Relationships among Root Traits, Nitrogen Availability, and Mineral-Associated Organic Carbon

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ABSTRACT

Mineral-associated organic carbon (MAOC) is a vital component of soil health and ecosystem productivity, playing a key role in carbon (C) sequestration and nutrient cycling. This dissertation investigates how plant root traits, nitrogen (N) fertilization, and cover cropping influence soil organic carbon (SOC) and MAOC. In the first chapter, a greenhouse experiment with 30 herbaceous plant species found that non-N-fixing plants exhibited stronger positive correlations with MAOC compared to N-fixing species, which were more closely linked to SOC. Root biomass contributed to decreases in MAOC, while specific root length (SRL), and coarse root traits were found to significantly contribute to increases in MAOC, highlighting the importance of plant root architecture in stabilizing C. In the second chapter which focused on relationships among N-fertilization, root traits, and MAOC, moderate N fertilization (56 and 112 kg N/ha) was found to enhance total SOC (ΔSOC) and MAOC (ΔMAOC) accumulation in the system, while higher N inputs (168 kg/ha) reduced C gains. Notably, the use of stable isotopes allowed for the quantification of fresh C additions, with results that indicate plant-added MAOC (PA-MAOC) was influenced more by plant species and root traits, such as coarse root length and aboveground biomass, rather than N fertilization. In the third chapter, a long-term field study demonstrated the effectiveness of cover cropping in increasing both SOC and MAOC by 43–59% and 27–36%, respectively, compared to conventional and no-till systems without cover crops. Despite triennial additions of N fertilizer over nine years, no significant increases in SOC or MAOC was observed. Additionally, root biomass exhibited positive trends with MAOC. These findings suggest that cover cropping, combined with no till practices, plays a pivotal role in enhancing MAOC by minimizing soil disturbance and promoting root-driven C inputs. This research highlights the importance of integrating plant species selection, root morphological traits, N management, and conservation practices to optimize long-term C storage (i.e., MAOC)

and support sustainable soil management. Future studies should continue to include MAOC and particulate organic carbon fractions as these functional C sub pools may respond differently than bulk SOC pool. Including further studies on the interactions between root morphology, environmental factors, and C/N dynamics is necessary to develop more resilient agroecosystems capable of mitigating C losses and improving long-term soil health.

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GENERAL AUDIENCE ABSTRACT

This dissertation explores soil organic carbon, an important component of soil organic matter, which is vital for soil health and fertility. Soil organic matter promotes plant growth and agricultural productivity, and is critical for mitigating climate change by acting as a carbon sink, absorbing carbon dioxide from the atmosphere, which is why maintaining and building soil carbon is important. Soil carbon that is bound to minerals such as clays, is referred to as mineral-associated carbon (MAOC), which holds the largest pool of carbon on land and is often believed to persist over longer time-scales. Cover crops, their plant roots, and nitrogen fertilizer may have different relationships with MAOC when compared to total soil carbon, and these responses are not well understood. To address some of these knowledge gaps, this dissertation measured MAOC, plant root traits such as root size and structure, among different types of plants commonly used in agriculture and land reclamation practices. Results from this work show that root traits play an important role in increasing MAOC during short-term plant growth, with different effects depending on whether the plants can fix nitrogen from the air. Nitrogen fertilization was found to strongly impact MAOC, with moderate levels increasing amounts of MAOC, but low or too much nitrogen caused losses. Additionally, plant traits like root and stem biomass had a stronger influence on fresh carbon inputs from plants to the soil versus nitrogen alone. Cover crops proved to be a highly effective strategy for improving MAOC storage over 9 years, while additional nitrogen fertilizer had little long-term effect. These findings highlight the importance of balancing plant selection, fertilization, and sustainable practices to maintain healthy, carbon-rich soils.

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Chapter 1 - Introduction

Soil organic matter (SOM) is a fundamental component of soil that consists of decomposed plant and microbial residues in varying states of breakdown. It is involved in nutrient supply and cycling, water infiltration and holding capacity, aggregate stability, and enhancing microbial activity and biodiversity (Lehmann and Kleber, 2015; Paul, 2016). SOM decomposition releases nutrients like nitrogen (N) and carbon (C) that contribute to plant productivity and maintain soil fertility (Johnston et al., 2009). Loss of SOM can weaken soil structure, making it more susceptible to erosion and compaction (Zhang et al., 2005). Climate change can exacerbate SOM loss, for instance, warming accelerated microbial respiration and carbon mineralization, leading to loss of soil organic carbon (SOC), which is a primary component of SOM (Lu et al., 2013; Poeplau et al., 2020). Beyond climate implications, loss of SOC is a large threat to sustainable agriculture and food production (Sanderman et al., 2017; Mayer et al., 2022).

Carbon sequestration refers to the concept of capturing atmospheric CO_2 and storing it in soil to help mitigate climate change (Janzen, 2015). Soils can store more carbon than atmosphere and land vegetation combined (Sokol et al., 2022). However, agricultural activities such as conventional tillage practices, and land-use change can disrupt SOM structure and reduce SOC by increasing microbial decomposition (Nunes et al., 2020). Nitrogen additions through fertilization can enhance SOC sequestration by promoting plant biomass production, in turn, providing organic matter inputs to the soil (Chen et al., 2020).

Nitrogen is a critical component for plant growth, essential for forming proteins, enzymes, and nucleic acids which support photosynthesis, cell development, and other functions (Leghari et al., 2016). In soils N availability can regulate microbial activity, influencing SOC decomposition and nutrient cycling (Novoa and Loomis, 1981). It is essential that N management be observed to build SOC, as insufficient N can limit plant productivity and reduce C inputs (Santner et al., 2009). Understanding the balance of nitrogen inputs is key to promoting carbon sequestration in agricultural soils without causing soil degradation or environmental pollution (Griffiths et al., 2012; Bei et al., 2022).

Plants contribute to SOC pools through root exudates, aboveground litter, and root turnover (Lehmann and Kleber, 2015). Root morphology plays a critical role in determining how carbon is deposited in the soil profile. Root traits such as root length, diameter, and specific root length (SRL) influence plants' ability to explore a volume of soil and acquire nutrients (Guo et al., 2008). Fine roots (< 2 mm) are primarily responsible for nutrient and water uptake, and have a short lifespan, readily turning over for decomposition, and contributing to labile carbon pools. Coarse roots provide structural support and persist longer, contributing more to stable carbon inputs (Zhang and Wang, 2015; Wen et al., 2019). Roots with high SRL produce more root length per unit biomass, and may increase the potential for root exudation and carbon inputs to the soil (Ma et al., 2018). Longer and denser root systems can increase carbon inputs by distributing organic matter deeper through the soil profile, and potentially promote SOC storage in deeper layers (Gao et al., 2024).

The use of plants in cover cropping has been shown to increase SOC levels and improve soil fertility by contributing plant residues and enhancing microbial activity (Jian et al., 2020). Legumes and other N-fixing plants provide additional nitrogen through symbiotic relationship with fungi and bacteria, which further supports increases in SOC (Huang et al., 2021). Understanding plant-soil interactions and root morphology relationships with SOC is essential to help identify management practices that could optimize root contributions to SOC sequestration.

While these dynamics discussed with regards to SOC are important, SOC has been studies for decades. Mineral-associated organic carbon (MAOC) is a fraction of SOC, representing a stable fraction that is bound to mineral surfaces, making it more resistant to chemical and physical decomposition processes (Lehmann and Kleber, 2015). MAOC may form directly from plant inputs, but also firms through microbial transformation of organic matter, resulting in low-molecular weight compounds with low C/N ratios that sorb onto mineral particles (Cotrufo et al., 2019). This fraction of SOC is thought to have a much longer turnover time, persisting for decades to centuries, and plays a critical role in long-term carbon storage (Lavallee et al., 2020). However, emerging research suggests that MAOC is more dynamic and can be influences by factors such as root exudates, litter quality, and N availability resulting in priming effects (Kuzyakov, 2002; Jilling et al., 2021). For example, high quality litter with low C/N ratios can enhance MAOC formation, while high C/N litter can induce loss of MAOC due to priming effects, which may occur when microbes are N-limited and must mine N from existing the existing SOM pools (Poeplau et al., 2023)

Understanding how plant root traits and N management affect MAOC cycling is crucial for improving land management practices. By identifying relationships between plant roots, N-availability, and MAOC, we can develop strategies that may enhance long-term carbon sequestration and improve soil health in managed systems. This research aims to fill knowledge gaps by examining the interconnected roles of these factors.

The work presented in this dissertation highlights the complex interactions between plant traits, nitrogen inputs, and soil organic carbon (SOC) fractions, particularly mineral-associated organic carbon (MAOC). Chapter 1 aims to determine whether short periods of growth (e.g. a single growing season) by herbaceous plant species representative of land management systems result in changes in MAOC, and whether plant root traits are connected to any observed differences in MAOC. In a controlled greenhouse experiment, a variety of herbaceous plant species were grown over 12-weeks. Root traits (e.g. biomass, length, surface area, diameter, and more) were quantified to draw any relationships to MAOC, as many annual herbaceous plants are used as cover crops in agriculture and in reclamation. Review of potential changes induced over these shorter timescales may be useful in assessing land management practices in these systems.

Building from Chapter 1, Chapter 2 approaches the relationship between MAOC, N-fertilization, and root morphology in short-term growing periods in two ways. Utilizing a controlled greenhouse mesocosm experiment and stable isotope techniques, it is possible to determine which factors are responsible for changes in existing MAOC versus factors related to freshly added Plant-C. As the response with N-fixing plants differed from non N-fixing plants, different levels of N-fertilization which may be commonly used in agricultural practices, were cumulatively applied so as to determine whether varying levels of N-fertilization illicit different responses in the relationship between root traits and MAOC.

Chapter 3 aims to understand whether MAOC is affected by agricultural management practices, specifically cover cropping, tillage, and N-fertilization. In the Eastern Shore of Virginia, a 9-year corn cropping system experiment with varied cover crop management and N-treatments was sampled to assess MAOC concentrations across treatments. While MAOC wasn’t sampled at the start of the experiment, data at the end of the experiment allows for the comparison between tillage, cover crop treatment, and N-fertilizer additions. This research is crucial because it not only clarifies how MAOC responds to common agricultural practices but also informs strategies that protect soil fertility and promote long-term carbon sequestration.

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Chapter 2

Diverging effects of root morphology and nitrogen on mineral-associated organic carbon and soil organic carbon

Abstract

Soil organic matter (SOM) plays a vital role in terrestrial ecosystem function. The loss of soil organic carbon (SOC) due to land-use change and climate effects poses significant threats to soil health. Plant photosynthetic inputs are a primary mechanism for building SOM, but knowledge about how best to manage plant production to build stable long-term SOC pools remains lacking. This study investigates the relationship between plant root traits and SOC, particularly focusing on mineral-associated organic matter (MAOM), which is essential for long-term carbon (C) sequestration. I hypothesized that specific root morphological traits are related to changes in SOC and mineral-associated organic carbon (MAOC) pools during plant growth. A 12-week greenhouse experiment was conducted using 30 herbaceous plant species commonly grown in managed ecosystems. Soil and root samples were analyzed for C content and root traits, including biomass, volume, surface area, diameter, and specific root length (SRL). Results showed that non nitrogen (N)-fixing plants exhibited stronger correlations with MAOC compared to N-fixing plants, which exhibited stronger correlations with SOC. Among non-N-fixing plants, root biomass, SRL, and coarse root traits were correlated with MAOC, indicating these root characteristics play a crucial role in increasing MAOC stabilization in soils. These findings highlight the importance of favoring certain plant species and root traits to optimize land management practices for C sequestration and soil health improvement.

Introduction

Soil organic matter (SOM) is a fundamental component in terrestrial ecosystems, involved in nutrient supply and cycling, water infiltration and holding capacity, aggregate stability, and enhancing microbial activity and biodiversity (Kleber et al., 2015; Paul, 2016). These benefits are particularly important in land management systems such as agriculture and restoration where decomposition of SOM provides carbon (C), nitrogen (N), and other nutrients to support plant productivity (Johnston et al., 2009; Wood et al., 2016). A study processing changes in SOC stocks between 2001-2015 estimates a total global loss of ~3.1 Pg C (Prăvălie et al., 2021) due to the conversion of grassland to agricultural systems, deforestation, land degradation, and unsustainable agricultural practices (Del Galdo et al., 2003; Conant et al., 2017; Chang et al., 2021; Nave et al., 2024). Climate further exacerbates ecosystem responses. For instance, warming can increase microbial respiration, C mineralization, and loss from soil (Lu et al., 2013; Poeplau et al., 2020). Not only is this problematic to soil health and ecosystem function, but the release of soil C to the atmosphere contributes to increased CO_2 concentrations, promoting a positive climate-soil C feedback (Scharlemann et al., 2014; Crowther et al., 2016). Additionally, loss of SOM can weaken soil structure and make soils more susceptible to erosion and compaction associated with land management practices (Zhang et al., 2005; Liu et al., 2019). The largest pool of terrestrial organic C (> 1500 Pg C) and a significant fraction of total SOM is referred to as mineral-associated (Sokol et al., 2022).

Mineral-associated organic carbon (MAOC) is crucial for long-term SOC storage, as it is stabilized by association with minerals and thereby less accessible to decomposers and enzymes, persisting over longer timescales in soil (Lehmann and Kleber, 2015; Cotrufo et al., 2019). However, emerging evidence suggests that MAOM may be more dynamic than previously thought, cycling on shorter timescales and influenced by factors such as above and below-ground litter quality, plant root exudates, and plant signaling (Chen and Stark, 2000; Veen et al., 2019, Cotrufo and Lavallee, 2022; Poeplau et al., 2023). Plant-soil interactions are complex, with evidence that plants can influence the net soil C balance (Dijkstra et al., 2021). It is well documented that plants can contribute to increasing SOM by adding organic materials (e.g., aboveground litter, root biomass, root exudates) (Cotrufo et al., 2015). However, interactions between plant functional traits, especially belowground traits such as root morphological features (e.g., root length, diameter, volume), specific root length, or nutrient uptake strategies, and different fractions of SOC are less clear (Kumar and Singh, 2024). For example, litter inputs may result in the mineralization of old MAOC (Poeplau et al., 2023), while other studies have shown that high quality (low C/N) litters aid in OM stabilization and formation of MAOC (Cotrufo et al., 2013; Lavallee et al., 2018). While research is growing considerably in this area, there is still limited understanding of how roots of different plant species influence MAOC cycling, especially regarding to root morphological traits.

Plant roots are a major contributor to soil nutrient cycling via inputs from root decomposition and rhizodeposition (Silver and Miya, 2001; Thivierge et al., 2016). Root diameter plays a key role in root foraging strategies and in determining the capacity of a plant to exploit a volume of soil (Ma et al., 2018). Herbaceous plant roots are often categorized as fine (< 2.0 mm) or coarse (> 2.0 mm), which are associated with different resource acquisition strategies. Fine roots are used for nutrient, water, and oxygen uptake, where coarse roots are support plant structure and the fine root network, as well as delivering nutrients and water to shoots (Zhang and Wang, 2015; Thivierge et al., 2016; Wen et al., 2019). Fine roots with high specific root length (SRL; cm g^{-1}) - a measure used to indicate root length production efficiency - exhibit more rapid growth, resource acquisition, and shorter life spans, while coarse roots with lower SLR have slower growth, resource acquisition, and longer life spans (Guo et al., 2008). Root length, in contrast, regulates the root’s absorbing surface area, impacting the plant’s competitive ability to explore and exploit soil. Longer root systems and higher root biomass contribute to increased C inputs throughout the soil volume or within specific horizons because of their spatial distribution and association with increased exudation rates (Fisher et al., 1994; He et al., 2021; Gao et al., 2024). Thus, plant root functions related to length, surface area, biomass, diameter, can play an important role in determining where and how quickly MAOC is likely to be formed or depleted in the soil profile (Slessarev et al., 2020). By understanding how root traits impact carbon cycling, we can better identify land management practices that promote sequestration of atmospheric C to mitigate climate change and improve soil health.

This study aims to determine whether MAOC changes over a short duration of plant growth in herbaceous plant species, and whether plant root traits are connected to any observed differences in MAOC. The hypothesis is that specific root functional traits are more strongly correlated with resulting concentrations of organic C in soil, and that these relationships will differ when evaluating ΔMAOC versus ΔSOC. To test this hypothesis, I grew a variety of herbaceous plant species commonly used in managed ecosystems such as cover cropping and restoration for 12 weeks and quantified a variety of root traits to explore how they affected resulting bulk SOC and MAOC. As many annual herbaceous plants are used as cover crops in agriculture and in restoration, review of potential change induced over these shorter timescales may be useful in assessing land management practices in these systems.

Methods

Soil Preparation and Plant Selection

A custom soil media used in this experiment was comprised of a 3:2 ratio of locally sourced and screened topsoil amended with bark and leaf compost (Pine Ridge Nursery, Roanoke, VA) and medium grain sand (0.25 – 0.5 mm). Amended soil media and sand were homogenized in a 1-cubic yard batch mixer (Pack Manufacturing, McMinnville, TN) and sieved to 4 mm. A group of thirty herbaceous plant species commonly used in managed ecosystems were selected to encompass a variety of several functional traits (Table 1).

Table 1: Selected plants arranged by family. Seeds were acquired from Ernst Conservation Seeds Inc.[1], Nature's Seed[2], Sow True Seeds[3], Southern Exposure[4], and Hilo Hemp Seed[5].

Life Form (N=30)	**Species**
Cool grass n=10	Annual Rye (*Lolium multiflorum*)[1]
	Barley (*Hordeum vulgare* L.)[2]
	Oat (*Avena sativa*)[1]
	Orchard Grass (*Dactylis glomerata*)[1]
	Perennial Rye (*Lolium perenne*)[1]
	Rye S. cereal (*Seculae cereal*)[1]
	Tall fescue (*Festuca arundinacea*)[1]
	Timothy (*Phleum pretense*)[1]
	Triticale (*x Triicosecale*)[2]
	Wheat (*Triticum aestivum*)[2]
Legume n=9	Alfalfa (*Medicago sativa*)[1]
	Birdsfoot trefoil (*Lotus corniculatus* L.)[1]
	Cowpea (*Vigna unguiculata*)[4]
	Crimson Clover (*Trifolium incarnatum*)[1]
	Hairy vetch (*Vicia villosa*)[1]
	Soybean (*Glycine max*)[1]
	Spring pea (*Pisum sativum*)[2]
	Sunn hemp (*Crotalaria juncea L.*)[4]
	White clover (*Trifolium repens*)[1]
Warm grass n=4	German millet (*Setaria italic*)[1]
	Pearl millet (*Pennisetum glaucum*)[2]
	Sudangrass (*Sorghum x drummondii*)[2]
	Teff (*Eragrostis tef*)[1]
Forb n=4	Buckwheat (*Fagopyrum esculentum*)[1]
	Hemp (*Cannabis sativa*)[5]
	Phacelia (*Phacelia tanacetifolia*)[3]
	Sunflower (*Helianthis annuus*)[1]
Brassica n=3	Mustard (*Synapis alba*)[2]
	Radish (*Raphanus sativus var. longipinnatus*)[1]
	Turnip (*Brassica rapa)*[1]

Experimental Design

Plants were grown in 5 L tree pots, (12.5 cm wide by 30 cm deep; Stuewe & Sons, Inc.) and filled with 4.5 L of soil. There was one pot for each plant species, with replicates by life forms (total N=30; Table1), as well as a no-plant control pot. Leguminous species including alfalfa, birdsfoot trefoil, crimson clover and white clover were inoculated with Rhizobium leguminosarum biovar trifolli (Exceedâ Super Legume Inoculant @ 170 g per 22.68 kg seed) while spring pea and hairy vetch were inoculated with Rhizobium leguminosarum biovar viceae (Exceedâ Super Legume inoculant @ 71 g per 22.68 kgs seed). Seeds were planted directly into each pot and seedlings were selectively terminated upon reaching 5-7 cm in height for grasses, or when the first set of true leaves emerged, to leave one individual plant in each pot. Variation exists in plant species growth rate and response based off of several environmental factors (Vollmer et al., 2023). Plants in this study were grown over a 12-week period in a greenhouse under ambient light with day and night temperatures ranging between 20 – 22°C and 16 – 18°C, respectively. Plants were watered every 2-3 days through week 11 to allow soil to dry prior to sampling.

Sampling

At 12 weeks, soil from each pot was emptied into a container and the whole, intact plant was separated from the soil. Aboveground biomass was clipped at the collar where stem and roots connect and stored in paper bags. Roots were gently washed with water to remove soil, collected in gallon plastic bags, and covered with enough deionized water to keep roots fully submerged until further analysis (Jaloviar et al., 2008). Roots were stored at 4°C up to a week post-harvest before analysis of physical attributes (Himmelbauer et al., 2004). Bulk soil was homogenized, subsampled into quart bags, and then allowed to air dry prior to fractionation and analysis.

Plant Analysis

Full root systems were carefully teased apart in a transparent acrylic scanning tray filled with deionized water and scanned with an Epson flatbed scanner (Epson Perfection V800/V850) connected to 2017 WinRHIZO® root-scanning software (Regent Instruments Inc., QC, Canada). Root systems too large for scanning trays were sub-sectioned longitudinally into smaller sections, one section was scanned and scaled by mass to represent the total root system (Bouma et al., 2000). Root structural traits were quantified (Table 2). Total length, root volume, and surface areas were determined for each plant by diameter class (10 classes of 0.5 mm increments). Values were summed to quantify fine (< 2 mm) and coarse (> 2 mm) root categories. Analytical correction was applied to scans to reduce error by identifying and excluding non-root debris. After root-scanning analysis, above and below ground plant material was oven-dried at 65°C and weighed. Sub-sectioned root masses used for scanning were scaled by biomass to quantify the total plant values for root length, surface area, and volume. Subsamples of oven-dried roots were then ball milled to obtain C and N concentrations using a continuous flow isotope ratio mass spectrometer with an elemental analyzer front end (Isoprime 100 EA-IRMS, Elementar, Ronkonkoma, NY, USA).

Soil Analysis and Fractionation

Functional fractionation of bulk SOC into POM and MAOM provides soil C subpools with differing chemical and physical characteristics as well as varied rates of turnover (Elliott, 1986; Six et al., 1998; Lavallee et al., 2020). To separate and quantify C associated with MAOM, bulk soil was separated by particle size (modified from Six et al., 1998); defined here as the C associated with the silt and clay (< 53 µm) size fraction. A 10 g subsample of air-dried soil was sieved through a 53 µm screen using deionized water. The suspensions passing through the screen were centrifuged in 50 mL falcon tubes at 4000 g for 15 min, after which the supernatant was decanted. The remaining solids were collected and dried at 65° for 24 hr, and then ground using an agate mortar and pestle and stored until analysis. Total SOC was determined from a subsample of bulk soil, which was sieved to 2 mm and then ball-milled prior to elemental analysis for C and N.

