

Long-term Effects of Cover Crops on Soil Organic Matter Composition

by

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Abstract

Cover crops (CCs), as a long-term sustainable agricultural practice, play a key role in enhancing soil organic matter (SOM). Cover crops add SOM directly through the decomposition of aboveground residues and root exudates, and indirectly by stimulating microbial activity. Decomposition of different cover crop residues has distinct functional traits and contributes differently to SOM and water-extractable organic matter (WEOM) production and composition. The objective of this study was to quantify how different cover crop species and soil depth affect the composition of SOM and WEOM at two long-term field sites in the southeastern United States– the Tennessee Valley Research and Extension Center (TVREC) and the Wiregrass Research and Extension Center (WREC), using spectroscopic techniques. Soil samples were collected in November 2024 at depths of 0-10 and 10-30 cm following 7 years of continuous cover crop management. This study included winter fallow, cereal rye (*Secale cereale* L.), crimson clover (*Trifolium incarnatum* L.), and radish (*Raphanus sativus* L.) as monocultures and a mixture of the three species. WEOM was then extracted from the soil samples to characterize dissolved organic carbon (DOC) concentration, total nitrogen (TN), UV-Vis absorbance, and fluorescence properties. FTIR (Fourier Transform Infrared) spectroscopy was used to characterize the SOM composition of the samples. Results showed no significant differences in SOM functional groups or WEOM composition among cover crop treatments, whereas clear compositional separation was observed between sites and soil depths ($p < 0.05$). Clover increased TN in the 0-10 cm depth at both sites; responses in the 10-30 cm depth were evident only at TVREC. DOC concentrations were higher at the WREC site under legume-based treatments, but this effect was not observed at the TVREC site. Although cover crop treatments at these sites improved soil health, surface SOC levels, and the soluble nitrogen pool, compositional patterns were primarily governed by differences in climate, soil texture, mineralogy, crop rotation history, and tillage management. These findings emphasize the need for long-term

studies to disentangle species-level cover crop effects from dominant site- and management-driven controls on SOM and WEOM dynamics.

Artificial Intelligence (AI) Use Disclosure Statement

In the preparation of this thesis, the following Artificial Intelligence (AI) tools were used: Gemini 3 Flash and ChatGPT. These tools were used primarily to troubleshoot LaTeX formatting for the nomenclature and front matter, and for debugging R scripts for statistical analysis. The author acknowledges full responsibility for the intellectual content of this work and has ensured that all AI-assisted sections have been reviewed and revised for accuracy and appropriate academic style. All AI-generated content was reviewed and validated for relevance, appropriateness, and accuracy before incorporation into the final document to maintain the scholarly integrity of this research.

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List of Abbreviations

CC	Cover Crop
SOM	Soil Organic Matter
SOC	Soil Organic Carbon
DOM	Dissolved Organic Matter
WEOM	Water-Extractable Organic Matter
DOC	Dissolved Organic Carbon
TN	Total Nitrogen
DON	Dissolved Organic Nitrogen
WEOC	Water-Extractable Organic Carbon
WEON	Water-Extractable Organic Nitrogen
POM	Particulate Organic Matter
MAOM	Mineral-Associated Organic Matter
MBC	Microbial Biomass Carbon
EEM	Excitation-Emission Matrix
UV-Vis	Ultraviolet-Visible Spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy
SUVA	Specific Ultraviolet Absorbance
HIX	Humification Index
FI	Fluorescence Index
BNF	Biological Nitrogen Fixation
CEC	Cation Exchange Capacity
TVREC	Tennessee Valley Research and Extension Center
WREC	Wiregrass Research and Extension Center
POXC	Permanganate Oxidizable Carbon
Mg ha ⁻¹	Megagrams per hectare
kg N ha ⁻¹	Kilograms of nitrogen per hectare

Chapter 1

Introduction

Cover crops are the close-growing crops that protect and improve the soil between periods of normal crop production (Blanco-Canqui et al., 2015; Soil Science Society of America, 2008), and they are widely recognized for improving soil health (Adetunji, Ncube, Mulidzi, & Lewu, 2020; Benedict, Cogger, & Andrews, 2014). They provide multiple ecosystem services, including erosion control, enhanced water infiltration, reduced runoff, improved nutrient retention, and increased soil organic matter (Battany & Grismer, 2000; Steenwerth & Belina, 2008). Among these benefits, SOM is a crucial, ubiquitous soil component that regulates sorption–desorption processes, cation exchange, and organo–mineral complexation, thereby influencing soil fertility, productivity, and physical structure (Ding et al., 2006). SOM is formed through decomposition and humification processes driven by microorganisms acting on plants and animal residues Corvasce, Zsolnay, D’Orazio, Lopez, and Miano (2006a). Cover crops can enhance SOM either by adding direct carbon inputs (above- and belowground biomass) or indirectly by stimulating soil microbial activity (Steenwerth & Belina, 2008).

SOM represents the overall carbon pool in soil, which includes both stable and labile fractions (Kalbitz & Kaiser, 2008). Dissolved organic matter (DOM) represents the most active and mobile form of SOM (Corvasce et al., 2006a). Although DOM is soluble and mobile, it can become stabilized in soil through sorption to minerals and co-precipitation with Al/Fe. These stable DOM contribute to the long-term buildup of SOM, especially in mineral subsoils (Kalbitz & Kaiser, 2008). Therefore, DOM acts as a link between fresh organic inputs and stable soil carbon. WEOM

is an operationally defined, labile, and active portion of SOM (Haney, Haney, Smith, Harmel, & White, 2018; Rinot, Borisover, Levy, & Eshel, 2021). Although it accounts for only a small fraction of total SOM (Armenise, Redmile-Gordon, Stellacci, Ciccicarese, & Rubino, 2013; Chantigny, 2003; Rinot et al., 2021), it plays a crucial role in biological turnover (Rees & Parker, 2005; Rinot et al., 2021). WEOM refers specifically to the organic matter pool extracted from the solid soil phase during a laboratory procedure, whereas DOM is the broader functional term for organic matter present in a dissolved state in a liquid medium. The quantity and composition of WEOM are influenced by land use and management practices (Chantigny, 2003; Rinot et al., 2021). Characterizing WEOM serves as a sensitive indicator of changes in quantity and quality of labile SOM caused by different land uses and soil-management practices (Chantigny, 2003; Haney et al., 2018; Jones, Simfukwe, Hill, Mills, & Emmett, 2014; Kalbitz, Schmerwitz, Schwesig, & Matzner, 2003; Rinot et al., 2021)

Previous studies have revealed that spectroscopic approaches are powerful tools for characterizing soil WEOM in terms of both quantity and quality (Bünemann et al., 2018; Fellman, D'Amore, Hood, & Boone, 2008; Jones et al., 2014; M. P. Schmidt & Martínez, 2019). Conventional extraction-based methods quantify the amount of organic matter recovered but do not show the differences in chemical composition or lability. Spectroscopic techniques like excitation-emission matrix (EEM) fluorescence analysis, infrared spectroscopy, and ultraviolet-visible spectroscopy (UV-Vis), provide detailed characterization of specific components within WEOM and molecular composition insights that cannot be obtained from bulk measurements alone. Hence, spectroscopy improves the detection of differences among labile organic matter pools and enhances the interpretation of biogeochemical processes through WEOM composition (Griffin, 2008; Li & Hur, 2017b; Rinot et al., 2018; Ros, Hanegraaf, Hoffland, & Van Riemsdijk, 2011).

The soils in the southeastern United States regions are dominated by highly weathered Ultisols and had history of prolonged degradation due to intensive tillage, erosion, and continuous row-crop production under warm, humid climatic conditions (Sikora & Moore, 2014; Triplett &

Dick, 2008). These conditions have reduced SOM. Cover crops, as a conservation agriculture practice, have been adopted in the region to restore soil productivity and improve soil health indicators (Causarano, Franzluebbers, Reeves, & Shaw, 2006; Franzluebbers, 2005). Previous studies conducted at two research stations in Alabama - Tennessee Valley (TVREC) and Wiregrass (WREC) evaluated the short-term effects of cover crop monocultures and mixtures on dynamic soil health indicators (Decker, Gamble, Balkcom, Johnson, & Hull, 2022; Johnson, Gamble, Balkcom, & Hull, 2021). Those studies found that 2-4 years of cover crop adoption improved selected indicators, such as soil organic carbon (SOC) and permanganate oxidizable carbon (POXC), and reduced soil strength, especially in the finer-textured TVREC soils. But, responses at the coarser-textured WREC site were minimal or inconsistent. Radish monoculture rarely improved soil health relative to fallow, and mixtures did not outperform monocultures. These findings suggest that soil type strongly mediates the short-term benefits of cover crops in southeastern Ultisols.

Despite those findings, it is poorly understood about how cover crop species identity influences the chemical composition of SOM and the dynamics of WEOM. Previous work at these sites has focused on bulk C fractions, aggregate stability, and compaction metrics, leaving a gap in understanding carbon transformation pathways and the composition of SOM pools. Evaluating SOM functional group composition and WEOM characteristics provides more meaningful insights into carbon transformation processes that may not be reflected in total SOC concentrations.

This study was a part of a long-term field experiment in the Alabama region. This experiment evaluates the effects of three winter cover crop species—cereal rye (*Secale cereale* L.), crimson clover (*Trifolium incarnatum* L.), and radish (*Raphanus sativus* L.)—and their mixture within a cotton (*Gossypium hirsutum* L.)-legume crop rotation. The objective of this study was to assess treatment- and depth-dependent variations in the composition of WEOM and SOM across different cover crop systems after 7 years of continuous management. Specifically, we quantified DOC, TN, and optical properties, fluorescence intensities, and SOM composition using FTIR at two soil depths (0-10 cm and 10-30 cm) to determine whether CC species influence WEOM composition in contrasting Ultisols.

1.1 Hypothesis

We hypothesized that: (i) Cover crop treatments would alter WEOM and SOM composition relative to fallow following seven years of continuous management due to greater organic inputs from residues and roots; (ii) rye, radish, clover, and their mixtures would generate distinct WEOM and SOM signatures reflected in differences in DOC, DON, and optical indices due to differences in their residue functional traits and contributions of different organic carbon inputs; and (iii) surface soils (0-10 cm) would exhibit greater WEOM concentrations and more pronounced compositional characteristics than 10-30 cm depth, due to the cover crop residue inputs and their retention in 0-10 cm depth.

1.2 Thesis Structure

To address the research objectives and hypothesis, the thesis begins with a literature review (Chapter 2), followed by the analysis of soils under different cover crop treatments and depths to characterize SOM and WEOM using spectroscopic techniques (Chapter 3), results and discussion (Chapter 4 and 5), and conclusion (Chapter 6).

Chapter 2

Literature Review

2.1 Cover Crops

2.1.1 Definition and Functional Role of Cover Crops

Cover crops are close-growing crops that provide soil protection and soil improvement between periods of normal crop production (Adetunji et al., 2020; Blanco-Canqui et al., 2015; Soil Science Society of America, 2008). These crops are grown during the bare fallow periods between the harvest of one cash crop and the sowing of the next, and they are not intended for any economic or market purposes (Rivière, Béthinger, & Bergez, 2022; Wagg et al., 2021).

CCs are also known as green manure crops and catch crops, and they play an important role in providing the agroecosystem services (Steenwerth & Belina, 2008). Benefits of growing cover crops include soil health improvement (Benedict et al., 2014), erosion control, enhanced water infiltration, reduced runoff, improved nutrient retention, increased SOM (Battany & Grismer, 2000; Steenwerth & Belina, 2008), moisture conservation (Fageria, Baligar, & Bailey, 2005; Morse, 1993), weed suppression (Sturm, Peteinatos, & Gerhards, 2018), reduced disease and insect attack (Ristaino, Parra, & Campbell, 1997; Sustainable Agriculture Network, 1998), and increased crop yields (Triplett, Dabney, & Siefker, 1996).

Beyond these agronomic advantages, cover crops provide continuous soil cover and improve soil quality by adding carbon to the soil (Reicosky & Forcella, 1998). Generally, cover crops are chemically terminated, and their residues are left on the soil surface. These residues contribute

to aboveground biomass (litter inputs), which undergoes decomposition and adds carbon (C) and nitrogen (N) to the soil (Quintarelli et al., 2022). Along with these surface residues, cover crops also supply belowground soil C through their living root inputs (commonly referred to as rhizodeposits). Rhizodeposits, when released from roots, get readily assimilated by rhizosphere microbes and contribute to the formation of mineral-associated SOC (Bradford, Keiser, Davies, Mersmann, & Strickland, 2013; Cotrufo, Wallenstein, Boot, Deneff, & Paul, 2013; Kallenbach, Frey, & Grandy, 2016; Sokol, Kuebbing, Karlsen-Ayala, & Bradford, 2019). Despite their smaller quantity, root-derived inputs are 2-13 times more efficient for SOC formation and microbial biomass accumulation than aboveground residues (Sokol et al., 2019; Somenahally et al., 2018). Also, they contribute relatively more to the stable soil C pools than the shoot-derived C (Sokol et al., 2019).

2.1.2 Types of Cover Crops

Cover crops are commonly classified into three main groups: legumes, grasses/cereals, brassicas. Each group species contributes differently to soil health, biomass production, residue quality, and nutrient supply. These differences among the group species affect microbial activity and organic matter inputs, thereby leading to variations in SOC status (D. W. Reeves, 2018). When combined as mixtures, these groups can complement each other's functional traits, providing more diverse and balanced benefits such as improved biomass production, enhanced nutrient cycling, and greater soil organic matter inputs.

A) Legumes

Leguminous cover crops fix the atmospheric nitrogen through Biological Nitrogen fixation (BNF) and thereby increase the soil N content (Quintarelli et al., 2022; D. W. Reeves, 2018). These leguminous cover crops supply 50–200 kg N ha⁻¹, which varies with species type and management practices, and thereby reduce the need for inorganic N fertilizers. Crimson clover (*Trifolium incarnatum* L.) and hairy vetch (*Vicia villosa* Roth) are most commonly used legume cover crops.

Approximately 16% of the total nitrogen in crimson clover is present in its root biomass, highlighting the importance of belowground inputs to soil nitrogen cycling (D. W. Reeves, 2018).

Leguminous cover crop residues have low, narrow Carbon to Nitrogen (C:N) ratios ranging from 9:1 to 25:1. These low C:N ratio residues decompose quickly and supply high-quality organic inputs to the soil and microbes (Ibewiro, Sanginga, Vanlauwe, & Merckx, 2000; M. J. Lynch, Mulvaney, Hodges, Thompson, & Thomason, 2016; D. W. Reeves, 2018), but they produce a lower amount of biomass (approximately 0.5–4 Mg ha⁻¹). While legumes generally have smaller seed sizes and lower seeding rates than small grains, the total cost of establishing legume cover crops remains higher due to elevated seed prices per unit, slower early-season growth, and more specific establishment requirements (Roberts & Swinton, 1996; Snapp et al., 2005).

The lower lignin content and lower C:N ratio cause the rapid residue decomposition. Previous studies have shown that nearly 75-80% of the residue nitrogen is released within 8 weeks after the termination of the cover crops. This rapid mineralization increases the availability of soluble N and labile organic compounds in the soil during the early stages of decomposition (Waggoner, Cabrera, & Ranells, 1998).

B) Grasses / Cereals

Grasses, also known as cereal or small-grain cover crops, are commonly utilized for their rapid establishment and high biomass production. This group of cover crops includes species like cereal rye, wheat, oats, and barley. They are more widely adopted than the legumes because of their higher biomass production (D. W. Reeves, 2018). They provide greater ground cover and surface residue, thereby increasing the amount of input addition. These crops have a fibrous root system and can take up N from the soil during non-cropping periods and slowly release it during the cropping period as residue decomposes, thereby preventing N from leaching. The total N accumulated within the aboveground biomass varies by species, typically ranging from 25 to 50 kg N ha⁻¹, and is subsequently released back into the soil as the residue decomposes during the following cropping season (Dinnes et al., 2002; Fageria et al., 2005).

Cereal rye CC has a higher C:N ratio (36:1), cellulose (331 g kg⁻¹), and hemicellulose (279g kg⁻¹) content. These result in slower decomposition of residues (Wagger et al., 1998). D. W. Reeves (2018) noted that cover crop residue quality depends on the termination time and growth stage. If the CCs are terminated at early stages, it would result in narrower C:N ratios, faster decomposition, and lower surface residue. Usually, grass cover crops are terminated at later growth stages, yielding residues with wide C:N ratios (exceeding 30:1). These residues decompose more slowly and cause short-term nitrogen immobilization during the subsequent cropping season (D. W. Reeves, 2018).

C) Brassicas

The Brassica group of cover crops belongs to the Brassicaceae family and has recently gained popularity in the U.S. regions. Brassica species like *Brassica juncea* (L.) Czern. (brown or Oriental mustard), *B. nigra* L. (black mustard), *B. rapa* L. (turnip, rapeseed, canola), *B. napus* L. (rapeseed, canola), *Raphanus sativus* L. (forage, oilseed, or daikon radish), and *Sinapis alba* L. (white or yellow mustard) are commonly used as CCs (Gieske et al., 2016). These species have biofumigation and allelopathic effects, which are regulated by glucosinolate compounds. Glucosinolates are sulfur-containing compounds that, upon hydrolysis, produce active and toxic compounds and are effective against some soil-borne weeds, insect pests, and pathogens (Haramoto & Gallandt, 2004). Unlike legumes, Brassicas are fast-growing crops that produce a higher amount of biomass, with dry matter production ranging from 3000 to 5000 kg ha⁻¹ (Dean & Weil, 2009; Gieske et al., 2016; Isse, MacKenzie, Stewart, Cloutier, & Smith, 1999; G. Wang, Ngouajio, & Charles, 2009), and are able to survive in frost (G. Chen et al., 2007). Their rapid growth and ability to scavenge residual soil mineral N, and unique biochemical properties made these species to gain attention as cover crops in temperate agricultural regions (Jahanzad et al., 2016).

Some species in this group have long taproots (such as turnip, radish, rapeseed, and canola), and others have shallow fibrous roots (such as cabbage, cauliflower species). Tap roots penetrate deep into the soil and scavenge nitrate from deeper soil horizons, whereas fibrous root take up N

from the top soil horizons. N uptake by brassica cover crops ranges from 50 to 100 kg N ha⁻¹ (Gieske et al., 2016). C:N ratios of brassicas are low (9-14 in autumn), that promotes rapid decomposition and nitrogen mineralization. Some non-persistent brassica species (like white mustard and fodder radish) die during the winter due to cold environment, allowing much of that nitrogen to be lost through leaching, and their residues become recalcitrant, with higher C:N and greater lignin content. On the other hand, hardy brassicas like winter rape maintain a low C:N (~9 in spring) and release N more steadily in spring (G. Chen et al., 2007; Gieske et al., 2016; Thorup-Kristensen, 1994).

D) Multi-species or Mixtures

Although monoculture cover crops provide important agronomic benefits, multi-species mixtures have attracted attention for their ability to combine complementary functional traits and potentially enhance overall ecosystem services (Chapagain, Lee, & Raizada, 2020; Finney, White, & Kaye, 2016). A multi-species mixture is a combination of grasses (for biomass and erosion control), legumes (for N fixation, low C:N ratio, and rapid decomposition), and brassicas (for biofumigation, fast growth, and subsoil exploration) in different proportions to achieve all desired benefits (G. Chen et al., 2007; Finney et al., 2016; Snapp et al., 2005; Stavi, Lal, Jones, & Reeder, 2012). Several studies have reported that cover crop mixtures provide positive agronomic and economic benefits. The literature reports that legume-cereal or brassica-cereal mixtures produce large amounts of high-quality residues across a wide range of niches (Snapp et al., 2005). Decomposition of cover crop mixture residues has increased microbial biomass and functional diversity when compared to monocultures (Drost, Rutgers, Wouterse, De Boer, & Bodelier, 2020). A combination of legume and non-legume cover crops also improved SOC concentration (Adetunji et al., 2020). Couédel, Alletto, and Justes (2018); Couédel, Alletto, Kirkegaard, and Justes (2018) reported that crucifer-legume mixtures provided multiple ecosystem services, including pest control and higher biomass.

Some studies have found limitations or no benefits of growing the cover crop mixtures. Studies reported that multi-species cover crop mixtures did not produce a higher amount of biomass than monocultures. However, they may provide greater protection against adverse weather conditions or disease than monoculture species. Finney et al. (2016) discovered that while mixtures improved weed control and decreased nitrate leaching, they had no effect on corn crop yields. Some monocultures outperformed the mixtures in terms of overall functionality. R. G. Smith, Atwood, and Warren (2014) found that cover crop mixtures increased the yields but did not improve biomass stability, weed suppression, or subsequent oat (*Avena sativa* L.) yield. The C:N ratio of the cover crop mixtures depends on the proportional contributions of the individual species. If the mixtures contain a greater proportion of legumes, then they exhibit lower C:N ratios, leading to faster residue breakdown and N mineralization. If the mixtures have a higher proportion of grasses, then they have higher C:N ratios, thereby slowing down decomposition and improving N immobilization (Finney et al., 2016). Overall, parameters like study duration, species composition, and functional complementarity affect the performance of cover crop mixtures.

2.1.3 Benefits of Cover Crops

Integration of cover crops into cash crop production cycles will help in moving from single-crop benefits to multiple-crop benefits (Schipanski et al., 2014). Cover crops provide numerous ecosystem services such as nutrient cycling, soil fertility, pollination, climate, water regulation, including erosion control, enhanced water infiltration, reduced runoff, improved nutrient retention, and increased SOM, productivity (Battany & Grismer, 2000; Lundgren & Fergen, 2011; Ryan, Mirsky, Mortensen, Teasdale, & Curran, 2011; Schipanski & Drinkwater, 2011; Steenwerth & Belina, 2008; Tonitto, David, & Drinkwater, 2006). Overall, cover crops enhance the soil quality by improving all the physical, chemical, and biological properties (Dabney, Delgado, & Reeves, 2001).

Soil Physical Benefits

Cover crops improve the soil physical properties through the addition of above- and belowground biomass and providing soil cover through vegetation and residues. CCs improve SOM, water infiltration, soil aggregation, macropore formation through root growth (D. W. Reeves, 2018) and thereby reduce evaporation, moisture loss (Bruce, Hendrix, & Langdale, 1991; Haruna et al., 2020; D. W. Reeves, 2018).

Apart from the above mentioned physical benefits, CCs also protect the soil from being eroded. During rainfall, cover crops provide protection through their living canopies and as surface mulch after termination (Dabney et al., 2001). Living canopies intercept raindrops and reduce their kinetic energy, in turn reducing surface sealing and runoff. After termination, residues act as mulch, protecting the surface soil from erosion (Haruna et al., 2020). Additional physical benefits that cover crop provides include improved aggregate stability, regulation of soil temperature, and mitigation of soil compaction (Dabney et al., 2001).

Soil Chemical Benefits

Cover crops provide organic inputs either directly through above- and below-ground biomass or indirectly by stimulating soil microbial activity and increasing the SOM (Steenwerth & Belina, 2008). SOM is the key factor in soil chemical changes. Ding et al. (2006) and Corvasce et al. (2006a) reported that SOM is a crucial soil-reactive, ubiquitous component that is formed through processes called decomposition and humification. These processes are driven by microorganisms acting on plant residues, forming SOM, which thereby enhances soil fertility, productivity, and physical structure.

SOM represents the overall carbon pool in soil, which includes both stable and labile fractions (Kalbitz & Kaiser, 2008). SOC is a major constituent of SOM and is mainly derived from the cash crop and cover crop organic inputs (Reicosky & Forcella, 1998). SOC is one of the most important properties used to examine the effects of CCs on soil fertility (Adetunji et al., 2020). Studies have found that CCs increase SOC and thereby provide soil chemical benefits (Stavi et al., 2012).

Another study found that cover crops such as hairy vetch, crimson clover, pea, turnip (Brassica), oats, and rye significantly enhanced SOC concentrations compared to fallow plots (Dabney et al., 2001; Olson, Ebelhar, & Lang, 2014; Sainju, Singh, & Whitehead, 2002). SOC build-up depends on many factors, including location, climate, cover crop type, biomass quantity, materials applied before termination, initial soil C content, soil type, and tillage management (Blanco-Canqui et al., 2015).

