DISSERTATION

MECHANISMS AND MANAGEMENT FOR SOIL CARBON SEQUESTRATION

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ABSTRACT

MECHANISMS AND MANAGEMENT FOR SOIL CARBON SEQUESTRATION

Soil organic matter (SOM) holds more carbon (C) than the atmosphere and terrestrial aboveground biomass combined. SOM also provides many other co-benefits in the form of ecosystem services. The rate at which we lose or sequester more C in soils is of great importance for mitigating the rising atmospheric greenhouse gas concentrations as well as for maintaining the fundamental services that soils provide to humanity. Many of the mechanisms involved in accruing and storing soil C are not entirely clear, and factors like litter chemistry and minerology can all come into play when determining the sequestration potential of a specific ecosystem. Additionally, not all soil C is equal in its turnover time or in its ability to resist disturbance. Therefore it is crucial that we better understand how functionally different soil C pools form and persist in the soil environment. Several “climate smart” soil management practices have been analyzed for their potential to sequester more C. However there are still gaps in our knowledge regarding soil C sequestration and how it can be impacted by land use management. The southeast US is a region with particularly severe soil degradation from poor agricultural management, but also has a high potential for increased soil C sequestration and overall soil health. This dissertation looks at some potential mechanisms and management practices involved with storing more stable soil C in the southeastern US. Mechanisms include how litter chemistry and soil C saturation can enhance or inhibit soil C sequestration. Then, we evaluated management practices from pine plantations and grassland grazing in the

southeastern US to evaluate if improved management could increase soil C stocks, their distribution, and overall soil health.

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INTRODUCTION

Increasing atmospheric greenhouse gas concentrations, with the resulting climate change are some of the biggest challenges humanity is currently facing. Several greenhouse gas and climate change mitigation strategies have been proposed recently, ranging from engineered solutions to natural carbon sinks (Paustian et al., 2016; Chabbi et al., 2017). One of the most promising strategies includes managing soils for carbon (C) sequestration (Schimel et al., 1990; Chabbi et al., 2017). Soil organic matter (SOM) holds much more C than the atmosphere and terrestrial aboveground biomass (Ciais et al., 2013). Soil organic matter is also important because it provides many other co-benefits in the form of ecosystem services. For example SOM improves nutrient and water retention, supports microbial communities, and can help maintain successful crop yields, etc. (Balesdent et al., 2000; Smith et al., 2001; Grandy and Robertson, 2006). Therefore, the rate at which we lose or sequester SOM, specifically C, in soils is of great importance for mitigating the rising atmospheric greenhouse gas concentrations as well as for maintaining the fundamental services that soils provide to humanity (Schimel et al., 1990).

Many of the mechanisms involved in storing soil C are not entirely understtod as they are often dependent on the climate regime and the type of ecosystem (Rasmussen et al., 2018). Things like aboveground and root litter chemistry, soil C saturation deficit, and mineralogy can all come into play when determining the sequestration potential of a specific location (Cotrufo et al., 2013; Castellano et al., 2015; Rasmussen et al., 2018). Additionally, not all soil C is equal in its turnover time or in its ability to resist disturbance. For example, particulate organic matter

(POM) and mineral-associated organic matter (MAOM) are formed through different pathways and they reside in the soil for differing time periods (Cotrufo et al., 2015; Cotrufo et al., 2019). MAOM can eventually saturate in soils with low amounts of silt and clay, whereas POM does not saturate in these soils as it is not associated with silt and clay (Cotrufo et al., 2019). Therefore it is crucial that we better understand how each soil C pool forms and persists in the soil environment. It is also important to understand which types of C to manage for depending on the C sequestration potential of the location.

Several climate smart soil management practices have been analyzed for their potential to accrue more C (Paustian et al., 2016; Chabbi et al., 2017). However there are still gaps in our knowledge regarding soil C sequestration and how it can be impacted by land use management. Some management strategies that have been given attention more recently are grassland management, crop management, biochar amendments, enhanced root phenotypes, as well as restoration of degraded lands to name a few (Paustian et al., 2016). These strategies all have varying degrees of greenhouse gas mitigation potential as well as different potentials based on the areas in which they could be implemented. The southeast US is a region with particularly severe soil degradation. Due to years of poor agricultural management, those fields were no longer productive and have long since been abandoned (Schultz, 1997). However, the southeastern US is also a region with very high potential to restore soils and increase soil C and overall soil health due to the soil types and climate in this region.

This dissertation looks at some potential mechanisms involved in storing more stable soil C, specifically MAOM. The objective of this study was to understand how litter chemistry and soil C saturation can enhance or inhibit soil C sequestration. This dissertation also looks at

two management case studies where management practices have been implemented to improve degraded lands.

The objective of the first case study, was to assess whether pine plantations could be used as a potential sustainable bioenergy feedstock. Pine plantations have been planted across vast areas of land in the southeast United State since the 1950s (Jokela et al., 2010). This transition from cropland to forest land was due to severe degradation from poor agricultural practices (Schultz, 1997). We evaluated the sustainability of these systems by evaluating how management has impacted soil C stocks and their distribution.

The objective of the second case study was to evaluate grassland grazing management practices as a potential area where more C can be drawn down from the atmosphere to the soil. Conventional grazing has degraded many of the grasslands in the United States (Milchunas and Laurenroth 1993; Bailey et al., 1998; Conant et al., 2001; Teague et al., 2004; Steffens et al., 2008; Teague et al., 2016; Teague, 2018). However, newer management practices, specifically rotational grazing, could be a way to restore these grasslands and improve the soil environment (Conant and Paustian 2002; Franzluebbers and Stuedemann 2009; Teague et al., 2011; Teague et al., 2013; Machmuller et al., 2014; Byrnes et al., 2018; Teague et al., 2018). We compared conventional and rotational grazing to see if improved management could improve soil C stocks, their distribution, as well as overall soil health. Taken all together, this dissertation investigates potential mechanisms and management for soil C sequestration.

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CHAPTER 1: Exploring the role of litter chemistry and degree of carbon saturation deficit on soil organic matter formation

Overview 1.1

Soil organic matter (SOM) is the largest store of carbon (C) in terrestrial ecosystems, representing 2/3 of the total terrestrial C pool. The rates at which soils store or lose C could either mitigate or exacerbate atmospheric CO_2 concentrations. New frameworks have been proposed regarding the mechanisms controlling the formation and persistence of SOM, which point to the role of both plant litter chemistry and soil C saturation deficit as major controls. However, these mechanisms have not been tested with a comprehensive approach. We incubated isotopically labeled litters with different chemistries and tracked the fate of the litter-derived C and N as they formed SOM fractions characterized by different mechanisms of stabilization (i.e., inherent litter chemical recalcitrance or mineral-association) in two soils with contrasting degrees of C saturation. We hypothesized that labile litter components (i.e., hot water-extractable fraction) would contribute more to mineral-associated organic matter (MAOM), in soils with a higher C saturation deficit. Whereas recalcitrant litter components would contribute more to particulate organic matter (POM). After one year of incubation, litter types with the highest proportion of hot water-extractable components were preferentially decomposed. Additionally, these litter types contributed more to MAOM as opposed to litter types with more recalcitrant components, which contributed more to POM, supporting our hypothesis. Contrary to our hypothesis, these differences were only apparent in the soil type with a lower soil C saturation deficit and were also dynamic throughout the 12 month

incubation. Our results confirm the role of litter chemistry in determining pathways of new SOM formation in the short term, but less so that of mineral C saturation deficits.

Background 1.2

Soil organic matter (SOM) is the largest store of carbon (C) in terrestrial ecosystems, representing 2/3 of the total terrestrial C pool (Ciais et al., 2013) SOM is important in ecosystems as it supplies plants with nutrients, maintains soil structure, and helps control the exchange of CO_2 with the atmosphere (Balesdent et al., 2000; Smith et al., 2001; Grandy and Robertson, 2006). The rates at which soils store or lose C could either mitigate or exacerbate increases to the atmospheric CO_2 concentrations (Schimel et al., 1990). Mediating SOM formation and storage under future predicted climate change has been the focus of much study recently (IPCC 2014). To better understand how the soil C balance may change in the future, we need to understand how different types of plant materials and soil conditions will interact with climatic and biotic factors in determining the stabilization of C in soils.

Litter decomposition and SOM formation are strongly influenced by litter chemistry (Von Lützow et al., 2006; Swift et al., 1979). Certain attributes of litter can influence the rate at which it is decomposed. Litter residues with high C:Nitrogen (N) ratio, lignin:N ratio, lignin:cellulose ratio, and/or high lignin content have been found to degrade more slowly in numerous studies (Melillo et al., 1982; Taylor et al., 1989). It was thought for many years that lignin was a recalcitrant component of organic matter and persisted in soils due to selective preservation (Schlesinger, 1977; Melillo et al., 1982). However, more recently, the importance of lignin in long-term SOM formation has changed. It is now widely accepted that lignin is not necessarily a large contributor to stabilized SOM pools and instead, stable, mineral-associated

SOM is derived from microbial biomass and byproducts (Kiem and Kögel-Knaber, 2003; Grandy and Neff, 2008; Miltner et al., 2009; Carrington et al., 2012). Microbes can utilize different litter components with more or less efficiency depending on their chemistry. Simple compounds found in the water-extractable fraction of litters can be used 2-7 times more efficiently by microbes than the lignin fraction of litters (Bahri et al., 2008; Dijkstra et al., 2011). The use efficiency of these litter fractions can directly affect the amount of litter that is mineralized into CO_2 or stabilized as SOM (Cotrufo et al., 2013).

Factors other than litter chemistry are also important for SOM formation and stabilization, specifically, organo-mineral interactions and physical protection within aggregates (Von Lützow et al., 2006). SOM accumulates and stabilizes more readily in soils with high clay contents through physical and chemical mechanisms such as aggregation, interactions with mineral surfaces, and entrapment in soil pores (Von Lützow et al., 2006). The ability of soils to physically protect C is lowered in soils with larger particle sizes, like sandy soils (Grandy and Neff, 2008). In fact, the amount of C stored in the silt and clay fraction is related to the proportion of silt and clay found in the soil and is different across many ecosystems. However, given continuous C inputs, soils will eventually reach a C stabilization limit, also known as C saturation (Six et al., 2002; Stewart et al., 2008). For example, a soil that is further from C saturation (i.e. soils with a large proportion of silt and clay combined with low SOM) has a C saturation deficit and it is, thus, thought to have the potential to accrue the most C.

New frameworks have been proposed regarding the mechanisms controlling the formation and persistence of SOM, which point to the role of both plant litter chemistry and

soil C saturation deficit as major controls (Kalbitz et al., 2000; Six et al., 2002; Cotrufo et al., 2013; Castellano et al., 2015; Cotrufo et al., 2015). For example, Cotrufo et al. (2013) proposed the microbial efficiency-matrix stabilization (MEMS) framework, hypothesizing that labile litter components are more efficiently used by microbes and will therefore accrue as microbial biomass, leading to stable SOM in soils with high matrix potential, whereas more recalcitrant litter will be used less efficiently by microbes and will contribute more to CO_2 production. Additionally, Castellano et al. (2015) hypothesized that plant litter chemistry will have an effect on SOM stabilization only when there is a C saturation deficit. These proposed mechanisms controlling the formation and persistence of SOM are receiving a lot of new attention and several studies have been performed to test them (Cordova et al., 2018; Kallenbach et al., 2016). However, none of these studies address in a comprehensive mechanistic way, both the effect of litter chemistry and soil C saturation deficit on POM and MAOM formation, as this study does.

This study analyzes the fate of litters of different chemistries as they form SOM fractions characterized by different mechanisms of stabilization (i.e., POM by inherent litter chemical recalcitrance or MAOM by protection through mineral-association), in a topsoil and a subsoil with contrasting degrees of C saturation. We hypothesize that labile litter components (i.e. hot water-extractable fraction and non-lignin encrusted celluloses) will be preferentially decomposed and contribute more to MAOM in the subsoil with a higher C saturation deficit. In order to test these hypotheses, we incubated isotopically (^{13}C) labeled leaf and root litters with different chemistries in two soils that vary in C saturation deficit, and tracked the fate of the litter-derived C as it stabilized into POM and MAOM fractions.

Methods 1.3

To generate a broad variety of litter chemistries, we used above and below ground litter produced from a grass (*Sorghum bicolor*) and a conifer tree (*Pinus taeda*). All litter types were produced in an isotopic labeling chamber to enrich their ^{13}C content. All litter types were applied to the surface of topsoil and a subsoil from the same soil pedon. These soils had similar textures, but different C saturation deficits. The soils and the remaining litters were harvested at three time points over the course of 12 months of incubation: 1, 6, and 12 months. The remaining bulk litter was analyzed and then fractionated into α-cellulose, acid unhydrolyzable (AUR), hemicellulose, and water-extractable fractions. The bulk soil was also analyzed and then fractionated into two functionally different organic matter fractions (Lavallee et al., 2020): particulate organic matter (POM) and mineral associated organic matter (MAOM). Both the soil and the litter samples were analyzed for ^{13}C enrichment with an elemental analyzer coupled with an isotope ratio mass spectrometer (EA-IRMS, Costech coupled to a VG Isochrom continuous flow IRMS, Isoprime Inc., Manchester, UK) to trace the litter throughout the soil and determine the relative contribution of litter to each SOM fraction.

Sorghum bicolor (Sorghum grain variety BTx623) and *Pinus taeda* (Loblolly Pine) plants were germinated and then transplanted, where they were grown in a continuous isotopic labeling chamber (4 atom% ^{13}C-CO_2 atmosphere) located at the Colorado State University (CSU). Temperature and humidity were controlled for with an air conditioner unit and a dehumidifier. A more detailed description of the isotope-labeling chamber is outlined in Soong et al. (2014).

The experiment followed a fully factorial design with two treatments: litter type and soil C saturation, and three time harvests, with all combinations in 4 replicates. The two different plant species, as well as a no litter control, represented five litter type treatments: control (no litter), loblolly pine needles and roots, and sorghum leaves and roots (Table 1.1). Soil C saturation treatments included two soils harvested from the CSU Horticulture Field Research Center (40.610012, -104.993979) in 2015. One of the soils was from the topsoil (0-30cm depth) and the other was from the subsoil (60-90cm depth) of the same soil column. These soils had similar textures, but contrasting degrees of C saturation (Table 1.2). To ensure similar microbial communities, a soil inoculum was created from each soil type and then applied to the opposite soil type before the start of the incubation (adapted from Soong et al., 2015a). Briefly, 50g of air-dried soil was brought up to 60% water-filled pore space and incubated for one week. After incubation, 1g of soil was added to 100ml of deionized water and shook for 2 hours and then filtered over a 20 µm nylon filter. Finally, 1ml of inoculum filtrate was added to the opposite soil type. There were three harvests for all the litter type treatments. The three harvests included 1, 6, and 12 months. Each treatment combination had four replicates per harvest, for a total of 40 treatments and 120 experimental units.

Table 1.1 Summary of relative proportion of litter chemical constituents by mass for *Sorghum bicolor* (Sorghum grain variety BTx623) and *Pinus taeda* (Loblolly Pine) leaves and root litters used in the incubation experiment.

	Sorghum		**Loblolly**	
	leaves	**roots**	**needles**	**roots**
hot water-extractable	29%	19%	28%	17%
hemicellulose	30%	30%	24%	12%
α-cellulose	34%	36%	24%	32%
acid unhydrolyzable (AUR)	7%	15%	24%	39%
Lignocellulose Index (LCI)	0.10	0.19	0.33	0.47
Carbon:Nitrogen	44	76	38	65

Table 1.2 Summary of each soil type (C saturation calculated from regression equation for cultivated soils relating silt & clay proportion to silt & clay associated C: y= 0.2x + 7.18, where x= % silt & clay, Six et al., 2002).

				Silt & Clay g C/kg soil		
	% C (bulk soil)	% Silt & Clay	% C (silt & clay)	Current C saturation status	Maximum C saturation	Current C saturation deficit
0-30cm	2.11	70.26	1.48	14.81	21.23	6.42
60-90cm	1.08	55.20	0.60	5.96	18.22	12.26

Soil + litter incubations took place in wide mouth gallon-sized Mason jars. The jars were incubated at a constant temperature of 25°C in the dark. These soils were chosen because they had sufficient, yet similar, proportions of silt and clay combined with different amounts of organic matter. 50g of homogenized air-dried soil was added to each Mason jar. Air-dried soil was brought up to 60% water filled pore space to ensure that the microbial activity was not inhibited (Linn and Doran, 1984). Litter application depended on the C content of each litter type. Each litter type was cut into 2cm pieces where necessary and 1.0g of litter C was added directly to the surface of the soil in each jar.

At each harvest time point, the remaining litter was separated from the soil by picking out all noticeable litter from the soil. Then, a subsample of the soil was fractionated into two SOM fractions: POM and MAOM by size after aggregate dispersion (Cotrufo et al., 2019). A 10g subsample of oven-dried soil was dispersed with glass beads and 0.5% sodium hexa-metaphosphate for 18 hours to break up aggregates. Before the soil was fractionated into POM and MAOM, all inorganic C, in the form of carbonates, were removed (adapted from Follett et al., 1997). After carbonate removal, soil was wet sieved through a 53µm sieve to separate the POM (>53µm) from the MAOM (<53µm). Total mass recovery was always within 5% of the initial mass.

A subsample of the litter was fractionated into four chemical fractions: α-cellulose, acid

unhydrolyzable (AUR), hemicellulose, and hot water-extractable (HWE), following Soong et al., 2015. The cellulose and AUR fractions were extracted using the Acid Detergent Fiber method (Van Soest and Wine, 1968). The hemicellulose fraction was extracted using the Neutral Detergent Fiber method (Van Soest et al., 1991). The water-soluble fraction was extracted by the hot water extraction method (Tappi, 1981). The lignocellulose index (LCI), which is the proportion of structural litter components in the AUR fraction, was determined by dividing the AUR fraction by the AUR + α-cellulose + hemicellulose fractions.

