswanath Dari for his encouragement of all my endeavors, his selfless dedication to mentorship, and his generosity in sharing his project with me.

I am very grateful to the other members of my committee: Dr. Kristin Phillips for her openness to us learning from each other and her willingness to provide a social perspective to my work, and Dr. Eric Lonsdorf for his valuable guidance on how to better communicate my ideas.

This work would not have been possible without the countless hours of lab work put in by my colleagues in the Sihi Lab, namely Jack Kagan and Gwen Read, and the mentorship provided by Milon Barmon, Yaxi Du, and Dr. K. Taylor Cyle. I also greatly appreciate the research support and advice given to me by Kristan Majors and Dr. John Wegner.

I also acknowledge the valuable feedback provided by friends, including Nick Chang, Yulia Gu, and Bella Roeske. Many more of my friends and family provided encouragement during this project, which I could not have completed without.

Funding for this project was granted by the Emory University Department of Environmental Sciences' Lester Research grant. This study was supported in part by the Emory HPLC Bioanalytical Core (EHBC), which is subsidized by the Emory University School of Medicine and is one of the Emory Integrated Core Facilities. Additional support was provided by the Georgia Clinical & Translational Science Alliance of the National Institutes of Health under Award Number UL1TR002378. The content is solely the responsibility of the authors and does not necessarily reflect the official views of the National Institutes of Health.

Table of Contents

1. Introduction / Background 1

1.1 *Climate Change and Agriculture* 1

1.2 *Climate-Smart Agriculture* 1

1.2.1 Cover Crops 2

1.2.2 Amendments 4

1.2.3 Tillage 4

1.2.4 Agroforestry 4

1.3 *Soil Health and Carbon Cycling* 6

2. Materials & Methods 9

2.1 *Experimental Design & Sample Collection* 9

2.1.1 Oxford Farm, Emory 9

2.1.2 University Research Farm, North Carolina A&T 10

2.2 *Treatment Details: Oxford Farm: Amendment* 10

2.2.1 Amendments 10

2.2.2 Tillage 11

2.3 *Treatment Details: NCA&T* 11

2.3.1 Agroforestry 11

2.3.2 Tillage 11

2.3.3 Alley Cover Crop 12

2.4 *Laboratory Analyses* 13

2.5 *Statistical Analyses* 15

3. Results 15

3.1 *Oxford Farm* 18

3.1.1 Microbial Biomass 18

3.1.2 Enzyme Kinetics 19

3.1.3 Necromass 21

3.1.4 Correlation Matrix 22

3.2 *NCA&T* 23

3.2.1 Microbial Biomass 23

3.2.2 Respiration 24

3.2.3 Enzyme Activities 26

3.2.4 Necromass 31

3.2.5 Total C & N 34

3.2.6 Correlation Matrix 35

4. Discussion 36

4.1 *Microbial Biomass* 36

4.2 *Respiration* 38

4.3 *Enzyme Activities & Kinetics* 40

4.3.1 Enzyme Kinetics (Oxford Farm) 40

4.3.2 Enzyme Activities (NCA&T) 41

4.4 *Necromass* 43

4.5 *Correlation Matrices* 45

5. Societal Implications of Climate-Smart Practices 47

6. Conclusions 48

7. References 50

8. Appendix A: Additional Statistical Outputs 66

9. Appendix B: Correlation Matrices 77

List of Figures

Figure 1. Conceptual diagram of soil carbon cycle proxies within the context of climate-smart agriculture and framework of the microbial carbon pump. 8

Figure 2. Map of study sites contextualized by ecoregions of the Southeastern U.S 9

Figure 3. Effect of tillage treatments on microbial biomass C (a) and microbial biomass N (b) at Oxford Farm 18

Figure 4. Effect of amendment treatments on microbial biomass N at Oxford Farm 18

Figure 5. Effect of amendment treatments by tillage treatment on microbial biomass C (a) and microbial biomass N (b) at Oxford Farm. 19

Figure 6. Effect of amendment treatments by tillage treatment on Vmax of BG (a) and Km of BG (b) at Oxford Farm. 20

Figure 7. Effect of amendment treatments on Vmax of PHOS (a) and Vmax of BG (b) at Oxford Farm. . 20

Figure 8. Effect of amendment treatments on Vmax of NAG at Oxford Farm. 21

Figure 9. Effect of tillage treatments on Km of LAP at Oxford Farm. 21

Figure 10. Effect of amendment treatments by tillage treatment on total necromass (microbial residual carbon) at Oxford Farm 22

Figure 11. Correlation matrix showing positive (blue) and negative (red) correlations between soil carbon cycle proxies measured at Oxford Farm. 23

Figure 12. Effect of agroforestry tree type on microbial biomass carbon (a) and microbial biomass nitrogen (b) at NCA&T 23

Figure 13. Effect of tillage method on microbial biomass carbon at NCA&T 24

Figure 14. Effect of agroforestry tree type on microbial respiration (carbon dioxide) at NCA&T 25

Figure 15. Effect of cover crop type on microbial respiration (carbon dioxide) at NCA&T. 25

Figure 16. Effect of tillage method on microbial respiration (carbon dioxide) at NCA&T. 26

Figure 17. Effect of agroforestry tree type by tillage method on microbial respiration (carbon dioxide) at NCA&T 26

Figure 18. Effect of agroforestry tree type by enzyme on potential extracellular enzyme activity at NCA&T 27

Figure 19. Effect of cover crop type on potential extracellular enzyme activity of BG at NCA&T.....28

Figure 20. Effect of cover crop type on potential extracellular enzyme activity of NAG at NCA&T.....28

Figure 21. Effect of cover crop type on potential extracellular enzyme activity of PHOS at NCA&T. 29

Figure 22. Effect of cover crop type on potential extracellular enzyme activity of PHOS with pecan agroforestry at NCA&T. 29

Figure 23. Effect of cover crop type on potential extracellular enzyme activity of PHOS (a) and BG (b) with pine agroforestry at NCA&T. 30

Figure 24. Effect of tillage method by enzyme type on potential extracellular enzyme activity at NCA&T 31

Figure 25. Effect of agroforestry tree type on total necromass (a) and bacterial necromass (b) at NCA&T. 31

Figure 26. Effect of cover crop type on total necromass for all agroforestry (a) and pecan agroforestry (b) at NCA&T. 32

Figure 27. Effect of cover crop type on fungal necromass for all agroforestry (a) and pecan agroforestry (b) at NCA&T 32

Figure 28. Effect of cover crop type on bacterial necromass for all agroforestry (a) and pecan agroforestry (b) at NCA&T 33

Figure 29. Effect of tillage method on total necromass (microbial residual carbon) at NCA&T 33

Figure 30. Effect of agroforestry tree type on total carbon (a) and total nitrogen (b) at NCA&T 34

Figure 31. Effect of cover crop type on total carbon (a) and total nitrogen (b) at NCA&T.....34

Figure 32. Effect of tillage method on total carbon (a) and total nitrogen (b) at NCA&T.....35

Figure 33. Correlation matrix showing positive and negative correlations between soil carbon cycle proxies measured at NCA&T. 36

Figure A1. Correlation matrix chart visualizing correlations between soil carbon cycle proxies for Oxford Farm 77

Figure A2. Correlation matrix chart visualizing correlations between soil carbon cycle proxies for NCA&T 78

List of Tables

Table 1. Effect of amendment and tillage treatments on soil carbon cycle proxies at Oxford Farm 16

Table 2. Effect of agroforestry, cover crop, and tillage treatments on soil carbon cycle proxies at NCA&T. 17

Table A1. Post-hoc Tukey HSD multiple comparison of means of soil carbon cycle proxies across amendment treatments at Oxford Farm 66

Table A2. Post-hoc Tukey HSD multiple comparison of means of soil carbon cycle proxies across cover crop treatments at NCA&T.....68

1. Introduction / Background

1.1 Climate Change and Agriculture

As human populations grow, finding a way to sustainably and equitably feed the population without depleting natural resources will be of paramount importance. Human-induced climate change may further exacerbate issues of global food security (Godfray et al., 2010). The atmospheric concentration of carbon dioxide (CO_2), a potent greenhouse gas, has increased at an uncharacteristically high rate over the last century due to human activities. This has contributed to global warming, which threatens to destroy economies, degrade natural environments, and further socioeconomic inequalities (Diffenbaugh & Burke, 2019). Modifying the balance between carbon (C) sources, which emit greenhouse gasses to the atmosphere, and C sinks, which store them away from the atmosphere, will be crucial to mitigating climate change.

Agriculture is particularly important to consider under a changing climate because warmer temperatures and the disruption of global precipitation patterns could threaten the world's food production systems and endanger the role of soil in climate change mitigation (Tilman et al., 2011). Soils have the potential to act as a C sink by sequestering C from atmospheric CO_2 and storing it in the soil (Don et al., 2023). In fact, soil organic carbon (SOC) contains more C globally than the atmosphere or vegetation, making it the largest terrestrial C pool (Stockmann et al., 2013). Even small disturbances to the soil system, such as climate change or intensive agriculture, could result in large amounts of C being released from the soil (Davidson & Janssens, 2006; Goh, 2004). Certain agricultural practices can increase productivity and resilience while enhancing the ability of soil to act as a C sink, which are classified as climate-smart agriculture (CSA) (*Climate-Smart Agriculture*, 2021; Das et al., 2022).

1.2 Climate-Smart Agriculture

CSA is an approach developed by the Food and Agriculture Organization (FAO) of the United Nations in 2010 to increase food security, bolster resilience to climate change, and reduce or remove

greenhouse gases when possible (FAO, 2010). CSA includes a number of sustainable practices such as cover cropping, application of organic amendments, conservation tillage, and agroforestry.

1.2.1 Cover Crops

Cover crop practices are useful to small-scale organic farmers interested in sustainable agriculture because they can offer economic, ecological, and soil health benefits ("Benefits of Cover Crops," 2007). Research into the utility of these practices for C sequestration and soil health is essential for its scientific and policy implications, such as in determining which practices should be associated with government subsidies to offset farmer efforts or in establishing an economic and environmental basis for agricultural C credits. Generally, cover crops can enhance soil structure and stability, improve soil health, and reduce greenhouse gas fluxes (Kaye & Quemada, 2017). Different types of cover crops can provide different benefits, and the cover crop chosen in a particular scenario will depend on the needs of the farmer and the environmental context. For example, while cereal crops may provide the largest amount of biomass, legumes provide nitrogen (N) fixation and brassicas provide easily accessible nutrients (Snapp et al., 2005).

Rye (*Secale cereale*) is a particularly popular grass cover crop due to its suitability for late-season planting (through winter hardiness and rapid germination), the allelopathic effect of its residues on suppressing weed germination, and its appropriateness for a roll-crimp mulch system (Magdoff & van Es, 2021). Rye performs many of the vital services that cover crops are known for, including erosion prevention, N scavenging, and organic matter addition ("Cereal Rye," 2007). Mixing rye with legumes such as clover can be a climate-friendly way to offset the N-immobilization caused by rye while still maintaining yield and soil nutrient supply, especially over nutrient application as an alternative ("Cereal Rye," 2007; Sainju et al., 2005). Legumes are typically less receptive to rye's allelopathic effects, making weed suppression and N fixation potentially compatible goals ("Cereal Rye," 2007). Mixing legumes with rye can achieve this benefit, which would not otherwise be possible due to rye's vulnerability to cold weather ("Cereal Rye," 2007).

Oats (*Avena sativa*) are another popular grass cover crop, particularly for cool-season planting, due to their inability to overwinter, providing an easily used mulch in the spring (Magdoff & van Es, 2021). Oats provide several key benefits of cover crops, such as protection from erosion, weed suppression, and scavenging of excess nutrients ("Oats," 2007). Oat-clover mixtures are used to provide quick cool-season cover, with the oat stems trapping snow and conserving moisture (Magdoff & van Es, 2021). Oat-vetch is another common mix, because oats can assist the relatively slow growth of legumes (i.e., hairy vetch, clover) while reducing weeds, offering value as legume nurse crops or companion crops ("Oats," 2007).

Tillage radish (*Raphanus sativus*), which belongs to the family Brassicaceae (although not the genus Brassica), is a brassica, which are often known informally as cruciferous vegetables ("Brassicas and Mustards," 2007). Brassica are commonly used due to their abilities to prevent erosion, offer weed and pest suppression, reduce soil compaction, and scavenge nutrients ("Brassicas and Mustards," 2007; Lawley et al., 2012). Brassica generally express fast fall growth, making them prime cool-season cover crops ("Brassicas and Mustards," 2007). Tillage radish in particular has the potential to capture large amounts of N from deep in the soil profile ("Brassicas and Mustards," 2007). Radish is recommended to be mixed with small grains, such as rye or oat ("Brassicas and Mustards," 2007). Mixing radish with legumes such as vetch can provide additional biological N fixation (Blesh et al., 2019). There is a risk of brassicas crowding out other species due to their competitiveness, which necessitates careful consideration of seeding rate ("Brassicas and Mustards," 2007).

Goal-oriented mixes of cover crops were selected to evaluate how particular cover crop strategies relevant to sustainable agricultural considerations also contributed to soil C cycling. While each of these cover crops have specific purpose-related value and suitability constraints, their contributions to soil health and C storage are also important to consider in a systems-minded ecological approach to agricultural management. For example, while oats typically do better than rye in hot weather ("Cereal Rye," 2007), a farmer might want to weigh the effects on soil health of their cover crop options if they're all suitable for a given climate.

1.2.2 Amendments

Organic amendments provide additional C inputs to the soil system, often enhancing biological health and nutrient availability (Lima et al., 2009). Besides contributing directly to soil C, application of organic amendments such as compost have been shown to increase microbial activities and plant growth, thereby enhancing soil biology and productivity (Thangarajan et al., 2013). The application of biochar (C-rich biomass pyrolyzed in the absence of oxygen) can increase soil C storage, reduce soil C emissions, and modify microbial habitat (Chen et al., 2023). Application of organic amendments is also an efficient waste recycling method, carrying with it both economic and environmental benefits (Lima et al., 2009).

1.2.3 Tillage

Conservation tillage practices, which refer to reductions in the depth and intensity of tillage, can reduce soil erosion and lead to greater microbial diversity and stability, thereby promoting soil health and microenvironment (Wang et al., 2017). Conservation tillage practices usually result in a higher proportion of crop residues being maintained on the soil surface, thus working synergistically with cover cropping to create sustainable agricultural systems (Wang et al., 2006).

Conservation tillage is also an important strategy in balancing increasing demand for food production with sustainable land management due to its ability to increase crop yields in comparison with conventional tillage (Busari et al., 2015). Specifically, research indicates that minimum tillage and no-till may offer the best balance between soil environment, crop yield, and overall environmental impact (Busari et al., 2015).

1.2.4 Agroforestry

Agroforestry, which involves the incorporation of trees and shrubs into agricultural systems, can offer benefits on ecosystem, economic, and societal levels. This includes enhancing biodiversity and resilience, acting as a buffer to reduce runoff intensity and erosion, and improving soil fertility and properties (Fahad et al., 2022; Parewa et al., 2018). A range of types of agroforestry systems exist,

including the practice of alley cropping which involves the placement of crops in rows between trees or shrubs. The diversification of agricultural products provided by agroforestry can benefit small-scale farmers by providing them with resilient livelihoods in the face of ecosystem-scale disruption such as climate change (Muthuri et al., 2023). On the soil level, agroforestry has demonstrated potential to enhance microbial dynamics and generally foster C sequestration (Rolo et al., 2023). In the Intergovernmental Panel on Climate Change's Land-Use, Land-Use Change and Forestry report, agroforestry was deemed to be the land use with the highest potential for C sequestration (IPCC, 2000; Jose & Bardhan, 2012).

Agroforestry systems often seek to optimize the balance of benefits provided by agriculture and commercial tree production (for such products as fruits, nuts, or timber). To accomplish these purposes, certain species may be more relevant than others in regional or site-specific contexts. For example, pecan (*Carya illinoinensis*) is important to the Southeast for economic and environmental reasons. Pecan production is currently centered in the Southeast, with Georgia in the lead (Peña, 1995). Recent work points to a trend of pecan growers in the Southeast increasing production density, which may indicate a demand for incorporating more pecan trees in a finite amount of space (Wells, 2014). Pecan trees have been noted to show potential for high carbon sequestration in their aboveground and belowground biomass (Cambareri et al., 2023). Incorporating pecan trees in agroforestry can thus provide simultaneous benefits to long-term carbon storage and food production, making this an important pathway towards sustainable agriculture (Cambareri et al., 2023).

Loblolly pine (*Pinus taeda*), another tree species integral to the Southeastern U.S.'s ecology and economy, comprises a high proportion of regional forested area, representing over 50% of the Southeast's standing pine resources (Rauscher & Johnsen, 2004; Winandy et al., 2022). Native pines grown in the Southeast are notable for their extraordinarily high rates of growth, which may contribute to this abundance (Borders & Bailey, 2001). Loblolly pine provides a high yield per unit land, grows well with other species, and is a leading timber species in the U.S. (Schultz, 1997). Research has demonstrated that loblolly pine plantations also have a high potential for C sequestration, even in managed stands (Rauscher

& Johnsen, 2004). This makes them key candidates for finding ways to meet reduction goals for U.S. greenhouse gas emissions (Zhao et al., 2023). This is important in considering co-benefits of climate-smart agricultural management, such as C sequestration and its possible endorsement through C credits.

1.3 Soil Health and Carbon Cycling

Soil health, with a focus on SOC, can be evaluated to determine and compare the efficacy of CSA practices (Jat et al., 2019). Soil health is a soil's capacity to maintain ecological services and function, including the regulation of water and pollutants, support of animal and plant life, and cycling of nutrients (*Soil Health*, n.d.). Soil health can be conceptualized as C existing within the soil in a number of discrete pools. A large portion of the C that enters the soil is decomposed by microbes, and is emitted from the soil as CO_2 through microbial respiration (Zhu et al., 2020). On the other hand, when soil microbes die, the C that they're composed of is stored in the soil as stable C (Zhu et al., 2020). Stable C from microbial residues can be stored in the soil for long periods of time, contributing to C sequestration.

Soil health can be represented by a soil's physical, biological, and chemical properties. In particular, biological properties can indicate the extent to which soil is acting as a C source or sink through the balance between microbial respiration and contributions to stable C (Fierer et al., 2021; Suman et al., 2022). This follows from the concept of the soil microbial carbon pump, in which buildup of dead microbial matter (or necromass) contributes to C transformations from labile (e.g., particulate carbon in plant residues) to stable (e.g., mineral-associated organic carbon) forms (Liang et al., 2017; Zhu et al., 2020). Measurements of soil microbes and the extracellular enzymes involved in their decomposition activities can demonstrate where a system lies within this framework (Gómez et al., 2020).

Biological parameters are beneficial to measure in comparison to large long-term pools of soil C and N due to their ability to change on shorter timescales and act as early indicators of direction and magnitude of changes in soil C and nutrient cycling (Smith, 2004). Microbial biomass C (MBC) and microbial biomass N (MBN) represent the total mass of microbes present, which can assist in understanding releases of C and N from the soil as part of nutrient mineralization. Because extracellular

enzymes facilitate nutrient cycling and decomposition by microbes, measuring their activity and kinetics can inform the capacity for necessary reactions tied to sustainable nutrient cycling (Gómez et al., 2020; Norris et al., 2020). This can show differences between mineralization of particular nutrients. As biological indicators of microbial residues, quantification of amino sugars can identify the total and type of necromass (fungal or bacterial) present in a soil, providing insight into contributions to stable C (Chen et al., 2023). Measurements of microbial respiration can estimate soil carbon availability and microbial activity, which is important for accounting for C emissions during decomposition. Measurements of these biological parameters can be contextualized within total soil C and N pools to distinguish microbial contributions from other organic sources and contextualize function-specific subsets of total pools, thereby parsing out biological involvement in soil C and nutrient cycling under CSA practices (Figure 1).

This project seeks to accomplish two goals: (1) to evaluate the impacts of climate-smart agricultural practices on soil health and soil C and nutrient cycling; and (2) to assess the utility of biological soil parameters as early indicators of long-term changes in soil C pools. By comparing two field experiments employing CSA practices at different points in their development, this project is able to monitor short-term dynamics in soil C and nutrient cycling and develop metrics for their interpretation.

I hypothesized that CSA practices will influence measured soil carbon cycle proxies. Specifically, I hypothesized that cover crops would increase microbial biomass (predominantly MBC for cereal- and brassica-based cover crops, and MBN for legume-based cover crops), respiration, enzyme activities (predominantly C-degrading enzymes for cereal- and brassica-based cover crops, and N-degrading enzymes for legume-based cover crops), microbial necromass, and total pools. I also hypothesized that application of amendments would increase microbial biomass, respiration (more under compost than under biochar or biochar-compost), enzyme activities, necromass, and total pools. Furthermore, I hypothesized that all soil C cycle proxies would increase under reduced or no-tillage, and under pecan over pine agroforestry. In addition, I hypothesized that the response of enzyme kinetic parameters (V_{max} and K_m) would be greatest under the perennial inputs of agroforestry. Lastly, I hypothesized that changes

in microbial biomass, respiration, and enzyme kinetics would occur over the shortest timescales, while changes in necromass and total pools would occur over longer timescales.