Beyond C inputs, cover crops also serve as essential sources of macronutrients (such as N) and micronutrients (such as Fe and Zn). A few examples include Dube, Mutanga, Sibanda, Seutloali, and Shoko (2017) reported that residues from winter oats and grazing vetch increased P concentration in surface soil and SOM in the Eastern Cape of South Africa. Increase in SOM improves soil cation exchange capacity (CEC) and the soil's ability to retain macronutrients such as calcium, potassium, and magnesium (Newman, Wright, Mackowiak, Scholberg, & Cherr, 2007). Other studies have directly quantified micronutrient dynamics, proving that winter cover crops can influence soil zinc and copper pools, warranting further investigation (Dube et al., 2017). Beyond this, cover crops also regulate C:N ratios and nutrient cycles (such as the carbon and nitrogen cycles), thereby influencing soil chemical properties (Adetunji et al., 2020).

Soil Biological Benefits

Cover crops provide above- and belowground organic inputs that serve as energy and substrates for microorganisms and thereby increase microbial growth, activity, and community diversity (Kaleem Abbasi, Mahmood Tahir, Sabir, & Khurshid, 2015; Mullen, Melhorn, Tyler, & Duck, 1998; Murungu, Chiduza, Muchaonyerwa, & Mkeni, 2011). Improvements in the soil's physical and chemical properties also lead to increased microbial activity and stability (Mullen et al., 1998).

Few studies have found that cover crop residue and root exudates increase microbial biomass and functional diversity, thereby enhancing biological properties. Studies reported by Blanco-Canqui et al. (2015), Kabir and Koide (2000) and Mukumbareza, Muchaonyerwa, and Chiduza (2015) state that cover crops such as crimson clover, pea, wheat, and vetch improve microbial

biomass carbon (MBC), arbuscular mycorrhizal fungi abundance, bacterial populations, and soil enzyme activities relative to fallow soils, across diverse environments and management systems. Buyer, Teasdale, Roberts, Zasada, and Maul (2010) reported greater soil microbial biomass under rye-vetch cover crops, with rye-based treatments exhibiting higher microbial functional diversity than other treatments. However, the magnitude of these biological response strongly depends on the characteristics of the residue, particularly its quality and quantity.

Cover crop mixtures promote greater soil microbial diversity than single-species cover crops or monocultures (Drost et al., 2020). Legumes and non-legume cover crop mixtures have improved microbial activity (Adetunji et al., 2020). These findings indicate that microbial responses to cover cropping depend strongly on species composition, residue characteristics, and biomass inputs, rather than on cover crop presence alone.

Other biological benefits that cover crops provide are enhancing the soil faunal activity, especially earthworm populations, which improve soil structure through burrowing and cast formation, and weed suppression through competitive interactions or species-specific allelopathic effects (Sturm et al., 2018). Cover crops also increase soil enzymatic activities, such as β -glucosidase, phosphatase, and urease, thereby enhancing nutrient cycles of carbon, phosphorus, and nitrogen (Mbuthia et al., 2015).

2.1.4 Cover Crops Adoption in U.S. Agriculture

Cover cropping is a sustainable agricultural practice whose adoption has increased over the past decades; however, overall adoption remains low, increasing from 3.4% in 2012 to only 5.1% of total U.S. cropland in 2017 (Baumgart-Getz, Prokopy, & Floress, 2012; Dessart, Barreiro-Hurlé, & Van Bavel, 2019; Wallander, Smith, Bowman, & Claassen, 2021). Even though it has gained attention as the best sustainable practice, its adoption rate remained low (Basche & Roesch-McNally, 2017; Martins, Rejesus, Reberg-Horton, & Myers, 2021; Myers & Watts, 2015).

Cover crop adoption rates across different parts of the U.S. depend on many factors. A study in the Midwest region of the U.S. by Popovici et al. (2023) demonstrated that in Iowa, Illinois,

and Indiana, cover crop adoption among adjacent counties differed mainly due to social and institutional factors such as farmer perceptions, conservation agency involvement, local technical expertise, and public-private partnerships, rather than climatic factors. In Western regions of the U.S., cover crop adoption was based on farm characteristics, such as larger farms are more likely to use cover crops, and farms with higher income are less likely to adopt cover crops. Cost and limited knowledge are identified as barriers to the adoption in these regions (Golden et al., 2023). In the northeastern U.S., regions such as Maryland, Pennsylvania, New York, and Vermont, an incentive-based program played a crucial role in increasing the adoption of cover crops (Chami et al., 2023). In the Southern Great Plains, semi-arid conditions and water limitations were the main factors that prevented farmers from adopting cover crops (Ghimire et al., 2018). Despite these barriers, the adoption rate has reached approximately 11% of farms in this region (Jamar et al., 2025; T. Wang, Xu, Kolady, Ulrich-Schad, & Clay, 2021).

Overall, the 2017 Census of Agriculture indicated that less than 13% of cropland farms nationwide used cover crops (Conservation Technology Information Center (CTIC), 2017; U.S. Department of Agriculture, National Agricultural Statistics Service (USDA-NASS), 2019). Lee and McCann (2019) stated that, although cover crop use has increased over time, the overall adoption rate remained low.

2.1.5 Cover Crop Interactions with Cash Crops or Benefits of Cash Crops

Cover crops benefit cash crops by improving soil structure, nutrient availability, and biological activity, thereby enhancing crop productivity. But the yield magnitude depends upon the type of cover crop and cash crop species type, their management, and termination time (Fageria et al., 2005). Cover crops serve as a nutrient management tool, regulating nutrient supply and intake (Ruffo & Bollero, 2003). Leguminous cover crop provides the N for subsequent cash crop and reduces the use of inorganic N fertilizers (U. Singh, Sainju, et al., 2004). Along with N supply, they can also enhance phosphorus (P) uptake (Cavigelli & Thien, 2003). Grass CCs can also reduce nitrate leaching (Meisinger & Vough, 1991).

Cover crops also benefit the cash crop by regulating pest, insect, and weed populations. Cover crops provide favorable conditions for the growth of beneficial insects and predators, which, in turn, act on harmful insect pests and reduce their populations (Network, 1998). However, sometimes they can increase pest population also. Cover crops also suppress weeds directly by competing for resources such as sunlight, water, and nutrients, or indirectly through allelopathic effects (Hoffman, Smeada, & Moyer, 1996; Moyer et al., 2000).

Cover crops benefit subsequent cash crops by altering residue quality and regulating soil nitrogen availability. Residues with wide C:N ratios (small-grain cover crops) can temporarily immobilize soil nitrogen. This immobilization is more pronounced under no-tillage systems that require careful nitrogen management (Aulakh, Doran, Smith, Somda, & Torbert, 1991; Doran & Smith, 1991; Somda et al., 1991). In contrast, cover crop residues with narrower C:N ratios (legume) promote rapid nitrogen mineralization and increased nitrogen availability to succeeding cash crops (Torbert & Reeves, 1991).

2.1.6 Long-term (≥ 7 Years) Effects of Cover Crop Management Practice

Improvements in the soil's physical, chemical, and biological properties are reported under long-term cover crop management. Some soil indicators require several years of management before measurable changes occur, whereas some other properties respond more rapidly. But their response magnitude strongly depends on cover crop species, management practices, soil texture, and climate (Blanco-Canqui & Jasa, 2019; Mbutia et al., 2015). Some responses occur within short periods (approximately 1-5 years), whereas changes in more stable properties, such as soil organic carbon, takes long time (≥ 10 years) of continuous cover crop management, depending on site conditions and management practices (Poeplau & Don, 2015; Wood & Bowman, 2021).

Several field studies have shown that long-term cover cropping improved soil properties. For Example, Blanco-Canqui et al. (2015) reported that long-term cover cropping increased water-stable macroaggregate formation, reduced soil bulk density, and enhanced infiltration rates. Similarly, Simon, Obour, Holman, and Roozeboom (2022) observed improvement in soil structure in a

no-till wheat-sorghum system in ≥ 10 years of cover crop land compared with fallow. Poeplau and Don (2015) demonstrated that SOC sequestration under cover crops is greater and becomes more consistent in the long term. Jiménez-González et al. (2023) reported increased SOC stocks and improved SOM quality after ten years of cover crop management in a semi-arid region. A global meta-analysis found that the average SOC increase is about 15.5% and the sequestration rate is about $0.56 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$, indicating that detectable SOC gains occur over decadal timescales (Jian, Du, Reiter, & Stewart, 2020). Long-term cover cropping enhanced microbial biomass, enzyme activity, nutrient cycling efficiency, and aggregation (Mbuthia et al., 2015). A review by Salisu, Ampim, Oyebamiji, Kotochi, and Imoro (2025) also concluded that long-term cover cropping enhanced microbial-mediated soil processes and overall soil quality indices relative to short-term trials. These studies provide evidence that long-term cover cropping improves soil physical, chemical, and biological properties, thereby enhancing overall soil quality.

In addition, a few other studies found that long-term cover cropping produced limited or no significant differences in soil physical, chemical, or biological properties. They indicate that SOC, bulk density, and aggregation do not consistently respond to long-term cover cropping, particularly in coarse-textured, semi-arid, or water-limited environments (Acharya, Ghimire, & Acosta-Martínez, 2024; Blanco-Canqui et al., 2015; Mbuthia et al., 2015).

2.1.7 Environmental & Site-Specific Influences on Cover Crop Residue Decomposition

Cover crop residue decomposition depends on factors such as climate, soil texture, and residue quality. According to Quemada and Cabrera (1997); Stott, Elliott, Papendick, and Campbell (1986); Thapa et al. (2022), climatic factors influence the residue decomposition rate, with higher rates observed under high moisture and temperature conditions. Soil texture also affects residue decomposition rate, with decomposition occurring more quickly in sandy soils with frequent rainfall (Thapa et al., 2021). Residue quality, such as the C:N ratio and the lignin-to-nitrogen ratio (L:N), also influences decomposition rate, along with the cover crop growth period and site. The lower C:N and L:N ratios result in faster decomposition, and vice versa (Thapa et al., 2022).

Soil texture and mineralogy are also major factors that influence decomposition and carbon stabilization of cover crop residues. Residues decompose more quickly in coarse-textured or sandy soils and retain the derived carbon as unstabilized particulate organic matter. In contrast, fine-textured soils rich in clay and silt retain higher carbon in mineral-associated organic matter (MAOM) due to their greater reactivity and sorption capacity (Kaštovská et al., 2024). Saggiar, Parshotam, Sparling, Hart, and Feltham (1997) also investigated the roles of soil texture and mineral composition in accelerating the decomposition of cover crop residues. A total of 12 to 25% carbon from residues has stabilized for a period of 5 years, particularly in clay-rich soils, compared to silt loam soils. A study by Angst et al. (2021); Saggiar et al. (1997) further demonstrated that soils rich in mineral sands and clay have a higher capacity to retain carbon from residues than soils dominated by clay. Longer carbon residence times have been observed in smectitic and amorphous clays, reflecting higher carbon fixation on mineral surfaces. Clay- and silt-rich soils promote greater retention of litter-derived carbon by enhancing microbial carbon-use efficiency and stabilizing microbial residues within aggregates and mineral-associated organic matter pools, whereas sand-rich soils experience higher carbon losses due to limited sorptive capacity and weaker organo-mineral interactions (Angst et al., 2021; Saggiar et al., 1997). Laboratory incubation experiments have established that soils rich in montmorillonite or oxide decompose carbon from litter and stabilize carbon from residues in MAOM, as compared to kaolinites and low-reactivating soils (Angst et al., 2021; Elias et al., 2024; M. Singh, Sarkar, Bolan, Ok, & Churchman, 2019). These studies have thus established that soil texture is a major factor influencing residue decomposition and carbon stabilization.

2.2 Soil Organic Matter and Carbon Pools

SOM is the organic fraction of the soil consisting of plant and animal residues at various stages of decomposition (Soil Science Society of America, 1997). It represents the end product of humification and decomposition processes, during which soil microorganisms transform plant and animal

inputs into organic compounds with varying degrees of chemical complexity and stability (Stevenson, 1986). The majority of SOM is obtained from plant-derived carbon through root exudation, root turnover, and aboveground residue decomposition. But the magnitude or contribution of SOM depends on the type of species, management practices, and ecosystem (Cotrufo et al., 2021, 2013).

SOM comprises a continuum of organic materials ranging from fresh plant debris to highly processed organic compounds. Plant debris is the organic material with a particulate size larger than 2000 μm , whereas organic residue is the fresh organic material with a size in the range of 53 μm to 2000 μm . These fractions include decomposing plant and animal tissues, as well as a wide range of biomolecules such as amino acids, carbohydrates, lipids, waxes, and organic acids, along with their intermediate breakdown products.

SOM is an important component of the C cycle, with 58% of it being SOC (Field & Raupach, 2004; Schlesinger, 1990). SOM is divided into pools to better understand their complex behavior. SOM is mostly present in the form of particulate organic matter (POM), mineral-associated organic matter (MAOM), and dissolved organic matter (DOM) (Lavallee, Soong, & Cotrufo, 2020). Among them, DOM is the smallest pool, and it represents only 1-2% of the total SOM (von Lützow et al., 2007). MAOM is operationally defined as organic material smaller than 20-63 μm , typically associated with clay and silt particles (Totsche et al., 2018). Particulate organic matter is referred to as non-water extractable organic material larger than 20-63 μm , lighter than 1.6-1.85 g cm^{-3} . Sometimes OM, which is heavier and larger than 20-63 μm , is called heavy POM or sand-sized SOM. This shows distinct behavior compared to light POM and MAOM (Soong et al., 2016).

DOM represents the most active and mobile fraction of SOM and is operationally defined as water extractable organic matter that passes through a 0.45 μm filter (Corvasce et al., 2006a; von Lützow et al., 2007). DOM originates from multiple sources, like plant leachates, microbial exudates, and SOM decomposition. Even though DOM accounts for a small proportion of total SOM, studies say that low-molecular-weight carbon inputs as DOM are efficient precursors of SOM formation (L. M. Lynch, Machmuller, Cotrufo, Paul, & Wallenstein, 2018; Strickland, Wickings, &

Bradford, 2012). The major sources of DOM in the surface soils are the decomposition of above-ground residues, whereas the subsoil source is the decomposition of root litter and root exudates (Cotrufo et al., 2013; Gmach, Cherubin, Kaiser, & Cerri, 2020).

WEOM represents a specific operational fraction of DOM and refers to the organic matter extracted under gentle conditions using ultra-pure water at ambient temperature and filtered through 0.45 μm pores (A. Zsolnay, 1996; Á. Zsolnay, 2003). The WEOM magnitude is thus generally larger than that of DOM (A. Zsolnay, 1996). It is labile (easily decomposable), mobile, biologically available, and an active portion of the dissolved carbon pool (Haney et al., 2018; Rinot et al., 2021). WEOM is a heterogeneous mixture containing soluble and nano-colloidal organic compounds that move freely in soil solution or are loosely adsorbed onto mineral surfaces (Á. Zsolnay, 2003). Even though WEOM accounts for only a small fraction of total SOM, it plays a very crucial role in biological turnover, pedogenesis, carbon stabilization, and nutrient cycling (Armenise et al., 2013; Christ & David, 1996; Marhuenda-Egea et al., 2007; Rinot et al., 2021).

WOEM is the reactive fraction of SOM and is highly sensitive to land-use and management practices (Chantigny, 2003; Haney et al., 2018; Rinot et al., 2021). Because most organic matter transformations occur in the water-soluble phase, WEOM responds rapidly to changes in organic inputs and microbial activity and can directly reflect ongoing SOM transformations (Caricasole, Provenzano, Hatcher, & Senesi, 2010; Said-Pullicino, Erriquens, & Gigliotti, 2007). It has been widely used as an indicator of organic matter transformation, stability, and maturity in systems such as composting and vermicomposting (Lv, Xing, Yang, Qi, & Lu, 2013; Marhuenda-Egea et al., 2007; Tang, Yu, Liu, Xu, & Shen, 2011). These characteristics make WEOM an “early-warning” indicator of management-induced changes in soil carbon status. DOM and WEOM act as crucial links between fresh organic inputs, such as plant residues and cover crops, and the formation of more stable SOM pools, particularly in mineral soils and subsoil horizons (Kalbitz & Kaiser, 2008).

2.3 Cover Crop Impacts on Soil Carbon Pools and Fractions

2.3.1 Transformation of Cover Crop Residues Through the Carbon Pools

The transformation of cover crop residues into stable SOC initiates with the biochemical fragmentation and depolymerization of inputs by soil microbes. This process converts complex biopolymers, such as cellulose and lignin, into a critical intermediate pool known as DOM. DOM is the water-soluble, low-molecular-weight monomers and biopolymers that exhibit high mobility and reactivity in the soil solution. These dissolved compounds are readily accessible to microbial communities and play a central role in the microbial carbon pump (MCP). Microbial assimilation of DOM and subsequent transformation into microbial biomass and necromass contribute to SOC formation. This significantly enhances the carbon use efficiency (CUE) of microbes, allowing a greater proportion of carbon to be incorporated into microbial necromass rather than lost to the atmosphere as CO_2 via respiration. Ultimately, these carbon inputs are partitioned into two distinct pools according to their stabilization mechanisms: POM and MAOM. POM consists of undecomposed plant and fungal fragments that are physically protected through occlusion within soil aggregates (oPOM). MAOM is formed when microbial necromass and DOM compounds bond chemically to silt and clay particles through physicochemical protection or mineral sorption (Cotrufo et al., 2013; Kallenbach et al., 2016; Moukanni, Brewer, Gaudin, & O'Geen, 2022).

2.3.2 Species-Specific Effects on Carbon Inputs

Cover crop species differ in both the magnitude and chemical composition of carbon inputs to soil. The amount of C supplied by cover crops is measured using aboveground and belowground biomass production and residue quality indices such as the C:N ratio, which together influence the quantity and fate of carbon entering SOM pools. Grass cover crops, such as cereal rye and oats, produce greater aboveground biomass, resulting in higher total carbon inputs (Mirsky, Curran, Mortensen, Ryan, & Shumway, 2011). Grasses have higher C:N ratios, greater concentrations of cellulose and hemicellulose, and higher lignin concentrations than legumes (Wagger et al., 1998).

Residue chemistry measurements conducted by Thapa et al. (2023) showed that grass cover crops contained lower proportions of labile carbohydrate fractions than legume residues.

Legume cover crops are characterized by a lower C:N ratio and produce less total biomass, thus contributing less carbon overall. However, their carbon inputs are lower in lignin, hemicellulose, and cellulose concentrations and higher in more labile carbohydrate structures, making them decompose faster and release nitrogen more quickly (Kocira et al., 2020; Thapa et al., 2023; Wagger et al., 1998). Brassica cover crops are fast-growing species that can produce greater biomass and are particularly effective at scavenging residual mineral nitrogen from the soil. Brassica residues contain sulfur-rich compounds called glucosinolates, along with carbohydrates, lipids, proteins, crude fiber, and ascorbic acid. The C:N ratio, root systems, and decomposition behavior of brassica residues vary widely across species and growth stages, thereby influencing their contribution to soil carbon dynamics (Dean & Weil, 2009; Gieske et al., 2016; Haramoto & Gallandt, 2004; Isse et al., 1999; G. Wang et al., 2009).

In multispecies mixtures, both the quantity and composition of carbon inputs are determined by the dominant functional group. Mixtures with more grass species tend to have more residue characteristics similar to those of grass monocultures, including higher biomass production and higher C:N ratios, whereas legume-dominated mixtures more closely resemble legume residue quality (Finney et al., 2016). These differences in residue quantity and chemistry regulate decomposition pathways and microbial processing, thereby influencing the partitioning of carbon into POC and its subsequent transformation into dissolved forms, including WEOM, which can be further stabilized as MAOM.

2.3.3 Depth Effects on SOM Dynamics

Cover crops affect vertical stratification of SOM through differences in carbon input distribution, biological activity, and stabilization processes. In surface soils, greater input amounts serve as a substrate, stimulating microbial activity. This promotes the accumulation of relatively labile carbon fractions, particularly POC, along with increased microbial biomass, enzyme activity, and

the formation of water-stable aggregates in surface soils, making them richer in SOM than subsoil. However, the extent to which this biologically driven stratification is expressed is strongly regulated by management practices. In no-till systems, limited mechanical disturbance preserves surface-enriched SOM and aggregate stability, whereas tillage redistributes residues, disrupts aggregates, and reduces SOM accumulation in the surface layer (Franzluebbers, 2002).

According to (Franzluebbers, 2002), SOM and microbial activity stratification are well-known characteristics of soils, particularly under no-till management. Stratification is an indicator of soil quality and refers to the vertical and spatial separation of soil properties. Franzluebbers (2002) demonstrated that SOM stratification ratios in no-till systems increased SOC levels and biologically mediated activities near the soil surface due to greater carbon inputs and reduced disturbance compared with the subsoil.

The study by Gibbs (2020) indicates that soil depth and temporal variability exert stronger influences on microbial activity and N content than cover crop species or mixtures do. Although cover crops stimulated biological activity at greater depths, these effects were not specific to the treatments, as cover crops and control plots produced similar responses.

Beyond management effects, soil texture and mineralogy additionally affect the SOC stratification. Clay-rich soils with more reactive minerals provide greater surface area for organo-mineral interactions, promoting the stabilization of microbially processed carbon as MAOM, particularly at subsurface depths. In contrast, coarse-textured sandy soils, characterized by lower clay content and fewer reactive mineral surfaces, rely more on particulate organic matter and aggregate protection for carbon retention, leading to weaker organo-mineral stabilization and greater temporal variability in SOC (Cotrufo et al., 2013; Lavalley et al., 2020; Six, Conant, Paul, & Paustian, 2002; Wagai, Kajiura, & Asano, 2020).

2.3.4 Roles and Interconnections

Carbon inputs from cover crops are processed through a continuum of soil carbon pools, from labile WEOM and microbial biomass to more stabilized forms such as POM and MAOM, with distinct mechanistic linkages (Table 2.1).

2.4 Water Extractable Organic Matter

2.4.1 Extraction

Previously, DOM was collected in situ using lysimeters or suction cups, which primarily collect organic matter from soil macropores, leaving micropores and failing to fully represent the total organic matter (A. Zsolnay, 1996). To avoid that limitation, laboratory-based extractions are used to quantify WEOM. These laboratory extractions include DOM present in soil macropores as well as organic compounds mobilized from mesopores, micropores, and weak mineral associations due to soil disturbance during extraction (Chantigny, 2003). They have higher WEOM concentrations than in situ DOM, representing the total mobile and active soil organic matter.

Different extraction methods have been followed to obtain the WEOM. Those methods mainly differ in extractant composition, soil-to-solution ratio, agitation intensity, and temperature. Some studies used distilled or deionized water as an extractant to minimize chemical alterations in soil and capture environmentally relevant soluble organic matter (Strosser, 2010). Q. Chen et al. (2021) used distilled water for WEOM extraction, along with higher temperatures and vigorous shaking, to obtain soluble organic matter. They stated that these conditions may increase the release of intracellular and mineral-associated organic compounds from soil. Other studies used diluted salt solutions, such as CaCl_2 (concentrations ranging from 0.01 to 10 mM), to represent the soil solution ionic strength and to reduce soil colloids dispersion (Sun et al., 2017). These salt-based extractions are considered more conservative and better represent in situ soil solution chemistry.

Table 2.1: Roles of soil carbon pools and their mechanistic linkage to cover crop inputs

Pool	Role	Linking it to cover crops
WEOM (Water-extractable organic matter)	WEOM is a labile, active C pool that supplies energy to microorganisms and also helps in regulating the carbon (C) cycle (Y. Wang et al., 2021).	Cover crops provide residual carbon inputs that decompose, producing water-soluble compounds. Those compounds are referred to as WEOM (L. M. Lynch et al., 2018; Strickland et al., 2012) (Reicosky & Forcella, 1998).
Microbial biomass Carbon (MBC)	MBC is the living, labile component of soil organic matter (SOM) that regulates nutrient transformation and organic matter turnover. It contains 1-3% of total soil carbon and helps in carbon stabilization through microbially mediated processes (J. L. Smith & Paul, 1990).	Cover crop residues, after decomposition and the formation of WEOM compounds, serve as a substrate for microorganisms, thereby stimulating microbial activity and promoting microbial-derived C sequestration (Moukanni et al., 2022).
Microbial necromass	Microbial necromass is an extracellular compound released by microorganisms or by dead microbial cells/tissues. It is the major component of the SOM and the terrestrial C pool (Buckeridge, Creamer, & Whitaker, 2022).	After microbial biomass formation, cell death and lysis occur, along with hyphal fragmentation and the release of extracellular biopolymers (EPS), proteins, enzymes, polysaccharides, and DNA, leading to the formation of necromass. This necromass has a stronger affinity for mineral surfaces and forms MAOM. It also leads to stable soil organic carbon (SOC) storage (7% of total SOC) (Moukanni et al., 2022)
POM (Particulate organic matter)	POM is referred to as non-water extractable organic material composed of partially decomposed plant structures. It is a labile SOC pool that acts as a precursor to stable SOC fractions (Moukanni et al., 2022).	Cover crops decompose via fragmentation and depolymerization, forming POM. This POM pool contains C, either in free form or occluded within aggregates, forming micro- and macroaggregates. These aggregates provide the physical protection to SOC (Moukanni et al., 2022).
MAOM (Mineral-associated organic matter)	Formation of MAOM occurs when plant-origin C compounds (such as dissolved organic compounds) and microbial metabolites are stabilized by sorption to clay and silt mineral surfaces (Lavalley et al., 2020; Moukanni et al., 2022).	Through continuous residue inputs and rhizodeposition, cover crops stimulate microbial processing of plant-derived carbon, increasing the production of dissolved organic compounds and microbial metabolites that are subsequently stabilized on mineral surfaces as MAOM, thereby resulting in long-term SOC storage (Cotrufo et al., 2013).

