THESIS

ASSESSING THE EFFECTS OF GRAZING AND LAND USE CHANGE ON SOIL CARBON STOCKS IN PASTURES OF THE VIRGINIA BLUE RIDGE

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ABSTRACT

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The impact of livestock on our climate and the environment has been a hot topic since the 2006 FAO report "Livestock's Long Shadow" (Steinfeld et al., 2006) which estimated that livestock are responsible for 18% of anthropogenic greenhouse gas emissions and stimulated a great stir in the popular press. Numerous life cycle assessments have studied the environmental costs of various livestock species and have found beef and dairy to have particularly high greenhouse gas emissions (de Vries and de Boer, 2010). These assessments are often unable to include soil carbon changes caused by land-use change or management, perhaps because these data are limited and vary widely by region. The results of this study present data from the first known long-term study of grazing impacts on soils along the Virginia Blue Ridge.

In this study, I sampled nine pastures on five farms to study both management and landuse change. I found significant increases in soil organic carbon on eight of the nine pastures with evidence that land-use and management change drove these increases. A portion of these data were an in-depth chronosequence on one farm, within which, data from our preferred approach (repeated measures) conflicted with the results of the chronosequences that we collected in 1999 and 2010. This conflict should serve as a cautionary tale to ecologists using space-for-time substitution.

Averaged across all sites (all of which are grazed), we found soil carbon in the top 20 cm of soil increased 1.2 ± 0.2 Mg/ha/yr for the 11 years between 1999 and 2010. This provides clear evidence that pasture-raised beef and dairy production can offset at least part of its

ii

environmental impact. These results are valuable to policy makers and consumers that are interested in reducing the impacts our food system has on climate change and the environment.

TABLE OF CONTENTS

ABSTRACT	ii
CHAPTER 1: INTRODUCTION	1
Grazinglands Impact the Carbon Cycle:	1
Land Use Change alters soil organic matter:	3
Management Intensive Rotational Grazing:	4
Mechanisms for MIRG-induced productivity gains:	5
Carbon Dynamics:	7
Aggregation is linked to turnover time:	8
Previous Work:	9
Study Purpose and Approach:	10
LITERATURE CITED	12
CHAPTER 2: THE EFFECTS OF LAND USE CHANGE ON SOIL ORGANIC CA BULK SOIL AND PHYSICAL SOIL FRACTIONS	ARBON IN 17
Methods:	19
Site Description and History:	
Sampling protocol and processing:	
Water-stable aggregate isolation:	
Statistical Analysis:	
Results:	24
Physical soil fractions:	
Discussion:	
LITERATURE CITED	36
CHAPTER 3: SOIL ORGANIC CARBON CHANGES IN PASTURES WITH MANAGEMENT-INTENSIVE ROTATIONAL GRAZING	
Methods:	41
Previous work:	
Study Area:	
Sample Collection:	
Statistical Analyses:	
Results:	47
Changes through time:	

MIRG vs. other pasture management:	
Discussion:	
LITERATURE CITED	

CHAPTER 1: INTRODUCTION

Soil organic matter (SOM) functions much like a "bank" (or credit union if you prefer) by storing nutrients which provide "dividends" such as improved water infiltration, enhanced water storage and increased cation exchange capacity. The more we withdraw from the bank (releasing nutrients to plants and microbes), the fewer dividends it is able to pay. The soil's bank of organic matter is in constant flux; plant inputs provide "deposits", while microbial decomposition is the primary "withdrawal". Management is an important determinant of the balance between these deposits and withdrawals (West and Post, 2002). Tillage, for example – used to prepare the seed bed, control weeds, and relieve compaction – has been shown to cause a withdrawal from SOM stocks by redistributing substrates, aerating the mixture and promoting microbial decomposition (Post and Kwon, 2000). It can also promote erosion which causes a loss of SOM along with the mineral soil.

Globally, soils contain an estimated 1,500-2000 Pg C in the top meter compared to 780 Pg C in the atmosphere (Houghton, 2007). Annually, land use change causes soils to contribute a significant 0.4 Pg C yr⁻¹ (12.5%) of the atmosphere's 3.2 Pg C yr⁻¹ net increase (Houghton, 2007). Understanding how humans influence these stocks and flows can help us make management decisions that improve food security and mitigate climate change (Lal, 2004; Lal et al., 2007). In most cases, these decisions will require balancing various trade-offs of food and fiber versus carbon dioxide, methane and nitrous oxide (Janzen, 2007). Original research and independent validation are needed to better understand these trade-offs.

Grazinglands Impact the Carbon Cycle:

Grasslands, savannas and shrublands make up around 23-30% of the earth's terrestrial

biomes by area and store an estimated 27% of the earth's soil carbon (Prentice et al., 2001). Temperate grasslands cover about 9% of the earth's terrestrial area and store an estimated 11% of the earth's soil carbon (Schlesinger, 1986). Pastures are often considered separately because they require occasional disturbance and reseeding of forage species, which leads some to group them with croplands. Collectively, grasslands, pastures and savannas can all be termed "grazinglands." Moderate livestock grazing has been shown to promote SOC sequestration relative to having or grazing exclusion (Conant et al., 2001; Derner, 2007; Liebig et al., 2005; Rice and Owensby, 2001). Hafner et al. (2012) indicate at least two reasons for this: a) lower total C inputs by ungrazed grasslands which leads to b) increased reliance by microbial communities upon medium and long-term C stocks as a substrate. Light and moderate rates of grazing also promote increased soil nitrogen and which decreases soil carbon to nitrogen ratios (Franzluebbers et al,. 2000; Franzluebbers and Stuedemann, 2003; Schuman et al., 1999). Planting legumes (Conant et al., 2001) and reducing grazing pressure during drought (Derner and Schuman, 2007) are two other pasture management strategies that have been shown to significantly increase SOC. Other practices with the potential to sequester carbon include earthworm inoculation, sowing new forage grasses, (Conant et al., 2001) and rotational grazing (Conant et al., 2003a).

Grazing research in the United States has taken place primarily on native grasslands rather than pastures, both because they cover more acres and are readily available to federal research agencies (62 Mha are federally owned (Lubowski et al. 2006) vs. 165 Mha private (USDA, 2009) compared to 48 Mha pasture, of which all is private (USDA, 2009)). Accordingly, management impacts on carbon sequestration in pastures remains under-studied (Olander et al., 2011).

Land Use Change alters soil organic matter:

Land use conversion to cropland (Haas et al. 1957; Schlesinger 1986; Davidson and Ackerman 1993; Kern and Johnson 1993) and overgrazing (Fearnside and Barbosa, 1998; Abril and Bucher, 1999) are noted as major causes of carbon losses from grazingland soils. However, these losses from grazinglands are reversible (Conant and Paustian, 2002; Conant et al., 2001), though the potential for grazinglands to sequester carbon is still poorly understood (Scurlock and Hall, 1998; Jones, 2010; Olander et al., 2011). When land is converted to cropland, a combination of deforestation, fire and tillage can all cause plant productivity to cease temporarily, while microbial respiration may continue relatively unhindered. Historically, land use change from 1850 to 2000 is estimated to have caused the net loss of $154 \pm 15 \text{ Pg C}$ (Houghton, 2010). These losses are dominated by forest and grassland conversion to cropland.

Converting tropical forest to pasture has been shown to mostly increase SOC stocks (Guo and Gifford, 2002), although these gains generally do not aboveground biomass loss is still a large contributor to historic changes in the terrestrial carbon pool. Guo and Gifford (2002) found that SOC increased by about 8% with the most substantial increases (24%) in areas with greater than average rainfall (2000-3000 mm). Sites with less than 1000 mm MAP tended toward slight losses in SOC while more intermediate sites (1000-2000 mm) tended toward slight gains (Guo and Gifford, 2002). Correlations with mean temperature were not mentioned by the authors.

In this study, I analyzed a forest-to-pasture chronosequence in Louisa County, VA that combines repeated measures with a standard paired-site analysis to build a complete picture of in-situ carbon dynamics. This provides an important update to a field that suffers from a relative lack of data.

Management Intensive Rotational Grazing:

Management-intensive rotational grazing (MIRG) is a practice designed to optimize yearround forage production and usage by densely stocking sections of pasture (paddocks) for short periods of time before moving livestock to a new paddock (Williams and Hall, 1994). Figure 1 illustrates how paddocks might be arranged. The practice is intended to allow longer recovery times for forage species (Dalrymple, 2002), while also keeping forage at heights that are considered optimal for growth (Beetz and Rinehart, 2004). It provides managers reserves of forage and gives the manager more control over utilization, which can sometimes lead to overgrazed paddocks without careful monitoring and management.



Figure 1: Management Intensive Grazing: In the center diamond, 4 paddocks are labeled A-D. A. was grazed first and is recovering. B. was recently grazed and is will begin recovering after plant growth starts back up. C. is currently being grazed. D. will be grazed next.