Data Analysis

Plant-induced changes in SOC (ΔSOC) and MAOC (ΔMAOC) (g C / 100g soil) were calculated as the difference in C between a planted pot and the no-plant control at the end of the 12-week growing period. Data was tested for normality, and outliers were tested using IQR and quantile robust fit. Sudangrass was found to be an outlier in all factors (except diameter), so it was removed from subsequent analyses. Pearson's correlation (*r)* was used to assess linear correlation between root traits and ∆SOC and ∆MAOC, and then simple linear regression was used to further analyze the predictive relationship between root traits and soil C variables. Regressions were tested for homogeneity of the residuals using Shapiro-Wilks test. ANOVA was used to test root C/N between N-fixing and non N-fixing groups. All data analysis was completed using JMP software (JMP® Pro, Version 17.0. SAS Institute Inc., Cary, NC, 1989-2024.).

Table 2: p-values and R^2 for linear regression models between soil carbon and root traits for all plants, and then grouped by N-fixing and non N-fixing species. Bold values indicate significant relationships, while bold with asterisk indicates marginally significant relationships between plant and no-plant control after 12-weeks in Bulk SOC or MAOC. "ALL" indicates p-values when all plants were analyzed; results for the N-fixing and non N-fixing groups are reported for both Bulk

		Root Volume			Root Surface Area			Root Length			Root Biomass	SRL	Avg Diameter
		Total	Fine	Coarse	Total	Fine	Coarse	Total	Fine	Coarse			
Bulk SOC	All	0.86 (0.0)	0.93 (0.0)	0.64 (0.02)	0.97 (0.0)	0.75 (0.0)	0.46 (0.02)	0.94 (0.0)	0.91 (0.0)	0.45 (0.02)	**0.09*** (0.10)	0.15 (0.08)	0.26 (0.05)
	N-fixing	0.98 (0.0)	0.88 (0.0)	0.25 (0.18)	0.84 (0.0)	0.72 (0.02)	0.25 (0.18)	0.55 (0.05)	0.54 (0.06)	0.30 (0.15)	**0.08*** (0.38)	**0.10*** (0.34)	0.18 (0.24)
	No N-fixing	0.97 (0.0)	0.95 (0.0)	0.97 (0.0)	0.96 (0.0)	0.94 (0.0)	0.99 (0.0)	0.88 (0.0)	0.87 (0.0)	0.98 (0.0)	0.46 (0.03)	0.90 (0.0)	0.41 (0.04)
MAOC	All	**0.05** (0.14)	0.40 (0.01)	**0.067*** (0.14)	0.17 (0.07)	0.45 (0.02)	**0.028** (0.17)	0.55 (0.01)	0.60 (0.01)	**0.046** (0.15)	**0.01** (0.22)	0.11 (0.09)	**0.066*** (0.12)
	N-fixing	0.76 (0.01)	0.80 (0.01)	0.56 (0.05)	0.75 (0.01)	0.79 (0.01)	0.57 (0.05)	0.77 (0.01)	0.77 (0.01)	0.60 (0.04)	0.71 (0.01)	0.61 (0.04)	0.99 (0.0)
	No N-fixing	**0.058*** (0.19)	0.33 (0.05)	**0.05** (0.20)	0.19 (0.09)	0.50 (0.02)	**0.02** (0.25)	0.61 (0.01)	0.67 (0.01)	**0.04** (0.22)	**0.01** (0.36)	**0.004** (0.37)	**0.053*** (0.19)

Results

All Plants

Root traits demonstrated numerous negative correlations with ΔMAOC, but not with bulk ΔSOC. Specifically, significant ($p < 0.05$) relationships with ΔMAOC were observed for total root volume, coarse root surface area, coarse root length, and root biomass. Marginally significant ($p < 0.1$) relationships were observed with coarse root volume and average diameter. With ΔSOC, which in this study, represents all SOC in the bulk soil, there were no significant relationships observed with any root traits, and only root biomass was found to be marginally significant (Table 2).

Root biomass exhibited a negative relationship with ΔMAOC (Figure 1a); as did all other correlated factors with $p < 0.05$ (Appendix 1). Meanwhile, although only marginally significant, the trend between root biomass and ΔSOC was opposite, with increased root biomass resulting in positive ΔSOC (Figure 1b).

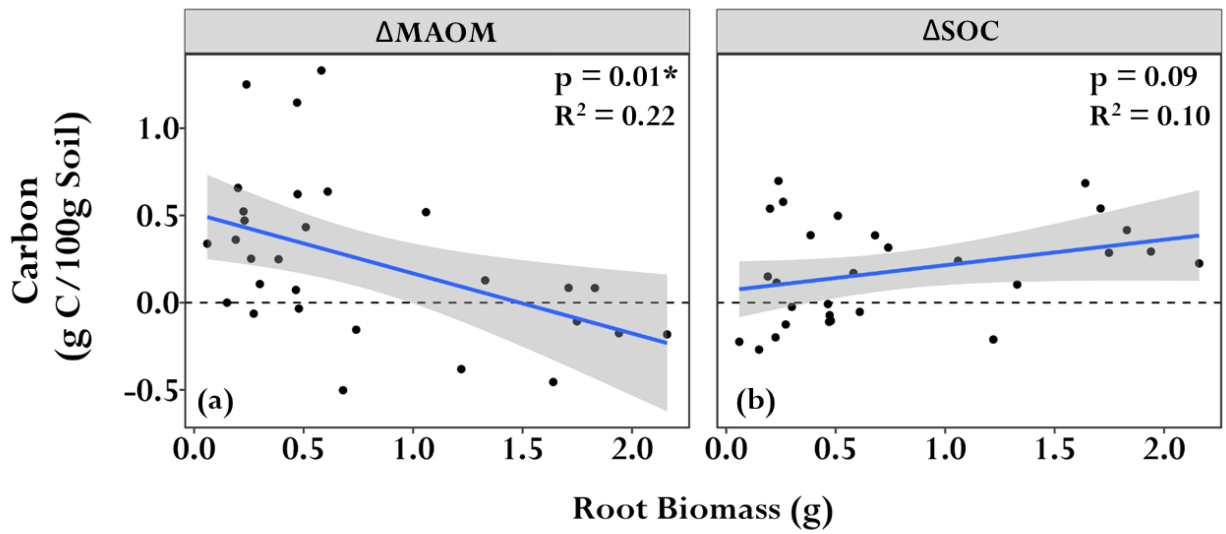

Figure 1: Linear regressions with confidence interval (blue line with grey shading) for root biomass (g) in both ΔSOC (right) and ΔMAOC fraction (left) across all 30 plant species (n=1 per species). Amount of soil carbon (g C / 100 g soil) reported as the difference between planted pot and the no-plant control after 12 weeks.

N-fixing vs. non N-fixing plants

After analyzing all plant species together (Table 2: All), N-fixing and non N-fixing plants were analyzed separately due to differences in nitrogen dynamics based on lifeform from symbiotic N-fixing relationships that may influence C cycling in plant-microbe-soil interactions (Table 2: No N-fixing v. N-fixing). Direct analysis of root C/N between non N-fixing and N-fixing plants shows the two groups are different, with C/N means of 21.6 and 13.9 respectively (ANOVA, $p = 0.0008$; Figure 2).

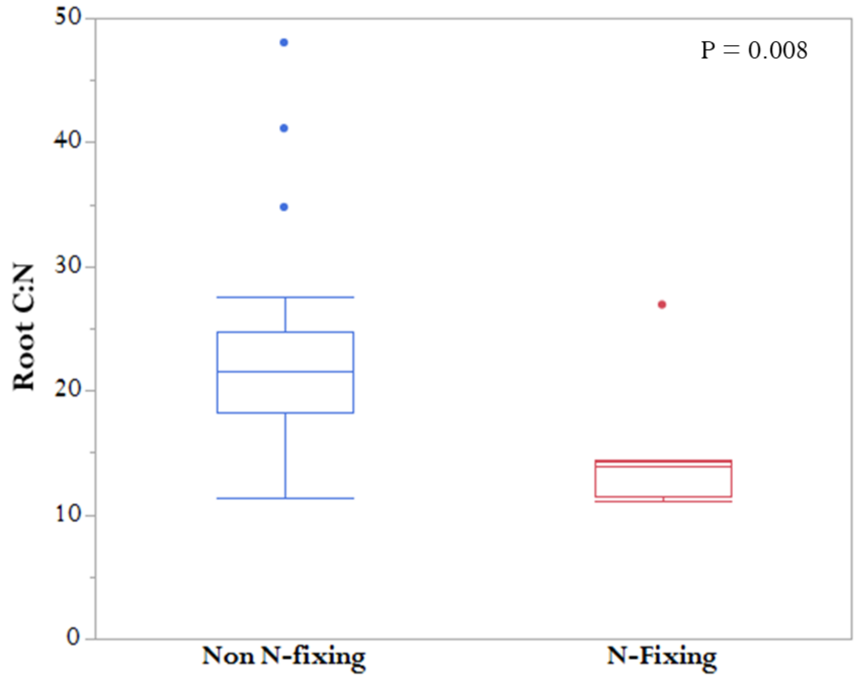

Figure 2: One-sided t-test analysis for root C/N values grouped by non N-fixing and N-fixing plant lifeforms.

For ΔMAOC, the relationships of root traits in non N-fixing plants were significant, with the R^2 of the model increasing from the model with all plants, to indicate a better fit (Table 2: MAOC no N-fixing). In contrast, none of the root factors were significant to ΔMAOC in N-fixing plants (Table 2: MAOC N-fixing).

Root biomass exhibited a similar trend after splitting the data; it remained significant in non N-fixing plants (Figure 3a), and became non-significant with N-fixing plants (Figure 3b). For ΔSOC, after splitting groups, root biomass was marginally significant ($p = 0.08$), exhibiting a positive trending relationship with ΔSOC, but only for N-fixing plants (Figure 3d). In ΔSOC, while the root factors remained insignificant, the R^2 values increased between ΔSOC and N-fixing plants, and decreased with non N-fixing plants. This data suggests that root traits as controlling factors are limited to non N-fixing plants in ΔMAOC, and to N-fixing plants in ΔSOC.

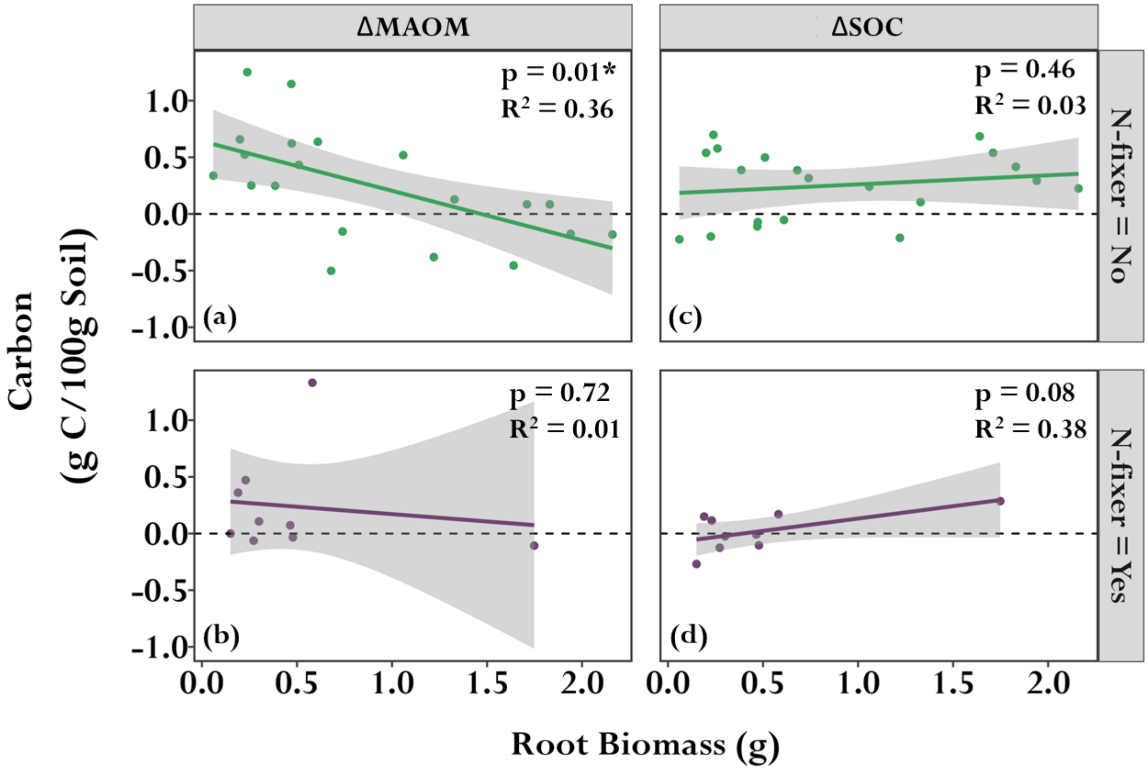

Figure 3: Linear regressions with 95% confidence interval (blue line with grey shading) for root biomass (g) in both SOC (right) and MAOM fraction (left). Panels represent a split between plant function as N-fixing (bottom, n=9) or not (top, n=21). Amount of soil carbon (g C / 100 g soil) reported as the difference between planted pot and the no-plant control after 12 weeks.

Specific Root Length and Root Diameter

When all plants were analyzed together, SRL was not correlated with ΔMAOC or ΔSOC (Table 2: ALL). However, when subgroups were analyzed separately, SRL was positively correlated with ΔMAOC ($p = 0.004$) among non N-fixing plants (Figure 4a), and marginally negatively correlated with ΔSOC ($p = 0.10$) in N-fixing plants (Figure 4b). N-fixing capacity was not statistically related with ΔSOC and SRL (Figure 4c & 4d).

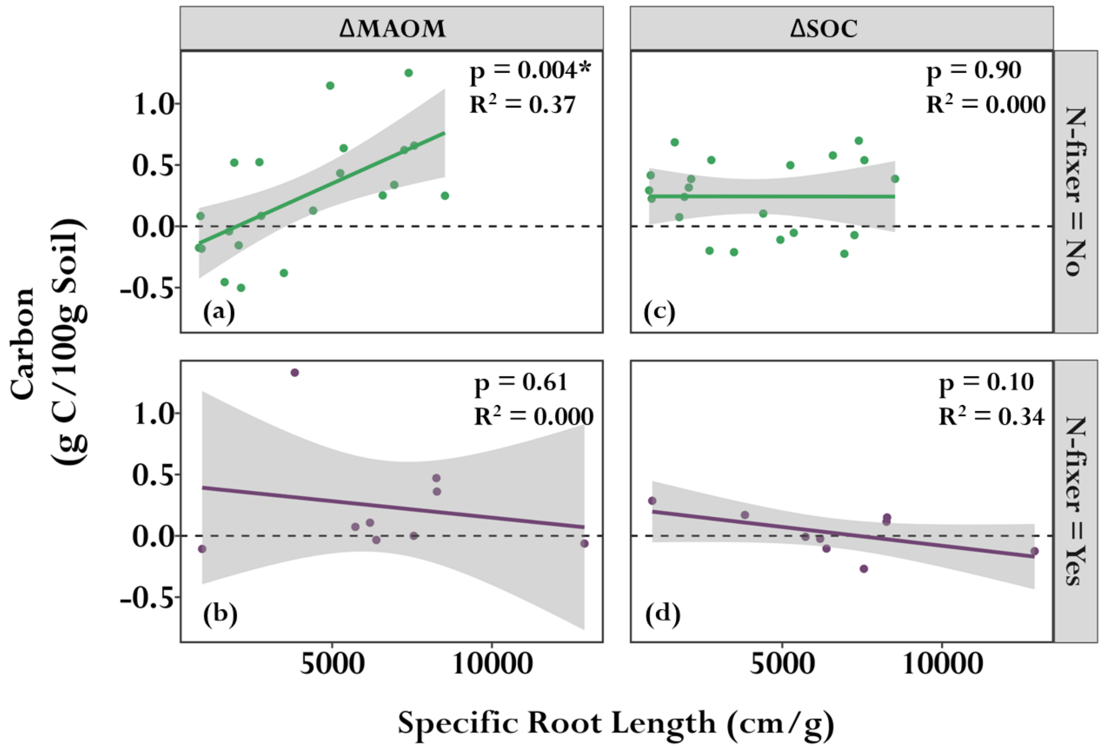

Figure 4: Linear regressions with 95% confidence interval (green and purple lines with grey shading) for specific root length (cm/g) in both SOC (right) and MAOM fraction (left). Panels represent a split between plant function as N-fixing (bottom, n=9) or not (top, n=21). Amount of soil carbon (g C / 100 g soil) reported as the difference between planted pot and the no-plant control after 12 weeks.

SRL exhibited a negative relationship with average root diameter across all plants (Figure 5). SRL maintained a negative relationship with root diameter (Figure 6a) and exhibited a positive relationship with ΔMAOC in non N-fixing plants (Figure 4a). This implies that fine roots, associated with higher SRL, led to increased ΔMAOC. Furthermore, as root diameter increased (i.e. roots become coarser), SRL (Figure 4 and 6a) and ΔMAOC (Figure 7a) both decreased. This observation is consistent with the negative correlations observed between coarse root length, surface area, and root volume and ΔMAOC for non N-fixing species; as the coarse fraction of root length, surface area, and root volume increase, ΔMAOC decreased (Appendix 1). This is another example of differing dynamics between ΔSOC and ΔMAOC, as the relationship between

SRL and root diameter does exhibit similar responses with ΔSOC as they do with ΔMAOC and regardless of N-fixing capacity of plant species (Figures 4b and 6b).

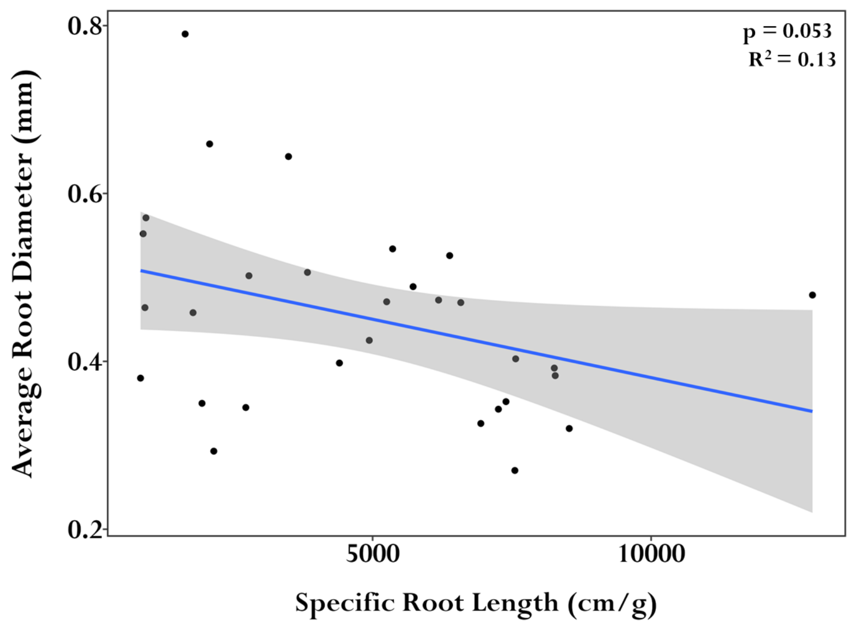

Figure 5: Linear regression with 95% confidence interval (blue line with grey shading) showing the relationship between specific root length (cm/g) and average root diameter (mm) for all 30 plant species (n=1 per species) at 12 weeks.

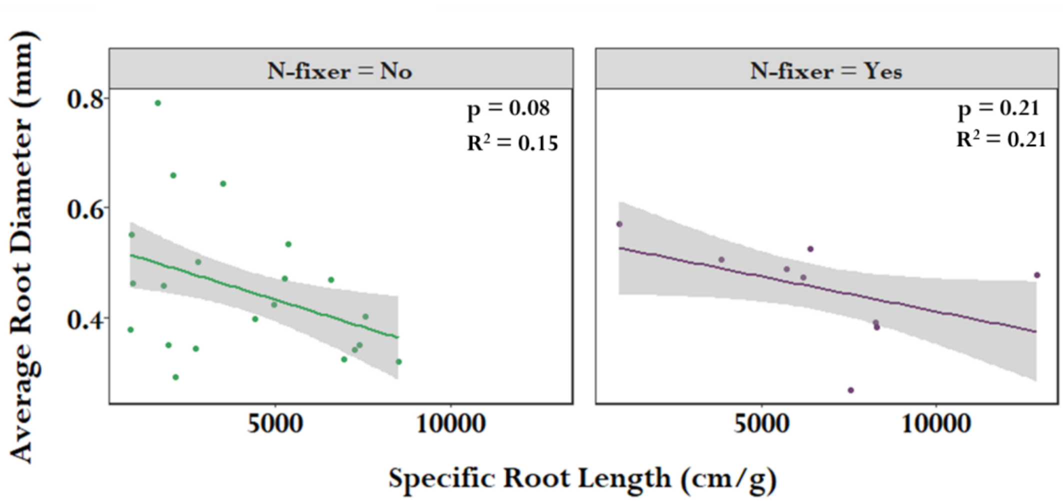

Figure 6: Linear regressions with 95% confidence interval (green and purple lines with grey shading) for the relationship between specific root length (cm/g) and average root diameter (mm) grouped by N-fixing function (N-fixer n=9; non N-fixer n=21) of plants at 12 weeks.

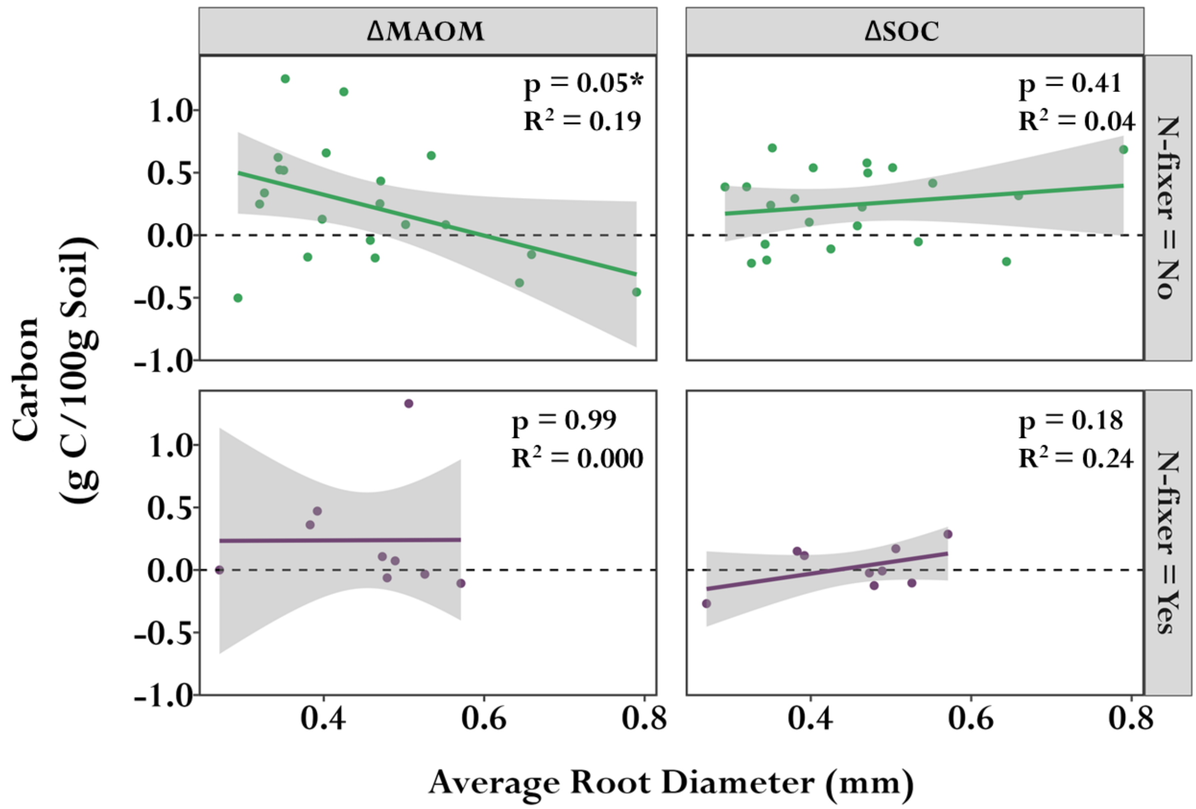

Figure 7: Linear regressions with 95% confidence interval (green and purple lines with grey shading) for average root diameter (mm) in both SOC (right) and MAOM fraction (left). Panels represent a split between plant function as N-fixing (bottom, n=9) or not (top, n=21). Amount of soil carbon (g C / 100 g soil) reported as the difference between planted pot and the no-plant control after 12 weeks.