The initial litter components, bulk soils, and soil fractions were oven-dried at 60 °C, weighed, and then analyzed for %C and δ^{13}C with an elemental analyzer coupled with an isotope ratio mass spectrometer (EA-IRMS, Costech coupled to a VG Isochrom continuous flow IRMS, Isoprime Inc., Manchester, UK). A 2-endmember mixing model was used to determine the proportion of each soil fraction that was derived from the added labeled litter C and the proportion derived from the native SOM (Fry, 2006):

$$f_{enriched\ litter} = \frac{\left(\delta^{13}C_{sample} - \delta^{13}C_{native\ SOM}\right)}{\left(\delta^{13}C_{enriched\ litter} - \delta^{13}C_{native\ SOM}\right)}$$

Where $\delta^{13}C_{sample}$ is the ^{13}C value in delta notation of soil sample for each harvest time point, $\delta^{13}C_{enriched\ litter}$ is the initial ^{13}C litter values and $\delta^{13}C_{native\ SOM}$ is the average of the ^{13}C natural abundance values of the control soils (i.e., no litter added, n=4) harvested at the same time point.

We assessed the effects of litter type and degree of soil C saturation deficit and their interactions on the % litter remaining, litter-derived bulk soil C, litter-derived POM C, and litter-

derived MAOM C at all three harvest time points using a general linear model. We also analyzed litter-derived bulk soil C, litter-derived POM C, and litter-derived MAOM C for differences between harvest time points. Finally, we compared each of these variables for differences between harvests. We used R software (R version 3.3.1; R Core Team, 2016). Necessary log transformations were performed when the data was non-normal or had unequal variance.

Results 1.4

There was a significant effect of litter type on the percent litter remaining at all three harvest time points (Table 1.3; Figure 1.1). Pine roots consistently had significantly more mass remaining than any of the other litter types (p-value <0.01). After 12 months, the sorghum leaf treatment had 11.8% to 37.9% less mass remaining than any of the other litter types. There was no significant difference in percent litter remaining between the soil C saturation deficit treatments at the 1 or the 12 month harvests (Table 1.3; Figure 1.1). However, there was a difference in the percent litter remaining between the soil treatments in the 6 month harvest (Table 1.3; Figure 1.1). After 6 months, the topsoil had less litter remaining compared to the subsoil (46.4% and 50.6% respectively, p-value= 0.07, Table 1.3; Figure 1.1).

The more labile litter components such as the cellulose and hemicellulose (holocellulose) were preferentially decomposed. After only 6 months of incubation, all litter types had lost between 65% and 90% of the holocellulose fraction (Figure 1.2). The HWE fraction decreased throughout the incubation, but did not completely disappear after 1 year (Figure 1.2). Whereas the relative proportion of AUR components remained constant or even increased throughout the 12 months of decomposition (Figure 1.2).

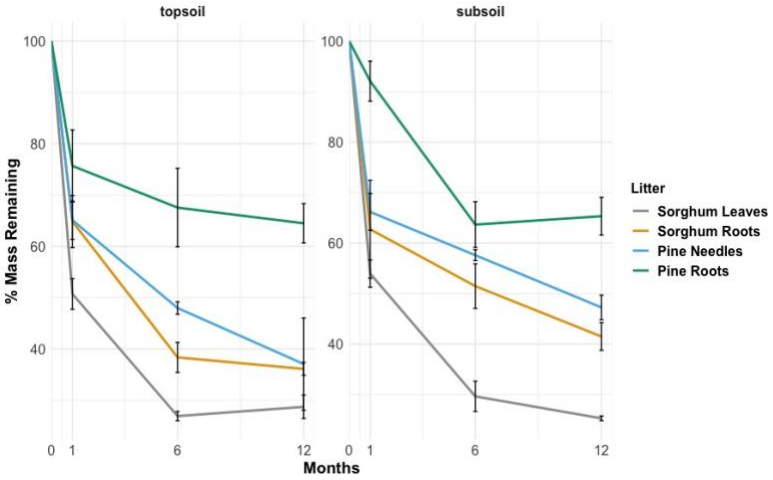

Figure 1.1 Average percent mass remaining ± standard errors for each litter type and soil carbon saturation deficit treatment after 1 month, 6 month, and 12 month harvests.

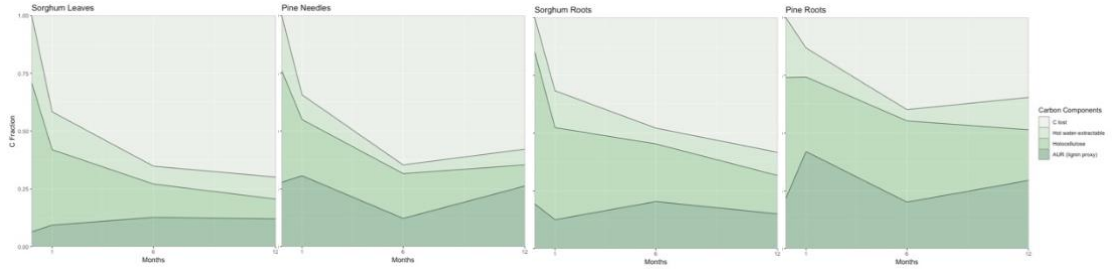

Figure 1.2 Litter carbon component distribution by litter type over 12 months.

Overall, there was very little SOM formation at each harvest time point (Figure 1.3). There was a consistent significant litter type effect on bulk SOM formation at the 1, 6, and 12 month harvests (Table 1.3, p-values=0.01, 0.03, and <0.01, respectively). The sorghum roots and the pine roots treatments had greater litter-derived bulk SOM C at all three harvests compared to the sorghum leaves and the pine needle treatments (Figure 1.3, p-values=0.04, 0.02, and <0.01, respectively). Both root treatments are also the two litter types that had the lowest proportion of HWE components and the highest C:N ratios (Table 1.1). There was a significant soil type effect on bulk SOM formation after the 1 month harvest, with more litter-derived bulk SOM C in the topsoil compared to the subsoil (Figure 1.3, p-value=0.01).

Additionally, the 6 month harvest had the highest litter-derived bulk SOM C compared to the 1 month and 12 month harvests (p-value<0.01).

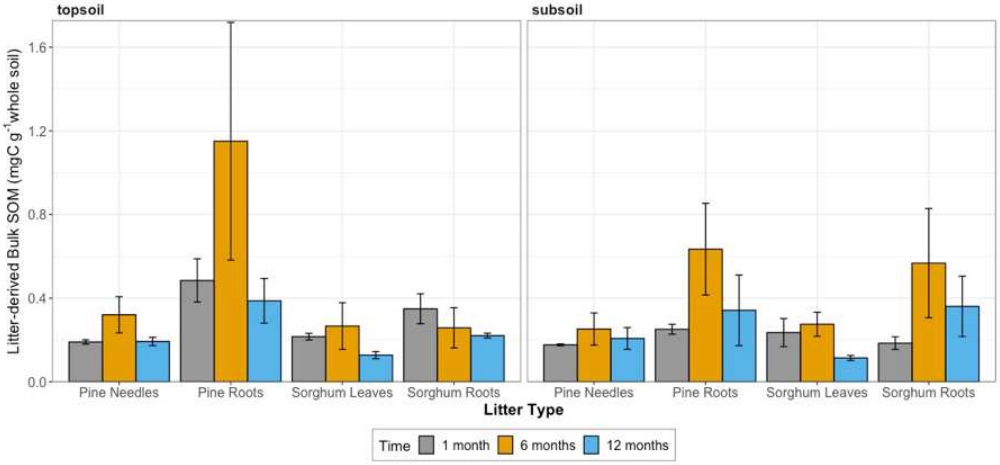

Figure 1.3 Average litter-derived SOM (mg C/g dry soil) for each litter and soil types after 1, 6, and 12 month harvests, ± standard errors.

There was very little of the litter C found in the POM fraction (Figure 1.4). There was a significant litter type effect on litter-derived POM throughout the incubation (Table 1.3). Litter-derived C in the POM fraction was significantly higher in sorghum roots and pine roots litter treatments compared to the sorghum leaves and the pine needle treatments at all three harvests (Figure 1.4, p-values <0.01 consistently). There was only a significant soil type effect on litter-derived POM C after the 1 month harvest (Table 1.3, p-value=0.04). After 1 month, more litter C was found in the POM fraction in the topsoil compared to the subsoil (Figure 1.4). There were no differences in the amount of litter-derived POM C between harvest time points.

The amount of litter C that formed MAOM was small (Figure 1.5). There was a significant litter treatment effect after the 1 month harvest (Figure 1.5, p-value <0.01). After 1 month of incubation, the sorghum leaves and the pine needle treatments had greater litter-derived MAOM C compared to the sorghum roots and the pine roots treatments (Figure 1.5, p-value

<0.01). Both the sorghum leaves and the pine needles were also the two litter types that had the highest proportion of HWE and holocellulose components as well as the lowest C:N ratios (Table 1.1). There were no significant litter treatment effects on litter-derived MAOM C, after the 6 month and 12 month harvests. At the 6 month harvest there was a significant soil type effect, with higher MAOM formation in the topsoil compared to the subsoil (Figure 1.5, Table 3, p-value <0.01). There were no significant soil type effects after the 1 month and 12 month harvests.

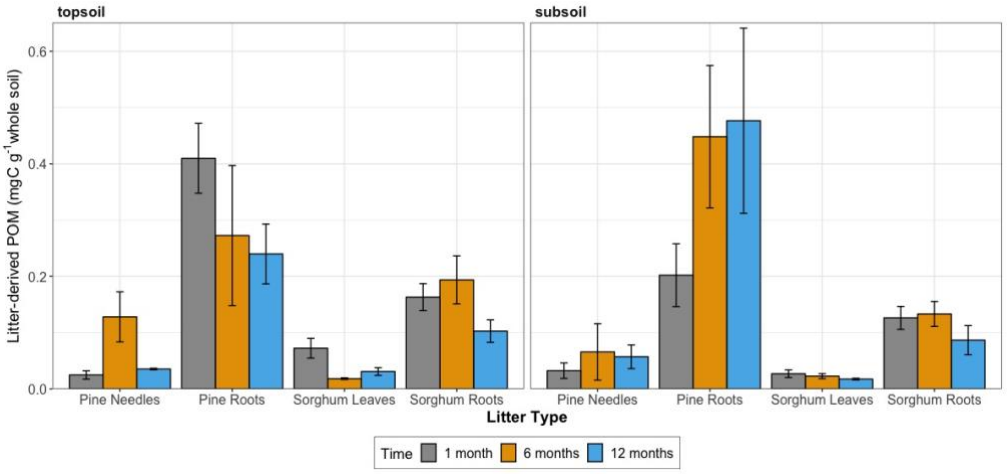

Figure 1.4 Average litter-derived POM (mg C/g dry soil) for each litter and soil type after 1, 6, and 12 month harvests ± standard errors.

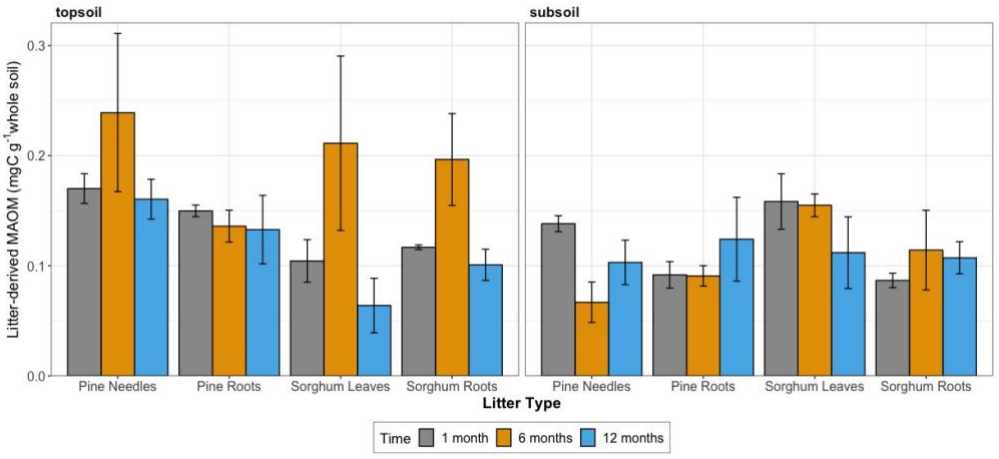

Figure 1.5 Average litter-derived MAOM formation for each litter type and soil carbon saturation deficit treatment after 1, 6, and 12 month harvests ± standard errors.

Table 1.3 The general linear model treatment effect p-values. Significant treatment effects are in bold.

Response	Treatment	Harvest		
		1 month	6 months	12 months
% Litter Remaining	Litter	**<0.01**	**<0.01**	**<0.01**
	Soil	0.29	0.07	0.26
	Litter*Soil	0.35	0.17	0.39
Litter-derived C in bulk soil	Litter	**0.01**	**0.03**	**<0.01**
	Soil	**0.01**	0.92	0.79
	Litter*Soil	0.12	0.35	0.70
Litter-derived C in POM	Litter	**<0.01**	**<0.01**	**<0.01**
	Soil	**0.04**	0.38	0.81
	Litter*Soil	0.44	0.12	0.36
Litter-derived C in MAOM	Litter	**<0.01**	0.56	0.37
	Soil	0.13	**<0.01**	0.82
	Litter*Soil	**<0.01**	0.29	0.28

Discussion 1.5

Litter chemistry was an important determining factor for the amount of litter mass remaining at each harvest. Pine roots had significantly more mass remaining likely due to having the greatest amount of AUR components (Table 1.1). These components, conventionally identified with lignin, are known to be the most recalcitrant (Melillo et al., 1982; Taylor et al., 1989). Additionally, the pine roots were the litter type with the least amount of HWE and cellulose components. Sorghum leaves had significantly less mass remaining compared to the other litter types at all three harvest time points. This is likely because the sorghum leaves were the litter type with the smallest proportion of AUR components and the largest proportion of HWE components (Table 1.1). Other studies have also shown that litter types with a greater proportion of celluloses are lost more rapidly than those with a greater proportion of lignin (Melillo et al., 1982; Taylor et al., 1989; Cotrufo et al., 2015). The celluloses, across all litter types, were preferentially decomposed (Figure 1.2). The celluloses were decomposed to a lesser degree in the pine root litter type. This result could be due to the pine roots having a higher LCI and more lignin encrusted celluloses, which are much more difficult to break down (Cotrufo et al., 2015; McKee et al., 2016). In all litter types the HWE fraction decreased, but

remained steady throughout the incubation. This fraction can be produced during cellulose decomposition and via microbial decomposition byproducts (Soong et al., 2015), which is why this fraction did not completely disappear. Additionally, the proportion of AUR components can oftentimes increase during decomposition. This is because the other components, like celluloses, are being decomposed preferentially and because the AUR fraction can be created throughout decomposition via microbial byproducts (McKee et al, 2016). These trends were reflected in all of our litter types across all harvest time points (Figure 1.2).

Soil type, and therefore C saturation deficit, had less of an effect on the amount of litter remaining. There was no difference in the amount of mass remaining between the two soil types at the 1 or 12 month harvest. However, at the 6 month harvest the soil with a lower soil C saturation deficit (topsoil) had less litter remaining than the soil with a higher soil C saturation deficit (subsoil). This effect is likely more of a depth effect and less so a C saturation deficit effect. Topsoil is known to have higher microbial biomass and is therefore more active than subsoil (Fierer et al., 2003; Tessier et al., 1998) and, despite our best efforts to inoculate the soils with a similar microbial community, it could be that the inoculation did not fully take and the topsoil treatment had more starting microbial biomass than the subsoil treatment. Therefore, it is possible that the topsoil was a more active soil, which would lead to the observed greater decomposition and less litter remaining.

Litter chemistry had an effect on the amount of litter-derived C that was found in the bulk SOM. Overall, the litter types with the least amount of HWE components and the greatest C:N ratios (sorghum and pine roots) formed the most litter-derived SOM. This was consistent at all three harvest time points. This finding is highly dependent on the SOM distribution (POM vs.

MAOM). There was significant fragmentation of the roots and these recoveries were then allocated to the POM fraction in high amounts.

The soil C saturation deficit treatment had less of an effect on the amount of litter-derived SOM that was formed during the incubation. There was no difference in bulk SOM formation across soil treatments during the 6 and 12 month harvests. As stated above, the topsoil could have had a higher starting microbial biomass which may have led to more SOM formation in the topsoil treatment compared to the subsoil treatment. Additionally, each soil type also had low starting organic matter content well under their C saturation limits to ensure C-stabilizing potential for the duration of the incubation, and therefore unlikely that either soil type would reach saturation during the incubation (Six et al., 2002).

Consistent with our hypothesis, there was significantly more POM formed under the litter types with the least amount of HWE components and the greatest C:N ratios (sorghum and pine roots), following the same trend of the bulk SOM. As stated above, there was significant fragmentation of the roots which were then allocated to the POM fraction in high amounts, causing these significant differences. This finding aligns with our hypothesis that recalcitrant litter types would contribute more to POM. Other studies have shown that recalcitrant litters contribute to the POM fraction disproportionately (Cotrufo et al., 2015; Adair et al., 2008; Castellano et al., 2015; Haddix et al., 2016).

Litter chemistry played a significant role in MAOM formation, only in the topsoil with a lower C saturation deficit. Litter types with the highest proportion of HWE components formed the most MAOM in the topsoil. This is similar to the findings of other recent studies using similar approaches (Cotrufo et al., 2015; Lavallee et al., 2018; Liang et al., 2017, further

confirming that more MAOM is formed under labile litter types, that can be used more efficiently by microbes (Cotrufo et al., 2013; Moorhead et al., 2013). There was no litter chemistry effect in the subsoil treatment. We predicted that labile litters would contribute more to MAOM in the subsoil with a greater soil C saturation deficit. For all three litter treatments combined, the topsoil treatment had more litter-derived MAOM at all three harvest time points. However, this was most pronounced and only significant at the 6 month harvest. This indicates that soil C saturation deficit may be less of a determining factor in MAOM formation than previously thought.