Compared to continuous grazing, researchers have shown that MIRG improves forage quantity (Oates et al., 2011) and quality (Walton et al., 1981). Although on semi-arid rangelands MIRG has shown productivity gains ranging from negligible to negative (Briske et al., 2008), in another meta-analysis Holechek et al. (1999) reported 20-30% increases in forage production on "more humid range types." Of the few studies that compare continuous grazing and MIRG on humid and sub-humid pastures, results have been more positive. Oates et al. (2011) recently compared MIRG to continuous grazing, hay harvests and no management and found productivity gains of 35-65% throughout a two year experiment. They also found MIRG promoted better availability during the summer and fall seasons in addition to more consistent and slightly better forage quality (Oates et al. 2011). Further studies have found some productivity benefits from rotational grazing in terms of forage quantity and quality (Paine et al., 1999), milk production (Ernst et al., 1980) and beef production (Jacobo et al., 2006). Results are not consistent, however, and other studies have not found any productivity benefits from MIRG (Arriaga-Jordan and Holmes, 1986; Pulido and Leaver, 2003). Of these studies, Oates et al. (2011) had the most similar climate and species composition to our study along the Virginia Blue Ridge.

It is important to note multiple authors indicate that stocking rates are often judged to have a larger impact on grassland productivity than grazing system (Briske, 2008; Holechek et al., 1999; Fales, 1995). McMeekan and Walshe (1963) suggested that although stocking rate seemed to have a greater impact on productivity than MIRG, the optimal stocking rate was 5-10% higher under controlled rotational grazing than uncontrolled continuous grazing.

Mechanisms for MIRG-induced productivity gains:

A variety of mechanisms have been put forward to explain productivity gains of MIRG. Using a theoretical approach of the logistic growth patterns of pasture, Morley (1986) determined that certain grazing intervals enhance pasture growth. Optimizing pasture growth might be considered as simple as maintaining grass height so as to maintain linear growth predicted by a logistic growth model. However, plant physiology complicates this picture. After defoliation, many species exhibit root die-off and temporary cessation of growth (Crider, 1955). Root die-off

and root exudation (Paterson and Sim, 1999; Holland, 1995) in turn stimulate N mineralization (Hamilton and Frank, 2001; Hamilton et al., 2008). It would be valuable to know how this process is altered by rotational grazing, perhaps through altered timing and scale of grazing. Indeed, this "pump priming" effect may form some theoretical basis for the "compensatory growth" theory.

Managers use rotational grazing to control for longer or shorter rest periods on sections of pasture, often using longer rest periods during seasons of slow growth (e.g. winter) and shorter rest periods during periods of fast growth (e.g. spring). This rest period is one way to prevent plant stress during the period of stalled growth that follows defoliation (Crider, 1955). Compensatory growth (reviewed by Belsky, 1986) is a controversial theory that plants directly responded to defoliation with more total aboveground production than otherwise would have been observed.

Compared to more extensive management such as continuous grazing or hay production, MIRG may provide indirect benefits to pasture productivity. For example, Carlassare and Karsten (2002) found that altered timing of cattle grazing promotes legumes and taller grasses, such as white clover (*Trifolium repens* L.) and orchardgrass (*Dactylis glomerata* L.), two species that were the dominant planted forages in our sites across Virginia. Walton et al. (1981) also found that rotational grazing benefits the taller grasses and legumes in their study. Similarly, Paine et al. (1999) found that taller grass species and legumes were more prevalent on MIRG than continuously grazed pastures. Of studies that showed yield boosts from MIRG, Oates et al. (2011) had orchardgrass and legumes present while the Pulido and Leaver (2003) only mentions perennial ryegrass (*Lolium perenne*). The Briske et al. (2008) and Holechek et al. (1999) metaanalyses include data from range types that are dominated by mixed and shortgrass prairie and

likely have less percent coverage by legumes or cool season tallgrasses like orchardgrass. No similar meta-analysis exists to my knowledge regarding forage and animal productivity changes caused in temperate humid pastures more common in the Eastern US, which may be an opportunity for future researchers.

Carbon Dynamics:

Primary productivity and microbial respiration drive organic matter import and export processes in soil. Weather and macrobiota facilitate the processes of translocation and transformation of organic matter. Each of these processes function in an environment that is conditioned by the five state factors of soil formation (Jenny, 1941): climate, topography, biota, parent material and time. Human influence is the "sixth factor" of soil formation which can influence or dictate each of the other factors and processes, while also altering the processes that influence soil development within the context of these state factors. Together these factors determine everything about a soil and provide context that can help understand these processes.

A variety of mechanisms can change the turnover time of carbon in the soil matrix; "biological, physical and chemical stabilization" became widely recognized as the primary trio responsible for carbon stabilization in soils (Jastrow and Miller, 1998). Through the years, many quantitative studies of organic soil carbon have focused on 'recalcitrance': a property varying definitions and usage in the literature. I think the term is best defined as the property of a substrate that slows its microbial respiration in laboratory experiments. The property has been correlated with molecule size and complexity. For example, lignin (a complex compound found in wood) content is sometimes used as a surrogate for recalcitrance.

During the 80s, procedural advances in the laboratory made it possible to study other soil properties that help stabilize organic carbon. For example, wet-sieving allows researchers to

isolate pools of carbon according to size of water-stable "crumbs" of soil, which are more formally known as "aggregates". Over the years, many studies have found these physical structures to be an important determinant of organic matter stabilization. Simultaneously, growing numbers of researchers are questioning that 'recalcitrance' is a significant driver of the longevity of organic matter in soil (Dungait et al., 2012). Schmidt et al. (2011) framed the discussion:

> "By moving on from the concept of recalcitrance and making better use of the breadth of relevant research, the emerging conceptual model of soil organic carbon cycling will help to unravel the mysteries surrounding the fate of plant- and fire- derived inputs and how their dynamics vary between sites and soil depths, and to understand feedbacks to climate change."

I would even argue that numerous lab-studies of recalcitrance have been the product of tunnel vision, while management and related pathways that shorten or extend turnover time have not received nearly enough attention.

Aggregation is linked to turnover time:

Over the years, a variety of models have been developed to describe how management influences soil carbon. Of these CENTURY (Parton et al. 1993) notably features "theoretical" pools of carbon, which cycle – that is, build up and break down – at different rates. These theoretical pools (labeled "active," "slow," and "passive") provided sufficient model optimization to successfully simulate SOC within 25% during validation (Parton et al., 1993). Attempts to incorporate in-situ data into these models met limited success, perhaps largely because the most popular mechanism – "recalcitrance" – has been difficult to validate experimentally (Dungait et

al., 2012).

Previous Work:

Methods

Most researchers use a paired site analyses or chronosequence approach to study the effects of management or land use change on SOC (Conant et al., 2001). Because both types of analysis substitute space-for-time, both can suffer from even slight differences in site properties, including natural variability and non-steady state dynamics when the land use change of interest was initiated (Murty et al., 2002; Sanderman and Baldock, 2010). Spatial variability is also a challenge for researchers investigating SOC stocks. For example, SOC in soil cores collected less than 5 meters apart on a conventionally tilled farm in Tennessee varied from less than 1% to as much as 50%, with typical variation on the order of 10% (Conant 2003b). A recent study by Blanco-Canqui and Lal (2008) received criticism (Franzluebbers, 2009) for a paired site approach in which they compare no-tillage and conventional tillage based on three cores within a single field. While most studies would not be based on one "pseudoreplicate" – an unreplicated treatment upon which standard statistical analyses can be performed with little real world meaning in terms of treatment differences – it highlights the challenge of replicating specific management practices on multiple fields and multiple soil types.

Rotational Grazing

I am aware of only two studies that have investigated the effect of MIRG on SOC in pastures (Conant et al., 2003a; Sanford et al., 2012). Conant et al. (2003a) found 20% higher SOC in MIRG sites than hay and continuously grazed sites, while Sanford et al. (2012) found no change in SOC over time. The results from these studies could conflict because they were in different locations. Conant et al. (2003a) investigated these practices in Virginia while Sanford et

al. (2012) studied management in Wisconsin. The results could also conflict because the researchers used different methods. For example, Conant et al. (2003a) compared four sets of paired sites while Sanford et al. (2012) only investigated one site by repeating measures over time. Neither study can be considered a definitive investigation of the effects on MIRG relative to controlled grazing with a similar stocking rate. Conant et al. (2003a), investigated pastures of Virginia on ultisols and alfisols where the soils formed under forest while Sanford et al. (2012) investigated MIRG on a mollisol in Wisconsin that was tallgrass prairie before tillage in the mid-19th century. The sites in Virginia have a history of grazing since the 1950s (Conant et al., 2003a), while the site in Wisconsin was used for dairy forage production approximately 1860 until Sanford et al. (2012) first sampled in 1989.

Land-use Change

Numerous studies have compared soil carbon in tilled cropland to grazinglands, generally finding substantially higher SOC on grazinglands (Conant et al., 2001; Post and Kwon, 2000). Forest to pasture conversion has been fairly well studied in the tropics, where results are variable but tend to show slight increases in SOC (Guo and Gifford, 2002). Results have been similarly variable in the studies done in the sub-tropical and temperate Southeastern US, though they have shown average losses in SOC after forest-to-pasture conversion (Franzluebbers, 2005; Conant et al., 2004).