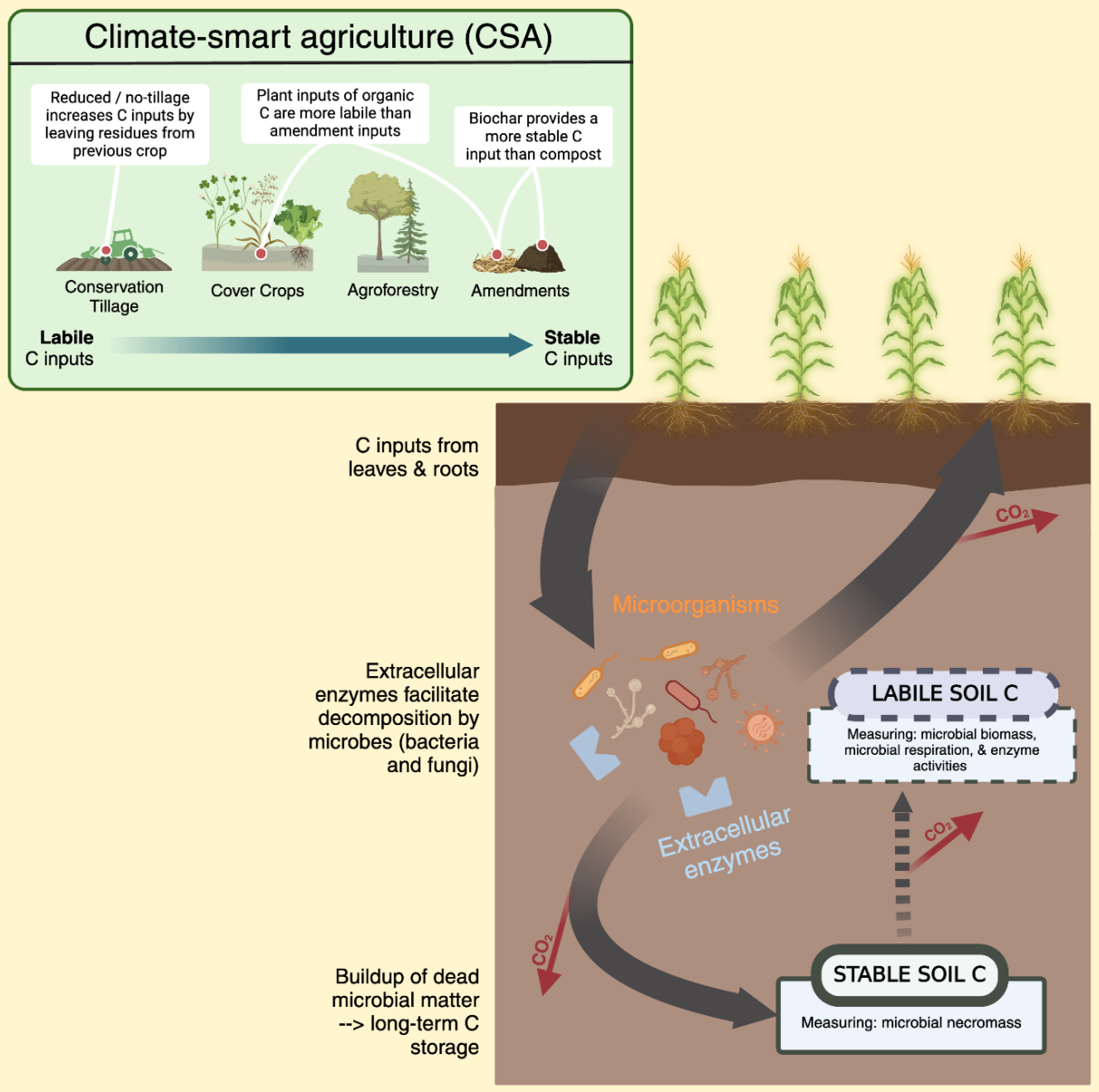

Figure 1. Conceptual diagram of soil carbon cycle proxies within the context of climate-smart agriculture and framework of the microbial carbon pump. Red arrows indicate output of CO_2 (carbon dioxide), while black arrows indicate the transfer of materials (carbon) through the soil system. Figure adapted from Liang et al., 2017 and created using BioRender.com.

2. Materials & Methods

2.1 Experimental Design & Sample Collection

Soil samples were collected from two farms within the Southeastern US, one representative of an annual vegetable production system and the other of a perennial agroforestry system. While soil order, ecoregion, and experimental design were consistent, plot arrangement differed between the two field sites.

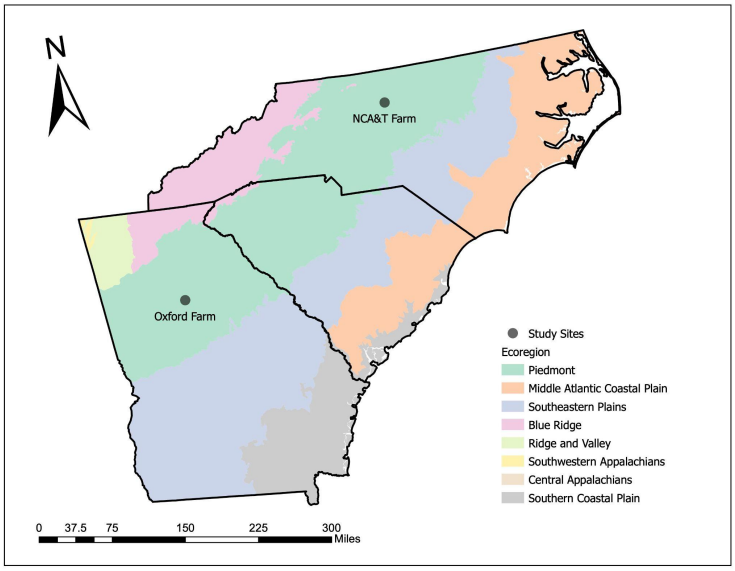

Figure 2. Map of study sites contextualized by ecoregions of the Southeastern U.S. The Piedmont region is generally characterized by eroded, clay-like soils with low infiltration rates (Markewich et al., 1990). Figure created in ArcGIS Pro using ecoregion data provided by the EPA (US Environmental Protection Agency, 2012).

2.1.1 Oxford Farm, Emory

Soil samples were collected from an annual vegetable production system (Oxford Farm), an organic farm located in Oxford, GA in the Southeastern U.S. (lat: 33.623, long: -83.867). The farm is situated on a red clay soil representing Ultisols, within the Pacolet series of the taxonomic class fine, kaolinitic, thermic Typic Kanhapludults (Soil Survey Staff, n.d.). This series is characterized by its very deep, well drained, moderately permeable soils (Natural Resources Conservation Service, 2008). Two treatments (amendment type and tillage depth) were implemented on 20 ft. by 5 ft. experimental plots. Cover crops were applied continuously across all plots. Pre-season samples were collected in May 2023, and post-harvest samples were collected in October 2023. Sampling was done manually with a hand-held

soil sampler (~soil core, inner diameter: 1 inch) at a depth of 0-10 cm. 5 composite field samples (each consisting of ~10-12 individual soil samples) were collected per plot, transferred to the laboratory and stored in a refrigerator at 4 °C until further analyses.

2.1.2 University Research Farm, North Carolina A&T

Soil samples were collected from a perennial agroforestry system (the University Research Farm at North Carolina Agricultural & Technical [NCA&T] State University), a certified organic farm located in Greensboro, NC in the Southeastern U.S. (lat: 36.070, long: -79.731). The farm is also situated on a red clay soil representing Ultisols, within the Enon series of the taxonomic class fine, mixed, active, thermic Ultic Hapludalfs (Soil Survey Staff, n.d.). This series is characterized by its deep, well drained, slowly permeable soils taking place on tops or sides of ridges in the Piedmont region (Natural Resources Conservation Service, 2022). Three treatments (agroforestry tree type, alley cover crop type, and tillage depth) were implemented on experimental plots. The entire experiment was conducted in 0.75 acres of land with plot dimensions of 95 ft. by 15 ft. with 25 ft. wide alleys. Within 15 ft. of the width, each plot was divided into two divisions (6 ft. each) and implemented no-tillage and minimum tillage into each 6 ft. (width-wise). An alley width of 25 ft. was chosen within a range of 20-45 ft. alleys as a common practice for agroforestry systems in the Southeastern U.S. Sampling was done with an automated tractor-mounted soil sampler at a depth of 0-15 cm. 6 field composite samples were collected for the pecan field. Similarly, a total of 4 composite samples were collected consisting of 10-12 randomly selected locations within each treatment from the pine field. Upon collection, soil samples were transferred to the lab and stored in a refrigerator at 4 °C until further analyses.

2.2 Treatment Details: Oxford Farm: Amendment

2.2.1 Amendments

Three levels of amendment treatment were applied to Oxford Farm plots: compost, co-composting with biochar (i.e., compost + biochar), and no amendment (control). Biochar and compost

treatments were chosen based on affordability and preference for trusted vendors of local farmers. Organic fertilizers, composed of feather, bone, and mined potash, were applied on the field following University of Georgia recommendations based on soil testing, and reduced to account for the nutrient input provided by the biochar or biochar + compost amendment. The compost amendment applied was Vermont Compost at a rate of 18 tons/acre. The combined biochar and compost amendment applied was Wakefield Biochar + Compost at a rate of 10 tons/acre biochar and 18 tons/acre compost.

2.2.2 Tillage

Two different tillage depths were implemented at Oxford Farm: deep tillage (DT; depth 10-15 cm.) by heavy disking with a tractor and shallow tillage (ST; 3-4 cm.) by shallow disking or harrowing with a no-till drill.

2.3 Treatment Details: NCA&T

2.3.1 Agroforestry

At the NCA&T research site, pecan (*Carya illinoinensis*) and loblolly pine (*Pinus taeda*) trees were planted in rows as seedlings in 2015, with some plots designated as non-agroforestry controls in which trees were not grown. Trees were permitted to grow with minimum intervention limited to tillage as needed for field maintenance and yearly unregulated application of cover crop.

2.3.2 Tillage

Two tillage treatments were established in fall 2021 within all but the control plots to compare no-till and minimum tillage practices. Here, minimum tillage refers to a disturbance depth of no more than 7-10 cm. A focus on conservation tillage was chosen due to the regional relevance of associated methods due to their ability to reduce soil erosion and agricultural water use (Raczkowski et al., 2009; Sullivan et al., 2007) and the need to distinguish the effects of individual practices within the conservation framework.

2.3.3 Alley Cover Crop

Within agroforestry plots, an alley-cropping system was established by planting trees in rows, within which cover crops were grown as quasi-cash or production crops. For crop termination, each alley was broken up into two sections lengthwise using a roller-crimper or a flail mower, ensuring that crop residues were left on the surface post-termination. Cover crop maintenance methods were chosen based on tillage-alternatives suggested for large-scale organic farmers (Rodale Institute, n.d.). With the roller-crimper, stems are cut so that cover crop residues are left on the soil surface as mulch for cash crops to grow through (Rodale Institute, n.d.). To ensure the roller-crimper (300 pounds) was heavy enough to successfully crimp the cover crop, it was filled with 100 gallons of water (834 pounds), for a total weight of 1,134 pounds.

Cover crop experimental treatments were established in fall 2021, in which blocks of different region- and purpose-specific cover crops were implemented in all but non-cover crop control plots. Crop planting and termination occurred in two cycles each year, and were differentiated using the distinction of warm-season (cash) and cool-season (cover) crops. Cool-season cover crops were generally planted in early fall and allowed to grow until termination in late May, through a period of winter dormancy. Soil sampling was then conducted post-termination. Warm-season cash crops (usually corn [*Zea mays*] for silage) were grown until termination mid-September, after which cool-season cover crops were planted again to continue the cycle.

Cover crop species were chosen based on region-specific popularity and function to provide information about a portfolio of individual cover crops as well as relevant combinations for farmers interested in alleviating downsides associated with use of individual crops. Choices of cover crop were made using the Cover Crop Chart provided by the USDA's Agricultural Research Service (U.S. Department of Agriculture, 2023). Within each agroforestry regime, legumes, grasses, and brassicas were evaluated both as mono-cover crop and as cover crop mix treatments. Selection of cover crop species was done by consulting local expertise to determine which cover crop options were viable for application in

this particular Southeastern agroforestry system. As common annual cereals, rye and oat were chosen to represent grass cover crops. Leguminous cover crops were represented by hairy vetch and clover, and brassicas were represented by tillage radish. Within the plots using pecan for agroforestry, 6 cover crop applications were evaluated: rye monocrop, oat monocrop, rye-vetch mix, rye-clover mix, oat-vetch mix, and oat-clover mix. Within the plots using pine for agroforestry, 4 cover crop applications were evaluated: tillage radish monocrop, radish-oat mix, radish-rye mix, and radish-vetch mix. Seeds were planted within recommended ranges at rates of 90 lbs./acre for rye, 120 lbs./acre for oat, 20 lbs./acre for clover, 30 lbs./acre for vetch, and 15 lbs./acre for radish.

2.4 Laboratory Analyses

Laboratory analyses for soil health parameters of all samples was done in the Sihi Biogeochemistry Lab within the Department of Environmental Sciences at Emory University. Soil samples were air dried, ground, and sieved through a 2-mm sieve for total C and N analyses. Field moist soil samples were used for all other analyses, and were ground through a 2-mm sieve with roots and other debris removed before measurement. The following parameters were analyzed on Oxford Farm samples: MBC & MBN, potential extracellular enzyme activities and kinetics, and microbial necromass. The following parameters were analyzed on NCA&T samples: total C & N, MBC & MBN, potential extracellular enzyme activities, microbial necromass, and potential C mineralization.

Total C and N were measured by combustion with an elemental analyzer using argon as carrier gas for analysis of elemental percent C and percent N (EPA, 1983). This was done using the Thermo Scientific Flash*Smart* NC SOIL instrument (Krotz et al., 2017). Samples collected in 2023 were measured following an identical method by the University of Georgia Extension's Agricultural & Environmental Services Laboratory (University of Georgia Extension, n.d.).

MBC and MBN were measured using the chloroform-fumigation direct extraction method, in which one soil subsample fumigated with chloroform and one non-fumigated subsample were extracted in K_2SO_4 (Hobbie, 1998; Vance et al., 1987). The difference in total organic C and total dissolved N

between fumigated and non-fumigated subsamples was measured with a Shimadzu TOC-L analyzer (Spohn et al., 2016), and was converted to MBC and MBN using measured gravimetric soil water content (Hobbie, 1998) and a kEC (correction factor) value of 0.45 (Beck et al., 1997). Gravimetric soil water content was measured by weighing samples pre- and post-drying at 105°C for 24 hours (Spohn et al., 2016).

Potential extracellular enzyme activities were measured following a high-throughput fluorometric method (Bell et al., 2013). Accordingly, fluorescent dye-bound substrates were introduced to samples to visualize catalyzation of substrate degradation by relevant C-, N-, and P-degrading enzymes. Relevant enzyme substrates (4-Methylumbelliferyl β-D-glucopyranoside [BG], 4-Methylumbelliferyl N-acetyl-β-D-glucosaminide [NAG], and 4-Methylumbelliferyl phosphate [PHOS]) were measured on samples from NCA&T, and this analysis was expanded to include calculation of enzyme kinetics and a wider range of enzyme substrates (BG, NAG, PHOS, L-Leucine-7-amido-4-methylcoumarin hydrochloride [LAP], and 4-Methylumbelliferyl-β-D-xylopyranoside [XYL]) for samples from Oxford Farm. The substrate BG targets labile C, NAG targets N and some C, LAP targets N, PHOS targets phosphorus (P), and XYL targets stable C. Enzyme kinetics were determined by fitting a 2 parameter Michaelis-Menten model and calculating V_{max} (the maximum velocity of reaction achieved) and K_m (the Michaelis constant).

Microbial necromass was estimated through extraction of amino sugars by hydrolysis, followed by high-performance liquid chromatography (HPLC) to detect muramic acid, mannosamine, glucosamine, and galactosamine (Indorf et al., 2011). HPLC was performed by the Emory HPLC Bioanalytical Core using pre-column OPA derivatization HPLC coupled to an electrochemical detector (*Services*, n.d.). Total microbial necromass was calculated, and further subdivided into fungal C and bacterial C using average conversion factors for their relative amino sugar indicators (Indorf et al., 2011).

Potential C mineralization was measured as CO_2 produced by soil over a 24-hour period after rewetting samples that had been dried overnight at 50°C (Haney et al., 2018; Haney & Haney, 2010). Measurement was conducted using a Qubit Q-S151 CO_2 gas analyzer (*Q-S151 CO2 Analyzer 0-2000ppm*, 2020).

2.5 Statistical Analyses

Statistical analyses were done using R version 4.3.2 with a significance value of $\alpha = 0.05$ (R Core Team, 2021). One-way ANOVA were performed using amendment and tillage as factors for the Oxford Farm site, and agroforestry tree type, cover crop type, and tillage treatments as factors for the NCA&T site. Tukey's HSD test was performed to identify differences in means when multiple comparisons were deemed necessary (i.e., for amendment treatments in Oxford Farm plots and for cover crop type in NCA&T plots) due to significant ANOVA results. Two-way ANOVA were performed to analyze main effects of and interactions between amendment with tillage for the Oxford Farm site, and agroforestry tree type with tillage and cover crop type with tillage for the NCA&T site. Correlation matrices were constructed to reveal relationships between treatments using the corrplot and chart.Correlation functions in R.

3. Results

Table 1 and Table 2 indicate results of ANOVA on soil C cycle proxies at each of the two sites. Lower tillage intensity increased MBC at both sites (Tables 1 & 2), pecan agroforestry led to higher MBC, respiration, and BG activity than pine at NCA&T (Table 2), and cover crop type affected respiration and enzyme activity at NCA&T (Table 2). Significant differences in necromass only occurred across cover crop types within plots using pecan agroforestry (Table 2).

Table 1. Effect of amendment and tillage treatments on soil carbon cycle proxies at Oxford Farm.

Parameter			Treatment		
			Amendment	Tillage	Amendment * Tillage
Microbial biomass	MBC		0.22	0.01*	0.07˙
	MBN		0.07˙	0.01*	0.09˙
Enzyme Kinetics	Km	BG	0.53	0.72	0.09˙
		LAP	0.98	0.06˙	0.46
		NAG	0.16	0.92	0.75
		PHOS	0.32	0.17	0.63
		XYL	0.17	0.26	0.80
	Vmax	BG	0.10˙	0.44	0.03*
		LAP	0.38	0.23	0.79
		NAG	0.08˙	0.67	0.26
		PHOS	0.04*	0.73	0.29
		XYL	0.51	0.56	0.94
Necromass	Total necromass		0.28	0.36	0.91
	Fungal necromass		0.28	0.53	0.82
	Bacterial necromass		0.33	0.16	0.96

BG: 4-Methylumbelliferyl β-D-glucopyranoside, LAP: L-Leucine-7-amido-4-methylcoumarin hydrochloride, NAG: 4-Methylumbelliferyl N-acetyl-β-D-glucosaminide, PHOS: 4-Methylumbelliferyl phosphate, XYL: 4-Methylumbelliferyl-β-D-xylopyranoside.

See Appendix A for post-hoc Tukey HSD multiple comparison testing of soil carbon cycle proxies across amendment treatments.

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, . $p < 0.1$.

Table 2. Effect of agroforestry, cover crop, and tillage treatments on soil carbon cycle proxies at NCA&T.

Parameter		Treatment						
		Agroforestry Tree Type	Cover Crop Type (Pecan & Pine)	Cover Crop Type (Pecan)	Cover Crop Type (Pine)	Tillage	Tree Type * Tillage	Cover Crop Type * Tillage
Microbial biomass	MBC	0.02*	0.24	0.25	0.72	0.03*	0.41	0.92
	MBN	0.84	0.57	0.18	0.58	0.44	0.12	0.71
24-hour microbial respiration		<0.001***	<0.001***	0.04*	0.22	0.71	0.09·	0.27
Enzyme activities	BG	<0.001***	<0.001***	0.59	0.10·	0.47	<0.001***	<0.001***
	NAG	0.54	0.10·	0.02*	0.63	0.67	<0.001***	<0.001***
	PHOS	<0.001***	<0.001***	<0.001***	0.007**	0.76	0.009**	<0.001***
Necromass	Total	0.58	0.50	0.03*	0.53	0.85	0.70	NA
	Fungal	0.97	0.58	0.04*	0.62	0.83	0.84	NA
	Bacterial	0.09·	0.20	0.03*	0.35	0.91	0.35	NA
Total pools	Total C	<0.001***	<0.001***	<0.001***	0.01*	0.56	0.57	0.85
	Total N	<0.001***	0.01**	0.27	0.23	0.68	0.87	0.82

NA: Test not performed due to sample size limited to one season of data.

BG: 4-Methylumbelliferyl β-D-glucopyranoside, NAG: 4-Methylumbelliferyl N-acetyl-β-D-glucosaminide, PHOS: 4-Methylumbelliferyl phosphate.

See Appendix A for post-hoc Tukey HSD multiple comparison testing of soil carbon cycle proxies across cover crop treatments.

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, . $p < 0.1$.

3.1 Oxford Farm

3.1.1 Microbial Biomass

At Oxford Farm, shallow tillage resulted in significantly higher MBC (Table 1, Figure 3a, p = 0.01) and MBN (Table 1, Figure 3b, p = 0.01) as compared to deep tillage. Amendment treatments (biochar, biochar & compost) had a weak effect on MBN (Table 1, Figure 4, p = 0.07), did not cause significant differences in MBC (Table 1, p = 0.22), and weak interactions between amendment and tillage treatments were observed for MBC (Table 1, Figure 5a, p = 0.07) and MBN (Table 1, Figure 5b, p = 0.09).

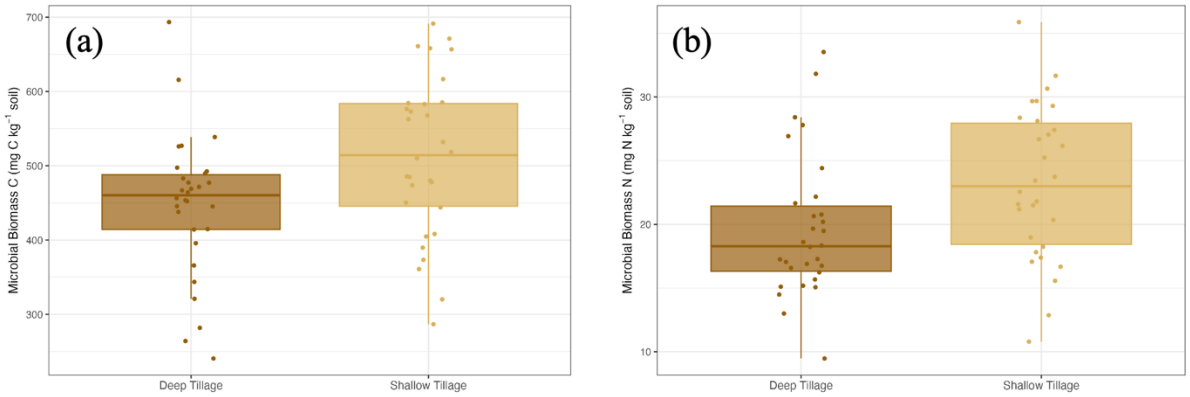

Figure 3. Effect of tillage treatments on microbial biomass C (a) and microbial biomass N (b) at Oxford Farm.

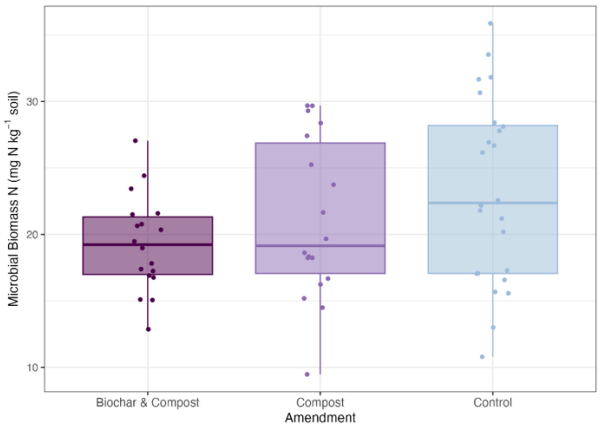

Figure 4. Effect of amendment treatments on microbial biomass N at Oxford Farm.

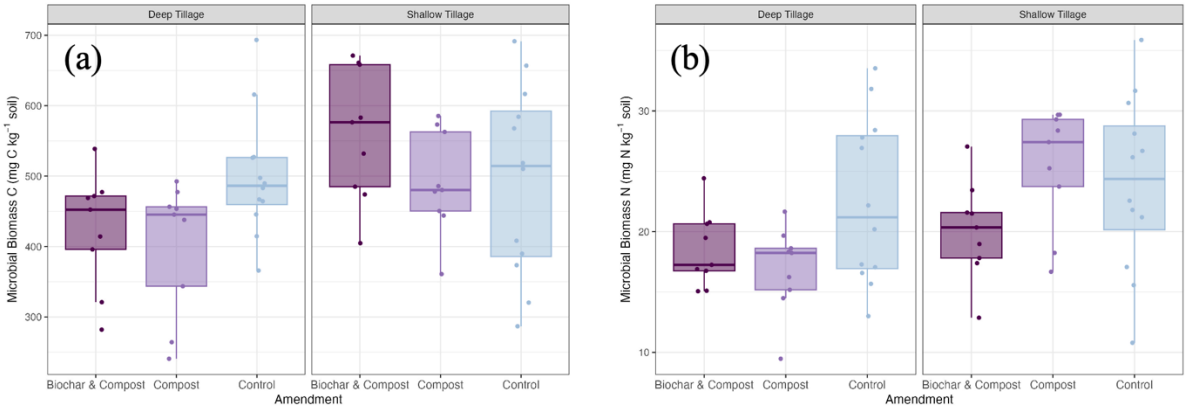

Figure 5. Effect of amendment treatments by tillage treatment on microbial biomass C (a) and microbial biomass N (b) at Oxford Farm.