Previous studies have reported that the extraction method influences both the quantity and composition of WEOM, but the magnitude of the difference is smaller. Guigue et al. (2014) compared three WEOM extraction techniques - water extraction, column leaching, and pressurized hot-water extraction. They found that soil characteristics had a stronger influence on WEOM quality than the extraction procedure. The pressurized hot-water extraction showed higher C concentration and mobilized organic compounds of lower aromaticity and greater stability, while differences in fluorescence composition and biodegradability among extraction methods were modest. These findings indicate that although extraction procedures may alter WEOM composition to some extent, soil properties remain the dominant control on WEOM characteristics (Strosser, 2010).

2.4.2 Chemical Composition of WEOM

According to the literature, water-extractable organic matter (WEOM) originates from multiple sources, including plant litter and crop residues, green manures, root exudates, manure inputs, and the decomposition of SOM (Kalbitz, Solinger, Park, Michalzik, & Matzner, 2000). The chemical composition of WEOM is influenced by soil type and its physicochemical properties, ecosystem characteristics, management practices, and land-use history. Literature reports that the crop residues are the major source of WEOM in agricultural cropland systems (Chang, Gao, Zhou, & Cao, 2021). Among these inputs, green manure (a subset of cover crops) application has altered the WEOM composition and structure, making it more susceptible to microbial decomposition than any other source (P. Sharma et al., 2017c). The cultivation and incorporation of crop residues increases soil fertility and crop production, promotes carbon accumulation, and alters rhizosphere microbial communities, thereby influencing both the quantity and chemical composition of WEOM (Chang et al., 2021). Spectroscopic analyses have shown that WEOM contains carboxylic acids, alcohols, phenolic compounds, fatty acids, aliphatic and aromatic carbon structures, and amide-containing compounds (Chang et al., 2021). This wide range of WEOM composition is a result of different crop residues decomposition, microbial processing, and sorption of OM onto soil mineral

surfaces (Kalbitz et al., 2000). Hence, WEOM composition provides insights into sources of OM and transformation pathways.

From the previous studies, we can conclude that the WEOM composition varies depending on the origin of the organic material. Differences in aromaticity, hydrophobicity, humification degree, and molecular weight distribution have been observed among WEOM extracted from crop residues, municipal solid waste compost, pig slurry, sewage sludge, and biochar. These differences alter the availability of WEOM and the adsorption of organic compounds onto soil mineral surfaces. It has been reported that WEOM derived from green manure showed greater bioavailability than WEOM from pig manure or sewage sludge, due to differences in the molecular weight. Similarly, the adsorption properties of WEOM onto soil minerals are also influenced by their molecular weight (Chang et al., 2021; Gigliotti, Kaiser, Guggenberger, & Haumaier, 2002; Ohno, Chorover, Omoike, & Hunt, 2007; Ohno, Sleighter, & Hatcher, 2018; Zhou & Wong, 2000).

Different cover crop species and green manures influence the chemical characteristics of WEOM through differences in residue composition and decomposition behavior. A study conducted by Chang et al. (2021) reported that spectroscopic analysis of soils following Chinese milk vetch, radish, ryegrass, hairy vetch, february orchid, and rye crops, WEOM showed a wide range of functional groups, including carboxylic acids, phenolic compounds, aliphatic and aromatic carbon structures, fatty acids, and amide groups. Among those species, Chinese milk vetch WEOM had the highest water-extractable organic carbon (WEOC) concentrations and a greater abundance of carboxylic acid functional groups, whereas inorganic sulfate functional groups were detected only in radish due to the presence of glucosinolate compounds. Compositional indices like aromaticity index, humification index, fluorescence index, and FTIR absorbance ratios indicated that WEOM from Chinese milk vetch and hairy vetch was characterized by lower aromaticity and humification degree, and greater bioavailability (Chang et al., 2021). Plant-derived WEOM also contains lignin, which is more stable, and carbohydrate, N-rich compounds, which readily decompose (Kalbitz et al., 2000).

Beyond differences in organic inputs, WEOM composition is regulated by several soil management factors (e.g., tillage, fertilization, liming, plant species, and field crops), land use (forest, grassland, and arable), and environmental factors (e.g., climate, landscape, and hydrology), including its stabilization. Stabilization of WEOM depends on its interaction with soil minerals, as sorption and co-precipitation processes involving aluminum and iron oxides that retain aromatic compounds. Hence, soil texture and mineralogy play a key role in controlling mineral interactions and thereby affect the persistence and vertical distribution of WEOM components (Kalbitz et al., 2000).

2.4.3 WEOM Quantity and Quality Analysis

WEOM is analysed based on its quantity and quality, which together determine its functional role in soil biogeochemical processes. WEOM quantity refers to the size of the extractable organic matter pool and is generally quantified as water-extractable organic carbon (WEOC) (Akagi & Zsolnay, 2008; Strosser, 2010). In some studies, water-extractable organic nitrogen (WEON) and the biodegradable fraction of WEOC (BWEOC) have also been measured to characterize the labile organic matter pool (Akagi & Zsolnay, 2008; Embacher, Zsolnay, Gattinger, & Munch, 2008). Whereas WEOM quality describes the chemical nature, degree of transformation, and decomposability of the extractable organic matter.

WEOM quantity is determined through carbon concentration measurements, WEOM quality is mostly assessed using optical techniques, including ultraviolet-visible (UV-Vis) absorption and fluorescence spectroscopy (Coble, 1996; Kalbitz et al., 2003; Á. Zsolnay, 2003). Spectroscopic indicators such as specific UV absorbance at 254 nm ($SUVA_{254}$) and 280 nm ($SUVA_{280}$) are widely used to determine aromaticity and hydrophobicity of WEOM. Absorbance ratios such as E3/E3 and E4/E6 provide information on molecular size and decomposition (He et al., 2017; Jaffrain, Gérard, Meyer, & Ranger, 2007). Fluorescence-based indices, such as the humification index (HIX) and Fluorescence index(FI), have been used to determine the degree of humification and the source of DOM, respectively. Biological indices derived from fluorescence emission ratios have been used to

indicate the contribution of autochthonous microbial activity (Cuss & Guéguen, 2015; M. Zhang, He, & Zhao, 2015). In addition to these indices, short-term incubation experiments have been used to quantify the biodegradable fraction of WEOM to assess its decomposability (Akagi & Zsolnay, 2008; McDowell et al., 2006).

Studies have shown that WEOM quantity and quality are influenced by the environmental and management practices. Seasonal variability exerts a stronger influence on WEOM concentrations (quantity) than on compositional attributes, whereas WEOM quality is more strongly controlled by soil type and site characteristics. Embacher et al. (2008) reported temporal variability in WEOM quality in soils, with higher absorptivity in Luvisols and higher humification in Chernozems, whereas the biodegradable WEOM fractions remained constant across soils. Statistical analyses also indicated that WEOM quality is primarily governed by site-specific soil properties, whereas quantity is more sensitive to seasonal dynamics (Embacher et al., 2008).

The quantity and quality of WEOM are also influenced by land use, soil management, and environmental conditions. Changes in land use, such as fertilization, application of organic and inorganic amendments, liming, and tillage practices, have all been shown to affect WEOM levels and composition, although responses are not always consistent across studies (Chantigny, 2003; M. Zhang et al., 2011; Zhao, Zhang, & He, 2013). Environmental factors such as soil moisture, temperature, and wetting-drying or freeze-thaw cycles also regulate WEOM dynamics (Chow, Tanji, Gao, & Dahlgren, 2006). Hence, WEOM quality, which is reflected in parameters such as aromaticity, humification, and soluble organic carbon, has frequently been used in the literature as a sensitive indicator of soil organic matter dynamics rather than WEOM concentration (Embacher et al., 2008; M. Zhang et al., 2011). Overall, assessing both the quantity and quality of WEOM provides a more detailed understanding of soil organic matter behavior.

2.4.4 Key Roles of WEOM

Water-extractable organic matter (WEOM) plays multiple process-based roles in soil systems, linking carbon and nutrient cycling processes across solid and solution phases. A summary of the key roles of WEOM is presented in Table 2.2.

2.4.5 Sensitivity of WEOM to Land Use and Management Practice

Land use and management practices alter the soil's physical, chemical, and biological properties, which in turn affect the quantity and composition of DOM and WEOM. The literature indicates that land use is the primary long-term driver of DOM/WEOM quantity and composition by controlling vegetation type and organic matter inputs, while management practices and environmental factors contribute to these effects and to short-term variability (Chantigny, 2003).

Mant studies utilized UV-Vis and fluorescence spectroscopy demonstrate that land-use and specific management practices-including amendment history and soil mineralogy, exert a more dominant control over WEOM quality than simple ecosystem classification (Rinot et al., 2021). Furthermore, the application of mid-IR regressions has refined our ability to predict the molecular attributes and functional group distribution within aqueous soil extracts across varying agricultural intensities (Nasonova, Olshansky, Siebecker, Polubesova, & Chefetz, 2022; Rotbart, Rinot, Olshansky, et al., 2025). Consequently, the distinction between labile and persistent compounds is increasingly understood through high-resolution spectral fingerprints rather than traditional humic-fraction categories. In the long term, WEOM concentrations reflect the size of the SOM pool, suggesting that WEOM production is controlled by long-term organic inputs and vegetation rather than short-term environmental fluctuations (Chantigny, 2003; Kalbitz et al., 2000; A. Zsolnay, 1996). Changes in land use, such as shifts among forest, grassland, and arable systems, influence WEOM quantity and composition by altering litter quality, root inputs, microbial activity, and organomineral interactions. Despite this, the factors controlling land-use-induced changes in WEOM remain incompletely understood, as multiple soil and environmental factors often interact simultaneously under field conditions.

Table 2.2: Key process-based roles of water-extractable organic matter (WEOM) in soil systems

Biogeochemical Function	Role of WEOM	Key References
Link between solid and solution phases	Represents soil organic matter released from the solid phase into the soil solution, where it becomes available for transport, microbial processing, mineralization, or re-stabilization.	(A. Zsolnay, 1996).
Energy source	Supplies labile organic carbon, which acts as substrate for microbial metabolism, driving carbon turnover, CO ₂ production, microbial mineralization, and also contributes to stable mineral-associated organic matter pools.	(Q. Chen et al., 2021; Smolander & Kitunen, 2002)
Nutrient flux (organic N)	Transports WEON (Water Extractable Organic Nitrogen) and influences nitrogen availability and microbial nutrient cycling.	(Q. Chen et al., 2021)
Mineral stabilization and Vertical transport	Sorbs to soil minerals and co-precipitates with Fe and Al oxides, leading to selective retention and stabilization of aromatic and humified compounds. Also facilitates downward movement of DOC and other nutrients, contributing to subsoil carbon inputs.	(Kalbitz et al., 2000)
Pollutant transport	Forms complexes with heavy metals and organic pollutants, influencing their mobility, availability, and transport within soils.	(Kalbitz et al., 1997; Marschner, 1999; Temminghoff, Van Der Zee, & De Haan, 1997)
Mineral weathering	Enhances mineral dissolution through organic ligand interactions, promoting the release of plant-available nutrients.	(Raulund-Rasmussen, Borggaard, Hansen, & Olsson, 1998)
Soil formation	Helps in the pedogenic processes through organomineral interactions and long-term chemical transformations.	(Dawson, Ugolini, Hrudfiord, & Zachara, 1978; Petersen, 1976)
Indicator of soil quality	Responds rapidly to changes in land use and management, making WEOM a sensitive proxy for shifts in soil biogeochemical functioning.	(Embacher, Zsolnay, Gattinger, & Munch, 2007; Kalbitz et al., 2000)

Management practices highly influence WEOM quantity and composition, as it represents the most labile, active fraction of soil organic matter. Crop rotation, a management practice, has become a major source of WEOM sensitivity in agricultural soils. Rotations with perennial legumes increased WEOC and WEON concentrations more than monoculture annual cropping (Barber & Martin, 1976; Chantigny, 2003; Xu, Wilson, Saiers, & Entz, 2013). Management practices like crop rotation, cover cropping, and green manures alter residue quantity, quality, and root exudation patterns across different crop species. These changes promote microbial activity and accelerate organic matter turnover, thus changing both the quantity and composition of WEOM (Barber & Martin, 1976; Chantigny, 2003; Xu et al., 2013).

WEOM dynamics are also influenced by nutrient management, but the results are inconsistent and site-specific. Application of organic amendments (such as manure or poultry litter) increased WEOC and WEON due to direct input of soluble organic compounds, whereas mineral fertilization may increase, decrease, or have no effect on WEOM depending on soil conditions, microbial nutrient demand, and background organic matter status (Chantigny, 2003; M. Zhang et al., 2011). Other management factors, such as liming, fertilization, and tillage, are secondary controls that affect WEOM concentrations and composition (Andersson, Valeur, & Nilsson, 1994; Chantigny, 2003; Leinweber, Schulten, & Körschens, 1995; McDowell, Currie, Aber, & Yano, 1998; Rochette & Gregorich, 1998; Simard, Evans, & Bates, 1988; A. Zsolnay & Görlitz, 1994). Spectroscopic analysis indicated that management factors alter WEOM composition by altering the values of the aromatic index, humification index, and the humic acid, fulvic acid, and protein-like fractions (Sun et al., 2017; M. Zhang et al., 2011).

Although studies indicate that management practices affect WEOM, the mechanisms underlying these effects are poorly understood. Most of the literature cannot trace whether the observed changes result from increased or decreased WEOM production, microbial consumption, immobilization through mineral complexes, or leaching to lower soil depths. The varying techniques used across studies, such as different extractants, extraction extents, and sample collection methods, complicate interscientific comparisons of WEOM solutions (Chantigny, 2003). The WEOM

content, therefore, is a sensitive indicator of management change; its response is highly dependent on interactions among soil, vegetation, and environmental controls, masked by intricate soil-plant-environment interactions.

2.4.6 Influence of Soil Depth and Environmental Factors on WEOM Quantity and Composition

A strong vertical differentiation is seen in WEOM quantity and composition within soil profiles. It is due to the shifts in organic matter sources, microbial processing, and mineral interactions with increasing depth (Corvasce et al., 2006a; Kalbitz et al., 2000). Surface soils are rich in WEOM, which is derived from recent plant inputs, and they contain higher proportions of aromatic and hydrophobic compounds associated with lignin degradation products (Cannavo, Dudal, Boudenne, & Lafolie, 2004; Corvasce et al., 2006a; Hassouna, Massiani, Dudal, Pech, & Theraulaz, 2010a). In contrast, deeper soils are characterized by WEOM, which is enriched in hydrophilic, aliphatic, and more humified structures (Cannavo et al., 2004; Corvasce et al., 2006a; Dudal, Sévenier, Dupont, & Guillon, 2005; Hassouna et al., 2010a). Cannavo et al. (2004) reported that WEOM in deep calcareous soils contained high levels of recalcitrant hydrophilic structures, either produced in situ or transported downward from surface layers with minimal changes, while Dudal et al. (2005) suggested that the preservation of these structures leads to relatively homogeneous complexation properties throughout the soil profile. Spectroscopic analyses showed that WEOM aromaticity and humification indices decreased with depth, whereas fluorescence intensity increased, indicating transport of structurally simple molecules and selective retention of complex aromatic compounds by minerals (Corvasce et al., 2006a). In deeper soil horizons, WEOM shows a tendency to interact more strongly with clay minerals, whereas in surface soil it is less strongly controlled by soil texture (Corvasce et al., 2006a). Although subsoil WEOM tends to be more persistent, several studies report that its biodegradability is similar to that of surface WEOM. Thus, persistence at depth is controlled by mineral protection and low microbial activity, rather than by inherent chemical recalcitrance (Fontaine et al., 2007; M. W. I. Schmidt et al., 2011; Toosi, Clinton, Beare, & Norton, 2012).

Environmental conditions regulate WEOM production, mineralization, transport, and stabilization through interactions among biological, hydrological, and geochemical processes (Boyer & Groffman, 1996; Christ & David, 1996; K. Kaiser, Guggenberger, Haumaier, & Zech, n.d.; Tipping et al., 1999). The lack of correlation of WEOM properties and climate factors has been observed in field studies, likely due to the simultaneous operation of multiple counteracting processes (Kalbitz et al., 2000; Mertens et al., 2007). WEOM dynamics are controlled by hydrological processes that regulate vertical transport and residence time, whereas biological processes play a secondary role over longer time scales (Kalbitz et al., 2000). Adsorption to mineral surfaces is the primary mechanism controlling WEOM retention in mineral subsoils, while the contribution of microbial degradation of both soluble and mineral-bound WEOM is likely underappreciated (Kalbitz et al., 2000). Field studies show that temporal variability in WEOM is seen in surface soils and is closely linked to rainfall events and changes in soil moisture, whereas deeper horizons exhibit strong temporal stability due to limited hydraulic flow and effective organo-mineral interactions (Embacher et al., 2008). Soil texture and drainage further influence WEOM behavior, with poorly drained clay soils promoting organic matter accumulation and depth-specific WEOM composition that can persist for decades following land-use change (Laganière, Angers, & Paré, 2010; Weller, Browne, Hosen, & Febria, 2026). Overall, environmental controls affect WEOM indirectly by modulating the balance among production, transport, stabilization, and microbial accessibility rather than by imposing uniform effects across soil profiles (Kalbitz et al., 2000).

2.5 Spectroscopic Tools for WEOM Characterization

2.5.1 Significance of Spectroscopy in WEOM analysis

Spectroscopic analysis is a scientific field that provides information about the structure, quantity, and composition of compounds by measuring interactions between matter and electromagnetic radiation. Different spectroscopic types are present, each of which has particular significance.

They are infrared spectroscopy, ultraviolet-visible spectroscopy (UV-Vis), X-ray spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry, raman spectroscopy, fluorescence spectroscopy, and atomic emission spectroscopy. Identifying and quantifying compounds/molecules is the most common use of spectroscopy in soil chemistry and environmental chemistry (A. Sharma, Naz, Singh, & Tripathi, 2021).

Water-extractable organic matter (WEOM) is a chemically complex and heterogeneous fraction of soil organic matter. Many studies have revealed that spectroscopic approaches are powerful tools for characterizing soil WEOM in terms of both quantity and quality (Bünemann et al., 2018; Fellman et al., 2008; Jones et al., 2014; M. P. Schmidt & Martínez, 2019). Conventional extraction-based methods quantify the amount of organic matter recovered but do not show the differences in chemical composition or lability. Spectroscopic techniques like excitation-emission matrix (EEM) fluorescence analysis, infrared spectroscopy, and UV-Vis, provide detailed characterization of specific components within WEOM and molecular composition insights that cannot be obtained from bulk measurements alone. Hence, spectroscopy improves the detection of chemical composition of labile organic matter pools and enhances the interpretation of biogeochemical processes through WEOM composition (Griffin, 2008; Li & Hur, 2017b; Rinot et al., 2018; Ros et al., 2011).

Over the past decades, spectroscopy has been widely used in DOM analysis to broaden our understanding of DOM by bridging gaps across different research areas. These techniques are widely used, rapid, sensitive, low-cost, and nondestructive. Also, UV-Vis absorption spectra and fluorescence spectroscopy provide one and three-dimensional data (i.e., excitation, emission, and intensity) for easy understanding. These advantages made spectroscopic methods widely popular for DOM studies (Li & Hur, 2017b).

2.5.2 UV-Vis Spectroscopy

UV-visible spectroscopy accounts for the ultraviolet (UV) and visible regions of the electromagnetic spectrum. UV light ranges from 200 to 400 nanometers, while visible light ranges from 400

to 700 nanometers. Different spectral features are produced when molecules absorb UV or visible light through electronic transitions. UV-Vis spectroscopy is based on the Beer-Lambert law, expressed as

$$A = \varepsilon cl, \quad (2.1)$$

where A is absorbance, ε is the molar absorptivity, c is the concentration of the absorbing species, and l is the optical path length. This law helps quantify a sample's concentration by measuring its light absorption (A. Sharma et al., 2021).

UV-Vis spectroscopy is widely used to indirectly analyze the aromaticity, molecular weight, and humification degree of soil DOM (Weishaar et al., 2003). Water-extractable organic matter is commonly characterized using UV-Vis absorbance metrics, either as absorbance at a single wavelength (Kukkonen, 1992; Pennanen, 1982; Pettersson, Ephraim, & Allard, 1994) or as ratios between two wavelengths to qualitatively describe the curvature of the absorption spectrum (Chin, Aiken, & O'Loughlin, 1994; Kukkonen, 1992; Stevenson, 1982). Among these indices, specific ultraviolet absorbance at 254 nm ($SUVA_{254}$) is widely used as a proxy for aromatic carbon content. $SUVA_{254}$ represents the absorbance at 254 nm normalized to dissolved organic carbon (DOC) concentration and is positively associated with the relative abundance of aromatic and humic-like compounds (Weishaar et al., 2003).

SUVA measured at different wavelengths provides insights into DOM properties. SUVA at 254 nm ($SUVA_{254}$), 260 nm ($SUVA_{260}$), and 280 nm ($SUVA_{280}$) indicates aromaticity, hydrophobicity, and molecular weight of DOM molecules, respectively (Chin et al., 1994; Dilling & Kaiser, 2002; Weishaar et al., 2003). Higher $SUVA_{254}$ values generally indicate more chemically complex and terrestrially derived WEOM, whereas lower values suggest a greater contribution of microbially derived or aliphatic organic matter.

$$SUVA_{254} (\text{L}, \text{mg}^{-1}, \text{m}^{-1}) = \frac{\text{Abs}_{254} (\text{cm}^{-1})}{\text{DOC} (\text{mg}, \text{L}^{-1})} \cdot 100 \text{ cm} \cdot \text{m}^{-1} \quad (2.2)$$

where Abs_{254} was obtained by dividing the optical density measured at 254 nm by the optical path length (1 cm).

Spectral absorption ratios, such as E2:E3 and E4:E6, are also used to characterize the chemical properties of DOM. The E2:E3 ratio is defined as the absorbance at 250 nm divided by that at 365 nm and is inversely related to the average molecular weight of DOM, with lower values indicating larger, more condensed organic molecules (Li & Hur, 2017b).

$$E2:E3 = \frac{Abs_{250}}{Abs_{365}} \quad (2.3)$$

A higher specific UV absorbance is associated with lower E2:E3 ratios in organic compounds such as humic and fulvic acids. This increase in specific absorbance, coupled with a decrease in absorption ratios, indicates that the compound is humified, more aromatic, and of increasing molecular weight.

E4:E6 is most commonly defined as the absorbance ratio at 465 to 665 nm and has long been used by soil scientists to characterize humic and fulvic acids and, more broadly, soil organic matter (Y. Chen, Senesi, & Schnitzer, 1977).

$$E4:E6 = \frac{Abs_{465}}{Abs_{665}} \quad (2.4)$$

Early interpretations proposed the E4:E6 ratio as an indicator of aromatic condensation in humic substances (Kononova, 1966; Schnitzer & Khan, 1978). Subsequent studies demonstrated that E4:E6 is more strongly related to molecular weight than aromaticity alone, with lower ratios corresponding to larger and more condensed organic molecules (Y. Chen et al., 1977). Despite its long history of use, direct molecular-level evidence supporting E4:E6 as a definitive indicator of aromatic condensation remains limited, especially for fulvic acids, and interpreting this index requires caution. As a result, E4:E6 has received less emphasis in recent dissolved organic matter studies compared with other optical indices, particularly in systems with low DOC concentrations. Higher E4:E6 values are typically associated with low-molecular-weight, microbially derived, and

more labile DOM fractions, whereas lower E4:E6 values suggest high-molecular-weight, highly condensed, and more humified DOM components (Ukalska-Jaruga, Bejger, Debaene, & Smreczak, 2021).

