ECOSYSTEM C STOCKS AND THE IMPACTS OF LAND USE INTENSIFICATION IN SUBTROPICAL GRAZING LAND ECOSYSTEMS

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2014
To my mom Xiaolan, my dad Yanan, and my husband Chong
ACKNOWLEDGMENTS

First I would like to thank my advisor Dr. Maria Lucia Silveira. As chairman of the supervisory committee, her effective and creative guidance on my PhD scientific research, including developing the program of courses, determining the research design, conducting field and lab experiments, data interpretation and dissertation writing is greatly appreciated. Thanks are also expressed to my co-advisor Dr. Kanika Sharma Inglett for her guidance and suggestions on my PhD program work, especially on the experiments conducted in the wetland biogeochemistry lab. Thanks also go to other committee members, Dr. Stefan Gerber, Dr. Lynn Sollenberger and Dr. Ted Schuur for their input on my PhD program and dissertation writing.

Financial support from Southern Region USDA Program on Sustainable Agriculture Research and Education (SARE) is greatly appreciated. Special thanks also due to Dr. K. Ramesh Reddy, chair of the Soil and Water Science Department, and Dr. John Arthington, director of the Range Cattle Research and Education Center.

Acknowledgement is expressed to Ms. Cynthia Holley and Ms. Yu Wang for their instructions and help with my experiments. Statistical support from the IFAS team, particularly James Colee is appreciated. Thanks also go to Mr. Michael Sisk, the department coordinator and all the other support staff from Soil and Water Science Department and Range Cattle and Research Station for their assistance during my PhD program.

The help from other students with my field and lab work, data processing and interpretation and dissertation writing is greatly appreciated, especially fellow graduate student Julius Adewopo, Hiran Marcelo, Mariana Azenha, Anna Normand, Jing Hu, Francisca Hinz, Katelyn Foster, Joshua Papacek, Debjani Sihi, and undergraduate
students Guilherme Buonadio, Henrique B Brunetti and post-doc fellow Paulo Martins and Lucy Ngatia.

The friendship and company of Luana, Bluna, Joao, Menglong, Xiaofeng, Jin, Jian, Chong, Xiang, Yi, Xiaofei, Jianye was appreciated as they made my life in Ona and Gainesville delightful.

Sutie is deeply grateful to her family for their great love, support and encouragement to achieve her educational goals and to make sure that her life and her career fulfilling.
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<tr>
<td>C</td>
<td>Carbon</td>
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<td>CiPOM</td>
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Land use often leads to changes in ecosystem carbon (C) cycling, including major impacts on soil C stocks. Considerable efforts have been placed on understanding land use change impacts on soil C responses in temperate regions; however, much less research has been conducted in subtropical ecosystems. Grazing land management practices such as grazing intensity and nutrient management are expected to affect plant community and soil characteristics; however the direction (either positive or negative effects) and extent that these practices affect ecosystem C responses have not been fully evaluated. In this study, we investigated the impacts of grazing land intensification on ecosystem C and microbial community responses. Treatments consisted of three land use types: native rangeland (less intensively managed), silvopasture and sown pasture (more intensively managed). The impacts of grazing land intensification on above- and below-ground plant biomass, litter biomass, soil organic C (SOC), and soil microbial community structure and activity were evaluated. Silvopasture exhibited the greatest above-ground C biomass (59 Mg ha\(^{-1}\)) compared with native rangeland and sown pasture (4 and 2 Mg ha\(^{-1}\), respectively). The
greatest proportion of ecosystem C was associated with SOC (average of 77% of total ecosystem C). Grazing land intensification promoted SOC accumulation (76 Mg ha\(^{-1}\) for native rangeland vs. 100 and 110 Mg ha\(^{-1}\) for silvopasture and sown pasture at 0 to 90 cm depth). However, data also demonstrated that labile C increased with grazing land intensification. Particulate organic C (POC) at 0 to 20 cm soil depth increased from 17 to 28 Mg ha\(^{-1}\) with the conversion of native rangeland to sown pasture. Similarly, light-free (LF) C fraction also increased in the sown pasture (33 g kg\(^{-1}\) soil) compared with native rangeland (16 g kg\(^{-1}\) soil). Microbial biomass and β-Glucosidase activity followed a similar pattern. For instance, average microbial biomass at 0 to 20 cm depth in native rangeland and silvopasture was 213 mg kg\(^{-1}\) compared with 334 mg kg\(^{-1}\) in the sown pasture. Data indicated that conversion of native rangelands into more intensively-managed pastures can promote SOC in subtropical ecosystems; however, intensification can also affect soil microbial activity and mineralization of SOC.
CHAPTER 1
BACKGROUND AND INTRODUCTION