Study Purpose and Approach:

The purpose of my work was to investigate how grazing affects soil organic matter and compare space-for-time substitution with repeated measures sampling, which are the two main approaches that are used to study how management and land-use change affect soil. In this study, I have re-sampled a series of pasture sites over time with examples that include crop-to-pasture conversion, changes in management on old (more than 40 yr) pastures and forest-to-pasture conversion. On one farm in Louisa county, I examined how conversion from forest to pasture and crop-to-pasture conversion affect soil organic matter stocks. I also investigated how management intensive rotational grazing affects SOM at sites in three different counties. All of the sites where we investigated the effects of MIRG had been maintained as pasture for at least 40 years before taking baseline samples in 1999.

My primary hypotheses are: 1) In temperate areas, forest-to-pasture conversion through deforestation, tillage, and seeding, causes an initial loss in soil organic matter. After this conversion, soil organic matter accumulates to equal or even exceed what levels present in the unmanaged forest. 2) Well-managed grazing of temperate, sub-humid pastures promotes soil organic matter accumulation. To test these hypotheses, I measured SOM down to 50 cm depth at sites where baseline samples had been collected. I used a repeated measures approach, which provides significant advantages to studies that collect samples only once providing including the original work upon which this study was based.

These efforts are unique because few authors have investigated changes in SOM using a repeated measures approach. Our results are necessary because they help fill a gap which exists on how forest-to-pasture conversion and the implementation of rotational grazing impact soil carbon in sub-humid, temperate ecosystems. These areas, while less extensive than tropical or semi-arid systems, can be highly productive and may play a significant role in the global carbon cycle.

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CHAPTER 2: THE EFFECTS OF LAND USE CHANGE ON SOIL ORGANIC CARBON IN BULK SOIL AND PHYSICAL SOIL FRACTIONS

Livestock have been identified as the source of 18% of the world's greenhouse gas emissions, (including 37% of methane and 65% of nitrous oxide emissions) and a major contributor to water quality degradation (Naylor et al., 2005; Steinfeld et al., 2006; de Vries and de Boer, 2010). Despite these impacts, livestock are difficult to replace in developing economies where they are an essential source of protein and nutrients as well as an economic resource (Steinfeld et al., 2006; Janzen, 2011). This has led many researchers to suggest revisiting mixed crop-livestock production and well-managed grazing systems as a way to maintain or boost production while also conserving soil and closing nutrient cycles (Steinfeld et al., 2006; Herrero et al., 2010; FAO, 2009; Franzluebbers et al., 2012; Janzen, 2011). But we must consider options carefully because some studies have found that energy intensive production systems (e.g., confined animal feeding operations) have lower greenhouse gas emissions than pasture-based production (González et al., 2011; de Vries and de Boer, 2010). Pelletier et al. (2010) also found that pasture-based systems with "average" management had higher emissions than feedlot systems, but suggested well-managed systems would perform better, especially ones that avoid outside feed and fertilizer inputs or sequester carbon through land-use change or management.

When analyzing greenhouse gas emissions that are caused by livestock, it is important to understand how soil carbon stocks respond to land-use and management. For some time, researchers concentrated their efforts in the tropics, which hold substantial carbon reserves and have been the front line in humanity's struggle to balance growth and ecosystem services. Guo

and Gifford (2002) analyzed 74 studies that focused on the effects of land-use change in the tropics and found substantial soil organic carbon (SOC) losses after forest-to-cropland conversion, while native forest-to-pasture conversion and crop-to-pasture conversion both seem to increase SOC (Guo and Gifford, 2002). Other regions have received less attention (Houghton and Goodale, 2004), but pastures of the Southeastern US have shown fairly large sequestration rates. For example, newly planted pastures in Texas, Alabama and Georgia sequestered about one ton of carbon per hectare per year over periods ranging from 5-60 years (Franzluebbers, 2005). Further, the eastern United States is a promising area for pasture plantation and increased production after years of cropland abandonment, which claimed about half of the region's agricultural lands between 1920 and 2000 (Ramankutty et al., 2010).

To understand the impacts of land-use change on soil carbon reserves—a massive pool that can take decades to measurably change—researchers usually use a paired-site or chronosequence approach to simulate the effects of time (Conant et al., 2001). In both approaches, researchers carefully select sites with similar topography, parent material and other major soil factors with the hope that a relative comparison of sites provides an accurate assessment of how soil organic matter stocks change over time. Multiple studies point out weaknesses common to both "space-for-time" approaches (Murty et al., 2002; Sanderman and Baldock, 2010). The two main weaknesses are: 1) Space-for-time studies are vulnerable to uncontrollable variation between sites; for example, higher initial carbon stocks in any field are difficult to attribute to anything other management differences. 2) Space-for-time substitution depends on the assumption that sites are at equilibrium; for example, if two pastures with differing land-use history are accumulating carbon at different rates, space-for-time substitutions have no way to capture this process. Both approaches make up for these problems by being fairly

easy to replicate, but land-use changes and following management regimes are as different as managers themselves. Both studies (Murty et al., 2002; Sanderman and Baldock, 2010) suggest taking multiple measurements on the same farm over time as the best way to determine what is happening.

This study revisits the efforts of Conant et al. (2004) which analyzed a forest-to-pasture chronosequence in temperate, sub-humid farm in Virginia. In a chronosequence studied in 1999, little difference was found between pastures conversions of varying ages (Conant et al., 2004). Resampling these sites in this study has allowed me to build a more rigorous assessment of the effects of land use conversion and management on soil organic matter stocks and what changes occurred. It also allowed me to determine what mechanisms were protecting organic matter and how various pools of soil carbon and nitrogen change over time. To do this, I used a simple procedure that physically separates macroaggregates from microaggregates and non-aggregated silt & clay fractions of the soil.

Methods:

Site Description and History:

I collected soil samples on two farms in Louisa County, VA, USA. The first farm (38.07°N, 78.11°W) contained a four field forest-to-pasture chronosequence as well as crop-to-pasture conversion. The second farm (38.01°N, 78.17°W), also in Louisa county, contained an additional crop-to-pasture conversion. I will refer to these fields using a shorthand that includes information about the initial and subsequent land-use and what year land-use changed. For example, the one crop-to-pasture conversion on our first farm was converted to pasture in 1983 and will be referred to as "CP83".

On the first farm, differences in slope, aspect, site history, soil texture (silty loam) and

soil series (Typic Hapludult) were minimized to ensure that recent land use change was the primary factor influencing SOC. For example, in the sites that we selected for analysis, we chose to sample fairly flat upland sections. The chronosequence contained three pasture sites, two of which were converted from forest in the same manner: trees were harvested, slash material was consolidated and burned in place then buried, followed by orchard grass (*Dactylis glomerata*) and clover (*Trifolium repens*) seeding. The third site was cultivated for a few years before being converted to pasture; this occurred more than 40 years before the initial (1999) sampling (Conant et al., 2004). A forested site ("FOR") served as an estimate of baseline SOC stocks. In 1999, sites across the forest-to-pasture chronosequence were identified with conversion dates of 1996 (FP96), 1985 (FP85), and sometime during the 1950s (FP50). The fifth site (CP83) was under long-term cultivation until 1983 when it was converted to pasture that was initially grazed by sheep. Cultivation started on this field around the end of the Civil War in 1865. In 1999, the oldest trees on the forested site were 25 years old, though the site was likely under forest for much longer (B. Wayson, personal communication). The forest was composed of mixeddeciduous species including beech (Fagus sp.), maple (Acer sp.), and oak (Quercus sp.). The soil was not re-sampled at the forested site in 2010.

Stocking rates were similar across the entire farm and were consistent through time. In both 10-year periods leading up to 1999 and 2010, the farm was stocked at 1.9 AUE/ha. Sheep were the primary livestock 1984-1994, dairy cattle 1995-2006 and beef cattle after 2006. Stocking rates increased gradually from 1.65 in 1992 to 2.70 in 2002 and dropped to an average of 1.48 for years 2006-2010.

"CP86" is another pasture in the same county on another farm (38.01°N, 78.17°W), that is underlain by Poindexter loam (Typic Hapludalf). Like CP83, this farm also has a long history

as cropland. It was put into tobacco production in the 1790s which involved frequent tillage by moldboard plow. This field remained in cultivation until 1986 when it was converted to pasture. A less intensive rotational grazing regime was implemented on this farm around 1999. The producer had been experimenting with home-made compost tea applications which he sprayed for three consecutive years during the mid-2000s. The stocking rate on this farm is much lower, about half that of the other farm.