Discussion

Results in this study demonstrate that root morphological traits likely influence short-term cycling of MAOC. While there were several root trait factors with negative correlations to ΔMAOC, root biomass emerged as the most consistent significant trait affecting both ΔSOC in bulk soil and ΔMAOC. Most notable though, were the differing relationships with each carbon pool depending on whether or not plant species were N-fixing. Plant species identified as non N-fixing had stronger relationship with ΔMAOC, and N-fixing species exhibited stronger relationships with ΔSOC. While ΔSOC in the non N-fixing group (Figure 3c) did not exhibit significant relationships with any root traits, the decreasing trend in ΔMAOC and steady ΔSOC suggests that organic carbon may be increasing in the particulate organic matter (POM) fraction, which is made up of low density, minimally decomposed fragments of plant and other organic biotic materials (Lavallee et al., 2020). Although POM was not measured in this study, it is important to consider how the dynamics in POM may be changing. For example, if SOC in bulk soil is neutral or increasing, and MAOC is decreasing, then the assumption that C is increasing in another soil fraction, such as POM is likely. For example, there is existing evidence which shows that fresh litter additions can promote mineralization of old MAOM, and that these same additions result in increasing POM over the same timeframe (Poeplau et al., 2023).

Rhizosphere Priming Effects

There are two important implications of the observed decreases in ΔMAOC among non N-fixing plant species (Figure 3a). Firstly, the presence of plants did not always result in a net increase of C in soil and, secondly, MAOC pools can change measurably in relatively short time frames. A likely explanation for the observed effect was insufficient N availability to meet nutrient demands of soil microbes, leading to C mineralization. Plant roots are sensitive to environmental conditions around them and may receive cues which promote directional growth (Monshausen and Gilroy, 2009), or in limited below-ground resources, an increase in root biomass versus aboveground biomass (Poorter and Nagel, 2000). Higher root biomass can contribute to increased carbon inputs to the soil via increased exudation rates (Ryser, 2006). Plants may also, intentionally or incidentally, exude high C/N residues, which can stimulate priming of microbial SOM mineralization as N-limitation increases (Jilling et al., 2019). Root C/N impacts ΔMAOC because of differences in C/N, which the data in this study supports. Legumes have lower C/N ratios than grass species due to increased N compounds (Sorensen and Thorup-Kristensen, 2003; Redin et al., 2018) which leads to higher decomposition rates during root turnover, and release of N, which can decrease N limitations in the soil resulting in increased SOC, or reduced SOC loss when compared to non-legume plants (Virk et al., 2022). N-fixing plants are known to supplement nutrient availability in soil through root associated rhizobial relationships, which play a key role in SOC accumulation (Fornara and Tilman, 2008; Rasmussen et al., 2012).

Higher C/N inputs in non-legume species can induce priming to provide increased available nutrients that could increase plant or microbial growth. Microbes, which respond to the N-limitation, upregulate the production of extracellular enzymes to degrade SOM and access N contained within for use, resulting in mineralization and loss of carbon. This process is referred to as the rhizosphere priming effect (Kuzyakov et al., 2000; Dijkstra & Chen, 2007). Priming could also explain the lack of differences in ΔMAOC in the N-fixing group (Figure 2b). Within the N-fixing group, there may be adequate nitrogen availability due to symbiotic relationships with N fixing microbiota and decomposition of roots as described above. In turn, if the plants would not need to allocate resources to promote higher root biomass to help with nutrient acquisition. While this study did not focus on C/N explicitly, the data do align with studies showing the lower C/N in legumes (ANOVA, means of 14.5 vs. 23.5) which supports the notion of differing C/N availability and N-limitations which may induce priming.

Lower root biomass was observed in N-fixers (mean = 0.49 g) compared to non N-fixing species (mean = 1.13 g). The stability of MAOC in N-fixing species, despite variations in root biomass, underscores the importance of considering nitrogen supply from plants in moderating carbon cycling processes. Plant selections that incorporate N-fixing plants, or other soil management practices aimed at enhancing nitrogen availability, could help prevent the priming and short-term loss of MAOC after planting in soil ecosystems.

Root Biomass

There is ample evidence that suggests root biomass is correlated with increasing SOC stocks (Menichetti et al., 2015; Cotrufo et al., 2015; Poeplau et al., 2021). In this study, that trend was only observed for SOC in the N-fixer group (Figure 2d), where root biomass corresponded to positive ΔSOC. In addition to differing C/N ratios in roots, higher root biomass in N-fixers may increase rhizodeposition of N (Wang et al., 2021), in turn, decreasing mineralization of SOC. In this study, the root biomass of N-fixers was actually lower than non N-fixing species, which may indicate that lower proportion of root biomass means that these plants have a lower demand for nutrients (Wang et al., 2022).

While root biomass does exhibit some influence on both ΔSOC and ΔMAOC over short time scales, whether it will maintain the same relationship over longer periods of growth is unknown and could vary based on environment and other factors. The observed decrease in MAOC could just be an initial dip as a result of early plant growth and increasing microbial activity. Further, dynamics between root biomass and MAOC may vary widely when considering the entire life cycle of plant additions, and how those plants are managed seasonally and over years. The differing impacts of root biomass on MAOC between N-fixing and non N-fixing species reinforces existing knowledge that nitrogen availability is a crucial factor in carbon management. For instance, non N-fixing species with high root biomass might be much less effective at maintaining MAOC. While increased root biomass may typically be seen as a benefit to soil health, it may not always lead to increased carbon retention in certain soil fractions. Improving our understanding of dynamic root relationships and the synergistic effects of species-rich plant mixtures could help inform species selection and planting timelines in cover cropping and restoration practices with the aim to balance carbon sequestration and nutrient management (Wuest et al., 2021).

Specific Root Length and Root Diameter

Plants with high SRL exhibit higher rates of nutrient uptake due to more root length given per soil volume, where low SRL plants exhibit a more conservative growth strategy (Kramer-Walter et al., 2016). A positive correlation has been shown between the amount of root exudates and higher SRL due to fine roots (Meier et al., 2020), and (Huang et al., 2021) found that priming effects increase with higher SRL, which was associated with decreased SOC, but that 72% of the root-derived C was in the particulate organic C pool. On the other hand, in grassland and crop species, increasing root diameter may also lead to increased root exudation, potentially resulting in increased microbial activity and priming mechanisms (Wen et al., 2019) which could reduce SOC. The results observed in this study suggest that high SRL and low diameter roots have a positive relationship with ΔMAOC, which is inverse of what has been observed with SOC and highlights differences in the dynamics of different soil carbon pools. It is possible that losses in SOC are primarily occurring in POC, while gains in MAOC may be occurring. The loss of C in POC would need to be greater than increases in MAOC to result in net loss of SOC, which highlights why additional research focusing on SRL and root diameter with relation to MAOC and POC is necessary.

Conclusion

I examined the role of root morphological traits in influencing SOC and MAOC concentrations among herbaceous species commonly used in agricultural and managed ecosystems. Results indicate that root morphological traits have unique relationships with MAOC over relatively short periods of plant growth that are common in these systems (e.g., cover crop seasons). What is also apparent from this research is that the direction and magnitude of effects that root traits exhibited with SOC and MAOC differed from each other and between non N-fixing and N-fixing species. This work underscores the importance of considering dynamics between plant-soil interactions in soil carbon studies. Plant function, such as N-fixing capacity, and other traits have different relationships depending on how soil is analyzed, such as total SOC versus MAOC. Therefor plant selection may have a significant impact on findings in studies such as this, and should be carefully considered.

The findings in this study also highlight several knowledge gaps requiring future research. Firstly, future studies should aim to quantify POM alongside MAOM to better understand the full spectrum of soil carbon dynamics in relation to root functional traits. Secondly, identifying the specific mechanisms by which root functional traits influence MAOC, especially in relation to exudation rates, quality, and nitrogen interactions in varying root diameters would improve understanding of how root systems can be leveraged to influence soil carbon sequestration. Lastly, while this study provides valuable insights from relatively short time periods in controlled conditions, long-term field studies that incorporate plant species diversity, land management practices, and environmental factors are crucial to maximize the benefits of the work.

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Chapter 3

Effects of nitrogen fertilization on the relationship between root morphological traits and mineral associated organic carbon

Abstract

Nitrogen (N) fertilization plays a crucial role in mediating how plant growth impacts soil organic carbon (SOC) dynamics, particularly in relation to mineral-associated organic carbon (MAOC), a key reservoir for long-term carbon (C) storage. This study examines how N fertilization and root morphological traits of herbaceous plants influence MAOC formation. Using a potted mesocosm design, eight non-leguminous plant species with distinct root traits were grown under four N-fertilization levels (0, 56, 112, and 168 kg/ha) for 11 weeks. Plant contributions to SOC and MAOC were traced using ^{13}C stable isotope labeling. Results indicate that N fertilization was the dominant factor affecting total SOC and MAOC pools, with the more moderate N additions (56 and 112 kg/ha) promoting C increases, while higher N (168 kg/ha) led to a decline in C accumulation. In contrast, plant-added MAOC (PA-MAOC) had no main or interaction effects due to N fertilization, instead, PA-MAOC was related to plant trait factors such as root biomass, coarse root length, and aboveground biomass. Plant species differences also contributed to PA-MAOC variability. Relationships between root morphological traits and MAOC shifted with N treatment, with positive correlations observed at moderate N levels (112 kg/ha), but negative correlations emerging under N-limited conditions. These results underscore the nuanced role of N fertilization in soil C stabilization and highlight the importance of plant traits in governing plant-derived C additions to MAOM. This study suggests that strategies for enhancing soil C sequestration should consider both the influence of N fertilization and the role of plant root traits in shaping carbon storage within the MAOM fraction.

Introduction

Different pools of soil organic carbon (SOC), in bulk soil are functionally separated based on whether they are associated with minerals or not (i.e., mineral associated organic carbon (MAOC) vs. particulate organic carbon (POC)). MAOC and POC have characteristics which may differ due to these associations, such as turnover times and chemical attributes (Sokol et al., 2022). In particular, MAOC represents organic C thought to be protected by sorption to mineral surfaces, with a residence time of decades to centuries, which is made up of low molecular weight compounds with low C/N ratios (Lavallee et al., 2020). MAOC is also a critical reservoir for nutrients, accounting for the majority of soil C and N (Gentsch et al., 2015). There is increasing evidence that environmental and management factors affect these protections and that MAOC cycling is much more nuanced and dynamic than initially thought. For example, old and newly generated MAOC cycled faster based on wet-dry cycling in the presence of simulated root exudates (Jilling et al., 2021). It has also been shown that litter quality (C/N ratio) of roots resulted in increasing loss of MAOC as root C/N increases over a 2 year duration (Poeplau et al., 2023). As a long-term storage pool for SOC, management of MAOC may be key to managing SOC stabilization and minimizing carbon losses to the atmosphere. There are few studies which have included plant root dynamics and MAOC cycling, and most focus on some form of C/N quality in litters and roots exudates. Assessing how root traits may relate to MAOC dynamics under differing levels of N availability may provide further insight useful in land management practices.

Nitrogen (N) is an essential nutrient required for plant growth and development due to its role in forming proteins, enzymes, and nucleic acids, which are essential for plant metabolism and structural components of cells (Leghari et al., 2016). Nitrogen stored as proteins in plants facilitates critical functions such as photosynthesis, energy transfer, and cell growth (Fernandes and Rossiello, 1995). Availability of N in soil is another factor that aids in shaping the soil microbiome, which in turn contributes to plant uptake of N as soil microbes contribute to N-cycling through mineralization, nitrification, and denitrification (Novoa and Loomis, 1981). Nitrogen also directly influences root physiology by regulating root morphology, nutrient uptake, and metabolic activity (Glass, 2003). Conditions of N in soil influence root-soil interactions, affecting root elongation, branching, and the absorption of nutrients (Ötvös et al., 2021). Additionally, N availability impacts root-to-shoot growth ratios, with low N levels encouraging root development to explore new nutrient sources, whereas high N levels may suppress root growth in favor of shoot development (Glass, 2003; Santner et al., 2009).

Anthropogenic manipulations of soil N, specifically the addition of N fertilizers and cultivation of N-fixing plants, is a common practice in land management strategies. Nitrogen addition to supports plant growth and associated ecosystem services such as soil C sequestration, improvements in soil health, and climate change mitigation (Tisdale, et al., 1956; Khan et al., 2007; Ladha et al., 2011; Kumari and Maiti, 2022). Such N additions alter soil C/N stoichiometry, a pivotal relationship influencing ecosystem function (Griffiths et al., 2012; Abrar et al., 2021). Specifically, fertilization strategies have demonstrated the ability to alter soil nutrient dynamics and promote C sequestration in both topsoil and subsoil layers, highlighting the interconnectedness of C and N in sustaining soil health and fertility (Griffiths et al., 2012; Abrar et al., 2021). However, there is also evidence that N can have adverse effects on C sequestration; incubation studies have shown that N fertilizers can promote accelerated mineralization of SOC by inducing higher microbial activity at lower C/N ratios (positive priming effect) (Kuzyakov, 2002; Bei et al., 2022).

Nitrogen additions, through fertilization or natural cycling, can enhance soil organic carbon (SOC) stabilization by promoting plant biomass production, which in turn provides litter inputs via shoots and leaves, and may increase root production and exudates inputs that contribute to SOM pools (Chen et al., 2020). Plants are the primary contributors of organic carbon (C) into soil through plant senescence, aboveground litter inputs, and root turnover, all of which undergo decomposition processes imposed by microbes and other macro and microflora (Lehmann and Kleber, 2015). Additionally, plants release root exudates into the soil, providing a readily available source of C for microbial processing (Kuzyakov and Domanski, 2000).

Stable isotopes may be used to gain additional information about C and N cycling in ecosystems by analyzing the $\delta^{13}C$ isotopic signature of plant inputs and existing SOM (Kayler et al., 2011; Werner et al., 2012). Labeling plant materials with ^{13}C can trace how above-ground plant litter decays and becomes distributed in the soil (Bromand et al.,2001; Cotrufo et al., 2015). Further, isotopic techniques have been used to trace how litter quality of some plants affects

MAOM formation in plant shoots versus roots; results from Lavallee et al., (2018) , found that shoots contributed more C and N to MAOM versus roots in *Andropogon gerardii*. In the global initiative to reduce C loss to the atmosphere, understanding the dynamics of stabilized SOM versus mineralized SOM, especially with relation to MAOM as there is a greater potential for long-term C depletion, but also the opportunity for increasing C stocks.

Applying $^{13}CO_2$ at regular intervals in a closed chamber can uniformly enrich plants, microbes, and soil in ^{13}C. Atmospheric $^{13}CO_2$ is taken up by the plant via photosynthetic processes, becoming stored in aboveground plant tissues, but is also released in ^{13}C heavy compounds through exudation and root turnover (Swinnen, 1993). Tracking C additions in this way may help distinguish different C stabilization pathways, allowing for the separation of inputs and other processes which contribute to soil C. Removing any aboveground litter inputs, allows for the estimation of plant-added C inputs directly from root exudates and root turnover. Analyzing ^{13}C distribution patterns for different species of plants during their growing period may yield information about MAOM associations and their processing history.

This study aims to help improve understanding of N-additions and plant root morphology in relation to MAOC. The objectives in this study are; (i) to determine whether varying levels of N-fertilization illicit different responses in MAOC (e.g., whether different levels of N result in an increase or decrease of MAOC); (ii) determine whether plants or N additions directly contribute to MAOC over short time scales via isotope tracing; and (iii) identify plant root morphological traits that may be directly linked to changes in the MAOC pool.

By testing multiple levels of N-fertilization, I hypothesize that adding N-fertilizer will result in increased MAOC compared to zero N added, but also that too much N addition (e.g. 168 kg/ha) may result in a decrease of MAOC when compared to the 56 and 112 kg/ha treatments due to lowering the C/N ratio in soil, driving a higher demand for C. In the two approaches used to assess differences in MAOC (e.g. ΔMAOC, the total difference of MAOC between plant and no plant pots, and PA-MAOC, MAOC added directly from plants not accounting for changes to existing MAOC or microbial inputs), I hypothesize that N fertilization will be the driving factor when assessing the entire system (ΔMAOC) due to the strong influence of microbes to C cycling dynamics. For PA-MAOC, while N fertilization will likely affect plant growth, I hypothesize that plant species and root attributes will determine how much PA-carbon is added to the system.

Methods

This study approaches C changes in SOC and MAOC in two different ways. First is the more traditional approach, where changes in C at the end of the experiment is determined to represent change in the entire system as influenced by plant inputs, microbial activity, and other cycling processes. In the second approach, isotopic labeling by growing plants in an enriched ^{13}C atmosphere, is used to separate recent carbon inputs from plants from the decomposition of existing SOM (McCloskey et al., 2021).

To address the study objectives, a completely randomized potted mesocosm experiment was implemented in a greenhouse. The experiment consisted of a no plant control, eight herbaceous plant species common to reclamation and cover cropping management strategies with and four levels of N-additions (0, 56, 112, 168 kg/ha) as a treatment, with three replicates per treatment, resulting in a total of N = 108 pots. . This approach ensures that observed differences in plant responses are primarily attributed to fertilization treatments rather than spatial variability within the greenhouse. Plants were also labeled with ^{13}C to distinguish freshly added Plant-C from decomposition of existing SOC. To quantify plant traits, aboveground biomass, root biomass, and several root morphological attributes were measured. To quantify effects on different SOC pools, bulk soil was fractioned by particle size to obtain the fine mineral fraction (< 53 μm). Root biomass, bulk soil, and the fine mineral soil fraction were all measured separately for C, N, and ^{13}C.

Soil Preparation and Plant Selection

Soil was collected from Virginia Tech's Kentland Farm, in Blacksburg, VA, USA. Soil was collected from the top 30 cm of the soil profile and sieved in the field through a 4 mm screen to remove rocks, large clay aggregates and organic material (e.g. plants and root systems). Soil exhibited a silty clay loam texture and did not contain carbonates. Field sieved soil was mixed in a soil batch mixer (Pack Manufacturing, McMinnville, TN) with coarse sand in a 2:1 soil to sand mixture. The purpose of mixing with sand was to promote drainage and reduce breakage when harvesting plant root systems.

Eight different non-legume cover crop species were selected for varying belowground architectural traits (e.g., total root length, root diameter, root biomass), and visual assessment of root structure to include plants with traits spanning a range of root biomass and average root diameter as quantified from the greenhouse experiment in Chapter 2 (Table 1). Three of 12 total pots for each plant species and no plant control were randomly assigned to one of four N-treatments, resulting in N = 108 pots. Seeds were planted directly into 1 gal pots (diameter 20 cm, height 18 cm) filled with 3 L of soil media. Plants were terminated upon reaching 5-7 cm in height, leaving one individual per pot. Plants were grown for 11 weeks under ambient light conditions and watered as necessary with day and night temperatures ranging between 20-22°C and 16-18°C respectively. Weeds were removed from pots twice a week and checked for pests. The experiment was terminated at the end of 11 weeks.

Table 1: Plant group, family, and root habit description of plant species used in this study.

Plant Species	Plant Group and Family
Oat (*Avena sativa*)	Cool season grass (Poaceae)
Annual Rye (*Lolium multiflorum*)	Cool season grass (Poaceae)
Buckwheat (*Fagopyrum esculentum*)	Pseudocereale (Polygonaceae)
Mustard (*Synapis alba*)	Brassica (Brassicaceae)
Tall Fescue (*Festuca arundinacea*)	Cool season grass (Poaceae)
Triticale (*x Triticosecale)*	Cool season grass (Poaceae)
Radish (*Raphanus sativus* var. *longipinnatus*)	Brassica (Brassicaceae)
Rye S. Cereale (*Secale cereale*)	Cool season grass (Poaceae)

^{13}C Pulse Labeling

In this study, $^{13}CO_2$ was used as an isotopic tracer to track the migration of photosynthetically derived carbon from plant to soil in order to help determine whether root architectural traits are related to soil carbon stabilization within bulk soil and MAOM. Acrylic chambers measuring 76 cm x 76 cm x 81 cm were modified to house a USB powered PC fan and Tyvek tubing ports to introduce labeled CO_2 (Moore-Kucera and Dick, 2008; Denis et al., 2019). All holes were sealed with silicone to prevent air leakage. The bottom edge of the chamber was placed on weather stripping to seal and isolate the internal atmosphere during labeling. There were four labeling sessions, with the first labeling event 2 weeks after plant emergence and thinning, and each successive labeling event 2 weeks after the previous. The purpose of multiple pulse labeling events was to ensure labeled carbon was added across multiple growth stages for increased homogeneity and allocation of C throughout the plant (Moore-Kucera and Dick, 2008;

Denis et al., 2019). Otherwise, quantification of C fluxes can be biased due to differences between growth stages and contribution of labile and stable fractions (Kuzyakov and Domanski, 2000; Slaets et al., 2020; Bromand et al.,).

To account for environmental gradients within the greenhouse, 9 plants were randomly selected for each chamber and labeling event and then redistributed throughout the space at the end of each labelling event to minimize location-based microclimate effects. Plants were selected this way for each of three labeling events throughout the week (n = 18 pots per 2 hr labeling duration, total of n = 36 pots labeled per day). To generate 600 ppm of 10‰ $^{13}CO_2$, 1.72 g of $NaH^{13}CO_3$ (sodium bicarbonate) was added to a flask plugged with a 2-hole stopper, one hole is for a thistle funnel to add HCl, the other for Tyvek tubing connected to the chamber, to introduce the $^{13}CO_2$ gas. 1 M HCl was added to the flask in excess to generate $^{13}CO_2$ gas, the funnel hole was plugged immediately after HCl addition, and then the solution was agitated with a stir bar to help mix the solution. The USB fan was used to circulate the chamber air for the entirety of each labeling event.