3.1.2 Enzyme Kinetics

Results indicated that amendment application and tillage treatments were generally not influential on enzyme K_m values or V_{max} of LAP, NAG, or XYL (non-significant results not shown in figures). However, the V_{max} of BG showed a significant interaction between amendment and tillage treatments (Table 1, Figure 6a, $p = 0.03$), in which V_{max} increased with biochar + compost under deep tillage, and decreased with amendment treatments under shallow tillage. The K_m of BG showed a weak interaction between amendment and tillage treatments (Table 1, Figure 6b, $p = 0.09$). V_{max} of PHOS was influenced by amendment treatment (Table 1, Figure 7a, $p = 0.04$), with the compost treatment significantly increasing V_{max} of PHOS as compared to the biochar & compost treatment ($p = 0.04$). There was weak evidence that amendment treatment impacted V_{max} of BG (Table 1, Figure 7b, $p = 0.10$) and V_{max} of NAG (Table 1, Figure 8, $p = 0.08$), and that tillage impacted K_m of LAP (Table 1, Figure 9, $p = 0.06$). Amendment and tillage treatments were not found to have a significant effect on K_m of the other enzymes measured (PHOS or XYL).

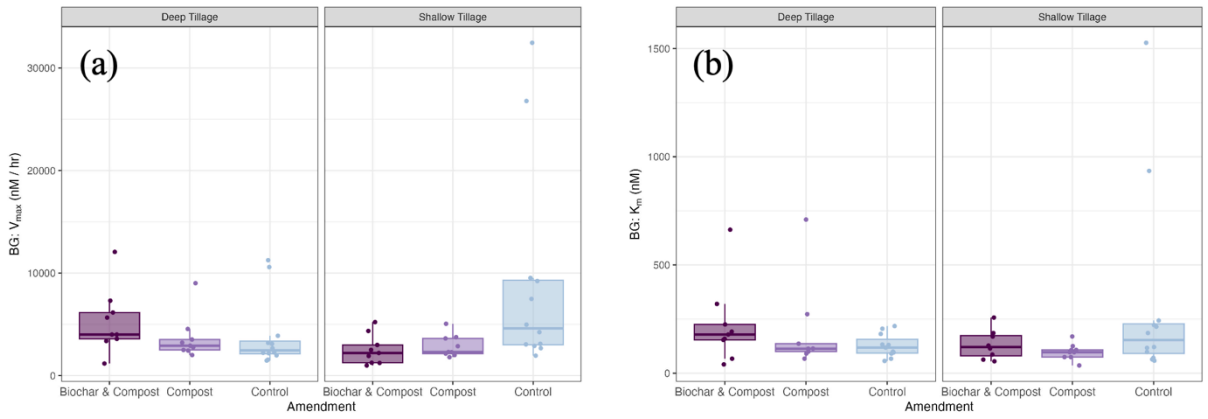

Figure 6. Effect of amendment treatments by tillage treatment on Vmax of BG (a) and Km of BG (b) at Oxford Farm. BG: 4-Methylumbelliferyl β-D-glucopyranoside.

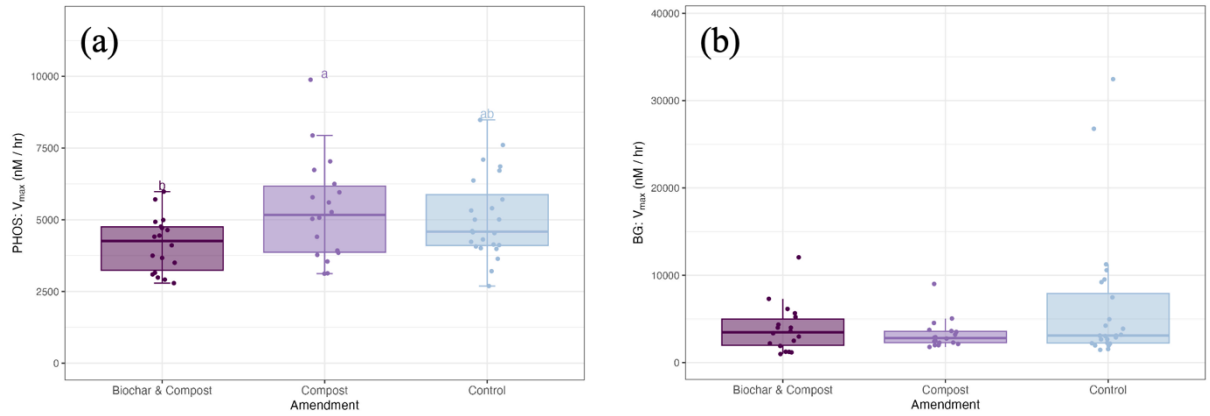

Figure 7. Effect of amendment treatments on Vmax of PHOS (a) and Vmax of BG (b) at Oxford Farm. PHOS: 4-Methylumbelliferyl phosphate, BG: 4-Methylumbelliferyl β-D-glucopyranoside.

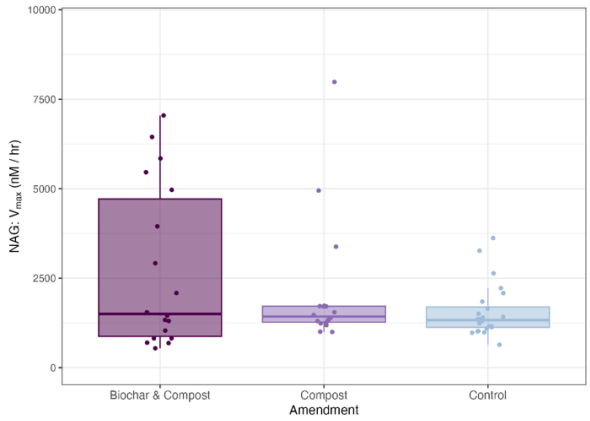

Figure 8. Effect of amendment treatments on Vmax of NAG at Oxford Farm. NAG: 4-Methylumbelliferyl N-acetyl-β-D-glucosaminide.

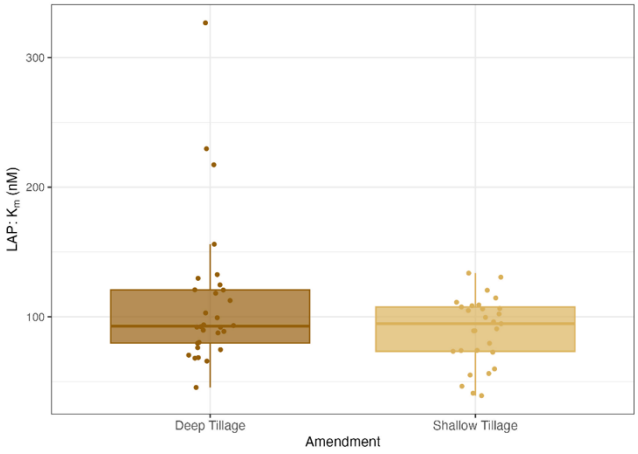

Figure 9. Effect of tillage treatments on Km of LAP at Oxford Farm. LAP: L-Leucine-7-amido-4-methylcoumarin hydrochloride.

3.1.3 Necromass

Changes in total, fungal, or bacterial necromass were not found to be caused by amendment treatments (Table 1, $p = 0.28$, $p = 0.28$, and $p = 0.33$, respectively) or tillage treatments (Table 1, $p = 0.36$, $p = 0.53$, and $p = 0.16$, respectively). Interactions between amendment and tillage treatments were also not observed for total, fungal, or bacterial necromass (Table 1, Figure 10, $p = 0.91$, $p = 0.82$, and $p = 0.96$, respectively).

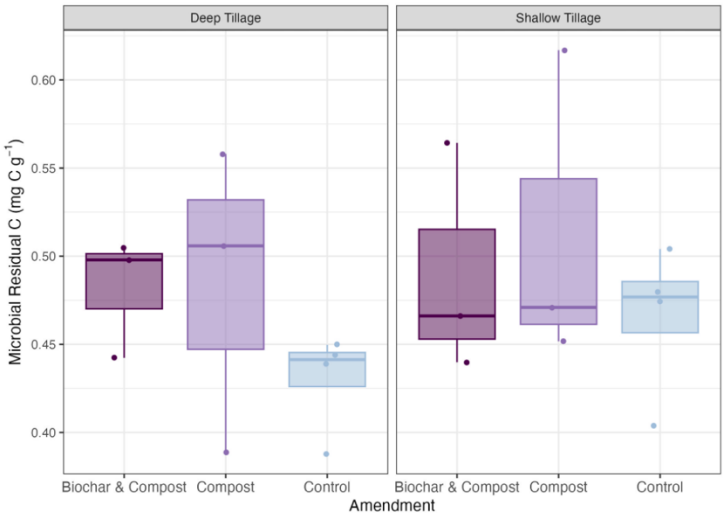

Figure 10. Effect of amendment treatments by tillage treatment on total necromass (microbial residual carbon) at Oxford Farm.

3.1.4 Correlation Matrix

A correlation matrix for soil C cycle proxies measured at Oxford Farm showed strong positive correlations between V_{max} of NAG and necromass and V_{max} of NAG and XYL (Figure 11). While BG kinetics showed a strong negative correlation with MBC and a moderate negative correlation with MBN, PHOS kinetics showed a moderate positive correlation with both MBC and MBN. V_{max} and K_m of BG were strongly negatively correlated with both V_{max} and K_m of PHOS.

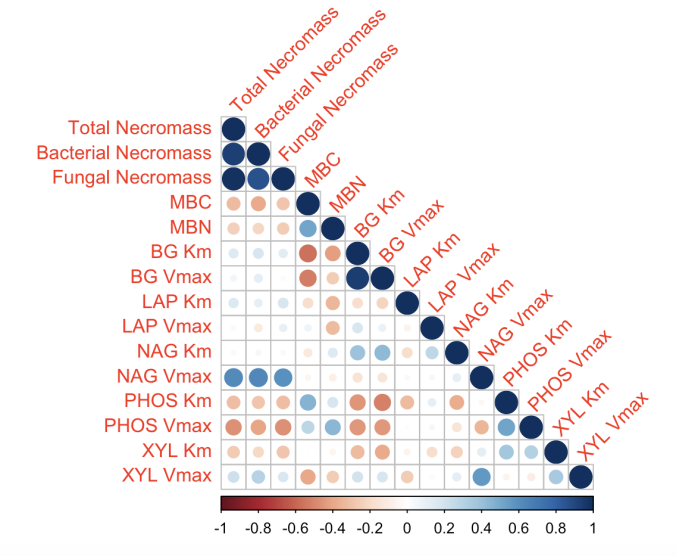

Figure 11. Correlation matrix showing positive (blue) and negative (red) correlations between soil carbon cycle proxies measured at Oxford Farm.

3.2 NCA&T

3.2.1 Microbial Biomass

Use of pecan trees over pine trees for agroforestry led to significantly more MBC (Table 2, Figure 12a, p = 0.02), but MBN did not change significantly (Table 2, Figure 12b, p = 0.84).

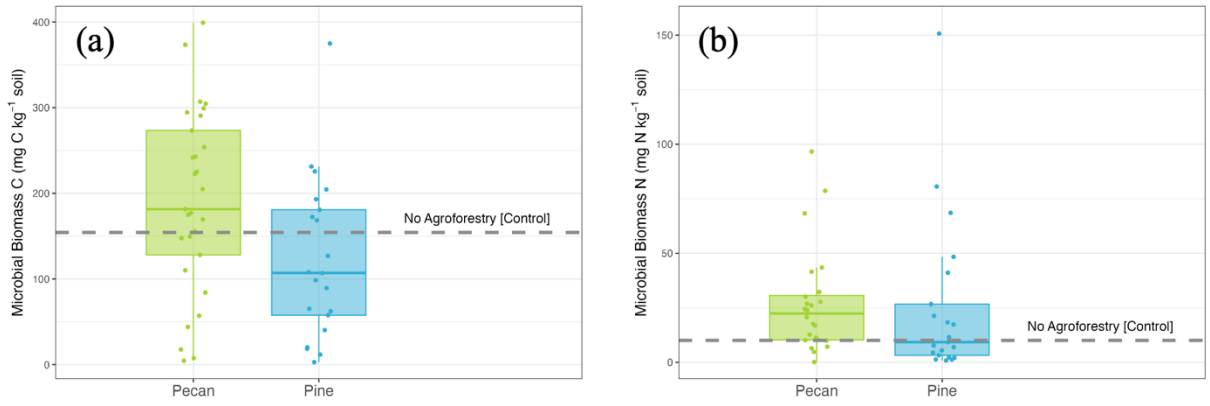

Figure 12. Effect of agroforestry tree type on microbial biomass carbon (a) and microbial biomass nitrogen (b) at NCA&T.

Cover crop type did not have a significant impact on MBC or MBN within plots using pecan trees for agroforestry (Table 2, p = 0.25 and p = 0.18, respectively), plots using pine trees (Table 2, p = 0.72 and p = 0.58, respectively), or the two plots considered together (Table 2, p = 0.24 and p = 0.57, respectively).

At NCA&T, no-till did result in significantly higher MBC over minimum tillage (Table 2, Figure 13, p = 0.03). This was not the case for MBN, which was not significantly affected by tillage treatment (Table 2, p = 0.44). Interactions were not found between tree type and tillage treatments for MBC or MBN (Table 2, p = 0.41 and 0.12, respectively), or between cover crop type and tillage treatments for MBC or MBN (Table 2, p = 0.92 and 0.71, respectively).

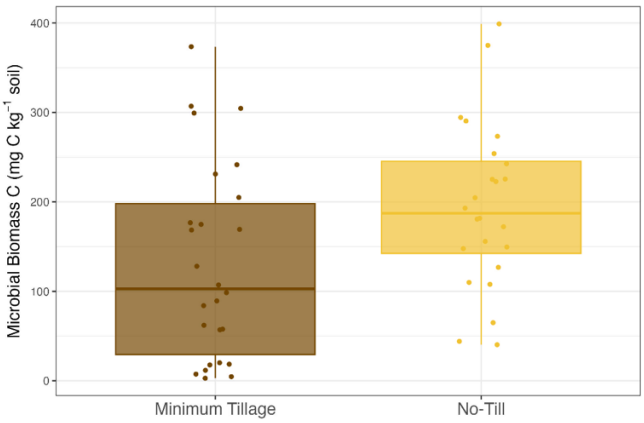

Figure 13. Effect of tillage method on microbial biomass carbon at NCA&T.

No significant interaction was observed between cover crop type and tillage for MBC (Table 2, p = 0.41) or MBN (Table 2, p = 0.92).

3.2.2 Respiration

Incorporating pecan trees for agroforestry caused a significant increase in microbial respiration as compared to pine trees (Table 2, Figure 14, p < 0.001).

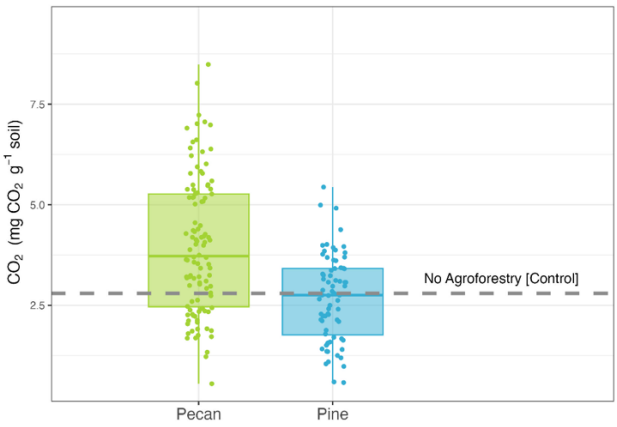

Figure 14. Effect of agroforestry tree type on microbial respiration (carbon dioxide) at NCA&T.

Application of different cover crops led to significant changes in microbial respiration when considering plots using pecan and pine agroforestry together (Table 2, Figure 15, $p < 0.001$), with some indications that mixed cover crops led to higher respiration than mono cover crops, and that cereal-legume mixes led to higher respiration than brassica mixes.

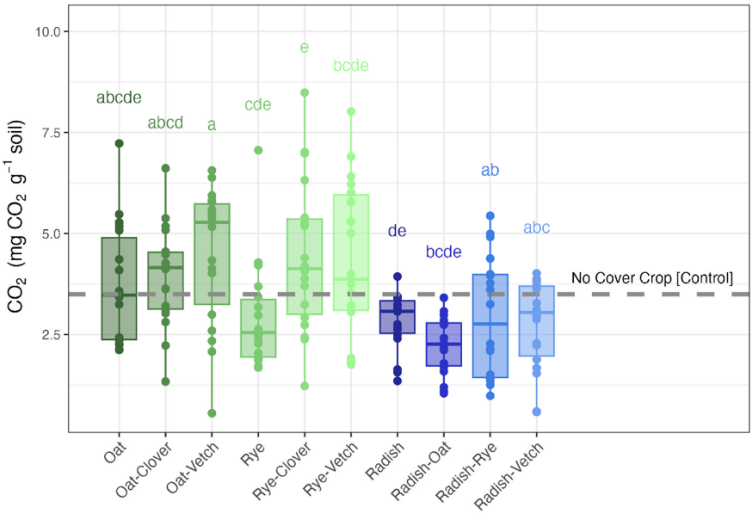

Figure 15. Effect of cover crop type on microbial respiration (carbon dioxide) at NCA&T. Shades of green (left) correspond to pecan agroforestry, and shades of blue (right) correspond to pine agroforestry.

Tillage treatments were not noted to have a significant effect on microbial respiration (Table 2, Figure 16, $p = 0.71$).

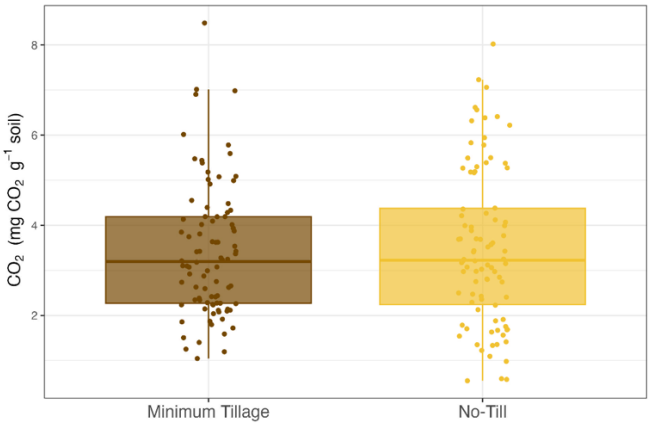

Figure 16. Effect of tillage method on microbial respiration (carbon dioxide) at NCA&T.

Weak evidence of an interaction was found between tree type and tillage (Table 2, Figure 17, $p = 0.09$), although not between cover crop type and tillage (Table 2, $p = 0.27$) for microbial respiration.

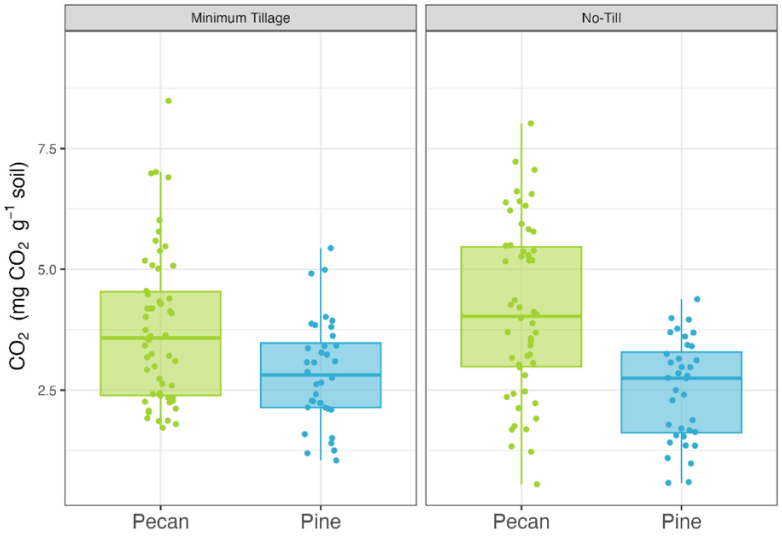

Figure 17. Effect of agroforestry tree type by tillage method on microbial respiration (carbon dioxide) at NCA&T.

3.2.3 Enzyme Activities

Activity of BG was significantly increased under agroforestry with pecan trees as compared to pine trees (Table 2, Figure 18, $p < 0.001$). The opposite trend was observed for PHOS, in which activity was significantly increased under pine as compared to pecan agroforestry (Table 2, Figure 18, $p < 0.001$).

On the other hand, activity of NAG wasn't significantly affected by agroforestry tree type (Table 2, Figure 18, $p = 0.54$).

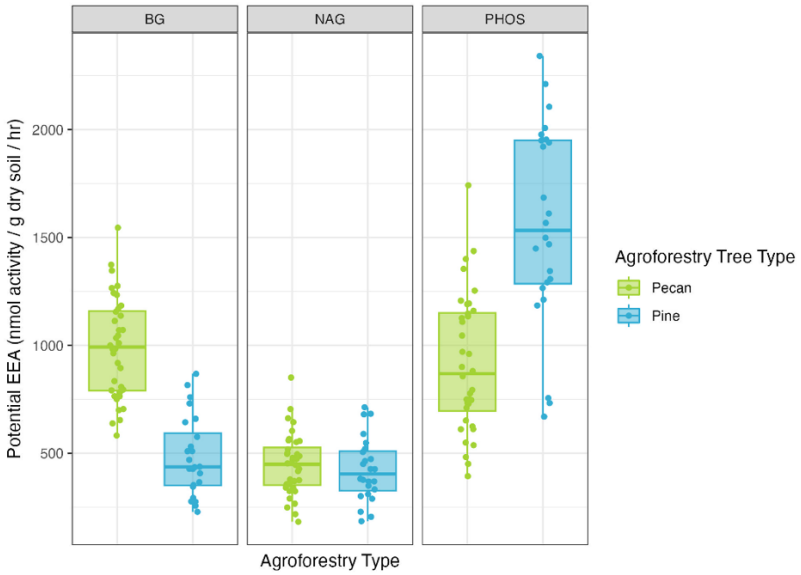

Figure 18. Effect of agroforestry tree type by enzyme on potential extracellular enzyme activity at NCA&T. BG: 4-Methylumbelliferyl β-D-glucopyranoside, NAG: 4-Methylumbelliferyl N-acetyl-β-D-glucosaminide, PHOS: 4-Methylumbelliferyl phosphate.