Sampling protocol and processing:

I intensively sampled each of the five pasture sites and one forested site using a scheme based on the one used by the Canadian Prairie Soil Carbon Project (Ellert et al. 2001, 2002). Within each field, I sampled three "microsites," i.e. a replicate consisting of six regularly-aligned cores. Each microsite was oriented in the same direction and arrangement, which was offset by half a meter for the second sampling (see Figure 2), The northeastern-most core was marked using differential GPS (Magellan, San Dimas, Calif.), and a relocatable Skotchmark EMS magnetic ball marker (3M Corporation, Austin, Tex.) was buried at 1 m depth to enable future relocation and resampling. The ball marker allowed for utmost precision when relocating each microsite's northeastern corner. By resampling each of the pasture sites in 2010, I created a chronosequence with seven data points, including one point for the forested site in1999, and two points each for the 1999 and 2010 samplings of the three converted pastures.



Figure 2: Microsite sampling diagram. An offset of 0.5 m was used to avoid disturbance effects caused by ssoil removal and eventual partial inversion.

A Giddings hydraulic soil coring rig was used to collect 6.5-cm- diameter soil cores to a depth of 50 cm. Soil samples were split into three segments (0–10, 10–20, and 20–50 cm), returned to the laboratory, and weighed. Surface litter and above-ground vegetation were removed. Samples were passed through an 8-mm mesh sieve by gently breaking the soil along planes of least resistance. All visible root material was removed by hand picking during the 8-mm sieving. Soils were then air-dried for 48 to 96 hours. Next, I created composite samples from the six cores within a given depth from each microsite by mixing an equal amount of soil (40g \pm .2g) from each of the six individual cores. This process resulted in one sample for each of the

three depths, which I set aside for the aggregation analysis described below. I then sieved a 50g subsample from each air-dried sample to pass a 2-mm sieve. Samples were then oven-dried at 60°C for 72 h, and ground to fine powder using a roller mill (Arnold and Schepers, 2004). Soil C and N concentrations were determined for total soil with a LECO CHN-1000 autoanalyzer (LECO Corporation, St. Joseph, MI). Bulk density was calculated using volume of sample collected and the weight of soil in the sample. Fresh sample weight was corrected for soil moisture (determined by oven-drying a subsamples at 105°C) and subtracting root and rock mass from both 8-mm and 2-mm sievings. Root matter collected during the 8-mm sieving was ashed using a muffle furnace. Root matter was also collected during the 2-mm sieving and was dried and stored, but not analyzed for C. Samples that were collected in 1999 contained no inorganic soil carbon (carbonates), so I assumed for this analysis that carbonates were absent.

Water-stable aggregate isolation:

Beginning with the composite samples that we set aside earlier, I used wet-sieving to isolate physical soil fractions in a method similar to that used by Conant et al. (2004). Wet-sieving isolated three sizes of water stable aggregates: >2mm (larger macro-aggregates), 250 μ m – 2mm (small macro-aggregates), 53 μ m – 250 μ m (microaggregates). Fractions smaller than 53 μ m were classified as unaggregated or non-water-stable silt- and clay-associated OM. Further dispersion and isolation of intra-macroaggregate POM or microaggregates was not performed for this study.

Statistical Analysis:

Because our microsites precisely overlapped the original sample locations, we were able to use a paired test with paired microsites instead of a standard t-test or ANOVA. By using paired

t-tests, I was able to address much of the standard error and treatment effects that have the potential to confuse or limit the analysis of aggregate changes over time. Although these paired t-tests describe within field change over time stocks should only be compared with caution, the changes are more readily comparable, especially within the chronosequence.

To complement the within-field paired t-test analyses, I used a mixed model (Proc Mixed, SAS 9.2, SAS Inc., Cary, NC) to better understand how time since land use conversion affected the entire chronosequence. The model included random effects estimation to account for some random variation that could not be controlled by careful site-selection. I used this model to provide a linear estimate of the rate of change in soil carbon and nitrogen stocks against time since land-use conversion and how this interacted within individual fields over time. I also attempted quadratic and logistic regressions.

Results:

Organic soil carbon stocks (SOC) increased at all depths in each of five pastures that were converted from other land-uses. The average increase was 11.4 ± 2.1 Mg C ha⁻¹ ($32.5\%\pm7.5\%$) in the top 50 cm of soil (Table 1). The average was 12.7 ± 3.6 Mg C ha⁻¹ among forest-to-pasture conversions and 9.5 ± 0.5 Mg C ha⁻¹ among crop-to-pasture conversions (Table 1). In five out of six points across the forest-to-pasture chronosequence, SOC stocks were greater than or equal to the unmanaged forest (35.0 ± 4.1 Mg C ha⁻¹), averaging 44.3 ± 2.2 Mg C ha⁻¹ (n=5) (Table 1). The one point lower than the unmanaged forest was FP96 in 1999 with 31.6 ± 2.8 Mg C ha⁻¹ (Table 1).

Soil organic carbon increased significantly in the top 50 cm in pastures FP96 (18.7 \pm 2.3 Mg C ha⁻¹), CP83 (10.1 \pm 0.3 Mg C ha⁻¹), CP86 (9.0 \pm 1.1 Mg C ha⁻¹), and FP50 (6.3 \pm 0.7 Mg C ha⁻¹) (Table 1). Increases in FP85 were greater than the average change (12.9 \pm 5.1 Mg C ha⁻¹) (Table

1), but not significant, though the top 0-10 cm in FP85 did increase significantly (6.3 ± 1.2 Mg C ha⁻¹) (Table 1). It was also interesting that the crop-to-pasture conversion (CP83) gained about the same SOC as a forest-to-pasture conversion of about the same age (FP85) (Table 1). Expressed as a proportion of 1999 SOC stocks, SOC increased 59.1% \pm 7.4% (FP96), 36.7% \pm 14.5% (FP85), 26.2% \pm 0.1% (CP86), 15.1% \pm 2.7% (FP50).

Table 1: 0-50 cm soil carbon stocks and changes. Asterisks (*) indicate a significant (P<0.05) difference between 1999 and 2010 data.

	SOC Stocks (SE)			ΔSOC (SE)		
Site	1999	2010	$(Mg C ha^{-1})$	$(Mg C ha^{-1} yr^{-1})$	(% yr ⁻¹)	
Forest	35.0 (4.1)					
For→Pas 96	31.6 (2.8)*	50.4 (4.9)*	18.7 (2.3)*_	1.7 (0.2)*	5.4% (0.7%)*	
For→Pas 85	35.3 (1.7)	48.3 (3.4)	12.9 (5.1)	1.2 (0.5)	3.3% (1.3%)	
For→Pas 50	41.7 (2.7)*	48.0 (1.9)*	6.3 (0.7)*	0.6 (0.1)*	1.4% (0.2%)*	
Crop→Pas 83	38.3 (0.4)*	48.4 (0.1)*	10.1 (0.3)*	0.9 (0.0)*	2.4% (0.1%)*	
Crop→Pas 86	35.1 (0.6)*	44.1 (1.3)*	9.0 (1.1)*	0.8 (0.1)*	2.3% (0.3%)*	

Among all five land-use conversions, 0-10cm SOC stocks increased $39.6\pm7.1\%$ (Table 2), while 0-50cm stocks increased $32.5\pm7.5\%$ (Table 1). The average increase for the top ten cm was 6.6 ± 1.1 Mg C ha⁻¹ (Table 2). Within the chronosequence, SOC stocks were uniformly greater in the top 10 cm of forest-to-pasture conversions (21.4 ± 1.7 Mg C ha⁻¹) than the control forest (13.7 Mg C ha⁻¹) (Table 2). The largest increase in SOC (10.7 Mg C ha⁻¹) occurred between 3 and 14 years since forest to pasture conversion in FP96 (Table 2).

 $\Delta SOC (SE)$ SOC Stocks (SE) %C (SE) %C (SE) Site 1999 2010 $(Mg C ha^{-1})$ 1999 2010 13.7 (1.4) 2.3 (0.1) Forest For→Pas 96 16.4 (2.4)* 27.1 (1.3)* 10.7 (1.1)* 2.2 (0.3)* 3.7 (0.2)* 17.0 (0.6)* 23.3 (0.8)* 6.3 (1.2)* 1.9 (0.1)* 2.5 (0.1)* For→Pas 85 20.1 (2.0) 24.3 (0.8) 4.2 (1.9) 2.3 (0.1) 2.7 (0.0)_ For→Pas 50 Crop→Pas 83 16.5 (0.4)* 22.6 (0.7)* 6.2 (0.9)* 2.2 (0.1)* 3.3 (0.1)* Crop→Pas 86 15.0 (0.3)* 20.5 (1.0)* 5.6 (0.8)* 1.5 (0.0)* 2.1 (0.1)*

Table 2: 0-10 cm soil carbon stocks, changes and percentages. Asterisks (*) indicate significant (P < 0.05)differences in soil C stocks between 1999 and 2010.