MAOM formation appeared to be quite dynamic in the topsoil treatment, whereas the subsoil treatment was relatively static. The apparent increase in MAOM formation at the 6 month harvest coincides with the breakdown of celluloses from the litter residues after 6 month of decomposition (Figure 1.2). These carbohydrates likely formed weak mineral bonds (Von Lützow et al., 2006; Kogel-Knabner et al., 2008), because after 1 year, these mineral-associations were already dissociated from the mineral surfaces. However, this dynamic was only observed in the topsoil. As stated above, the topsoil is richer in microbial biomass and is typically more active (Fierer et al., 2003; Tessier et al., 1998) which may have led to more MAOM formation in the topsoil treatment compared to the subsoil treatment. This could also be why we did not observe a disassociation of MAOM in the subsoil treatment. Others have shown that certain plant products can aid in the desorption of organic matter associated with silt and clay (Jilling et al., 2018). This indicates that MAOM may not be as stable in the more active soil layers as compared to its apparent persistence in deeper soil layers. Additionally, the minerology can play a big role in the strength of organic matter bonding types (Von Lützow et

al., 2006; Kogel-Knabner et al., 2008). We did not test for differences in mineralogy at depth, but this could be another reason for the differences in the disassociation of MAOM.

Overall, we found that after one year of incubation, litter types with the highest proportion of HWE components were preferentially decomposed. Additionally, these litter types contributed more to MAOM as opposed to litter types with more recalcitrant components contributing more to POM, supporting our hypothesis. Contrary to our hypothesis, these differences were only apparent in the topsoil, with a lower soil C saturation deficit, and were also dynamic throughout the 12 month incubation. These results confirm the role of litter chemistry in determining pathways of new SOM formation, but less so that of mineral C saturation deficits.

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CHAPTER 2: Soil organic matter pools under management intensification of loblolly pine plantations

Overview 2.1

Early thinning of loblolly pine plantations can potentially deliver sustainable feedstocks for biofuel/bioenergy. However, the management intensification for increased productivity and the removal of additional biomass from these plantations could reduce carbon (C) inputs belowground and therefore reduce overall ecosystem C storage. Increased fertilization could also affect C stocks, and their relative distribution between soil organic matter (SOM) fractions. We analyzed soil C stocks as a function of soil type and different pine plantation management systems across the Western Gulf region of the United States. Additionally, we analyzed SOM fractions with inherently different stabilization mechanisms and potential C persistence. We found no significant differences in bulk soil C stocks across management intensities or soil types. The early thinning treatment had no effect on the C distribution across each soil organic matter fraction. However, proportionally more C was found in mineral- associated organic matter and less in particulate organic matter in the more intensive management regime treatment, possibly due to higher below ground nutrient inputs and enhanced microbial activity. Our results suggest that management intensification to support biofuel production from loblolly pine plantations will not affect soil C stocks, but may increase their persistence. This study demonstrates that, from a soil C perspective, early thinning of intensively managed loblolly pine plantations has potential as a sustainable biofuel feedstock.

Background 2.2

Globally, forests have a strong climate change mitigation potential because they play a large role as a carbon (C) storage sink, absorbing 25-50% of annual CO_2 emissions from fossil fuel combustion through primary production (US Climate Change Science Program, 2008). Additionally, forests contain 861 Pg of total C worldwide, with approximately 379 Pg of forest C found in soils alone (Pan et al., 2011). Therefore, the impacts of forest use and management on soil organic matter (SOM) stocks can have a major impact on the net C footprint of these systems. Recently, forests have been identified as a potential source of sustainable cellulosic biofuel feedstock provided that production of other commodities (i.e., timber, pulp, and paper) are not substantially affected. However, it is still unclear if forests managed for biofuel production can be a net C sink, since we don't know if more intensive forest management, including additional fertilization and thinning required to produce more aboveground biomass for biofuel production, will move these forests from a net C sink into a net C source.

Among the most abundant managed forests in the United States are pine plantations. These plantations cover over 30 million acres of land in the southeastern United States, most of which are occupied by loblolly pine (*Pinus taeda*) (Jokela et al., 2010; Munsell and Fox, 2010). Loblolly pine is an important species both economically and ecologically. Economically, pine plantations provide over 40 billion dollars of revenue to the region annually through timber, paper, and pulp production (Jeffries, 2016). Many of the current loblolly pine plantations in the southeastern United States resulted from reforestation of abandoned, poor quality agricultural lands where other tree species had little success (Schultz, 1997). Loblolly pine can grow and thrive on these marginal soils, which has led to its success and dominance in the region. Pine

plantation management improvements have rapidly intensified over the past 60 years, increasing yields by up to 400% (Jokela et al., 2010). Among loblolly pine plantations, management commonly includes practices such as site preparation, planting, fertilization, competition control, thinning, and final harvest (Jokela et al., 2010; Fox et al., 2007). These management practices can impact C stocks by influencing plant growth, decomposition, belowground inputs, and aboveground forest floor accumulation. However, it is still unclear how soil type and management practices impact soil C storage in these systems. This lack of knowledge has raised questions about the true climate benefits of loblolly pine plantation management for biofuel production as well as the potential impact on soil nutrient cycling and other ecosystem functions for sustainable forest productivity.

As improvements in management increase pine plantation productivity, more biomass can be removed during early thinning. The use of pine plantations to produce biofuels, using these early thinnings in a rotation length regime that also produces traditional products of pulpwood, chip-n-saw, and sawtimber can potentially deliver sustainable biofuels, without the displacement of other forest products. However, the removal of additional biomass from these plantations could reduce C inputs belowground and therefore decrease overall ecosystem C storage. On the other hand, higher nutrient input through intensified fertilization could stimulate plant C inputs, promote organic matter decomposition, and increase stabilization processes that favor microbial transformation of organic matter into the MAOM pool with longer residence times (Averill et al., 2018). While many studies have evaluated the impact of pine plantation management on tree growth and overall yield (Zhao et al., 2011; Vogel et al., 2011; Jokela et al., 2010; Fox et al., 2007; Samuelson et al., 2004), far fewer studies have

assessed soil C dynamics in pine plantations managed for biofuel production. In other systems, particularly agricultural, it is well documented that management, like tillage and fertilization, can impact soil SOM stocks (Paustian et al., 2000; Mosier et al., 1998). Additionally, SOM stocks can be affected by the removal of biomass as well as by other forms of forest management (Johnson, 1992). Yet, it is still not entirely understood if more intensive management of loblolly pine leads to an overall net soil C sink or source.

This study aims to determine if intensifying pine plantation management and/or conducting early thinnings for biofuel production has an impact on soil C stocks in loblolly pine plantations. More specifically, we wanted to determine if intensifying pine plantation management has an impact on soil C stocks and their distribution among SOM pools with different mechanisms of C stabilization and persistence. We measured loblolly pine plantation soil C stocks in the Western Gulf Region of the United States across a range of different soil types and management intensities and then separated SOM by size and density fractionation into three different SOM pools: 1) free light fraction (fLF)- plant residues at the early stages of decomposition (Christensen, 2001) not protected within aggregates, 2) particulate organic matter fraction (POM)- defined as more decomposed plant residues and microbial products often attached to soil aggregates or occluded within aggregates (Golchin et al., 1997), and 3) mineral-associated organic matter (MAOM)- more amorphous, non-POM that is sorbed to mineral surfaces and is usually microbial in origin (Clemente et al., 2001). We hypothesized that a) plantations with heavier early thinning would have lower soil C stocks than plantations with no or low levels of thinning, and b) any differences in soil C stocks would be due to SOM fractions that are not associated with minerals and therefore less persistent and more

vulnerable to disturbance. We also hypothesized that management intensification, through adding more nutrients to the soil, could counterbalance changes in SOM by stimulating plant inputs and microbial SOM processing, resulting in relatively higher C stocks in the MAOM fraction.

Methods 2.3

We sampled 12 Plantation Management Research Cooperative loblolly pine plantation sites across the Western Gulf Culture Density Study in the Western Gulf region of the United States (Table 2.1; Kane et al., 2015; Zhao et al., 2016). Experimental sites were established between years 2001-2003 on previously managed loblolly pine plantations. Four broad soil groups, based on drainage and depth of restrictive layers were represented with three replicate installations each (Table 2.1; Kane et al., 2015). At each installation, we took four replicate soil cores from six treatment plots (0.105 hectares each), down to 100 cm. The six treatment plots represented three thinning intensities across two management intensities. For the thinning intensities, treatments included a light thinning (from 1730 to 1112 trees per hectare), heavy thinning (from 1730 to 494 trees per hectare), and an un-thinned control (1730 trees per hectare). Plots were thinned when their stand density index reached 55% of the maximum stocking density (Reineke, 1993). Thinning was completed by early 2010 and 2012. For the management intensities, treatments included an operational, 'business as usual' treatment with low levels of nitrogen (urea) + phosphorus (DAP/TSP) fertilization along with herbicide application and an intensive treatment with higher and more frequent levels of fertilization as well as herbicide application (Kane et al., 2015). Thus, our experimental design included four soil types: A (poorly-drained, <50 cm to restrictive layer), B (poorly-drained, >50cm to

restrictive layer), C (well-drained, <50cm to restrictive layer), and D (well-drained, >50cm to restrictive layer); two management intensities (operational and intensive); and three thinning levels (no thinning, light thinning, and heavy thinning), with four replicates for a total of 72 sample plots and 288 soil cores.

Soil sampling took place either in December 2016 (sites 1, 2, 3, 8, 10, and 12) or in March 2017 (sites 4, 5, 6, 7, 9, and 11) to align with operational management in the region. At each of the six treatment plots per site, we randomly chose four replicate soil sampling locations. First, we removed and collected the aboveground forest floor using a 20x20cm quadrant. Then we collected cores (5 cm diameter, 1 m deep) using an ATV mounted hydraulic soil probe (Giddings Machine Company Inc., Windsor CO), alternatively we used a manual corer of the same dimensions. We separated the mineral soil cores into 0-15, 15-30 and 30-50, and 50-100cm depth layers and then weighed them. We took a subsample of each soil and put it in a moisture can to determine field moisture content. All forest floor samples, soil samples, and moisture cans were then shipped to Colorado State University where they were refrigerated at 4°C until they could be processed and analyzed. Forest floor samples and moisture cans were de-quarantined using a heat treatment in an oven at 105°C and then weighed. This allowed us to determine the dry forest floor weight and the soil moisture content. We sieved all soils to 8 mm and then de-quarantined them using heat treatment as described above. We separated litter and root fragments > 2mm from the mineral soil and then separately assayed them along with coarse rock fragments. This allowed a determination of bulk density for each individual core and depth layer (Table 2.1). Additionally, each 0-15cm soil depth was analyzed for pH,

using a pH electrode, and texture, using the particle size method (Gee and Bauder, 1986; Table 2.1).

Root fragments were dried and then combusted in a muffle furnace to determine the ash-corrected root weight. A subsample of each mineral soil depth was then sieved to 2 mm, oven-dried at 105°C, pulverized and analyzed for %C on an elemental analyzer (LECO tru-SPEC, Leco Corp., St. Joseph, MI, USA). We determined soil C stocks by depth layer from concentrations and bulk density values. Roots and forest floor litter were also oven-dried, pulverized and analyzed for %C on the elemental analyzer mentioned previously.

To further increase our ability to detect soil C stock changes and to investigate trajectories of diagnostic soil C pools (Del Galdo et al. 2003, Six et al. 2002), we separated the surface layer soil samples (0-15cm) by size and density into SOM fractions, which represent organic matter pools with inherently different mechanisms of formation and differing persistence in soil (von Lutzow et al. 2006). These included: fLF, POM, and MAOM. We adapted our fractionation method from Poeplau et al., 2018 to separate different C pools in our soil samples. Briefly, a 10g oven-dried subsample of soil was density fractionated by adding 35 ml of 1.85 g cm^{-3} sodium polytungstate (SPT) to the soil and centrifuging at 3400 rpm for 30 minutes. After centrifugation, the fLF (<1.85 g cm^{-3}) was aspirated from the rest of the soil and rinsed. The remaining soil was also rinsed and then physically separated into POM (>53 μm) and MAOM (<53 μm) by wet sieving.

We analyzed each DOM fraction for total organic C content using a TOC analyzer (Shimadzu TOC 5000, Shimadzu Corp., Kyoto, Japan). All other fractions were then oven-dried and analyzed for %C as described above for bulk soils.

Table 2.1 Site descriptions of each installation with site ID, location, planting year, average annual precipitation (mm), site index, soil texture (%sand, %silt, %clay), 0-15cm average bulk density, soil pH, soil taxonomy, and broad soil group.

Site ID	Location (County/Parish, State)	Year Planted	Average Annual Precipitation (mm)	Site Index*	Soil Texture (%sand, %silt, %clay)	0-15cm Bulk Density (g/cm^3)	Soil pH	Soil Taxonomy	Soil Group (drainage, depth to restrictive layer)
1	Jasper, TX	2001	1270-1370	26.2	(62, 33, 5)	0.97	3.67	Paleudult	Poorly-drained, <50cm
2	Nacogdoches, TX	2001	1170-1270	25.0	(70, 26, 4)	0.98	4.33	Hapludult	Well-drained, <50cm
3	Newton, TX	2001	1370-1525	21.6	(88, 7, 5)	1.08	5.42	Paleudalf	Well-drained, >50cm
4	Bradley, AR	2001	1320-1420	23.2	(53, 43, 4)	1.19	3.32	Endoaquult	Poorly-drained, >50cm
5	Ashley, AR	2001	1370-1420	23.5	(13, 72, 15)	1.01	3.24	Fragiaqualf	Poorly-drained, >50cm
6	Livingston, LA	2001	1525-1625	26.8	(28, 59, 13)	1.09	3.28	Glossaqualf	Poorly-drained, <50cm
7	Lamar, MS	2001	1575-1625	26.2	(75, 19, 6)	0.96	4.04	Paleudult	Well-drained, >50cm
8	Bradley, AR	2002	1320-1420	21.6	(41, 52, 7)	0.97	3.63	Endoaquult	Poorly-drained, >50cm
9	Howard, AR	2002	1320-1370	26.8	(62, 35, 3)	0.99	3.67	Paleudult	Well-drained, >50cm
10	San Augustine, TX	2003	1170-1270	24.1	(67, 24, 9)	0.89	3.85	Hapludalf	Well-drained, <50cm
11	Sabine, LA	2003	1320-1420	22.6	(27, 36, 37)	0.92	3.90	Hapludalf	Well-drained, <50cm
12	Calhoun, AR	2003	1320-1370	24.7	(39, 46, 15)	1.02	3.94	Endoaquult	Poorly-drained, <50cm

* Estimated site index expressed as the height (meters) of the dominant and codominant trees at age 25

We assessed the effects of soil type, thinning, and management intensity on bulk soil C stocks and each SOM fraction using a general linear mixed-effects model using a significant alpha level of $p<0.01$. There was no inorganic C in our highly acidic soils (Table 1). Soil group, thinning, and management intensity were considered categorical fixed effects. Site and all replicates were considered categorical random effects. Time since establishment (Table 1) was also tested as a random effect; however, it was removed from the model due to non-significance. We used R software (R version 3.3.1; R Core Team, 2016) with the lme4 package (Bates et al., 2015). Necessary transformations were performed when the data was non-normal or had unequal variance. Bulk core soil organic C stocks were log transformed. Bulk 0-15cm soil organic C stocks, root C, and forest floor C were square root transformed. No necessary transformations were needed on the SOM fraction data.

Results 2.4

There were no significant main treatment effects or significant treatment interactions on total root C stocks at the entire core depth (0-100cm) nor at the 0-15cm depth (Table 2.2 and Table 2.3). In both the 0-100cm depth and the 0-15cm depth, numerically the estimated root C stocks were greater in the light thinning compared to the heavy and no thinning intensity treatments (18% and 22% higher, respectively; Figure 2.1) but differences were not significant. There were no clear trends in root C stocks between the management intensity treatments (Figure 2.1). In the 0-15cm depth, the soil types with a deep restrictive layer had numerically higher average root C stocks (36% higher) than the soil types with a shallow restrictive layer but the differences were not significant. When the 0-100cm depth was analyzed, the soil types with

a deep restrictive layer had a non-significant 60% greater root C than soil types with a shallow restrictive layer (p-value= 0.13).

Both management intensity and thinning intensity had a significant effect on the amount of forest floor C (Table 2.2, Figure 2.1). Averaged across management intensities, the light thinning intensity treatment had significantly more forest floor C than the heavy thinning intensity treatment (10.18 ± 0.39 compared to 8.86 ± 0.37 MgC/ha; p-value= 0.05, Figure 2.1). Additionally, the intensive management treatment had 20% more forest floor C than then operational management treatment when averaged across thinning treatments (10.52 ± 0.32 compared to 8.78 ± 0.31 MgC/ha, Figure 2.1). There was no significant impact of soil type on forest floor C.

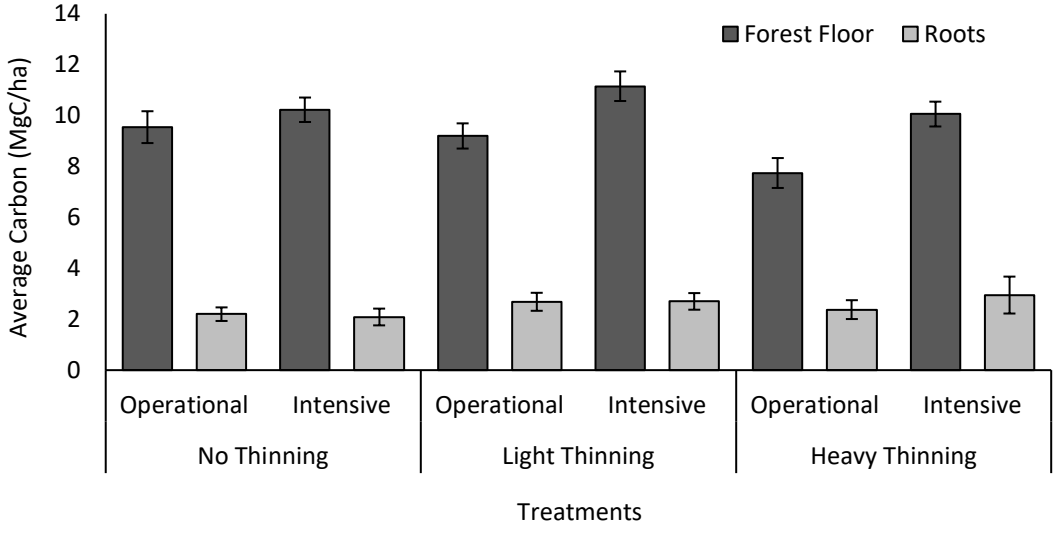

Figure 2.1 Average carbon stocks (MgC/ha) ± standard errors, for forest floor, and roots to a depth of 100cm across management and thinning intensities.