The spectral slope (S) is sensitive to light radiation and can reflect the photodegradation process of DOM, and the spectral slope ratio (SR) can reflect the structure and composition of DOM (Hansen et al., 2016; Helms et al., 2008; F. Zhang et al., 2022). Spectral slope (S) describes how quickly absorbance decays as wavelength increases in a UV-Vis spectrum of dissolved organic matter (DOM). The spectral slope ratio (SR) compares short- and long-wavelength slopes to better diagnose DOM source and processing. The ratio of S₂₇₅₋₂₉₅ divided by S₃₅₀₋₄₀₀ spectral slope (S) quantifies how fast DOM absorbance decays with wavelength and reflects molecular size and aromaticity. Absorbance ratios are historically the earliest form of spectral slope (Helms et al., 2008; Korak & McKay, 2024). While UV-Vis absorption spectroscopy provides less detailed compositional information than fluorescence-based methods, it captures a larger proportion of the CDOM pool. This makes UV-Vis a widely applied and accessible tool for evaluating CDOM concentration, quality, and source-related reactivity (Li & Hur, 2017b).

2.5.3 Fluorescence Spectroscopy

Fluorescence spectroscopy is widely used to obtain information on fluorescent components and their amount in DOM. This helps to obtain both quantitative and qualitative data (Murphy et al., 2010). Fluorescence is obtained when a molecule absorbs energy and an electron is excited to a higher energy state. As the electron gets back to its ground state, part of the absorbed energy is released as emitted light. The specific excitation and emission wavelengths at which this light is produced indicate the characteristics of the molecule's chemical structure (Fellman, Miller, Cory, D'Amore, & White, 2009).

Excitation-emission matrix (EEM) fluorescence spectroscopy combines emission scans across multiple excitation wavelengths and provides information on the number, type, and relative abundance of fluorescent components in WEOM/DOM (Stedmon, Markager, & Bro, 2003). It is a

nondestructive method that reveals the types of components in the DOM, including humic-like, fulvic-like and proteinaceous substances (Fellman, Hood, & Spencer, 2010; Stedmon & Markager, 2005; Stedmon et al., 2003; Yamashita, Maie, Briceño, & Jaffé, 2010). Sometimes, the multiple fluorescent components in EEMs overlap, leading to poor interpretation and a lack of understanding of fluorescence. DOM (FDOM) and initially, multivariate techniques such as Principal Component Analysis (PCA) were used to analyze EEM data; however, this approach requires data unfolding, complicating the model. To address these issues, Parallel Factor Analysis (PARAFAC), a three-way decomposition method, is better suited to EEMs because it directly accounts for their three-dimensional structure and can eliminate overlap among fluorescence peaks (Cory & McKnight, 2005a; Fellman et al., 2009; Murphy et al., 2011; Stedmon et al., 2003). PARAFAC emerged as a powerful tool for resolving the underlying structures in multi-way datasets such as fluorescence EEMs (Murphy, Stedmon, Graeber, & Bro, 2013). PARAFAC identifies the individual fluorescent components of WEOM, such as humic-like and protein-like substances, which reflect the quality and sources of organic matter (Murphy et al., 2013; Stedmon & Markager, 2005). There are many studies that used the EEM + PARAFAC approach to identify the molecular composition of agricultural soil DOM/WEOM, as EEM fluorescence detects changes in soil WEOM that are induced by different types of soils and different types of irrigation water (Borisover, Lordian, & Levy, 2012), different cropping systems, organic amendments, and fertilization regimes (Ohno & Bro, 2006; Ohno, He, Tazisong, & Senwo, 2009; Rinot et al., 2018; P. Sharma et al., 2017a, 2017b) and soil depth (Corvasce et al., 2006a; Fellman et al., 2008; Hassouna, Massiani, Dudal, Pech, & Theraulaz, 2010b; Rinot et al., 2021; M. P. Schmidt & Martínez, 2019).

PARAFAC modeling generally involves five main steps: (1) importing raw EEM data, (2) preprocessing, (3) exploratory data analysis, (4) model validation, and (5) interpretation of results. Data are first transferred from the instrument to analytical software such as R. The preprocessing step involves data correction, elimination of non-trilinear data, and signal normalization. Exploratory analysis involves identifying outliers and developing preliminary models. Step 4 yields the final PARAFAC model, where the EEM dataset is decomposed into individual fluorescence

components defined by their excitation–emission loadings and intensities. These components represent mathematical fluorophores describing the underlying DOM structure. Subsequently, the components are interpreted by comparing their spectral characteristics with published literature and assigning them to groups such as humic-like or protein-like substances. This classification is a literature-based interpretation rather than a direct output of the PARAFAC model and tracks the fluorescence intensity at the maximum (“Fmax”) for each component. Fmax represents the maximum fluorescence intensity of an independent component and is calculated from its excitation and emission loadings and sample score, providing values on the same scale as the original EEMs. Fmax does not measure concentration, but it can be used to compare how the same component or component ratios change across samples (Murphy et al., 2013).

Fluorescence measurements are both simple and complex, as they require multiple steps to calibrate the instrument and obtain high-quality data. To ensure comparability across samples, instrument-specific corrections should be made to minimize spectral bias caused by optical components and excitation-source variability. Fluorescence intensities should be standardized using quinine sulfate or the Raman signal of pure water as reference standards (Stedmon & Bro, 2008; Stedmon et al., 2003). The limitation of this instrument is that it does not provide direct, complete information on the chemical structure/composition and concentrations of organic compounds in DOM, as only a small portion of total DOM is considered in the fluorescence measurement. Fluorescence characteristics change with changes in temperature (Baker, 2005), oxygen amount (Fulton et al., 2004), and pH levels (Mobed, Hemmingsen, Autry, & McGown, 1996). Measurements can be influenced by inner-filter effects, as samples are highly absorbed by inner filters, and by fluorescence quenching caused by metals such as iron (McKnight, Scott, Hrncir, & Lovley, 2001). Some other limitations include restrictions on excitation wavelength ranges (Hudson, Baker, & Reynolds, 2007), difficulties in analyzing or interpreting DOM fluorescence data (Fellman et al., 2009; Stedmon & Bro, 2008).

2.5.4 Coble Peaks and Fluorescence Indices

Coble peaks are commonly used to provide a rapid, qualitative interpretation of fluorescence EEM spectra and to classify major fluorescent fractions of DOM. Peak A (Ex ~260 nm, Em ~380–460 nm) and peak C (Ex ~350 nm, Em ~420–480 nm) represent humic-like fluorescence and are commonly observed in soil and aquatic DOM. In contrast, peak B (Ex ~275 nm, Em ~310 nm) and peak T (Ex ~275 nm, Em ~340 nm) correspond to tyrosine-like and tryptophan-like fluorescence, respectively, and are indicative of proteinaceous and microbially derived organic matter (Coble, 1996).

Literature indicates many indices that are derived from fluorescence spectroscopy, such as Quantum yield, Humification index, Biological index, Fluorescence index, and Specific fluorescence intensity. My thesis mainly focuses on two indices: the Humification index (HIX) and the Fluorescence index (FI). HIX is the ratio of the total fluorescence intensity at emission wavelengths 435–480 nm to that at 300–345 nm at an excitation wavelength of 254 nm. HIX reflects the degree of humification of dissolved or soil organic matter, with higher values indicating more humified, complex organic compounds (Ohno, 2002; A. Zsolnay, Baigar, Jimenez, Steinweg, & Saccomandi, 1999).

$$\text{HIX} = \frac{\sum_{\lambda=435}^{480} F(254, \lambda)}{\sum_{\lambda=300}^{345} F(254, \lambda) + \sum_{\lambda=435}^{480} F(254, \lambda)} \quad (2.5)$$

There are multiple formulas to calculate the HIX. In 1999, (Kalbitz, Geyer, & Geyer, 1999) and (A. Zsolnay et al., 1999) independently proposed different definitions of HIX. Kalbitz et al. (1999) defined HIX based on synchronous fluorescence scans, while A. Zsolnay et al. (1999) defined HIX using integrated regions of emission spectra. The emission-based definition later became more widely used due to its easier data collection from EEMs and improved instrument capabilities. Ohno (2002) then refined the emission-based approach to reduce statistical bias, but all the HIX definitions aim to capture changes in DOM humification (Korak & McKay, 2024).

FI is the ratio of emission intensity at 450 nm to 500 nm at an excitation of 370 nm. FI indicates the source and nature of DOM (McKnight, Scott, et al., 2001).

$$FI = \frac{\text{Fluorescence Intensity at (Ex/Em) = (370 nm/450 nm)}}{\text{Fluorescence Intensity at (Ex/Em) = (370 nm/500 nm)}} \quad (2.6)$$

Later, another calculation approach was proposed: the ratio of emission intensities at 450 and 500 nm was revised to 470 and 520 nm, incorporating instrument-specific spectral corrections. Because spectral correction is now standard practice, the revised method is generally recommended. FI was developed to distinguish between microbial and terrestrial sources of fulvic acids, with higher FI values indicating microbially derived DOM and lower values reflecting terrestrially derived, more aromatic material (Cory, Miller, McKnight, Guerard, & Miller, 2010; Korak & McKay, 2024; McKnight, Scott, et al., 2001).

2.6 FTIR spectroscopy

FTIR spectroscopy is used to determine how infrared (IR) radiation interacts with a sample that is in solid, liquid, or gaseous form. This spectroscopy records the frequencies at which the sample absorbs IR light, along with the corresponding absorption intensities. This is because each functional group absorbs radiation at characteristic frequencies; these absorption bands provide information about the sample's chemical composition. The component intensity can be inferred from the strength of its absorption. The resulting FTIR spectrum will be displayed as a two-dimensional plot of absorption intensity versus frequency (or wavenumber) (Maria, 2012).

The principle of an FTIR instrument is that the IR radiation emitted from the source reaches the beam splitter, where it is divided and directed toward the two mirrors. One mirror will be at the stationary position, and the other mirror will be in motion with constant velocity. The IR beams first get reflected by the mirrors, then recombine at the beam splitter, pass through the sample, and finally reach the detector. This allows all infrared wavelengths to be measured simultaneously.

Because the two beams travel different optical path lengths before recombining, their recombination produces constructive and destructive interference, generating an interferogram. After the combined beam passes through the sample, the detector captures this signal. The recorded interferogram is then processed by a computer, which applies a Fourier transform to convert it into a conventional IR spectrum of the sample (Blum & John, 2012; Maria, 2012; B. C. Smith, 2011).

FTIR spectroscopy is now widely used for the characterization SOM structure and composition. The main advantage of this FTIR in soil analysis is its high sensitivity to polar bonds and associated functional groups. The method is rapid, inexpensive, non-destructive, and requires a minimal sample amount (Margenot, Calderón, & Parikh, 2016). FTIR spectroscopy has two primary applications in SOM research, they are compositional characterization, and quantitative analysis. It is sensitive to organic functional groups containing C, N, O, H, P, and S, making it well-suited for identifying SOM chemical composition and complementing techniques such as NMR spectroscopy and pyrolysis mass spectrometry (Margenot, Parikh, & Calderón, 2023). FTIR spectroscopy involves quantifying SOM fractions using chemometric calibration modeling. These calibration approaches make it possible to accurately determine not only the total organic carbon but also smaller pools of carbon that are of equal interest to biogeochemistry, such as the labile carbon and black carbon. Chemometric methods are low-cost and high-throughput compared to laboratory analytical methods and have significantly altered the field of SOM science, especially through their application to spatially-explicit mapping of SOM (Margenot et al., 2023).

For SOM analysis, mid-infrared (MIR) radiation in the range of 4000-400 cm^{-1} is commonly used through diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy. DRIFT is the method of sample introduction to the IR beam that characterizes SOM by measuring mid-infrared absorbance associated with polar chemical bonds in organic functional groups, including aliphatic, aromatic, phenolic, amide, and polysaccharide structures (Essington, 2015; Margenot, Calderón, Bowles, Parikh, & Jackson, 2015a). The DRIFT technique helps identify changes in the SOM functional groups that occur through decomposition and mineralization (Chefetz, Hader, & Chen, 1998; Hsu & Lo, 1999).

Numerous studies have used DRIFT techniques to detect how management practices alter SOM quality and composition with residue management, cropping systems, and decomposition processes. For example, P. Sharma et al. (2017a) used FTIR to quantify depth-related changes in SOM composition in an organically managed soil following multiple compost applications. Rath et al. (2022) compared SOM composition through DRIFT in tomato-corn rotations after 25 years of differing C and nutrient management practices in soils (Aranda et al., 2011; Parikh, Goyne, Margenot, Mukome, & Calderón, 2014; Rath et al., 2022; P. Sharma et al., 2017a; Veum, Goyne, Kremer, Miles, & Sudduth, 2014).

2.6.1 Soil sample preparation for FTIR analysis

In order to obtain the quality DRIFT spectra, sample preparation and pretreatment are necessary. Those include: 1. reduction in particle size, 2. demineralization with hydrofluoric acid, and 3. sample dilution with halide salts. Particle size reductions include grinding and sieving of the soil sample, which increases homogeneity, reduces light scattering, and minimizes spectral artifacts associated with specular reflectance (Margenot et al., 2023). Large soil particles, such as quartz-rich sand fractions, enhance non-diffuse reflection, which can complicate spectral interpretation and subtraction procedures (Raphael, 2011; J. B. Reeves, Francis, & Hamilton, 2005). Grinding soils to finer and more uniform particle sizes reduces scattering effects, minimizes artifacts, and improves spectral reproducibility. It also reveals the organic functional groups protected within intra-aggregates (Barthès et al., 2016). Excess grinding also increases the highly reflective mineral surfaces, alters mineral structures, degrades organic compounds, and thereby masks SOM signals (Nguyen, Janik, & Raupach, 1991; Stumpe, Weihermüller, & Marschner, 2011). Some studies stated that the particle size of 250-100 μm is ideal because the aperture of most FTIR spectrometers is 1000 μm (Deiss, Margenot, Culman, & Demyan, 2020; Le Guillou et al., 2015; J. B. Reeves & Smith, 2009). Stumpe et al. (2011) reported that grinding soils to <1.0 mm generally provides improved spectral quality without introducing significant artifacts.

Mineral absorbances often dominate the organic functional groups. So chemical demineralization using hydrofluoric acid (HF) often helps to dissolve the mineral and quartz substances, resulting in the high-quality organic absorbance features (Margenot, Calderón, Magrini, & Evans, 2017a). But it has several limitations, such as altering spectral baselines and spectral shape, altering the SOM chemical structure, and loss of organic carbon due to acid (Gonçalves et al., 2003; Mathers, Xu, Berners-Price, Senake Perera, & Saffigna, 2002; Sleutel, Leinweber, Ara Begum, Kader, & De Neve, 2009). Dilution of soil samples with halide salts, such as KBr, is commonly used to obtain high-quality organic compounds. Dilution reduces the non-linear absorbance and peak-inversion effects that are associated with highly absorbing mineral components, particularly Si-O vibrations below 1100 cm^{-1} (Nguyen et al., 1991; J. B. Reeves, McCarty, Calderon, & Hively, 2012). But this dilution sometimes reduces SOM-related peaks (Demyan et al., 2012; Margenot et al., 2017a) and is less advantageous in improving chemometric prediction of SOM fractions.

FTIR spectra obtained using DRIFT should follow some standardised procedures in order to minimise the atmospheric interference and variability associated with the sample handling. Background or reference spectra should be collected using empty sample holders or infrared-transparent materials such as KBr, using the same acquisition parameters as the soil samples, and should be monitored to account for changes in ambient temperature and humidity (Deiss et al., 2020; Fultz, Moore-Kucera, Calderón, & Acosta-Martínez, 2014). Atmospheric CO_2 and water vapor interference should be reduced by collecting spectra under controlled conditions or by applying software-based or manual subtraction using reference spectra acquired with identical detector settings, including spectral range, resolution, and scan number (Margenot et al., 2023).

2.6.2 Spectral Subtractions

Overlapping of mineral absorbances with organic functional groups is the major issue in SOM composition. To address this issue, spectral subtraction is used to enhance the absorbance of organic functional groups. These spectral subtractions are performed mathematically using the

formula mentioned below:

$$S_{\text{sub}} = S_{\text{spl}} - (S_{\text{ref}} \times \text{SF}) \quad (2.7)$$

where S_{sub} is the subtraction spectrum, S_{spl} is the sample spectrum, S_{ref} is the reference (usually mineral background) spectrum, and SF is the subtraction factor. This formula subtracts the absorbance at each wavelength of mineral spectra from the absorbance at the same wavenumber of mineral + organic spectra. This subtraction yields spectra with the organic peaks, which would be easier to characterize. The subtraction factor is important for obtaining the subtracted spectra, as it can sometimes alter spectral features. SF ranges between 0.1 and 1.0, with SF at 0.1 increasing the absorbance of mineral bands and at 1.0 SF increasing the absorbance of organic bands (Margenot et al., 2016, 2023).

DRIFT spectral subtraction is used not only to improve SOM interpretation but also to complement molecular analyses, identify SOM removed during fractionation, and fingerprint SOM for forensic applications, although it is less useful for quantifying SOM using chemometrics modeling. Spectral subtractions also have some limitations, such as artifacts that arise from particle-size-dependent light scattering, interactions between organic and mineral components, and inaccuracies in background spectra or instrument wavenumber alignment, which can lead to incomplete or non-linear removal of mineral and organic absorption bands (F. Calderón, Haddix, Conant, Magrini-Bair, & Paul, 2013; Griffiths & De Haseth, 2007; Weis & Ewing, 1998).

To perform the spectral subtractions, mineral reference spectra are needed. These mineral reference spectra can be generally obtained from the same soil sample by removing the SOM. SOM can be removed by either ashing or by chemical oxidation. ashing at high temperatures (≥ 500 °C) removes most of the SOM but disturbs the bands at $3700\text{-}3600\text{ cm}^{-1}$ and $1100\text{-}900\text{ cm}^{-1}$ through thermal alteration (Ernakovich, Wallenstein, & Calderón, 2015; Margenot, Calderón, Bowles, Parikh, & Jackson, 2015b; Margenot, Calderón, Magrini, & Evans, 2017b). This ashing method of subtraction facilitates the comparison of SOM composition among samples and has proven useful in diagnostic and forensic applications (M. Kaiser, Ellerbrock, & Gerke, 2007; Margenot et

al., 2015a). But ashing at high temperatures alters mineral structures through dehydration, dehydroxylation, and interlayer collapse, which leads to peak shifts, peak losses, or peak broadening that appear as artifacts in subtraction spectra (Frost & Vassallo, 1996; Padilla et al., 2014; Suarez, Southard, & Parikh, 2015). Lower ashing temperatures can reduce mineral alteration but may not completely remove SOM, leading to poor results. (Padilla et al., 2014; J. B. Reeves, 2012).

Chemical oxidation is another method for removing SOM and obtaining mineral backgrounds, as it preserves mineral structure without artifacts or alterations (Margenot et al., 2015b; J. B. Reeves, 2012). J. B. Reeves (2012) hypothesized that these chemical oxidations are a better alternative to ashing. Common chemical oxidants used to remove SOM include sodium hypochlorite (Margenot et al., 2015b), hydrogen peroxide, and sodium hyposulfite (Adegoroye, Uhlik, Omotoso, Xu, & Masliyah, 2009; Mikutta, Kleber, & Jahn, 2005; Siregar, Kleber, Mikutta, & Jahn, 2005; von Lützow et al., 2007). But these chemicals remove only 70-80% of SOM without altering minerals, whereas ashing removes 95% of SOM but often causes mineral alterations. The choice between chemical oxidation and ashing depends on study objectives, with chemical methods used when preserving mineral structure is critical and ashing preferred when near-complete SOM removal is required (Margenot et al., 2016, 2023).

2.7 Linking Spectroscopic Signatures to Cover Crops Soil WEOM Chemistry

Combining UV-Vis, Fluorescence, and FTIR spectroscopy provides a better assessment of WEOM chemistry than a single analytical technique alone. As WEOM is a complex, heterogeneous mixture with rapid turnover, multi-spectroscopy analysis provides insights into the WEOM signature. The literature indicates that numerous studies have successfully integrated multiple spectroscopic tools to investigate WEOM and SOM dynamics and to evaluate the effects of land use and management practices on soil carbon chemistry. P. Sharma et al. (2017a) combined FTIR spectroscopy to characterize SOM functional group composition with UV-Vis and fluorescence spectroscopy to assess WEOM chemical properties in soils receiving different compost application rates. Similarly, Rath et al. (2022) integrated total organic carbon (TOC) and total nitrogen (TN) analyses

with FTIR spectroscopy to evaluate changes in soil carbon storage, organic matter composition, and molecular structure under cover-crop and compost management systems. Corvasce, Zsolnay, D’Orazio, Lopez, and Miano (2006b) used UV absorbance, fluorescence spectroscopy, and derived optical indices to characterize WEOM composition along deep soil profiles, highlighting the value of integrated spectroscopic approaches for understanding vertical variability in WEOM chemistry.

UV-Vis spectroscopy provides key insights into WEOM aromaticity (e.g., $SUVA_{254}$), molecular weight and size distribution (e.g., E2:E3 ratios and spectral slopes), source characteristics, and degree of degradation (e.g., slope ratios). EEMs + PARAFAC resolves WEOM origin (fluorescence index, FI), humification extent (humification index, HIX), and the relative abundance and intensity of distinct molecular fractions (PARAFAC components). FTIR spectroscopy characterizes the functional group chemistry of SOM, including aliphatic, aromatic, polysaccharide, amide, and phenolic structures, thereby providing molecular-level information on organic matter chemistry and transformation processes (F. J. Calderón, Mikha, et al., 2011; Li & Hur, 2017b; Parikh et al., 2014).

Cover crops, as a sustainable management practice, contribute carbon and organic matter inputs to soil through both above- and belowground biomass. These inputs alter microbial activity, residue decomposition dynamics, and organomineral interactions, thereby changing the quantity and composition of WEOM and SOM. The literature also consistently reports that WEOM is highly sensitive to land use and management practices. To capture these management-induced changes, a range of spectroscopic techniques is employed, as they provide insights into the chemical composition, molecular structure, and transformation pathways of WEOM and SOM. By characterizing the molecular structure and humification state of these organic pools, spectroscopic indices provide a sensitive indicator of nutrient cycling efficiency and the potential for long-term carbon stabilization. These insights enable researchers and land managers to evaluate soil health not just by the total quantity of carbon, but by its functional quality, such as the soil’s capacity to supply plant-available nitrogen or build resilient, aggregate-protected organic matter in response to specific management regimes.

Additionally, Shepherd and Walsh (2007) proposed a diagnostic surveillance framework based on infrared spectroscopy to provide a real-time assessment of soil health, crop condition, and environmental quality, especially in resource-limited farming systems. Their framework emphasizes the role of spectroscopy in providing fast, reproducible, and cost-effective screening of soil properties, allowing early identification of soil constraints and timely management interventions. The authors highlight the potential to deploy portable, field-based infrared spectroscopy systems, enabling smallholder farmers to access soil and crop diagnostics via mobile or handheld devices. This helps for a rapid assessment of soil organic matter quality, nutrient status, and management impacts, so that they can make decisions on cover crop selection, residue management, organic amendments, and fertilizer application.

Chapter 3

Materials and Methods

3.1 Site Description and Experimental Design

This study was conducted in the southeastern United States, with one site located in Belle Mina, AL (34°41'22.4" N, 86°53'01.7" W) at the Tennessee Valley Research and Extension Center (TVREC), with an average annual rainfall of 1283 mm and a temperature of 17.5°C. The second site was located in Headland, AL (31°30' N, 85°17' W) at the Wiregrass Research and Extension Center (WREC) with an average annual rainfall of 1503 mm and a temperature of 19.7°C. At TVREC, soils were classified as Dewey silt loam (fine, kaolinitic, thermic Typic Paleudult) and Ooltewah silt loam (fine-silty, mixed, active, thermic Fluvaquentic Dystrudept), while soils at WREC were classified as Lucy loamy sand (loamy, kaolinitic, thermic Arenic Kandiodult). The crop rotation during the experiment was cotton-soybean (*Glycine max* (L.) Merr.) at TVREC and cotton-peanut (*Arachis hypogaea* L.) at WREC, reflecting the dominant cropping systems in those regions. Before the experiment began, TVREC had been under cotton, corn (*Zea mays* L.), or soybean cultivation for over 10 years, with wheat (*Triticum aestivum* L.) planted in winter during two of those years (2014 and 2016). Additionally, land had been managed as no-till for over 20 years and remained no-till throughout the study. WREC had historically been under a cotton-peanut rotation and was managed with conventional tillage. At the start of the experiment, it was converted to strip tillage, with a 10-cm-wide tilled strip within the crop row. Peanut harvesting at WREC caused some soil disturbance.