When considering combined agroforestry regimes (pecan and pine together), differences in cover crop type caused significant differences in activity of BG and PHOS (Table 2, Figures 19 and 21, $p < 0.001$ and $p < 0.001$, respectively), but only a small effect in activity of NAG (Table 2, Figure 20, $p = 0.10$).

When plots using pecan and pine trees for agroforestry were considered together, there were indications that some cover crop mixtures led to increases in BG activity over mono cover crops, and that cover crop mixes with cereals as a base led to higher BG activity than those with radish (a brassica) as a base (Figure 19).

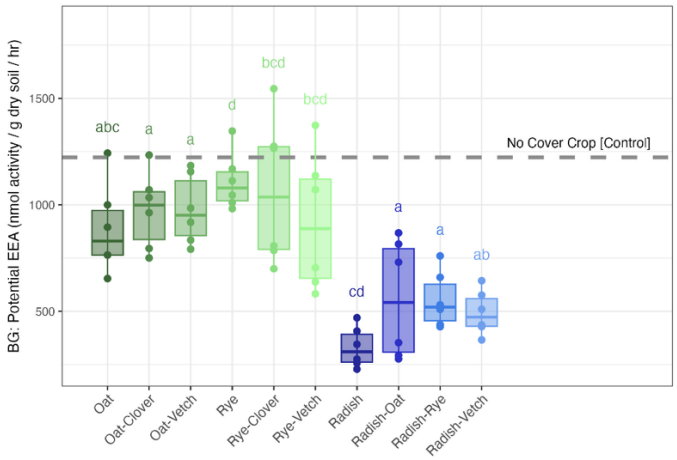

Figure 19. Effect of cover crop type on potential extracellular enzyme activity of BG at NCA&T. BG: 4-Methylumbelliferyl β-D-glucopyranoside. Shades of green (left) correspond to pecan agroforestry, and shades of blue (right) correspond to pine agroforestry.

While NAG activity showed a similar general trend to BG activity, there was less of a difference in activity between cover crops with cereals as a base and radish as a base (Figure 20).

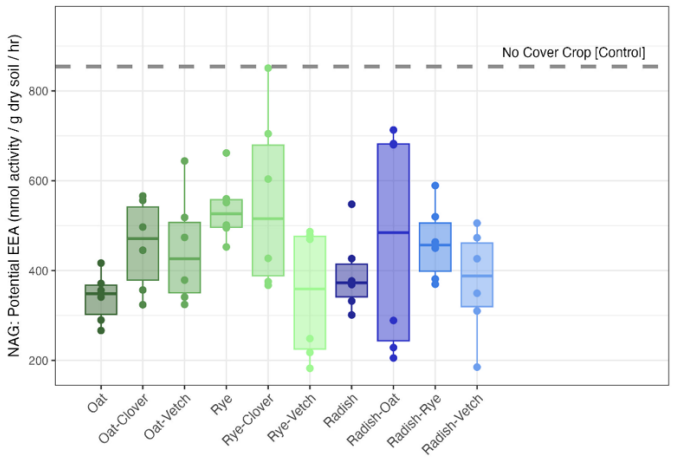

Figure 20. Effect of cover crop type on potential extracellular enzyme activity of NAG at NCA&T. NAG: 4-Methylumbelliferyl N-acetyl-β-D-glucosaminide. Shades of green (left) correspond to pecan agroforestry, and shades of blue (right) correspond to pine agroforestry.

In the same plots considering pecan and pine agroforestry together, results pertaining to PHOS activity indicated an opposite trend in which cover crop mixes involving radish as a base led to

consistently higher activity than mixes using grasses as a base (Figure 21). There was not a clear trend in activity as differentiated by cover crop mixes and mono cover crops.

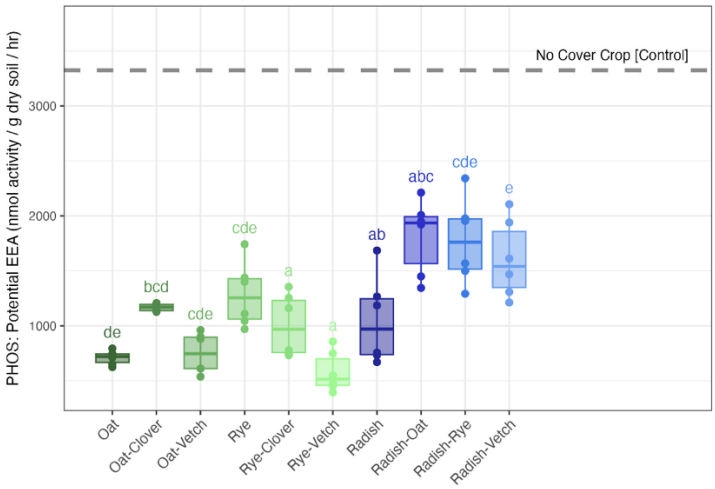

Figure 21. Effect of cover crop type on potential extracellular enzyme activity of PHOS at NCA&T. PHOS: 4-Methylumbelliferyl phosphate. Shades of green (left) correspond to pecan agroforestry, and shades of blue (right) correspond to pine agroforestry.

Within plots using only pecan agroforestry, different cover crop types caused significant changes in activity of NAG (Table 2, $p = 0.02$) and PHOS (Table 2, Figure 22, $p < 0.001$), but not BG (Table 2, $p = 0.59$). Results indicated that for PHOS activity considering cereal-based cover crops, the effect of mono vs. mixed cover crops may depend on the cover crop base itself.

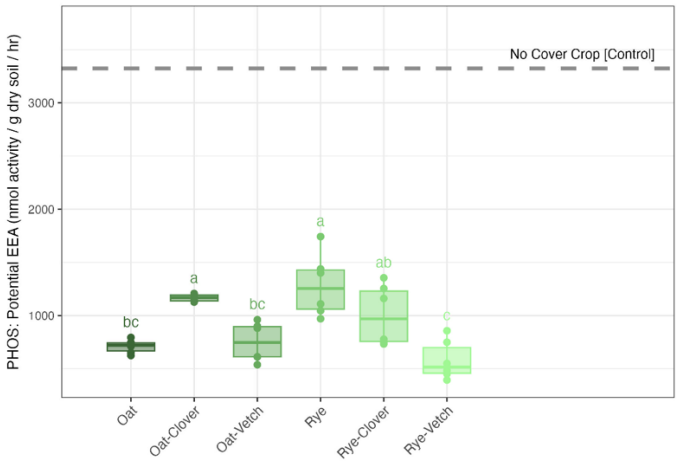

Figure 22. Effect of cover crop type on potential extracellular enzyme activity of PHOS with pecan agroforestry at NCA&T. PHOS: 4-Methylumbelliferyl phosphate.

Within the plots using only pine agroforestry, significant changes due to cover crop type occurred in PHOS activity (Table 2, Figure 23a, $p = 0.007$), and weak evidence of treatment effect was seen in BG activity (Table 2, $p = 0.10$), but not in NAG activity (Table 2, Figure 23b, $p = 0.63$). In particular, PHOS activity, and to a lesser extent BG activity, showed an increasing trend with brassica-based cover crop mixes over mono brassica cover crops.

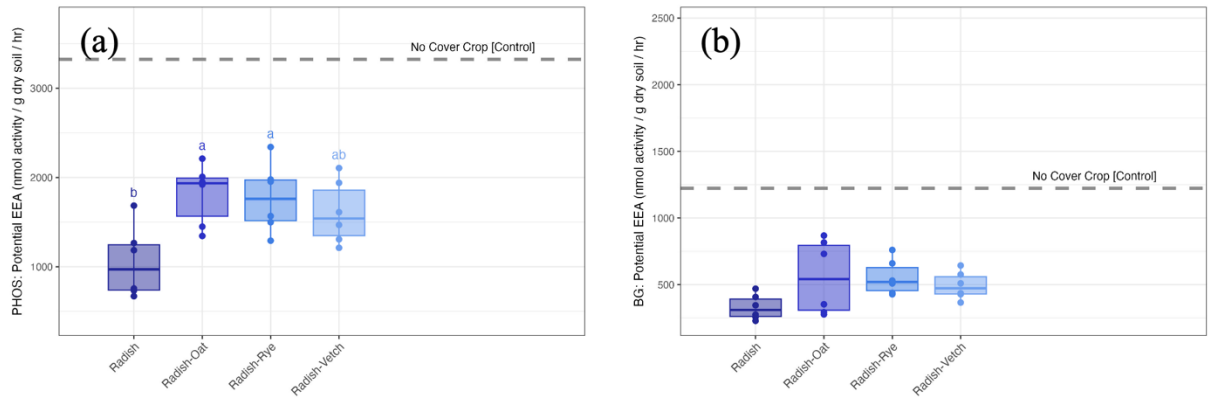

Figure 23. Effect of cover crop type on potential extracellular enzyme activity of PHOS (a) and BG (b) with pine agroforestry at NCA&T. PHOS: 4-Methylumbelliferyl phosphate, BG: 4-Methylumbelliferyl β-D-glucopyranoside.

Tillage treatments were not found to cause significant changes in BG, NAG, or PHOS activity (Table 2, Figure 24, $p = 0.47$, $p = 0.67$, and $p = 0.76$, respectively).

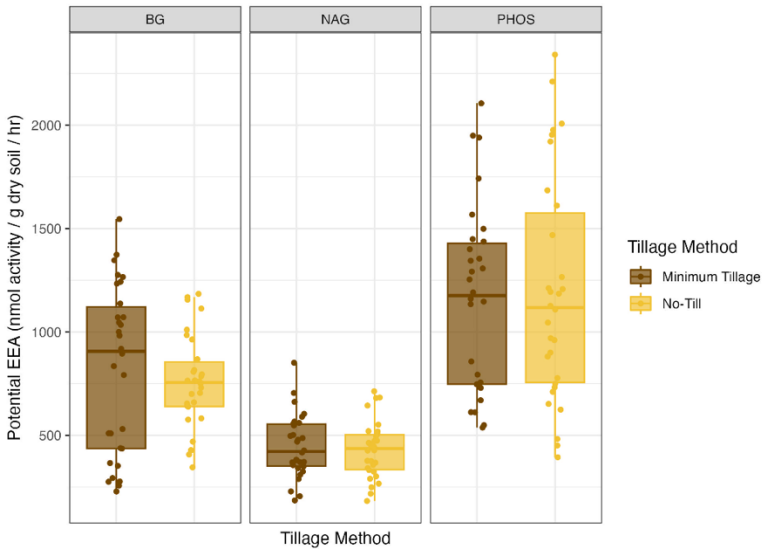

Figure 24. Effect of tillage method by enzyme type on potential extracellular enzyme activity at NCA&T. BG: 4-Methylumbelliferyl β-D-glucopyranoside, NAG: 4-Methylumbelliferyl N-acetyl-β-D-glucosaminide, PHOS: 4-Methylumbelliferyl phosphate.

3.2.4 Necromass

While weak evidence was found for pecan trees resulting in higher bacterial necromass than pine trees (Table 2, Figure 25b, p = 0.09), agroforestry tree type was not found to cause significant changes in total or fungal necromass (Table 2, Figure 25a, p = 0.58 and p = 0.97, respectively).

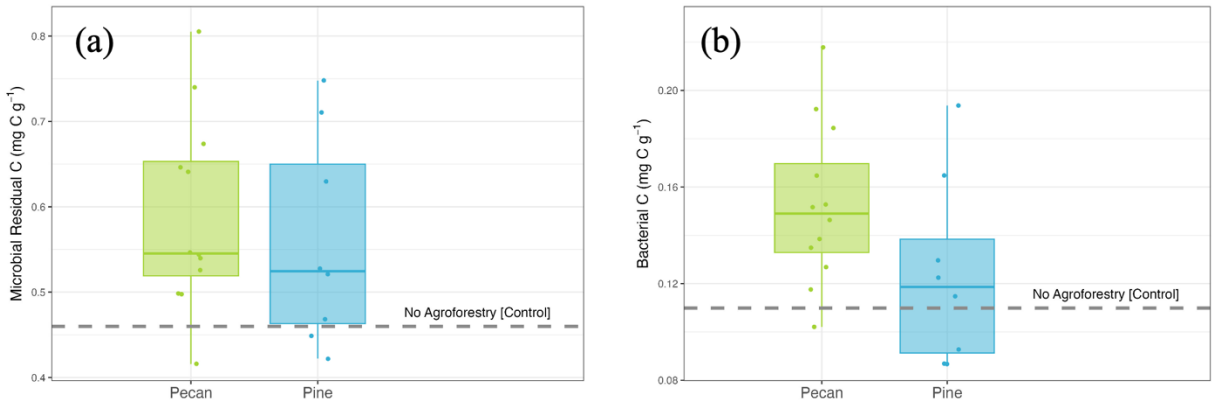

Figure 25. Effect of agroforestry tree type on total necromass (a) and bacterial necromass (b) at NCA&T.

While different types of cover crops did not cause significant changes in total necromass in plots using pine agroforestry (Table 2, p = 0.53) or pecan and pine when considered together (Table 2, Figure

26a, p = 0.50), significant differences in total necromass were observed in plots using pecan agroforestry (Table 2, Figure 26b, p = 0.03).

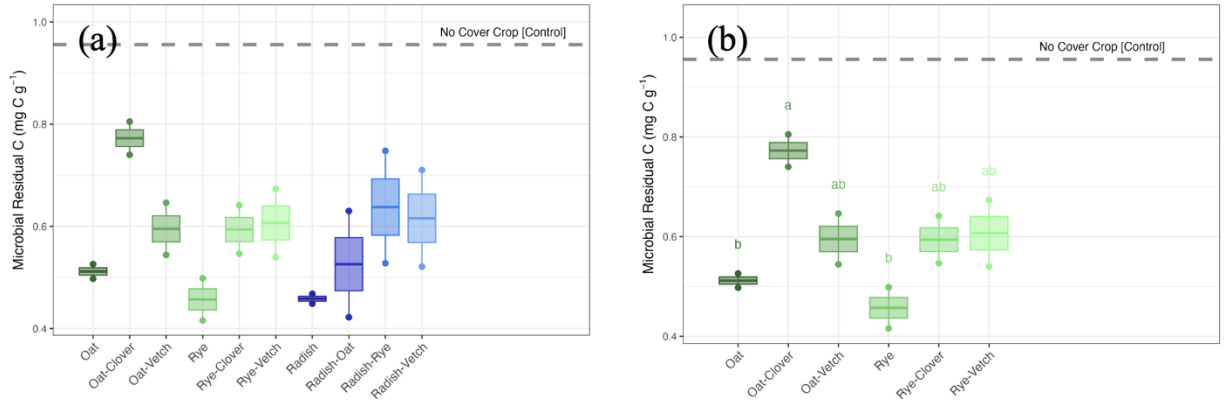

Figure 26. Effect of cover crop type on total necromass for all agroforestry (a) and pecan agroforestry (b) at NCA&T. Shades of green (left) correspond to pecan agroforestry, and shades of blue (right) correspond to pine agroforestry.

Similar to with total necromass, cover crop type only caused significant changes in fungal necromass in the plots using pecan agroforestry (Table 2, Figure 27, p = 0.04).

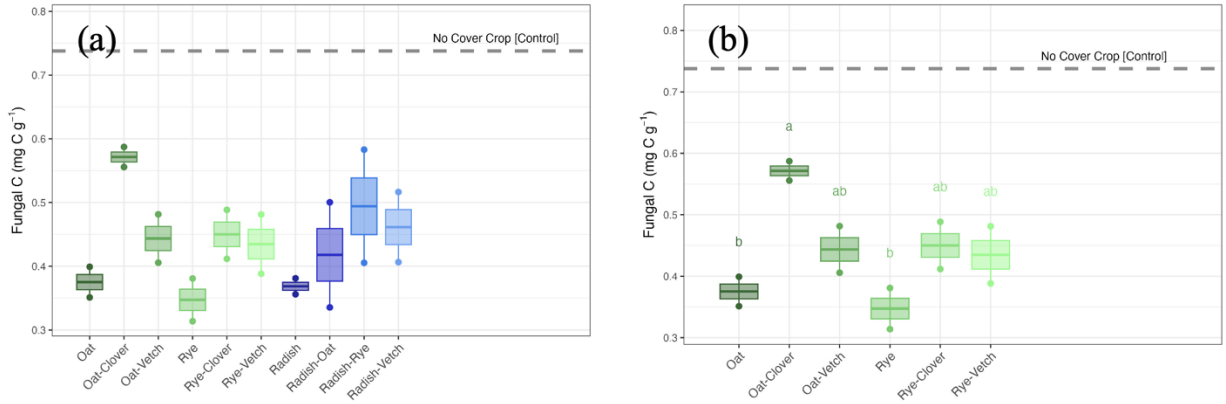

Figure 27. Effect of cover crop type on fungal necromass for all agroforestry (a) and pecan agroforestry (b) at NCA&T. Shades of green (left) correspond to pecan agroforestry, and shades of blue (right) correspond to pine agroforestry.

Following the same trend, significant changes in bacterial necromass due to cover crop type only occurred in plots under pecan agroforestry (Table 2, Figure 28, p = 0.03).

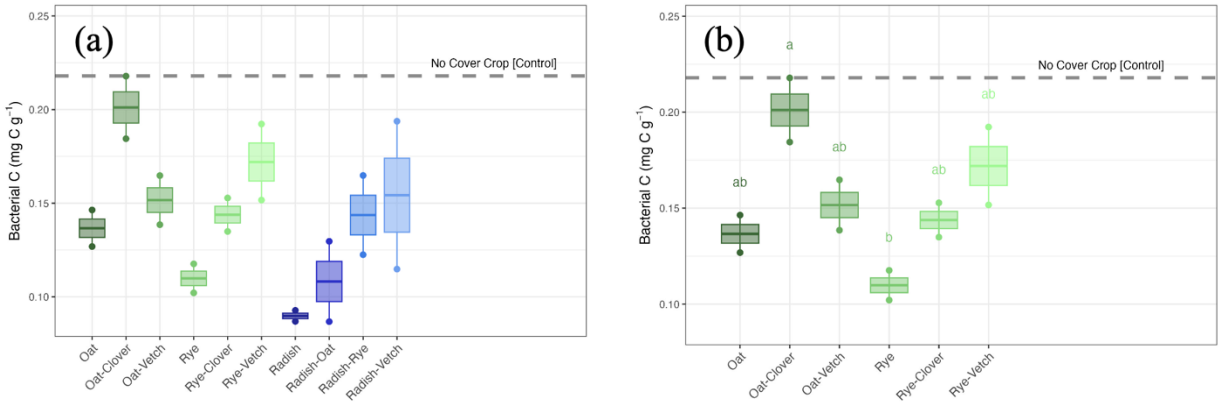

Figure 28. Effect of cover crop type on bacterial necromass for all agroforestry (a) and pecan agroforestry (b) at NCA&T. Shades of green (left) correspond to pecan agroforestry, and shades of blue (right) correspond to pine agroforestry.

Tillage treatments did not cause significant changes in total, fungal, or bacterial necromass (Table 2, Figure 29, p = 0.85, p = 0.83, and p = 0.91, respectively).

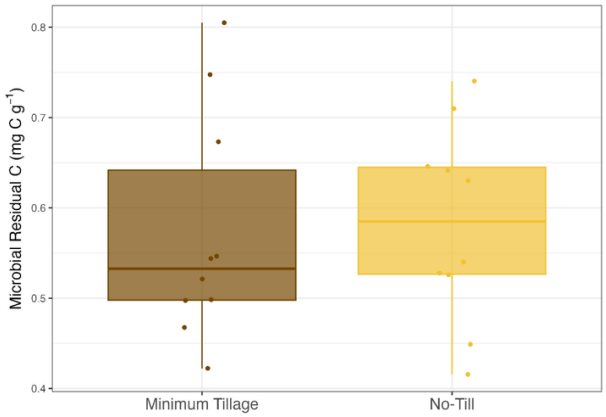

Figure 29. Effect of tillage method on total necromass (microbial residual carbon) at NCA&T.

Interactions were not found between agroforestry tree type and tillage on total, fungal, or bacterial necromass (Table 2, p = 0.70, p = 0.84, and p = 0.35, respectively). Interactions between cover crop type and tillage on necromass were not able to be performed due to a small sample size limited to only one season of data.

3.2.5 Total C & N

Agroforestry with pecan trees as compared to pine trees led to significantly higher total C ($p < 0.001$, Table 2, Figure 30a) and total N ($p < 0.001$, Table 2, Figure 30b).

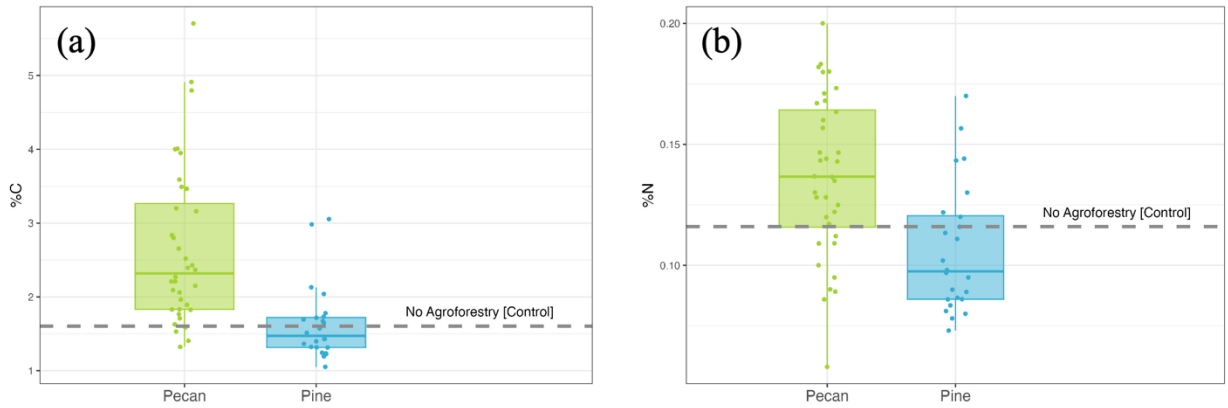

Figure 30. Effect of agroforestry tree type on total carbon (a) and total nitrogen (b) at NCA&T.

Total C differed significantly by cover crop type ($p < 0.001$, Table 2), with some indications that cover crop mixes incorporating legumes led to higher total C than as mono cereal or brassica cover crops (Figure 31a). Total N also differed significantly by cover crop type ($p = 0.01$, Table 2, Figure 31b), showing roughly the same trend as total C.