There were no significant main treatment effects on bulk C stocks when analyzed at the entire core depth (0-100cm) nor at the 0-15cm depth (Table 2.2 and 2.3). Overall, soil type had no significant impact on C stocks. Averaged across the management intensity treatments and

soil types, the bulk C stocks to 100 cm depth for the no thinning, light thinning, and heavy thinning treatments were 41.89 ± 1.44, 38.86 ± 1.18, and 39.92 ± 1.55 Mg C/ha, respectively (Figure 2.2). Mean C stocks to 100 cm depth did not significantly differ between the operational and intensive management intensities (39.94 ± 1.09 and 40.35 ± 1.20 Mg C/ha; Figure 2.2).

While there were no significant main treatment effects, there was a significant management and thinning intensity interaction effect on total core soil C stocks (Table 2.2). The operational management treatment had greater, yet non-significant, soil C stocks compared to the intensive management treatment in the no thinning and light thinning intensity treatments (Figure 2.2). However, in contrast, the intensive management treatment had significantly higher C stocks (15%) than the operational management treatment in the heavy thinning intensity treatment (42.74 ± 2.57 compared to 37.10 ± 1.68 Mg C/ha; p-value= 0.05; Figure 2.2).

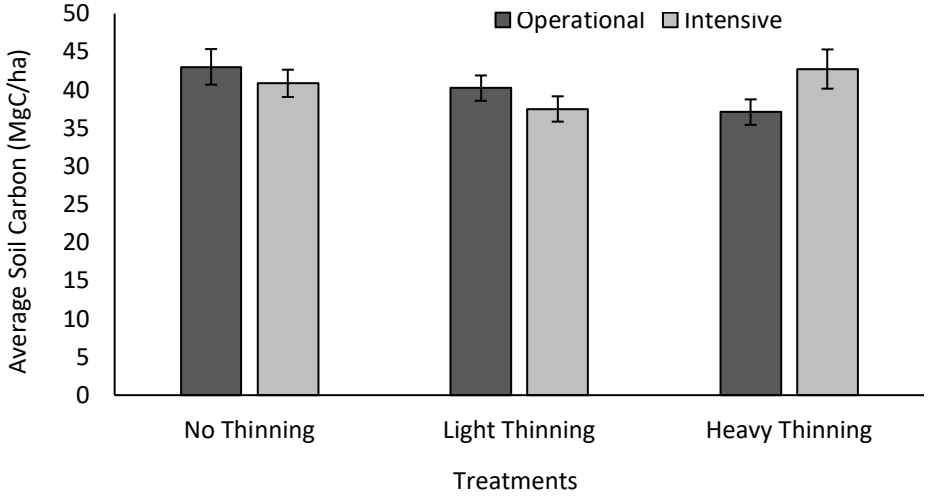

Figure 2.2 Average soil carbon (C) stocks (MgC/ha) ± standard errors down to 100cm across management and thinning intensities. No significant main treatment effects on soil C stocks.

There was a significant interaction between management intensity and thinning intensity on soil C at the 0-15cm depth (p-value= 0.03; Table 2.3). Top soil C stocks were

significantly greater in the operational management treatment compared to the intensive management treatment in the light thinning intensity treatment (15.40 ± 0.72 and 12.62 ± 0.60 MgC/ha, respectively; p-value= 0.02, Figure 2.3) but not in the other thinning intensity treatments. There was also a significant interaction between management intensity and soil type on soil C stocks at the 0-15cm depth (Table 2.3). The intensive and operational management treatments had similar top soil C stocks in all soil types except in the well-drained, shallow restrictive layer soil (15.02 ± 0.75 compared to 14.30 ± 0.66 MgC/ha). Whereas the well-drained, shallow restrictive layer soil type had significantly (29% higher) soil C stocks in the operational management treatment compared to the intensive management treatment (17.55 ± 0.84 and 13.64 ± 1.17 MgC/ha, respectively; p-value <0.01).

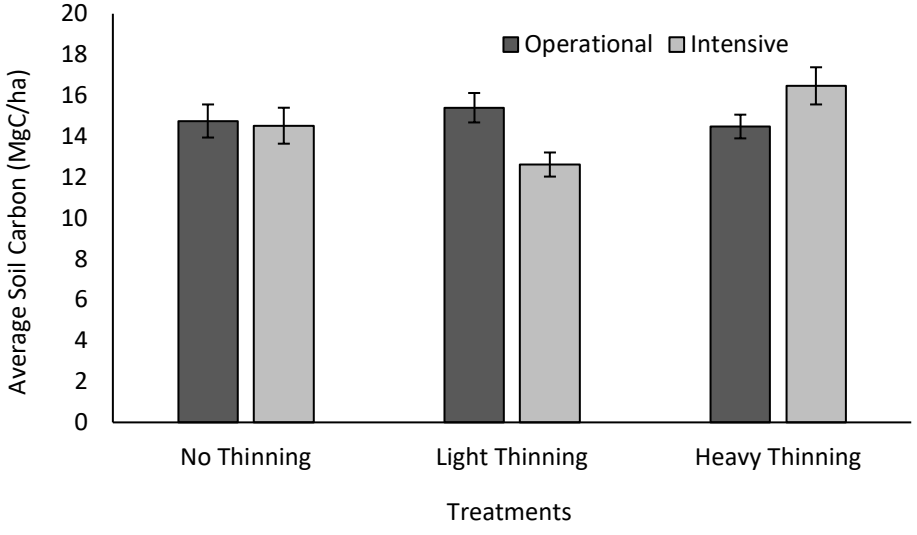

Figure 2.3 Average soil carbon (C) stocks (MgC/ha) ± standard errors in the 0-15cm depth across management and thinning intensities. No significant main treatment effects.

While there were no significant main treatment effects on bulk soil C stocks in the 0-15cm depth, there was a significant management intensity effect on some SOM fractions. There were no differences in the relative amount of total soil C found in the fLF across the operational

and intensive management treatments. However, there were small but significant differences in the relative distribution of C found in POM and MAOM across the operational and intensive management treatments (Figure 2.4). The operational management treatment had a significantly higher relative amount of soil C found in POM than the intensive management treatment (18.3% ± 0.43% compared to 16.6% ± 0.38%). Whereas the intensive management treatment had a significantly higher relative amount of soil C found in MAOM than the operational management treatment (55.3% ±0.87% compared to 53.3% ± 0.91%).

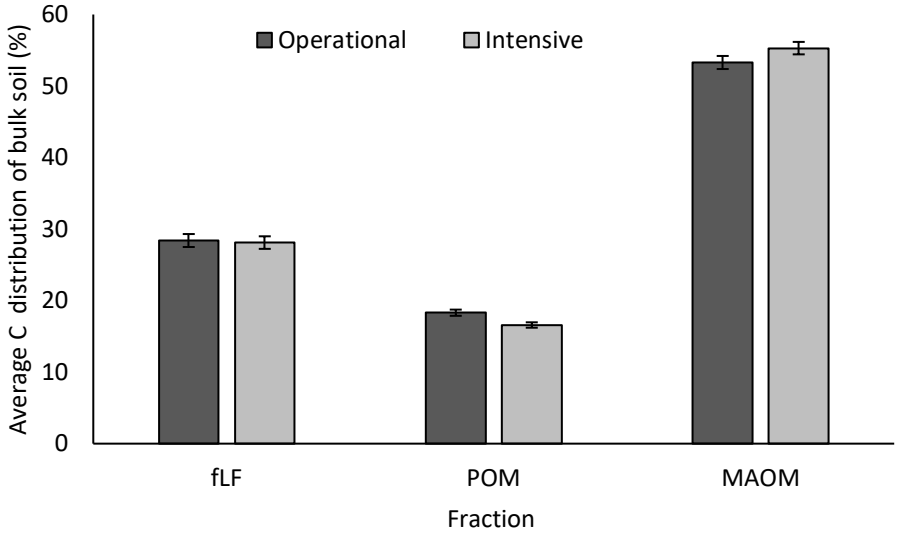

Figure 2.4 Average distribution ± standard errors of total soil C among soil organic matter fractions: free light fraction (fLF), occluded particulate organic matter (POM), and mineral-associated organic matter (MAOM) by management intensities.

Table 2.2 Linear mixed-effects model results for carbon stocks (Mg C/ha) down to 100 cm in soil, root and forest floor. Predictor effects include management intensity (Manage), thinning intensity (Thin), soil type (Soil), as well as all two-way interactions. Effects with p <0.1 are emphasized in bold.

	Roots (Mg C/ha)				Forest Floor (Mg C/ha)				Soil (Mg C/ha)			
Effect	Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F
Manage	1	42.01	0.55	0.46	1	43.05	16.73	**<0.01**	1	43.06	0.02	0.89
Thin	2	43.30	0.91	0.41	2	43.32	3.97	**0.03**	2	43.36	0.49	0.62
Soil	3	7.99	1.06	0.42	3	7.99	0.12	0.94	3	7.99	2.20	0.17
Manage x Thin	2	43.04	0.20	0.82	2	3.05	1.23	0.20	2	43.05	2.80	**0.07**
Thin x Soil	6	43.26	1.22	0.32	6	43.28	1.83	0.11	6	43.31	1.08	0.39
Manage x Soil	3	43.03	0.25	0.86	3	43.04	0.28	0.84	3	43.05	0.67	0.58

Table 2.3 Linear mixed-effects model results for carbon stocks (Mg C/ha) in the 0-15cm depth in soil and roots. Predictor effects include management intensity (Manage), thinning intensity (Thin), soil type (Soil), as well as all two-way interactions. Effects with p <0.1 are emphasized in bold.

	Roots (Mg C/ha)				Soil (Mg C/ha)			
Effect	Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F
Manage	1	42.99	0.72	0.40	1	43.01	0.73	0.40
Thin	2	43.51	1.86	0.17	2	43.29	1.53	0.23
Soil	3	7.97	1.32	0.33	3	7.99	0.09	0.97
Manage x Thin	2	43.04	0.07	0.93	2	43.04	4.02	**0.03**
Thin x Soil	6	43.44	1.10	0.79	6	43.25	0.63	0.71
Manage x Soil	3	43.05	0.01	1.00	3	43.03	3.84	**0.02**

Discussion 2.5

We found no significant differences in root C stocks across soil type, thinning intensities, or management intensities. To the best of our knowledge no other study has directly quantified the impact of early thinning intensity on root C stocks in these systems. However, the effect of fertilization on root biomass in pine plantations is well documented. Similar to our findings, others have also demonstrated that fertilization has no significant impact on coarse root (1-2mm) biomass (King et al., 2002), while nitrogen and phosphorus additions can either decrease fine root biomass (Samuelson et al., 2009; Vogel et al., 2015) or increase fine root biomass (King et al., 2002). The discrepancies in fine root biomass findings are likely due to the rapid turnover of fine roots as well as the wide range of methods used and the difficulty of measuring and quantifying root biomass (King et al., 2002). For this study, we used a method designed for accurate soil C stocks determination rather than root C stocks, thus we did not differentiate between coarse and fine roots.

While there was no management effect on root biomass, we found the intensive management significantly increased forest floor C, while heavy thinning tended to reduce forest floor C, as hypothesized. The decreases in forest floor C with heavy thinning could be explained by a significant loss of forest floor debris from harvesting (i.e., thinning) operations (Harding and Jokela, 1994). Not only have other studies measured significantly more forest floor production with fertilizer inputs (Drum, 2014; Vogel et al; 2011; Harding and Jokela, 1994), but have also recorded significantly more total aboveground biomass with fertilizer inputs (Zhao et al., 2016; Jokela et al., 2010; Sword Sayer et al., 2004) which would likely increase litter inputs. Interestingly, even though we saw significantly more forest floor C with greater fertilizer N

inputs, this trend did not carry over into an increase in soil C stocks. Additionally, since we did not see significant decreases in root C, the lack of management effect in soil C stocks cannot be attributed to any changes in root C. A possible explanation for the lack of management effect on soil C stocks could be due to the negative influence of fertilization events on the abundance of soil fauna (Gunnar and Thompson, 1979; Lohm et al., 1977). These faunal communities play a critical role in litter fragmentation, the creation of the fLF and the POM fraction, and the incorporation of litter products into the soil environment, and could explain why the increases in forest floor C was not translated into increases in bulk soil C stocks.

Many studies have also reported no significant impact of fertilization on bulk soil C stocks (Harding and Jokela, 1994; Johnson and Curtis, 2001; Leggett and Kelting, 2006; Sartori et al., 2007). We also found no significant differences in bulk soil C stocks with increased fertilization. We saw small increases in soil C stocks under the intensive management treatment, highlighting that management intensity did not significantly reduce bulk soil C stocks in these systems. Additionally, we saw an increase in MAOM likely due to a measured increase in decomposition from added nutrient inputs (Richter et al., 1999; Trettin et al., 1999) especially if nutrient additions do not significantly increase soil acidity (Averill et al., 2018). We did not see any differences in soil acidity across our management treatments. The greater soil and forest floor C stocks under intensive management indicate that these plantation systems are likely most productive with additional nutrient inputs (Table 1; Figure 2; Zhao et al., 2016; Drum, 2014; Vogel et al; 2011; Jokela et al., 2010; Harding and Jokela, 1994; Sword Sayer et al., 2004.) Our results also showed that any decreases in soil C were from SOM fractions that are not mineral-associated. These findings suggest that nutrient inputs may increase microbial

efficiencies which contributes to greater MAOM formation and stabilization. Overall, the addition of fertilizer may move loblolly pine plantations towards becoming a larger C sink due to the negligible changes in soil C together with large increases in aboveground woody biomass (Samuelson et al., 2009; Lai et al., 2002; Maier et al., 2004).

We hypothesized that more intensive biomass removals would decrease soil C stocks, but our results show that early thinning practices have no significant impact on soil C stocks (Figure 2.1 and 2.2). Instead of decreases in soil C, we saw no differences in soil C with thinning intensity. These results are in accordance with others that show that some biomass removals do not negatively affect soil C stocks (Johnson and Curtis, 2001; Huntington and Ryan, 1990), and can often increase soil C stocks. For example, thinning has been found to increase bulk soil C stocks by 13-35% through the decomposition of coarse roots that are left behind following a thinning (Selig et al., 2008; Powers et al., 2005). Additionally, the opening of the canopy following thinning can allow for new growth, which can positively contribute to soil C (Peterson et al., 1997; Wetzel and Burgess, 2001). Our findings may not have shown significant increases because the coarse roots left after the thinning did not have adequate time to decompose, as coarse roots can take over 10 years before reaching 50% mass loss (Garrett et al., 2008). Additionally, our sampling design did not include coarse, structural roots.

We consistently saw that any management effects on soil C were not impacted by soil type. Often in these systems, differences in soil C stocks are a function of soil drainage and soil texture (Leggett and Kelting, 2006) with higher soil C stocks in poorly-drained clayey soil types compared to well-drained sandy soil types. This can often be attributed to higher compaction in poorly-drained clayey soils, significantly increasing bulk density, which can inflate soil C stocks

(Powers et al., 2005). However, we saw no significant differences in bulk densities between the poorly-drained clayey and the well-drained sandy soils, which is likely why we did not have measured differences in soil C stocks across these soil types.

Our results also showed that management intensity had an impact on the SOM fraction distribution. In support of our hypothesis, we found that higher fertilization inputs increased the stable MAOM pool and decreased the less stabilized POM pool. Vogel et al., 2015 also demonstrated that fertilization can increase soil C stocks in the heavy soil fraction, which is the soil fraction that is more stable and less susceptible to decomposition compared to its more labile soil C counterparts. Once again, this could be due to an increase in decomposition of the POM with added nutrient inputs (Averill et al., 2018; Richter et al., 1999; Trettin et al., 1999). Nitrogen is an essential and limiting nutrient for decomposition (Staaf and Berg, 1982) and often increases in nitrogen availability can stimulate microbial activity (Averill et al., 2018; Shimel and Weintraub, 2003) which has been measured through increases in microbial respiration (Samuelson et al., 2009) and the MAOM pool (Averill et al., 2018). Since the MAOM pool is mostly made up of microbial products (Clemente et al., 2011), this could explain why we saw decreases in the POM pool and increases in the MAOM pool.

Overall, our results suggest that management intensification to support biofuel production using feedstocks from early thinning of loblolly pine plantations may not adversely affect soil C stocks, and may possibly increase soil C stabilization in the more persistent MAOM fraction. Additionally, our soil organic C results were consistent across all soil types investigated, which provides further evidence that these findings could be widespread across the pine plantations of the Southeast United States. This study demonstrates that, from a soil C

stock perspective, early thinnings from intensively managed loblolly pine plantations have potential as a sustainable biofuel feedstock.

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CHAPTER 3: Adaptive multi-paddock grazing increases soil carbon and nitrogen stocks and shifts their distribution in southeastern U.S. grazing lands

Overview 3.1

Conventional continuous grazing has led to significant losses of carbon (C) in grassland soils. By promoting grazing management that improves soil C sequestration and soil health, grasslands have a large potential to help alleviate rising atmospheric CO_2 as well as increase the sustainability of soil across a vast area of land. Previous research has shown that rotational grazing, specifically adaptive multi-paddock (AMP) grazing can increase soil C stocks in these grasslands systems, but the extent and mechanisms are unknown. Our research analyzed soils from 10 grasslands in the southeast United States representing either AMP or continuous grazing grassland management. We quantified soil C stocks as well as the distribution of C among soil organic matter (SOM) pools with varying mechanisms of formation and persistence in soils. Our findings show that the AMP grazing sites had over 13% more soil C and 9% more soil nitrogen compared to the continuous grazing sites. Adoption of AMP grazing lead to an increase in the soil of 9 tC/ha and 1 tN/ha over 1 meter depth relative to conventional grazing. Additionally, the increase was mostly in the mineral associated organic matter fraction, suggesting long term persistence of the accrued C on AMP grazing farms. These findings provide evidence that AMP grazing is a powerful management strategy to sequester C in soil and mitigate rising atmospheric CO_2 levels.