Treatments evaluated in this study included winter fallow, cereal rye (*Secale cereale* L.), crimson clover (*Trifolium incarnatum* L.), and radish (*Raphanus sativus* L.) monocultures, along with mixture treatment of three cover crop species arranged in a randomized complete block design (RCBD) with four replicates. Plot sizes at TVREC are 10.7 m long \times 8.2 m wide, and at WREC are 10.7 m long \times 7.3 m wide. Seed rates for cereal rye, crimson clover, and radish monocultures were maintained at 100, 22, and 9 kg ha⁻¹, respectively, until 2021, and were subsequently reduced to 40, 20, and 8 kg ha⁻¹, respectively. Similarly, three-species mixtures with seed rates of cereal rye, crimson clover, and radish at 34, 11, and 4 kg ha⁻¹, respectively, were maintained until 2021 and later adjusted to 20, 10, and 4 kg ha⁻¹, respectively. The germination rate was more than 80% for all seeds, and the seeding rates were not adjusted for germination. Clover seeds were pre-inoculated with *Rhizobium leguminosarum biovar trifolii*. All cover crops were drilled in 19-cm rows with a Great Plains 1205NT drill at WREC and a Great Plains 3P606NT drill at TVREC. Every year, at both locations, cover crops were chemically terminated, leaving their residues on the soil surface. Cover crops did not receive any irrigation, and soil pH is managed annually according to recommendations from the Alabama Cooperative Extension System.

3.2 Soil Sampling and Extraction of Water Extractable Organic Matter (WEOM)

Soil samples were collected from both sites in November 2024, seven years after the introduction of cover crop trials. All samples were taken 2-3 weeks after the cash crop harvest and seven months after the cover crop termination. Samples were collected from two depths: 0-10 cm and 10-30 cm using a soil probe. To account for field heterogeneity, approximately 10 subsamples were randomly collected from each plot and combined to form a single sample. The samples were then air-dried at room temperature, ground finely with a coffee grinder, and stored in Ziplock bags for further analysis. Soil pH, texture, and EC (electrical conductivity) data are presented in table 3.1.

WEOM extraction was performed using a 1:5 (w/v) soil-to-water ratio. 10 g of soil was mixed with 50 mL of ultra-pure water (18.2 M Ω) and placed on a reciprocal shaker at 150 rpm for 24 hours in the dark at room temperature (25°C). The mixture was then centrifuged at 14,000 rpm

Table 3.1: Basic properties of soil at TVREC and WREC sites.

Location	Soil depth (cm)	Texture (%)			pH ^a	EC ^b (dS m ⁻¹)
		Sand	Silt	Clay		
TVREC	0-10	15	63	22	6.2	3.2
TVREC	10-30	12	63	25	5.9	1.3
WREC	0-10	83	11	6	6.2	1.6
WREC	10-30	84	10	6	5.7	0.7

^aDetermined in a 1:1 (w/v) soil:water extract.

^bElectrical conductivity measured in a 1:1 (w/v) soil:water extract.

for 10 minutes using a refrigerated centrifuge (at 25°C) to separate the solid and liquid phases. The supernatant was then filtered under vacuum using a 0.45 μm cellulose acetate membrane filter. The filtered solution was stored in pre-combusted amber glass bottles to prevent photodegradation. These solutions were subsequently used to characterize the properties of WEOM.

3.3 Characterization of WEOM

3.3.1 Characterization of WEOM Solutions Using Organic Carbon, Total Nitrogen, and C/N Ratio

DOC and TN concentrations of all WEOM solutions were quantified using a Multi N/C TOC analyzer (Analytik Jena, Jena, Germany). The concentrations were expressed on a dry-soil basis in mg kg^{-1} . The C/N ratio was then calculated by dividing the DOC by TN concentrations.

3.3.2 Characterization of WEOM Solutions Using Ultraviolet-Visible Absorbance and Fluorescence Spectroscopy

The WEOM solutions were analyzed for UV-Vis absorbance using a JASCO V-750 UV-Vis spectrophotometer (JASCO, Easton, MD) over the wavelength range of 200 nm to 800 nm. EEM fluorescence spectra of WEOM were measured using a JASCO FP-8500 spectrofluorometer (JASCO, Easton, MD). The excitation wavelengths were scanned from 250 nm to 585 nm at 5 nm intervals, and the emission wavelengths were scanned from 260 nm to 600 nm at 2 nm intervals. Fluorescence-free ultrapure water blanks were routinely measured for background correction, scattering removal,

and Raman normalization (Murphy et al., 2013). To minimize the “inner filter effect” (IFE), WEOM solutions with UV absorbance exceeding 0.1 at 254 nm were diluted using fluorescence-free ultrapure distilled water. A dilution factor is determined for each sample to maintain absorbance ≤ 0.1 at 254 nm, and this factor is then used to calculate the sample’s fluorescence intensities (Gilchrist & Reynolds, 2014; Lakowicz, 2006).

3.3.3 Characterization of WEOM Solutions Using Spectroscopic Indices

Optical indices were derived from spectroscopic analyses to further characterize the compositional properties of WEOM solutions obtained from different cover crop treatments and depths. Two indices were calculated from absorbance spectra: i) Specific UV absorbance at 254nm (SUVA₂₅₄) and ii) E2:E3 ratio. Two indices are calculated from EEMs: i) Fluorescence Index (FI) and ii) Humification index (HIX).

3.4 EEM Pre-processing and PARAFAC Modeling

EEM fluorescence data were decomposed into principal fluorophores using the `staRdom` package (Pucher et al., 2019) in R (R Core Team, 2025) to separate WEOM components through PARAFAC. The analytical workflow followed the procedures described by (Murphy et al., 2013) and (Pucher et al., 2019). Data preprocessing included baseline correction of absorbance spectra (Li & Hur, 2017a), spectral correction using instrument-specific correction files (DeRose & Resch-Genger, 2010), subtraction of ultrapure water blanks to remove background fluorescence, and correction for inner-filter effects based on sample absorbance (Kothawala, Murphy, Stedmon, Weyhenmeyer, & Tranvik, 2013). Fluorescence intensities are normalized to Raman units by dividing the integrated Raman peak area of ultrapure water (Lawaetz & Stedmon, 2009). First- and second-order Rayleigh and Raman scatter bands are removed and interpolated to ensure smooth spectral surfaces (Elcoroaristizabal, Bro, García, & Alonso, 2015). The corrected and normalized EEM datasets are then used to construct a PARAFAC model using the `eem.parafac()` function, applying non-negativity constraints for all modes and multiple random initializations to

ensure model stability. Model validation involved split-half analysis, residual examination, exclusion of outliers, and core consistency diagnostics to assess model reliability and component uniqueness. The resulting fluorescence components are visualized and interpreted based on their excitation and emission loadings and subsequently compared with reference spectra from the Open Fluor database. The relative abundance of each component is expressed as the absolute fluorescence intensity (F_{\max}). To evaluate the effects of treatments and soil depth, the absolute intensities (F_{\max}) of the PARAFAC components were statistically compared.

3.5 Fourier Transform Infrared Spectroscopy

FTIR spectra were recorded using Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT). Soil samples were ashed in a furnace at 400°C for 4 hours to obtain the mineral fraction. Spectra of both whole (unashed) and ashed soils were recorded using a JASCO-FP8000 FTIR spectrometer over the wavelength range of 600–4000 cm^{-1} , with 64 scans per sample and a resolution of 4 cm^{-1} . Spectral subtractions were performed to improve the FTIR operational characterization of the relative composition of SOM functional groups (Margenot et al., 2015a). Nguyen et al. (1991) stated that subtractions are useful for enhancing organic bands within 2000–1600 cm^{-1} . Ashing the samples to remove organic matter, followed by subtracting the resultant mineral fraction spectra from the original whole-soil spectra, will enhance the organic bands, further allowing for easier identification and characterization of soil organic functional groups (F. J. Calderón, Mikha, et al., 2011). Baseline correction and normalization of spectra were performed in R (R Core Team, 2025) using the `prospectr` package (Stevens & Ramirez-Lopez, 2025). Spectral subtractions were carried out in R for each sample using a subtraction factor, following (Margenot et al., 2016):

$$S_{\text{sub}} = S_{\text{spl}} - (S_{\text{ref}} \times \text{SF}) \quad (3.1)$$

where S_{sub} is the subtraction spectrum, S_{spl} is the sample spectrum, S_{ref} is the reference (usually mineral background) spectrum, and SF is the subtraction factor. The subtracted spectra were

then analyzed for the peaks and shoulders corresponding to organic functional groups. To quantify the differences between the treatments and depth of SOM decomposition and recalcitrance, two indices are calculated: Index 1 is the ratio of absorbance of aromatic groups to the aliphatic groups, and Index 2 is the ratio of absorbance of C- to O- containing functional groups (Chefetz et al., 1998; Ding, Novak, Amarasiriwardena, Hunt, & Xing, 2002; Hsu & Lo, 1999; Veum et al., 2014). For the TVREC site, Index 1 and Index 2 were derived for all treatments at both soil depths, as shown in Equations 3.2 and (3.3).

$$\text{Index}_1 = \frac{\text{abs}_{1650} + \text{abs}_{1550}}{\text{abs}_{2930} + \text{abs}_{1450}} \quad (3.2)$$

$$\text{Index}_2 = \frac{\text{abs}_{2930} + \text{abs}_{1650} + \text{abs}_{1450}}{\text{abs}_{1550} + \text{abs}_{1270}} \quad (3.3)$$

For the WREC site, Index 1 and Index 2 were derived for all treatments at both soil depths, as shown in Equations 3.2 and (3.3).

$$\text{Index}_1 = \frac{\text{abs}_{1650} + \text{abs}_{920} + \text{abs}_{800}}{\text{abs}_{2930} + \text{abs}_{1440}} \quad (3.4)$$

$$\text{Index}_2 = \frac{\text{abs}_{2930} + \text{abs}_{1650} + \text{abs}_{1440} + \text{abs}_{920} + \text{abs}_{800}}{\text{abs}_{3400} + \text{abs}_{1300} + \text{abs}_{1160} + \text{abs}_{1025}} \quad (3.5)$$

Principal Component Analysis was performed on the subtracted FTIR spectra to explore patterns in organic functional group composition across cover crop treatments and soil depths.

3.6 Statistical Analysis

All data analysis and graphical representations were performed in R (R Core Team, 2025). A separate statistical analysis was carried out for each site. Analysis of variance (ANOVA) was performed using the linear mixed-effects model (`lmer`) to determine how the spectroscopic indices and PARAFAC components affect the different cover crop treatments and depths WEOM. Treatment and depth were treated as fixed effects, and replication as a random effect. Type III ANOVA

with Kenward-Roger approximation was used to obtain F - and p -values for the fixed effects. Mean comparisons among the treatments was performed using Tukey's post hoc HSD test, with differences considered significant at $\alpha \leq 0.05$ probability level. Principal Component Analysis was performed to determine the correlation among the spectroscopic indices and also FTIR spectra.

Chapter 4

Results

4.1 Dissolved Organic Carbon, Total Nitrogen and C:N Ratio

Clover treatment showed the higher DOC concentration in the 0-10 cm depth only at WREC. *F*- and *p*-values from the linear mixed effects ANOVA for all the parameters are reported in Table 4.1. Depth had a very strong negative effect at both sites ($p < 0.001$), with DOC concentrations decreasing with increasing depth. No significant differences were observed among treatments at either site. At TVREC, DOC concentrations were 110 to 125 mg kg⁻¹ in 0–10 cm depth, and 60 to 70 mg kg⁻¹ in 10–30 cm depth (Table 4.2; Figure 4.1a). At WREC, treatment × depth interaction was significant ($F = 3.8$, $p = 0.025$), indicating that treatment effects varied with depth. Clover significantly increased the DOC concentrations (117.8 ± 12.3 mg kg⁻¹) in the 0–10 cm depth when compared to Fallow (83.8 ± 9.4 mg kg⁻¹) and Rye (85.4 ± 2.0 mg kg⁻¹) but at the 10- 30 cm depth, DOC concentrations were similar across all the treatments, ranging from 46 to 49 mg kg⁻¹(Table 4.2; Figure 4.1b).

Total Nitrogen (TN) concentrations differ among the treatment and depth at both sites ($p < 0.05$). Across all treatments, TN concentrations decreased significantly with increasing soil depth (Figure 4.1c-d). At the 0–10 cm depth, Clover exhibited the highest TN concentration (with 16.0 ± 0.6 mg kg⁻¹ in TVREC and 16.3 ± 2.7 mg kg⁻¹ in WREC), followed by the Rye/-Clover/Radish (RCR) mixture treatment (13.3 ± 1.0 mg kg⁻¹ in TVREC and 14.4 ± 0.7 mg kg⁻¹ in WREC) (Table 4.2). At 10–30 cm depth, TN concentrations at TVREC were higher under Clover (10.3 ± 0.8 mg kg⁻¹) and RCR (9.8 ± 1.0 mg kg⁻¹), reflecting a similar trend as 0–10 cm

Table 4.1: Results of Linear Mixed Effect Model ANOVA showing the effects of treatment, depth, and their interaction on DOC, TN, C:N ratio, and spectroscopic indices at TVREC and WREC sites. Values represent F - and p -statistics. Significance was determined at the $\alpha \leq 0.05$ level.

TVREC														
Effects	SUVA		DOC		TN		E2:E3		C:N		HIX		FI	
	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Treatment	1.400	0.284	0.160	0.956	8.020	0.001	2.920	0.057	2.000	0.146	1.120	0.383	0.880	0.501
Depth	200.0	0.000	85.26	0.000	75.45	0.000	47.13	0.000	9.420	0.008	192.92	0.000	68.91	0.000
Treatment:Depth	2.400	0.098	0.150	0.960	1.050	0.414	1.900	0.162	0.220	0.922	1.420	0.276	0.800	0.544

WREC														
Effects	SUVA		DOC		TN		E2:E3		C:N		HIX		FI	
	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Treatment	0.500	0.739	2.260	0.112	3.330	0.038	0.333	0.851	1.190	0.344	0.580	0.684	2.130	0.127
Depth	27.30	0.000	214.16	0.000	169.86	0.000	25.59	0.000	34.61	0.000	0.150	0.707	0.400	0.535
Treatment:Depth	0.700	0.593	3.790	0.025	4.510	0.014	1.370	0.289	0.770	0.557	0.130	0.968	2.190	0.120

Note. SUVA = specific ultraviolet absorbance ($L\ mg^{-1}\ m^{-1}$); DOC = dissolved organic carbon ($mg\ kg^{-1}$); TN = total nitrogen ($mg\ kg^{-1}$); C:N = carbon-to-nitrogen ratio; HIX = humification index; FI = fluorescence index; E2:E3 = ratio of absorbance at 250 nm to 365 nm.

Table 4.2: Mean (\pm SE) values of SUVA, DOC, TN, E2:E3 ratio, C:N ratio, HIX, and FI across treatments and depths at TVREC and WREC. Different letters indicate Tukey's HSD groupings ($\alpha < 0.05$).

Treatment	Depth (cm)	SUVA	DOC	TN	E2:E3	C:N ratio	HIX	FI
(a) TVREC Site								
Radish	0–10	0.7 \pm 0.0 a	118.3 \pm 19.2 a	10.4 \pm 0.2 bcde	6.2 \pm 0.2 b	11.4 \pm 1.7 a	0.8 \pm 0.0 a	2.0 \pm 0.0 b
Radish	10–30	0.4 \pm 0.0 b	61.2 \pm 3.0 c	7.2 \pm 0.4 e	9.2 \pm 1.0 ab	8.6 \pm 0.8 a	0.7 \pm 0.0 b	2.1 \pm 0.0 ab
Fallow	0–10	0.7 \pm 0.0 a	121.9 \pm 14.5 a	11.5 \pm 0.6 bc	5.7 \pm 0.1 b	10.5 \pm 0.9 a	0.8 \pm 0.0 a	1.9 \pm 0.0 b
Fallow	10–30	0.4 \pm 0.0 b	62.8 \pm 2.6 bc	7.6 \pm 0.4 de	8.2 \pm 1.5 b	8.4 \pm 0.4 a	0.6 \pm 0.0 b	2.0 \pm 0.0 ab
Rye	0–10	0.9 \pm 0.0 a	111.4 \pm 16.3 ab	11.2 \pm 1.4 bcd	5.5 \pm 0.1 b	10.7 \pm 2.4 a	0.8 \pm 0.0 a	2.0 \pm 0.0 b
Rye	10–30	0.4 \pm 0.0 b	64.2 \pm 1.6 bc	7.9 \pm 0.5 cde	9.2 \pm 1.3 ab	8.2 \pm 0.6 a	0.6 \pm 0.0 b	2.1 \pm 0.0 a
Rye/Clover/Radish	0–10	0.8 \pm 0.0 a	123.9 \pm 5.1 a	13.3 \pm 1.0 ab	6.3 \pm 0.1 b	9.5 \pm 0.7 a	0.8 \pm 0.0 a	2.0 \pm 0.0 b
Rye/Clover/Radish	10–30	0.4 \pm 0.0 b	64.5 \pm 7.5 bc	9.8 \pm 1.0 bcde	12.2 \pm 0.6 a	6.7 \pm 0.8 a	0.6 \pm 0.0 b	2.1 \pm 0.0 a
Clover	0–10	0.8 \pm 0.0 a	121.6 \pm 2.9 a	16.0 \pm 0.6 a	5.6 \pm 0.1 b	7.7 \pm 0.4 a	0.8 \pm 0.0 a	2.0 \pm 0.0 b
Clover	10–30	0.4 \pm 0.0 b	68.5 \pm 8.3 bc	10.3 \pm 0.8 bcde	7.6 \pm 0.96 b	6.7 \pm 0.8 a	0.6 \pm 0.0 b	2.1 \pm 0.0 ab
(b) WREC Site								
Radish	0–10	1.7 \pm 0.1 a	93.9 \pm 1.7 ab	10.4 \pm 1.1 bc	4.2 \pm 0.2 a	9.5 \pm 1.4 abcd	0.9 \pm 0.0 a	1.6 \pm 0.0 a
Radish	10–30	2.2 \pm 0.0 a	47.3 \pm 3.1 c	4.3 \pm 0.5 d	3.9 \pm 0.1 a	11.4 \pm 1.1 ab	0.9 \pm 0.0 a	1.6 \pm 0.0 a
Fallow	0–10	2.0 \pm 0.2 a	83.8 \pm 9.3 b	8.8 \pm 0.8 cd	4.0 \pm 0.2 a	9.5 \pm 0.6 abcd	0.9 \pm 0.0 a	1.6 \pm 0.0 a
Fallow	10–30	2.3 \pm 0.2 a	46.7 \pm 3.8 c	4.3 \pm 0.3 d	3.8 \pm 0.3 a	10.9 \pm 0.6 abcd	0.9 \pm 0.0 a	1.6 \pm 0.0 a
Rye	0–10	1.8 \pm 0.1 a	85.4 \pm 2.0 b	11.5 \pm 1.0 abc	4.5 \pm 0.1 a	7.6 \pm 0.5 bd	0.9 \pm 0.0 a	1.6 \pm 0.0 a
Rye	10–30	2.2 \pm 0.1 a	49.4 \pm 2.2 c	4.6 \pm 0.4 d	3.8 \pm 0.1 a	11.0 \pm 0.6 ac	0.9 \pm 0.0 a	1.6 \pm 0.0 a
Rye/Clover/Radish	0–10	1.7 \pm 0.1 a	109.8 \pm 9.9 ab	14.4 \pm 0.7 ab	4.4 \pm 0.1 a	7.6 \pm 0.3 abcd	0.9 \pm 0.0 a	1.7 \pm 0.0 a
Rye/Clover/Radish	10–30	2.4 \pm 0.1 a	47.2 \pm 2.4 c	4.8 \pm 0.3 d	3.8 \pm 0.1 a	10.0 \pm 0.8 abcd	0.9 \pm 0.0 a	1.6 \pm 0.0 a
Clover	0–10	1.6 \pm 0.1 a	117.8 \pm 12.9 a	16.3 \pm 2.7 a	4.2 \pm 0.1 a	7.4 \pm 0.4 cd	0.9 \pm 0.0 a	1.6 \pm 0.0 a
Clover	10–30	2.4 \pm 0.1 a	48.9 \pm 2.7 c	4.8 \pm 0.3 d	3.8 \pm 0.2 a	10.4 \pm 0.8 abcd	0.9 \pm 0.0 a	1.6 \pm 0.0 a

Values are means \pm standard error; letters indicate Tukey groupings (Tukey's HSD, $\alpha < 0.05$).

Units: SUVA ($L\ mg^{-1}\ m^{-1}$); DOC and TN ($mg\ kg^{-1}$ soil); E2:E3 (unitless); C:N ratio (unitless); HIX (unitless); FI (unitless).

Abbreviations: DOC, dissolved organic carbon; TN, total nitrogen in WEOM extract; SUVA, specific UV absorbance; HIX, humification index; FI, fluorescence index.

depth. In contrast, at WREC, TN concentrations among treatments at 10–30 cm depth were not significantly different ($p > 0.05$) (Table 4.1).

The C:N ratio at both sites ranged from 6 to 11 which are very low. C:N ratio remained stable across all treatments, with no significant treatment differences ($p > 0.05$) at both sites. At TVREC, depth had a significant effect on C:N ratio ($p < 0.05$) according to ANOVA; however, Tukey's HSD post hoc test, being a conservative method, did not detect significant pairwise differences, resulting in the same letter assignment (a) for all treatments and depths (Table 4.2; Figure 4.1e). At WREC, 10–30 cm depth has higher C:N ratios (10–12) compared to the 0–10 cm depth (7–10) with significant difference in depth ($p < 0.05$) and is inversely correlated to TVREC C:N ratios (Figure 4.1f).

4.2 Spectroscopic Indices

4.2.1 Specific UV Absorbance (SUVA) and E2:E3 Ratio

No significant differences were noted among the treatments at either depth across both sites (Table 4.1). SUVA values at the TVREC were in the range of 0.4 to 0.8 L mg⁻¹ m⁻¹. Significant difference between the depths ($p < 0.001$) was observed with higher SUVA values at 0–10 cm depth (0.7–0.9 L mg⁻¹ m⁻¹) compared to the 10–30 cm depth (0.4–0.5 L mg⁻¹ m⁻¹) (Figure 4.2a). At WREC, SUVA values were higher, with a range of 1.5 L mg⁻¹ m⁻¹ to 2.5 L mg⁻¹ m⁻¹. Although ANOVA indicated a significant depth effect, Tukey's conservative adjustment grouped both depths, indicating no detectable pairwise separation (Figure 4.2b).

At TVREC, the treatment effect yielded a near-significant p -value ($p = 0.057$), with the RCR treatment exhibiting the highest E2:E3 ratio. The depth effect was also significant, with higher ratios at the 10–30 cm depth compared to the 0–10 cm depth. At both depths, RCR treatment had the highest E2:E3 ratio, which was more pronounced at the deeper depth (12.2 ± 0.6 at 10–30 cm and 6.3 ± 0.1 at 0–10 cm). At WREC, there were no significant differences among treatments; however, depth had a significant effect in the ANOVA, although Tukey's HSD test did not detect

pairwise differences. Overall, the E2:E3 ratio ranged from 5 to 12 at TVREC and from 3 to 5 at WREC (Figure 4.2c + 4.2d).

4.2.2 Humification Index (HIX) and Fluorescence Index (FI)

HIX mean values ranged between 0.6-0.8 at TVREC and 0.8-0.9 at WREC. At TVREC, there were no significant differences among the treatments, and the depth effect was significant with slightly higher HIX values (≈ 0.8) observed at 0–10 cm compared to 10–30 cm. Whereas in WREC, neither treatment, depth, nor their interaction showed significant effects (Table 4.1 ; Figure 4.2e and 4.2f).

Fluorescence Index mean values at TVREC were between 2.0–2.1, whereas values at WREC were lower, ranging from 1.6 to 1.7. At TVREC, only depth is the significant factor, with subsoil exhibiting slightly higher FI values. There were no significant differences among the treatments at either depth across both sites, and depth differences were not significant at WREC (Table 4.1 ; Figure 4.2g and 4.2h).

4.3 PARAFAC

EEM processing and PARAFAC modeling identified five independent fluorescent components (Components 1-5) (Figure 4.3 and 4.4). The absolute intensities (F_{\max}) of the PARAFAC components were statistically compared, and the corresponding p-values for each component are presented in Table 4.4. Table 4.5 and Figure 4.5 illustrate the relative contribution of each component under different treatments and depths. Components 1 and 2 did not differ significantly among the treatments but showed significant depth-related variation at both sites. At the TVREC site, these component intensities decreased with increasing depth, whereas at the WREC site, their intensities increased with increasing depth. Components 3 and 4 also showed no treatment effects at either site; however, depth effects varied by location. At TVREC, there was no significant difference between the depths, whereas in WREC, there was a significant difference in depth, with intensities

higher at 10–30 cm. Component 5 likewise showed no treatment differences, but depth effects were significant at both sites, with intensities increasing consistently with depth.