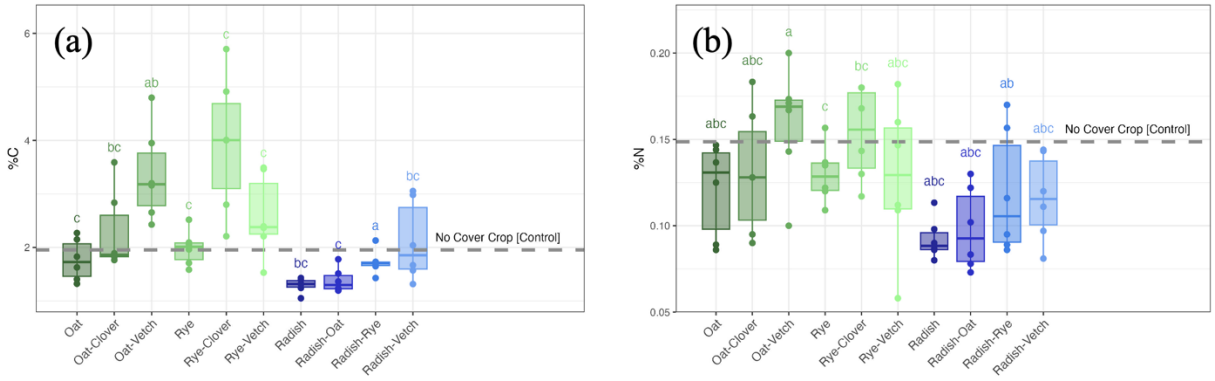

Figure 31. Effect of cover crop type on total carbon (a) and total nitrogen (b) at NCA&T. Shades of green (left) correspond to pecan agroforestry, and shades of blue (right) correspond to pine agroforestry.

Differences due to conservation tillage method were not observed for total C ($p = 0.56$, Table 2, Figure 32a) or total N ($p = 0.68$, Table 2, Figure 32b).

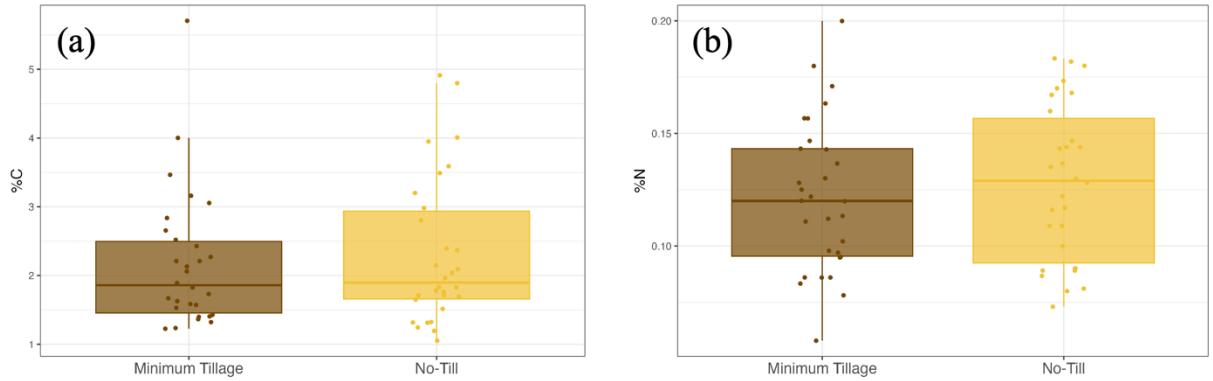

Figure 32. Effect of tillage method on total carbon (a) and total nitrogen (b) at NCA&T.

Interactions were not found between tree type and tillage ($p = 0.57$, Table 2) or cover crop type and tillage ($p = 0.85$, Table 2) for total C. Interactions were also not found between tree type and tillage ($p = 0.87$, Table 2) or cover crop type and tillage ($p = 0.82$, Table 2) for total N.

3.2.6 Correlation Matrix

A correlation matrix for soil C cycle proxies measured at NCA&T showed strong positive correlations between BG activity and MBC, NAG activity and MBN, and PHOS activity and NAG activity (Figure 33). PHOS activity showed a strong negative correlation with respiration, and NAG activity showed a weaker negative correlation with respiration. The opposite is true for BG activity, which showed a weak positive correlation with respiration. Respiration was more strongly correlated with necromass, especially bacterial necromass, than with MBC or MBN. Total C and N were positively correlated with necromass (particularly bacterial necromass), respiration, microbial biomass C and N, and BG activity.

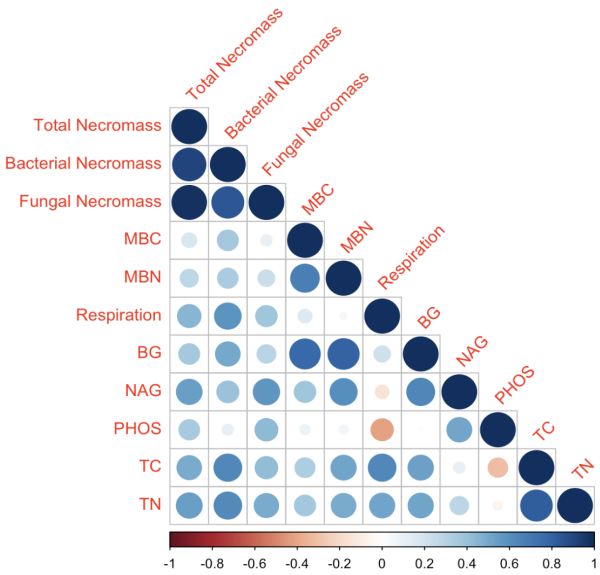

Figure 33. Correlation matrix showing positive and negative correlations between soil carbon cycle proxies measured at NCA&T.

4. Discussion

4.1 Microbial Biomass

Our finding that MBC and MBN increased at both Oxford Farm and NCA&T as a result of reducing tillage (Tables 1 and 2, Figures 3 and 12) was in line with the general consensus in the literature. Results from a meta-analysis have indicated that MBC and MBN are generally greater under no-till than with tillage (Balota et al., 2003; Zuber & Villamil, 2016), including in other Ultisol systems (Alvear et al., 2005; Bini et al., 2014). Reducing or eliminating tillage has been noted to stimulate microbial biomass through preventing microclimate degradation and increasing formation and stabilization of macroaggregates (Zuber & Villamil, 2016). Less disturbance could maintain a slow release of labile C from decomposing plant residues that could sustain microbial biomass more in a reduced (or no) tillage system than an intensive tillage system subjected to a temporary flush of labile C (and microbial activity) (Balota et al., 2003). This result is also promising from an economic perspective, as other studies in the Southeast U.S. within the same soil type have documented positive correlations between microbial

biomass and crop yield, likely due to greater cycling of essential nutrients through microbial biomass (Insam et al., 1991).

Our observation of no significant relationship between amendment and MBC and only a small effect of amendment on MBN at Oxford Farm (Table 1, Figure 4) can be attributed to properties of the amendments themselves and soils in question (Li et al., 2020). While some studies reported that application of biochar increased MBC (Pokharel et al., 2020), others reported no effect of biochar on microbial biomass (Foster et al., 2016; Galvez et al., 2012). Biochar may not provide substantial enhancement of microbial activity when pyrolyzed at too high temperature (i.e., 700°C) (Zhang et al., 2014). Application of biochar through the biochar-compost mix appeared to increase MBC more under shallow tillage than deep tillage, which may be due to increased contact of microbial habitats with the amendment (biochar+compost>compost) that can sustain a slow release of organic C to match with microbial demand. Application of compost has also been demonstrated by others to increase MBC and MBN (Perucci, 1990). The small effect observed here of amendment on MBN as compared to MBC (Table 1, Figure 4) indicated that N may be more sensitive to management than C in highly weathered Ultisols in the southeast U.S. (Bini et al., 2014). However, considerable uncertainty in interpretations of these measurements is expected as the response of MBC and MBN to compost could also depend heavily on the properties of the compost (Jedidi et al., 2004).

In general, incorporating agroforestry into agroecosystems modifies the soil microclimate, which impacts microbial dynamics and nutrient cycling (Amatya et al., 2002). Growing trees in tree-crop combinations has been found to increase soil microbial biomass (Chander et al., 1998). Another study in the Midwest U.S. reported that agroforestry increased MBC and MBN as compared to crop rotations without trees due to the woody perennial nature of the C inputs (Eddy & Yang, 2022). Our observation at NCA&T of higher MBC under pecan agroforestry (Table 2, Figure 12) is in agreement with others noting it to be a beneficial practice. For example, a study in the Southern U.S. found that a pecan-cotton alley cropping system resulted in higher microbial biomass than cotton monocrop without trees (Lee & Jose, 2003). Our finding that pine agroforestry does not provide a dramatic increase in microbial biomass was

also in line with another study that reported lower microbial biomass in an agroforestry system with Monterey pine (*Pinus radiata*) trees than in comparable grassland soils (Saggar et al., 2001). The difference in contributions to microbial biomass between our two agroforestry regimes may indeed be caused by differences in aboveground biomass that affect leaf litter inputs, and substrate degradability. Pine and other evergreen trees produce difficult to degrade litter, while pecan and other deciduous trees generally produce litter that is vulnerable to be more rapidly decomposed (Polyakova & Billor, 2007). Given that litter quality influences microbial biomass (Ndaw et al., 2009), differences in litter quality between pecan and pine trees likely contributed to observed differences in microbial biomass between their respective agroforestry regimes in our study.

Although significant differences in microbial biomass by cover crop type were not observed at NCA&T (Table 2), this may be due to the relatively recent start (3 years) of cover crop incorporation, considering that cover crops have been shown by others to increase MBC and MBN (Muhammad et al., 2021). One study found greater microbial biomass in the surface layer when radiata pine (*Pinus radiata* D. Don) pasture systems included ryegrass (*Lolium perenne*) as an understory than without ryegrass (Amatya et al., 2002), pointing to the utility of agroforestry and alley cropping as climate-smart farming techniques. While no significant interaction was observed between cover crop type and tillage for microbial biomass (Table 2), others have found increases in microbial biomass in no-till crop rotation systems following high-residue crops, and the opposite in tilled plots (Granatstein et al., 1987).

4.2 Respiration

At NCA&T, similar to with MBC, higher microbial respiration was noted in pecan than in pine trees (Table 2, Figure 14), likely due to higher microbial activity fueled by labile plant C input in pecan. This was especially the case under no-till, and represented by the weak interaction effect observed between tree type and tillage method. Agroforestry plots at NCA&T have been established for longer than cover crop or tillage treatments, potentially explaining the differences observed. While not many studies comparing the effects of agroforestry type on microbial respiration using similar methods were found in

the literature, short-term alley cropping was noted to increase soil respiration in another pecan agroforestry system in the Southeast U.S. (Lee & Jose, 2003).

Our observation that microbial respiration differed by cover crop type (Table 2, Figure 15) fits with what others have found at multiple agricultural field sites (Crookston et al., 2023). A field experiment in Georgia found higher CO_2 flux rates from plots using clover as a cover crop than rye, both in no-till and conventionally tilled plots (Hendrix et al., 1988), which corresponds to our observed increases in respiration with legume cover crop mixes in pecan agroforestry. Another field study done in Georgia also demonstrated that legume cover crops and legume-rye mixes increased soil respiration in comparison to just rye (Muhammad et al., 2021; Sainju et al., 2007). Leguminous cover crops have been shown to generally increase microbial activity (Dinesh et al., 2009), likely in response to their ability to increase nutrient availability and thus plant biomass production (Lange et al., 2015).

Significant differences in respiration by cover crop type were only observed when subset to just the plots using pecan agroforestry (Table 2), not to just those using pine, which could be because the higher C input from pecan litter as discussed earlier permits the differences between cover crops to be more readily observed.

Our finding that tillage treatment didn't lead to a significant difference in respiration (Table 2, Figure 16) could be somewhat surprising, as tillage has been shown to stimulate release of CO_2 through soil aeration and the exposure of OM to potential attack by microbes (Bini et al., 2014). However, previous findings have been mixed as one field experiment in Georgia did find significantly higher CO_2 efflux from no-till than conventionally tilled soils (Hendrix et al., 1988). Furthermore, at NCA&T, no-till was only compared to minimum tillage, as opposed to conventional tillage. As such, this result provides evidence for conservation tillage practices maintaining CO_2 losses from soils, or at least not causing significant increases in CO_2 losses. This is relevant in weighing the benefits of conservation tillage (e.g., more weed suppression than no-till system, etc.) against perceived shortcomings, such as increased loss of CO_2 when compared to no-till. In addition, tillage treatments started relatively recently (~3 years), and enough time for significant differences in respiration to manifest may not have elapsed.

4.3 Enzyme Activities & Kinetics

4.3.1 Enzyme Kinetics (Oxford Farm)

Of the enzymes surveyed at Oxford Farm, Vmax of BG and PHOS were influenced by CSA treatments (Table 1, Figures 6 and 7), suggesting that these two enzymes may act as reliable indicators of soil health across both sites. While effects of amendment and tillage on enzyme kinetic parameters of BG are not well documented, our finding of an interaction between amendment and tillage on V_{max} of BG (Table 1, Figure 6a) aligns with other work showing that soil amendments impact enzyme kinetic parameters (Raiesi & Khadem, 2019). Generally, organic fertilizers improve macroaggregation of soil particles, which can protect SOM from being broken down by soil organisms and extracellular enzymes (Ye et al., 2019). One study found that application of relatively labile plant litter increased V_{max} values, including for BG and LAP, while the application of relatively stable compost generally suppressed V_{max} (Morrissey et al., 2014). Similar to our findings (Table 1), this study did note that differences in K_m by compost treatment did not occur (Morrissey et al., 2014).

Biochar application has also been documented to affect activity of BG, and one study showed that maize residue biochar application led to an increase in V_{max} (representing increased enzyme concentration) and a decrease in K_m (signaling an increase in substrate affinity) in a sandy loam soil (Raiesi & Khadem, 2019). However, in a clayey soil, maize residue biochar application did not affect K_m, indicating that the effect of biochar application on BG was more dependent on soil properties (Raiesi & Khadem, 2019). This dependency on soil properties rather than management practices may help explain the interaction observed between amendment and tillage treatments on K_m of BG (Table 1, Figure 6b), as well as the weak evidence for the impact of amendment on V_{max} of BG (Table 1, Figure 7b). In another study, while biochar inhibited the activity of BG, LAP, and NAG, there were indications that both compost and biochar-compost may strengthen BG, LAP, and NAG activities (Zhao et al., 2022).

Our finding of a decrease in V_{max} of PHOS with biochar & compost as compared to compost alone (Table 1, Figure 7a) and weak evidence of the impact of amendment on V_{max} of NAG (Table 1,

Figure 8) is in line with findings from another field experiment, which noted reduced activities of BG and PHOS, and increased activities of NAG when amended with biochar (Foster et al., 2016). However, application of biochar has also been shown to increase activity of alkaline phosphatase, although effects are known to vary across soil type and biochar properties (Pokharel et al., 2020).

Another consideration is that a sorption effect has been identified through a laboratory experiment as a mechanism by which certain types of biochar cause decreases in activity of BG and PHOS, because types of biochar with large surface areas are able to sorb nutrients and other organic molecules (Foster et al., 2018). To some extent, enzyme activities in agricultural soils indicate the system's capacity to display sustainable nutrient cycling, such that the decomposition of organic inputs can slowly release essential elements to match crop demand, thus reducing the need for external fertilizer application. For this reason, a potential decrease in enzyme activities with certain CSA practices is relevant due to the implication of reduced soil C and nutrient cycling (Foster et al., 2018).

While a significant relationship was not found between amendments and enzyme kinetic parameters of NAG, LAP, or XYL (Table 1), other work suggests that this may not be surprising. In one field experiment, biochar was found to have no effect on activity of LAP (Foster et al., 2016; Galvez et al., 2012) or XYL (Foster et al., 2016). Other results indicate that the effect of biochar on N cycling enzymes such as LAP depends on the rate of biochar addition (Foster et al., 2016; Wang et al., 2015). This may explain the weak evidence of tillage treatment affecting K_m of LAP (Table 1, Figure 9), which would impact the rate at which amendments were made available to soil microbes. Studies have shown that the effect of biochar on XYL also varies substantially and depends partially on initial conditions (Foster et al., 2016).

4.3.2 Enzyme Activities (NCA&T)

The C-degrading enzyme BG is generally considered a good indicator of soil changes from management (Sanchez et al., 2019). Our observation at NCA&T that pecan agroforestry led to higher activity of BG, a C-degrading enzyme, while pine agroforestry led to higher activity of PHOS, a P-

degrading enzyme, may be due to differences in quality and type of nutrient inputs from each tree (Table 2, Figure 18). As discussed earlier, the broadleaf litter content provided by pecan trees may be more easily degradable in comparison to the needleleaf litter provided by pine trees. Additionally, BG activity has been found to decrease when soil moisture decreases (Wang et al., 2022). This is relevant because agroforestry systems may stimulate competition for nutrients and water between trees and pasture (Amatya et al., 2002) or crops. Our observation that tree type did not influence activity of the N-degrading enzyme (NAG) (Table 2, Figure 18) was opposite what was anticipated, as agroforestry with pecan trees has been shown in other studies to increase NAG activity (Wang et al., 2022).

Our finding that cover crop type affected activity of BG (Table 2, Figure 19) is consistent with multiple studies that have found enzyme activities to be greater with mixed legume-nonlegume cover crops rather than purely legume or nonlegume cover crops (Muhammad et al., 2021; Mukumbareza et al., 2016). Others in nearby regions have also found a general sensitivity of enzyme activities to legume cover crops, particularly for enzymes indicative of C cycling and N mineralization (Farmaha et al., 2022). This could explain the increase observed in BG activity with legume mixes (Table 2, Figure 19). This observation also corresponds to results of a field experiment in North Carolina, in which researchers found that crimson clover and hairy vetch cover crops resulted in higher enzyme activity of BG and NAG (Farmaha et al., 2022; Liang et al., 2014). Our result is further corroborated by a Louisiana field experiment, where BG increased in the first year of cover crop application with legumes (including clover and hairy vetch) in comparison with fallow and grass / brassica mono and mixed cover crops (rye, forage radish, and rye with forage radish) (Farmaha et al., 2022). NAG, which is linked to C and N cycling in soils, has been found to respond to management in similar ways to BG in some experiments (Acosta-Martínez et al., 2007) but did not show direct responses to cover crop treatments in a field experiment in Louisiana (Sanchez et al., 2019). This validates our finding that NAG was not changed significantly by cover crop type when considering pecan and pine sites combined (Table 2, Figure 20).

Multiple studies have suggested that legumes release more phosphatase enzymes in comparison to non-legumes (Makoi et al., 2010) due to their need for additional P for improved nodulation and

efficient biological N fixation (Adetunji et al., 2021; Zhong et al., 2022). This may explain our observation of higher PHOS activity under radish-vetch than radish alone (Table 2, Figures 21 and 23a), as does a greenhouse experiment that found higher phosphatase activity under legumes than cereal or brassica, possibly demonstrating a higher potential ability of legumes to mobilize P (Maltais-Landry, 2015). However, the same trend was not observed in PHOS activity for cereal-based cover crop mixes. One study comparing mycorrhizal to non-mycorrhizal cover crops found an increase of ~30-35% in PHOS activity using oilseed radish (*Raphanus sativus* L. var. *oleiferus Metzg*) as a cover crop (Kunze et al., 2011). Forage radish is another non-mycorrhizal brassica cover crop (White & Weil, 2010), so this finding could explain the increase observed in PHOS activity under radish-based cover crops.

A significant effect of tillage method on enzyme activity was not observed (Table 2, Figure 24), in contrast to what was expected due to enzyme activities having been shown to generally be greater under no-till than with tillage for BG (Mankolo et al., 2012; Zuber & Villamil, 2016), NAG (Ekenler & Tabatabai, 2003), and phosphatase (Mankolo et al., 2012) in other studies. C- and N-cycling enzyme activity in particular have been noted to increase under no-till as compared to in tilled systems (Alvear et al., 2005), perhaps due to more favorable microclimate and protection against disturbance for fungal hyphae, as fungi are essential to C and nutrient cycling and related enzyme activity (Zuber & Villamil, 2016).

4.4 Necromass

Microbial necromass composes up to 80% of the organic C in soil, making microbial contributions to SOM essential to consider while evaluating soil health under different management practices (Liang & Balser, 2011; Ye et al., 2019). A large portion of SOM turnover and thus C sequestration is dependent upon the properties of soils and the fertilizers used on them (Stockmann et al., 2013; Ye et al., 2019). Although differences in microbial biomass from treatments were observed at Oxford Farm (Table 1, Figures 3 and 4), our observations of necromass suggest that the same trends did not carry over to accumulation of stable C forms within our study period (Tables 1 and 2, Figures 10, 25-

29). Contrary to this finding, trends in necromass have been documented in the literature, although treatments were generally applied for longer than have been at Oxford Farm. A long-term field experiment done in an Ultisol found that while fertilization increases amino sugars in general, application of manure may contribute to SOM accumulation and stabilization to some extent through increasing fungal necromass in particular, which was favored over bacterial necromass (Ye et al., 2019). However, this study applied fertilizers for 27 years, suggesting that differences in necromass may require longer timescales than the one season of data produced so far at Oxford Farm.

At NCA&T, while activity of live microbes increased under pecan agroforestry, there was only weak evidence indicating that pecan agroforestry resulted in higher bacterial necromass than pine, and neither total nor fungal necromass changed appreciably under different tree types (Table 2, Figure 25). While comparable studies of microbial necromass by agroforestry tree type were not found in the literature, other results have demonstrated that plant species influences the composition of soil bacterial necromass (Menpadi et al., 2023). The lack of significant results in total and fungal necromass at NCA&T (Table 2) may also have to do with the limited amount of time elapsed since the onset of treatments.

Our results at NCA&T suggest that, at least within pecan agroforestry, total, fungal, and bacterial necromass may be increased under cover crop mixes over mono cover crops (Table 2, Figures 26-28). Cover crops provide plant residues to the soil at varying quality. They can favor bacterial growth when they have high N content (or a low C/N ratio), while lower-quality residues contribute more to fungal growth (Bossuyt et al., 2001; Muhammad et al., 2021). Cover crops characterized by high litter quality (i.e., legumes) have been demonstrated to contribute more to accumulation of microbial necromass C than those characterized by low litter quality (i.e., grasses) (Hollister et al., 2013), which may explain the differences we've observed. An agricultural field study at multiple orchard sites in China found that legume cover crops led to higher amounts of microbial necromass (Hu et al., 2023), further supporting our observation of higher necromass when legumes were incorporated into cereal-based cover crops (Figures 26-28).

A long-term cover crop field experiment in Pennsylvania suggested that extra N inputs supplied by legumes may inhibit fungal growth and hinder the accumulation of fungal necromass (Zhang et al., 2022). The same study also found higher concentrations of fungal necromass C under grass monoculture than brassica monoculture, which they attribute to the anti-fungal and non-mycorrhizal properties of brassicas (Hollister et al., 2013; Zhang et al., 2022). This may explain our observed increase in fungal C from radish monocrop to radish cover crop mixes (Figure 27a), although these differences were not deemed to be statistically significant.

Similar to microbial respiration and enzyme activities, significant differences in necromass between tillage treatments were not observed (Table 2, Figure 29), perhaps due to the fact that more disruptive tillage methods were not considered, and thus even expected differences would be slight.

4.5 Correlation Matrices

The biological proxies observed in this study provided varying degrees of useful information, and they appear to react to changes in soil management and conditions at varying rates and degrees of agreement with each other and with total pools. At both sites, MBC acted as a reliable indicator of how reducing tillage can spur biological activity, with results being consistent across sites (Tables 1 and 2) and with others' findings (Carter, 1986). On the other hand, significant differences in necromass were only observed between cover crop types in the NCA&T plots in pecan agroforestry (Tables 1 and 2).