Global C cycling is greatly influenced by anthropogenic activities including land use change (Houghton, 1995; Watson, 2000). Ecosystem responses to land-use management include changes in SOC stocks (Conant et al. 2001; Franzluebbers and Stuedemann, 2002; Lal, 2005; Shrestha and Stahl, 2008) and soil microbial community structure and composition (McKinley et al., 2005; Bradley et al., 2006; Wang et al., 2006; Rousk, and Bååth, 2007; Stark et al., 2008; Denef et al., 2009).

Previous research has documented both positive and negative impacts of land use change on ecosystem C. While land-use conversion and management can result in C losses and subsequent increase in greenhouse gas emissions (Eswaran et al., 1993; Houghton, 2003), positive effects of land use on SOC sequestration have also been observed if proper management practices are implemented (Schimel, 2000; Silver et al., 2000; Conant et al., 2001; Houghton, 2003; Arevalo et al., 2009; Arevalo et al., 2011; Cantarello et al., 2011; Cusack et al., 2011). Proper management practices often include the introduction of trees or productive plant species, such as C4 grasses into grazing ecosystems (Fisher et al., 1994; Schimel et al., 1995; Conant et al., 2001; Montagnini and Nair, 2004; Haile et al., 2008; Casals et al., 2014). Additionally, SOC sequestration can also be promoted with proper soil fertility management (Van Cleve and Moore, 1978; Malhi et al., 1991 and 1997; Sumner et al., 1992; Conant et al., 2001; Johnson and Curtis, 2001), grazing intensity (Abril and Bucher, 1999; Wright et al. 2004), and rotational stocking (Conant et al., 2003).

Despite the large number of studies focused on understanding ecosystem responses to land use changes, important questions regarding the direction and extent
that changes occur have not been fully elucidated. For instance, while N fertilization may increase C inputs, it can also affect microbial community structure and activity, and subsequently soil C accumulation and stabilization. Additionally, most previous studies addressing ecosystem C responses to land use management were focused on temperate ecosystems with much less effort on subtropical regions. Florida grasslands have unique climate and soil characteristics, thus, it is important to better understand ecosystem C and soil microbial responses to land use intensification in this region. Moreover, grazing lands worldwide are increasingly subjected to intensification to meet global demand for food driven by growing human population. However, management practices intended to promote forage and animal production can often have impacts on ecosystem C. Both the intensity and type of management applied to maximize productivity will affect the direction and extent that changes in ecosystem C occur.

In this study, we evaluated the long-term impacts of grazing land intensification (conversion of extensively managed native rangelands into more intensively managed silvopasture and sown pastures) on ecosystem C, including total ecosystem C stocks and distribution among the various above- and below-ground pools, SOC stocks and characteristics, and microbial community structure and enzyme activity. Silvopasture and sown pasture have been established on a native rangeland for more than 20 years and have been consistently managed to mimic management intensity commonly used in the region. Intensification was defined as increased use of N fertilization, greater stocking rate, and introduction of trees and highly productive perennial grass species.
The overall objective of this research was to investigate the impacts of grazing land intensification on ecosystem C and microbial community responses in a subtropical region. The specific goals of this study were:

1) To determine the long-term effects (> 20 yr) of grazing land intensification on ecosystem C stocks and distribution among the various above- and below-ground pools (Chapter 3);

2) To quantify and characterize SOC and aggregate size fractions in subtropical grazing lands subjected to different levels of management (Chapter 4);

3) To evaluate microbial community and process responses to grazing land intensification (Chapter 5).

The central hypothesis was that management practices intended to increase plant and animal production such as converting native rangelands into silvopasture and sown pastures control production inputs, distribution, and quality and, therefore, have major impacts on ecosystem C dynamics and soil microbial community structure. The specific hypotheses tested in this study were:

1) Ecosystem C stocks increase with grazing land intensification because of greater nutrient inputs and introduction of more productive plant species.