Organic soil nitrogen stocks (SON) behaved similarly to SOC, increasing for both the top 50 cm and top 10 cm in all pastures with average increases of 1.19 ± 0.03 Mg N ha⁻¹ and 0.70 ± 0.06 Mg N ha⁻¹ respectively (Table 3). In the forest-to-pasture chronosequence, there were no examples where SON stocks were lower than the unmanaged control forest. Among all pastures, only FP96 SON stocks did not increase significantly, although its median 0-50cm SON stocks increased a similar amount $(1.18\pm0.51$ Mg N ha⁻¹) as the other fields (Table 3). Soil N stock (0-50 cm) increases were greatest for: FP85 $(1.27\pm0.32$ Mg N ha⁻¹), followed by FP50 $(1.13\pm0.27$ Mg N ha⁻¹) and CP83 $(1.13\pm0.23$ Mg N ha⁻¹) (Table 3). Expressed in proportion to 1999 stocks, N stocks increased 43.9%±18.7% in FP96, 41.7%±10.4% in FP85, 34.5%±8.1% in FP50 and 30.7%±6.3% in CP83. Soil N stocks in the 0-10cm layer were more than twice as large in pastures than the control forest, averaging 1.76 ± 0.12 Mg N ha⁻¹ compared to 0.61 Mg N ha⁻¹ (Table 3).

	0-50 cm SON	Stocks (SE)	ΔSON (SE)	0-10 cm SON	Stocks (SE)	ΔSON (SE)
Site	1999	2010	$(Mg N ha^{-1})$	1999	2010	$(Mg N ha^{-1})$
Forest	2.3 (0.3)			0.6 (0.1)		
For→Pas 96	2.7 (0.5)_	3.9 (0.3)_	1.2 (0.5)_	1.2 (0.4)_	2.2 (0.1)_	0.9 (0.4)_
For→Pas 85	3.1 (0.1)*	4.3 (0.3)*	1.3 (0.3)*	1.3 (0.1)*	1.9 (0.1)*	0.6 (0.1)*
For→Pas 50	3.3 (0.2)*	4.4 (0.0)*	1.1 (0.3)*	1.4 (0.2)*	2.0 (0.1)*	0.7 (0.2)*
Crop→Pas 83	3.7 (0.0)*	4.8 (0.2)*	1.1 (0.2)*	1.5 (0.1)*	2.2 (0.1)*	0.6 (0.1)*
Crop→Pas 86	4.2 (0.1)*	5.5 (0.2)*	1.3 (0.3)*	1.6 (0.0)*	2.2 (0.0)*	0.6 (0.0)*

Table 3: 0-50 cm and 0-10 cm soil nitrogen stocks as well as changes in stocks between 1999 and 2010. Asterisks (*) indicate a significant (P<0.05) difference between samples collected in 1999 and 2010. All data are in Mg N ha⁻¹.

Nitrogen increased faster than carbon, increasing $50.9\% \pm 6.2\%$ and $36.1\% \pm 2.9\%$ in the top 10 and 50 cm, respectively, compared to carbon, which increased $39.6\% \pm 7.1\%$ and $32.5\% \pm 7.5\%$ in the top 10 and 50cm, respectively. This resulted in lower C:N ratios in pastures than the forested control site. The average C:N ratio among forest-to-pasture sites was 12.3 in 1999 and 11.7 in 2010. C:N ratios were even lower on crop-to-pasture sites, dropping from an average of 9.4 in 1999 to 9.1 in 2010. The forested site C:N ratio was 15.2.

Averaged across all fields, bulk density increased for the 20-50cm depth, slightly decreased at the 0-10cm depth, and slightly increased for the 10-20cm depth. In the top ten centimeters bulk density decreased $1.2\% \pm 3.0\%$ on average (data not shown). For the 10-20cm depth, bulk density increased an average of $3.9\% \pm 1.8\%$. Bulk density increased 20-50cm deep, averaging $15.7\% \pm 1.3\%$ for all four sites with significant (*P*<0.05) increases in FP50 and CP83 (Table 4).

Table 4: 20-50cm 1999 and 2010 bulk density and percent carbon as well as change in SOC stocks. Asterisks (*) indicate a significant (P<0.05) difference between 1999 and 2010. Units are in g/cm³ and exclude root and rock weights, which resulted in lower than expected bulk densities in gravelly soils.

	Bulk Density (SE)		%C	(SE)	ΔSOC (SE)
Site	1999	2010	1999	2010	$(Mg C ha^{-1})$
Forest	0.82 (0.02)		0.54 (0.05)		
For→Pas 96	0.79 (0.02)_	0.92 (0.02)_	0.39 (0.03)_	0.48 (0.05)_	3.9 (1.0)*
For→Pas 85	0.85 (0.01)	1.00 (0.04)	0.33 (0.07)	0.47 (0.06)	5.6 (1.8)*
For→Pas 50	0.85 (0.03)*	1.00 (0.04)*	0.48 (0.03)_	0.46 (0.05)_	1.4 (1.0)_
Crop→Pas 83	0.84 (0.01)*	0.94 (0.02)*	0.49 (0.02)	0.54 (0.01)	2.9 (0.7)*
Crop→Pas 86	1.03 (0.05)_	1.07 (0.08)_	0.38 (0.01)*	0.42 (0.01)*	1.6 (0.9)_

Once random factors were accounted for, our mixed linear model showed no evidence (P>0.05) that time since land-use conversion (TLCD) correlated with SOC across all years (Table 5). There was better, but still not significant, evidence that SOC increased within the 1999 chronosequence (0.28±0.05 Mg C ha⁻¹ yr⁻¹, P>0.05) after a 10.5±3.8% loss in SOC after clearing (Table 5). This rate is 48% of the rate observed in a repeated measures analysis at FP50, which accrued SOC slower than any other site.

Solution for Fixed Effects							
Effect	mgmt	Year	Estimate	Standard Error	DF	t Value	$\Pr > t $
Intercept			50.32	1.1707	1.00	42.980	0.015
year		1999	-18.87	1.4639	1	-12.890	0.049
year		2010	0.00			-	
mgmt	for		3.663	1.326	1	2.760	0.221
mgmt	pas		0.000				
tlcd			-0.046	0.03224	1	-1.410	0.392
tlcd*year		1999	0.278	0.04559	1	6.090	0.104
tlcd*year		2010	0				

Table 5: Proc mixed model output for the forest-to-pasture chronosequence.

Physical soil fractions:

Across the forest to pasture chronosequence, 0-10cm SOC was consistently larger in the two smallest size classes in pastures than the unmanaged forest (Figure 3). Although sometimes larger (Figure 3) and often showing the largest changes within a field (Figure 4), macroaggregate SOC was not consistently greater in pastures than the unmanaged forest (Figure 3). Decreases in large and small macroaggregate C were accompanied by increased in microaggregate C between year 0 (FOR) and year 3 (FP96) (Figure 4).



Forest-to-pasture Chronosequence



Macroaggregate pools of carbon changed faster than any other pool of carbon. Substantial changes were apparent in every pasture and were significant in FP96 (small macroaggregates increased 6.3 ± 1.1 Mg C ha⁻¹) and FP50 (large macroaggregates increased 3.3 ± 0.6 Mg C ha⁻¹) (Figure 4). In FP50, these increases were offset by significant decreases in microaggregate C (-2.7\pm0.3 Mg C ha⁻¹), which was the largest relative change in any pool (Figure 4). For comparison, microaggregate C in FP50 decreased 82% compared to small macroaggregates, which increased an average of 37%. Averaged across all fields, SOC in large macroaggregates increased 1.10 ± 0.82 Mg C ha⁻¹, SOC in small macroaggregates increased 3.57 ± 1.09 Mg C ha⁻¹, SOC in microaggregates increased 0.22 ± 0.98 Mg C ha⁻¹, and SOC in silt and clay associated fractions increased 0.19 ± 0.23 Mg C ha⁻¹ (Figure 4).



Figure 4: Within-field changes in C within aggregate fractions. Changes were calculated as: 2010 stock - 1999 stock. Asterisks (*) indicates significant (P < 0.05) differences in a paired t-test comparing 1999 and 2010. Error bars represent standard error.

Discussion:

Soil carbon accumulated in these pastures at an average rate of 1.15 ± 0.33 Mg C ha⁻¹ yr⁻¹, which is much faster than our 1999 chronosequence (0.23 Mg C ha⁻¹ yr⁻¹) would have led me to expect. The rate of increase was slower in a pasture approximately 50 years old (0.57 Mg C ha⁻¹ yr⁻¹) and faster in a newly converted (3 years old at the start of sampling) pasture (1.70 Mg C ha⁻¹ yr⁻¹). The crop-to-pasture conversion also had high rates of carbon accumulation (0.91 Mg C ha⁻¹ yr⁻¹). While large compared to many studies (the worldwide native-to-pasture average C sequestration rate was only 0.35 Mg C ha⁻¹ yr⁻¹ (n=42)), these rates are comparable to the woodland- and grassland-to-pasture conversions analyzed by Conant et al., (2001), which averaged 1.0 Mg C ha⁻¹ yr⁻¹. That the chronosequence would have vastly underestimated the rate of carbon accumulation highlights the need to use the repeated measures approach (Conant et al., 2001).