Some researchers have found a positive correlation between MBC and soil enzyme activities (Chavarría et al., 2016). Previous research on Ultisols has noted close associations between MBC, MBN, and enzyme activities of BG in particular (Alvear et al., 2005), which is in contrast to our finding of a negative correlation between BG kinetics and both MBC and MBN at Oxford Farm (Figure 11). However, a correlation between BG activity and MBC was observed at NCA&T (Figure 33). This inconsistency could be due to different C and nutrient economy of soil at each site. At Oxford Farm, PHOS kinetics were positively correlated to MBC and MBN (Figure 11), which may point to microbes becoming more P-limited when their C needs are fulfilled through decomposition of organic matter at that

site. On the other hand, relatively high plant litter input in the agroforestry system may have increased the supply of C substrates, which could have stimulated microbial biomass and production of BG.

Other studies have demonstrated correlations between enzyme activities and soil C and N pools, as well as microbial biomass (Waldrop et al., 2000), similar to what was observed at NCA&T (Figure 33). Specifically, NAG activity was correlated with soil organic C (SOC) (Ekenler & Tabatabai, 2003), BG activity was correlated with total N and SOC (Mankolo et al., 2012), and a correlation has been observed between LAP activity and total N (Foster et al., 2016). However, some have found BG and phosphatase activity to not be directly related to total N content (Waldrop et al., 2000). The positive correlations at NCA&T between BG activity and MBC, and NAG activity and MBN (Figure 33), likely represent extracellular enzyme-mediated microbial degradation of C- and N-based substrates, respectively (Ashraf et al., 2021).

Our finding of a stronger correlation between respiration and necromass than respiration and microbial biomass (Figure 33) can be understood using the aforementioned conceptual framework of the microbial C pump: the process of transforming labile soil C to stable soil C releases CO_2 as necromass accumulates (Liang et al., 2017). While this correlation was not found to be strong in our study, soil respiration has been noted to be correlated to microbial biomass in other agroforestry systems (Lee & Jose, 2003).

A correlation between total N and MBN, which we observed, has also been previously documented (Jedidi et al., 2004). In addition, other studies have also found BG activity to also be positively correlated to total C, due to its role in soil C cycling (Eivazi & Tabatabai, 1990). The positive correlation we observed between total C and both bacterial and fungal necromass has been found by others as well (Wang et al., 2021). This correlation may be another indication in support of the microbial C pump (Liang et al., 2017), whereby accumulation of microbial necromass is reflected in the total C within the soil system.

5. Societal Implications of Climate-Smart Practices

CSA offers a flexible, context-specific framework, and practices that contribute to resilience and resource use efficiency (Lipper et al., 2014). On a global level, CSA can support rural livelihoods and climate resilience through enhancing productivity and profitability of small-scale local food systems (U. Das & Ansari, 2021; Imran et al., 2018; Lipper et al., 2018). In 2021, about 89% of all U.S. farms were small-scale family farms, operating on nearly 50% of U.S. agricultural land (Whitt et al., 2022). As such, there is immense potential in building and enhancing integrated small-scale resilient farming systems.

Climate-smart practices such as conservation tillage can be economically beneficial to farmers, although this is largely dependent on site-specific factors (Uri, 2000). While conservation tillage methods can mean less pre-season labor investment due to a reduction in number of occurrences and passes in which tillage equipment is used, labor savings may be slightly offset when additional chemical application is needed (Christensen, 1985). However, some research demonstrates that this increase in cost is often negligible (Christensen, 1985).

While upscaling these practices has clear utility as a strategy to promote global development and sustainability, it's important to note that many small-holder farmers may already inadvertently practice these techniques (Fanen & Olalekan, 2014). Furthermore, impact of farming techniques on rural livelihoods varies based on the level of involvement and investment they necessitate, the difference in yields spurred by these techniques, political context, and the existence of incentives (Fanen & Olalekan, 2014; Waaswa et al., 2021). CSA presents opportunities for smallholder farmers, including women and indigenous groups, who are especially affected by climate change impacts (FAO, 2021). For instance, a case study in Somalia revealed that while rural women were already applying practices that fit under the CSA umbrella, they were generally excluded from decision-making processes in agriculture (FAO, 2021). A key takeaway from this project was the need for programs supporting rural women in promoting both CSA and gender equality.

In addition, the impact on farms from adoption of these practices is also highly dependent on agricultural and socio-economic contexts (FAO, 2021; Lubwama, 1999; Waaswa et al., 2021). For

example, an International Fund for Agricultural Development program in Moldova had to take into account the region's heavy reliance on subsistence rainfed agriculture in identifying CSA practices that could contribute to sustainable development goals while being applicable for the regional context (FAO, 2021). Although often region- and site-specific, these concerns must be considered when comparing or recommending certain practices. For this study, decision-making regarding CSA treatments was done considering local conditions and in consultation with local experts and stakeholders. This approach prioritized small-scale farmers by providing them with information on the treatments that would be most realistic to apply.

6. Conclusions

Soil biological parameters can serve as informative proxies of soil carbon cycling, and can provide early indications of differences between effects of CSA techniques. Microbial biomass and respiration offered the clearest responses to soil management, and to tillage practices in particular. On a longer timescale, extracellular enzymes act as reliable indicators, particularly in assessing practices that supply nutrient inputs, the degradation of which by specific enzymes can be monitored. Enzyme activities also appear to be sensitive enough to reveal interactions between multiple practices. Microbial necromass functions as a less sensitive indicator in our sites likely due to the short duration of our study, although still a relevant one to assess indications of long-term C storage.

This study has provided an evaluation of soil biological proxies for soil carbon cycling, and insight into the relative utility of CSA techniques with high potential for adoption in the Southeast U.S. Over short (<3 season) time scales, cover cropping holds promise for enhancement of labile and stable forms of C, and conservation tillage is useful in increasing microbial biomass, which is a first step towards maximizing C storage. However, compost and biochar amendments may not lead to noticeable differences in soil C cycling or accumulation of stable forms of C in the short term. Over medium (5-10 season) time scales, agroforestry provides perennial inputs that spur accrual of labile C but may, along with other parameters investigated here, require longer periods of time to create distinct increases in

stable C. Further work remains to link long-term C storage and sequestration with management techniques like CSA on more immediate time scales. While the parameters considered here are situated in the context of Ultisols in the Southeast U.S., these findings demonstrate how understanding the relative utility of early indicators of soil C cycling is useful for assessing land management practices.

7. References

Acosta-Martínez, V., Mikha, M. M., & Vigil, M. F. (2007). Microbial communities and enzyme activities in soils under alternative crop rotations compared to wheat–fallow for the Central Great Plains. *Applied Soil Ecology*, *37*(1), 41–52. https://doi.org/10.1016/j.apsoil.2007.03.009

Adetunji, A. T., Ncube, B., Meyer, A. H., Olatunji, O. S., Mulidzi, R., & Lewu, F. B. (2021). Soil pH, nitrogen, phosphatase and urease activities in response to cover crop species, termination stage and termination method. *Heliyon*, *7*(1), e05980. https://doi.org/10.1016/j.heliyon.2021.e05980

Alvear, M., Rosas, A., Rouanet, J. L., & Borie, F. (2005). Effects of three soil tillage systems on some biological activities in an Ultisol from southern Chile. *Soil and Tillage Research*, *82*(2), 195–202. https://doi.org/10.1016/j.still.2004.06.002

Amatya, G., Chang, S. X., Beare, M. H., & Mead, D. J. (2002). Soil properties under a Pinus radiata – ryegrass silvopastoral system in New Zealand. Part II. C and N of soil microbial biomass, and soil N dynamics. *Agroforestry Systems*, *54*(2), 149–160. https://doi.org/10.1023/A:1015076607090

Ashraf, M. N., Jusheng, G., Lei, W., Mustafa, A., Waqas, A., Aziz, T., Khan, W.-D., Shafeeq-ur-Rehman, Hussain, B., Farooq, M., Wenju, Z., & Minggang, X. (2021). Soil microbial biomass and extracellular enzyme–mediated mineralization potentials of carbon and nitrogen under long-term fertilization (> 30 years) in a rice–rice cropping system. *Journal of Soils and Sediments*, *21*(12), 3789–3800. https://doi.org/10.1007/s11368-021-03048-0

Balota, E. L., Colozzi-Filho, A., Andrade, D. S., & Dick, R. P. (2003). Microbial biomass in soils under different tillage and crop rotation systems. *Biology and Fertility of Soils*, *38*(1), 15–20. https://doi.org/10.1007/s00374-003-0590-9

Beck, T., Joergensen, R. G., Kandeler, E., Makeschin, F., Nuss, E., Oberholzer, H. R., & Scheu, S. (1997). An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C. *Soil Biology and Biochemistry*, *29*(7), 1023–1032. https://doi.org/10.1016/S0038-0717(97)00030-8

Bell, C. W., Fricks, B. E., Rocca, J. D., Steinweg, J. M., McMahon, S. K., & Wallenstein, M. D. (2013).

High-throughput fluorometric measurement of potential soil extracellular enzyme activities. *Journal of Visualized Experiments*, *81*, e50961. https://doi.org/10.3791/50961

Benefits of Cover Crops. (2007). In *Managing Cover Crops Profitably*. SARE Outreach. https://www.sare.org/publications/managing-cover-crops-profitably/benefits-of-cover-crops/

Bini, D., Santos, C. A. dos, Bernal, L. P. T., Andrade, G., & Nogueira, M. A. (2014). Identifying indicators of C and N cycling in a clayey Ultisol under different tillage and uses in winter. *Applied Soil Ecology*, *76*, 95–101. https://doi.org/10.1016/j.apsoil.2013.12.015

Blesh, J., VanDusen, B. M., & Brainard, D. C. (2019). Managing Ecosystem Services with Cover Crop Mixtures on Organic Farms. *Agronomy Journal*, *111*(2), 826–840. https://doi.org/10.2134/agronj2018.06.0365

Borders, B. E., & Bailey, R. L. (2001). Loblolly Pine—Pushing the Limits of Growth. *Southern Journal of Applied Forestry*, *25*(2), 69–74. https://doi.org/10.1093/sjaf/25.2.69

Bossuyt, H., Denef, K., Six, J., Frey, S. D., Merckx, R., & Paustian, K. (2001). Influence of microbial populations and residue quality on aggregate stability. *Applied Soil Ecology*, *16*(3), 195–208. https://doi.org/10.1016/S0929-1393(00)00116-5

Brassicas and Mustards. (2007). In *Managing Cover Crops Profitably* (3rd ed.). SARE Outreach. https://www.sare.org/publications/managing-cover-crops-profitably/nonlegume-cover-crops/brassicas-and-mustards/

Busari, M. A., Kukal, S. S., Kaur, A., Bhatt, R., & Dulazi, A. A. (2015). Conservation tillage impacts on soil, crop and the environment. *International Soil and Water Conservation Research*, *3*(2), 119–129. https://doi.org/10.1016/j.iswcr.2015.05.002

Cambareri, G., Frusso, E. A., Herrera-Aguirre, E., Zoppolo, R., Leite, F. F. G. D., Beltrán, M., Martins, C., & Mendoza, C. (2023). Contribution of pecan (Carya illinoinensis [Wangenh.| K. Koch) to Sustainable Development Goal 2 under the dual perspective of carbon storage and human nutrition. *Frontiers in Soil Science*, *3*. https://www.frontiersin.org/articles/10.3389/fsoil.2023.1092003

Carter, M. R. (1986). Microbial biomass as an index for tillage-induced changes in soil biological properties. *Soil and Tillage Research*, *7*(1), 29–40. https://doi.org/10.1016/0167-1987(86)90005-X

Cereal Rye. (2007). In *Managing Cover Crops Profitably*. SARE Outreach. https://www.sare.org/publications/managing-cover-crops-profitably/nonlegume-cover-crops/cereal-rye/

Chander, K., Goyal, S., Nandal, D. P., & Kapoor, K. K. (1998). Soil organic matter, microbial biomass and enzyme activities in a tropical agroforestry system. *Biology and Fertility of Soils*, *27*(2), 168–172. https://doi.org/10.1007/s003740050416

Chavarría, D. N., Verdenelli, R. A., Serri, D. L., Restovich, S. B., Andriulo, A. E., Meriles, J. M., & Vargas-Gil, S. (2016). Effect of cover crops on microbial community structure and related enzyme activities and macronutrient availability. *European Journal of Soil Biology*, *76*, 74–82. https://doi.org/10.1016/j.ejsobi.2016.07.002

Chen, Y., Sun, K., Yang, Y., Gao, B., & Zheng, H. (2023). Effects of biochar on the accumulation of necromass-derived carbon, the physical protection and microbial mineralization of soil organic carbon. *Critical Reviews in Environmental Science and Technology*, *0*(0), 1–29. https://doi.org/10.1080/10643389.2023.2221155

Christensen, L. A. (1985). Economics of Conservation Tillage in the Southeast. In *Proceedings of the 1985 Southern Region No-Till Conference*. USDA-ARS, Southern Piedmont Conservation Research Center. https://scholar.googleusercontent.com/scholar?q=cache:_kAI-ZJ2aJAJ:scholar.google.com/+conservation+tillage+southeast&hl=en&as_sdt=0,11

Climate-Smart Agriculture. (2021, April 5). World Bank. https://www.worldbank.org/en/topic/climate-smart-agriculture

Crookston, B. S., Yost, M. A., Bowman, M., Veum, K., & Stevens, J. R. (2023). Microbial respiration gives early indication of soil health improvement following cover crops. *Journal of Soil and Water Conservation*, *78*(3), 272–281. https://doi.org/10.2489/jswc.2023.00015

Das, S., Chatterjee, S., & Rajbanshi, J. (2022). Responses of soil organic carbon to conservation practices including climate-smart agriculture in tropical and subtropical regions: A meta-analysis. *Science of the Total Environment*, *805*, 150428. https://doi.org/10.1016/j.scitotenv.2021.150428

Das, U., & Ansari, M. A. (2021). The nexus of climate change, sustainable agriculture and farm livelihood: Contextualizing climate smart agriculture. *Climate Research*, *84*, 23–40. https://doi.org/10.3354/cr01648

Davidson, E. A., & Janssens, I. A. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, *440*(7081), Article 7081. https://doi.org/10.1038/nature04514

Diffenbaugh, N. S., & Burke, M. (2019). Global warming has increased global economic inequality. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(20), 9808–9813. https://doi.org/10.1073/pnas.1816020116

Dinesh, R., Ghoshal Chaudhuri, S., Sheeja, T. E., & Shiva, K. N. (2009). Soil microbial activity and biomass is stimulated by leguminous cover crops. *Journal of Plant Nutrition and Soil Science*, *172*(2), 288–296. https://doi.org/10.1002/jpln.200700300

Don, A., Seidel, F., Leifeld, J., Kätterer, T., Martin, M., Pellerin, S., Emde, D., Seitz, D., & Chenu, C. (2023). Carbon sequestration in soils and climate change mitigation—Definitions and pitfalls. *Global Change Biology*, *n/a*(n/a), e16983. https://doi.org/10.1111/gcb.16983

Eivazi, F., & Tabatabai, M. A. (1990). Factors affecting glucosidase and galactosidase activities in soils. *Soil Biology and Biochemistry*, *22*(7), 891–897. https://doi.org/10.1016/0038-0717(90)90126-K

Ekenler, M., & Tabatabai, M. A. (2003). Tillage and residue management effects on β-glucosaminidase activity in soils. *Soil Biology and Biochemistry*, *35*(6), 871–874. https://doi.org/10.1016/S0038-0717(03)00094-4

Emory HPLC Bioanalytical Core. (n.d.). *Services*. Emory University. Retrieved February 18, 2024, from https://cores.emory.edu/hplc/services/index.html

Fahad, S., Chavan, S. B., Chichaghare, A. R., Uthappa, A. R., Kumar, M., Kakade, V., Pradhan, A.,

Jinger, D., Rawale, G., Yadav, D. K., Kumar, V., Farooq, T. H., Ali, B., Sawant, A. V., Saud, S., Chen, S., & Poczai, P. (2022). Agroforestry Systems for Soil Health Improvement and Maintenance. *Sustainability*, *14*(22), 14877. https://doi.org/10.3390/su142214877

Fanen, T., & Olalekan, A. (2014). Assessing the role of climate-smart agriculture in combating climate change, desertification and improving rural livelihood in Northern Nigeria. *African Journal of Agricultural Research*, *9*(15), 1180–1191. https://doi.org/10.5897/AJAR2013.7665

FAO. (2010). *"Climate-Smart" Agriculture: Policies, Practices and Financing for Food Security, Adaptation and Mitigation*.

FAO. (2021). *Climate-smart agriculture case studies 2021—Projects from around the world*. FAO. https://doi.org/10.4060/cb5359en

Farmaha, B. S., Sekaran, U., & Franzluebbers, A. J. (2022). Cover cropping and conservation tillage improve soil health in the southeastern United States. *Agronomy Journal*, *114*(1), 296–316. https://doi.org/10.1002/agj2.20865

Fierer, N., Wood, S. A., & Bueno de Mesquita, C. P. (2021). How microbes can, and cannot, be used to assess soil health. *Soil Biology and Biochemistry*, *153*, 108111. https://doi.org/10.1016/j.soilbio.2020.108111

Foster, E. J., Fogle, E. J., & Cotrufo, M. F. (2018). Sorption to Biochar Impacts β-Glucosidase and Phosphatase Enzyme Activities. *Agriculture*, *8*(10), Article 10. https://doi.org/10.3390/agriculture8100158

Foster, E. J., Hansen, N., Wallenstein, M., & Cotrufo, M. F. (2016). Biochar and manure amendments impact soil nutrients and microbial enzymatic activities in a semi-arid irrigated maize cropping system. *Agriculture, Ecosystems & Environment*, *233*. https://doi.org/10.1016/j.agee.2016.09.029

Galvez, A., Sinicco, T., Cayuela, M. L., Mingorance, M. D., Fornasier, F., & Mondini, C. (2012). Short term effects of bioenergy by-products on soil C and N dynamics, nutrient availability and biochemical properties. *Agriculture, Ecosystems & Environment*, *160*, 3–14. https://doi.org/10.1016/j.agee.2011.06.015

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., Pretty, J., Robinson, S., Thomas, S. M., & Toulmin, C. (2010). Food Security: The Challenge of Feeding 9 Billion People. *Science*, *327*(5967), 812–818. https://doi.org/10.1126/science.1185383

Goh, K. M. (2004). Carbon sequestration and stabilization in soils: Implications for soil productivity and climate change. *Soil Science and Plant Nutrition*, *50*(4), 467–476. https://doi.org/10.1080/00380768.2004.10408502

Gómez, E. J., Delgado, J. A., & González, J. M. (2020). Persistence of microbial extracellular enzymes in soils under different temperatures and water availabilities. *Ecology and Evolution*, *10*(18), 10167–10176. https://doi.org/10.1002/ece3.6677

Granatstein, D. M., Bezdicek, D. F., Cochran, V. L., Elliott, L. E., & Hammel, J. (1987). Long-term tillage and rotation effects on soil microbial biomass, carbon and nitrogen. *Biology and Fertility of Soils*, *5*(3), 265–270. https://doi.org/10.1007/BF00256912

Haney, R. L., & Haney, E. B. (2010). Simple and Rapid Laboratory Method for Rewetting Dry Soil for Incubations. *Communications in Soil Science and Plant Analysis*, *41*(12), 1493–1501. https://doi.org/10.1080/00103624.2010.482171

Haney, R. L., Haney, E. B., Smith, D. R., Harmel, R. D., & White, M. J. (2018). The soil health tool—Theory and initial broad-scale application. *Applied Soil Ecology*, *125*, 162–168. https://doi.org/10.1016/j.apsoil.2017.07.035

Hendrix, P. F., Han, C.-R., & Groffman, P. M. (1988). Soil respiration in conventional and no-tillage agroecosystems under different winter cover crop rotations. *Soil and Tillage Research*, *12*(2), 135–148. https://doi.org/10.1016/0167-1987(88)90037-2

Hobbie, S. (1998, May 5). *Chloroform fumigation direct extraction (CFDE) protocol for microbial biomass carbon and nitrogen*. Vitousek Biogeochemistry Lab. https://web.stanford.edu/group/Vitousek/chlorofume.html

Hollister, E. B., Hu, P., Wang, A. S., Hons, F. M., & Gentry, T. J. (2013). Differential impacts of brassicaceous and nonbrassicaceous oilseed meals on soil bacterial and fungal communities.