Plants were exposed for 2 hour intervals (Bromand et al., 2001) and then moved from the chamber back to the greenhouse. During labeling CO_2 (ppm), temperature, and relative humidity (RH) were monitored and recorded in 5 second intervals using an Autopilot portable CO_2 monitor (Hydrofarm, Shoemakersville, PA, USA). During labeling events between 6 am and 8:30 am, average temperature and RH were 27°C and 75% respectively. For labeling events between 8:30 and 11 am, average temperature and RH were 35.7°C and 68% respectively. These measurements were for monitoring purposes to check the relative starting and end conditions of the chamber during labeling, and to measure ambient levels of CO_2 pre-labeling, confirm a CO_2 spike after labeling addition, and to measure the decrease of CO_2 over the labeling duration and make sure CO_2 levels did not drop below critical thresholds. To minimize increased humidity in labeling chambers, which may lead to increased condensation interfering with light passing into the chamber, and may reduce plant transpiration rates (Gisleröd et al., 1987), plants were not watered prior to labeling, and all labeling occurred between 6 am and 11 am.

Nitrogen Fertilization

For each fertilization application, granular NH_4NO_3 was dissolved in deionized water to provide (0, 14, 28, and 42 kg/ha) of mineral N-fertilizer. The fertilizer solution was added, after labeling, to each plant pot in the associated treatment units. Ammonium nitrate (NH_4NO_3) was selected for this experiment because is one of the most widely used fertilizers for priming effect studies (Feng and Zhu, 2021). Plants were fertilized in the manner described above, four times, every other week, over an 11-week period.

Sampling

Watering of plants ceased at the end of 10 weeks to allow for the soil medium to dry out in the final week before harvesting. Aboveground biomass was clipped at the root collar and stored in paper bags for later drying. Roots were gently washed with water to remove soil, and then stored in gallon bags covered with ~100 mL of deionized water, which were stored at 4°C until scanning to obtain quantitative data on physical attributes (Himmelbauer et al., 2004).Bulk soil was homogenized in a large container, subsampled into quart bags, and then allowed to air dry prior to fractionation and analysis.

Plant Analysis

Aboveground material was oven dried at 65°C for 48 hours and weighed. Roots were carefully teased apart in a transparent acrylic scanning tray filled with deionized water for scanning on an Epson flatbed scanner (Epson Perfection V800/V850) connected to 2017 WinRHIZO® root-scanning software (Regent Instruments Inc., QC, Canada). Roots that were too large for scanning trays (n = 44) were sub-sampled longitudinally from the crown into smaller sections with similar coarse and fine root makeup. One sub-sample per root system was scanned and scaled by mass to represent the total root system (Bouma et al., 2000). Total length, root volume, and surface areas were determined for each plant by diameter class (10 classes of 0.5 mm increments). Values were summed to quantify fine (< 2 mm) and coarse (> 2 mm) root categories. Analytical correction was applied to scans to reduce error by identifying and excluding non-root debris. After analysis, roots were oven-dried at 65°C for 48 hours and weighed. Sub-sample root masses used for scanning were scaled by biomass to quantify the total plant values for root length, surface area, and volume. Oven-dried roots and above ground biomass were then ball milled separately for %C, %N, and $\delta^{13}C$ analysis using a continuous flow isotope ratio mass spectrometer with an elemental analyzer front end (Isoprime 100 EA-IRMS, Elementar, Ronkonkoma, NY, USA).

Soil Analysis and Fractionation

All soil samples were sieved to 2 mm and then ball-milled to be used for elemental and isotopic analysis. To obtain the MAOM fraction, bulk soil was fractioned by size, collecting the <53 µm silt and clay fraction (modified from Six et al., 1998). A 10 g subsample of air-dried soil was sieved through a 53 µm screen using DI water. The samples were centrifuged at 4000 g for 15 min, water was decanted from the tube, the fine soil fraction was collected dried at 65°, and then ground into a fine powder using an agate mortar and pestle. Fine fraction soils were analyzed for %C, %N, and $\delta^{13}C$ on an EA-IRMS (Isoprime 100 EA-IRMS, Elementar, Ronkonkoma, NY, USA).

Soil C Calculations

To determine the absolute difference in C (ΔSOC and ΔMAOC) at 11 weeks between planted and unplanted pots, a simple mass balance calculation was used for each N treatment group (N-treat: 0, 56, 112, 168 kg/ha). Where the concentration of MAOM or SOC in unplanted pots within each treatment group are subtracted from the concentration of MAOM or SOC in planted pots within each N treatment group.

All isotopic plant and soil samples are expressed in the relative δ‰ scale, according to the equation:

$$\text{(i)}\quad \delta^{13}C = [(^{13}C/^{12}C)_{sample} / (^{13}C/^{12}C)_{standard}) - 1] \times 1000$$

To determine the amount of plant-added SOC (PA-SOC) and MAOC (PA-MAOC) formed, the following calculation was used (Hayes, 2004; Dijkstra and Cheng, 2007):

$$\text{(ii)}\quad C_{gain} = C_{Psoil} \frac{\delta^{13} C_{NPsoil} - \delta^{13} C_{Psoil}}{\delta^{13} C_{NPsoil} - \delta^{13} C_{root}}$$

Where C_{Psoil} is the total amount of SOC in planted pots at 11 weeks, and $\delta^{13}C_{NPsoil}$, $\delta^{13}C_{Psoil}$, and $\delta^{13}C_{root}$ are the $\delta^{13}C$ values for the soil with no plant, planted soil, and of root biomass at the 10-

week mark of the experiment. Plant-derived SOC represents the amount, if any, of traceable carbon contributed to the system directly from the plant.

Statistical Analysis

To assess the effects of different N-fertilization treatments on MAOC and root attributes, a completely randomized design was employed. Each species and no plant control had n = 12 pots. Three individual pots from each species and the no plant control group were randomly assigned to each N-treatment level (0, 56, 112, 156 kg N/ha). Mixed-effect models and ANOVA were used to evaluate interactions and the effect of plant species and N-fertilization treatment to plant attributes, ΔSOC and ΔMAOC at 11 weeks. To evaluate relationships in plant root attributes, aboveground biomass, and ΔMAOC, a multi-variate correlation matrix with correlation coefficients was created and all pair-wise factors were tested with Spearman's ρ test. All plant factors greater or less than 0.33 were used in linear mixed-effects models using standard least squares to determine the relationship between root attributes and ΔMAOC.

Zero-inflated gamma models were used to analyze the relationship between root attributes and both PA-SOC and PA-MAOC. The isotopic mass balance data for plant-added carbon resulted in a heavy skew of zero values because not every plant had measurable additions of C. The zero-inflated gamma model takes into account the excess of zeros in the dependent variable and was justified for use because the probability > ChiSquare on the distribution parameters was < 0.0001. Main effects for all independent variables were evaluated, and then all significant factors were added to the model; using forward selection with AICc validation methods, prediction formulas were saved and tested using ANOVA to select the best-fit model based on R^2 and RASE. Analyses were performed using JMP software (JMP® Pro, Version 17.0. SAS Institute Inc., Cary, NC, 1989-2024.)

Results

Plant biomass results (A1) indicate there were no statistically significant interaction effects between plant species and N treatment and the response of aboveground biomass, root biomass, or the ratio of aboveground biomass to root biomass. However, plant species was a significant main effect (Table 2).

Table 2: The effects of plant species, N treatment and their interaction for plant biomass response: aboveground biomass, root biomass, and the ratio of aboveground biomass and root biomass (AG Bio:Root Bio).

Source	Aboveground Biomass (g)	Root Biomass (g)	AG Bio:Root Bio
Plant	<0.0001	<0.0001	<0.0001
N Treatment	0.929	0.185	0.057
Plant x Treatment	0.332	0.145	0.147

$\delta^{13}C$ Enrichment of Plants and Soil

The average isotopic composition ($\delta^{13}C$) of C3 plant roots is typically around -27‰ relative to Vienna Pee Dee Belemnite (VPBD) standard (Pessenda et al., 1996), but can range between −22‰ and –36‰ depending on plant species and environmental factors (Kohn, 2010). Isotopic results indicate that pulse labeling enriched plant root biomass in $\delta^{13}C$‰, though these results varied by plant species (Table 3), and successful enrichment was determined based on the $\delta^{13}C$‰ values being higher than -27‰. Since $\delta^{13}C$‰ varies widely for soils, for the purpose of this experiment, soil pools with plants were compared to the $\delta^{13}C$‰ of bare soil in this experiment.

Table 3: Mean $\delta^{13}C$ (‰) values for roots, SOC, and MAOM soil fraction for each N fertilization treatment at the end of 11 weeks (n = 3 for each species by treatment level).

	Root δ13C (‰)				SOC δ13C (‰)				MAOM δ13C (‰)			
Nitrogen Treatment (lbs/ac)	*0*	*56*	*112*	*168*	*0*	*56*	*112*	*168*	*0*	*56*	*112*	*168*
Buckwheat	9.61	2.83	2.75	7.25	-27.57	-26.12	-26.17	-25.92	-25.66	-25.41	-25.50	-25.37
Annual Rye	-18.40	-15.47	-17.12	-13.24	-27.92	-27.72	-27.27	-27.49	-25.90	-25.89	-25.76	-25.76
Mustard	-10.68	-11.67	-8.46	-6.95	-28.12	-27.47	-26.98	-27.43	-25.95	-25.89	-25.80	-25.86
Oat	-9.54	-5.69	0.01	-5.73	-27.99	-27.50	-27.63	-27.44	-25.92	-25.88	-26.07	-25.88
Radish	-4.73	-9.62	-7.94	-4.71	-26.96	-26.59	-26.81	-26.80	-25.43	-25.49	-25.56	-25.54
Rye S. Cereale	-9.62	-12.75	-10.59	-4.52	-27.55	-27.47	-27.51	-27.65	-25.68	-25.95	-25.73	-25.89
Tall Fescue	-15.82	-13.84	-16.19	-12.55	-28.18	-27.68	-27.44	-27.34	-26.12	-25.94	-25.99	-25.85
Triticale	-3.36	-11.65	-4.72	-15.27	-27.53	-27.33	-27.68	-27.37	-25.94	-26.01	-25.78	-25.90

Whole System Carbon

Differences between total C stocks in planted and unplanted pots at 11 weeks in bulk soil (ΔSOC) and the fine mineral fraction < 53 μm (ΔMAOC) were negative under 0 kg/ha N additions, while the 56 and 112 kg/ha N treatments had higher and positive mean C differences. In the 168 kg/ha N treatment, mean ΔSOC and ΔMAOC differences decrease when compared to the 56 and 112 kg/ha treatments, averaging close to zero (Figure 1; Table 4: ΔSOC and ΔMAOC).

Table 4: Mean C (g C/100g Soil) in each N treatment for ΔSOC, ΔMAOC, which represent the difference in C between plants and no plant controls at 11 weeks. Mean C (g C/100 g Soil) for PA-SOC and PA-MAOC values are based on isotopic mass balance, zero values indicate that no plant-added C was within the detectable limits.

	ΔSOC (g C/100g Soil)				**ΔMAOM-C (g C/100g Soil)**				**PA-SOC (g C/100g Soil)**				**PA-MAOM-C (g C/100g Soil)**			
Nitrogen Treatment (lbs/ac)	*0*	*56*	*112*	*168*	*0*	*56*	*112*	*168*	*0*	*56*	*112*	*168*	*0*	*56*	*112*	*168*
Buckwheat	-0.088	0.024	0.120	-0.090	-0.166	0.019	0.090	-0.020	0.003	0.042	0.036	0.028	0.010	0.017	0.012	0.014
Annual Rye	-0.112	0.051	0.024	-0.039	-0.146	-0.033	0.206	-0.019	0	0.003	0.015	0	0.002	0	0.004	0.001
Mustard	-0.047	0.079	0.114	-0.053	-0.068	0.071	0.080	0.068	0	0.015	0.023	0.002	0.003	0.001	0.002	0
Oat	-0.172	0.018	0.107	-0.030	-0.061	0.113	0.026	-0.030	0	0.009	0	0	0.003	0.002	0	0
Radish	-0.123	0.060	0.101	0.020	0.092	0.189	0.252	0.037	0.015	0.050	0.032	0.020	0.026	0.018	0.015	0.009
Rye S. Cereale	-0.054	0.014	0.083	-0.104	-0.141	0.032	0.052	0.032	0	0.013	0.007	0	0.010	0	0.006	0
Tall Fescue	0.010	0.084	0.168	0.010	-0.134	0.066	0.105	0.003	0	0.002	0.008	0.003	0	0	0	0
Triticale	-0.219	0.131	0.110	-0.030	-0.096	-0.003	0.245	-0.103	0	0.037	0.001	0.003	0.001	0	0.004	0

Plant species had no significant interaction with N treatments for ΔMAOC (p = 0.749) or ΔSOC (p = 0.96). Neither ΔMAOC nor ΔSOC differed by plant species (ANOVA, p = 0.18, p = 0.57 respectively). Linear regressions for aboveground biomass and all root morphological traits were non-significant factors in relation to ΔSOC and ΔMAOC (Table 5).

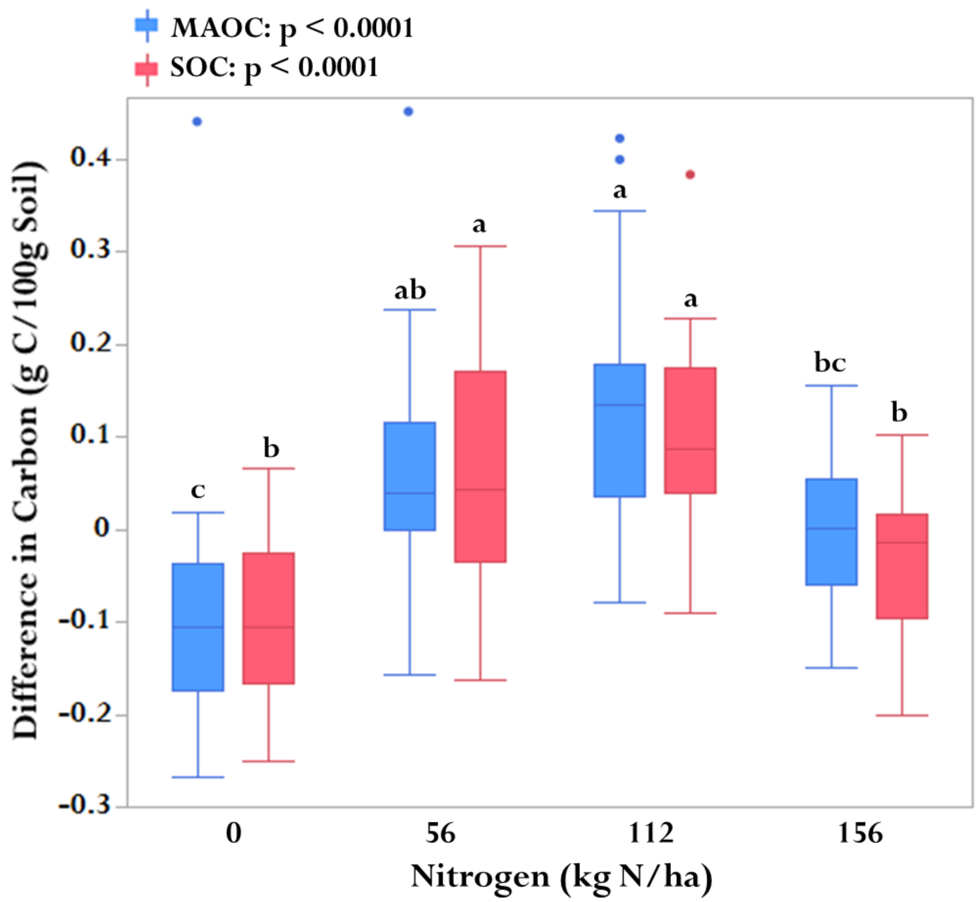


Figure 1: The response to N treatments for ΔSOC (red) and ΔMAOC (blue) averaged across all plant species. Tukey HSD was used for post-hoc testing of N treatments within each group (ΔSOC and ΔMAOM) and means followed by different lowercase letters within a carbon type indicate statistically significant differences (p < 0.05).

Table 5: Linear regression and ANOVA results for ΔSOC and ΔMAOC between having a plant and no plant; bold values indicate significance $p < 0.05$.

		Plant Type	N Fert	Aboveground Mass	Root Volume	Root Surface Area			Root Length			Root Mass	SRL	Avg Diameter
						Total	Fine	Coarse	Total	Fine	Coarse			
ΔSOC	p-value	0.76	**<0.0001**	0.47	0.40	0.43	0.19	0.18	0.38	0.38	0.24	0.37	0.998	0.44
	R^2	0.05	0.40	0.006	0.008	0.007	0.02	0.02	0.009	0.009	0.02	0.01	$1.06e^{7}$	0.007
ΔMAOM-C	p-value	0.18	**<0.0001**	0.83	0.26	0.30	0.80	0.933	0.51	0.51	0.89	0.50	0.88	0.22
	R^2	0.12	0.30	0.018	0.015	0.013	0.0008	$8.21e^{5}$	0.005	0.005	0.0002	0.005	0.0003	0.018

Nitrogen fertilization and C/N ratios

The C/N ratios of bulk SOC and the MAOM fraction observed in this study showed significant differences between N treatment groups, with C/N generally decreasing as N inputs increased (Figure 2). The C/N ratio of soil microbes is generally around 10:1, while the ideal ratio of their substrate is approximately 24:1 (Robertson and Groffman, 2007). In this study, the initial C/N values of the soil media for SOC and MAOM were 7.4 and 10.9 (estimate of variance 4.1 and 0.01 respectively). At 11 weeks, differences in C/N were observed, even in the N=0 treatment, indicative of plant and microbial processes influencing soil C/N stoichiometry. The trends in C/N between SOM and MAOM are opposite each other. The C/N of SOC starts at 7.4, increasing at N = 0 kg/ha, and then decreasing in each N treatment group back towards the starting baseline, whereas the C/N of MAOM starts at 10.9 (e.g. more C than N) and then decreases at N = 0 kg/ha, moving further away from the baseline (e.g. increasing N relative to C) (Figure 2).

There was a significant interaction between plant species and N treatment for MAOM (Figure 3, $p = 0.04$). For bulk SOC, only the main effect of N treatment was significant (Figure 4, $p < 0.0001$).

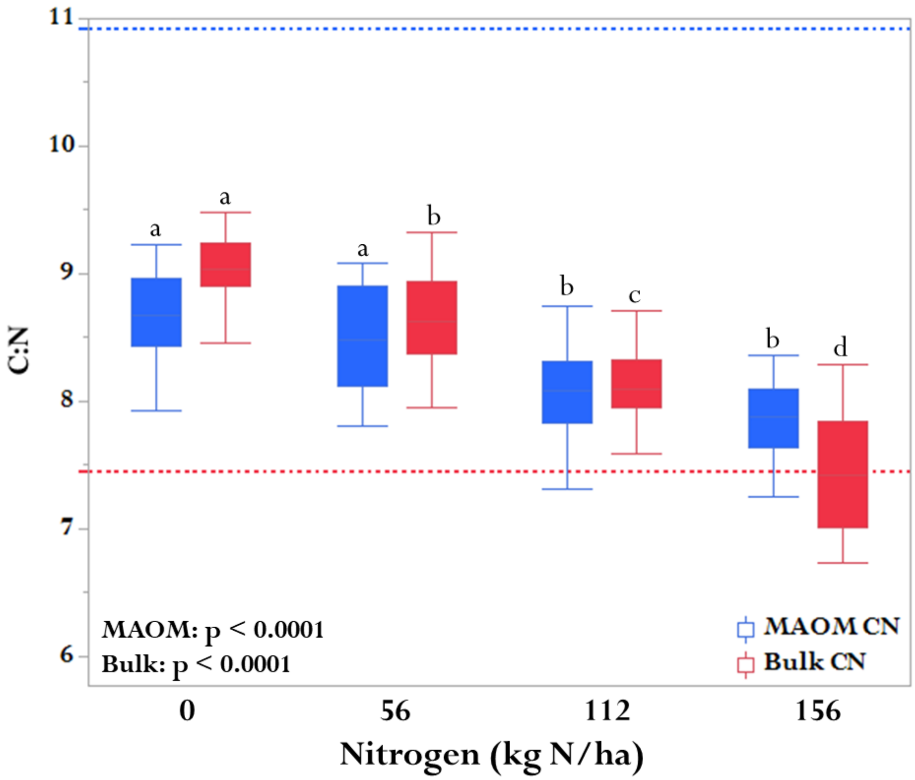


Figure 2: The response to N treatments for C/N ratios of MAOM and SOM averaged across all species. Dashed lines represent C/N ratio of SOC (red) and MAOM (blue) at the start of the experiment. Tukey HSD was used for post-hoc testing of N treatments within each group (MAOM and Bulk) and means followed by different lowercase letters within a carbon type indicate statistically significant differences ($p < 0.05$).