2) Compared with native rangelands, sown pastures and silvopastures exhibit greater SOC stocks because of the greater C inputs.

3) Soil C mineralization and microbial activity increase as grazing land management increases because of greater N availability and greater inputs of labile C. Soil microbial community structures change in respond to the conversion of vegetation type and management strategies.
CHAPTER 2
LITERATURE REVIEW

Global Climate Change and Greenhouse Gas Emissions

In the third report of Intergovernmental Panel on Climate Change (IPCC) (2001), global climate change refers to the changes in measure of climate such as temperature and precipitation caused by natural processes and anthropogenic activities. This IPCC report indicated that, in addition to increased average surface temperature, global change has also promoted a decrease in extent of snow and ice cover, and a rise of average sea level. Despite considerable debate, most climate scientists agree that anthropogenic activities can increase greenhouse gas emissions to the atmosphere, which is regarded as the dominant factor affecting climate change (IPCC, 2001, 2007). In the fourth IPCC (2007) report, it was indicated that human-induced activities such as burning of fossil fuel and, to a lesser extent, agriculture and changes in land-use have increased the levels of carbon dioxide (CO$_2$) and nitrous oxide (N$_2$O) in the atmosphere. For example, atmospheric CO$_2$ concentration was ~280 ppm in the pre-industrial period while it increased to 379 ppm in 2005. Approximately 20% of the CO$_2$ emissions since 1990 have been associated with land-use change (average of 5.9 Gt CO$_2$ per year). Although the consequences of unprecedented levels of greenhouse gas are difficult to predict, it is certain that they will have major impacts on earth’s temperature and hydrological cycle.

Grasslands and Grazing Lands: Importance to Greenhouse Gas Mitigation

Terrestrial ecosystems can act as an important sink or source of atmospheric C and play an important role in climate change regulation (Schimel et al., 2001). Grasslands are important as they occupy a large area in terrestrial ecosystems,
covering nearly one third of the land surface (~50 ×106 km²) in the world (White et al., 2000). Grasslands are grass-dominated ecosystems with grasses, grass-like plants, shrubs, and forbs vegetation and include shrub lands, lands with forbs, and planted pastureland and rangeland for grazing (USDA-NRCS, 2009).

A number of important ecosystem services, which can provide ecological benefits and economic values, are often associated with grasslands (USDA-NRCS, 2009). For example, healthy grasslands support the livestock industry and recreational opportunities for humans and can provide diverse habitat and food sources for wildlife, greenhouse gas mitigation, water quality protection, and groundwater recharge. Grasslands can also store soil organic matter and enhance C sequestration, protect soil from wind and water erosion, and sustain plant, and animal biodiversity. Previous studies reported the benefits associated with SOC accumulation in grasslands. For instance, Reeder et al. (1998) observed an increase in SOC after 5 years of conversion from croplands to grasslands in a sandy loam soil.

Grazing lands occupy ~30% of the area in the United States (634 million acres) (USDA-NRCS, 1997; Follett et al., 2001). According to the USDA-NRCS, ~63% (401 million acres) of grazing lands are rangelands, ~21% (130 million acres) are pasturelands and ~10% (64 million acres) are grazed forest land (USDA-NRCS, 1997). Rangelands represent the predominant grazing land type in the United States, and the native vegetation includes grass, forbs, and shrubs suitable for grazing. Conversely, pasturelands consist of introduced plant species that are often more intensively managed than native rangelands. Grazed forest lands consist of widely spaced trees and understory vegetation which is suitable for grazing.
A significant body of literature has demonstrated that grazing management can have major impacts on important ecosystem processes such as nutrient cycling and SOC storage (Ingram et al., 2008). Grazing land can offset ~ one fifth of CO$_2$ released by other land uses (Follett and Reed, 2010). However, important ecosystem processes, such as SOC storage and characteristics, litter and root decomposition rates, soil N stocks and mineralization rate can be affected by grazing land management (Naeth et al., 1991; Shariff et al., 1994; Frank et al., 1995; Abril and Bucher, 1999; Schuman et al., 1999; Ingram et al., 2008).