FEMS Microbiology Ecology, *83*(3), 632–641. https://doi.org/10.1111/1574-6941.12020

Hu, Q., Jiang, T., Thomas, B. W., Chen, J., Xie, J., Hu, Y., Kong, F., Yang, Y., Chen, X., Zhang, Y., & Shi, X. (2023). Legume cover crops enhance soil organic carbon via microbial necromass in orchard alleyways. *Soil and Tillage Research*, *234*, 105858. https://doi.org/10.1016/j.still.2023.105858

Imran, M. A., Ali, A., Ashfaq, M., Hassan, S., Culas, R., & Ma, C. (2018). Impact of Climate Smart Agriculture (CSA) Practices on Cotton Production and Livelihood of Farmers in Punjab, Pakistan. *Sustainability*, *10*(6), Article 6. https://doi.org/10.3390/su10062101

Indorf, C., Dyckmans, J., Khan, K. S., & Joergensen, R. G. (2011). Optimisation of amino sugar quantification by HPLC in soil and plant hydrolysates. *Biology and Fertility of Soils*, *47*(4), 387–396. https://doi.org/10.1007/s00374-011-0545-5

Insam, H., Mitchell, C. C., & Dormaar, J. F. (1991). Relationship of soil microbial biomass and activity with fertilization practice and crop yield of three ultisols. *Soil Biology and Biochemistry*, *23*(5), 459–464. https://doi.org/10.1016/0038-0717(91)90010-H

IPCC. (2000). *Land Use, Land-Use Change, and Forestry* (R. T. Watson, I. R. Noble, B. Bolin, N. H. Ravindranath, D. J. Verardo, & D. J. Dokken, Eds.). Cambridge University Press. https://www.ipcc.ch/report/land-use-land-use-change-and-forestry/

Jat, H. S., Datta, A., Choudhary, M., Sharma, P. C., Yadav, A. K., Choudhary, V., Gathala, M. K., Jat, M. L., & McDonald, A. (2019). Climate Smart Agriculture practices improve soil organic carbon pools, biological properties and crop productivity in cereal-based systems of North-West India. *Catena*, *181*, 104059. https://doi.org/10.1016/j.catena.2019.05.005

Jedidi, N., Hassen, A., van Cleemput, O., & M'Hiri, A. (2004). Microbial Biomass in a Soil Amended with Different Types of Organic Wastes. *Waste Management & Research*, *22*(2), 93–99. https://doi.org/10.1177/0734242X04043930

Jose, S., & Bardhan, S. (2012). Agroforestry for biomass production and carbon sequestration: An overview. *Agroforestry Systems*, *86*(2), 105–111. https://doi.org/10.1007/s10457-012-9573-x

Kaye, J. P., & Quemada, M. (2017). Using cover crops to mitigate and adapt to climate change. A review. *Agronomy for Sustainable Development*, *37*(1), 4. https://doi.org/10.1007/s13593-016-0410-x

Krotz, L., Leone, F., & Giazzi, G. (2017). *Elemental Analysis: NC Determination in Soils and Plants Using Argon as Carrier Gas*. Thermo Fisher Scientific. https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-42219-ea-nitrogen-carbon-soils-plants-an42219-en.pdf

Kunze, A., Costa, M. D., Epping, J., Loffaguen, J. C., Schuh, R., & Lovato, P. E. (2011). Phosphatase activity in sandy soil influenced by mycorrhizal and non-mycorrhizal cover crops. *Revista Brasileira de Ciência Do Solo*, *35*, 705–711. https://doi.org/10.1590/S0100-06832011000300005

Lange, M., Eisenhauer, N., Sierra, C. A., Bessler, H., Engels, C., Griffiths, R. I., Mellado-Vázquez, P. G., Malik, A. A., Roy, J., Scheu, S., Steinbeiss, S., Thomson, B. C., Trumbore, S. E., & Gleixner, G. (2015). Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications*, *6*(1), 6707. https://doi.org/10.1038/ncomms7707

Lawley, Y. E., Teasdale, J. R., & Weil, R. R. (2012). The Mechanism for Weed Suppression by a Forage Radish Cover Crop. *Agronomy Journal*, *104*(2), 205–214. https://doi.org/10.2134/agronj2011.0128

Lee, K.-H., & Jose, S. (2003). Soil respiration and microbial biomass in a pecan—Cotton alley cropping system in Southern USA. *Agroforestry Systems*, *58*(1), 45–54. https://doi.org/10.1023/A:1025404019211

Li, X., Wang, T., Chang, S. X., Jiang, X., & Song, Y. (2020). Biochar increases soil microbial biomass but has variable effects on microbial diversity: A meta-analysis. *Science of The Total Environment*, *749*, 141593. https://doi.org/10.1016/j.scitotenv.2020.141593

Liang, C., & Balser, T. C. (2011). Microbial production of recalcitrant organic matter in global soils: Implications for productivity and climate policy. *Nature Reviews Microbiology*, *9*(1), 75–75. https://doi.org/10.1038/nrmicro2386-c1

Liang, C., Schimel, J. P., & Jastrow, J. D. (2017). The importance of anabolism in microbial control over soil carbon storage. *Nature Microbiology*, *2*(8), 1–6. https://doi.org/10.1038/nmicrobiol.2017.105

Liang, S., Grossman, J., & Shi, W. (2014). Soil microbial responses to winter legume cover crop management during organic transition. *European Journal of Soil Biology*, *65*, 15–22. https://doi.org/10.1016/j.ejsobi.2014.08.007

Lima, D. L. D., Santos, S. M., Scherer, H. W., Schneider, R. J., Duarte, A. C., Santos, E. B. H., & Esteves, V. I. (2009). Effects of organic and inorganic amendments on soil organic matter properties. *Geoderma*, *150*(1), 38–45. https://doi.org/10.1016/j.geoderma.2009.01.009

Lipper, L., McCarthy, N., Zilberman, D., Asfaw, S., & Branca, G. (Eds.). (2018). *Climate Smart Agriculture: Building Resilience to Climate Change* (Vol. 52). Food and Agriculture Organization of the United Nations.

Lipper, L., Thornton, P., Campbell, B. M., Baedeker, T., Braimoh, A., Bwalya, M., Caron, P., Cattaneo, A., Garrity, D., Henry, K., Hottle, R., Jackson, L., Jarvis, A., Kossam, F., Mann, W., McCarthy, N., Meybeck, A., Neufeldt, H., Remington, T., … Torquebiau, E. F. (2014). Climate-smart agriculture for food security. *Nature Climate Change*, *4*(12), 1068–1072. https://doi.org/10.1038/NCLIMATE2437

Lubwama, F. B. (1999). Socio-economic and gender issues affecting the adoption of conservation tillage practices. In *Conservation tillage with animal traction: A resource book of the Animal Traction Network for Eastern and Southern Africa (ATNESA)*. https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=27b86afc8c3669f5e4afa448bb376946714bea3b

Magdoff, F., & van Es, H. (2021). Ch 10. Cover Crops. In *Building Soils for Better Crops*. SARE Outreach. https://www.sare.org/publications/building-soils-for-better-crops/cover-crops/

Makoi, J., Chimphango, S., & Dakora, F. (2010). Elevated levels of acid and alkaline phosphatase activity in roots and rhizosphere of cowpea (Vigna unguiculata L. Walp.) genotypes grown in mixed culture and at different densities with sorghum (Sorghum bicolor L.). *Crop and Pasture Science*, *61*. https://doi.org/10.1071/CP09212

Maltais-Landry, G. (2015). Legumes have a greater effect on rhizosphere properties (pH, organic acids

and enzyme activity) but a smaller impact on soil P compared to other cover crops. *Plant and Soil*, *394*(1), 139–154. https://doi.org/10.1007/s11104-015-2518-1

Mankolo, R., Reddy, C., Senwo, Z., Nyakatawa, E., & Sajjala, S. (2012). Soil Biochemical Changes Induced by Poultry Litter Application and Conservation Tillage under Cotton Production Systems. *Agronomy*, *2*(3), Article 3. https://doi.org/10.3390/agronomy2030187

Markewich, H. W., Pavich, M. J., & Buell, G. R. (1990). Contrasting soils and landscapes of the Piedmont and Coastal Plain, eastern United States. *Geomorphology*, *3*(3), 417–447. https://doi.org/10.1016/0169-555X(90)90015-I

Menpadi, M. M., M, M., & Singh, D. G. (2023). Microbial community composition and necromass are important determinants of the soil organic carbon in agroforestry tree species. *Indian Journal of Agroforestry*, *25*(2), Article 2. https://epubs.icar.org.in/index.php/IJA/article/view/127301

EPA. (1983). *Methods for Chemical Analysis of Water and Wastes* (p. 491). Office of Research and Development, United States Environmental Protection Agency. https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=30000Q10.txt

Morrissey, E. M., Berrier, D. J., Neubauer, S. C., & Franklin, R. B. (2014). Using microbial communities and extracellular enzymes to link soil organic matter characteristics to greenhouse gas production in a tidal freshwater wetland. *Biogeochemistry*, *117*(2), 473–490. https://doi.org/10.1007/s10533-013-9894-5

Muhammad, I., Wang, J., Sainju, U. M., Zhang, S., Zhao, F., & Khan, A. (2021). Cover cropping enhances soil microbial biomass and affects microbial community structure: A *meta*-analysis. *Geoderma*, *381*, 114696. https://doi.org/10.1016/j.geoderma.2020.114696

Mukumbareza, C., Muchaonyerwa, P., & Chiduza, C. (2016). Bicultures of oat (Avena sativa L.) and grazing vetch (Vicia dasycarpa L.) cover crops increase contents of carbon pools and activities of selected enzymes in a loam soil under warm temperate conditions. *Soil Science and Plant Nutrition*, *62*(5–6), 447–455. https://doi.org/10.1080/00380768.2016.1206833

Muthuri, C. W., Kuyah, S., Njenga, M., Kuria, A., Öborn, I., & Van Noordwijk, M. (2023).

Agroforestry's contribution to livelihoods and carbon sequestration in East Africa: A systematic review. *Trees, Forests and People*, *14*, 100432. https://doi.org/10.1016/j.tfp.2023.100432

Ndaw, S. M., Gama-Rodrigues, A. C., Gama-Rodrigues, E. F., Sales, K. R. N., & Rosado, A. S. (2009). Relationships between bacterial diversity, microbial biomass, and litter quality in soils under different plant covers in northern Rio de Janeiro State, Brazil. *Canadian Journal of Microbiology*, *55*(9), 1089–1095. https://doi.org/10.1139/W09-066

Norris, C. E., Bean, G. M., Cappellazzi, S. B., Cope, M., Greub, K. L. H., Liptzin, D., Rieke, E. L., Tracy, P. W., Morgan, C. L. S., & Honeycutt, C. W. (2020). Introducing the North American project to evaluate soil health measurements. *Agronomy Journal*, *112*(4), 3195–3215. https://doi.org/10.1002/agj2.20234

Oats. (2007). In *Managing Cover Crops Profitably* (3rd ed.). SARE Outreach. https://www.sare.org/publications/managing-cover-crops-profitably/nonlegume-cover-crops/oats/

Parewa, H. P., Saresh, N., Yadav, R. P., Meena, V. S., Meena, S. K., & Choudhary, A. (2018). Revisiting the agroforestry ecosystem for soil sustainability: Lessons from the past and objectives for the future. *Tropical Ecology*, *59*(4), 565–573.

Peña, J. G. (1995). *Pecan Production Trends: A Comparison of Production in the Southeastern and Southwestern United States*. *5*(3), 202–205. https://doi.org/10.21273/HORTTECH.5.3.202

Perucci, P. (1990). Effect of the addition of municipal solid-waste compost on microbial biomass and enzyme activities in soil. *Biology and Fertility of Soils*, *10*(3), 221–226. https://doi.org/10.1007/BF00336141

Pokharel, P., Ma, Z., & Chang, S. X. (2020). Biochar increases soil microbial biomass with changes in extra- and intracellular enzyme activities: A global meta-analysis. *Biochar*, *2*(1), 65–79. https://doi.org/10.1007/s42773-020-00039-1

Polyakova, O., & Billor, N. (2007). Impact of deciduous tree species on litterfall quality, decomposition rates and nutrient circulation in pine stands. *Forest Ecology and Management*, *253*(1), 11–18. https://doi.org/10.1016/j.foreco.2007.06.049

Qubit Systems. (2020). *Q-S151 CO2 Analyzer 0-2000ppm*. Instrumentation for Biological Sciences. https://qubitbiology.com/products/q-s151-co2-analyzer-0-2000ppm/

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

Raczkowski, C. W., Reyes, M. R., Reddy, G. B., Busscher, W. J., & Bauer, P. J. (2009). Comparison of conventional and no-tillage corn and soybean production on runoff and erosion in the southeastern US Piedmont. *Journal of Soil and Water Conservation*, *64*(1), 53–60. https://doi.org/10.2489/jswc.64.1.53

Raiesi, F., & Khadem, A. (2019). Short-term effects of maize residue biochar on kinetic and thermodynamic parameters of soil β-glucosidase. *Biochar*, *1*(2), 213–227. https://doi.org/10.1007/s42773-019-00016-3

Rauscher, H. M., & Johnsen, K. (2004). Carbon Sequestration in loblolly pine plantations: Methods, limitations, and research needs for estimating storage pools. In *Southern forest science: Past, present, and future* (p. 394).

Rodale Institute. (n.d.). *Organic No-Till*. https://rodaleinstitute.org/why-organic/organic-farming-practices/organic-no-till/

Rolo, V., Rivest, D., Maillard, E., & Moreno, G. (2023). Agroforestry potential for adaptation to climate change: A soil-based perspective. *Soil Use and Management*, *39*(3), 1006–1032. https://doi.org/10.1111/sum.12932

Saggar, S., Hedley, C. B., & Salt, G. J. (2001). Soil microbial biomass, metabolic quotient, and carbon and nitrogen mineralisation in 25-year-old Pinus radiata agroforestry regimes. *Soil Research*, *39*(3), 491–504. https://doi.org/10.1071/sr00012

Sainju, U. M., Schomberg, H. H., Singh, B. P., Whitehead, W. F., Tillman, P. G., & Lachnicht-Weyers, S. L. (2007). Cover crop effect on soil carbon fractions under conservation tillage cotton. *Soil and Tillage Research*, *96*(1), 205–218. https://doi.org/10.1016/j.still.2007.06.006

Sainju, U. M., Whitehead, W. F., & Singh, B. P. (2005). Biculture Legume–Cereal Cover Crops for

Enhanced Biomass Yield and Carbon and Nitrogen. *Agronomy Journal*, *97*(5), 1403–1412. https://doi.org/10.2134/agronj2004.0274

Sanchez, I. I., Fultz, L. M., Lofton, J., & Haggard, B. (2019). Soil Biological Response to Integration of Cover Crops and Nitrogen Rates in a Conservation Tillage Corn Production System. *Soil Science Society of America Journal*, *83*(5), 1356–1367. https://doi.org/10.2136/sssaj2019.02.0051

Schultz, R. P. (1997). *Loblolly Pine: The Ecology and Culture of the Loblolly Pine (Pinus Taeda L.)*. U.S. Department of Agriculture, Forest Service.

Smith, P. (2004). How long before a change in soil organic carbon can be detected? *Global Change Biology*, *10*(11), 1878–1883. https://doi.org/10.1111/j.1365-2486.2004.00854.x

Snapp, S. S., Swinton, S. M., Labarta, R., Mutch, D., Black, J. R., Leep, R., Nyiraneza, J., & O'Neil, K. (2005). Evaluating Cover Crops for Benefits, Costs and Performance within Cropping System Niches. *Agronomy Journal*, *97*(1), 322–332. https://doi.org/10.2134/agronj2005.0322a

Soil Health. (n.d.). Natural Resources Conservation Service. Retrieved November 16, 2023, from https://www.nrcs.usda.gov/conservation-basics/natural-resource-concerns/soils/soil-health

Spohn, M., Klaus, K., Wanek, W., & Richter, A. (2016). Microbial carbon use efficiency and biomass turnover times depending on soil depth – Implications for carbon cycling. *Soil Biology and Biochemistry*, *96*, 74–81. https://doi.org/10.1016/j.soilbio.2016.01.016

Stockmann, U., Adams, M. A., Crawford, J. W., Field, D. J., Henakaarchchi, N., Jenkins, M., Minasny, B., McBratney, A. B., Courcelles, V. de R. de, Singh, K., Wheeler, I., Abbott, L., Angers, D. A., Baldock, J., Bird, M., Brookes, P. C., Chenu, C., Jastrow, J. D., Lal, R., … Zimmermann, M. (2013). The knowns, known unknowns and unknowns of sequestration of soil organic carbon. *Agriculture, Ecosystems & Environment*, *164*, 80–99. https://doi.org/10.1016/j.agee.2012.10.001

Sullivan, D. G., Truman, C. C., Schomberg, H. H., Endale, D. M., & Franklin, D. H. (2007). Potential impact of conservation tillage on conserving water resources in Georgia. *Journal of Soil and Water Conservation*, *62*(3), 145–152.

Suman, J., Rakshit, A., Ogireddy, S. D., Singh, S., Gupta, C., & Chandrakala, J. (2022). Microbiome as a

Key Player in Sustainable Agriculture and Human Health. *Frontiers in Soil Science*, *2*. https://www.frontiersin.org/articles/10.3389/fsoil.2022.821589

Thangarajan, R., Bolan, N. S., Tian, G., Naidu, R., & Kunhikrishnan, A. (2013). Role of organic amendment application on greenhouse gas emission from soil. *Science of the Total Environment*, *465*, 72–96. https://doi.org/10.1016/j.scitotenv.2013.01.031

Tilman, D., Balzer, C., Hill, J., & Befort, B. L. (2011). Global food demand and the sustainable intensification of agriculture. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(50), 20260–20264. https://doi.org/10.1073/pnas.1116437108

Uri, N. D. (2000). An evaluation of the economic benefits and costs of conservation tillage. *Environmental Geology*, *39*(3/4), 238–248. https://doi.org/10.1007/s002540050004

U.S. Department of Agriculture. (2023, April 13). *Cover Crop Chart (v 4.0)*. Agricultural Research Service: Northern Great Plains Research Laboratory. https://www.ars.usda.gov/plains-area/mandan-nd/ngprl/docs/cover-crop-chart/

US Environmental Protection Agency. (2012). *Level III Ecoregions of EPA Region 4* [Vector digital data]. ftp://ftp.epa.gov/wed/ecoregions/reg4/reg4_eco_l3.zip

Vance, E. D., Brookes, P. C., & Jenkinson, D. S. (1987). An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, *19*(6), 703–707. Scopus. https://doi.org/10.1016/0038-0717(87)90052-6

Waaswa, A., Nkurumwa, A. O., Kibe, A. M., & Ng'eno, J. K. (2021). Understanding the socioeconomic determinants of adoption of climate-smart agricultural practices among smallholder potato farmers in Gilgil Sub-County, Kenya. *Discover Sustainability*, *2*(1), 41. https://doi.org/10.1007/s43621-021-00050-x

Waldrop, M. P., Balser, T. C., & Firestone, M. K. (2000). Linking microbial community composition to function in a tropical soil. *Soil Biology and Biochemistry*, *32*(13), 1837–1846. https://doi.org/10.1016/S0038-0717(00)00157-7

Wang, B., An, S., Liang, C., Liu, Y., & Kuzyakov, Y. (2021). Microbial necromass as the source of soil

organic carbon in global ecosystems. *Soil Biology and Biochemistry*, *162*, 108422. https://doi.org/10.1016/j.soilbio.2021.108422

Wang, R., Dorodnikov, M., Yang, S., Zhang, Y., Filley, T. R., Turco, R. F., Zhang, Y., Xu, Z., Li, H., & Jiang, Y. (2015). Responses of enzymatic activities within soil aggregates to 9-year nitrogen and water addition in a semi-arid grassland. *Soil Biology and Biochemistry*, *81*, 159–167. https://doi.org/10.1016/j.soilbio.2014.11.015

Wang, X.-B., Cai, D.-X., Hoogmoed, W. B., Oenema, O., & Perdok, U. D. (2006). Potential Effect of Conservation Tillage on Sustainable Land Use: A Review of Global Long-Term Studies. *Pedosphere*, *16*(5), 587–595. https://doi.org/10.1016/S1002-0160(06)60092-1

Wang, Y., Li, C., Tu, C., Hoyt, G. D., DeForest, J. L., & Hu, S. (2017). Long-term no-tillage and organic input management enhanced the diversity and stability of soil microbial community. *Science of the Total Environment*, *609*, 341–347. https://doi.org/10.1016/j.scitotenv.2017.07.053

Wang, Z., Zhou, M., Liu, H., Huang, C., Ma, Y., Ge, H. xin, Ge, X., & Fu, S. (2022). Pecan agroforestry systems improve soil quality by stimulating enzyme activity. *PeerJ*, *10*, e12663. https://doi.org/10.7717/peerj.12663

Wells, L. (2014). Pecan Planting Trends in Georgia. *HortTechnology*, *24*(4), 475–479. https://doi.org/10.21273/HORTTECH.24.4.475

White, C. M., & Weil, R. R. (2010). Forage radish and cereal rye cover crop effects on mycorrhizal fungus colonization of maize roots. *Plant and Soil*, *328*(1), 507–521. https://doi.org/10.1007/s11104-009-0131-x

Whitt, C., Miller, N., & Olver, R. (2022). *America's farms and ranches at a glance*. United States Department of Agriculture, Economic Research Service. https://doi.org/10.32747/2023.8141996.ers

Winandy, J. E., Barnes, H. M., & Morrell, J. J. (2022). Exploratory studies on effects of growth location and conditioning on treatability and permeability of southern pine lumber. *Maderas. Ciencia y Tecnología*, *24*. https://www.redalyc.org/journal/485/48575019041/html/

Ye, G., Lin, Y., Kuzyakov, Y., Liu, D., Luo, J., Lindsey, S., Wang, W., Fan, J., & Ding, W. (2019). Manure over crop residues increases soil organic matter but decreases microbial necromass relative contribution in upland Ultisols: Results of a 27-year field experiment. *Soil Biology and Biochemistry*, *134*, 15–24. https://doi.org/10.1016/j.soilbio.2019.03.018

Zhang, H., Voroney, R. P., & Price, G. W. (2014). Effects of Biochar Amendments on Soil Microbial Biomass and Activity. *Journal of Environmental Quality*, *43*(6), 2104–2114. https://doi.org/10.2134/jeq2014.03.0132

Zhang, Z., Kaye, J. P., Bradley, B. A., Amsili, J. P., & Suseela, V. (2022). Cover crop functional types differentially alter the content and composition of soil organic carbon in particulate and mineral-associated fractions. *Global Change Biology*, *28*(19), 5831–5848. https://doi.org/10.1111/gcb.16296

Zhao, D., Bullock, B. P., & Wang, M. (2023). Long-term dynamics of aboveground carbon stocks in managed loblolly pine plantations in the southeast United States. *Forest Ecology and Management*, *546*, 121384. https://doi.org/10.1016/j.foreco.2023.121384

Zhao, K., Wang, N., Jiang, S., Li, F., Luo, S., Chen, A., Li, H., Lin, X., Zhang, J., Zhang, L., Huang, H., & Luo, L. (2022). Potential implications of biochar and compost on the stoichiometry-based assessments of soil enzyme activity in heavy metal-polluted soils. *Carbon Research*, *1*(1), 29. https://doi.org/10.1007/s44246-022-00029-x

Zhong, Y., Tian, J., Li, X., & Liao, H. (2023). Cooperative interactions between nitrogen fixation and phosphorus nutrition in legumes. *New Phytologist*, *237*(3), 734–745. https://doi.org/10.1111/nph.18593

Zhu, X., Jackson, R. D., DeLucia, E. H., Tiedje, J. M., & Liang, C. (2020). The soil microbial carbon pump: From conceptual insights to empirical assessments. *Global Change Biology*, *26*(11), 6032–6039. https://doi.org/10.1111/gcb.15319

Zuber, S. M., & Villamil, M. B. (2016). Meta-analysis approach to assess effect of tillage on microbial biomass and enzyme activities. *Soil Biology and Biochemistry*, *97*, 176–187.

https://doi.org/10.1016/j.soilbio.2016.03.011

8. Appendix A: Additional Statistical Outputs

Table A1. Post-hoc Tukey HSD multiple comparison of means of soil carbon cycle proxies across amendment treatments at Oxford Farm. Pairwise comparisons of all proxies are shown, regardless of significance.