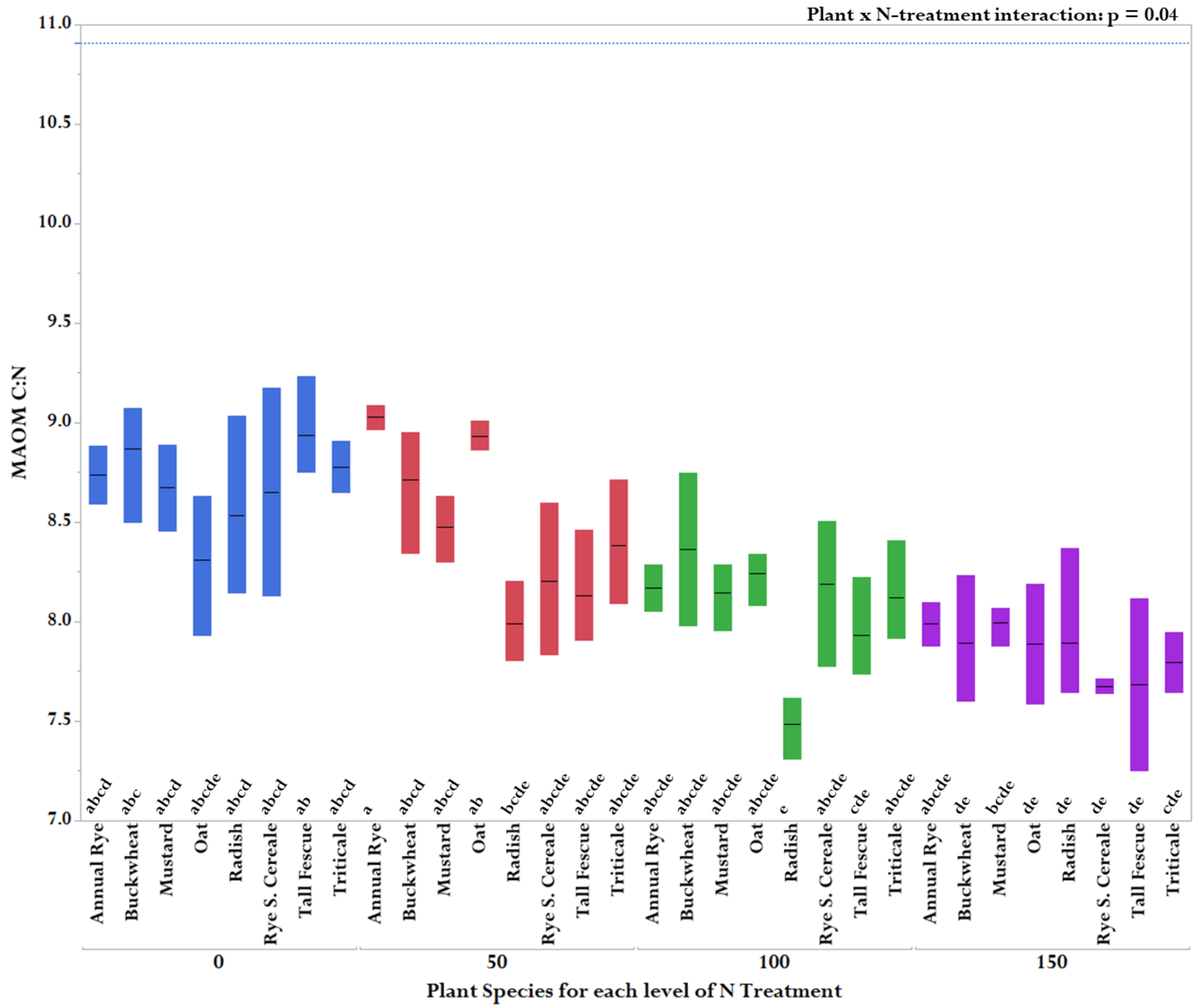


Figure 3: C/N ratios of MAOM in response to N treatments and plant species. Boxes display the expected variation of data for plant species by N treatment, black bars within boxes represent the mean. Dashed line represents C/N ratio of MAOM at the start of the experiment. Tukey HSD was used for post-hoc testing of plant species and N treatments and means followed by different lowercase letters indicate statistically significantly differences ($p < 0.05$).

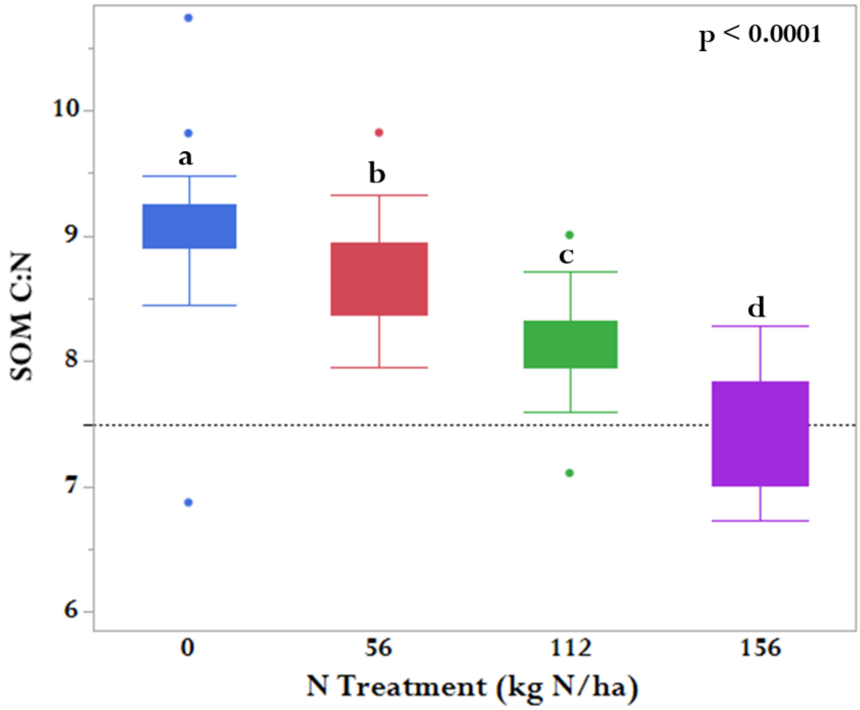


Figure 4: C/N ratios of bulk soil in response to N treatments. Dashed line represents C/N ratio of SOM at the start of the experiment. Tukey HSD was used for post-hoc testing of N treatments and means followed by different lowercase letters indicate statistically significant differences ($p < 0.05$).

Plant-added carbon

Carbon isotope tracing indicated that plants have the potential to contribute small amounts of C to the mineral soil fraction within 11 weeks. Further, not all plants contribute detectable amounts of C to the soil over this timeframe, and contributions vary based on levels of N fertilization (Table 4: PA-SOC and PA-MAOC). Due to non-detecatble amounts of PA-SOC and PA-MAOC in some pots, zero-inflated analyses were used to assess correlations between plant species, N-fertilization, and root morphological traits for both PA-MAOC and PA-SOC to account for zero heavy data. Analysis indicates positively correlated associations between PA-MAOC and several independent variables: plant species, aboveground biomass, root surface area (total, fine, and coarse), and root length (total, fine, and coarse). All traits were similarly significant for PA-SOC, with the exception of coarse surface area and coarse root length (Table 6).

Table 6: Zero-inflated model results for PA-SOC and PA-MAOC, bolded values indicate significance $p < 0.05$, and asterisk indicates marginally significant $p < 0.10$.

		Plant Type	**N Fert**	**Aboveground Mass**	**Root Volume**	**Root Surface Area**			**Root Length**			**Root Mass**	**SRL**	**Avg Diameter**
						Total	Fine	Coarse	Total	Fine	Coarse			
PA-SOC	p-value	**0.022**	0.506	**<0.0001**	0.061*	**0.021**	**0.021**	0.231	**0.010**	**0.010**	0.105	**0.003**	0.5545	0.592
	Generalized R^2	0.16	0.02	0.11	0.03	0.05	0.05	0.01	0.06	0.06	0.02	0.08	0.004	0.003
PA-MAOM-C	p-value	**0.0003**	0.466	**0.0002**	0.095*	**0.020**	**0.011**	**<0.0001**	**0.005**	**0.005**	**<0.0001**	**0.0003**	0.711	0.708
	Generalized R^2	0.26	0.03	0.11	0.03	0.05	0.06	0.13	0.07	0.07	0.13	0.11	0.002	0.002

Variables found to be independently significant were input to a larger model, to build a best fit model explaining which factors contribute to PA-MAOC. The final model for PA-MAOC (AICc = -206.1, $R^2 = 0.37$) resulted in root mass, aboveground biomass, coarse root length, and plant species as significant contributing factors. As root mass, aboveground biomass, and coarse root length increase, PA-MAOC increases (Figure 5a, b, c). Plant species was also a significant factor in the model, suggesting that inputs from different species of plants contribute to how much PA-MAOC is added, if any, at 11 weeks (Figure 5d). There were no significant interaction effects on PA-MAOC between plant species, N treatments, aboveground biomass, and root traits. While plant species had no effect in ΔMAOC, this is likely due to N fertilization having a greater overarching effect on the entire system where plant species and attributes are largely responsible for freshly added inputs to MAOC.

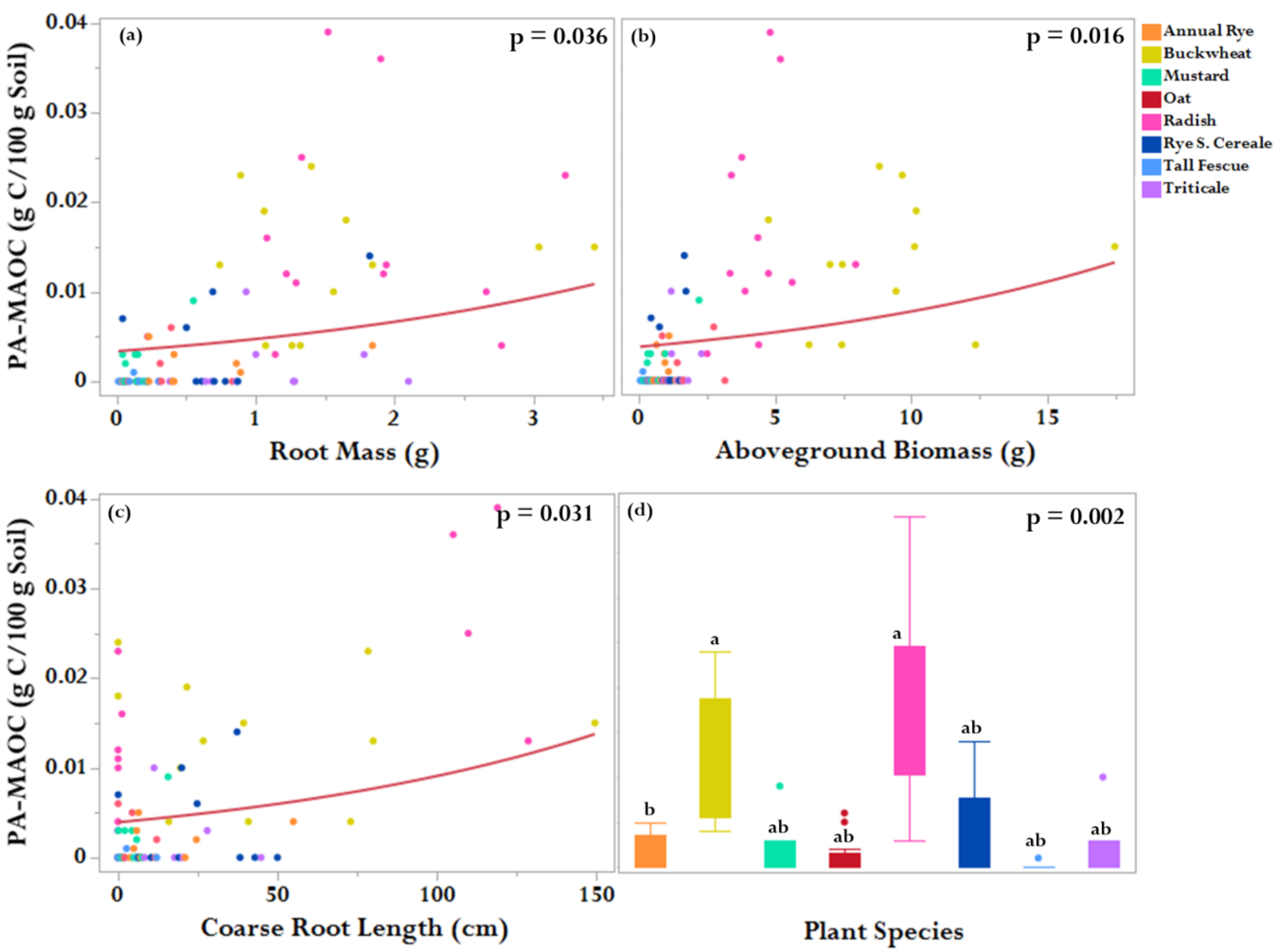


Figure 5: Relationship between PA-MAOC and plant traits from final zero-inflated model (a, b, c). The R^2 for the entire model is 0.37, and p-values are reported for each factor. Panel (d) shows plant species in relation to PA-MAOC. Tukey HSD post-hoc testing was used to test differences between groups. Values followed by different lowercase letters over the bars indicate statistically significant differences at $p < 0.05$ among plant species.

When compared to PA-MAOC, the final model for PA-SOC (AICc = -127.0, $R^2 = 0.15$) only exhibited a significant relationship with aboveground biomass, with increasing aboveground biomass resulting in increasing amounts of PA-SOC (Fig 6a). Fine root surface area was marginally significant (Figure 6b); but surface area ($p = 0.72$) and fine root length ($p = 0.20$), which were initially included, ended up being excluded in the final best fit model.

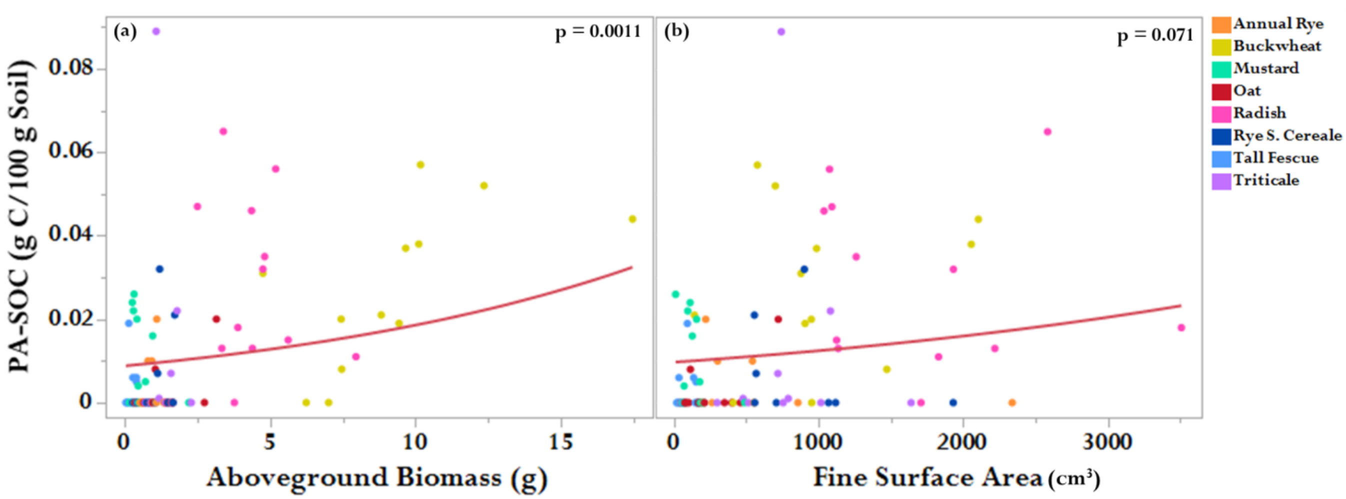


Figure 6: Correlations between significant plant traits and plant-added SOC concentrations as determined from zero-inflated gamma model and forward stepwise selection.

While N may not be a significant factor impacting PA-MAOC, I pose that potential covariance may exist between plant species and nitrogen on key root traits. Testing this on the zero-inflated model for PA-MAOC, indicates that there were no significant interaction effects between plant species and N treatment for either root biomass or aboveground biomass ($p = 0.15$ and $p = 0.33$ respectively). Plant species was the only significant factor influencing the amount of overall root mass ($p < 0.0001$) and aboveground biomass ($p < 0.0001$). However, coarse root length exhibited a significant interaction effect between N treatment and plant species ($p = 0.002$), suggesting that plant species response to nitrogen was varied in relation to the length of coarse roots.

Overall the zero inflated analysis showed that N treatments were a non-significant factor in how much PA-MAOC and PA-SOC was contributed to the system at the end of 11 weeks. Plant species, aboveground biomass, and some root traits were significant, but varied between PA-SOC and PA-MAOC.

Root morphology and C inputs

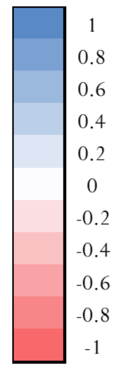
One of the aims of this study was to identify whether plant root morphological traits that may be directly linked to changes in the MAOC pool. Results showed that with regards to ΔMAOC and ΔSOC, N fertilization treatment was driving factor which explained differences in these carbon pools. A correlation matrix with Spearman's ρ was used to look at correlations between all plant physical data (e.g. aboveground biomass and root morphological traits) and ΔMAOC and ΔSOC.

First, all treatments were pooled together, which showed no correlations between ΔMAOC and any physical plant traits analyzed in this experiment. But, when splitting the data to test root traits for ΔMAOC and ΔSOC within each N treatment, results show significant positive correlations between plant-attributes and ΔMAOC in the N=112 kg/ha. Root volume, root length, fine root length, and fine root surface area were all positively correlated with ΔMAOC ($p < 0.5$). This would indicate that at certain levels of N-fertilization, some root traits may contribute to positive ΔMOAC (Table 6). When assessing ΔSOC across all treatments, there were significant ($p < 0.05$), but weak (Spearman $\rho > -0.33$) negative correlations between root length, fine root length, root surface area, and fine root surface area.

This may be driven largely by the fact that in the 0 kg/ha N treatment, there were several significant negative correlations between plant physical traits and ΔSOC: aboveground biomass, root biomass, root volume, root length, fine root length, root surface area, and fine root surface area (Table 6). These results indicate that during plant growth, in the absence of N fertilization, several plant traits are negatively correlation with ΔSOC.

Table 6: Spearman correlations values between ΔMAOC, ΔSOC and physical plant traits (aboveground biomass and root morphological traits). Significant correlations with p <0.05 are in bold; blank spaces indicate no significant correlation between variables. Results are shown per N treatments: 0, 56, 112, and 168 kg/ha.

	ΔMAOC					ΔSOC				
	All Treatments	0	56	112	168	All Treatments	0	56	112	168
Aboveground Biomass							**-0.460**			
Belowground Biomass							**-0.596**			
Average Root Diameter										
Specific Root Length (SRL)										
Root Volume				**0.460**			**-0.595**			
Root Length (RL)				**0.416**		**-0.221**	**-0.529**			
Fine RL				**0.419**		**-0.220**	**-0.529**			
Coarse RL										
Root Surface Area (RSA)						**-0.226**	**-0.575**			
Fine RSA				**0.423**		**-0.220**	**-0.548**			
Coarse RSA										



Discussion

The findings from this study highlight the nuanced relationship between N-fertilization and soil carbon dynamics. While moderate N additions (56 and 112 kg/ha) enhanced both MAOC and SOC, high N (168 kg/ha) appeared to destabilize MAOC and SOC, potentially triggering microbial activity that depletes stable carbon pools, aligning with previous findings. Interestingly, plant species and root morphological traits played a larger role than N-fertilization in determining plant-added inputs to MAOC, emphasizing the importance of plant-specific interactions in carbon stabilization.

Nitrogen and Priming Effects

Results from this study demonstrate that when assessing differences in MAOC (ΔMAOC) and SOC (ΔSOC) between unplanted and planted soil during short-term growing periods, N-fertilization is the dominant factor related to ΔMAOC and ΔSOC. Specifically, N additions at moderate levels of fertilization (56 and 112 kg/ha), resulted in increases of C. However, at higher N levels (168 kg/ha), both ΔMAOC and ΔSOC trended towards decreasing C and were more similar to the 0 kg/ha treatment. Thus, adding some amount of N supports positive increases in both MAOC and SOC, but results also support the hypothesis that excessive N may have detrimental effects on soil C and is consistent with findings from Zhang et al. (2016), who reported a 7–11% loss of SOC under high N fertilization, and (Wang et al., 2019), who observed increased carbon emissions in soils with excessive nitrogen and sufficient irrigation.

Nitrogen (N) has contrasting effects on soil organic matter (SOM) dynamics. While N fertilization supports plant growth and microbial activity, promoting SOM stabilization, imbalances in soil C/N stoichiometry can destabilize SOM, leading to carbon loss as CO_2, particularly in nutrient-poor soils, a phenomenon known as the priming effect (PE) (Kuzyakov et al., 2000; Fontaine et al., 2003; Chen et al., 2018). PE occurs when root exudates or decomposable plant inputs fuel microbial activity, accelerating SOM mineralization to access soil N. Stabilization and destabilization of SOM are influenced by two main pathways: an indirect microbially mediated pathway and a direct abiotic pathway. In the indirect pathway, root exudates (e.g., sugars) fuel microbial growth, increasing N limitation and triggering SOM degradation to access N (Keiluweit et al., 2015; Yuan et al., 2018; Jilling et al., 2021). However, when N is abundant, either naturally or through fertilization, microbes reduce SOM degradation by utilizing available N (Feng and Zhu, 2021). Excess N fertilization can destabilize SOM by altering C/N ratios, increasing microbial demand for carbon, and enhancing mineralization. Additionally, increased plant residue inputs from fertilization may alter microbial demands, which is not necessarily conducive to soil carbon sequestration (Jesmin et al., 2021).

To mitigate PE and protect MAOC, anthropogenic N additions may be beneficial. By alleviating N limitation, N inputs can suppress microbial mining and reduce the loss of MAOC (Feng and Zhu, 2021). Though N additions can result in decreased priming effects across many ecosystem types, nutrient additions do not always result in a decrease of priming effects and vary depending on ecosystem, experimental design, nutrient forms and rates, as well as carbon substrate types (Feng and Zhu, 2021). For instance, studies have shown that PE can increase SOM decomposition by up to 59.4% in pot experiments (Huo et al., 2017). Nevertheless, research suggests that nutrient additions can reduce the magnitude of PE by decreasing microbial mining for nutrients (Fontaine et al., 2003, 2004; Moore-Kucera and Dick, 2008; Denis et al., 2019). Balancing nitrogen inputs is therefore crucial to effectively managing SOM stability and preventing excessive carbon loss.

The response of MAOC to N-fertilization observed in this study aligns with the idea that PEs are largely driven by microbial mineralization to access nutrients within the MAOM fraction in response to inputs of readily decomposable C from root exudates, POM and other sources. Excessive N likely shifts microbial activity due to an imbalance between C and nutrient inputs necessary to meet microbial demands. The significant reduction in C at the highest N level suggests that microbes are decomposing organic matter to include MAOC, to meet their nutrient needs, particularly if labile sources are insufficient. Although POM was not directly measured in this study, these results align with previous research suggesting that POM serves as a readily accessible nutrient source for microbes, while MAOC acts as a reserve during nutrient limitation. This highlights one of the challenges and importance of balancing N inputs specifically to each system to avoid overstimulating microbial decomposition of more stable C pools, and to provide ample nutrients to support plant growth.

Priming and C/N Ratios

The response in C/N of SOC at may result from increasing C via plant inputs in the form of higher C/N root exudate inputs (Mastný et al., 2021); at 0 kg/ha, C/N increases, which corresponds with a negative response in ΔSOC and may indicate priming and mineralization of C. When N is added (56 & 112 kg/ha), N limitation decreases as evidenced by decreasing C/N, reducing C mineralization, allowing for increases in SOC (Kuzyakov et al., 2000). Microbial productivity becomes high due to a large abundance of N, which may shift microbes into mineralizing more soil C.

For MAOM, I hypothesized that C would be lost at N = 0 and N = 168 kg/ha, which was observed in our results for ΔMAOC (Figure 1). There has been evidence that root exudates can increase MAOC mineralization in just a few weeks (Jilling et al., 2021). The initial drop of C/N in the 0 kg N/ha treatment may indicate that decreasing C/N is a response of decomposition processes of root inputs which leave behind more N which is able to bind to minerals (Manzoni and Cotrufo, 2024). As N-treatment increases C/N continues to decrease, resulting in positive ΔMAOC due to decreased C-mineralization. Noting different response patterns in individual plants reinforces the notion that root exudates and microbial processing play a large role in these dynamics (Panchal et al., 2022; Wen et al., 2022).