**SOC Stocks and Pools in Grazing Lands**

**SOC Storage and Distribution**

Soil organic C consists of a heterogeneous pool of plant- and microbial-derived C compounds. Soil organic C accounts for ~ 50-58% of soil organic matter, which consists of biologically active and humified compounds such as root litter debris and soil organisms (Gregorich et al., 1994). Soil organic matter plays an important role in ecosystem function, such as providing plant nutrients, retention of water, supplying soil pores and aeration, reduction of soil erosion and run-off and supporting soil organisms (Weil and Magdoff, 2004). From a global perspective, SOC is important because it represents the largest terrestrial C pool. Approximately 1500 Pg of C is found in the top 1 m soil depth (Eswaran et al., 1995; Batjies 1996). The amount of SOC is about 2.5 times greater than that in the vegetation pool (550 Pg) and 2 times greater than the amounts present in the atmosphere (750Pg) (Post et al., 1990; Houghton, 1995; Hillel and Rosenzweig, 2009).

Accumulation and stabilization of SOC depend on the balance of C inputs and outputs. Soil organic C inputs derive from the absorption of C by plants via
photosynthesis, and conversion of living material into soil organic matter, while microbial and plant (above- and below-ground) respiration and decomposition represent the major C outputs (Zhu et al., 2010).

Different SOC pools, including active/labile C pool, slow C pool and resistant/passive C pool, can be operationally or conceptually defined according their turnover rates and characteristics (Parton et al., 1987; Allen et al., 2010). The active/labile C pool is associated with soluble fresh plant residues with turnover rate < 10 years and include material such as microbial and root exudates, fine roots, microbial biomass, and light fraction particulate organic C (Allen et al., 2010). The proportion of labile C pools relative to total SOC can vary widely depending on the soil type, management, and environmental conditions. For example, microbial and plant exudates account for ~ 0.5-5% of SOC but exhibit the fastest turnover rate (<0.1 year) (Parton et al., 1987; Allen et al., 2010). More easily decomposable C pools play a vital role as immediate C and nutrient sources for soil microbes and they can also promote the formation of soil microaggregates (Smucker et al., 2007). Microbial biomass represents ~ 5% of SOC and because the relatively fast turnover rate (< 5 year), this pool is an important source and sink of C and nutrients that can promote microbial activity and nutrient cycling (Parton et al., 1987; Dalal, 1998; Allen et al., 2010). Other labile C pools such as particulate organic C and light fractions account for ~ 30-40% of SOC with turnover rate of <10 years. Particulate organic C is considered an intermediate pool between active and passive pools (Cambardella and Elliott, 1992). Thus, POC is often used as an indicator of land use intensification impacts on soil C sequestration (Franzluebbers, 2002) and it is effective tool to evaluate the long-term impacts of land use intensification
on soil C sequestration (Franzluebbers, 2002). Labile C pools are typically more sensitive to land use change than SOC (Cambardella and Elliott, 1992; Sparling, 1992; Dalal and Chan, 2001; Allen et al., 2010).

Slow C pools with turnover rates of 10 to 200 years are humus or clay-sorbed C groups (Parton et al., 1987; Allen et al., 2010). Compared with labile C pools, slow C pools are less sensitive to land use since they often have more complex chemical structures and are physically protected in microaggregates, thus, they are less accessible to microorganisms (Allen et al., 2010). The resistant/passive C pool is charcoal C, which is ~30% of SOC and it is very stable with slow turnover rate (>100 year) (Allen et al., 2010).

Soil C stabilization and distribution into labile and passive pools vary greatly, depending on soil type, environmental conditions, and cultivation practices. For example, plant species composition can affect labile C inputs such as root exudates, dead root cells, and fine root turnover (Shamoot et al., 1968).

**SOC Accumulation in Grasslands and Grazing Lands**

Soil organic C stocks vary significantly depending on soil type and climatic conditions. The southeastern USA is characterized by warm and moist climatic condition that promote SOC decomposition; thus, SOC stocks in this region are relatively low compared with temperate ecosystems.