Introduction 3.2

Grasslands hold a lot of soil organic matter (SOM) averaging ~331 Mg SOM/ha down to a 1 meter depth (Schlesinger, 1977). Grasslands are also extensive in the U.S. and those that are

grazed cover 12% of total U.S. land (Census of Agriculture 2017). Grassland management improvements have been identified as a potential climate change mitigation strategy that could have a high impact due to its high potential area of adoption, sequestering up to 0.3 to 1.6 Pg CO_2 equivalents/year (Paustian et al., 2016). By promoting grazing management that improves soil C sequestration, grasslands have a large potential to help alleviate rising atmospheric CO_2 as well as increase the sustainability of the soil across a vast area of land.

Continuous grazing management has led to significant losses of soil carbon (C) and ecosystem function from grasslands (Teague, 2018; Conant et al., 2001). Specifically, conventional grazing management has led to altered nitrogen (N) cycling, increased erosion, and runoff of plant available water and nutrients (Pineiro et al., 2010; Milchunas and Laurenroth 1993). The cattle tend to congregate to nutritious patches and deplete the forage quickly (Teague et al., 2013; Barnes et al., 2008). That area will also experience high rates of erosion, bare patches of ground, and have a harder time regenerating (Teague et al., 2016; Teague et al., 2004; Bailey et al., 1998).

Early studies looking at rotational grazing have shown on a small-scale that this management can restore grassland ecosystem function and have beneficial impacts on soil as well. Specifically adaptive multi-paddock (AMP) grazing management, which utilizes small paddocks with high stocking densities for short periods of time, allows the soil to rest and the forage vegetation to replenish before the animals are returned (Conant et al., 2003). AMP grazing has shown to increase biodiversity, plant nutrition, cow health, among other benefits (Teague et al., 2016). The soil impacts are also promising, such as greater C stocks (Teague et

al., 2011; Conant and Paustian 2002; Machmuller et al., 2014), higher N stocks, more nutrients, and greater water infiltration (Teague 2018; Franzluebbers and Stuedemann 2009).

However, many of these studies were performed on a small-scale and produced results of varying magnitudes and directions (Briske et al., 2008; Teague et al., 2015),. This has made it difficult to understand where and when the improvements in management will have significant benefits to the soil environment. Additionally, these studies have not clearly investigated the effect of AMP grazing on the C distribution across SOM fractions. All pointing to the need for regional-scale studies across differing soil types, and environmental conditions, pairing AMP with conventional management on farm, and assessing soil C changes beyond the bulk soil, in functionally distinct SOM fractions.

All soil carbon is not created equally. Depending on the pathway of SOM formation and stabilization mechanisms, SOM can have varying times of persistence in the soil (Cotrufo et al., 2019; Cotrufo et al., 2015). For example, much of the particulate organic matter (POM) is derived from plant litter inputs and microbial products and has shorter residence time in the soil, whereas the majority of mineral-associated organic matter (MAOM) is microbially-derived and is considered the most persistent SOM pool (Clemente et al., 2011). Four SOM pools of particular importance and briefly include dissolved organic matter (DOM)- organic matter that is readily bioavailable, light POM- plant litter that is in the early stages of decomposition (Christensen 2001), heavy POM- more decomposed plant litter often protected by aggregation (Golchin et al., 1997), and MAOM- mostly microbial in origin and characterized by strong chemical bonds with minerals. Separating and quantifying SOM into functionally meaningful pools gives us more information about the mechanisms driving SOM accrual, its persistence,

and helps us to better understand the vulnerability of soil C to disturbance and management practices.

This study analyzed soils from five paired grazed grasslands in the southeast region of the United States, representing either AMP grazing management or continuous grazing (CG) management. Each of the pairs had two farms located across a fence line on neighboring farms to allow for a direct comparison of management strategies on the same soil type and similar facing slopes. We quantified soil C and N stocks as well as their distribution among SOM pools described above, i.e., DOM, light POM, heavy POM, and MAOM. The main objectives of this study were to determine if AMP grazing management increases soil C and N stocks and whether soil C storage shifted to deeper in the soil and to more persistent SOM physical fractions, in AMP grazing management relative to CG management.

Methods 3.3

We identified study sites after careful screening of AMP managed farms, which were paired with adjacent continuous managed farms. We selected study site locations to represent a latitudinal gradient from just south of Louisville, Kentucky through Southwestern Mississippi (Figure 3.1; Table 3.1). All AMP farms we selected had been using the identified grazing practice for a minimum of 10 years and all continuous grazers (CG) had also continuously grazed with unchanged practices for a decade or more. This represents five sets of paired grazed farms, with one neighbor practicing CG management and the other practicing AMP grazing management.

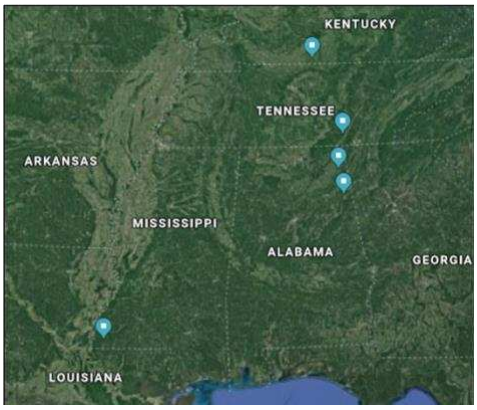

Figure 3.1 Map of farm pair locations. Each pair location contains one farm implementing adaptive multi-paddock grazing and one implementing continuous grazing.

Table 3.1 Farm pair locations, climate, soil, and management information.

Farm Pair	Farm Location	Soil Taxonomy	MAT	MAP	Grazing Practice	Inorganic N Fertilzation	Lime Additions	Herbicide Additions
1	Adolphus, Kentucky	Paleudult	13.75°C	131.57cm	AMP	no	yes	yes
					CG	no	no	no
2	Sequatchie, Tennessee	Dystudept/Paleudalf	14.17°C	143.15cm	AMP	no	no	no
					CG	yes	no	yes
3	Fort Payne, Alabama	Hapludult	15.11°C	141.96cm	AMP	no	yes	yes
					CG	yes	no	yes
4	Piedmont, Alabama	Paleudalf	15.67°C	135.23cm	AMP	no	yes	no
					CG	no	yes	no
5	Woodville, Mississippi	Fragiudalf	19°C	164.87cm	AMP	no	no	no
					CG	yes	yes	yes

At each grazed farm, we sampled two representative catenas. At each catena, we randomly established 3 longitudinal transects across the top of the catena, at the mid-slope of the catena, and at the toe-slope of the catena. We sampled six to eight representative soil cores contained in plastic sleeves along each transect with a Giddings unit down to 1 meter or as deep as was accessible. All sampling occurred within a period of six weeks, in the months of May-July 2018.

The intact soil cores were shipped to Colorado State University where we broke them down into meaningful horizons and/or depths. We first separated the A-horizon from the rest of the core by hand using a knife, and recorded at its depth. Then we separated the remaining

core by depth: the bottom of the A-horizon down to 30cm, 30-50cm, and lastly 50-100cm. We 8mm sieved each incremental core sample and removed and quantified rocks, roots, and noticeable litter. Next we took a representative soil sample for gravimetric water content and then we de-quarantined the sieved soils by heat treatment in a 110°C oven, according to APHIS protocol. After heat treatment, we 2mm sieved the soils where any remaining rock, root and litter fragment were removed and quantified. This allowed us to determine an accurate bulk density and root mass for each core depth increment.

To determine total C and N concentrations we ground and analyzed a subsample of soil by dry combustion on an elemental analyzer (Carlo Erba NA 1500). We also tested the soils for the presence of inorganic C using an acid pressure transducer connected to a voltage meter (Sherrod et al., 2002). If we found any inorganic C, it was quantified and removed from the total C amount to allow us to determine total organic C. Total organic C and N concentrations were scaled to total stocks using bulk density measurements determined during core processing.

We composited the 2mm sieved samples by transect to create a representative sample for each transect at each depth. There were six transects per grazed farm. There were 60 transects in total as well as 4 representative depths at each transect. This gave us a total of 240 composited samples for the SOM fractionation analysis. We fractionated each composited sample according to Mosier et al. (2019), and as described in Chapter 3, but modified to sample for the DOM fraction prior to the separation of light POM, heavy POM, and MAOM. Briefly, we added DI H_2O and shook for 15 minutes, then centrifuged for 15 minutes at 3400 rpm. Then we poured off the DOM fraction and analyzed for total organic C and total N on a Shimadzu TOC-L/TNM-L Analyzer(Shimadzu Corporation, Kyoto, Japan).

We assessed the effect of grazing management type and pair location on %C, %N, bulk density, and total soil C and N stocks, as well as the distribution of each SOM C stock between functionally distinct fractions (DOM, Light POM, Heavy POM, and MAOM) with a general linear mixed-effects model using a significant alpha level of $p < 0.05$. We used a nested block design as a random effect, with transect nested within catena. This allowed us to look at the overall effect of grazing management type across all farm pairs as well as the differences in grazing management type between each farm pair. R software was used for this analysis (R version 3.3.1; R core Team, 2016) with the lme4 package (Bates et al., 2015). We performed a combination of log and square root transformations when the data was non-normal distributed or had unequal variance. Additionally, we tested several factors associated with management and environmental differences between farms as covariates (Table 1). The covariate information was collected by our project partners through climate data and farmer interviews/surveys. We ultimately left out the covariates from the final model as none of them were significant.

Results 3.4

There was over 13% more total soil organic soil C across 1 meter depth on AMP farms compared to CG farms. Soils in AMP farms had on average 72.49 +/- 1.25 tC/ha while in CG farms had on average 64.02 +/- 1.04 tC/ha (Figure 3.2a; p-value= 0.02). The increase in soil C stocks was most pronounced in the A-horizon depth, but continued at each depth increment down to 1 meter (Figure 3.2a). There were two farm pairs where AMP grazing did not have significantly more soil C than CG (Figure 3.3a; Table 3.2). The other three farm pairs had significantly more soil C than CG (Figure 3.3a; Table 3.2).

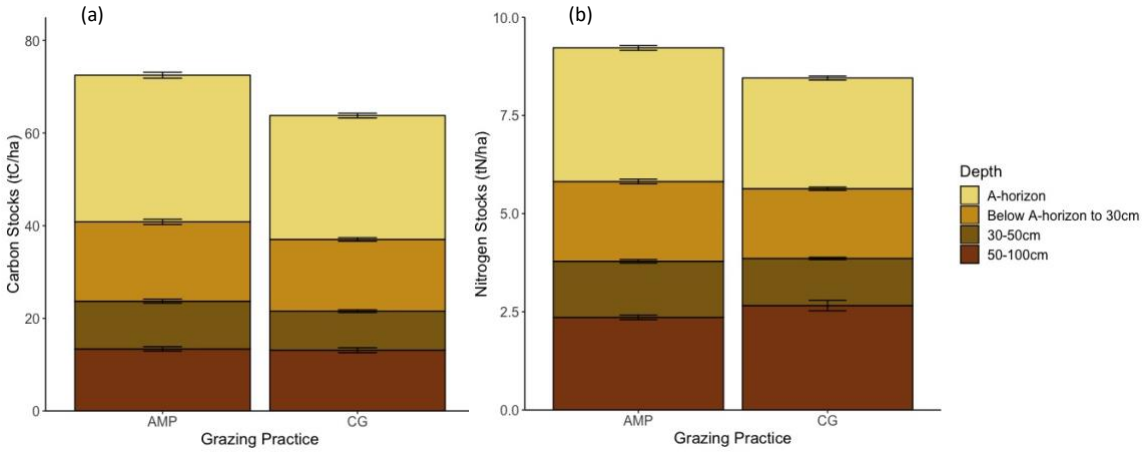

Figure 3.2 Total soil organic C stocks (a) and total soil N stocks (b) down to 1 meter +/- standard errors separated by core depth increment and by adaptive multi-paddock (AMP) and continuous (CG) grazing managements.

Overall standing root mass C stocks were relatively small compared to the total soil organic C stocks. When we averaged all farm pairs together, there was significantly more total standing root mass C on CG farms compared to AMP farms (p-value= 0.01). This result was driven by the two southernmost farm pairs, where on average CG farms had over 4 times more standing root biomass C than AMP farms. In the other three farm pairs, there was no difference in root biomass C between grazing types (Figure 3.3b). However, when standing root biomass C and total soil C were combined and averaged across farm pairs, AMP farms still had greater total belowground C than CG farms (75.79 +/- 1.31 and 71.01 +/- 1.09 respectively; p-value= 0.24).

Total soil N stocks were significantly greater in AMP farms relative to CG farms. There was on average over 8% more soil N in AMP farms compared to CG farms (Figure 3.2b; p-value<0.01). Soils in AMP farms averaged 9.26 +/- 0.14 tN/ha and in CG farms averaged 8.52 +/- 0.13 tN/ha. These results were consistent for all five farm pairs, but only statistically significant on three farm pairs (Figure 3.3c).

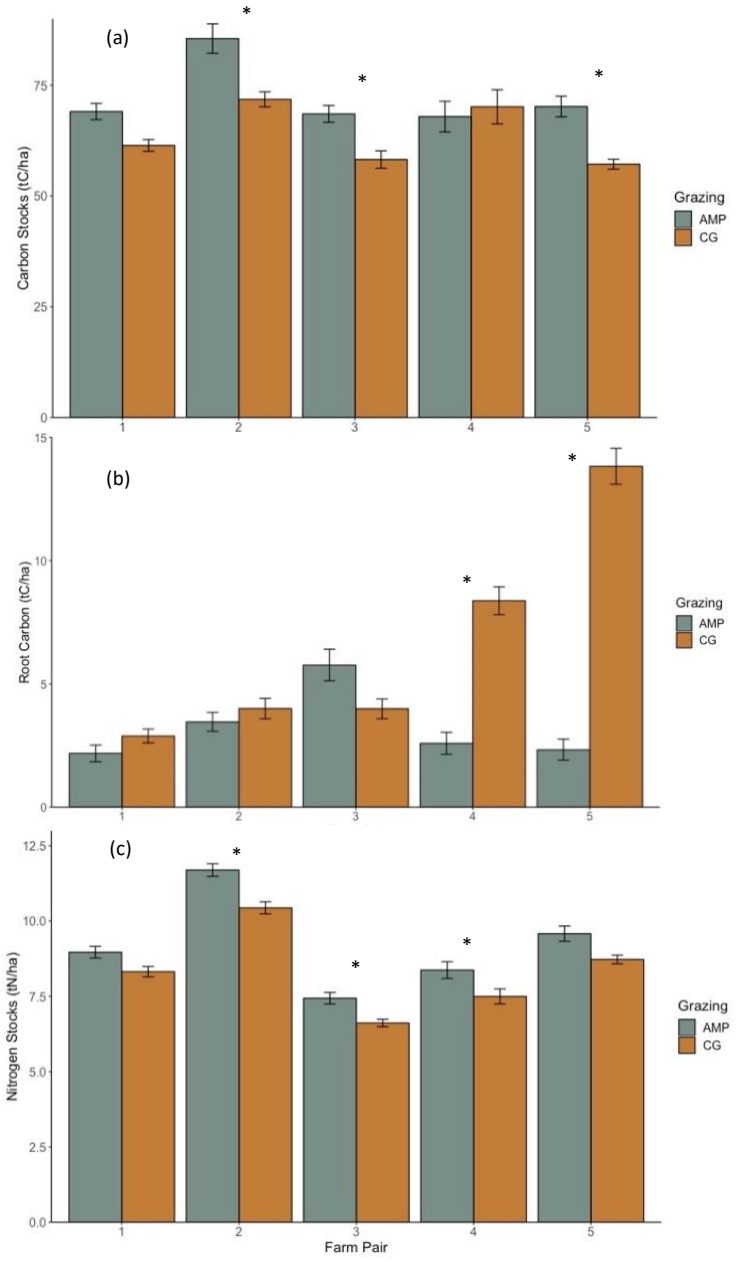

Figure 3.3 Total organic C stocks (a), total root biomass C stocks (b), and total soil N stocks (c) down to 1 meter +/- standard errors separated by farm pairs and by adaptive multi-paddock (AMP) and continuous (CG) grazing managements. Asterisks denote significant differences between farm pairs.

Differences in soil C and N stocks were the results of differences in C and N concentrations, not bulk density. We saw no significant differences in bulk density between grazing managements at any of the core depth increments except in the 50-100cm depth. In

the 50-100cm depth CG farms had higher bulk density than the AMP farms (p-value = 0.014). We saw significantly higher C and N concentrations on AMP farms compared to CG farms at every core depth increment except the 50-100cm depth. At this deepest depth, we found no differences in either C or N concentrations.

Soil C fractions shifted towards more persistent fractions in AMP farms down to 1 meter at all soil depth increments measured. Overall, there was 25% more C in the MAOM fraction on AMP farms compared to CG farms, with AMP having 56.14 +/- 1.98 tC/ha and CG having 44.82 +/- 1.01 tC/ha (Figure 3.4; Table 3.2; p-value <0.01). Additionally, there was over 15% more C in the heavy POM fraction on AMP farms compared to CG farms, with AMP having 9.80 +/- 0.36 tC/ha and CG having 8.47 +/- 0.27 tC/ha (Figure 3.4; Table 3.2; p-value= 0.02). There were no differences in the amount of C found in the light POM fraction (Figure 3.4; Table 3.2). We found significantly more DOM C on the AMP farms compared to CG farms (2.50 +/- 0.13 compared to 2.19 +/- 0.14 tC/ha, respectively), however this fraction only contributed 3% of the total soil (Figure 3.4; Table 3.2; p-value <0.01).