4.4 Fourier Transform Infrared Spectroscopy

Subtracted FTIR Spectra of the different cover crop treatments showed the clear, significant differences in spectral peaks between the two sites, indicating that site effects dominated the variability in the soil organic C functional group composition, whereas treatment and depth-related differences were minor. The FTIR peak assignments for both sites are presented in Table 4.6. The subtracted FTIR spectral plots of the different cover crop treatments at both soil depths and at both locations are presented in Figures 4.6 and 4.7. At the TVREC site, all the cover crop treatments, including fallow, exhibited the same set of peaks at both soil depths, indicating similar functional group composition across treatments and depths. Similarly, the WREC site also showed consistent peak patterns across all the treatments at both soil depths. PCA was performed on the subtracted FTIR spectra for each site individually. The resulting score plots showed overlap among samples, with treatments and depths intermingled and no detectable spectral separation (Figure 4.8, 4.9).

The combined PCA of both sites revealed a clear separation of samples between the two sites along PC1, which explained 65.2% of variance. Samples from TVREC clustered distinctly on the negative side of PC1, whereas those from WREC clustered on the positive side, indicating strong site-specific differences in soil organic functional group composition. Samples within each site showed extensive overlap, with treatments and soil depths intermingled throughout the score plot and no clustering along either PC1 or PC2 (Figure 4.10).

Index 1 values at TVREC ranged from 1.10 to 1.26, indicating that aromatic and aliphatic functional group contributions were similar across treatments, with only slight differences in their relative proportions. No consistent patterns were observed across the soil depths. Fallow had the lowest index value (1.13) at 0-10 cm, while Rye had the lowest index value (1.10) at 10-30cm, indicating slightly greater aliphatic C sources in these cases compared with other treatments. Index 2 values are higher at the TVREC than at the WREC, revealing that TVREC has a more abundant

Table 4.3: PARAFAC component characteristics, including excitation and emission maxima and the inferred nature of dissolved organic matter (DOM) for Components 1-5.

Component	Excitation Max (nm)	Emission Max (nm)	Nature of DOM
Component 1	260 (340)	430	reduced semiquinone-like component
Component 2	270 (370)	486	UVA humic-like, terrestrial (peak C)
Component 3	305	426	humic/quinone-like fluorophore
Component 4	290	382	terrestrial humic-like component (peak A)
Component 5	275	298	protein-like fluorophore (peak B)

Table 4.4: Results of Linear Mixed Effect Model ANOVA showing the effects of treatment, depth, and their interaction on Absolute intensities (F_{\max}) of PARAFAC components at TVREC and WREC sites. Values represent F - and p -statistics. Significance was determined at the $\alpha \leq 0.05$ level.

TVREC										
Effects	Comp. 1		Comp. 2		Comp. 3		Comp. 4		Comp. 5	
	F	p	F	p	F	p	F	p	F	p
Treatment	1.20	0.357	1.20	0.356	1.10	0.392	0.90	0.471	0.00	0.996
Depth	22.9	0.000	42.5	0.000	0.50	0.479	2.50	0.135	49.6	0.000
Treatment:Depth	1.30	0.307	1.30	0.310	1.00	0.434	1.50	0.267	2.80	0.067
WREC										
Effects	Comp. 1		Comp. 2		Comp. 3		Comp. 4		Comp. 5	
	F	p	F	p	F	p	F	p	F	p
Treatment	0.40	0.819	0.60	0.666	0.40	0.814	0.40	0.788	0.50	0.760
Depth	20.2	0.000	23.7	0.000	31.6	0.000	19.9	0.000	31.1	0.000
Treatment:Depth	0.40	0.785	0.50	0.766	0.30	0.878	0.40	0.813	0.60	0.676

Table 4.5: PARAFAC component Absolute Intensities (Fmax) for different treatments and depths. Values are means \pm standard error.

Treatment	Depth (cm)	Comp.1	Comp.2	Comp.3	Comp.4	Comp.5
(a) TVREC Site						
Radish	0–10	0.52 \pm 0.12	0.19 \pm 0.04	0.49 \pm 0.15	0.31 \pm 0.07	0.23 \pm 0.09
Radish	10–30	0.29 \pm 0.00	0.07 \pm 0.00	0.38 \pm 0.01	0.18 \pm 0.00	0.29 \pm 0.01
Fallow	0–10	0.39 \pm 0.02	0.15 \pm 0.00	0.35 \pm 0.03	0.22 \pm 0.01	0.18 \pm 0.03
Fallow	10–30	0.26 \pm 0.02	0.08 \pm 0.01	0.37 \pm 0.02	0.20 \pm 0.03	0.34 \pm 0.04
Rye	0–10	0.34 \pm 0.05	0.13 \pm 0.01	0.31 \pm 0.05	0.19 \pm 0.02	0.19 \pm 0.05
Rye	10–30	0.24 \pm 0.02	0.07 \pm 0.01	0.36 \pm 0.00	0.18 \pm 0.03	0.33 \pm 0.02
Rye/Clover/Radish	0–10	0.33 \pm 0.01	0.13 \pm 0.00	0.29 \pm 0.01	0.20 \pm 0.00	0.15 \pm 0.03
Rye/Clover/Radish	10–30	0.24 \pm 0.01	0.08 \pm 0.00	0.37 \pm 0.01	0.19 \pm 0.00	0.40 \pm 0.02
Clover	0–10	0.37 \pm 0.01	0.14 \pm 0.00	0.30 \pm 0.01	0.20 \pm 0.01	0.10 \pm 0.00
Clover	10–30	0.24 \pm 0.00	0.08 \pm 0.00	0.38 \pm 0.02	0.19 \pm 0.02	0.41 \pm 0.01
(b) WREC Site						
Radish	0–10	0.16 \pm 0.02	0.10 \pm 0.01	0.12 \pm 0.01	0.06 \pm 0.00	0.04 \pm 0.00
Radish	10–30	0.23 \pm 0.02	0.14 \pm 0.02	0.21 \pm 0.02	0.09 \pm 0.01	0.06 \pm 0.00
Fallow	0–10	0.15 \pm 0.02	0.10 \pm 0.01	0.12 \pm 0.02	0.06 \pm 0.01	0.05 \pm 0.01
Fallow	10–30	0.23 \pm 0.06	0.15 \pm 0.04	0.23 \pm 0.06	0.09 \pm 0.02	0.06 \pm 0.01
Rye	0–10	0.18 \pm 0.01	0.10 \pm 0.01	0.13 \pm 0.01	0.07 \pm 0.00	0.04 \pm 0.00
Rye	10–30	0.21 \pm 0.01	0.13 \pm 0.01	0.19 \pm 0.02	0.08 \pm 0.00	0.05 \pm 0.00
Rye/Clover/Radish	0–10	0.14 \pm 0.01	0.08 \pm 0.00	0.11 \pm 0.01	0.05 \pm 0.00	0.03 \pm 0.00
Rye/Clover/Radish	10–30	0.2 \pm 0.02	0.11 \pm 0.01	0.18 \pm 0.02	0.07 \pm 0.00	0.06 \pm 0.01
Clover	0–10	0.13 \pm 0.01	0.07 \pm 0.00	0.10 \pm 0.00	0.05 \pm 0.00	0.03 \pm 0.00
Clover	10–30	0.19 \pm 0.04	0.11 \pm 0.02	0.17 \pm 0.04	0.07 \pm 0.01	0.05 \pm 0.00

C-functional group composition. Among treatments, Rye had a significantly higher index 2 value (2.27) at 0-10cm compared to all other treatments, whereas it had the lowest value (2.09) at 10-30 cm, suggesting a shift from a stronger C-functional group composition in surface soil to more O-functional group signatures in the subsoil (Table 4.7).

At WREC, index 1 values were slightly higher than at TVREC. Rye had the highest index 1 values at both depths (1.52 at 0-10 cm and 1.71 at 10-30 cm), indicating a relatively greater contribution of aromatic functional groups compared with all other treatments. But the 10-30cm depth values were higher, suggesting a more aromatic composition in the subsoil. For index 2, the values were higher in the Rye/Clover/Radish mixture (1.38) at 0-10cm soil depth, and clover had a higher index value (1.34) at 10-30cm soil depth, indicating that these treatments contain SOM with a higher proportion of C-functional groups (Table 4.7).

4.5 Principal Component Analysis for the Spectroscopic Indices of WEOM

PCA was performed separately for each site. At both the sites, PCA was applied on indices derived from cover crop soil WEOM, which include dissolved organic carbon (DOC), total nitrogen (TN), absorbance at 254 nm (WL_{254}), specific UV absorbance at 254 nm ($SUVA_{254}$), humification index (HIX), fluorescence index (FI), the E2:E3 ratio, and the C:N ratio. PCA was used to summarize multivariate patterns and identify the main directions of variation in WEOM quantity and quality across cover crop treatments and soil depths at each location.

For the TVREC site, the first two principal components, PC1 and PC2, have eigenvalues greater than 1, and these components explain 68.6% and 15.4% of the total variation, respectively. PC1 was negatively correlated with DOC, HIX, $SUVA_{254}$, WL_{254} , and C:N Ratio, whereas positively associated with FI and E2:E3, indicating a gradient from aromatic, humified WEOM with higher C and N concentrations toward microbially derived WEOM with lower apparent molecular weight. PC2 was primarily driven by the C:N ratio, which loaded positively on this axis, and was opposed by TN, which loaded negatively. Along this axis, WEOM shifts from higher

Table 4.6: Assignment of functional groups to the peaks identified at both sites in the subtracted FTIR spectra of different cover crop treatment soils (based on (Coates, 2000; Margenot et al., 2015a; Parikh et al., 2014)).

Wavenumber (cm ⁻¹)	Functional Group / Assignment
TVREC	
2930	Aliphatic $\nu(\text{C-H})$ stretching (CH ₂ /CH ₃)
1650	Amide I or carbonyl $\nu(\text{C=O})$ stretching or aromatic $\nu(\text{C=C})$ stretching
1550	Amide II $\delta(\text{N-H})$ bending and $\nu(\text{C-N})$ stretching
1450	Aliphatic $\delta(\text{CH}_2/\text{CH}_3)$ bending
1270	Carboxyl or phenolic $\nu(\text{C-O})$ stretching; lignin-associated vibration
WREC	
3400	$\nu(\text{N-H})$ and $\nu(\text{O-H})$ stretching
2930	Aliphatic $\nu_{\text{as}}(\text{C-H})$ asymmetric stretching (CH ₂ /CH ₃)
1650	Amide I or carbonyl $\nu(\text{C=O})$ stretching or aromatic $\nu(\text{C=C})$ stretching
1440	Aliphatic $\delta(\text{CH}_2/\text{CH}_3)$ bending
1300	Carboxylate or phenolic $\nu_{\text{s}}(\text{C-O})$ symmetric stretching
1160	Polysaccharide $\nu(\text{C-O})$ stretching; tertiary alcohol $\nu(\text{C-O})$ stretch
1025	Polysaccharide $\nu(\text{C-O})$ stretching; primary alcohol $\nu(\text{C-O})$ stretch
920	Aromatic $\delta(\text{C-H})$ bending
800	Aromatic $\delta(\text{C-H})$ bending (less substituted)

Vibrational notation: ν = stretching vibration; ν_{as} = asymmetric stretching vibration; ν_{s} = symmetric stretching vibration; δ = bending vibration.

Table 4.7: Mean values of Index 1 (Aromatic/Aliphatic ratio) and Index 2 (C-functional/O-functional groups) at TVREC and WREC across two soil depths.

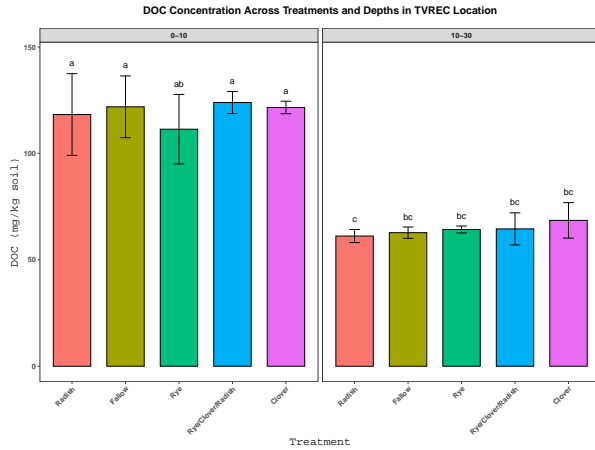
Treatment	TVREC				WREC			
	Index 1		Index 2		Index 1		Index 2	
	0-10	10-30	0-10	10-30	0-10	10-30	0-10	10-30
Radish	1.22	1.21	2.11	2.24	1.47	1.55	1.14	1.15
Fallow	1.13	1.16	2.11	2.25	1.48	1.63	1.22	1.10
Rye	1.24	1.10	2.27	2.09	1.52	1.71	1.16	1.12
Rye/Clover/Radish	1.19	1.26	2.12	2.27	1.40	1.50	1.38	1.19
Clover	1.26	1.12	2.16	2.24	1.46	1.38	1.19	1.34

C:N and lower TN (plant-like, C-rich material) to lower C:N and higher TN (N-rich, microbially influenced material) (Figure 4.11a)

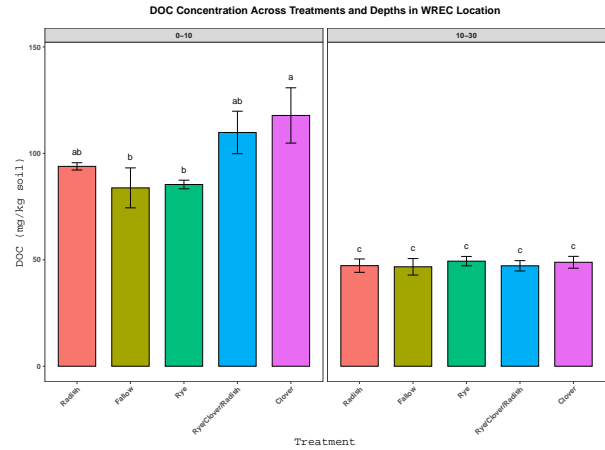
The PCA scores plot for TVREC clearly distinguished samples by soil depth rather than cover crop treatments along PC1 (Figure 4.11b). Samples from 0–10 cm cluster at negative PC1 scores, while most 10–30 cm samples cluster at positive PC1 scores. Since negative PC1 values correlate with higher DOC, SUVA₂₅₄, HIX, WL₂₅₄, and C:N ratio, the 0–10 cm soils contained more aromatic, humified, and C-rich WEOM. Conversely, the 10–30 cm soils shifted toward higher FI and E2:E3 (positive PC1), indicating relatively lower-molecular-weight and more microbially derived WEOM at depth. Different treatment samples were mixed within each depth cluster, suggesting that depth has a much stronger influence on WEOM composition than cover crop treatments at TVREC. Variation along PC2 was smaller and lacked a consistent pattern across treatments, although clover at 0–10 cm shifted toward lower PC2 scores, indicating WEOM with higher TN.

For the WREC site, the first three principal components had eigenvalues greater than 1 and together explained 79.9% of the total variance (48.8%, 18.2%, and 12.9% for PC1, PC2, and PC3, respectively). Subsequent interpretation focused on the first two components, PC1 and PC2, which accounted for 66.9% of the variation and summarized the dominant gradients in WEOM composition. The variable correlation biplot revealed that PC1 was negatively correlated with DOC, TN, WL₂₅₄, FI, and E2:E3, while positively correlated with SUVA₂₅₄ and the C:N ratio. PC1 defined the primary gradient separating WEOM quantity from compositional quality. The negative side of PC1 was associated with higher DOC, TN, and WL₂₅₄, as well as indices of fresher, lower-molecular-weight material (FI, E2:E3), whereas the positive side was dominated by SUVA₂₅₄ and the C:N ratio, indicating more aromatic, C-rich, humified WEOM. PC2 (18.2% of variance) defined a secondary gradient, which was largely independent of PC1. This axis was positively loaded with HIX and negatively loaded with FI and WL₂₅₄. Accordingly, higher PC2 scores correspond to more humified WEOM, whereas lower PC2 scores indicate fresher, microbially derived organic matter with higher FI and WL₂₅₄ (Figure 4.12a).

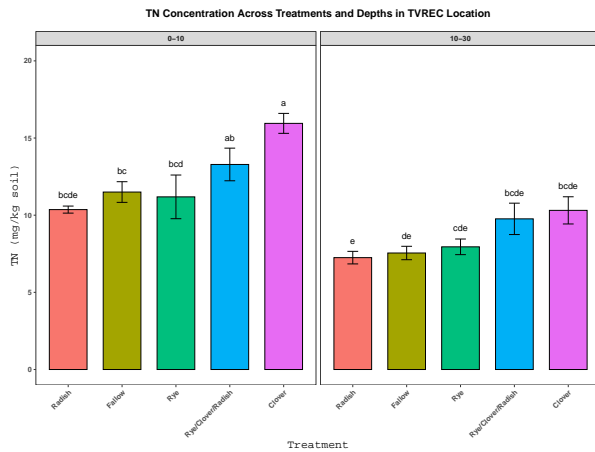
The WREC site PCA plots indicated that soil depth was the primary driver of multivariate variation in WEOM indices. Samples from 0-10 cm were clustered on the negative side of PC1, whereas most 10-30 cm samples occurred on the positive side, forming two partially overlapping but clearly distinguishable depth groups. Negative PC1 scores are associated with higher DOC, TN, and WL_{254} , indicating that the surface soil (0-10cm) has WEOM with a greater concentration and fresher, lower-molecular-weight character. In contrast, positive scores were correlated with higher $SUVA_{254}$ values and a higher C:N ratio, indicating that deeper soils have relatively more aromatic, C-enriched, and humified WEOM (Figure 4.12b).



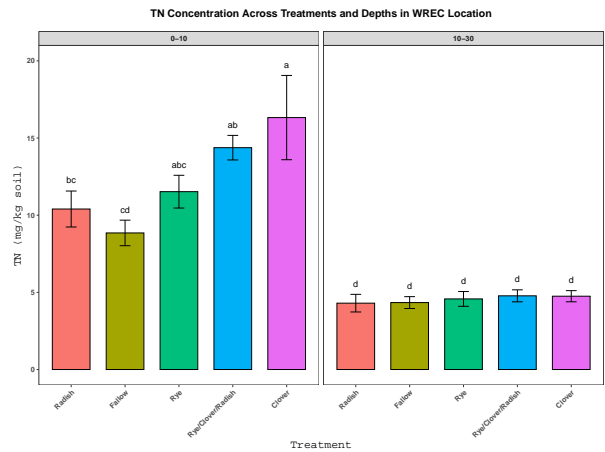
(a) DOC concentration at TVREC.



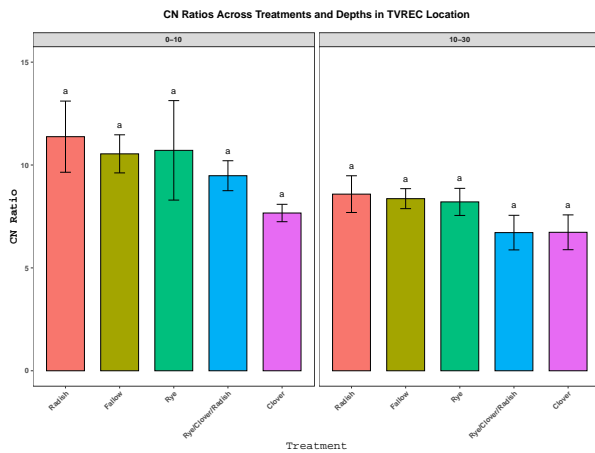
(b) DOC concentration at WREC.



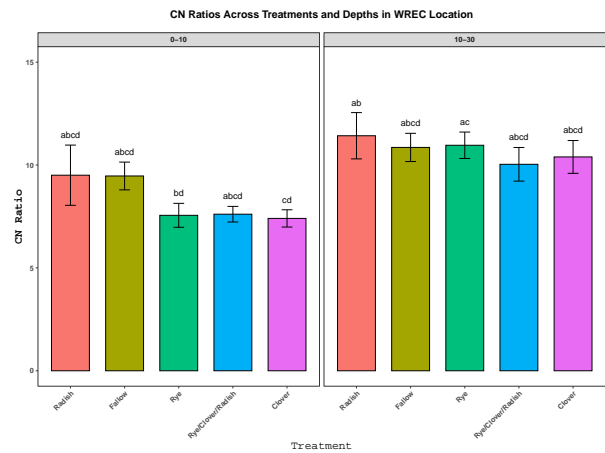
(c) TN concentration at TVREC.



(d) TN concentration at WREC.

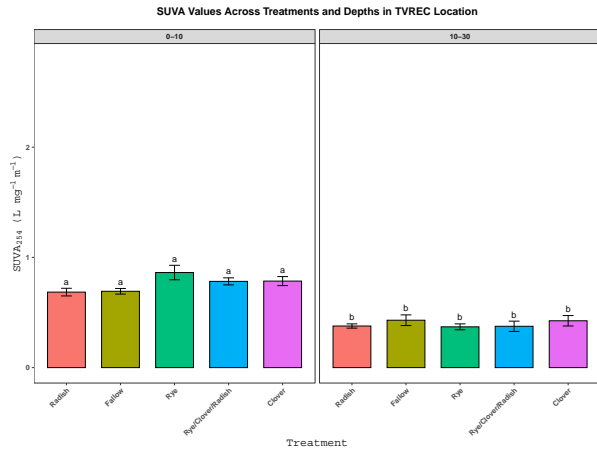


(e) C:N ratio at TVREC.

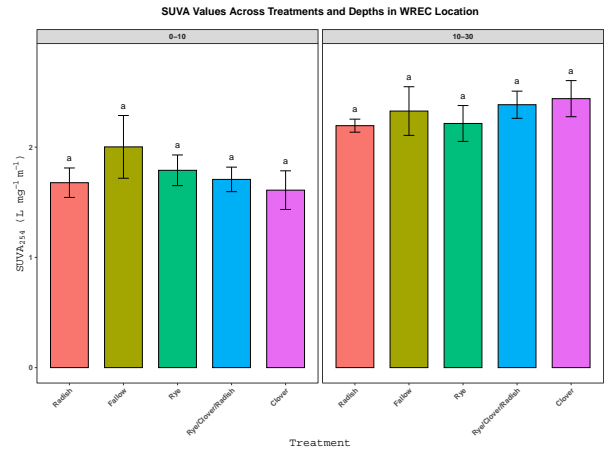


(f) C:N ratio at WREC.

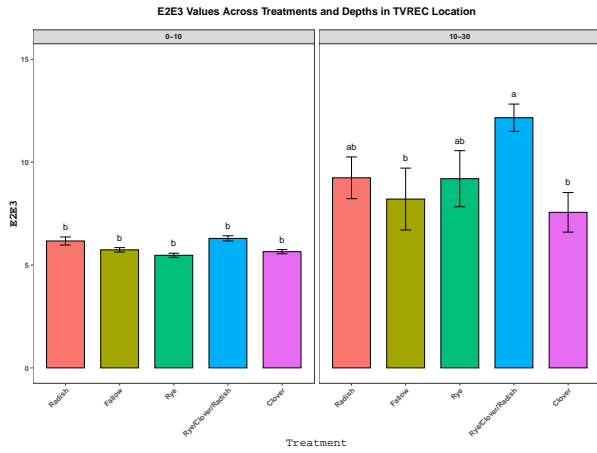
Figure 4.1: Mean DOC, TN, and C:N ratio across treatments and depths at both sites. Panels (a) and (b) show DOC concentrations at TVREC and WREC, (c) and (d) show TN concentrations, and (e) and (f) show C:N ratios. Error bars represent \pm SE ($n = 4$), and different letters indicate significant differences based on Tukey's HSD test ($p < 0.05$).



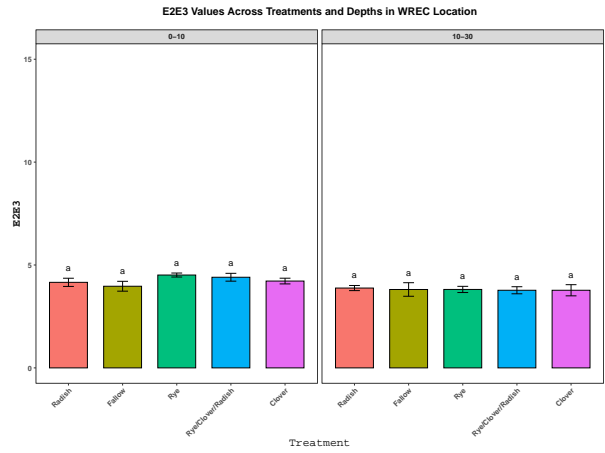
(a) SUVA at TVREC.