Figure 5 may help the reader better understand how much the chronosequence approach could have misled us. For example, the 1999 chronosequence showed a low rate of carbon accumulation, while the 2010 chronosequence showed no change (Figure 5). Traditionally, this would result in the interpretation that the ecosystem was approaching or had reached equilibrium around 15 years after conversion from forest. Indeed, when we set out for 2010 sampling, we expected new data to fit approximately along the lower (1999) chronosequence regression (Figure 5). However, sequestration rates over 0.5 Mg C ha⁻¹ yr⁻¹ in the ~50 year old pasture appear to invalidate that interpretation (Figure 5). That such a carefully selected chronosequence appears to have been invalidated by the repeated measures approach should encourage the scientific community to revisit sites around the world, particularly in dynamic, productive systems such as forest-to-pasture conversions in the tropics. Such an effort would be extremely useful in developing a new understanding of agroecosystems and how they respond to changes in

management and land use.



Figure 5: A scatterplot of total SOC vs time since land use conversion. I performed a regression for both 1999 and 2010 chronosequences, which are plotted with solid lines. 1999 and 2010 samplings in the same pasture are connected with dashed lines and are labeled with the rate of accumulation observed in that pasture.

Although I believe land-use-change is likely the primary driver of SOC accumulation on this farm, numerous other factors are likely contributing to the large increases I have observed. Crucially, well-managed pastures that receive regular additions of fertilizer have been shown to drive carbon sequestration 0.29 Mg C ha⁻¹ yr⁻¹ (2.2% yr⁻¹ by relative weight) (Conant et al., 2001) and lime is estimated to promote sequestration between 0.10 and 0.20 Mg C ha⁻¹ yr⁻¹ (Follett et al., 2000). In addition, these pastures are occasionally reseeded with legumes, which has been found to sequester an average of 0.75 Mg C ha⁻¹ yr⁻¹ (Conant et al., 2001).

Other factors could be affecting our results, but have received less attention in the literature, including rotational grazing and nitrogen deposition. Rotationally grazing has been shown to improve net primary productivity on some sites (Oates et al., 2011), but not shown to

increase SOC when repeated measures were used (Sanford et al., 2012). Another study observed substantial nitrogen deposition along the Appalachian mountains, which could add to the effects of fertilization (Tian et al., 2010).

In addition to the various factors that could have contributed to high sequestration rates, including both the sampling depth and regional factors. Sampling deeper than 20cm remains fairly uncommon, but is an important zone for changes in organic matter (Conant et al., 2001). Also, higher MAP:PET ratios (highly related to biome) correlated with amount of carbon sequestered (Conant et al., 2001). Regional factors could also be making an impact; Virginia appeared to have highly variable changes in NPP over the course of the 20th century (Tian et al., 2010) ranging from a 40% loss to a 70% gain.

Factors like sampling depth and region make it valuable to compare relative percent changes in addition to mass changes. In this forest-to-pasture conversion, sequestration rates start around 5% yr⁻¹ in the first 15 years as pasture and eventually drop to less than 2% around 50 years. By comparison, Conant et al. (2001) reported relative percent change around 4% for cropto-pasture conversion in areas where the native ecosystem was forest (but not rainforest). Averaged across all three forest-to-pasture conversion sites, the total relative percent increase was almost 40%, compared to an average increase of 8% reported by Guo and Gifford (2002). Rates were more similar (around 25%) in wetter sites of 2000-300mm mean annual precipitation (MAP), but not the wettest sites where a range of losses were observed (Guo and Gifford, 2002). The Guo and Gifford (2002) study is dominated by tropical sites with high precipitation and temperatures. In comparison to our sites in Virginia, these environmental factors could simultaneously cause physiological plant stress and stimulate microbial activity. Sequestration rates were initially higher than many crop-to-pasture conversion studies indicates is very

impressive, though it would be valuable to revisit many sites where researchers used space-fortime substitution.

Fractionation revealed that the largest changes in SOC occurred in macroaggregates. The only noticeable decrease in macroaggregates occurred following deforestation. Subsequently repeated measures analysis at the pasture sites revealed significant increases in small macroaggregate C for FP96 and FP50. This echoes results from our previous investigation at these fields (Conant et al., 2004) and the works of others, which have found that microaggregates and silt and clay associated SOC are resistant to tillage and disturbance while macroaggregates are easily disrupted (Elliott 1986; Six et al. 2000, 2002). The relatively large increases in microaggregate SOC after deforestation were likely caused by the release of microaggregates from macroaggregates that were disturbed during land use change.

In summary, it seems that temperate pastures have a large capacity to sequester carbon. Even more than 50 years after land-use conversion, well-managed pastures can sequester more than 0.5 Mg C ha⁻¹ yr⁻¹. In the top 20cm, crop-to-pasture conversions in Louisa County, VA sequestered a similar amount of carbon as the average reported by Eagle et al. (2012), while forest-to-pasture conversions sequestered more (Table 6). Other factors such as changes in regional climate or nitrogen deposition may have also contributed to these substantial increases in SOC. Finally, these results underscore the caution that we should take when interpreting results from space-for-time analyses. In addition, researchers should do their best to document location and sample layout so that it is possible to revisit and resample sites, particularly when dramatic changes in land-use or management occur.

Table 6: Mitigation potential in comparison to other management practices. Numbers in parenthenses are 95% confidence intervals. † Total forestland acres in states of VA and WV from USDA NRI Data (2009). Estimates from this study (indicated by an asterisk (*)) only include the top 20 centimeters because that is the depth to which most other studies measure. Our estimates do not include a range because of limited replication.

Management Practice	CO_2 Mitigation Potential (Mg CO_2-eq ha ⁻¹ yr ⁻¹)	Max Potential Area (Mha)	Estimate Source
Implement No-Till	1.22 (-0.24–3.22)	94	Eagle et al., 2012
Switch to Short- Rotation Woody Crops	2.51 (-7.34–13.26)	40	Eagle et al., 2012
Crop-to-Pasture Land Use Change	2.39 (0.40–4.18)	Uncertain	Eagle et al., 2012
Forest-to-Pasture Land Use Change	3.02*	23†	This Study
Crop-to-Pasture Land Use Change	2.40*	Uncertain	This Study

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CHAPTER 3: SOIL ORGANIC CARBON CHANGES IN PASTURES WITH MANAGEMENT-INTENSIVE ROTATIONAL GRAZING

Recent record-breaking droughts and storms across the United States have reignited the public debate about climate change and humanity's need to mitigate greenhouse gas emissions in housing, manufacturing, transportation and food production. Those that would curb emissions face a moving target as the world's populations of around 7 billion people and 20 billion livestock keep growing. Ruminants (e.g., cattle, goats, sheep, bison and buffalo) are an important puzzle piece that can convert indigestible forages into highly nutritious meat and dairy (Smil, 2000 and Garnett, 2009 in Janzen, 2011). At the same time, they consume and pollute significant quantities of water (Naylor et al., 2005; Herrero et al., 2009; FAO, 2009; de Vries and de Boer, 2010) while also contributing 9-18% of direct greenhouse gas emissions (Gill et al., 2010; Steinfeld et al., 2006). Accordingly, livestock are important to investigate for their role in mitigating climate change, conserving fresh water supplies while also maintaining or improving food security. Management options that can accomplish all three goals are uncommon "win-win-win" scenarios (Janzen, 2007), but there are significant opportunities worth exploring (Janzen, 2011; Franzluebbers et al., 2012).

Grazinglands present multiple opportunities, both because they are extensive and also because they are dynamic. Optimizing stocking rates seems to be applicable to the largest area, but another strategy shows great promise. Management-intensive rotational grazing (which I refer to alternately as MIRG or rotational grazing) has shown potential for both GHG mitigation and increased agricultural output on up to 48 million hectares of pasture in the United States (Olander et al., 2011). Terminology for rotational grazing practices is not always consistent, but

the basic idea is that a manager controls the movement of their livestock, often moving them to new sections of pasture multiple times per week, mimicking the movements of ruminants under threat of predation across native grasslands (Savory, 1983).

MIRG has provoked some controversy, largely because short-duration grazing has shown little or no productivity gains on arid and semi-arid rangelands (Holechek et al., 1999; Briske et al., 2008). However, by implementing MIRG on humid and sub-humid sites, researchers have increased forage production and quality (Oates et al., 2011; Paine et al., 1999), per unit area performance (Phillip et al., 2001) and beef production (Jacobo et al., 2006). Only one study has investigated MIRG impacts on soil organic carbon (SOC) finding SOC averaged 22% higher on MIRG sites compared to extensively managed sites (Conant et al. 2003a). Similarly, one study found 22% lower methane emissions per animal among rotationally grazed cattle in Louisiana (DeRamus, 2003). Together, these benefits could certainly represent a "win-win-win" option for mitigating climate without sacrificing production.

Knowing how soil organic matter (especially soil carbon) responds to changes in agroecosystem management is essential when we try to evaluate the relative cost benefit analysis of various management options. To study how soil organic matter changes, researchers typically use a paired site or chronosequence approach, which both substitute space in an effort to simulate the effects of time (Conant et al., 2001). This space-for-time substitution can suffer due to natural variability or if some sites are not at equilibrium when the management change of interest was initiated (Murty et al., 2002; Sanderman and Baldock, 2010). In this study, I have evaluated the same sites over an 11 year period (an approach called "repeated measures") which avoids these issues by tracking changes over time (Murty et al., 2002).