Proxy	(I) Amendment	(J) Amendment	Mean Difference (I-J)	Significance	95% Confidence Interval	
					Lower Bound	Upper Bound
Microbial biomass C (MBC)	Compost	Biochar-Compost	-46.409884	0.3937164	-131.44783	38.62806
	Control	Biochar-Compost	3.786522	0.9927929	-75.75919	83.33223
		Compost	50.196405	0.2899334	-29.34931	129.74212
Microbial biomass N (MBN)	Compost	Biochar-Compost	1.826955	0.6205054	-2.8743645	6.528274
	Control	Biochar-Compost	3.976957	0.0840071	-0.4207239	8.374639
		Compost	2.150003	0.4719351	-2.2476785	6.547684
BG K_m	Compost	Biochar-Compost	-35.13366	0.9021918	-230.77408	160.5068
	Control	Biochar-Compost	48.52204	0.8033743	-136.50146	233.5455
		Compost	83.65570	0.5139408	-98.38858	265.7000
LAP K_m	Compost	Biochar-Compost	-2.742067	0.9833700	-40.58778	35.10365
	Control	Biochar-Compost	-1.198627	0.9963584	-36.67223	34.27497
		Compost	1.543440	0.9937673	-33.34859	36.43547
NAG K_m	Compost	Biochar-Compost	-45.70409	0.4253295	-133.25182	41.84364
	Control	Biochar-Compost	21.35172	0.8004799	-59.36330	102.06674
		Compost	67.05581	0.1298170	-15.00455	149.11618

PHOS K_m	Compost	Biochar-Compost	-13.10959	0.7637473	-58.09936	31.88019
	Control	Biochar-Compost	-26.79440	0.2837144	-68.87848	15.28968
		Compost	-13.68481	0.7152150	-55.76889	28.39927
XYL K_m	Compost	Biochar-Compost	92.600714	0.2666740	-49.29832	234.49974
	Control	Biochar-Compost	-7.153312	0.9907721	-139.88770	125.58108
		Compost	-99.754026	0.1759559	-232.48841	32.98036
BG V_{max}	Compost	Biochar-Compost	-625.6606	0.9319043	-4832.0927	3580.772
	Control	Biochar-Compost	2556.1132	0.2697933	-1378.6438	6490.870
		Compost	3181.7738	0.1352791	-752.9832	7116.531
LAP V_{max}	Compost	Biochar-Compost	-1097.4505	0.3406604	-2965.430	770.5292
	Control	Biochar-Compost	-665.9694	0.6317720	-2413.304	1081.3656
		Compost	431.4811	0.8237636	-1315.854	2178.8160
NAG V_{max}	Compost	Biochar-Compost	-650.7713	0.4599349	-1959.261	657.71828
	Control	Biochar-Compost	-1162.0744	0.0661203	-2386.054	61.90552
		Compost	-511.3031	0.5766089	-1735.283	712.67685
PHOS V_{max}	Compost	Biochar-Compost	1208.6204	0.0389462	50.4917	2366.7491
	Control	Biochar-Compost	927.5049	0.1073625	-155.8253	2010.8352
		Compost	-281.1155	0.8073526	-1364.4457	802.2147
XYL V_{max}	Compost	Biochar-Compost	12.47656	0.9630058	-102.2988	127.25191
	Control	Biochar-Compost	-37.89128	0.6741386	-145.2538	69.47123

		Compost	-50.36784	0.5004152	-157.7303	56.99467
Total Necromass	Compost	Biochar-Compost	0.01275276	0.9195162	-0.07088578	0.09639129
	Control	Biochar-Compost	-0.03818456	0.4401937	-0.11642125	0.04005212
		Compost	-0.05093732	0.2449639	-0.12917401	0.02729937
Fungal Necromass	Compost	Biochar-Compost	0.007283595	0.9444680	-0.05065534	0.06522253
	Control	Biochar-Compost	-0.027629965	0.4101381	-0.08182688	0.02656695
		Compost	-0.034913559	0.2517020	-0.08911047	0.01928335
Bacterial Necromass	Compost	Biochar-Compost	0.005469163	0.8777425	-0.02326139	0.03419971
	Control	Biochar-Compost	-0.010554597	0.5822792	-0.03742957	0.01632037

Table A2. Post-hoc Tukey HSD multiple comparison of means of soil carbon cycle proxies across cover crop treatments at NCA&T. Pairwise comparisons are only shown of proxies with significant results.

Proxy	(I) Cover Crop Type	(J) Cover Crop Type	Mean Difference (I-J)	Significance	95% Confidence Interval	
					Lower Bound	Upper Bound
24-hour microbial respiration	Oat	Oat-Clover	-0.25668465	0.99994060	-1.26815870	1.78152800
		Oat-Vetch	-0.79256158	0.81253300	-0.73228177	2.31740493
		Rye	0.76551705	0.84182120	-2.29036040	0.75932630
		Rye-Clover	-0.74573845	0.86153940	-0.77910490	2.27058179
		Rye-Vetch	-0.62997633	0.94665210	-0.89486702	2.15481967
		Radish	0.87335432	0.71116910	-2.39819767	0.65148903
		Radish-Oat	1.45507329	0.07518570	-2.97991664	0.06977006
		Radish-Rye	0.75945876	0.84801730	-2.28430210	-2.28430210

		Radish-Vetch	0.93439515	0.62479180	-2.45923850	0.59044820
	Oat-Clover	Oat-Vetch	-0.53587693	0.98129960	-0.98896642	2.06072028
		Rye	1.02220170	0.49572860	0.50264165	0.50264165
		Rye-Clover	-0.48905380	0.99013490	-1.03578955	2.01389715
		Rye-Vetch	-0.37329168	0.99871670	-1.15155167	1.89813503
		Radish	1.13003897	0.34687040	-2.65488232	0.39480438
		Radish-Oat	1.71175794	0.01485580	-3.23660129	-0.18691459
		Radish-Rye	1.01614340	0.50457390	-2.54098675	0.50869994
		Radish-Vetch	1.19107980	0.27370730	-2.71592315	0.33376355
	Oat-Vetch	Rye	1.55807863	0.04080330	-3.08292198	-0.03323528
		Rye-Clover	0.04682313	1.00000000	-1.57166648	1.47802022
		Rye-Vetch	0.16258525	0.99999890	-1.68742860	1.36225810
		Radish	1.66591590	0.02031810	-3.19075925	-0.14107255
		Radish-Oat	2.24763487	0.00020240	-3.77247822	-0.72279152
		Radish-Rye	1.55202033	0.04236120	-3.07686368	-0.02717699
		Radish-Vetch	1.72695673	0.01336260	-3.25180008	-0.20211338
	Rye	Rye-Clover	-1.51125550	0.05424360	-0.01358785	3.03609884
		Rye-Vetch	-1.39549338	0.10431240	-0.12934997	2.92033672
		Radish	0.10783727	1.00000000	-1.63268062	1.41700608
		Radish-Oat	0.68955624	0.90930530	-2.21439959	0.83528711
		Radish-Rye	-0.00605829	1.00000000	-1.51878505	1.53090164
		Radish-Vetch	0.16887810	0.99999840	-1.69372145	1.35596525
	Rye-Clover	Rye-Vetch	0.11576212	0.99999990	-1.64060547	1.40908123

		Radish	1.61909277	0.02769320	-3.14393612	-0.09424942
		Radish-Oat	2.20081174	0.00030720	-3.72565509	-0.67596839
		Radish-Rye	1.50519720	0.05623250	-3.03004055	-3.03004055
		Radish-Vetch	1.68013360	0.01845680	-3.20497695	-0.15529025
	Rye-Vetch	Radish	1.50333065	0.05685770	-3.02817400	0.02151270
		Radish-Oat	2.08504962	0.00083440	-3.60989296	-0.56020627
		Radish-Rye	1.38943508	0.10772360	-2.91427843	0.13540827
		Radish-Vetch	1.56437148	0.03923800	-3.08921483	-0.03952813
	Radish	Radish-Oat	0.58171897	0.96770440	-2.10656232	0.94312438
		Radish-Rye	-0.11389557	1.00000000	-1.41094778	1.63873891
		Radish-Vetch	0.06104083	1.00000000	-1.58588418	1.46380252
	Radish-Oat	Radish-Rye	-0.69561454	0.90474880	2.22045788	2.22045788
		Radish-Vetch	-0.52067814	0.98465790	-1.00416521	2.04552149
	Radish-Rye	Radish-Vetch	0.17493640	0.99999780	-1.69977975	1.34990695
BG activity	Oat	Oat-Clover	-87.79293	0.99932	-320.85173	496.43759
		Oat-Vetch	-91.42561	0.99906	-317.21905	500.07028
		Rye	-224.35153	0.72133	-184.29314	632.99619
		Rye-Clover	-176.49405	0.91180	-232.15061	585.13871
		Rye-Vetch	-31.12889	1.00000	-377.51577	439.77355
		Radish	556.45470	0.00151	-965.09936	-147.81004
		Radish-Oat	330.66300	0.21046	-739.30766	77.98166
		Radish-Rye	332.64481	0.20390	-741.28947	75.99985
		Radish-Vetch	393.65448	0.06778	-802.29914	14.99018

	Oat-Clover	Oat-Vetch	-3.63269	1.00000	-405.01197	412.27735
		Rye	-136.55860	0.98181	545.20326	545.20326
		Rye-Clover	-88.70112	0.99926	-319.94354	497.34578
		Rye-Vetch	56.66404	0.99998	-465.30870	351.98063
		Radish	644.24763	0.00014	-1052.89229	-235.60297
		Radish-Oat	418.45593	0.04072	-827.10059	-9.81127
		Radish-Rye	420.43773	0.03904	-829.08240	-11.79307
		Radish-Vetch	481.44741	0.00982	-890.09207	-72.80274
	Oat-Vetch	Rye	-132.92591	0.98485	-275.71875	-275.71875
		Rye-Clover	-85.06843	0.99947	-323.57623	493.71310
		Rye-Vetch	60.29672	0.99997	-468.94138	348.34794
		Radish	647.88032	0.00013	-1056.52498	-239.23566
		Radish-Oat	422.08861	0.03769	-830.73327	-13.44395
		Radish-Rye	424.07042	0.03612	-832.71508	-15.42576
		Radish-Vetch	485.08009	0.00901	-893.72475	-76.43543
	Rye	Rye-Clover	47.85748	1.00000	-456.50214	360.78718
		Rye-Vetch	193.22263	0.85805	-601.86729	215.42203
		Radish	780.80623	0.00000	-1189.45089	-372.16157
		Radish-Oat	555.01452	0.00156	-963.65919	-146.36986
		Radish-Rye	556.99633	0.00148	-965.64099	-148.35167
		Radish-Vetch	618.00600	0.00029	-1026.65067	-209.36134
	Rye-Clover	Rye-Vetch	145.36516	0.97253	-554.00982	263.27951
		Radish	732.94875	0.00001	-1141.59341	-324.30409

		Radish-Oat	507.15705	0.00526	-915.80171	-98.51239
		Radish-Rye	509.13886	0.00501	-917.78352	-100.49419
		Radish-Vetch	570.14853	0.00105	-978.79319	-161.50386
	Rye-Vetch	Radish	587.58359	0.00066	-996.22826	-178.93893
		Radish-Oat	361.79189	0.12406	-770.43655	46.85277
		Radish-Rye	363.77370	0.11970	-772.41836	44.87096
		Radish-Vetch	424.78337	0.03558	-833.42803	-16.13871
	Radish	Radish-Oat	-225.79170	0.71410	-182.85296	634.43637
		Radish-Rye	-223.80990	0.72404	-184.83477	632.45456
		Radish-Vetch	-162.80022	0.94448	-245.84444	571.44489
	Radish-Oat	Radish-Rye	1.98181	1.00000	-410.62647	406.66285
		Radish-Vetch	62.99148	0.99996	345.65318	345.65318
	Radish-Rye	Radish-Vetch	61.00967	0.99997	-469.65433	347.63499
NAG activity	Oat	Oat-Clover	-117.352561	0.887321	-142.278180	376.983300
		Oat-Vetch	-106.580054	0.933814	-153.050680	366.210790
		Rye	-196.852704	0.288412	-62.778030	456.483440
		Rye-Clover	-214.708682	0.187108	-44.922060	474.339420
		Rye-Vetch	-6.995784	1.000000	-252.634960	266.626520
		Radish	-52.053200	0.999609	-207.577540	311.683940
		Radish-Oat	-126.284506	0.836943	-133.346230	385.915240
		Radish-Rye	-122.078283	0.861970	-137.552460	381.709020
		Radish-Vetch	-34.757574	0.999987	-224.873160	294.388310
	Oat-Clover	Oat-Vetch	10.772507	1.000000	-270.403250	248.858230

		Rye	-79.500143	0.990044	-180.130600	339.130880
		Rye-Clover	-97.356121	0.961599	-162.274620	356.986860
		Rye-Vetch	110.356778	0.919298	-369.987520	149.273960
		Radish	65.299362	0.997661	-324.930100	194.331380
		Radish-Oat	-8.931944	1.000000	-250.698790	268.562680
		Radish-Rye	-4.725722	1.000000	-254.905020	264.356460
		Radish-Vetch	82.594987	0.987000	-342.225730	177.035750
	Oat-Vetch	Rye	-90.272650	0.976325	-169.358090	349.903390
		Rye-Clover	-108.128628	0.928091	-151.502110	367.759370
		Rye-Vetch	99.584271	0.955835	-359.215010	160.046470
		Radish	54.526855	0.999431	-314.157590	205.103880
		Radish-Oat	-19.704451	1.000000	-239.926290	279.335190
		Radish-Rye	-15.498229	1.000000	-244.132510	275.128970
		Radish-Vetch	71.822480	0.995217	-331.453220	187.808260
	Rye	Rye-Clover	-17.855978	1.000000	-241.774760	277.486720
		Rye-Vetch	189.856921	0.336116	-449.487660	69.773820
		Radish	144.799504	0.703377	-404.430240	114.831230
		Radish-Oat	70.568198	0.995802	-330.198940	189.062540
		Radish-Rye	74.774421	0.993578	-334.405160	184.856320
		Radish-Vetch	162.095130	0.558386	-421.725870	97.535610
	Rye-Clover	Rye-Vetch	207.712899	0.223221	-467.343640	51.917840
		Radish	162.655483	0.553606	-422.286220	96.975260
		Radish-Oat	88.424177	0.979350	-348.054920	171.206560

		Radish-Rye	92.630399	0.971996	-352.261140	167.000340
		Radish-Vetch	179.951108	0.410479	-439.581850	79.679630
	Rye-Vetch	Radish	-45.057416	0.999881	-214.573320	304.688160
		Radish-Oat	-119.288722	0.877296	-140.342020	378.919460
		Radish-Rye	-115.082500	0.898427	-144.548240	374.713240
		Radish-Vetch	-27.761791	0.999998	-231.868950	287.392530
	Radish	Radish-Oat	-74.231306	0.993910	-185.399430	333.862050
		Radish-Rye	-70.025084	0.996037	-189.605660	329.655820
		Radish-Vetch	17.295625	1.000000	-276.926360	242.335110
	Radish-Oat	Radish-Rye	4.206222	1.000000	-263.836960	255.424520
		Radish-Vetch	91.526931	0.974090	-351.157670	168.103810
	Radish-Rye	Radish-Vetch	87.320709	0.981011	-346.951450	172.310030
PHOS activity	Oat	Oat-Clover	-457.331910	0.157528	-79.390259	994.054077
		Oat-Vetch	-40.949360	1.000000	-495.772807	577.671530
		Rye	-574.680840	0.026944	37.958667	1111.403004
		Rye-Clover	-295.262780	0.719065	-241.459386	831.984951
		Rye-Vetch	129.123300	0.998338	-665.845470	407.598867
		Radish	-339.653690	0.539579	-197.068478	876.375859
		Radish-Oat	-1104.514680	0.000001	567.792512	1641.236849
		Radish-Rye	-1062.494580	0.000001	525.772413	1599.216749
		Radish-Vetch	-898.148240	0.000047	361.426074	1434.870410
	Oat-Clover	Oat-Vetch	416.382550	0.259659	-953.104715	120.339621
		Rye	-117.348930	0.999216	-419.373242	654.071095

		Rye-Clover	162.069130	0.990980	-698.791295	374.653042
		Rye-Vetch	586.455210	0.022068	-1123.177379	-49.733042
		Radish	117.678220	0.999198	-654.400386	419.043950
		Radish-Oat	-647.182770	0.007489	110.460603	1183.904940
		Radish-Rye	-605.162670	0.015959	68.440504	1141.884840
		Radish-Vetch	-440.816330	0.194321	-95.905835	977.538501
	Oat-Vetch	Rye	-533.731470	0.052405	-2.990694	1070.453642
		Rye-Clover	-254.313420	0.856564	-282.408747	791.035589
		Rye-Vetch	170.072660	0.987350	-706.794831	366.649505
		Radish	-298.704330	0.705830	-238.017839	835.426497
		Radish-Oat	-1063.565320	0.000001	526.843151	1600.287487
		Radish-Rye	-1021.545220	0.000003	484.823051	1558.267387
		Radish-Vetch	-857.198880	0.000112	320.476712	1393.921048
	Rye	Rye-Clover	279.418050	0.777120	-816.140221	257.304115
		Rye-Vetch	703.804140	0.002560	-1240.526305	-167.081969
		Radish	235.027140	0.904890	-771.749313	301.695023
		Radish-Oat	-529.833840	0.055690	-6.888323	1066.556013
		Radish-Rye	-487.813750	0.104034	-48.908423	1024.535913
		Radish-Vetch	-323.467410	0.606343	-213.254762	860.189575
	Rye-Clover	Rye-Vetch	424.386080	0.236804	-961.108252	112.336084
		Radish	-44.390910	1.000000	-492.331260	581.113076
		Radish-Oat	-809.251900	0.000306	272.529730	1345.974066
		Radish-Rye	-767.231800	0.000725	230.509630	1303.953966

		Radish-Vetch	-602.885460	0.016609	66.163291	1139.607628
	Rye-Vetch	Radish	-468.776990	0.135352	-67.945176	1005.499160
		Radish-Oat	-1233.637980	0.000000	696.915814	1770.360150
		Radish-Rye	-1191.617880	0.000000	654.895714	1728.340050
		Radish-Vetch	-1027.271540	0.000003	490.549375	1563.993712
	Radish	Radish-Oat	-764.860990	0.000761	228.138822	1301.583158
		Radish-Rye	-722.840890	0.001764	186.118722	1259.563058
		Radish-Vetch	-558.494550	0.035243	21.772383	1095.216719
	Radish-Oat	Radish-Rye	42.020100	1.000000	-578.742268	494.702068
		Radish-Vetch	206.366440	0.955174	-743.088607	330.355730
	Radish-Rye	Radish-Vetch	164.346340	0.990044	-701.068507	372.375829

9. Appendix B: Correlation Matrices

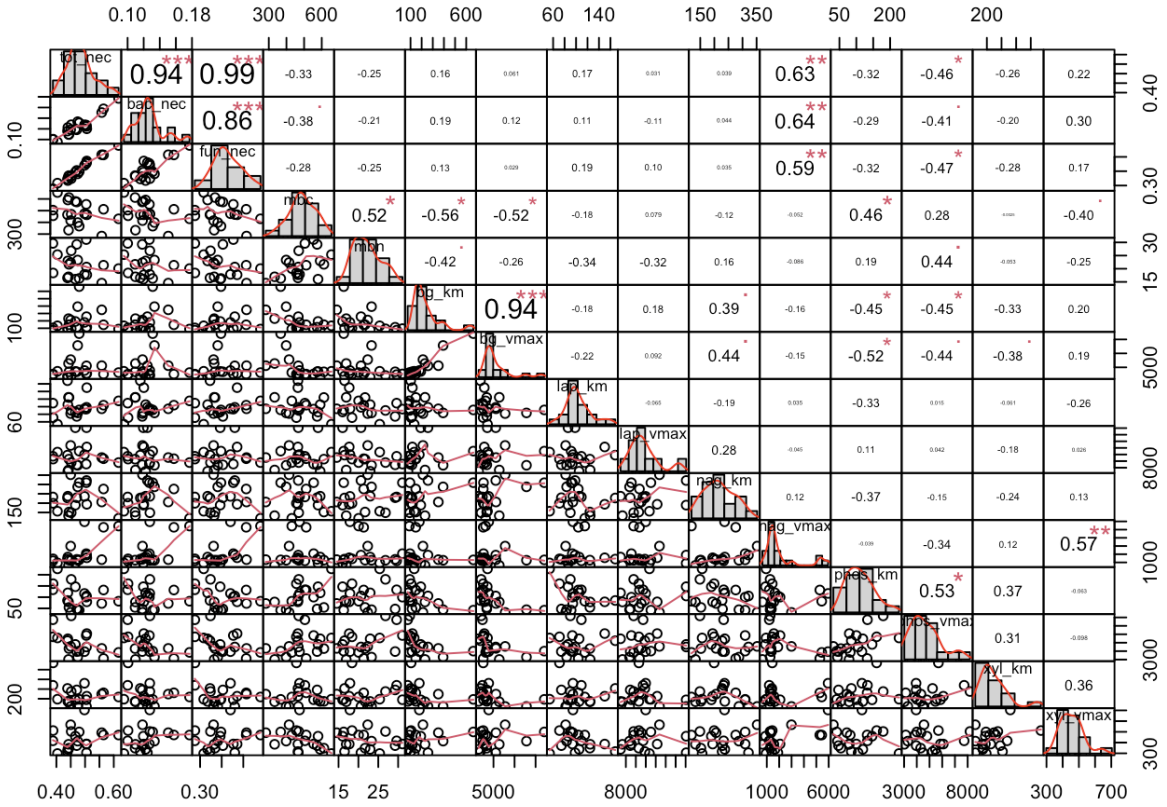

Figure A1. Correlation matrix chart visualizing correlations between soil carbon cycle proxies for Oxford Farm. The distribution of each variable is shown on the diagonal. Below the diagonal, bivariate scatter plots are shown with a fitted line. Above the diagonal, the value of the correlation is shown, along with stars to represent the significance level.

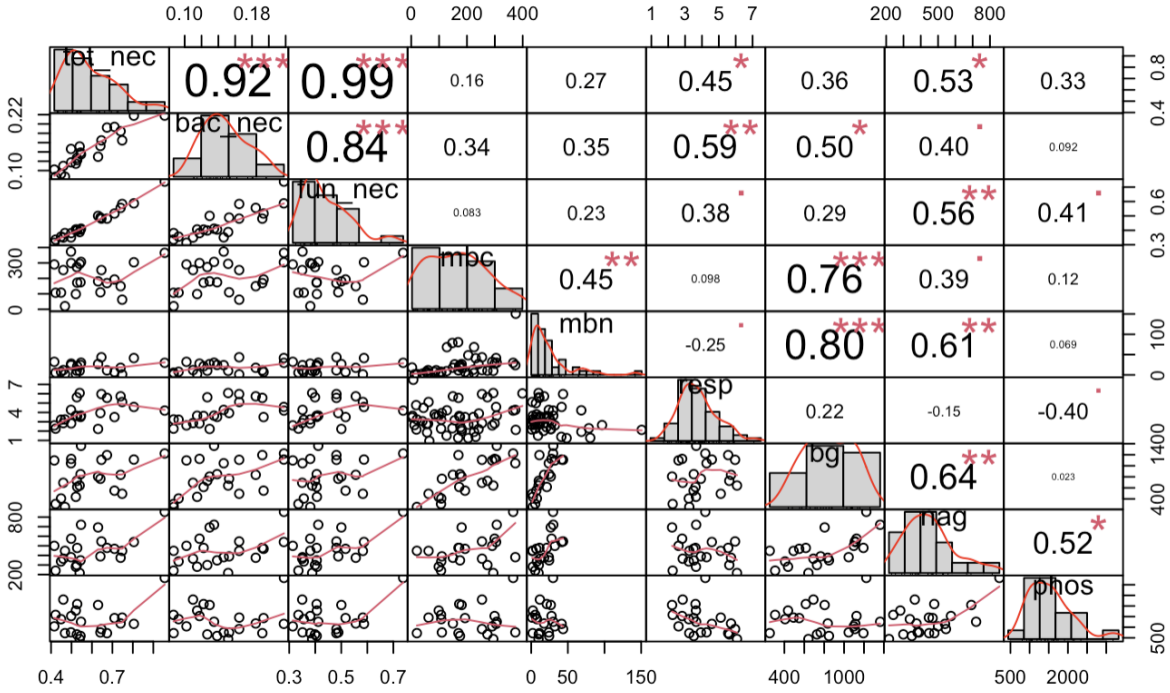

Figure A2. Correlation matrix chart visualizing correlations between soil carbon cycle proxies for NCA&T. The distribution of each variable is shown on the diagonal. Below the diagonal, bivariate scatter plots are shown with a fitted line. Above the diagonal, the value of the correlation is shown, along with stars to represent the significance level.