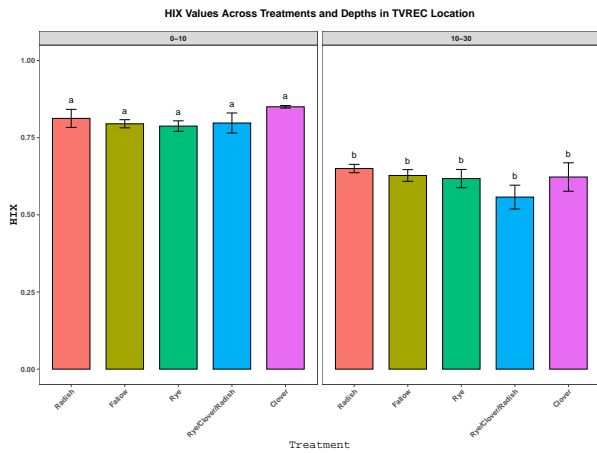
(b) SUVA at WREC.



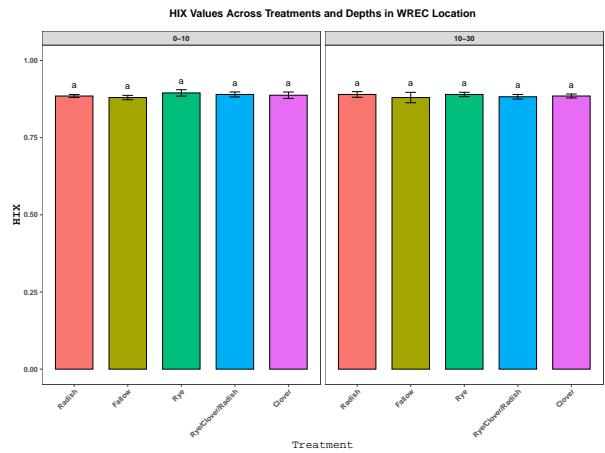
(c) E2:E3 ratio at TVREC.



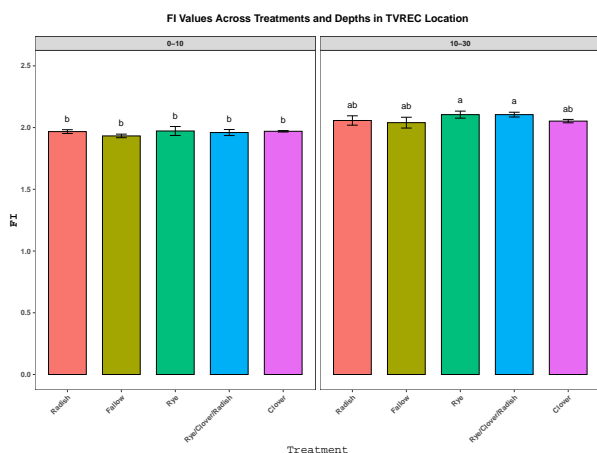
(d) E2:E3 ratio at WREC.



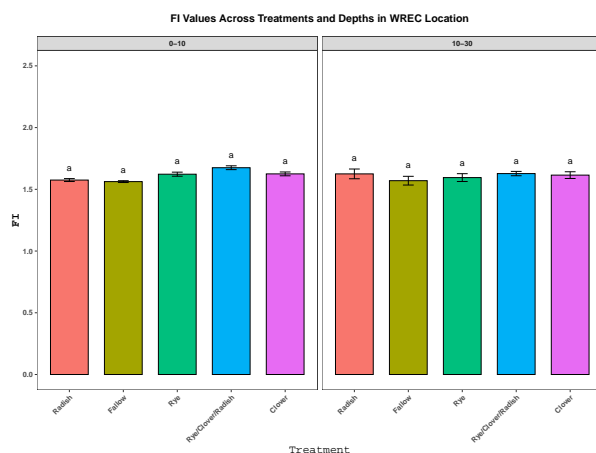
(e) HIX at TVREC.



(f) HIX at WREC.



(g) FI at TVREC.



(h) FI at WREC.

Figure 4.2: Spectroscopic indices across treatments and depths at both sites. Panels (a)–(h) show mean (\pm SE, $n = 4$) values of SUVA, E2:E3, HIX, and FI at TVREC (left) and WREC (right). Different letters indicate significant differences based on Tukey’s HSD test ($p < 0.05$).

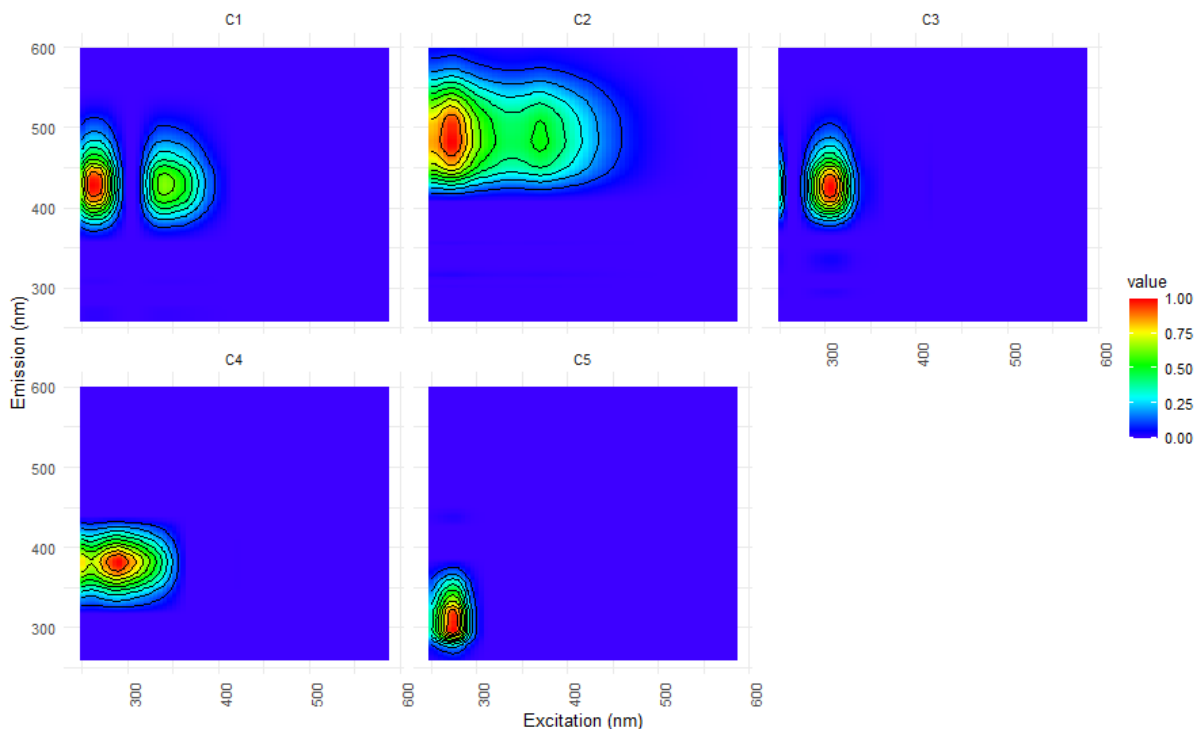


Figure 4.3: Contour plots of the five fluorescent components (C1-C5) identified using Parallel Factor Analysis (PARAFAC). The axes represent excitation and emission wavelengths in nanometers (nm), and the color scale indicates normalized fluorescence intensity.

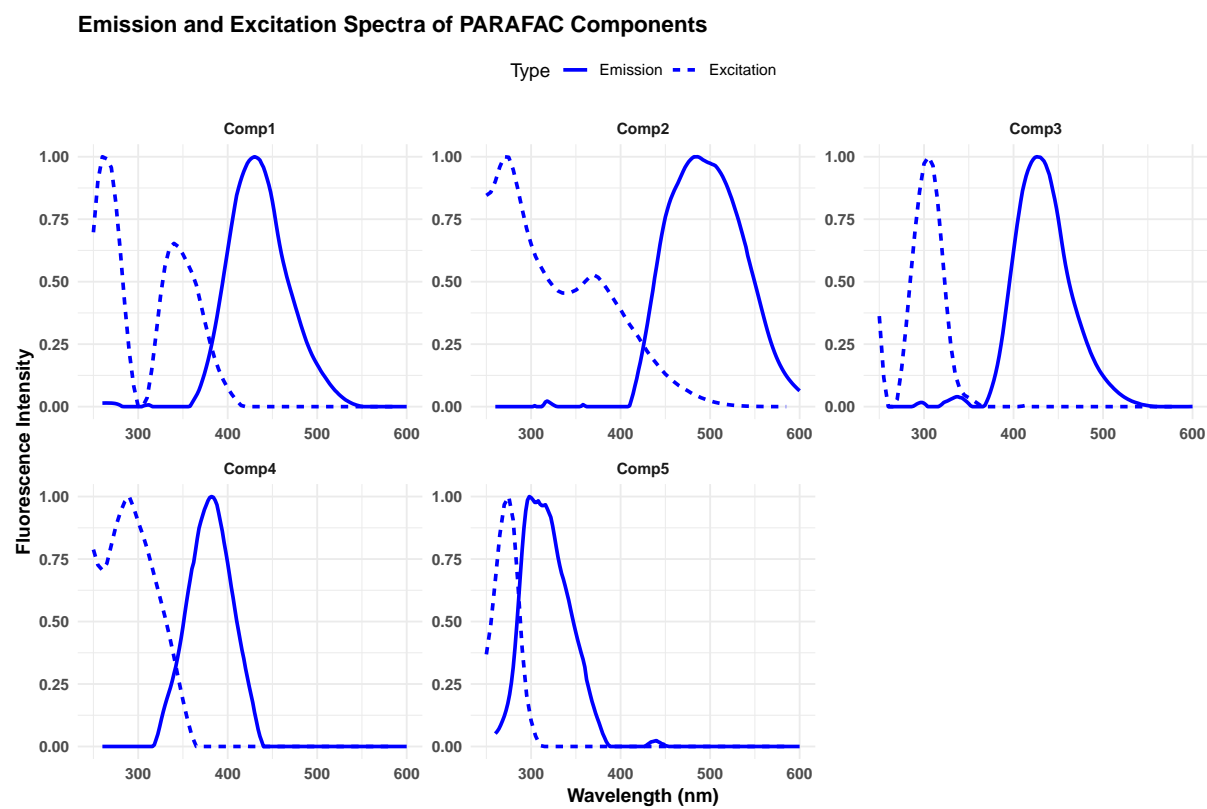


Figure 4.4: Excitation and emission maxima of the five fluorescent components (Components 1–5).

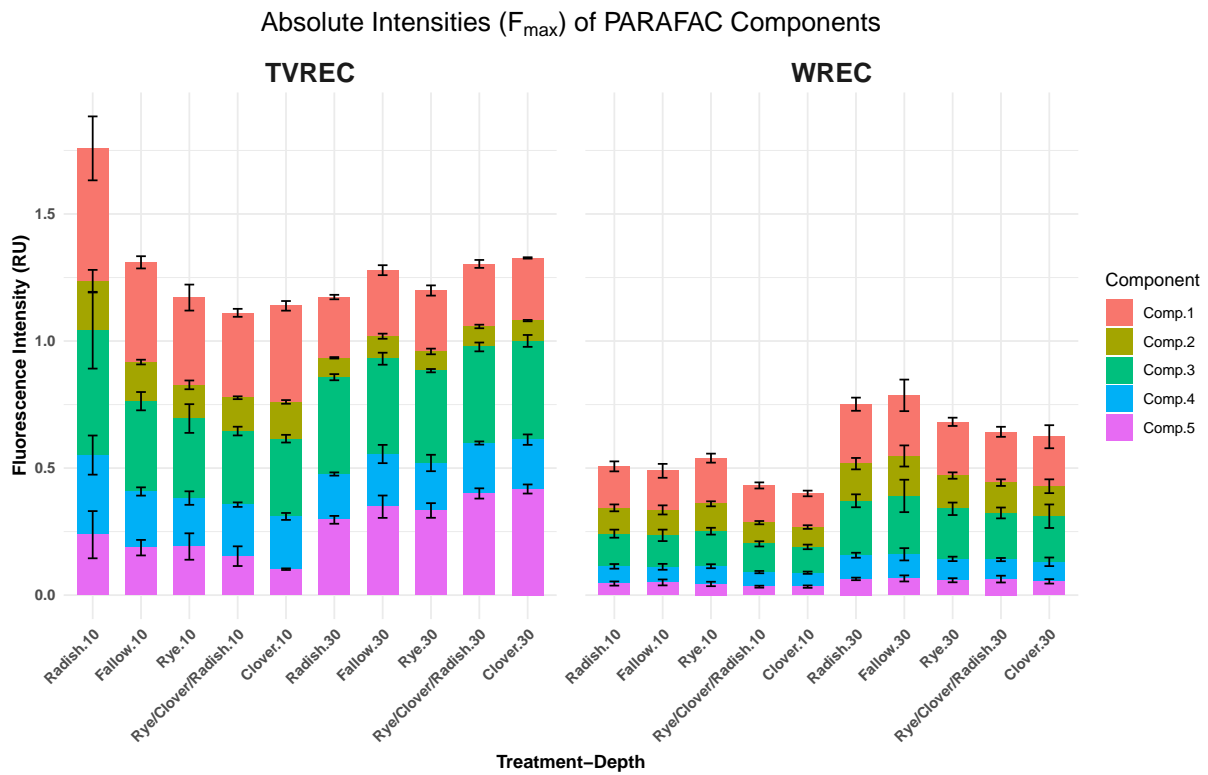
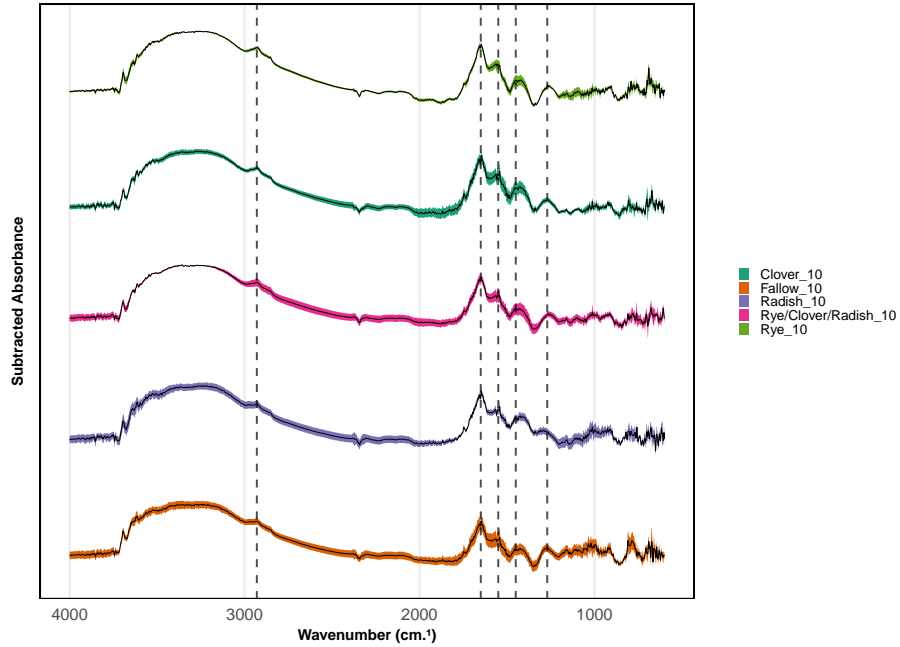


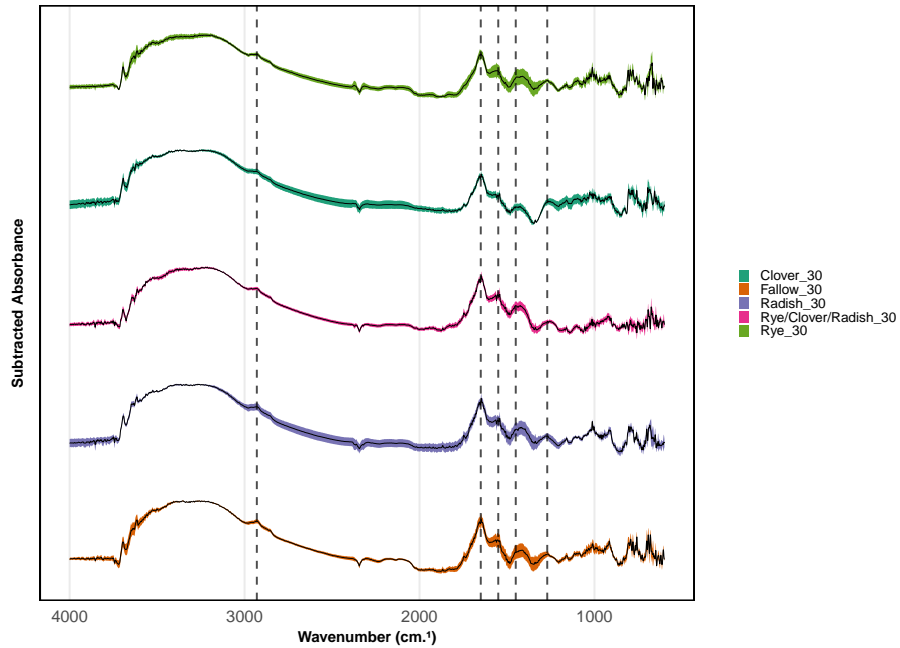
Figure 4.5: Absolute intensities (F_{\max}) of PARAFAC components across treatments and depths at TVREC and WREC. Error bars represent \pm standard error (SE) of the mean.

Subtracted FTIR Spectra of Different Cover Crop Treatments at TVREC (0–10 cm)



(a) TVREC (0-10 cm)

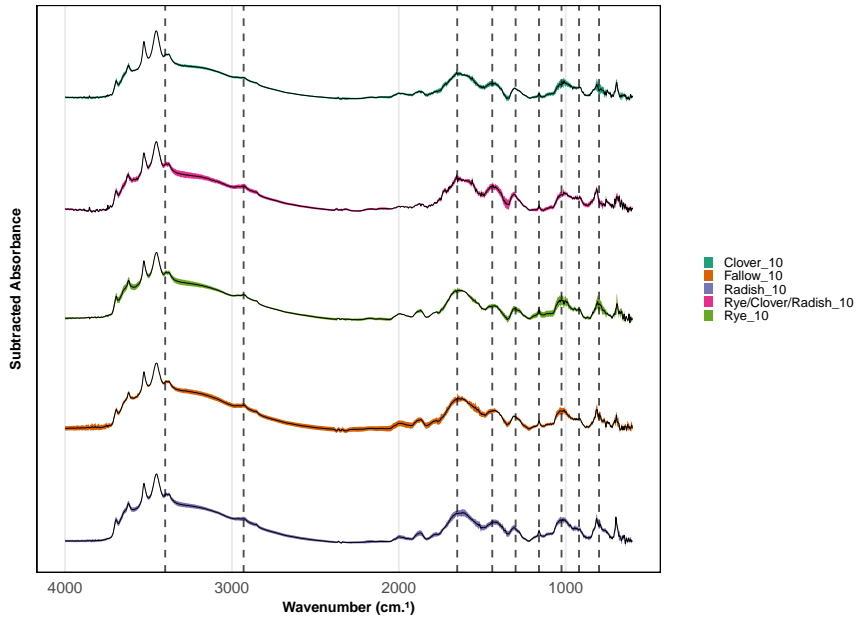
Subtracted FTIR Spectra of Different Cover Crop Treatments at TVREC (10–30 cm)



(b) TVREC (10-30 cm)

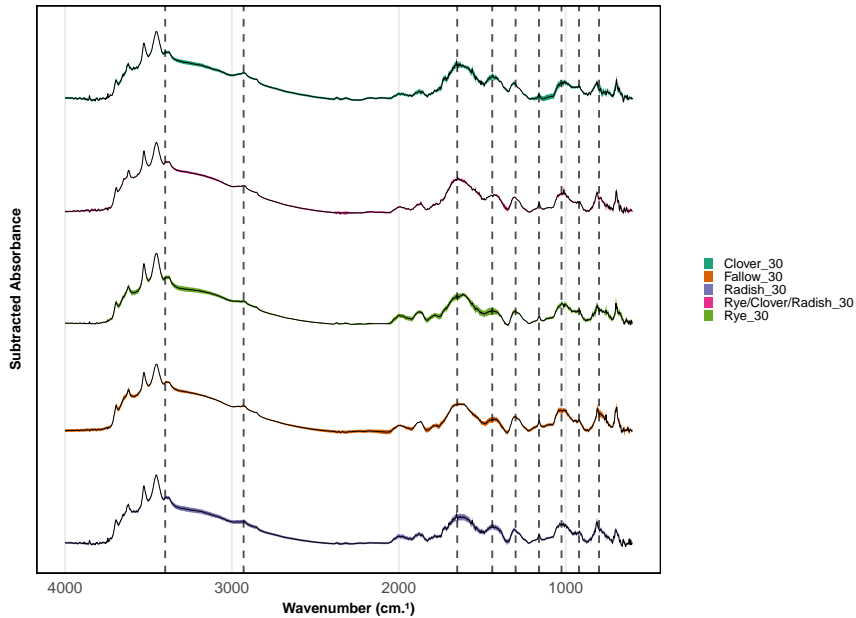
Figure 4.6: Subtracted FTIR spectra of different cover crop treatments at the TVREC site for two soil depths (0-10 cm and 10-30 cm). The black line represents the mean subtracted absorbance spectrum for each treatment, and the colored ribbons indicate the associated standard error (SE). The black vertical dashed lines mark the major FTIR absorption bands (spectral peaks).

Subtracted FTIR Spectra of Different Cover Crop Treatments at WREC (0–10 cm)



(a) WREC (0-10 cm)

Subtracted FTIR Spectra of Different Cover Crop Treatments at WREC (10–30 cm)



(b) WREC (10-30 cm)

Figure 4.7: Subtracted FTIR spectra of different cover crop treatments at the WREC site for two soil depths (0-10 cm and 10-30 cm). The black line represents the mean subtracted absorbance spectrum for each treatment, and the colored ribbons indicate the associated standard error (SE). The black vertical dashed lines mark the major FTIR absorption bands (spectral peaks).

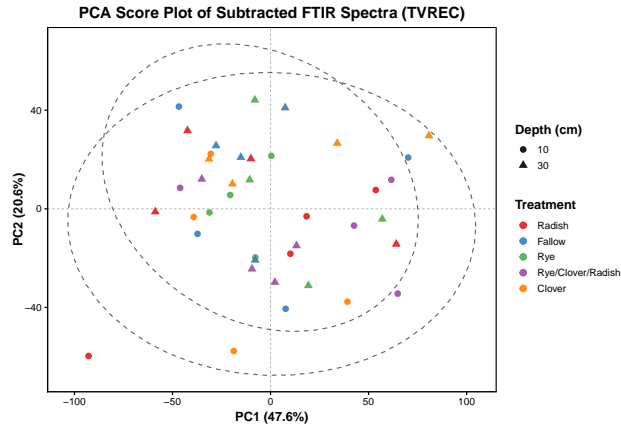


Figure 4.8: PCA score plot of subtracted FTIR spectra for the TVREC site.

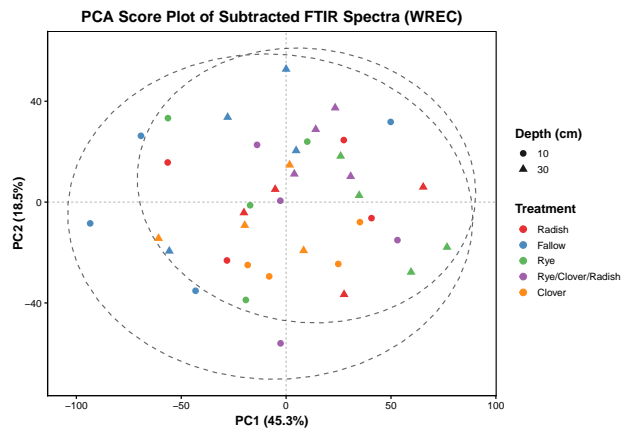


Figure 4.9: PCA score plot of subtracted FTIR spectra for the WREC site.