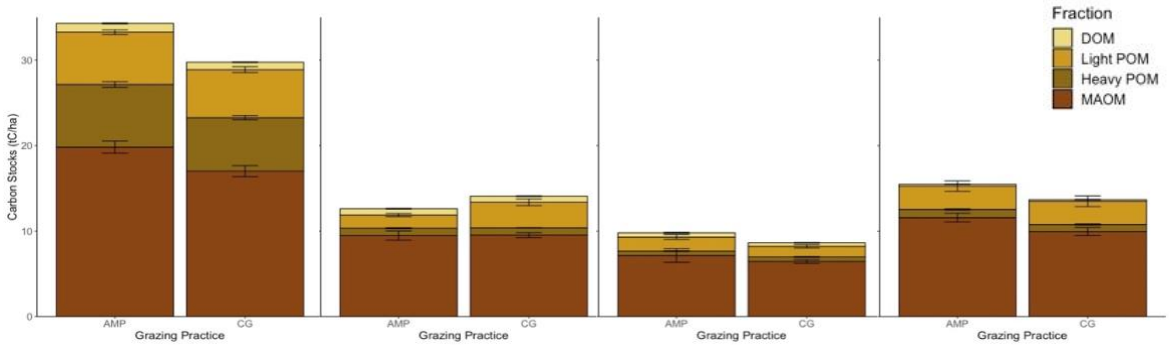

Figure 3.4 Soil C stocks (tC/ha) separated by fraction distribution for A-horizon (a), below A-horizon to 30cm (b), 30-50cm (c) and 50-100cm (d) +/- standard errors separated by adaptive multi-paddock (AMP) and continuous (CG) grazing managements.

Table 3.2 Farm pairwise comparisons of the differences between each response variable p-values as well as the general linear mixed effect model fixed effect p-values including all farm pairs. Significant differences are in bold. Red denotes when continuous grazing > adaptive multi-paddock grazing, black denotes when adaptive multi-paddock grazing > continuous grazing.

	Farm Pairwise Comparisons					Anova Results		
	Pair 1	Pair 2	Pair 3	Pair 4	Pair 5	Grazing	Location	Grazing*Location
Total soil organic carbon	0.1301	**0.0154**	**0.0213**	0.6927	**0.0033**	**0.0002**	**<0.0001**	0.16227
Standing root carbon	0.1016	0.4132	0.0829	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Total belowground carbon	0.2459	**0.0474**	**0.0192**	0.2491	0.9163	**0.0467**	**0.0035**	0.09
Total soil nitrogen	0.1408	**0.0098**	**0.0291**	**0.0149**	0.0591	**<0.0001**	**<0.0001**	0.9236
MAOM stocks	0.5816	**0.0468**	0.3459	**0.0222**	**0.0388**	**0.00259**	0.1298	0.5076
Heavy POM stocks	0.8772	0.8902	**0.0326**	0.1509	0.1558	**0.02539**	0.1061	0.4431
Light POM stocks	0.4046	0.241	0.506	0.9696	**0.0309**	0.9107	0.1573	0.1338
DOM stocks	**0.0002**	**0.0343**	0.7023	**0.0108**	0.3492	**0.00967**	**<0.0001**	**0.0014**

We also saw reductions in the A-horizon C:N ratios of the bulk soil and several of the SOM fractions. There were no differences in the A-horizon C:N ratio of the MAOM fraction (Table 3.3), but the A-horizon C:N ratio was lower in the heavy POM (13.18 +/- 0.43 compared to 14.91 +/- 0.43; p-value= 0.06), light POM (15.87 +/- 0.48 compared to 18.00 +/- 0.48; p-value=0.06), and DOM fractions (22.06 +/- 10.48 compared to 94.26 +/- 29.32; p-value= 0.03) on AMP farms relative to CG farms (Table 3.3). This trend of lower fraction C:N continued down to 50cm, but were much less pronounced and not statistically significant (Table 3.3).

Discussion 3.5

We observed that soils under AMP grazing had greater soil C stocks than soils under CG grazing. The soils C stock values were on average within the range of other reported soil C values of 35-51 tC/ha down to the 30-50cm depth of grassland soils in the southeast region of the US, as well as other grassland regions of the US and Australia (Machmuller et al., 2014; Hendrix et al., 1998; Conant et al., 2003; Stanley et al., 2019; Beare et al., 2014). In the majority of published studies, soils were only sampled to a depth of 30cm or 50cm, with none sampled down to 1 meter. Because we sampled soils deeper than previous studies, our overall total

Table 3.3 Average C:N ratios (with standard errors in brackets) of bulk soil and each SOM fraction (mineral-associated organic matter- MAOM, heavy particulate organic matter- Heavy POM, light particulate organic matter- Light POM, and dissolved organic matter- DOM) separated by incremental core depth. General linear mixed effect model p-value results (including all farm pairs) as well as farm pairwise comparisons of average C:N of bulk soil and each SOM fraction separated by incremental core depth. Significant differences are in bold. Red denotes when continuous grazing > adaptive multi-paddock grazing, black denotes when adaptive multi-paddock grazing > continuous grazing.

		C:N		Anova Results			Farm Pairwise Comparisons				
		AMP	CG	Grazing	Pair	Grazing*Pair	Pair 1	Pair 2	Pair 3	Pair 4	Pair 5
A-horizon	Bulk	9.27 (0.05)	9.66 (0.08)	**<0.0001**	**<0.0001**	**<0.0001**	0.2462	0.0777	**0.0387**	**<0.0001**	0.4561
	MAOM	8.53 (0.16)	8.47 (0.16)	0.675	**0.016**	0.3287	0.8855	0.1693	0.2491	0.7219	0.2359
	Heavy POM	13.13 (0.42)	14.87 (0.41)	**0.01787**	0.10967	0.03329	0.1307	0.1708	0.0721	**0.0058**	0.1182
	Light POM	15.82 (0.47)	17.94 (0.46)	**0.04418**	0.63585	0.06173	**0.5586**	0.8403	0.3362	**0.0037**	0.1442
	DOM	21.64 (10.14)	91.38 (28.44)	**0.03406**	0.19989	0.29436	**0.0242**	0.5929	0.433	0.4422	0.0972
to 30cm	Bulk	8.51 (0.10)	8.55 (0.10)	0.5519	**<0.0001**	**0.0005**	0.6204	0.8491	0.1938	**<0.0001**	0.1329
	MAOM	7.96 (0.15)	7.68 (0.11)	**0.032**	**0.0002**	**0.0023**	0.8622	**0.0013**	0.0637	**0.0254**	**0.0068**
	Heavy POM	12.87 (0.26)	14.03 (0.48)	**0.02907**	0.12006	**0.015**	0.2555	0.7273	0.4879	0.8177	**0.0005**
	Light POM	21.89 (1.67)	25.14 (2.63)	0.6724	0.4764	0.3033	0.6906	0.5097	0.1687	0.1375	0.5659
	DOM	11.13 (0.49)	11.78 (0.46)	0.4103	0.4124	**0.0071**	**0.007**	**0.0386**	0.9703	**0.011**	0.7329
30-50cm	Bulk	6.70 (0.11)	6.80 (0.12)	0.5789	<0.0001	0.1385	0.6104	0.8428	0.323	0.0522	0.1296
	MAOM	7.92 (0.38)	6.94 (0.16)	**0.0098**	**0.0003**	**0.00148**	0.2375	**0.0123**	**0.0027**	**0.002**	**0.0202**
	Heavy POM	13.85 (0.37)	14.06 (0.66)	0.8247	**0.02487**	0.06672	0.1749	0.9062	0.9248	**0.019**	0.1185
	Light POM	22.45 (2.98)	25.62 (2.39)	0.55003	0.1017	0.0943	0.1951	0.9911	0.0944	**0.0419**	0.7252
	DOM	15.67 (2.60)	12.51 (0.75)	0.886	**0.0064**	**0.0042**	**0.0017**	0.8516	0.4267	**0.0066**	0.9126
50-100cm	Bulk	5.72 (0.13)	5.32 (0.19)	0.1262	**<0.0001**	0.2691	**0.0456**	0.7815	0.2363	0.3004	0.3041
	MAOM	6.97 (0.55)	5.58 (0.23)	0.1026	**0.0012**	0.0929	0.759	0.41029	0.0624	**0.0358**	0.1215
	Heavy POM	14 46 (1.95)	12.15 (0.75)	0.3337	0.1014	0.4211	0.3957	0.7489	0.2839	0.0175	0.9449
	Light POM	20.69 (2.51)	21.67 (2.44)	0.8909	0.0859	0.2708	0.147	0.5596	0.2214	0.6401	0.2784
	DOM	3.15 (0.50)	3.37 (1.00)	0.5129	**<0.0001**	**<0.0001**	**0.0324**	**0.0136**	0.8198	**0.009**	0.3523

soil C stocks were higher than previously reported values (73-64 tC/ha). It was difficult to find other comparable studies, because of the diversity of grazing management types analyzed. However, despite these slight differences in grazing management practices, overwhelmingly, farms that implement rotational grazing in the southern US have higher soil C stocks compared to other conventional forms of grassland grazing management (Conant et al., 2003; Teague et al., 2011; Machmuller et al., 2014). In other regions such as Australia, the results show no significant differences in soil C stocks between rotational and continuous grazing due to low rainfall and poor vegetation productivity, difficulty capturing paddock heterogeneity, and confounding effects fertilizer application (Sandermand et al., 2015; Chan et al., 2010).

We observed large differences in root C stocks between farm pairs. At two of the five farms we observed that CG farms had much greater root C stocks than AMP farms. However, at the other three farms, there was no difference between grazing types and root C stocks. These results seem to be consistent with the aboveground vegetation types found at each farm. The farms with the greatest root C stocks are farms that were dominated by perennial grasses (Apfelbaum et al., in prep). Perennial grasses are known to have significantly greater root:shoot ratios compared to annual grasses, which would result in higher root biomass (Bray, 1963; Paustian et al., 1997). However, our grassland root C stocks did not parallel soil C stocks, suggesting that there could be faster root turnover on the farms with significantly lower root C stocks, which would contribute to the higher soil C stocks in these farms.

We consistently observed that AMP farms had higher soil N stocks than CG farms across the region. Interestingly, the only farms that utilized inorganic N fertilizers were those that were using CG grazing management. None of our AMP farms added inorganic N, whereas 3/5 of our CG farms implemented inorganic N inputs. AMP farms have cattle in greater concentrations at any given time, which more evenly distributes organic N cattle inputs from feces and urine to the soil (Teague et al., 2018). On a smaller scale, other studies have also found similar N stock values under most types of cattle grazing (Conant et al., 2003). Our findings confirm previous estimates of higher soil N stocks under rotational grazing versus continuous grazing (Conant et al., 2003). However, many studies found no differences in soil N stocks between grazing managements (Dubeux et al., 2006; Silveira et al., 2013; Altesor et al., 2006). This could be due to the fact that to only grazed versus ungrazed plots were being compared (Altersor et al.,

2006) or N stocks were compared across a gradient of N fertilization rates (Dubeux et al., 2006) and only short-term responses were measured (Silveira et al., 2013).

We consistently observed higher MAOM C stocks in the soils under AMP grazing compared to the soils under CG grazing. Based on the different pathways of MAOM formation, we know that higher quality inputs and the presence of more N can lead to increases in MAOM stocks (Cotrufo et al., 2015; Cotrufo et al., 2013). This is because microbes need N for metabolism and the majority of SOM has undergone some sort of microbial transformation (Miltner et al., 2002; Kallenbach et al., 2016). This points to the importance of N for microbial turnover and MAOM formation. Greater soil N stocks as well as lowered C:N ratios in the other fractions are likely the reason for why more MAOM was able to form and persist. Other studies have found mixed results when comparing MAOM fractions across grazing studies. These differences in MAOM stocks correlate with the differences in soil N stocks. For example, studies that found higher soil N stocks also saw higher MAOM stocks, whereas studies that showed no differences in soil N stocks also found no differences in MAOM stocks (Conant et al., 2003; Dubeux et al., 2006; Silveira et al., 2013; Altesor et al., 2006).

Similar to MAOM, we consistently saw higher heavy POM C stocks in the soils under AMP grazing relative to the soils under CG grazing. This result could be due to faster turnover as this pool is more active and also more vulnerable to microbial degradation and disturbance than the MAOM pool (Cotrufo et al., 2019). Additionally, the heavy POM pool had a lower C:N ratio under AMP grazing which means this pool has more accessible N and is more accessible to microbes (Averill and Waring, 2018; Shimel and Weintraub, 2003). Since more N is needed for microbial metabolism and ultimately MAOM formation, this finding indicates that the greater

proportion of N in the heavy POM fraction on AMP farms could lead to even MAOM formation (Clemente et al., 2011). Other studies have found mixed results when comparing heavy POM fractions across grazing studies which were influenced by things like different vegetation communities and fertilization (Conant et al., 2003; Dubeux et al., 2006; Altesor et al., 2006). However, of the studies, none had an identical soil fractionation scheme to the one we used here, which can make direct comparisons challenging. For example, SOM was only separated into POM and MAOM (Conant et al., 2003; Altesor et al., 2006) or SOM was only separated into light and heavy SOM (Dubeux et al., 2006).

We did not see any differences between grazing management types and light POM C stocks. This pool represents litter inputs that are rather recalcitrant and can often be in the early stages of decomposition (Christensen, 2001). While the relative quantities of light POM C were not different between grazing managements, the C:N ratios were lower under AMP grazing, indicating higher quality inputs that are more accessible to microbes, which could lead to faster turnover of light POM inputs as well as higher efficiencies in processing those inputs (Averill and Waring, 2018; Shimel and Weintraub, 2003).

Our findings show that the AMP grazing sites had over 13% more soil C and 9% more soil N compared to the continuous grazing sites. There was significantly more persistent C on AMP grazing farms compared to continuously grazed farms. Additionally, some of the soil organic matter fractions available for microbial transformation were of higher quality, with lower C:N ratios on AMP grazing farms relative to CG farms. There can be no long-term C sequestration without available N (van Groenigen et al., 2006; Averill and Waring, 2018). Therefore, the finding that AMP farms have both higher soil C and N stocks as well as lower C:N ratios in the

majority of SOM fractions relative to CG farms highlights the potential for AMP farms to sequester even more C. These findings provide strong evidence that AMP grazing management could be implemented at large scales as a way to sequester persistent C and mitigate rising atmospheric CO_2 levels.

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CHAPTER 4: Adaptive multi-paddock grazing improves soil health relative to continuous grazing in southeastern U.S. grazing lands

Overview 4.1

Soil health broadly refers to the capacity of soils to support food and fiber, all while providing and sustaining ecosystem services. Soil health has been severely compromised across grazing lands globally. In particular, continuous grazing management can impact soil health by reducing soil carbon stocks and other available nutrients, while creating bare vegetation patches that can increase erosion and runoff. In contrast, recently rotational grazing, specifically adaptive multi-paddock grazing (AMP), has been proposed as a regenerative grassland management that can improve several aspects of soil health such as soil carbon stocks, soil structure, as well as nutrient and water retention. Our research analyzed soils from 10 grasslands in southeast United States representing either AMP or continuous grazing management. We analyzed the A-horizons of these soils for physical, chemical, and biological soil health indicators across each management type. There were a number of soil health indicators, specifically chemical indicators, that were improved where AMP grazing management was implemented. These results provide strong evidence that AMP grazing management could be utilized to regenerate grassland soil health across a large area of conventionally managed grazing lands.

Background 4.2

Broadly, soil health refers to soils that are able to support food and fiber while also providing and maintaining ecosystem services (Larson et al., 1991; Doran and Parkin 1994; Kibblewhite et al., 2008). Soil health metrics encompass physical, chemical, and biological

properties of soil as they are all important for regulating a sustainable soil environment. Improving soil health can lead to increasing crop yield, water quality, drought and other extreme weather resilience, carbon (C) sequestration, and reducing greenhouse gas emissions (Kibblewhite et al., 2008; Byrnes et al., 2018). Thus, soil health has attracted a lot of attention, and several soil health initiatives have been developed to further research soil health indicators (Andrews et al., 2004; Moebius-Clune et al., 2016).

The Soil Health Institute is one organization of many that was created to promote sustainable soils by improving soil health. The institute created two lists of indicators to measure when monitoring soil health (*Soil Health Institute*). Their tier 1 list consists of metrics that are well documented regarding crop yields and have been responsive to management improvements, whereas Tier 2 consists of metrics that need more research and are less understood in terms of improving soil health (*Soil Health Institute*). Tier 1 includes, but is not limited to, soil pH, electrical conductivity, cation exchange capacity, % base saturation, extractable nutrients, texture, C and nitrogen (N) concentrations, water holding capacity, aggregation, bulk density, erosion rating, and C and N mineralization potentials. Tier 2 includes, but is not limited to, extracellular enzyme activities, phospholipid fatty acids, soil protein index, genomics, and reflectance.

Continuous grazing can have negative impacts on soil health, such as reducing soil C (Teague, 2018; Conant et al., 2001), increasing compaction and bulk density (Teague et al., 2016; Teague et al., 2004; Bailey et al., 1998; Steffens et al., 2008), and creating bare patches which affect plant productivity, erosion, and runoff of essential nutrients (Milchunas and Laurenroth 1993). Adaptive multi-paddock (AMP) grazing management practices involve having

cattle in dense concentrations for short periods of time. By having the cattle in greater concentrations, organic cattle inputs, specifically feces and urine, are more evenly distributed across the soil (Teague, 2018). The cattle are then quickly moved from the paddock, allowing the soil and vegetation to rest and recover from the grazing episode which can lead to improvements in soil health. For example, previous research has shown that AMP grazing can increase soil C (Teague et al., 2011; Conant and Paustian 2002; Machmuller et al., 2014), improve bulk density (Byrnes et al., 2018; Teague et al., 2013), soil structure (Teague, 2018), and nutrient & water retention (Franzluebbers and Stuedemann 2009), and reduce erosion (Teague et al., 2013).

Many of the studies that have analyzed the impacts of grazing on soil health have only looked at a few soil health indicators (Byrnes et al., 2018). To our knowledge, this is the first study to analyze and compare a large number of soil metrics, spanning both Tier 1 and Tier2 of the Soil Heath Institute test, between different grazing managements at a regional scale. This study analyzed soils from 5 paired grazed grasslands in the Southeast region of the United States, representing either AMP or continuous grazing (CG) management. For each pair the AMP and CG sites were located across a fence line on neighboring farms to allow for a direct comparison of management strategies on the same soil type and similar facing slopes. By analyzing soil health using such a large suite of physical, chemical, and biological metrics, we were able to determine whether AMP grazing relative to continuous grazing improves overall soil health. Additionally, we were able to better understand which suite of soil health indicators contributed to the biggest differences in grazing management.