The purpose of this project was to investigate the soil carbon dynamics in the relatively

uncharted pastures of the mid-Atlantic region in the Eastern United States. In particular, I was interested in assessing how rotational grazing affecting these sites. Using a repeated measurements approach provides a much more accurate assessment of SOC stock dynamics than our previous study, which used space-for-time substitution (Conant et al., 2003a). These results will be useful to anyone attempting to understand the complex interaction between livestock and the environment. In particular, policy analysts may find this study useful for understanding the role eastern pastures and rotational grazing could have on sequestration of atmospheric CO_2 as SOC.

Methods:

Previous work:

For this work I revisited sites sampled by Conant et al. (2003) in order to document changes over time under different types of grazing management. Four sets of site pairs (eight sites total) were identified so that climate, soils, and land use history were similar in each site pair to ensure that management was the main factor driving differences within each site pair. Previous sampling was conducted following sampling protocols developed for the Prairie Soil Carbon Balance study (Ellert et al. 2002). Conant et al. (2003a) sampled four pairs of one MIRG site and one extensively managed site, collecting samples from three locations, termed "microsites," in each field. I revisited those microsites, which were marked with a Skotchmark EMS magnetic ball marker (3M Corporation, Austin, TX), buried at 1 m depth to enable future relocation and resampling. Six cores were collected from each microsite according to the schematic in (Figure 6). In some cases, pastures had reverted to forest or been sold, which prevented resampling. This left two of the original site pairs and two unpaired MIRG sites for a total of 6 sites to re-sample in this study.



Figure 6: Microsite sampling diagram. An offset of 0.5 m was used to avoid disturbance effects caused by ssoil removal and eventual partial inversion.

In the Conant et al. (2003) study, the authors group continuously grazed and hayed pastures under the term "extensive management" a grouping that may have been inappropriate because grazing has important qualities that separates any form of grazing from hay production. Though both strategies decrease below-ground net primary productivity (Oates et al., 2011) and root biomass (Conant et al. 2003) relative to MIRG, grazing, not management intensity, significantly impacts microbial communities (Oates et al., 2012). In addition, hayed harvests remove a larger proportion of nutrients than grazing ruminant animals, which assimilate some nutrients but redeposit the rest as manure. This leads to decreased nutrient concentration in hayed pastures compared to grazed pastures (Mathews et al., 1994). In addition to differences in nutrient cycling, haying changes the pattern of defoliation. As opposed to diffuse incremental defoliation under continuous grazing, hay fields are cut to an even height during a discrete event. In some ways, this is similar to rotational grazing in which a patch of pasture is grazed to a more or less uniform height in a short period of time. For these reasons I will be clear when I am comparing continuous grazing or hayed treatments and avoid using the word "extensive" to group these treatments.

Study Area:

The sites that I analyzed represent a large portion of Virginia. Though not explicitly designed as a transect, the sites cover an almost 200 mile swath of Virginia that roughly follows the Blue Ridge Mountains. Table 7 describes key features of each farm including if and when MIRG was implemented as well as key environmental factors.

Louisa County

In Louisa County, I sampled three pastures on two farms that were not designed to represent a treatment pair due to differing soils and differences in management history. Farm "Louisa RG" (38.07°N, 78.11°W) is a long term pasture site on Nason silt loam soil (Typic Hapludult) in Louisa county. MIRG dairy production was implemented in 1984 after being managed extensively (i.e., some combination of hay and continuous grazed) since the 1950s. Since the original sample date, the manager of this farm ceased farming full-time and he has reduced his stocking rates and intensity of rotation.

Pulaski County

Two sites are located on one farm in Pulaski County, Virginia (37.12°N, 80.47°W). Both fields are underlain by Lowell silt loam soil (Typic Hapludalf) and had been extensively grazed prior to implementation of MIRG. The owner estimates they have been under pasture since the 1950s. MIRG was implemented in 1996 on both sites. The two sites are referred to as "Pulaski WG" and Pulaski "SH." Pulaski WG is used as for winter grazing and had a significant amount of thistle present when sampled in June, 2010. Pulaski SH is not grazed in the winter and has had one cutting of hay removed per year since 1996. In 2010, these sites experienced extremely dry conditions preventing me from sampling below 30 cm. Municipal sludge was applied on both fields more than once leading up to 1999. The manager estimated it is applied at a rate of 90 lbs N and 200 lbs P per acre.

Grayson County

In the case of Grayson County I am actually comparing two farms near the town of Galax, VA, one rotationally grazed site in Grayson County and one continuously grazed site in Carroll County. "Grayson RG" (36.58°N, 80.96°W) is a farm with a more aggressive implementation of MIRG than sites I sampled in other counties. On this farm, the producer rotated his herd more often and stocked his pastures at higher rates than any other site sampled. The farm also minimizes outside inputs such as fertilizer (although supplemental minerals and kelp meal are provided to the cattle). MIRG was implemented in 1994 after extensive pasture management since the 1950s. One microsite was covered in hay for the three years leading up to sampling and was excluded from our analysis, limiting the pseudoreplicates in this field to two.

The neighboring farm, "Grayson CG" (36.69°N, 80.81°W) is a continuously grazed site underlain by the same soil series (Chester loam, Typic Hapludult). The producer raises beef part-

time at this location and increased his stocking rate from about 45 or 50 units up to 55 or 60 units on 130 acres, a stocking increase of about twenty percent. He estimates about half are cow-calf pairs and about half are yearlings. He also cuts hay on about 40 acres and estimates that he is collecting about 800 bales per year leading up to the 2010 sampling, up from about 500 bales in the late 90s and early 2000s. This farm also minimizes fertilizer inputs, but applies lime approximately every five years. Grayson CG has comparable topography to Grayson RG and both were extensively managed pastures since conversion from forest in the 1950s. One marker ball could not be detected in 2010, limiting our number microsites at Grayson CG to two.

Site	Management	Date of	Stocking	MAP (mm)	MAT (°C)
		Implementation	Rate		
Louisa RG	MIRG +	1984	1.93	1087	13.2
	Moderate Grazing				
Pulaski SH	MIRG + Spring	1994	1.2	949	11.4
	Hay				
Pulaski WG	MIRG + Winter	1994	1.41	949	11.4
	Grazing				
Grayson RG	MIRG +	1996	2.88	1144	10.8
·	"Mob" Grazing				
Grayson CG	Cont. Grazing +	N/A	1.15	1144	10.8
	Increased Stocking				

Table 7: Site management history, stocking rates and climate data.

Forage species were actively managed at all sites and consisted of orchard grass (*Dactylis glomerata*), which was the dominant forage cover at most sites, with some Kentucky bluegrass (*Poa pratensis*) and white clover (*Trifolium repens*). Perennial ryegrass (*Lolium perenne*) was the dominant species at Grayson RG. Except at Grayson RG, some history of lime and fertilizer addition existed at all sites, though the timing and rates were not available at all sites.

Sample Collection:

To precisely relocate our original sample points, I used field maps, GPS locations and a 1420 EMS-iD Marker Locator (3M Corporation, Austin, TX) to find each sampling microsite. This technology allowed me to relocate the northeast corner of each microsite to within a few inches. At each microsite, I collected 6 cores. Soil samples were collected in April and June. I used Giddings and Concord hydraulic samplers with 6.5cm diameter sampling cores to at least 50 cm deep as conditions allowed. I removed as much surface litter as possible and split cores into 0-10 cm, 10-20 cm, 20-50 cm depths. Each sample was placed in a freezer bag (labeled by field, microsite, core, and depth) and placed in a cooler.

Samples were returned to the laboratory and weighed while wet. A subsample was weighed and dried to determine soil moisture. Samples were passed through an 8-mm mesh sieve by gently breaking the soil along the plane of least resistance. All visible root material that was caught by the sieve was removed by handpicking as the soil passed through the 8-mm sieving. Surface litter and aboveground vegetation were then removed. Soils were then air-dried for 48 to 96 hours. Bulk density (BD) was calculated using volume of sample collected and the weight of soil in the sample, after correcting sample weight of fresh samples for soil moisture and root and rock content.

Samples were then sieved to pass through 2-mm mesh screens. All visible root material was removed at this stage and the samples were then oven-dried at 60°C for 72 hrs. After drying, samples were ground to fine powder using a roller table (Arnold and Schepers, 2004). For 1999 samples, the clay content was determined by the hydrometer method and silt content by difference. Soil C quantity was determined for root material using the ignition loss method. Soils were analyzed with a LECO CHN-1000 autoanalyzer (LECO Corporation, St. Joseph, MI). Our

initial samples contained no inorganic soil carbon (carbonates), so I assumed for this analysis that carbonates were absent

Statistical Analyses:

I used paired t-tests to analyze whether soil properties changed between sampling dates. I treated microsites as replicates changing through time. Because Conant et al. (2003a) showed larger SOC stocks at rotationally grazed sites, I used one-sided tests for SOC, SON and %C. For tests we did not expect to change in a particular direction, including bulk density (BD) and soil C:N ratio, we used two-sided tests.