The observed differences in C/N ratios across N treatments and plant species highlight the delicate balance required to optimize soil microbial activity and organic matter turnover. Lower C/N ratios in soils with higher nitrogen inputs suggest more rapid nitrogen cycling, which may benefit short-term plant growth but could have long-term implications for soil health, carbon sequestration, and nitrogen retention in the system. These findings emphasize the importance of needing to manage nitrogen inputs to agricultural and other systems in order to maintain plant productivity and soil quality while minimizing negative environmental impacts.

Plant-added inputs

It is well known that N is a significant factor when assessing the whole soil system, to including microbial processes that are also occurring (Ladha et al., 2011; Qin et al., 2024; Manzoni and Cotrufo, 2024). But, in contrast to the results of ΔMAOC and ΔSOC, when assessing fresh, plant-added inputs and their influence on MAOC, N-fertilization was not significant, while plant species, aboveground biomass, and several root traits were related to how much C was added to MAOM. There were no interactions between N-fertilization and the measured plant traits; which is not to say that N treatments did not have any effect on plant growth or productivity, only that plants species and some specific traits were largely responsible for the additions to MAOC over the duration of this experiment.

Given the well-documented importance of N on plant physiology (Luo et al., 2020; Mu and Chen, 2021), it was initially predicted that N would have a significant effect on how much plant-added C was incorporated into SOM and MAOM. However, the results did not align with these expectations. Surprisingly, N was not a significant factor in determining PA-MAOC and PA-SOC. Root mass, aboveground biomass, coarse root length, and plant species were the most significant contributors to PA-MAOC. These results suggest that specific plant traits, or residues, rather than N availability, play a larger role in influencing how much PA-carbon is stabilized in the MAOM fraction. Further, MAOC and SOC showed distinctly different patterns when examining PA-carbon. For PA-SOC, the only relevant factor was aboveground biomass. These differences underline why studying soil carbon pools outside of SOC alone is imperative, as this research suggests that different mechanisms govern carbon dynamics in soil carbon pools.

The absence of a direct N effect on PA-MAOC may indicate a more nuanced relationship between N availability and plant root function, particularly in how roots adapt their physiology to seek out nutrient resources. This may suggest that N availability influences root traits indirectly, through plant responses to nutrient demand, rather than directly affecting carbon allocation to soil pools. Though, as these plants were only grown over a 10-week duration, the demand for N may be very low; grown over longer durations, N may become a more significant factor in PA-MAOC.

MAOC Stocks and Root Morphology

Root morphology and plant-species influenced PA-MAOC and PA-SOC, but morphological traits were largely overshadowed by N-fertilization when assessing ∆MAOC and ∆SOC dynamics. Though interaction effects between N-fertilization and root morphological traits were generally insignificant, it should be noted that there are some correlations at different levels of N-fertilization which exhibited positive or negative responses in carbon.

Root systems play a crucial role in plant growth and development, with nitrogen availability being a key driver of root development. Roots can sense internal and external changes in N which affects plant signaling and adaptation to the environment (Chen et al., 2024). Initial results in this study found that N-fertilization was the only significant factor related to ∆SOC and ∆MAOC, but an additional aim was to assess how different N treatments may impact the relationship between root traits and soil carbon fractions.

Correlation analyses using Spearman's ρ, showed that root traits had significant positive correlations with MAOC at moderate N fertilization levels. Specifically, in the 100 kg/ha treatment, root volume, root length, fine root length, and fine root surface area showed significant positive correlations with MAOC (correlation > 0.33; $p < 0.05$). The fine roots, which are primarily responsible for nutrient acquisition, seem to benefit from moderate N application; fine root production is increased in this scenario, where the plants are likely investing in root growth to enhance nutrient uptake. Under this condition, there is sufficient nitrogen to meet both plant and microbial needs, which reduced priming effects and promoted MAOC accumulation. Interestingly, these results are similar to findings assessing SOC, where root morphological traits such as root length, surface area, and root volume increased with moderate N fertilization but began to decrease at higher N levels (Chen et al., 2024). These results suggest that while moderate N fertilization stimulates root development and may support increasing MAOC, excessive N can have the opposite effect. While not significant to any root trait, the aforementioned statement is further supported in the data which shows correlations at the 150 kg/ha N treatment shifting from positive to negative. While there is no definitive answer as to what is causing this shift in our study, excessive N has been shown to reduce fine root production in favor of aboveground biomass growth (Zhang et al., 2017), which runs the risk of limiting plant growth due to lower nutrient acquisition capacity from an underdeveloped root system. Additionally, the observation that fine roots only show significant correlations with MAOC at moderate N levels is evidence that root carbon allocation may be optimized under certain nutrient conditions.

In contrast, SOC dynamics showed a different trend, particularly under the 0 kg/ha N treatment, which exhibited significant negative correlations between root traits and SOC and aboveground biomass. This aligns with established literature on priming effects and soil health in general, where it is well established that nitrogen-limited environments often experience SOC losses (Kuzyakov, 2002; Di Lonardo et al., 2019; Bastida et al., 2019). In this study, increasing aboveground biomass could be indicative of a greater demand for N to support plant growth, potentially leading to nitrogen limitations for the microbial community. When microbes face nitrogen shortages, they likely induce PE, leading to a net loss of SOC as they decompose more organic matter to meet their nitrogen demands. Additionally, increased root biomass in nitrogen-limited environments may reflect the plant's strategy of allocating more carbon to the root system to seek out scarce nutrients. Fine root length and surface area are closely related to nutrient acquisition in the soil (Zhang and Wang, 2015), and the negative correlations between these traits and SOC in nitrogen-limited conditions suggest that fine root production is a response to nutrient scarcity rather than a contributor to carbon sequestration.

The differing relationships between SOC and MAOC across N treatments reflect the varying contributions of different soil carbon fractions. This study is only reflective of a short-lived system, and may not represent dynamics in long-lived systems. Further, this study did not account for POC, which is a significant component of SOC, and the balancing of POC and

MAOC would explain the contrasting trends observed. SOC represents a broader pool that includes more labile carbon fractions, while MAOC is a more stable pool associated with mineral surfaces. Thus, N treatments may affect the balance between these fractions differently, with moderate nitrogen inputs promoting MAOC sequestration while nitrogen-limited conditions lead to SOC losses driven by microbial activity and nutrient competition.

Conclusion

Our findings reinforce the complexity of carbon-nitrogen interactions in soil systems, particularly the potential for high nitrogen inputs to disrupt the balance of carbon cycling processes and reduce long-term soil carbon storage. Overall, the findings from this study suggest that N-fertilization was the most significant factor in ΔMAOC dynamics, but not with PA-MAOC, which was driven by plant species, root biomass, coarse root length, and aboveground biomass. Roots not only have some importance in PA-MAOC, but fine root traits may play a crucial role in the accumulation of ΔMAOC under moderate N-fertilization. In nitrogen-limited environments and with excessive levels of nitrogen, carbon losses are more likely as plants and microbes compete for scarce resources. The distinct responses of SOC and MAOC to N treatments underscore the need to consider the balance between different soil carbon fractions in future studies. Lastly, while nitrogen plays an important role in plant growth, its effect on PA-carbon additions to soil pools appears to be limited and less understood. Instead, this study found that plant traits, particularly those related to root and shoot biomass, seem to have a more direct influence on how much carbon is stabilized in soil directly from plant inputs, particularly in the MAOM fraction. This emphasizes the importance of considering plant functional traits, rather than nitrogen availability alone, when assessing carbon sequestration in soils.

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Chapter 4

Long-term Effects of Cover Cropping, Tillage, and Nitrogen Fertilization on Mineral-Associated Organic Carbon in Agroecosystems

Abstract

Soil organic matter (SOM) and its constituent fractions, including mineral-associated organic matter (MAOM), are critical indicators of soil fertility and carbon sequestration potential in agroecosystems. This study evaluated the long-term effects of cover cropping, tillage, and cumulative nitrogen (N) fertilization on soil organic carbon (SOC) and MAOC after a 9-year corn cropping system experiment in sandy loam soils within the Eastern Shore of Virginia, USA. Cover crop treatments, including rye, vetch, and a mix of species, demonstrated significantly higher SOC and MAOC levels compared to conventional tillage (CT) and no-till (NT) systems without cover crops. MAOC increased 21 to 36 % in cover crop treated plots compared to CT and NT plots with no cover crops, and SOC increased 43 to 59% in cover crop treated plots. In contrast, cumulative N fertilization did not significantly impact SOC or MAOC concentrations. These results highlight the importance of using cover cropping in conjunction with NT systems, where reduced soil disturbance may assist in facilitating carbon stabilization. While N-availability may have important seasonal and long-term implications on MAOC, this study found that triannual additions of mineral N-fertilizer had no effect on SOC or MAOC. This study underscores the value of integrating cover crops into conservation agriculture practices for sustainable land management, though further research is recommended to explore the dynamics of different SOM fractions under long-term management, especially under varying soil and climate conditions.

Introduction

Soil health is “the continued capacity of a soil to function as a vital living ecosystem that sustains plants, animals, and humans” (NRCS, USDA). Soil organic matter (SOM) is a vital part of functioning soils consisting of a complex mixture of plant residues and microbial products in varying stages of decomposition (Weiglein et al., 2022). Additionally, because of its importance to soil structure, water holding capacity, and nutrient supply to micro- and macroflora (Lehmann and Kleber, 2015), SOM and more specifically, soil organic carbon (SOC) is largely measured as an assessment of soil health and to determine soil fertility. Carbon (C) inputs to soil are largely from root exudates, aboveground biomass, and in agricultural systems may include organic (e.g., manure, biochar, other plant materials) (Liptzin et al., 2022). Understanding the cycling of soil organic carbon (SOC) is of increasing interest due to concerns regarding how SOC pools will respond to global climate changes and land management practices. In agroecosystems, the balance of managing SOC stocks to support agricultural productivity, ecosystem health/resilience, while also sequestering more carbon in the soil is an ongoing challenge.

In agricultural management, soil fertility is often used in relation to soil and crop production that is managed by farmers at the field scale (Lehmann et al., 2020). Historically, the agricultural management of soils has led to loss of soil organic matter (SOM). While difficult to quantify, a data-driven statistical model estimates upwards of 133 Pg C lost from the soil as a result of historical agricultural land uses (Sanderman et al., 2017). Much of SOC lost is released as CO_2 to the atmosphere (Bölscher et al., 2020), though potential losses due to erosion (transportation) or land-use conversion such as native to agriculture use are also included in that. Challenges arise in managing SOC exists in agroecosystems due environmental factors such as varying precipitation, temperature, but also crop rotation, cover cropping, tillage, fertilization, which all individually have the potential to illicit change on inputs, outputs, and microbial productivity (Liptzin et al 2022). Practices used in intense agriculture to increase plant production may also be detrimental to soil health e.g. tillage which breaks up soil structure, impacting plant rooting depth and OM (Nunes et al., 2020).

Importance of Mineral Associated Organic Matter

In the current paradigm, particulate organic matter (POM) and mineral-associated organic matter (MAOM) have become accepted as important fractions in SOM with variable traits and functions that can be used to elucidate changes in SOM dynamics (Kleber et al., 2015; Cotrufo et al., 2015, 2019). POM represents a more dynamic, labile pool of SOM, which is mainly of plant origin in the early stages of decomposition. In contract, MAOM is microbially transformed via decomposition processes, which is then stabilized through sorption onto mineral surfaces in the soil. As a result, MAOM is generally more stable, with turnover rates ranging from decades to centuries (Lavallee et al., 2020; Cotrufo and Lavallee, 2022b). Additionally, as the conversion of POM to MAOM is microbially mediated, the interacting effects of soil nutrient availability and the stoichiometry of POM as well as the microbes themselves, further influence SOM dynamics (Jilling et al., 2021).

Tillage and Cover Cropping

Land management by tillage and cover cropping practices are common in agroecosystems with the intent to increase crop productivity and maintain or improve soil fertility. In agriculture SOC dynamics have been studied in response to tillage, cover cropping, nitrogen fertilization, and other land management processes in both short and long-term studies. It is well established that cover crops increase SOC and nutrient stocks compared to fallow fields, resulting in healthier and more productive soils (Poeplau and Don, 2015). No-till management, where soil is left undisturbed by tillage, generally leads to higher SOC levels, as minimal soil disturbance preserves existing SOM (Haddaway et al., 2017).

When considering factors related to increases of SOC, Jian et al. (2020) identified cover/cash crop type and soil texture as some of the most significant factors which contributed to increases in SOC. Some cover crops, such as soybean and other legumes are commonly used because they supply additional nitrogen to the soil via symbiotic relationships with plants and nitrogen fixation (Reckling et al., 2016), which helps enhance soil quality and crop productivity (Ladha et al., 2011; Yousaf et al., 2016). Incorporating crop residues into soils has also been effective in increasing SOM (Huang et al., 2007).

Nitrogen Fertilizer

Adding nitrogen fertilizer to supplement crop growth is also a common practice. Precision agriculture has advanced in such a way that farmers can add just enough nitrogen as needed, helping to prevent issues that stem from over fertilization. An 11 year experiment comparing conventional till and no-till systems with differing crop rotations to include mixes of corn, soy, and wheat showed that N fertilizer impacts were highly dependent on the crop and tillage system (Congreves et al., 2017).

Many studies have focused on SOC dynamics in relation to nitrogen (N) fertilization, and other management practices, N fertilizer applications have been commonly relied upon to increase crop yields and improve or help sustain soil fertility (Congreves et al., 2017). Research on the effects of long-term nitrogen fertilizer application have conflicting results. Some studies have found increased SOC stocks after long-term nitrogen fertilization; after 25 years of N fertilization, Wang et al. (2014) found that SOC levels increased 18% alongside a 26% increase in total soil nitrogen (TN), suggesting that long term N addition boosts SOC stocks along with crop yields. In the long term broadbalk wheat experiment in Rothamstead, UK, increasing rates of N fertilizer have been shown to increase the percent of SOC over the past 50 years (Poulton et al., 2024). Though results in this study were likely confounded due to differing treatments such as crop rotations and periods where fields were left fallow and N was not added. Increases in SOC were posited to be an indirect result of N fertilizer which increased crop yields and organic residue inputs to the system. Other studies have found no evidence of N fertilizer resulting in increased SOC, such as a 12-year experiment by Neff et al. (2002), which observed no significant changes in SOC, despite increasing plant productivity.

Much of the existing literature studying N fertilization treatments tends to focus more on the quality of N fertilizer, such as organic (e.g. manure, biochar, plant biomass) and mineral forms. A 35-year experiment on fertilizer type, mineral vs. organic, showed that fertilizer type may affect the turnover of MAOC. Mineral fertilizers resulted in longer mean residence times of carbon in MAOM, and organic fertilizers resulted in lower mean residence times for MAOC. In both pools, the concentration of MAOC remained constant, which suggests that higher turnover of MAOM with organic fertilizers is compensated for by high OM inputs from organic fertilizers

(Mayer et al., 2022). In a 25-year fertilization experiment, stable isotopes were used to assess MAOM and POM pools, which both increased in carbon due to N fertilization treatments, though there was evidence that different qualities of fertilization had varied impacts on SOC, with the strongest increase found in a mix of mineral and organic fertilizer treatment (Dou et al., 2016). There is also the potential that fertilization treatments may result in a loss of MAOC. In a 36-year study comparing conventional and organic farming plots with different fertilization treatments (e.g. organic and mineral fertilizers), losses of MAOC were observed in the treatments with no fertilizer (-27%), mineral fertilizer (-14%), mineral fertilizer and manure (-3%), with no change in the organic farming + manure (0%) (Mayer et al., 2022).

There are also other considerations with regards to understanding the effect of long-term N additions, to include: nutrient leaching from the system, groundwater contamination, eutrophication of waters, decline in biodiversity, and soil acidification (Kidd et al., 2017). When considering intensely managed systems, where short term effects may not be representative of long-term change, such experiments that include high N fertilization over a short duration may not be appropriate to extrapolate out to long-term impacts from chronic fertilization additions. While the current knowledge on SOC response to long term application of N fertilization is not conclusive to one system, all of these aforementioned N fertilizer studies highlight a limitation in relation to the current paradigm of SOC and different fractions of SOM. Studying a single pool of SOC does not account for changes that occur in the different pools that comprise SOC (e.g. POM and MAOM). It is probable that N additions could increase decomposition rates for some forms of OM, while decreasing others. This is why including MAOM and POM in long-term agroecosystem studies and how N fertilization affects these pools is critical. The connection between N fertilization and MAOM is even less understood when it comes to long-term nitrogen and cover crop studies. Understanding whether N additions trigger increased decomposition of the more stable MAOM fraction or whether they help preserve and build MAOM stocks may help inform better land management practices.

Plant Root Impacts

Morphologic root traits are directly linked to a plant's ability to acquire soil resources such as water and nutrients. Nitrogen additions in agriculture are mainly to help improve crop productivity, but a secondary outcome of N fertilization is plant growth; as plants are the primary source of C into the soil, with belowground inputs via root biomass and rhizodeposition (Hirte et al., 2018a). As root traits in plants are very plastic, N additions may also affect plant root biomass and belowground C inputs. Low N fertilization input systems have been found to yield similar or higher root biomass as compared to high-input systems (Chirinda et al., 2012). Extreme nutrient deficiencies in soil can impair root growth, but mild deficiency may enhance C allocation to roots, promoting soil exploration by the root system (Hirte et al., 2018b). Greater density of fine roots and fine root systems can increase the complexity of the soil pore network, which in turn enhances rhizosphere activity and C turnover (Mota Neto et al., 2024). Long term fertilization may shift plant species and change the amount of root biomass and root distribution, moving root biomass closer to the surface of the soil. In one such study, there was 50% less root biomass in the mineral soil of fertilized plots versus control plots, and plant root mass shifted from 30% at the near soil surface in unfertilized plots to 70% in fertilized plots (Mack et al., 2004). Plant roots may accelerate SOM decomposition and promote a different soil N economy (e.g. inorganic v. organic soil N) for microbes (Adamczyk et al., 2019), but there is also the potential that more root biomass can increase C inputs to the soil and help sequester C in the long term (Hirte et al., 2018a). Because root biomass is linked to C inputs, understanding how N fertilizer affects root biomass in relation to MAOC stocks may also provide value in informing land management decisions.

This study aims to address gaps in understanding how MAOC, a more stable SOC fraction, is affected by agricultural management practices, specifically cover cropping, and N fertilization. By focusing on a 9-year corn cropping system experiment with varied management treatments, I seek to determine the influence of these factors on MAOC levels and their interactions with root biomass and N fertility treatments. This research will not only clarify how MAOC responds to common agricultural practices, but may also inform strategies that protect soil fertility and promote long-term carbon sequestration.

Soil data were collected at the end of a 9-year experiment located in Painter, VA studying a corn crop system with different tillage, cover crop, and N fertility treatments, with the following objectives: i) to determine the influence of cover cropping and cumulative fertilization treatments on MAOC and ii) to assess the relationship between root biomass, cover crops, cumulative N fertility treatments, and MAOC. Specifically, I hypothesize that cover crops will increase MAOC compared to uncovered plots due to increased root biomass, and that nitrogen fertilization will have a more limited impact on MAOC stocks.

Methods

Site Description

Sampling for this experiment occurred in year 9 (2023) of an ongoing field experiment being conducted at the Eastern Shore Agricultural Research and Extension Center in Painter, VA. The average annual air temperature between 2020 and 2024 was a low of 10° and high of 21°F, and average annual precipitation is 119 cm (National Oceanic and Atmospheric Administration, 2024). The soil is predominately classified as Bojac sandy loam (coarse-loamy, mixed, semiactive, thermic Typic Hapludults), a well-drained soil found in the Coastal Plains with 0-2% slope (Soil Survey Staff, 2024).

Experimental Design

At this site, a split-plot design was used and replicated four times. Cover crop type was the whole plot factor, with five levels of cover crop treatment including conventional till (CT), no-till (NT), rye, vetch, mix (oat, triticale, rye, radish, rapeseed, crimson clover, Austrian winter pea, hairy vetch, phacelia). All treatments were NT managed, except for the CT, which was tilled each spring prior to corn planting using a disk harrow to a depth of 10 cm (Haymaker, 2024). Cumulative inorganic nitrogen fertilization treatment was the split plot factor with four treatment levels (0, 50, 100, 150 lb/ac). This gave a total of 80 plots: 5 cover crops x 4 fertilization levels x 4 replications = 80 plots. The plots, 3.6 m x 12.2 m with a 1.8 m alley, were established in the fall of 2014 and received the fertilizer treatments shown in Table 1.

Soil Sampling

Soil samples were collected using an 8 cm diameter by 8 cm deep soil corer. In each sub-plot, three random samples were collected by splitting the sub-plot into 6 sections, assigning each a random number 1 through 6 and then randomly selecting three numbers. Samples in each selected sub-section were collected in-row, next to plants, but not on top of a plant so as to avoid artificially inflating root biomass by collecting all root biomass of one plant. Samples were stored in gallon bags and homogenized for each plot resulting in an average soil volume of 1200 cm^3 per plot. Approximately 100 g of soil was subsampled from each plot; plant roots were removed and retained with the original field sample for root elutriation. All soil subsamples were air-dried, sieved to 2 mm and then ball-milled to be used for elemental and isotopic analysis. To obtain the MAOM fraction, the subsample was fractioned by particle size, with the < 53 µm fraction representing clays and silts modified from Six et al. (1998). First, 10 g of air-dried soil was sieved through a 53 µm screen using deionized water. Sieved water-soil solution was collected in 50 mL Falcon tubes, and then samples were centrifuged at 4000 g for 15 min. Water was decanted from the tube, the fine soil fraction was collected, tubes were rinsed and drained into each sample tin to collect all fine particles, and then sample tins were dried at 65°. Dried soils were ground into a fine powder using an agate mortar and pestle and stored for analysis at 65°C. Fine fraction soils were analyzed for %C and %N on an Elemental Vario Max CNS Elemental Analyzer (Elementar, Ronkonkoma, NY, USA).

Root Elutriation

To separate roots and other organic matter from mineral soil, a hydropneumatic root elutriator was used (Vienna Scientific Instruments GmbH, Alland, AT). Soil samples were split and placed in tubes of the washing system and washed for at least 5 minutes, or longer as necessary until water discharge became clear. Roots and other organic matter were collected in 0.5 mm sieves. After washing, non-root organic matter (e.g. insects, worms, corn husks) was picked from each sample and discarded. It is important to note, that while CT and NT treated plots did not have cover crops planted, they were not without belowground root mass, as they were allowed to grow weeds from the existing seedbank. Roots were dried at 65° for 48 hours or until dry mass remained constant. Roots were weighed and then ball milled for C & N analysis.

Statistical Analyses

Linear mixed-effects models and analysis of variances were conducted for a split-plot design where fixed effects were assessed using random effects of N fertilizer, cover crop, and replication. These analyses were used to evaluate the effect of cover crop treatments on the concentration of SOC and MAOC in the soil. Regression models were used to analyze the relationship between root biomass and the concentration of MAOC in the soil. Analyses were performed using JMP software (JMP® Pro, Version 17.0. SAS Institute Inc., Cary, NC, 1989-2024.).