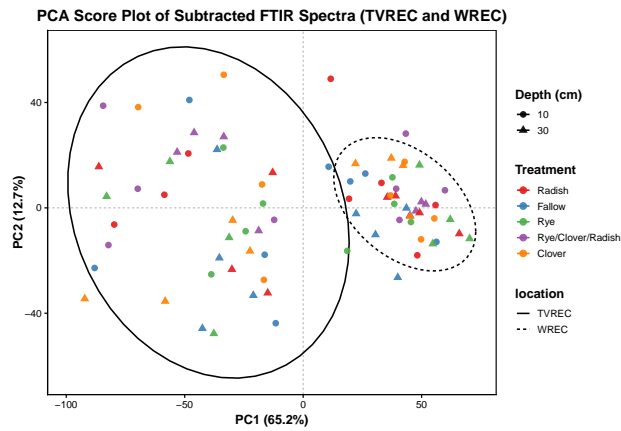
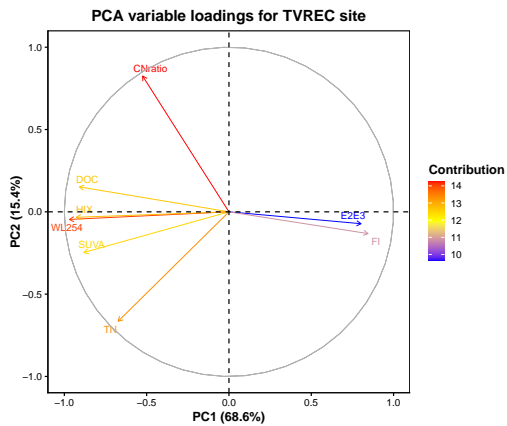
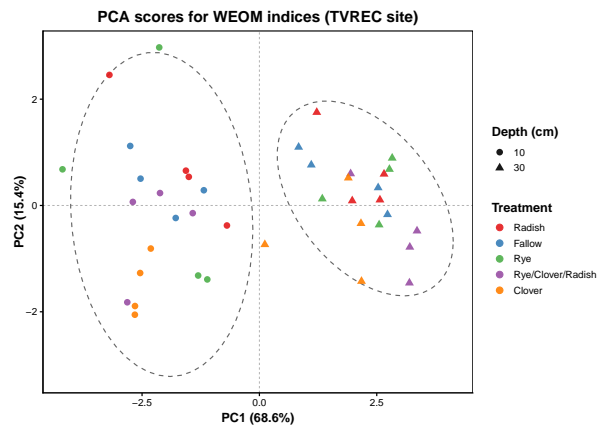


Figure 4.10: Combined PCA score plot of subtracted FTIR spectra for TVREC and WREC sites.

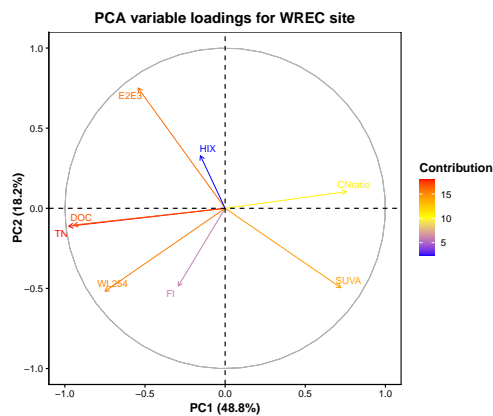


(a) PCA variable loadings for WEOM indices at TVREC.

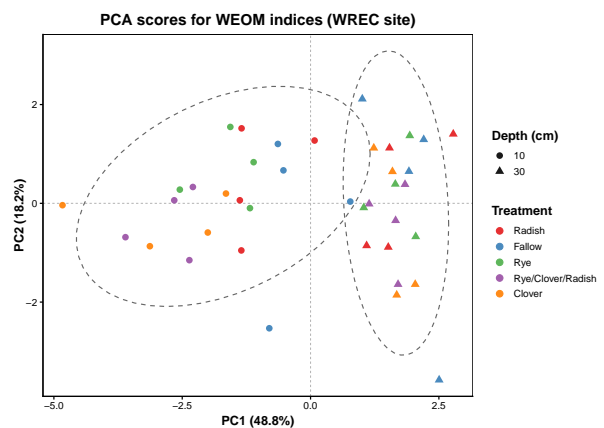


(b) PCA scores for WEOM indices at TVREC.

Figure 4.11: Principal component analysis of WEOM indices at the TVREC site. (a) Variable loadings for PC1 and PC2. (b) Sample scores colored by cover crop treatment and shaped by depth.



(a) PCA variable loadings for WEOM indices at WREC.



(b) PCA scores for WEOM indices at WREC.

Figure 4.12: Principal component analysis of WEOM indices at the WREC site. (a) Variable loadings for PC1 and PC2. (b) Sample scores colored by cover crop treatment and shaped by depth.

Chapter 5

Discussions

5.1 Site, Depth, and Physicochemical Controls on WEOM and DOC Dynamics

This study evaluated the effects of long-term cover cropping systems on the quantity and composition of WEOM and SOM using spectroscopic techniques across two contrasting agricultural sites. The soils in the southeastern United States are predominantly highly weathered Ultisols, with a long history of degradation from intensive tillage, erosion, and continuous row-crop production. Under the region's warm, humid climatic conditions, organic matter turnover is rapid, often leading to depleted SOM pools. We hypothesized that differences among cover crop species would result in diverse variation in WEOM and SOM composition due to differences in organic matter inputs and transformation pathways. However, soil depth and site-specific physicochemical properties emerged as the dominant factors influencing WEOM and SOM concentration and composition, whereas the effects of cover crop treatment were weaker than expected and site-specific. Our results indicate that while management can shift specific nutrient pools, the fundamental chemical composition of these soils remains heavily governed by their pedogenic history and high degree of weathering.

The TVREC and WREC sites differed in soil texture, mineralogy, and management history, which strongly influenced WEOM and SOM dynamics. The TVREC site is characterized by silt loam soils managed under long-term no-till (>20 years), whereas the WREC site consists of loamy-sandy soil under conservation tillage and experienced periodic disturbance associated

with peanut harvesting. These site differences are clearly reflected in the results, with distinct site-specific patterns in WEOM indices PCA, clear site separation in ash-subtracted FTIR-based PCA, and different depth responses in PARAFAC fluorescence components.

At TVREC, the silt loam matrix provides a larger reactive surface area composed of Fe and Al oxides and phyllosilicate minerals. These reactive sites facilitate the stabilization of organic compounds through inner- and outer-sphere complexation (Wagai et al., 2020). High sorption capacity and physical occlusion within stable microaggregates are key mechanisms that stabilize organic carbon in surface soils (Amelung et al., 2023; Six et al., 2002). This mechanism is evidenced by the sharp $\approx 50\%$ decline in DOC concentrations between the 0-10 cm and 10-30 cm depth at TVREC, indicating strong retention of dissolved organic matter near the surface and limited downward mobility. The WREC site was dominated by a quartz-rich sandy matrix that lacks these mineral anchors. They have limited reactive surface area, and the presence of large macropores promotes preferential flow paths that facilitate rapid vertical transport of WEOM and thereby reduce sorptive retention of DOC in surface soils (Jardine, Wilson, Luxmoore, & McCarthy, 1989; Neff & Asner, 2001). WREC exhibited lower surface DOC concentrations than the TVREC site, reflecting reduced dissolved carbon retention and more rapid turnover or transport of WEOM through the sandy soil profile. The lower C:N ratios observed at WREC further indicate faster organic matter turnover and a limited carbon pool, relative to the more persistent, carbon-rich organic matter retained at TVREC (Manzoni, Porporato, & Schimel, 2008).

5.2 Water-Extractable Organic Carbon Composition Derived from Optical Indices Differed Between the Sites and Depth

The TVREC site spectroscopic indices have resulted in lower SUVA values, relatively high HIX and FI values. Specific ultraviolet absorbance (SUVA) at 254 nm is used as an indicator of WEOM aromaticity, with higher values reflecting a greater proportion of aromatic, UV-absorbing compounds (Helms et al., 2008; Weishaar et al., 2003). Lower SUVA values indicate a WEOM pool depleted in aromatic structures. In silt loam soils, sorption of hydrophobic, aromatic compounds

to Fe- and Al-rich oxide phases and clay mineral surfaces will be greater, leading to their removal from the dissolved phase (K. Kaiser & Guggenberger, 2000; K. Kaiser, Guggenberger, & Zech, 2001). This selective removal of plant-derived aromatic compounds leaves a dissolved pool enriched in more polar, microbially processed material (Kalbitz et al., 2000), which aligns with the higher FI values (≈ 2.0 – 2.1) observed at TVREC.

Generally, fluorescence index (FI) distinguishes WEOM sources by differentiating microbially derived organic matter (FI ≈ 1.8 – 2.1) from terrestrially derived, plant-associated material (FI ≈ 1.2 – 1.6) (Fellman et al., 2010; McKnight, Boyer, et al., 2001). This information provides insight into the dominant origin and processing of WEOM, which influences microbial activity, substrate quality, and the potential pathways of nutrient cycling in soil. Fine-textured soils have smaller pores and lower saturated hydraulic conductivity, which slows water movement and increases the residence time of soil solution (Jarvis, 2007; Nimmo, 2021). This extended contact time enhances opportunities for microbial transformation of dissolved organic matter (K. Kaiser & Kalbitz, 2012; Kalbitz et al., 2000). Long-term no-till management at TVREC further promotes the development of a biologically active surface horizon, supporting sustained microbial activity and the accumulation of microbial metabolites and necromass in the dissolved organic matter pool (Hansen et al., 2016). These conditions led to the WEOM of TVREC soil samples, predominantly of microbial origin, with higher FI values. The HIX provides information on WEOM composition by indicating the degree of humification and the structural complexity of fluorescent organic matter. In my study, HIX was calculated using the scaled formulation, with values ranging from 0 to 1. Higher HIX (near 1) values generally reflect a greater proportion of humified, condensed, and structurally complex organic compounds, whereas lower values indicate a larger contribution of less-humified material (Ohno, 2002; A. Zsolnay et al., 1999). HIX values at TVREC were (≈ 0.6 in 10-30 cm soil and ≈ 0.8 in 0-10 cm soil), reflecting the intense humification and structural complexity of WEOM in the upper horizon, where fresh residue inputs and microbial reworking are most active. The decline in HIX with depth suggests that as WEOM moves downward, the more

humified and condensed fluorescent molecules are either selectively retained by the mineral matrix or diluted by less-humified material, a pattern typically seen in mineral-rich soils with strong organo-mineral associations (Kalbitz et al., 2000; Ohno, 2002; A. Zsolnay et al., 1999).

At WREC, higher SUVA values, particularly at 10-30 cm, indicate a greater contribution of aromatic, UV-absorbing compounds to the dissolved pool. Because SUVA reflects WEOM aromaticity (Helms et al., 2008; Weishaar et al., 2003). The weak sorptive capacity, aromatic WEOM is less selectively retained and can be transported vertically through the profile, resulting in higher SUVA values at deeper depths (Jiang et al., 2017; K. Kaiser et al., 2001; Kalbitz et al., 2000). This rapid preferential flow reduces contact time between WEOM and microbial communities, thereby reducing microbial reprocessing (Jiang et al., 2017; Neff & Asner, 2001). Furthermore, the periodic tillage associated with peanut production disrupts soil aggregates, redistributes organic inputs within the soil profile, and accelerates the decomposition of labile fractions (Six et al., 2002). These combined physical and hydrological drivers maintain a WEOM pool that is less processed by microbes, favoring the persistence of a terrestrial, plant-associated signature—as evidenced by the lower FI values we observed (1.6-1.7) (Hansen et al., 2016) and higher SUVA values at WREC. This higher abundance of plant-associated signatures provides the primary substrates (e.g., lignin, polyphenols) for the formation of humic substances, thereby driving the humification process (Kögel-Knabner, 2002), resulting in higher HIX values at WREC (≈ 0.9).

The E2:E3 ratio serves as an inverse proxy for dissolved organic matter molecular size, with higher values indicating a greater contribution of lower-molecular-weight, less condensed compounds (Helms et al., 2008; Weishaar et al., 2003). The contrasting depth patterns of E2:E3 between sites provide further evidence of the divergent WEOM transformation pathways. At TVREC, E2:E3 values increased with depth, indicating a downward enrichment of smaller, more fragmented WEOM. This pattern is characteristic of fine-textured, reactive soils where strong organo-mineral interactions selectively retain larger, aromatic compounds in surface horizons, allowing a more processed, lower-molecular-weight fraction to leach (K. Kaiser et al., 2001; Kalbitz et al., 2000). Long-term no-till management stabilizes this environment, promoting stratification

and allowing such molecular fractionation to develop over time (Six et al., 2002). In contrast, WREC exhibited uniformly low E2:E3 values with depth, signaling the persistence of larger, more condensed WEOM molecules. The coarse, quartz-dominated texture provides limited sites for sorptive fractionation, and rapid drainage limits the in-situ microbial reprocessing that would break down complex molecules (Kalbitz et al., 2000; Neff & Asner, 2001). Furthermore, periodic tillage disrupts aggregates and mixes organic matter across depths, actively homogenizing the WEOM pool and suppressing the development of vertical chemical gradients (Six et al., 2002).

5.3 Influence of Leguminous Cover Crops on Soil Nitrogen Dynamics in Long-Term Systems

The significant increase in TN concentrations observed under Clover and the Rye/Clover/Radish (RCR) mixture at both TVREC and WREC can be directly attributed to biological nitrogen fixation (BNF). Leguminous cover crops, such as clover, form symbiotic relationships with Rhizobium bacteria to fix atmospheric N_2 , providing a specialized internal source of nitrogen, and clover provides N from root inputs (D. W. Reeves, 2018). These N-rich inputs promote microbial assimilation and nitrogen incorporation into soil organic matter, thereby increasing TN concentrations, particularly in surface soils where biological activity and residue accumulation are greatest.

5.4 Dominance of Soil and Climatic Controls over Cover Crop Species Effects on Soil Organic Carbon and Soil Organic Matter

The absence of significant differences among cover crop treatments in this study was consistent with findings from multiple field-based studies demonstrating that cover crop effects on soil carbon pools are regulated by a suite of interacting factors beyond residue decomposition alone. Chu et al. (2017) reported no measurable differences in total SOC among multiple cover crop treatments during the early years of cover cropping, explaining the weak response to antecedent SOC levels, warm climatic conditions that favor rapid mineralization, and SOC stratification under no-tillage systems that can dilute treatment effects when sampled over broader depth intervals. Similarly, Acharya, Ghimire, Cho, Thapa, and Sainju (2022) found that five years of cover cropping with

multiple species and mixtures did not significantly alter soil profile C and N contents, with treatment effects confined mainly to specific surface layers and constrained by strong environmental and soil controls on carbon stabilization. Anuo, Cooper, Koehler-Cole, Ramirez, and Kaiser (2023) showed that even under multi-year no-till cover cropping, bulk SOC, N, and several SOM fractions did not differ among treatments, highlighting that cover crop impacts depend on multiple interacting controls, including biomass inputs, root-derived carbon contributions, climatic limitations on growing season length, residue placement under no-till, and sampling depth. WEOM, a component of SOM, is strongly controlled by its interactions with soil minerals through chemical bonding and physical aggregation (Sollins, Homann, & Caldwell, 1996). Together, these studies support the interpretation that, in long-term field systems, soil properties, climate, and management history can override species-specific cover crop effects, resulting in limited differentiation among treatments in soil carbon metrics.

5.5 PARAFAC Derived Fluorescent components of Cover Crops Water-Extractable Organic Matter

PARAFAC analysis resolved five distinct fluorescent components, of which four were humic-like (C1-C4), indicating that the WEOM pool was dominated by humic-like substances, with a smaller but significant protein-like fraction (C5). Humic-like substances are heterogeneous mixtures of plant- and microbially derived organic compounds and are operationally defined fractions of soil organic matter. These compounds are commonly associated with chemically complex and relatively slowly cycling carbon pools in terrestrial ecosystems (Kelleher & Simpson, 2006). The majority of soil organic matter consists of humic substances (approximately 40-60%), which have relatively slow turnover times (Romero, Engel, D'Andrilli, Miller, & Wallander, 2019). Previous studies using classical humus fractionation have demonstrated that cover crops and residue incorporation promote humification processes and improve humus quality, particularly by increasing humic acid fractions and the humification rate (Arlauskienė, Maikštėnienė, & Šlepetienė, 2010). The dominance of humic-like components (C1-C4) in the WEOM pool is due to the chemically

complex, aromatic, plant-derived organic structures, which are progressively transformed by microbes into redox-active moieties (Cory & McKnight, 2005b; Hernes, Bergamaschi, Eckard, & Spencer, 2009). In contrast, labile fractions like C5 (protein-like) are commonly associated with recently produced, labile organic matter derived from microbial biomass, root exudates, and fresh plant litter inputs, and are therefore characterized by rapid microbial utilization and turnover (Fellman et al., 2010; McKnight, Boyer, et al., 2001; Romero et al., 2019). It is subject to rapid microbial utilization and turnover, whereas humic-like fractions tend to persist due to their complex molecular structure and form stabilized associations with soil mineral surfaces via ligand exchange and hydrophobic interactions (K. Kaiser & Guggenberger, 2000; Kalbitz et al., 2000).

To assess the concentration differences of these distinct DOM fractions, we analyzed the absolute fluorescence intensities (F_{max}) of each PARAFAC component. Site differences and depth differences were dominant factors rather than treatment effects. The WEOM pool at the TVREC site showed higher fluorescence intensities for all components (C1-C5) than at the WREC site, regardless of depth or treatment. This suggests differences in the total pool size of fluorescent DOM, likely driven by contrasting site characteristics such as soil texture, mineralogy, native organic matter content, or historical land management (De Troyer, Merckx, Amery, & Smolders, 2014; Filep & Rékási, 2011). Statistical analysis confirmed that soil depth was a highly significant factor ($p < 0.001$) for most components at both locations, whereas the effect of cover crop treatment was not significant for any component (Table 4.4). At the TVREC site, higher F_{max} values for several humic-like components in surface soils likely reflect greater organic matter inputs, enhanced microbial activity, and stronger organo-mineral associations near the soil surface under long-term no-till management. In contrast, humic-like component intensities at the WREC site generally increased with depth, consistent with observations by (Romero et al., 2019), who reported enrichment of more processed and microbially transformed DOM fractions in subsurface horizons. This pattern suggests enhanced downward transport and redistribution of dissolved organic matter in coarser-textured or disturbed soils (K. Kaiser & Benner, 2012). The protein-like component (C5) also exhibited significant depth effects, with higher intensities in subsurface soils at both sites.

(Romero et al., 2019) similarly observed increased fluorescence efficiency and relative importance of microbially derived DOM with depth, which they attributed to root-derived inputs and microbial turnover combined with reduced microbial consumption in deeper soil layers.

Effects of cover crop treatments on PARAFAC component intensities were weak and consistently non-significant. This limited influence of management on DOM composition has been observed in other long-term agricultural systems. Romero et al. (2019) observed that PARAFAC-derived DOM composition differed minimally among contrasting cropping systems, despite measurable differences in soil organic carbon stocks, indicating that DOM quality may be buffered against management-induced change through microbial processing and soil physicochemical controls. Grebliunas, Armstrong, and Perry (2016) reported that the microbial community's processing of fresh cover crop residues is often so rapid that specific plant-derived signatures are ephemeral, leaving a bulk pool dominated by the stable, humified background of native soil organic matter.

5.6 Site-Specific Differences in Soil Organic Matter Functional Group Chemistry Identified by FTIR Spectroscopy and PCA

Subtracted FTIR analysis demonstrated that site-level controls exerted the strongest influence on SOM functional group composition, rather than the cover crop treatment and soil depth. This was clearly evident in the PCA analysis, where PC1 accounted for 65% of the variation between the sites. Similar observations were reported by (Margenot et al., 2015a), who found that differences in SOM functional group composition were evident among sites. Still, these differences primarily reflected inherent soil properties and long-term SOC accumulation rather than specific management treatments, whose effects appear to operate within narrower bounds that may be less detectable by bulk FTIR approaches. F. Calderón et al. (2013) demonstrated that differences in parent material and clay mineralogy strongly influence soil infrared spectra and can mask management-induced changes through mineral interference and SOM-mineral interactions. Within each site, the overlap in PCA score plots and consistent peak patterns (Figures 4.6 and 4.7) suggests that the "backbone" of SOM remained relatively stable across treatments. The lack of detectable separation among

treatments like Rye, Clover, or Fallow suggests that either (i) residue inputs were insufficient in magnitude to alter bulk SOM functional group composition, or (ii) rapid microbial processing converted diverse inputs into a uniform pool of microbial-derived organic matter, as described by (F. Calderón et al., 2013) and discussed by (Margenot et al., 2015a). The strong aliphatic C-H stretching (2930 cm^{-1}) and bending (1450 cm^{-1}) bands, pronounced in both sites, indicate the presence of organic macromolecules that may originate from plant cuticular waxes and microbial lipids (Kögel-Knabner, 2002; Margenot et al., 2015a). This aliphatic enrichment in OM usually occurs due to residue decomposition, such as with cover crops (Ait Baddi, Albuquerque, González, Cegarra, & Hafidi, 2004; Aranda et al., 2011; Hsu & Lo, 1999). The prominent peaks at 1650 cm^{-1} and 1550 cm^{-1} are associated with amide I (C=O stretch) and amide II (N-H bend/C-N stretch) vibrations, respectively, and are key indicators of proteinaceous materials and microbial necromass (F. J. Calderón, Reeves, Collins, & Paul, 2011; Parikh et al., 2014). Absorbance in the $1700\text{-}1500\text{ cm}^{-1}$ region has been associated with both amide functional groups linked to microbial biomass and carbonyl or aromatic C=C structures typical of humified SOM, depending on soil context and degree of organic matter processing (F. J. Calderón, Mikha, et al., 2011; Shirshova, Ghabbour, & Davies, 2006). Polysaccharide- and cellulose-associated functional groups are known to contribute to absorbance in the O-H stretching region near 3400 cm^{-1} , aliphatic C-H stretching between $2970\text{ and }2800\text{ cm}^{-1}$, and C-O stretching bands between approximately $1160\text{ and }1030\text{ cm}^{-1}$. Previous studies have shown that absorbance near $3400\text{ and }2870\text{ cm}^{-1}$ is associated with relatively labile carbon and tends to decline during decomposition of organic amendments (F. J. Calderón, McCarty, & Reeves, 2006). In addition, absorbance around 1100 cm^{-1} has been linked to polysaccharide binding agents that contribute to soil aggregation (Sarkhot, Comerford, Jokela, Reeves, & Harris, 2007).

In our study, the higher aromaticity (index 1- aromatic to aliphatic ratios) observed at the WREC site, compared to TVREC, is reflected in more intense aromatic C-H peaks, likely indicating more advanced decomposition of the bulk soil organic matter at that site (Chefetz et al., 1998; Hsu & Lo, 1999). This also aligns with the higher SUVA_{254} and HIX values observed at the

WREC site. Index 2 (C-functional group to O-O-functional group) values are higher in TVREC, indicating the higher proportion of C-H and C=C bonds relative to C-O bonds may indicate organic matter that is less polar and potentially more hydrophobic (Ding et al., 2002; Veum et al., 2014).

Chapter 6

Conclusion

This study examined the quantity and composition of WEOM and SOM in soils under long-term cover cropping at two locations in the southeastern United States. PCA of spectroscopic indices and ANOVA showed the significant depth differentiation in WEOM composition, whereas cover crop treatments had a very small influence. PCA of FTIR spectra indicated that SOM functional group composition varied between the sites rather than among treatments or depths. Fine-textured, reactive soils under long-term no-till management at TVREC had strong organo-mineral associations, enhanced microbial processing, and surface accumulation of WEOM and humified SOM. In contrast, coarse-textured, quartz-dominated soils subjected to periodic tillage at WREC had vertical transport of WEOM and deeper redistribution of aromatic and humified organic components. Hence, we conclude that the contrasting soil environments at the two sites followed different pathways for organic matter stabilization and transport due to differences in soil type, tillage, and climatic factors. We hypothesized that different cover crop species residues would cause distinct differences in organic matter inputs and transformation pathways; however, their effects on WEOM and SOM composition were relatively weak. The overall results demonstrate that leguminous cover crops and mixtures are highly effective tools for increasing soluble nitrogen pools in the top 30cm of soil within a 7-year timeframe, and soil mineralogy, hydrology, and depth-dependent processes primarily determine the stabilization and transformation of organic matter. However, cover crop species play a secondary role in regulating organic matter inputs and microbial processing, thereby influencing WEOM and SOM dynamics within soil-specific constraints.

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