Results:

Changes through time:

Over an 11 year period I observed significant increases in soil carbon (SOC) at each of five pastures that I sampled (Figure 7, Table 8). Averaged across all five sites, soil organic carbon stocks (SOC) increased an average 15.7 ± 2.9 Mg C ha⁻¹ in the top 20cm (Figure 7), a rate of 1.4 Mg C ha⁻¹ yr⁻¹. The smallest increases occurred (4.9 Mg C ha⁻¹) at Louisa RG, which had the longest history of rotational grazing and had slightly greater SOC stocks than other pastures in the same county. The largest increase was at Pulaski WG (21.4 Mg C ha⁻¹). On average, sites in Pulaski County increased more in the top 20cm than those in Grayson or Louisa counties, which increased 20.1 Mg C ha⁻¹, 16.8 Mg C ha⁻¹, and 6.5 Mg C ha⁻¹ respectively.



Figure 7: 0-20 cm SOC. Left bars in each pair represent SOC stocks from the 1999 sampling. Right bars represent 2010 SOC values. Errors bars are standard error. *Indicates significant at P<0.05.

At the five sites where I was able to sample down to 50cm, SOC stock changes at the surface accounted for $63\pm12\%$ (0-10 cm) and $82\pm16\%$ (0-20cm) of the total change (Table 8). The percentage contribution by the top ten centimeters was lower in Grayson County (46-55% of the 0-50cm change in the top 10cm than Louisa County) (Table 8). Sites in Pulaski and Grayson Counties had the greatest increases in soil carbon (Table 8).

Table 8: 1999, 2010 soil carbon stocks and changes in bulk soil for depths 0-50 cm and 0-10cm. Sites with a dagger symbol (\dagger) next to them report 0-20cm values because of conditions during sampling. Values with an asterisk (*) were shown to be significant in a paired t-test at p<0.05.

	0-50 cm SOC Stocks (SE)			0-10 cm SOC Stocks (SE)		
Site	1999	2010	Change	1999	2010	Change
Louisa RG	41.7 (2.7)*	48.0 (1.9)*	6.3 (0.7)*	20.1 (2.0)	24.3 (0.8)	4.2 (1.9)
Pulaski SH†	23.5 (0.1)*	37.3 (3.7)*	18.8 (4.2)*	23.5 (0.1)*	37.3 (3.7)*	13.7 (3.8)*
Pulaski WG†	29.5 (1.0)*	50.9 (1.6)*	21.4 (0.9)*	21.9 (0.9)*	39.4 (1.2)*	17.5 (0.4)*
Grayson RG	54.0 (2.0)*	78.6 (2.2)*	24.6 (0.2)*	23.5 (2.3)	37.0 (0.2)	13.5 (2.5)
Grayson CG	43.9 (1.1)*	66.9 (0.7)*	23.0 (0.4)*	20.4 (0.6)*	31.1 (0.2)*	10.7 (0.7)*

MIRG vs. other pasture management:

The only continuously-grazed site, Grayson CG, gained 23.05 Mg C ha⁻¹ (a rate of 2.10 Mg C ha⁻¹ yr⁻¹) in the top 50 cm (Table 8). The rotationally-grazed site paired with Grayson CG, Grayson RG, increased 7% more, totaling 24.6 Mg C ha⁻¹ (a rate of 2.24 Mg C ha⁻¹ yr⁻¹)(Table 8). At depth (20-50cm), Grayson RG SOC stocks increased by 5.6 Mg C ha⁻¹ (a 23% increase), compared to 8.4 Mg C ha⁻¹ (a 36% increase) for Grayson CG. On the other hand, if we compare relative percent increases, Grayson CG increased 4.8% yr⁻¹ while Grayson RG increased 4.1% yr⁻¹.

SOC stocks (0-20cm) for paired sites Pulaski SH (hayed) and WG (grazed year round) increased 18.8 Mg C ha⁻¹ (1.71 Mg C ha⁻¹ yr⁻¹) and 21.4 Mg C ha⁻¹ (1.95 Mg C ha⁻¹ yr⁻¹) respectively (Table 8). Expressed as a percent, Pulaski SH, with its history of harvest and removal of hay, accumulated 14% less carbon than Pulaski WG, where grazing extends into the winter (Table 8).

Discussion:

Significant increases in SOC were observed at each of six pasture sites that I analyzed, four of which increased at rates more than 1 Mg C ha⁻¹ yr⁻¹. Sites where MIRG implementation was the primary change in management (Louisa RG, Pulaski WG and Grayson RG) soil C increased by 1.37 Mg C ha⁻¹ yr⁻¹ in the top 20cm, with smaller changes in the oldest conversion. For comparison; Conant et al. (2001) found improved grazing (e.g., implementing moderate stocking rates after overgrazing or no grazing) led to a mean sequestration rate of 0.35 Mg C ha⁻¹ yr⁻¹ (n=45). Conant et al. (2001) also found that conversion from cultivation to pasture led to 1.01 Mg C ha⁻¹ yr⁻¹ (N=23). If we assume for a moment that SOC had reached some sort of equilibrium before MIRG was implemented in the 80s and 90s at our various sites, the large changes observed would indicate that MIRG leads to increases in SOC relative to that equilibrium. Indeed, implementation of MIRG led to a larger increase in SOC than conversion from cultivation or forest to pasture (Chapter 2).

Soil carbon stocks increased for Grayson RG at an annual rate of 2.24 Mg C ha⁻¹ yr⁻¹. For the Grayson CG, the rate was 2.10 Mg C ha⁻¹ yr⁻¹. The 0.18 Mg C ha⁻¹ yr⁻¹ difference amounts to 7%. The MIRG sites with moderate to high stocking rates (including Grayson RG and Pulaski CG), had an annual rate of 1.84 Mg C ha⁻¹ yr⁻¹ (0-20cm) compared to 1.33 Mg C ha⁻¹ yr⁻¹ for the continuously grazed site, a difference of 38%. In the original paired site analysis; Conant et al. (2003a) found approximately 24% more SOC in MIRG than CG.

Changes in other aspects of management complicate the comparison between rotational and continuous grazing. For example, the manager increased the stocking rate at Grayson CG after our 1999 sampling and it is hard to know if this sped up or slowed SOC accumulation. However, statistically similar rates of C uptake in spite of much higher stocking rates (Table 7) for Grayson RG than Grayson CG supporting the idea that MIRG increases the 'optimal' stocking

rate (Savory, 1983; McMeekan and Walshe, 1963) without a relative loss of SOC. At the same time, these results also indicate that both types of grazing can promote substantial gains in SOC.

A recent study performed at the University of Wisconsin-Madison investigated MIRG on a site with similar initial species composition as our sites in Virginia (Oates et al., 2011). The MIRG treatment produced approximately 40% more dry matter production than CG in their two year study (Oates et al., 2011). Belowground net primary productivity gains were less pronounced: around 10% in the first year and 30% in the second year (Oates et al., 2011). Interestingly, under MIRG they found the percent cover of the grass *Dactylis glomerata* and legume *Trifolium repens* increased, implying some fitness advantages were afforded these species, which were both present at our sites in Virginia (Oates et al., 2011). Increased cover by legumes is particularly significant because they seem to facilitate CO₂ uptake by grasslands (De Deyn et al., 2009; Conant et al., 2001) while also reducing methane production by cattle (McCaughey et al., 1999).

Few studies have shown C sequestration rates of more than 1 Mg C ha⁻¹ yr⁻¹ at more than one site. Indeed, Franzluebbers (2010) estimated newly planted perennial pastures could sequester 0.25 to 1.0 C ha⁻¹ yr⁻¹, but stressed the need for data across diverse pasture types. That our results can exceed those numbers in pastures more than 40 years old underscores that there are substantial mitigation opportunities available, but further studies in more areas are needed to confirm and quantify this finding.

Finally, this study highlights why repeated measures are an important tool for analyzing dynamic systems, but not without their own difficulties. Changing management (e.g., forestry or shifting stocking rates) can make interpreting results more difficult than a controlled study or ecosystem "snapshots" that substitute space-for-time. That said, incorporating a plan to revisit

sites regardless of funding adds marginal overhead to project costs (including 3M[™] EMS extended-range ball markers and additional labor necessary to install these markers deep enough to avoid disturbance). But the potential benefits of being able to revisit sites should dramatically outweigh the potential costs of misinterpreting chronosequence and paired site data, which could occasionally lead to perverse recommendations regarding goals of increasing production, protecting our freshwater supplies or mitigating climate change.

Solutions to global change are complex, and many may require trade-offs in terms of greenhouse gases and food production (Janzen, 2007). Here, however, substantial opportunities for C sequestration through grazing have been demonstrated without any evident decreases in production. In a continuous grazing site, increased stocking rates may have even helped boost C sequestration. Rotational grazing continues to offers promise, and is perhaps responsible for some portion of these gains in addition to possible reductions in methane (DeRamus et al., 2003) and improved production capacity (Oates et al., 2011).

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