Results

At the end of the 9-year experiment, the presence of cover crops had a significant effect on SOC, TN, MAOC, and MAOM-N (Table 2). SOC and MAOC (%) in the Mix, Rye, and Vetch plots resulting in higher concentrations of MAOC when compared to the NT control plot (ANOVA, $p < 0.0001$). TN (%) was higher in Vetch and Mix compared to CT and NT plots, and Rye was higher than the NT plot (ANOVA, $p < 0.0001$). MAOM-N (%) in Mix and Vetch were higher than the NT control (ANOVA, $p < 0.0001$). The cumulative nitrogen fertilization treatments had no significant effect on MAOC concentrations ($p = 0.742$), and there was no significant interaction between cover crop treatment and the cumulative nitrogen additions on MAOC concentration ($p = 0.899$) (Figure 1).

Table 2: Means of percent soil organic carbon (SOC), total nitrogen (TN), mineral-associated organic carbon (MAOC), and mineral-associated nitrogen (MAOM-N) in each treatment from 0-8 cm depth at the end of a 9-year cropping experiment. M represents the mean and SE represents the standard error of the mean. Letters indicate post-hoc testing with Tukey HSD (p < 0.05), means with different lowercase letters are significantly different within columns.

TREATMENT	% SOC		% TN		% MAOC		% MAOM-N	
	M	SE	M	SE	M	SE	M	SE
CT	0.68^{b}	0.029	0.06^{bc}	0.003	1.14^{b}	0.029	0.12^{bc}	0.006
NT	0.64^{b}	0.026	0.06^{c}	0.002	1.08^{b}	0.035	0.11^{c}	0.004
RYE	0.92^{a}	0.041	0.08^{ab}	0.003	1.37^{a}	0.04	0.14^{abc}	0.007
VETCH	0.91^{a}	0.055	0.09^{a}	0.005	1.43^{a}	0.048	0.16^{ab}	0.011
MIX	1.02^{a}	0.075	0.09^{a}	0.007	1.47^{a}	0.053	0.15^{a}	0.011

Cover crop treatments had a significant effect on SOC ($p = 0.0006$), with the Mix, Rye, and Vetch plots resulting in higher concentrations of SOC when compared to the CT and NT plots.

The cumulative nitrogen fertilization treatments had no significant effect on SOC ($p = 0.806$), and there was no significant interaction between cover crop treatment and the cumulative nitrogen additions on SOC ($p = 0.513$) (Figure 1).

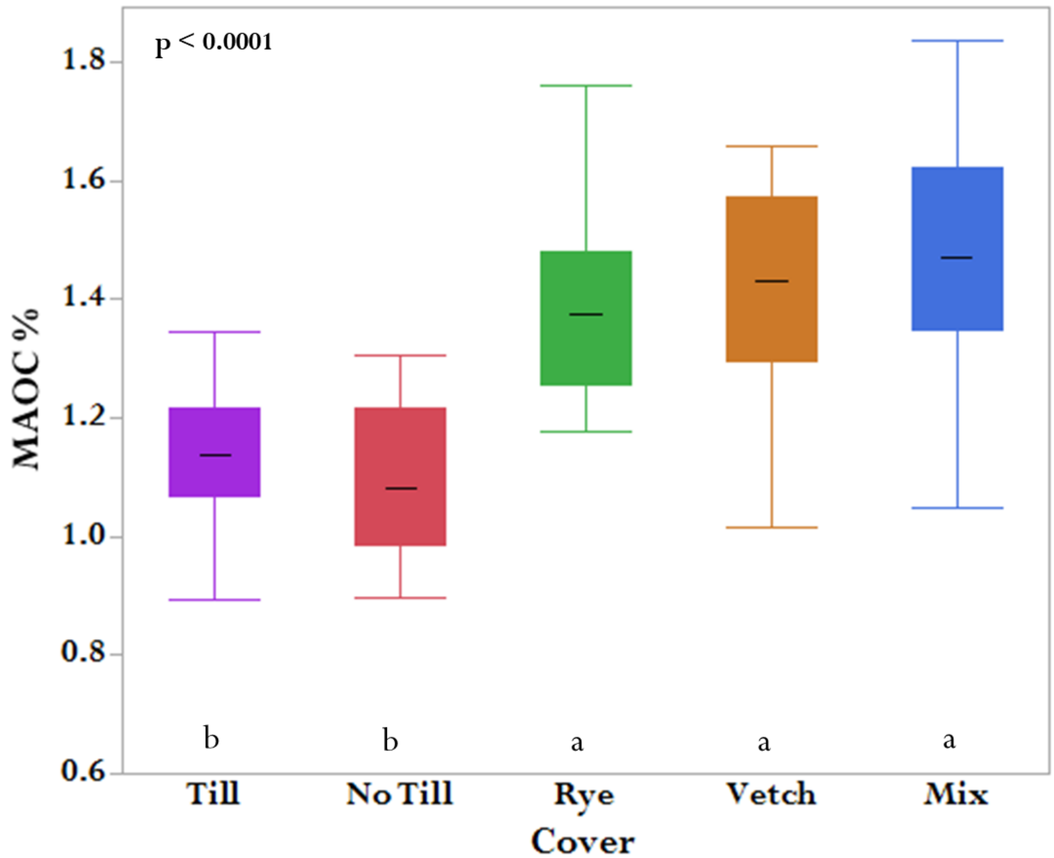

Figure 1: The effect of cover crop on MAOC (%). Boxes with whiskers display the expected variation of data for cover crop treatment, black bars within boxes represent the mean. Different lowercase letters above a cover crop treatment indicate a statistically significant difference using a Tukey HSD post-hoc test ($p < 0.05$).

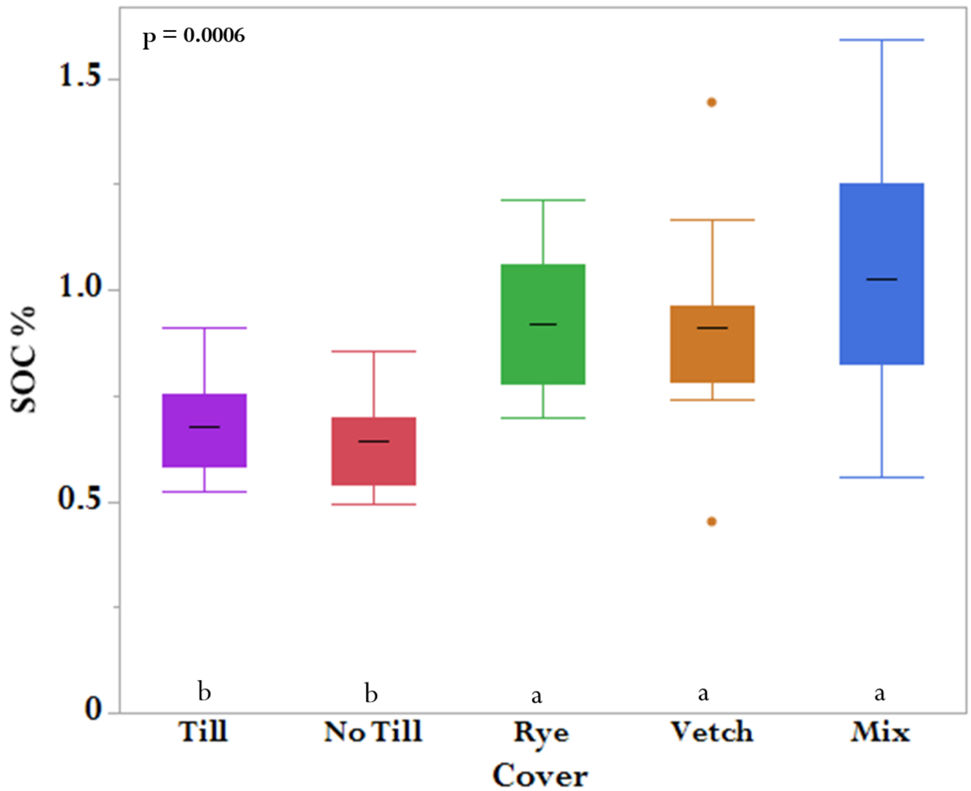

Figure 2: The effect of cover crop on SOC (%). Boxes with whiskers represent N-fertilization treatments and the expected variation of data for cover crop treatment, black bars within boxes represent the mean. Different lowercase letters above a cover crop treatment indicate a statistically significant difference using a Tukey HSD post-hoc test ($p < 0.05$).

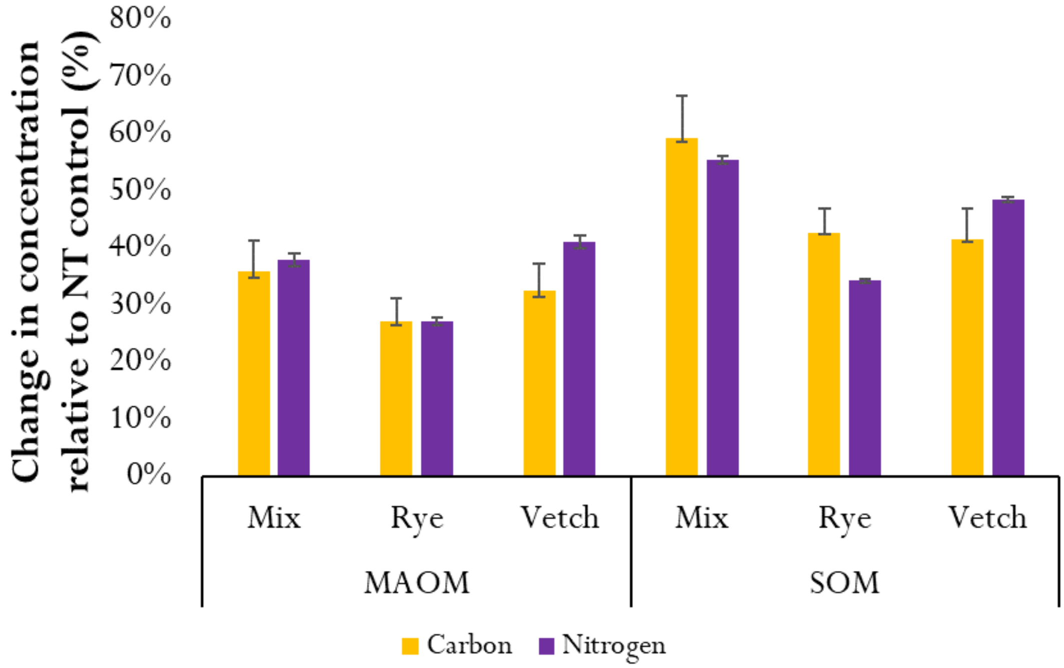

Figure 3: Change in concentration in bulk C and N means with standard error bars in MAOM and SOM as by each cover crop treatment (mix, rye, vetch) relative to NT treatments. ANOVA results indicate no significance between mix, rye, and vetch groups ($p < 0.05$).

Tillage impacts on SOM have been extensively researched, with no-till (NT) and cover crop practices generally recommended to improve SOM stocks over conventional tillage (CT) (Haruna and Nkongolo, 2019). Results of this study suggest that cover cropping helps build C and N in managed agricultural systems within a decade compared to NT alone. While ANOVA analysis indicated mix, rye, and vetch to have statistically more C and N than the NT control, there was no significance on the relative change in concentration between mix, rye, and vetch groups. Cover treatments did have 27-36% higher MAOC than NT plots. Additionally, there were also increases in MAOM-N of 27-41% in the NT (Figure 3).

The additional N-fertilization supplied every three years, to supplement plant productivity and soil fertility, did not result in significantly higher C stocks in either bulk SOC or the MAOC fraction ($p = 0.81$ and $p = 0.74$ respectively).

Root Biomass

Regression analyses showed a positive linear correlation between root biomass at the time of sampling and both MAOC (p = 0.0002, R^2 = 0.17) and SOC (p = 0.001, R^2 = 0.13) (Figure 5), indicating that regardless of N fertilizer or cover, MAOM concentrations were generally higher with more root biomass. Further analysis indicates that cover crop type was a significant indicator to the amount of root biomass in the plot with significant differences between treatment types (Figure 6). While NT and CT controls were not planted with cover crops, they did have root material from weeds. Vetch had similar root biomass to the Mix crop treatment, and had more root biomass than Rye, CT and NT. The mix cover treatment had more root biomass than the NT control. There was no significant difference between root biomass among different cumulative N fertilization treatments (p = 0.62).

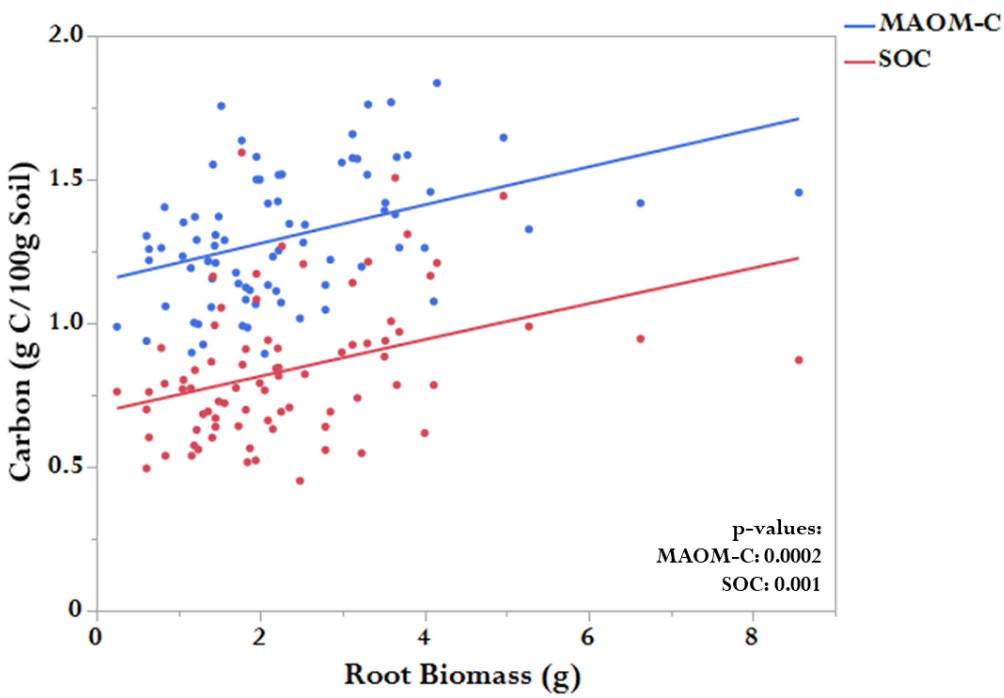

Figure 5: Linear regressions with 95% confidence for the relationship between root biomass (g), and SOC (red line) and MAOC (blue line) concentrations (g C/100 g soil) of all plots (N = 80).

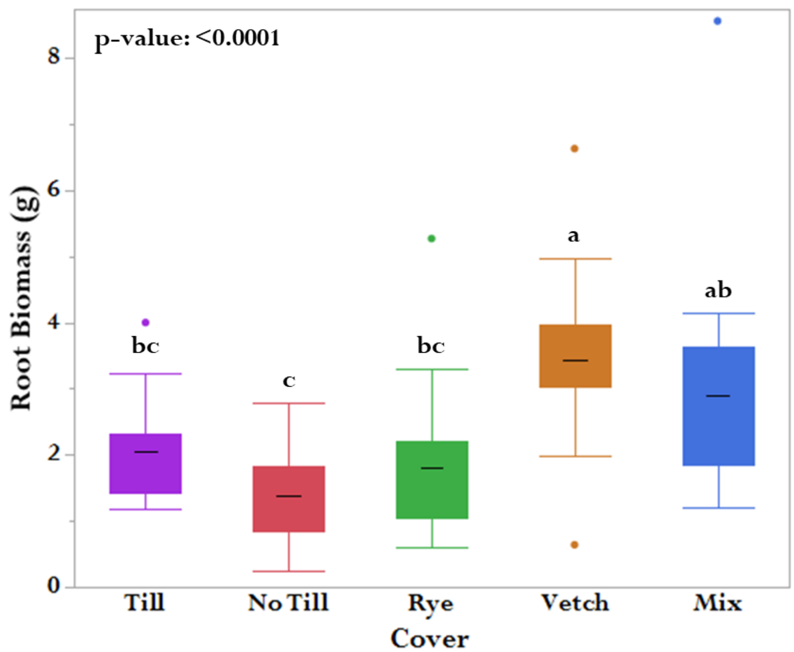

Figure 6: The effect of cover crop on root biomass (g). Boxes with whiskers display the expected variation of data for cover crop treatment, black bars within boxes represent the mean. Root Different lowercase letters above a cover crop treatment indicate a statistically significant difference using a Tukey HSD post-hoc test ($p < 0.05$).

Root biomass and cover crop were significant to concentrations of SOC and MAOC, and cover crop treatment had significant effects on how much root biomass was available. Further assessment to test these two factors and any interaction effects with carbon found that there was no interaction for either SOC ($p = 0.52$) or MAOC ($p = 0.09$) (Figure 7). For SOC, the slope of the mix cover treatment was significantly different between NT ($p = 0.0005$), CT ($p = 0.001$), and vetch ($p = 0.019$) while the slope of the rye cover treatment was significantly different from NT ($p = 0.009$) and CT ($p = 0.0003$). For MAOC mix, rye, and vetch were similar to each other, but significantly different from NT and CT ($p < 0.05$). Covariance between root biomass and cumulative N-fertilization was also assessed, with results indicating that N treatments were not a covariate to the relationship between root mass and SOC ($p = 0.27$) or MAOC ($p = 0.97$).

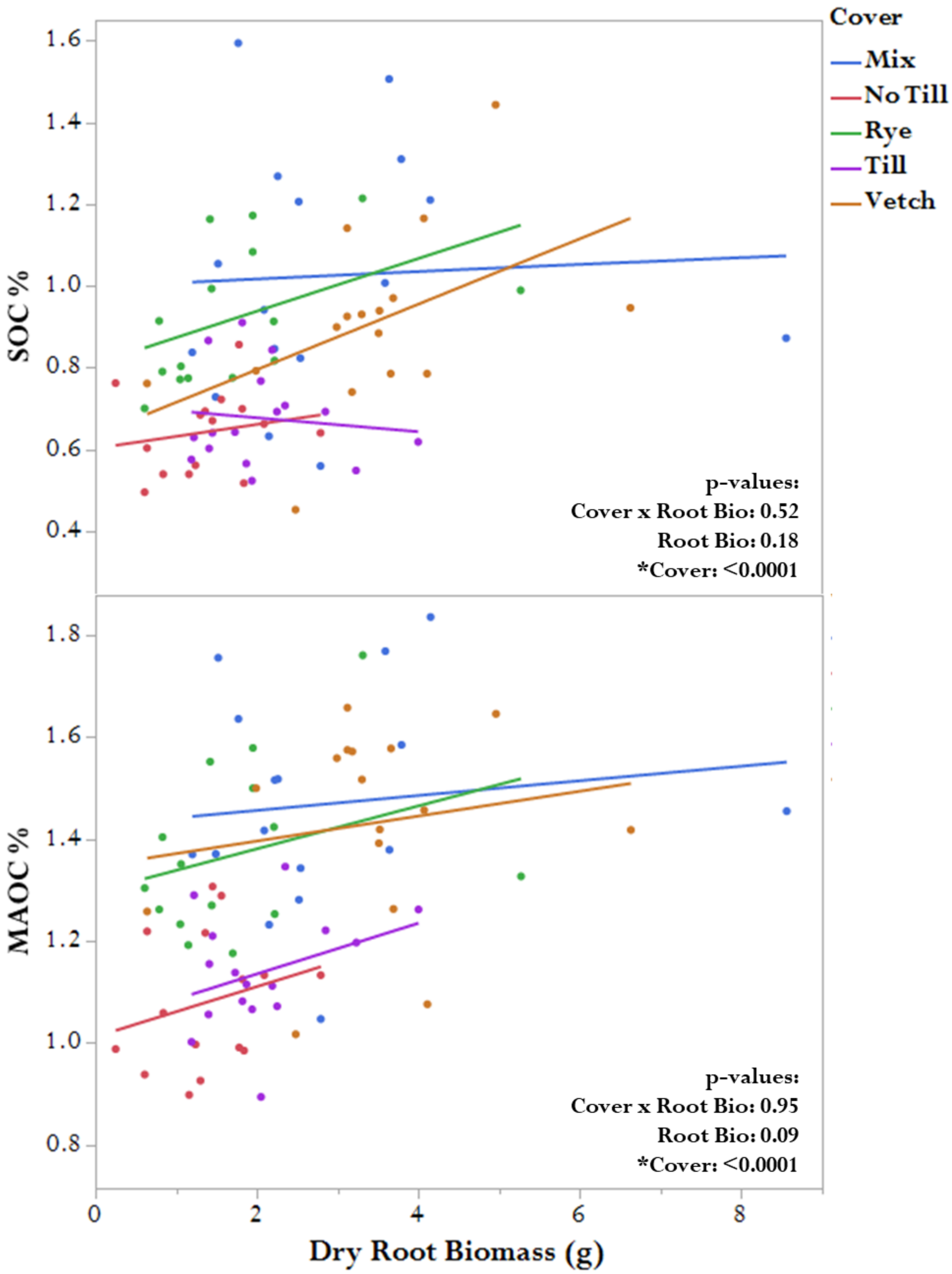

Figure 7: Linear relationship between soil carbon and root biomass, with Bulk SOC concentration (a) and MAOC (b) under different cover crop treatments. Different color lines represent different cover types. Inset p-values are the statistical results of the relationship between root biomass, cover treatment, and carbon.

Discussion

Efforts to improve SOC for sustainable agriculture and productivity, as well as sequestering carbon to offset losses of carbon to the atmosphere remain central to contemporary soil management strategies. However, one of the greater challenges is understanding how long-term agricultural practices such as cover cropping and nitrogen fertilization affect SOC dynamics to include different pools, such as MAOM. Analyzing SOC alone as a measure of soil fertility for agricultural purposes, does not capture C cycling within distinct pools of SOC, which vary in turnover rates and stability. Expanding studies to include these differing pools provides useful information into the specific impacts of long-term management on SOC stabilization and sequestration, as well as which SOM fractions may be most influences by specific management practices.

Overall, the findings in this study suggest that cover cropping, especially in NT systems, can significantly enhance SOC and MAOC sequestration, while variations in cumulative N additions supplied every 3 years had no significant effects on SOC or MAOC. Root biomass exhibited a positive relationship with C stocks, and while some cover crop treatments were significantly higher in root biomass, there was no actually no interaction between cover crop and root biomass in improving C stocks.

Cover crops and tillage

These results suggest several important observations about SOC and MAOC dynamics under different management practices. First, cover crop treatments all had higher, but similar means for SOC and MOAC compared to the CT and NT controls. Though this study did not focus on the differences between CT and NT explicitly, the results indicate that SOC and MAOC in these two practices did not differ. This result was unexpected as soil disturbance can increase the likelihood of SOC loss through loss of soil structure and increased access to O_2, which implies increased access to soil nutrients, which may induce positive priming effects, increasing microbial respiration and loss of carbon to the atmosphere (Chung et al., 2008). Soil disturbance can also increase the likelihood of nitrogen loss through volatilization, leaching, and erosion during precipitation events.