Methods 4.3

We identified our study sites after careful screening of AMP managed farms, which were paired with adjacent CG managed farms. We selected study site locations to represent a latitudinal gradient from just south of Louisville, Kentucky through Southwestern Mississippi (Table 3.1). All AMP farms selected had been using this grazing practice for a minimum of 10 years and all CG farms had also been under continuous grazing with unchanged practices for a decade or more. This represents 5 sets of paired grazed farms, with one neighbor practicing CG management and the other practicing AMP grazing management.

At each grazed farm, we sampled 2 representative catenas. At each catena, we randomly established 3 longitudinal transects across the top of the catena, at the mid-slope of the catena, and at the toe-slope of the catena. We took 6-8 representative cores along each transect. We used a Giddings unit to take soil cores down to 1 meter or as deep as was accessible.

The soil cores were then shipped intact to Colorado State University where they were broken down into meaningful horizons and/or depths. We first divided the soil cores separating the A-horizon from the rest of the core. We recorded the A-horizon depth and then used the depth as a metric of soil health and regeneration. Then we separated the remaining soil core by depth: the bottom of the A-horizon down to 30cm, 30-50cm, and lastly 50-100cm. For the soil health study we only used the A horizon samples because this depth is the most commonly used for soil health tests and it is the most active soil horizon where much of plant and soil organism activity takes place (Stott and Moebius-Clune, 2017). We 8mm sieved each sample while removing and quantifying all rocks, roots, and noticeable litter. We took a representative

soil subsample from each sample for gravimetric water content. We then de-quarantined the sieved soils by heat treatment in a 110°C oven. After heat treatment, we 2mm sieved removing and quantifying any remaining rocks, roots, and litter.

We composited the A-horizon samples by transect to create one representative sample for each transect. There were 6 transects per farm, resulting in a total of 69 composites A-horizon samples to analyze for soil health metrics. We took a portion of soil and placed them in a -80°C freezer for microbial analyses and N mineralization rates. We air-dried another portion for extractable nutrients, C mineralization rates, aggregate stability, pH, electrical conductivity, base saturation, and cation exchange capacity. We de-quarantined any remaining soil by heat treatment in a 110°C oven to determine gravimetric moisture contents and for further analysis of soil C and N stocks and soil organic matter (SOM) fraction distribution, as described in Chapter 3.

We determined texture by using a standard hydrometer method. Briefly, we shook 40g of 2mm sieved oven dried soil for 18 hours with sodium hexametaphosphate to break up aggregates. Then we added the soil slurry to a gravimetric flask and density was sampled at time 0, 40 seconds, and at 2 hours (Gee and Bauder, 1986). We determined water-stable aggregates by using a wet sieving procedure (Kemper and Roseneau, 1986). We determined aggregate stability on 4 aggregate sizes: >2mm, 2mm-250um, 250um-53um, and <53um. All aggregate size classes were corrected for rock and sand particles. We determined a mean-weight diameter (MWD) estimate by multiplying the proportion of each aggregate size class by the median diameter of each size class and then summing them together to get one value

(Kemper and Roseneau, 1986). Additionally, we measured available water holding capacity using the ceramic plate method measured at 1/3 bars and 15 bars (Klute, 1986).

We determined potential C mineralization rates by performing a 262 day incubation. We weight a 50g aliquot of 8mm sieved air-dried soil, brought it up to 60% water-filled pore space, and sealed it in a mason jar and sampled for time 0 CO_2 concentrations using an infrared gas analyzer (LI-COR 800, LI-COR Biosciences, Lincoln, NE, USA). We sampled each jar for CO_2 production and then flushed with CO_2 free air periodically at 20 different time points over the course of 262 days. We then summed the CO_2 produced at each sampling time point to determine cumulative CO_2 mineralization. We checked moisture contents throughout the incubation to make sure all soils maintained the same moisture content.

We determined baseline inorganic N concentrations as well as potential NH_4^+ mineralization rates by weighing out ~6g fresh soil in centrifuge tubes and then adding 40 ml 2M KCl. We then placed the tubes on a shaker for 1 hour. After shaking, we centrifuged the tubes to separate the soil from the KCl. We poured the supernatant through a #42 Whatman filter and captured the filtered solution in a vial and then analyzed for NH_4^+ and NO_3^- analysis to determine baseline inorganic N concentrations (Bundy and Meisinger, 1994; Alpkem Flow Solution IV Automated wet chemistry system; O.I. Analytical, College Station, TX). We used another set of centrifuged tubes to weigh out ~6g of fresh soil + 10ml DI H_2O to promote mineralization and prevent nitrification. We purged these tubes with N_2 and then capped for 7 days. After 7 days of incubation, we uncapped the tubes and added 30ml 2M KCl to each tube. We then placed the tubes on a shaker and analyzed as described above. We subtracted the 7

day NH_4^+ values from the NH_4^+ baseline concentrations to determine potential NH_4^+ mineralization rates.

We determined pH in water using a 1:2 soil to DI H_2O slurry and a pH electrode system (Thomas, 1996). We measured electrical conductivity using a 1:2 soil to DI slurry and an electrical conductivity meter system (Thoades, 1996). Additionally, we quantified cation exchange capacity, % base saturation, and extractable nutrients using a Mehlich 3 extractant (Sikora and Moore, 2014).

We assayed potential activities of 6 hydrolytic extracellular enzymes [α-Glucosidase (AG), β-Glucosidase (BG), Cellobiohydrolase (CB), and β-Xylosidase (XYL), which are involved in C-acquisition, N-acetyl glucosaminidase (NAG), which is involved in N-acquisition, and Acid phosphatase (PHOS), which is involved in P-acquisition] using the 96-well microplate fluorometric method modified from others (Lynch et al., 2018, Bell et al., 2013, Koyama et al., 2013, Wallenstein et al., 2009). Briefly, we combined 1.0 g of soil with 30 ml of 50 mM sodium acetate buffer corrected for soil pH. Soil slurries were shaken for 1 hour and then we added 800 µl subsamples to a deep 96-well microplate. We pipetted 200 µl of 200 µM fluorescing substrate for all substrates and incubated them for 3 hours at 25 °C. We also prepared standards for each soil slurry using a range of concentrations of 4-methylumbellifferone. When the incubation was complete, we centrifuged plates for 3 min at 1500 rpm and transferred 250 µl from each well into black 96-well plates. Substrate fluorescence was measured on a Tecan Infinite M200 microplate reader at an excitation wavelength of 365 nm and an emission wavelength of 450 nm (Tecan Trading AG,

Switzerland). Phospholipid fatty acids (PLFAs) were extracted and analyzed following established methods (Denef et al., 2007; Gomez et al., 2014).

We assessed the effect of grazing management type on each soil health indicator and farm pair location with a general linear mixed-effects model using a significant alpha level of $p < 0.05$. We considered catena as a block and used it as a random effect. We performed a combination of log and square root transformations when the data was non-normal or had unequal variance. Additionally, we tested several factors associated with management and environmental differences between farms as covariates, but they were ultimately left out of the final model as they were not significant. We also performed a principle component analysis to determine the relative contribution of each soil health indicator to grazing management differences. R software was used for this analysis (R version 3.3.1; R core Team, 2016) with the lme4 package (Bates et al., 2015) and the factoextra package (Kassambara, 2019).

Results 4.4

We found that the average depth of the A-horizon was slightly deeper on four out of five AMP farms compared to CG farms (Table 4.2). The average A-horizon depth was 12.61 +/- 0.27 cm on AMP farms compared to 11.75 +/- 0.21 cm on CG farms (Figure 4.1a; Table 4.2; p-value= 0.36). The depth of the A-horizon impacted our texture comparisons as the distribution of sand, silt, and clay varied across farms. However, none of these differences were statistically significant. On average, AMP farms had greater proportions of clay-sized particles whereas CG farms had greater proportions of sand-sized particles. AMP farms had a sand:clay ratio of 1.82 +/- 0.29 and CG farms had a sand:clay ratio of 2.26 +/- 0.28 (Figure 4.1b; Table 4.2; p-value 0.02). We found no significant differences in MWD across grazing management types or

between any farm pairs (Figure 4.1c; Table 4.2). When each aggregate size class was analyzed individually, we also found no significant differences.

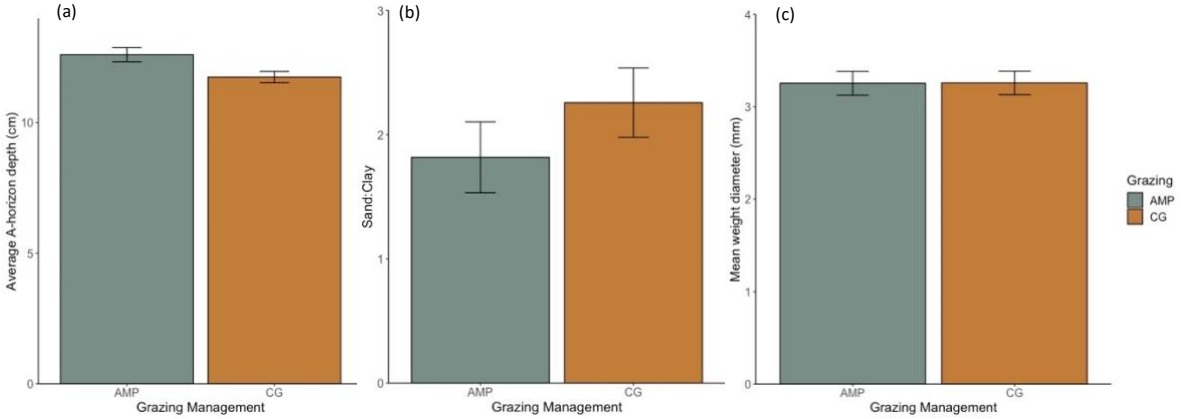

Figure 4.1 Comparison of average (a) A-horizon depth (cm), (b) sand:clay ratio, and (c) aggregate mean weight diameter (mm) +/- standard errors between adaptive multi-paddock (AMP) and continuous (CG) farms.

Additionally, we looked at water retention in a number of different ways. We compared initial moisture contents as well as available water holding capacity measurements across grazing managements. We did not see any significant differences in water holding capacity or moisture content between grazing managements (Table 4.2). However, in pair 4 CG had higher water holding capacity than AMP (Table 4.2). AMP farms had gravimetric moisture content of 17% +/- 0.00 and available water holding capacity of 0.21 +/- 0.01 (cm water/cm soil) compared to CG farms that had a gravimetric moisture content of 0.16 +/- 0.00 and available water holding capacity of 0.28 +/- 0.01 (cm water/cm soil).

We found the biggest differences between grazing management types in the chemical soil health properties. There was significantly higher pH, % base saturation, electrical conductivity, and cation exchange capacity at the AMP grazing sites compared to the continuous grazing sites. AMP farms had an average of 6% higher pH relative to CG farms (Table

4.2; p-value= 0.03). pH was used to calculate % base saturation, which was an average 16% higher in AMP compared to CG farms (Figure 4.2a; Table 4.2; p-value= 0.03). There was an increase in electrical conductivity and cation exchange capacity on AMP farms. Electrical conductivity averaged 0.23 +/- 0.01 (ds/m) on AMP farms compared to 0.15 +/- 0.01 (ds/m) on CG farms (Figure 4.2b; Table 4.2; p-value < 0.01). Cation exchange capacity was 33% higher on AMP farms with an average estimate of 15.62 +/- 0.89 (cmolc/kg soil) compared to 11.72 +/- 0.91 (cmolc/kg soil) on CG farms (Figure 2c; Table 4.2; p-value < 0.01). These findings were consistent on 3 out of 5 farm pairs and there was no differences in 2 farm pairs (Table 4.2).

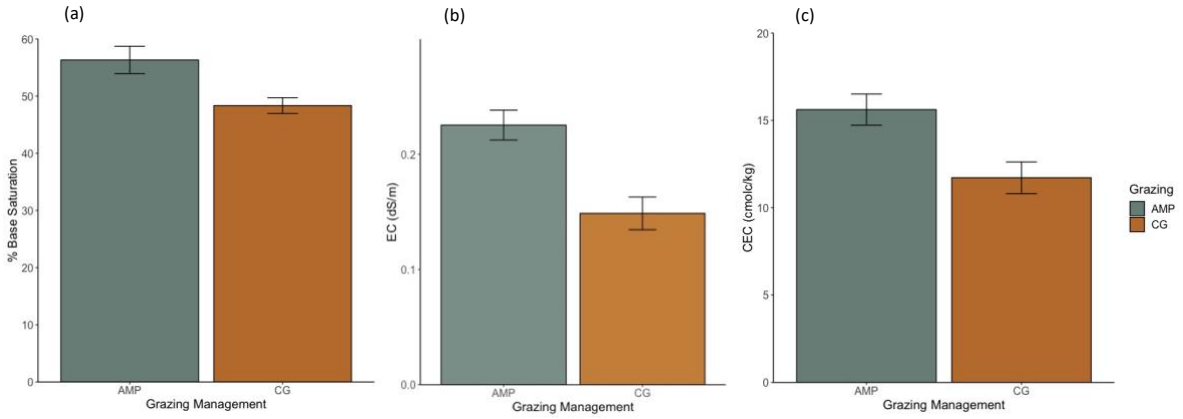

Figure 4.2 Comparison of average (a) % base saturation, (b) electrical conductivity (EC- ds/m), and (c) cation exchange capacity (CEC- cmolc/kg soil) +/- standard errors between adaptive multi-paddock (AMP) and continuous (CG) farms.

Overall, we found higher concentrations of extractable nutrients on the AMP grazing farms compared to the CG grazing farms. Of the nine extractable nutrients we analyzed, six were on average higher at the AMP sites, including Ca, Mg, K, Zn, Mn, and S (Table 4.1) However, only two extractable nutrients (calcium and potassium) were significantly higher on AMP grazing sites (Table 4.2; p-values < 0.01). These results were variable between each farm pair and were not consistent across all farm pairs (Table 4.2).

Table 4.1 Comparison of average extractable nutrient concentrations (mg/kg soil) with (standard errors) between AMP and CG farms.

	Ca	Mg	K	Na	Zn	Mn	S	Cu	Fe
AMP	1382.98 (114.96)	144.60 (11.22)	238.92 (18.41)	3.71 (1.03)	3.77 (0.34)	187.167 (24.21)	12.07 (0.25)	2.97 (0.18)	200.85 (21.31)
CG	831.01 (67.13)	116.10 (9.01)	148.82 (6.87)	4.12 (1.27)	3.45 (0.48)	167.37 (19.13)	11.55 (0.37)	4.10 (0.46)	204.41 (12.19)

There were no differences in the total amount of C that was mineralized over the 262 days incubation between grazing managements when they were normalized for starting soil C concentrations (Figure 4.3; Table 4.2). There was also no differences in the CO_2 respiration dynamics through time or between the cumulative CO_2 at any of the farm pairs (Figure 4.3; Table 4.2). However, potential N mineralization was higher on CG farms relative to AMP farms. During our incubation, 60% more organic N was mineralized into ammonium on CG farms compared to AMP farms (7.44 +/- 1.00 and 4.49 +/- 0.57 mg N/g N, respectively; Figure 4.4a; Table 4.2; p-value = 0.02). This result was consistent across all farm pairs, but only statistically significant at two farm pairs (Table 4.2). Initial concentrations of inorganic N also varied across grazing management types. CG farms had 29% more ammonium concentrations (Figure 4.4b; Table 4.2; p-value<0.01), whereas AMP farms had 55% higher nitrate concentrations (Figure 4.4b; Table 4.2; p-value= 0.06). Initial inorganic N concentrations varied between each farm pair and were not consistent across all farm pairs (Table 4.2).

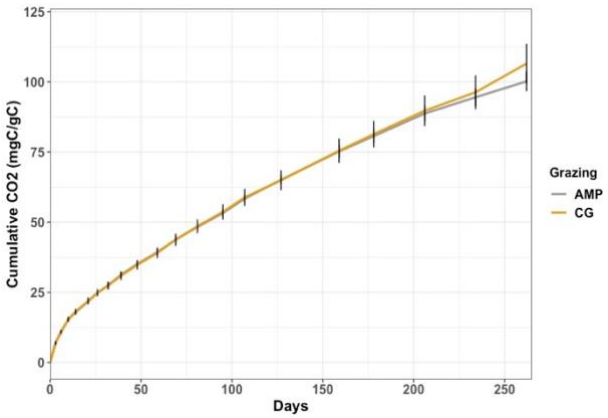

Figure 4.3 Comparison of the average potential C mineralization (mgC/gC) +/- standard errors over 262 days separated by adaptive multi-paddock (AMP) and continuous (CG) farms.

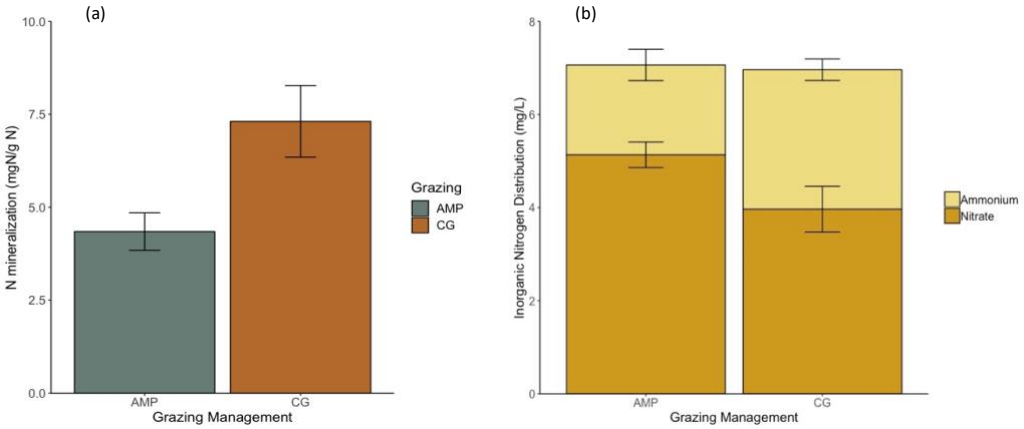

Figure 4.4 Comparison of (a) average N mineralization rates (mgN/gN/7 days) and (b) inorganic N distribution (mg/L) +/- standard errors between adaptive multi-paddock (AMP) and continuous (CG) farms.