I am unable to discern the change in MAOC from the beginning of this 9-year period as MAOC was not quantified at the start of the experiment. However, the broader long-term study includes findings reported by Haymaker (2024), who found that over 9-years, cover crop treatments had improved SOC by 22% to 65% compared to the no cover crop controls in 0-5 cm depth. These results are similar to those observed by Congreves et al. (2017), where gains in SOC and TN after 11 years of N fertilization resulted in a 31% increase of SOC, and 57% for TN. While different levels N fertilization were one of the treatments in that experiment, the observed gains of SOC and TN were highly dependent on crop and tillage system, which is similar to the findings in this study.

Future studies could expand on these findings by investigating SOC stabilization mechanisms across different SOM fractions over longer time scales, and examining the effects of different cover crop species under various tillage regimes to optimize carbon sequestration potential and soil fertility.

Nitrogen Fertilization

Soil organic matter is tied to decomposition and mineralization, ultimately stabilized via physical or chemical protection (Kleber et al., 2015; Mayer et al., 2022). The complexity of SOM in relation to all of these management factors, and how C, N, and P work together to regulate nutrient availability, makes it exceptionally difficult to ascertain SOC dynamics over the long term (Abrar et al., 2021). All are critical nutrients, whose interaction and stoichiometric ratio with one another, regulate nutrient availability to plants and microbes in the soil, with N being especially critical to crop productivity. As N can be a limiting factor in many of these processes, it has become commonplace to amend agricultural soils with N fertilizer while avoiding detrimental responses to SOM, as excessive N is not without some danger as it can readily leach into groundwater (e.g. nitrate), volatize to the atmosphere (e.g. nitrous oxide, nitric oxide), and acidify soil (Mueller et al., 2014).

Long-term application of fertilizers can increase SOC accumulation and potentially build MAOC stocks because the higher soil N availability supplements plant production and microbial activity (Jilling et al., 2021). Nitrogen in particular is often the limiting factor when it comes to soil health and productivity; too little nitrogen, and crop productivity decreases, along with SOC stocks. In part, this is due to a process referred to as priming (Kuzyakov et al., 2000; Qin et al., 2024). Microbes are partially responsible for cycling nitrogen into plant available forms to assist with plant growth in addition to using it as a resource for their own growth. When carbon is readily available from easily decomposable sources such as root exudate and other plant inputs, N becomes limiting, and microbes will seek out N in SOM, specifically in organic matter associated with minerals (Kleber et al., 2015; Keiluweit et al., 2015). It has been shown that nitrogen limitations result in a reduction of SOC and MAOC, and that nitrogen additions in some form are necessary to maintain productivity and aid in SOC sequestration (Jilling et al., 2018).

While the additional N-treatments were not added every year, the findings in this study are similar to Zhong et al. (2015) who found that concentrations of SOC, and the light (POM) and heavy (MAOM) fractions, were constant among differing N fertilization rates (0, 90, 180, and 270 kg ha^{-1}) after 10 years of N additions. Though it has also been found that long-term fertilization can increase crop yields with increasing N application rates, and that increasing N fertilizer also increases SOC in the top soil, though this may be attributed to increased inputs of root biomass and root exudates (Guo et al., 2012).

Roots

The relationship between N fertilization, root biomass distribution, and C cycling has been examined across a range of studies in both non-agroecosystems and agricultural contexts, revealing complex plant-soil-microbe interactions that shape carbon turnover and microbial dynamics. In our study, root biomass exhibited a direct relationship with MAOC, with increased root biomass resulting in greater amounts of MAOC, which was directly related to cover crop treatments, not the cumulative N fertilization treatments. In Hirte's (2018a) study on maize, findings demonstrated that root biomass response depended more on site conditions than fertilization intensity. Though this is not to say that N fertilization may not have impacts on root systems. Mack et al. (2004) observed that long-term fertilization in non-agroecosystems led to significant shifts in species composition and root biomass distribution, with fertilized plots losing approximately 2000 g C/m² over 20 years. Although total root biomass remained similar between treatments, fertilization shifted root distribution towards the soil surface, with 70% of root biomass concentrated in surface layers compared to 30% in control plots. This shift reduced root biomass in deeper mineral soil layers by 50% in fertilized plots, potentially impacting decomposition rates and nutrient accessibility in lower soil profiles.

Hirte et al. (2018a; b) further contributed to understanding root dynamics by showing that total root biomass and rhizodeposition were comparable between organic and conventional farming systems under wheat, though organic systems exhibited significantly higher rhizodeposition under corn. Mayer et al. (2022) also highlights the importance of evaluating root biomass across cover crop types and N fertilization levels, pointing to Hirte's findings, as described above, on the site-specific response of root biomass to N availability. Kazanski et al. (2018) provide additional insight from a 12-year N fertilization study on sandy soils, where no consistent effects were observed on soil C stocks, microbial respiration, or soil C pool sizes. However, they found strong correlations between microbial biomass, respiration, and root biomass C pools in response to N, indicating that soil C dynamics may be predominantly driven by fine root biomass under site-specific conditions. More **r**ecent findings by Mota Neto et al. (2024) emphasize the structural role of fine root density in enhancing rhizosphere complexity, suggesting that fine roots can increase soil pore network complexity and subsequently promote rhizosphere activity and C turnover. These findings highlight how cropping and management systems have differing effects on root dynamics, highlighting the interactions between N fertilization, root biomass distribution, and microbial processes, which are intricate and often site-dependent, with root architecture playing a crucial role in shaping soil C dynamics and microbial responses across ecosystems.

Conclusion

This study demonstrates that cover cropping substantially increases MAOC and SOC levels when compared to CT and NT without cover crops. All cover crop treatments examined in this study (vetch, rye, mix) resulted in notable increases of MAOC, indicating that cover cropping can be a key practice for enhancing C sequestration and promoting long-term soil fertility. In contrast, cumulative N fertilization applied at three-year intervals in addition to N fertilization at cash crop planting, and side-dressing treatments, did not significantly influence C concentrations. This suggests that, under the conditions studied at this site, additional N inputs may not be as critical for long-term carbon management in MAOM fractions compared to cover cropping and tillage strategies, though there may be some benefit to cash crop productivity. Due to a lack of initial soil MAOC data in this study it is unknown whether MAOC stocks increased or decreased by N-fertilization treatments and tillage/cover cropping over time. I am only able to infer that cropping and tillage result in different concentrations of MAOC in similar soils. Based on Haymaker et al., (2024) in this same system, there was an observed increase in bulk SOC among cover crop treatments after 9-years, but where the bulk of those changes occurred, whether in POM or MAOM, is unknown.

Future research should focus on understanding the long-term interactions between crop species, soil types, and management practices, but needs to incorporate comprehensive assessments of different SOM fractions, to include both particulate and mineral-associated forms. It is crucial for accurately evaluating the sustainability of agroecosystems. Due to confounding studies, there are likely other environmental factors such as precipitation, temperature, soil texture, and structure, which also impact C dynamics and question whether direct management of SOM pools is possible. This study underscores the importance of integrating tailored land management strategies to enhance soil health, while mitigating carbon losses and optimizing soil fertility in diverse agricultural landscapes.

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Chapter 5 Research Conclusions

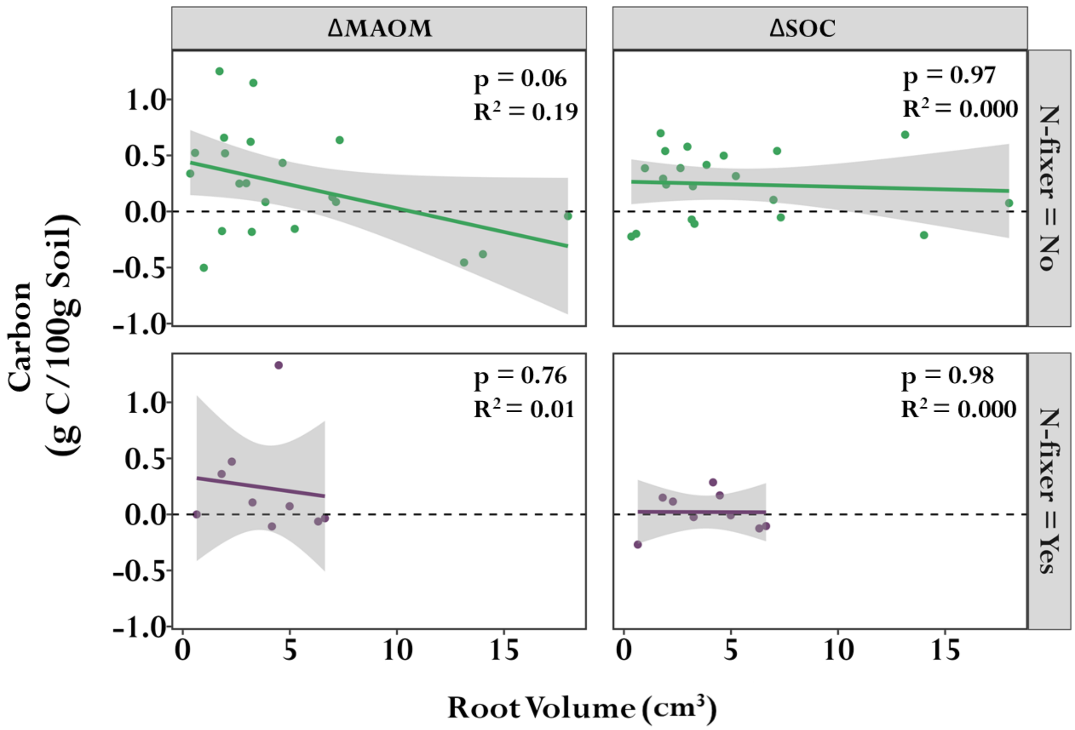
This dissertation underscores the complex interplay between plant traits, nitrogen inputs, and soil organic carbon fractions, particularly mineral-associated organic carbon (MAOC), in agricultural and managed ecosystems. Chapter 1 highlighted the importance of root morphological traits in influencing changes in SOC and MAOC during short-term plant growth cycles, such as cover crop seasons. The relationships between root traits and C dynamics differed between non-N-fixing and N-fixing species, with findings suggesting that SOC and MAOC exhibit different responses based on N-fixing capacity of plants.

Chapter 2 demonstrated that nitrogen fertilization was the dominant factor influencing ΔMAOC, which represents change in MAOC accounting for both old carbon and fresh carbon inputs, particularly at moderate application levels of N-fertilizer. However, excessive nitrogen inputs and nitrogen-limited environments resulted in MAOC losses, likely due to increased microbial competition for resources. In SOC with zero N-additions, root morphological traits are negatively correlated, resulting in SOC loss, though loss of MAOC was not connected to root morphology under any level of N-fertilization. In contrast, plant species, root biomass, coarse-root attributes and aboveground biomass were more influential in determining fresh inputs from plant-added MAOC (PA-MAOC), reinforcing the idea that plant root traits play a greater role in plant-added inputs than nitrogen availability alone over short time scales.

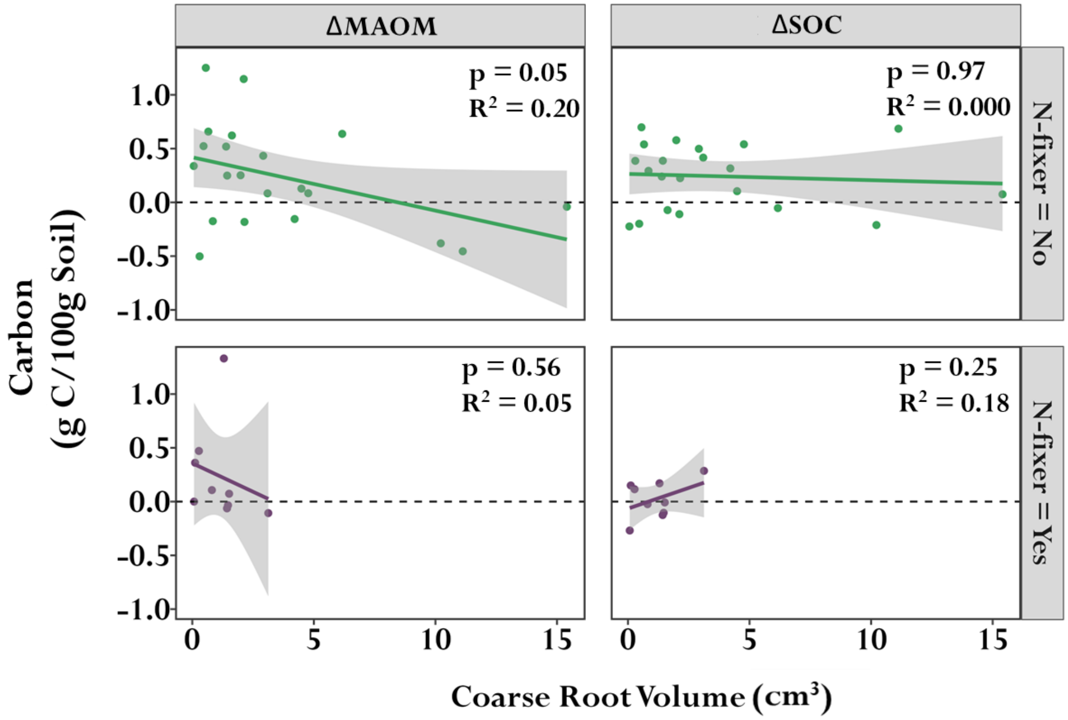
Chapter 3 confirmed that cover cropping significantly increased both MAOC and SOC compared to conventional and no-till systems without cover crops, while triennial N-fertilization applications had minimal impact on long-term carbon concentrations in MAOC. This finding suggests that cover cropping is a critical strategy for enhancing soil carbon sequestration and improving soil health in both SOC and MAOC. However, the absence of initial baseline MAOC data limited our ability to definitively quantify changes over time, highlighting the need for more comprehensive monitoring of SOM fractions, including particulate organic matter (POM) and MAOM.

Overall, this study extends what was previous known about MAOC cycling in relation to root traits and N-fertilization, but it further emphasizes the need to consider plant functional traits, soil management strategies, and nitrogen levels in tandem to optimize carbon sequestration. Future research should address the specific mechanisms by which root traits influence MAOM, particularly in relation to exudation rates, quality, and nitrogen interactions, and should also include analysis of the POM fraction. Lastly, while this study provides valuable insights from relatively short time periods in controlled conditions, long-term field studies that incorporate plant species diversity, land management practices, and environmental variables are essential for improving our understanding of MAOC dynamics and developing sustainable agroecosystem practices that enhance soil fertility while mitigating carbon losses.

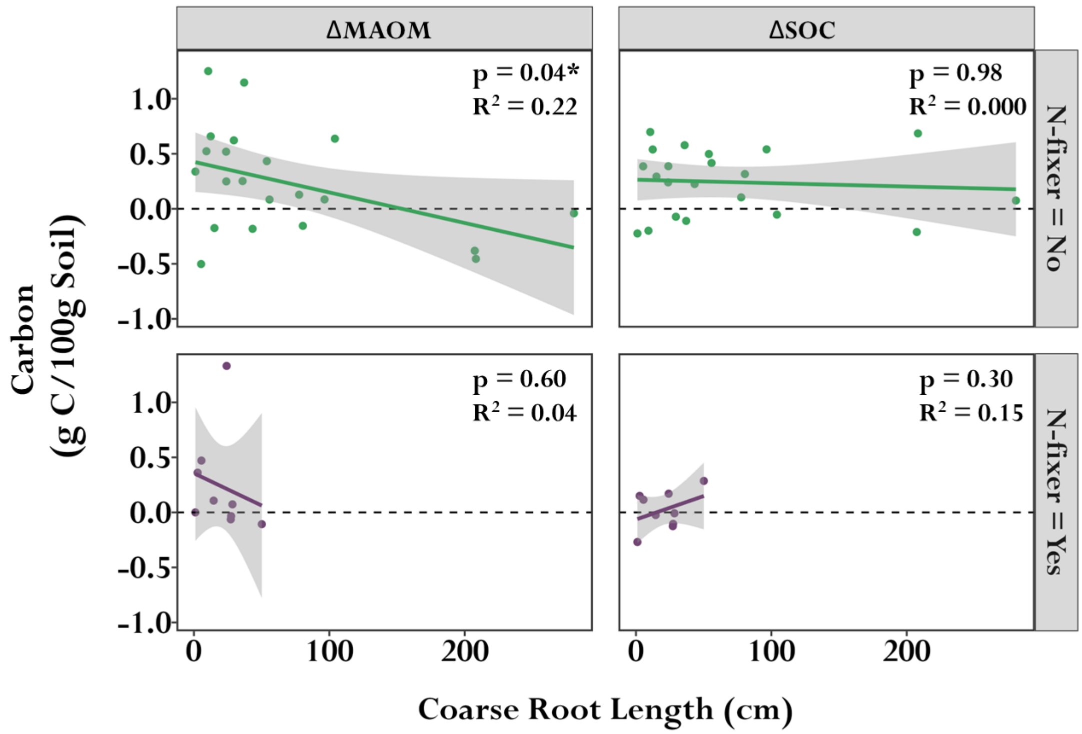
APPENDIX A: Supplementary Information for Chapter 2



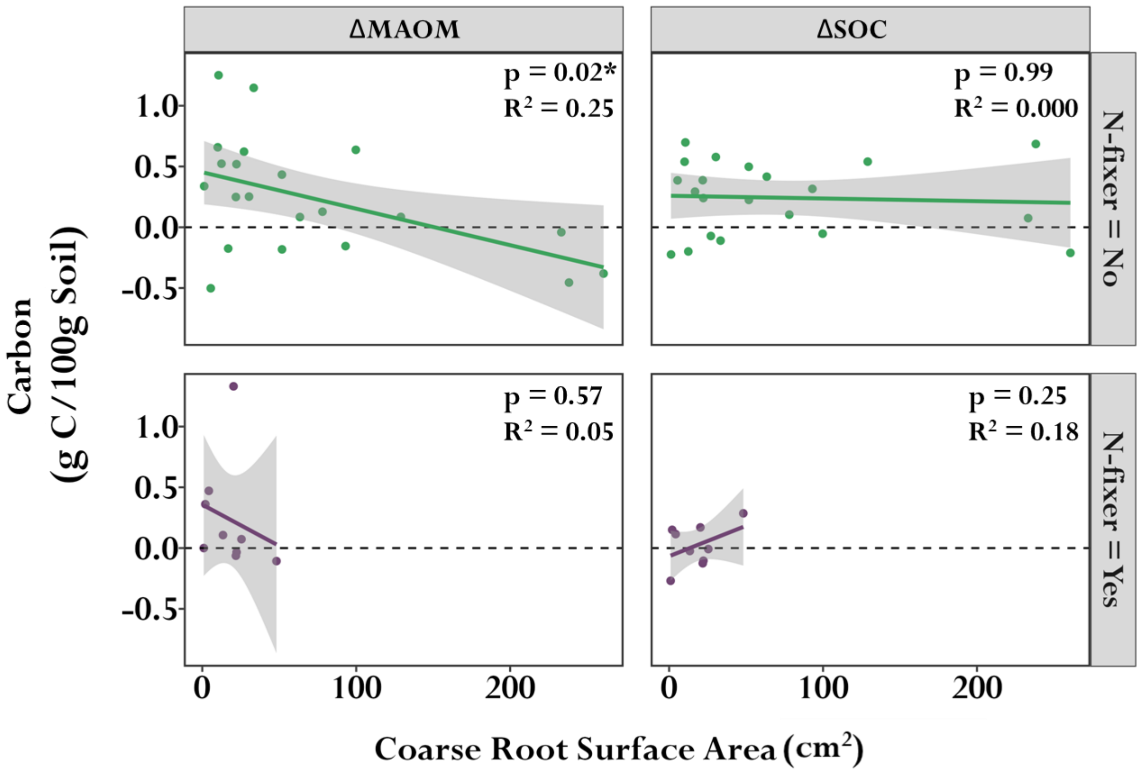
A1: Root volume (g) in both SOC (right) and MAOM fraction (left). Panels represent a split between plant function as N-fixing (bottom) or not (top). Amount of soil carbon (g C / 100 g soil) reported as the difference between planted pot and the no-plant control after 12 weeks.



A2: Coarse root volume (cm^3) in both SOC (right) and MAOM fraction (left). Panels represent a split between plant function as N-fixing (bottom) or not (top). Amount of soil carbon (g C / 100 g soil) reported as the difference between planted pot and the no-plant control after 12 weeks.



A3: Coarse root length (cm) in both SOC (right) and MAOM fraction (left). Panels represent a split between plant function as N-fixing (bottom) or not (top). Amount of soil carbon (g C / 100 g soil) reported as the difference between planted pot and the no-plant control after 12 weeks.



A4: Coarse root surface area (cm^2) in both SOC (right) and MAOM fraction (left). Panels represent a split between plant function as N-fixing (bottom) or not (top). Amount of soil carbon (g C / 100 g soil) reported as the difference between planted pot and the no-plant control after 12 weeks.

APPENDIX B: Supplementary Information for Chapter 3

A1: Biomass response of plant attributes per N fertilization treatment.

	Aboveground Biomass (g)				Average of Root Mass (g)				Ratio Aboveground:Root			
	0	50	100	150	0	50	100	150	0	50	100	150
Annual Rye	0.86	0.90	1.02	0.74	1.37	0.35	0.32	0.45	0.63	2.60	3.17	1.63
Buckwheat	6.90	8.34	10.48	11.24	1.28	1.92	1.26	1.97	5.39	4.35	8.34	5.71
Mustard	1.01	0.56	0.35	0.46	0.29	0.09	0.08	0.16	3.43	6.46	4.52	2.94
Oat	1.28	2.31	0.85	0.99	0.28	0.45	0.16	0.19	4.53	5.10	5.29	5.10
Radish	5.51	4.02	3.54	4.92	1.60	1.37	2.37	1.99	3.45	2.92	1.49	2.47
Rye S. Cereale	1.21	1.07	1.07	0.89	1.16	0.65	0.48	0.51	1.04	1.63	2.24	1.75
Tall Fescue	0.17	0.29	0.21	0.21	0.10	0.17	0.10	0.03	1.65	1.65	2.07	7.00
Triticale	1.82	1.43	1.02	1.25	1.94	1.14	0.77	0.66	0.94	1.26	1.33	1.89

APPENDIX C: Supplementary Information for Chapter 4

A1: Yearly fertilization schedule for plots to include yearly starting fertilizer, amount of side dressing at corn planting, and whether additional cumulative N fertilization has been applied in any given year.

Year	**Starter Fertilizer**	**Side dressed**	**Cumulative rate**
2015	No starter	135 lb N/acre	No
2016	No starter	135 lb N/acre	No
2017	30 lb N/acre starter	0, 50, 100, 150	Yes
2018	No starter	150 lb N/acre	No
2019	No starter	150 lb N/acre	No
2020	50 lb N/acre starter	0, 50, 100, 150	Yes
2021	No starter	200 lb N/acre	No
2022	No starter	200 lb N/acre	No
2023	50 lb N/acre starter	0, 50, 100, 150	Yes