We found no other statistically significant differences in the biological soil health indicators that we measured. However, we did find higher overall extracellular enzyme activities for each enzyme type measured on CG farms compared to AMP farms. When we combined all the C acquiring enzymes and summed them (BG, CG, XYL, and AG) we found that CG farms had 1.25 times more activity than AMP farms (169.75 +/- 23.91 compared to 134.78 +/- 9.98 nmol activity/g soil; Figure 4.5a; Table 4.2; p-value= 0.34). Additionally, CG farms had 1.32 times more N acquiring enzyme activity (78.46 +/- 14.86 compared to 59.20 +/- 4.07 nmol activity/g soil; Figure 4.5b; Table 4.2; p-value = 0.25) and 1.25 times more phosphorus acquiring

enzyme activity (244.80 +/- 25.16 compared to 195.92 +/- 17.37 nmol activity/g soil; Figure 4.5c; Table 4.2; p-values = 0.25 and 0.22, respectively). We found no differences between grazing management types in our PLFA microbial biomarker measurements. We analyzed our PLFA data for total PLFAs (AMP= 5.82 +/- 0.33 and CG= 5.89 +/- 0.37) as well as fungi:bacteria ratio (AMP= 0.09 +/- 0.00 and CG= 0.09 +/- 0.01 to name a few, all with nearly identical values between AMP and CG grazing farms (Table 4.2).

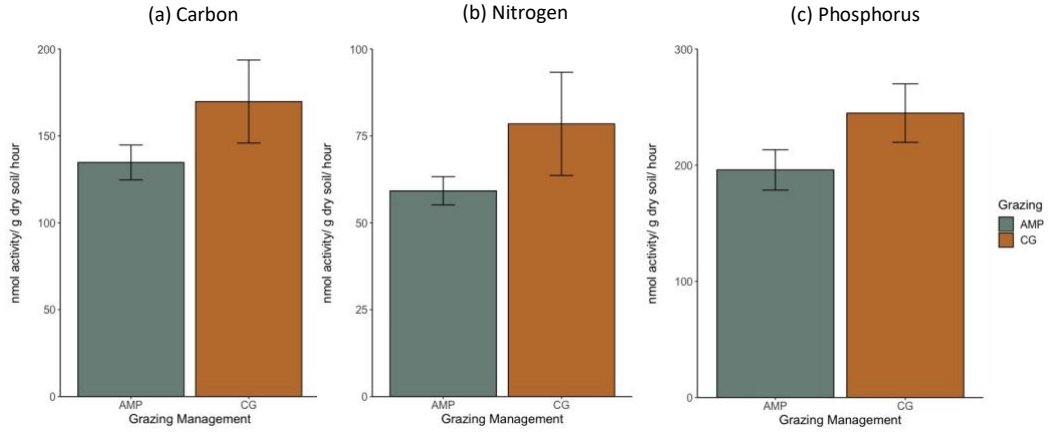

Figure 4.5 Comparison of average extracellular enzyme activities (nmol activity/ g dry soil) between adaptive multi-paddock (AMP) and continuous (CG) farms. (a) sum of carbon enzymes (BG+CG+AG+XYL), (b) nitrogen enzyme (NAG), and (c) phosphorus enzyme (PHOS).

We used a principle component analysis with all soil health indicators to determine the relative contribution of each soil health indicator to grazing management differences (Figure 4.5). This analysis showed a good separation between AMP grazing and CG grazing. PCA1 was able to capture 22.6% of the variability in grazing management practices and PCA2 was able to capture 14.6% of the variability (Figure 4.5). The biggest contributors to differences between the two grazing managements were several chemical indicators (base saturation, cation exchange capacity, electrical conductivity, Ca and Mg concentrations) and total N stocks. The next biggest contributors were total C stocks, mineral-associated organic matter C stocks,

dissolved organic matter C stocks, and NO_3^-, NH_4^+, and Fe concentrations. The lowest contributors were the biological indicators (PLFAs, enzyme activities, C mineralization).

Table 4.2 Farm pairwise comparisons of the differences between each response variable p-values as well as the general linear mixed effect model fixed effect p-values including all farm pairs. Significant differences are in bold. Red denotes when continuous grazing > adaptive multi-paddock grazing, black denotes when adaptive multi-paddock grazing > continuous grazing.

	Farm Pairwise Comparisons					Anova Results		
	Pair 1	**Pair 2**	**Pair 3**	**Pair 4**	**Pair 5**	**Grazing**	**Location**	**Grazing*Location**
A-horizon depth	0.8407	0.265	0.5385	0.8404	0.6771	0.3383	0.1043	0.8932
sand:clay	**0.0087**	**0.031**	0.8041	0.0778	0.2361	**0.0137**	**<0.0001**	0.057
Mean-weight diameter	0.4626	0.8995	0.3066	0.2914	0.1629	0.97578	**0.04645**	0.33489
Available water-holding capacity	0.6514	0.0618	**0.0015**	**0.0002**	0.6974	0.2647	**<0.0001**	**0.0005**
pH	**0.0122**	**<0.0001**	0.3551	**0.0047**	0.2245	**0.0004**	**<0.0001**	**0.0007**
Base saturation	**0.0122**	**<0.0001**	0.3551	**0.0047**	0.2245	**0.0004**	**<0.0001**	**0.0007**
Electrial conductivity	**0.0226**	**0.0245**	0.8971	**0.001**	0.4586	**0.0007**	**0.0004**	0.0654
Cation exchange capacity	0.4584	**0.0297**	0.2727	**0.004**	**0.0368**	**0.0007**	**<0.0001**	0.3072
Ca	0.1436	**0.0001**	0.2146	**<0.0001**	0.6885	**<0.0001**	**<0.0001**	**0.001**
Mg	0.062	**0.0491**	0.3926	**0.006**	0.1646	**0.0282**	**0.00033**	**0.0244**
K	**0.0002**	0.9881	0.2825	**<0.0001**	0.8165	**0.0001**	0.0993	**0.0008**
Na	**0.008**	**0.0223**	0.7	0.1987	0.8936	0.8266	**0.0479**	**0.01698**
Zn	0.2778	0.8361	0.936	0.4158	0.5393	0.2205	**0.0149**	0.93619
Mn	0.2714	0.0692	0.9966	0.2637	0.467	0.5729	**0.00017**	0.2086
S	**0.0477**	0.9184	**0.0186**	0.6706	**0.0085**	0.2132	0.078	**0.01423**
Cu	0.2088	**0.0324**	**0.0047**	**0.0183**	0.5409	0.1045	**0.02339**	**0.0075**
Fe	0.8048	**0.0079**	0.1738	**0.0028**	0.1425	0.1086	**<0.0001**	**0.0057**
Carbon mineralization rate	0.8081	0.9857	0.9806	0.3127	**0.0363**	0.5029	0.3661	0.2383
Nitrogen mineralization rate	0.7529	**0.0278**	0.9617	0.4475	**0.0044**	**0.008**	**0.0008**	0.1146
Ammonium concentrations	**0.0033**	**0.0005**	0.114	**0.0085**	0.3223	**0.00018**	**0.00039**	**0.01269**
Nitrate concentrations	0.7636	0.1505	0.0944	**0.002**	0.0764	**0.0211**	**0.00024**	**0.0196**
Carbon enzymes	0.1567	0.0879	0.385	0.5911	0.5536	0.2843	0.3569	0.2701
Nitrogen enzymes	0.4248	0.3356	0.414	0.9018	0.2387	0.3218	0.1808	0.6011
Phosphorus enzymes	0.1791	0.0837	0.2168	0.427	0.7884	0.2755	0.2392	0.2211
Total PLFA	0.9232	0.2319	0.3612	0.9156	0.4366	0.8026	**0.0037**	0.5533
Fungi:Bacteria	0.3917	0.4439	0.3189	0.5879	0.7954	0.9028	0.5857	0.5923

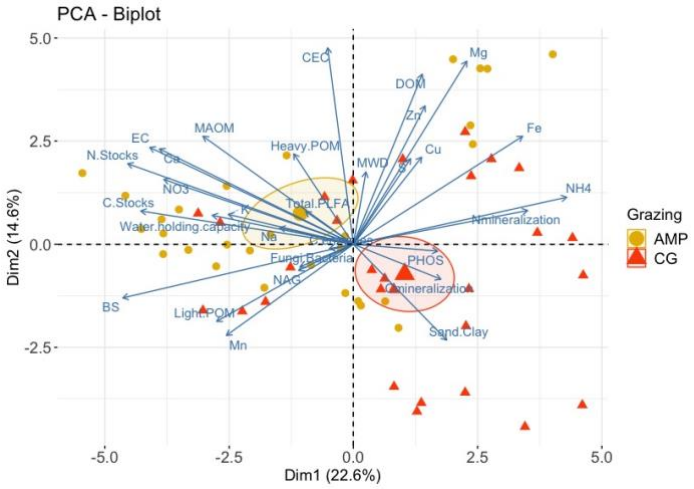

Figure 4.5 Principle component analysis of all measured soil health indicators contribution to the differences between AMP and CG grazing farms. The relative contribution of each indicator is reflected in the length and the direction of the arrows, with the longer arrows being most important. The data points are color-coded by grazing management with ellipses representing 95% confidence intervals.

Discussion 4.5

We focused our soil health study on the A-horizon, often considered topsoil, since it is the soil horizon that is most rich in organic matter and minerals (Stott and Moebius-Clune, 2017). It is one of the most active soil horizons where much of plant and soil organism activity takes place (Stott and Moebius-Clune, 2017). Having a deeper A-horizon implies that there is more available organic matter and minerals for plant and microbial activity on the AMP farms compared to the CG farms. Due to the depth of the A-horizon analyzed, we would expect to see differences in texture even though these sites are on similar soil types. Typically, the deeper in the soil profile, the more silt and clay is found (Keen and Rackowski, 1921). So, it was not surprising that we found a higher proportion of clay on the sites with a deeper A-horizon (Figure 4.1a).

We found no differences in the water-stable aggregate distribution between grazing managements, which resulted in no differences in the average MWD on AMP and CG farms. However, this information tells us that by having the cattle in higher densities on AMP farms, this does not negatively affect aggregate stability and there will still be significant structure in the soil. Additionally, microbial activity (specifically fungi) are very important for aggregate formation (Rillig, 2004; Van der Heijden et al., 2008). We didn't see any grazing management differences in our microbial indices, so it made sense that there were no differences in aggregate sizes between grazing managements. We didn't find any difference between grazing management and measurements of water content. However, other measurements of water holding capacity in the field, specifically infiltration measurements, found an order of magnitude more infiltration potential on AMP farms compared to CG farms (Apfelbaum et al. in prep).

We found the biggest differences between grazing management practices in the chemical soil health indicators. Electrical conductivity is typically used as a measure of salinity (Smith and Doran, 1996). This is considered an important soil health metric because high levels of salt can be very detrimental for vegetation growth (Smith and Doran, 1996). None of our farms were close to a dangerously high level (over 0.75 ds/m), but having higher electrical conductivity within the acceptable range for vegetation growth can have its advantages as it can influence the ease in which cations move through the soil profile (Smith and Doran, 1996). AMP farms seem to be better capable of holding onto nutrients and supplying plant-available nutrients based on the higher extractable nutrient, pH and % base saturation, and the cation exchange capacity data. Base saturation, pH, and cation exchange capacity are good indicators

of nutrient retention and availability (CUCE, 2007; Smith and Doran, 1996). Of the extractable nutrients measured, only Ca and K had significantly higher concentrations on the AMP farms. These are also the extractable nutrients that are used by plants in the greatest quantities (CUCE, 2007). Ca is crucial for plant growth and development and is also crucial in helping to regulate soil acidity (Rengel, 2002). K helps plants utilize N and water efficiently (Baligar et al., 2001), but can be easily leached out of sandier soils with low CEC (CUCE, 2007), which is likely why significantly less was found on CG farms. Additionally, Mg and Zn were also elevated on the AMP farms compared to the CG farms. These nutrients are important for plant metabolism, like photosynthesis (Bolan et al., 2002) as well as enzyme and protein synthesis (Lindsay, 1972).

We did not see any differences in the specific potential C mineralization rates between farms (Figure 4.3). This shows that the additional C accrued in the AMP soils (Chapter 3) has not accelerated mineralization rates and specific microbial respiration (i.e. the amount of C respired per unit of soil C) is the same across AMP and CG soils. This is an important finding because it highlights that even with more C present at the AMP farms (Chapter 3), the microbial community is not stimulated to respire more CO_2 per gram of C and could possibly be using the available C more efficiently, leading to more SOM formation. We saw much higher N mineralization rates at the CG farms relative to the AMP farms. One explanation is that N mineralization can be more rapid in sandy soils when compared to clayey soils due to greater physical N protection (Verberne et al., 1990). Another explanation can be linked to the amount of N in the soil, in another study component from the same farm pairs, we found that these soils had higher N stocks in AMP sites compared to CG sites (Chapter 3).

These potential mineralization rate measurements are often used as a proxy for microbial activity. However, we did not see any significant differences in our microbial PLFA biomarkers measurements or in our enzyme activity measurements. Even though these findings were not statistically significant, we still saw consistently higher enzyme activity on the CG farms relative to the AMP farms. These results are often difficult to interpret because it is impossible to say whether microbes are producing enzymes to find and acquire nutrients or if they are producing enzymes because those nutrients are easily available (Bell et al., 2013; Wallenstein et al., 2012). In this case, the results suggest that the microbial community might be producing more enzymes in the CG soils in order to find and acquire the resources that are less abundant than in the AMP soils.

Based on the PCA results in combination with our mixed-effects model ANOVA results, the chemical properties seem to be the biggest contributors to the differences in soil health across the two grazing management practices, whereas the biological properties seem to be the smallest contributors to the differences. There were a number of soil health indicators, specifically the chemical indicators, that were improved where AMP grazing management was implemented. This highlights the greater potential of AMP grazing management to supply and retain essential nutrients necessary for plant productivity. Additionally, where there were no significant differences, the soil health metrics were almost always similar between grazing management types and not lowered on AMP grazing farms. These results show that AMP grazing can have some significant soil health improvements without deleterious effects on any soil health metrics. These findings provide strong evidence that AMP grazing management

could be utilized as a way to improve grassland soil health across a large area of currently conventionally managed grazing lands.

CITATIONS 4.6

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CONCLUSIONS

The goal of this dissertation was to evaluate potential mechanisms and management practices involved with enhanced soil organic matter (SOM) formation and soil carbon (C) sequestration. The mechanisms evaluated included how litter chemistry and soil C saturation can enhance or inhibit soil C sequestration. The evaluation of management practices for improved soil C sequestration were from pine plantations and grassland grazing sites in the southeastern US.

Our findings from the mechanistic SOM formation study in Chapter 1 confirm the role of litter chemistry in determining pathways of new SOM formation in the short term, but less so that of mineral C saturation deficits. Overall, we found that after one year of incubation, litter types with the highest proportion of hot water-extractable components and highest C:Nn (N) were preferentially decomposed. Additionally, these litter types contributed more to persistent mineral-associated organic matter (MAOM) as opposed to litter types with more recalcitrant components contributing more to particulate organic matter (POM), supporting our hypothesis. Contrary to our hypothesis, these differences were only apparent in the topsoil, with a lower soil C saturation deficit, and were also dynamic throughout the 12 month incubation.

Our results from the evaluation of pine plantation management in Chapter 2 suggested that management intensification to support biofuel production from loblolly pine plantations may not adversely affect soil C stocks, and may possibly increase soil C stabilization in the more persistent MAOM fraction. We found no significant differences in bulk soil C stocks across pine plantation management intensities or soil types. The early thinning treatment had no effect on

the C distribution across each soil organic matter fraction. However, proportionally more C was found in MAOM and less in POM in the more intensive management regime treatment, possibly due to more available N, higher below ground nutrient inputs, and enhanced microbial activity. Additionally, our soil organic C results were consistent across all soil types investigated, which provides further evidence that these findings could be widespread across the pine plantations of the Southeast United States.

The evaluation of grassland grazing management on soil C and N stocks and their distribution in Chapter 3 showed that adaptive multi-paddock (AMP) grazing had over 13% more soil C and 9% more soil N compared to the continuous grazing. Overall, on average AMP grazing lead to the increase in the soil of 9 tC/ha and 1 tN/ha over 1 meter depth, as compared to conventional grazing. There was significantly more persistent C on AMP grazing farms compared to continuously grazed farms. Additionally, some of the soil organic matter fractions available for microbial transformation were of higher quality, with lower C:N ratios on AMP grazing farms relative to CG farms. These findings provide evidence that AMP grazing is a powerful management strategy to sequester C in soil and mitigate rising atmospheric CO_2 levels.

In Chapter 4, we found that there are a number of soil health indicators, specifically the chemical indicators, that can be improved where AMP grazing management is implemented. These results provide strong evidence that AMP grazing management could be utilized to regenerate grassland soil health across a large area of conventionally managed grazing lands. This highlighted the greater potential of AMP grazing management to supply and retain essential nutrients necessary for plant productivity. Additionally, Chapter 4 results showed that

AMP grazing can have some significant soil health improvements without deleterious effects on any soil health metrics. These findings provide strong evidence that AMP grazing management could be utilized as a way to improve grassland soil health across a large area of currently conventionally managed grazing lands.

Overall there is one central theme that unites this dissertation: nitrogen. The mechanistic study showed that the proportion of carbon to nitrogen matters for SOM formation, highlighting the importance of nitrogen for stable soil C sequestration. The case studies from the southeast US enhanced this understanding even further. The highly degraded lands investigated in this region had very little available nitrogen without extra inputs. The addition of nitrogen gave these sites the ability to transform organic matter more efficiently, creating more stable SOM. In both cases, more nitrogen shifted the distribution of SOM fractions to more MAOM. In the AMP grazing study, more nitrogen also resulted in an increase in total C as well as improved soil health. Taken together, this dissertation demonstrates that there are several land management practices in the southeastern US that can enhance soil C sequestration as well as the formation of persistent SOM. Even with the soils in much of the southeastern region of the US being severely degraded, we can make advancements in management to help restore these soils. And with continued improvement in our management practices, we can potentially provide even more food and fiber while maintaining healthy soils that provide other fundamental ecosystem services.