Greater SOC concentrations generally occur in the topsoil (upper 20 to 40 cm) and tend to decrease steadily with soil depth (Hillel and Rosenzweig, 2009). A relatively high proportion (90%) of ecosystem C in grassland ecosystems is associated with SOC (Burke et al., 1997). Soil organic C plays a vital role in grassland sustainability and the global C cycle (Parton, 1995).
Land Use and Ecosystem C

Impacts of Grazing Land Intensification on Ecosystem C and SOC Stocks

Anthropogenic activities, especially land use change, are important factors influencing global C cycle (Houghton, 1995; Watson, 2000). To respond to the rapid population growth rate and accompanying increased food demand, land use intensification is often aimed at achieving maximum production per unit of land. However, although land use intensification may result in increased crop yields and subsequent positive impacts on food security, it can also have detrimental effects on ecosystem function and terrestrial C equilibrium. Greenhouse gas emission caused by land use change during the period of 1850 to 2000 was ~ 156 Pg C (Houghton et al., 2003). Poor land management such as overgrazing and soil degradation can promote SOC loss and subsequent increases in CO₂ emissions to the atmosphere (Giardina et al., 2004, Mack et al., 2004; Su et al., 2005; Han et al., 2008). Conversely, proper management techniques which are frequently used to increase crop and animal production can also promote SOC accumulation (Abril and Bucher, 1999; Fearnside and Barbosa, 1998; Conant et al., 2001). These include conversion of native vegetation to productive sown pastures, increased use of fertilizer, and improved grazing management (Conant et al., 2001).

Grasslands have a great potential to store C in the soil. Conversion of native range or crop lands to reseeded grasslands in Wyoming resulted in greater above-ground biomass, litter accumulation, and SOC stocks, particularly when receiving N fertilizer (Reeder et al., 1998). Previous studies indicated that SOC accumulation can increase with introduction of C4 grasses or grass-legume mixtures (Fisher et al., 1994;
Fornara and Tilman, 2008). Fisher et al. (1994) studied SOC storage in pastures planted to deep-rooted C4 grasses in South American savannas and reported that as much as 100 to 507 Mt of C per year could be sequestered in grassland soils. These authors also indicated the SOC associated with grasslands represent an important strategy to offset the CO2 emissions caused by land use changes. Similarly, a 12-year study conducted in Minnesota also observed that the establishment of C4 grass can increase SOC by 193% compared to native vegetation (Fornara and Tilman, 2008).

Nitrogen availability is an important factor affecting SOC accumulation in grassland soils. A study conducted by Fisher et al. (1994) in South American Savannas found that introduction of C4 grasses and N-fixing legume species promoted forage production and N cycling. Similar results were also observed by Fornara and Tilman (2008). A number of studies demonstrated the positive effect of N addition on SOC sequestration (Van Cleve and Moore, 1978; Malhi et al., 1991 and 1997; Sumner et al., 1992; Johnson and Curtis, 2001). Increases in SOC with N fertilizer addition is mainly caused by greater biomass production and C returns (Johnson and Curtis, 2001). Warm-season grasses such as bermudagrass and bahiagrass respond well to N fertilization and forage production typically increases as N levels increase (Johnson et al., 2001; Silveira et al., 2013).

Proper grazing intensity is also an important factor affecting SOC accumulation and stabilization. Ecosystems subjected to grazing typically have greater SOC stocks compared with non-grazed systems (Schuman et al., 1999). Although no differences in SOC between heavy and light grazing was reported by Schuman et al. (2008), Ingram et al. (2008) studying SOC under different grazing intensities in Wyoming found that 10
years of heavy grazing resulted in 30% loss of SOC in the 0 to 60 cm soil depth. Similar results were also reported by Wright et al. (2004). These authors observed that pastures subjected to high grazing intensity had lower SOC stocks compared to pastures under low grazing intensity. While overgrazing can result in ecosystem degradation, low to moderate grazing intensity can have positive impacts on SOC and nutrient availability (Abril and Bucher, 1999; Franzluebbers et al., 2009). Soil N mineralization rates was also found to be greater in systems subjected to moderate grazing than that in non- or heavily-grazed ecosystems (Shariff et al., 1994).

Previous studies also suggested that rotational stocking has potential to facilitate SOC sequestration compared with continuous stocking (Conant et al., 2003). Soil organic C stocks in short-rotation grazed pastures in the southeastern United States were 22% greater than that in extensively grazed pastures (Conant et al., 2003).

**Silvopasture and Bahiagrass Pastures: Contribution to SOC Accumulation**

Silvopastures are agroforestry systems that incorporate trees in grass-dominated areas (Nair et al., 2007). They are widely used in United States because of the economic returns associated with timber and livestock production. The presence of trees in grass-dominated ecosystems affects a number of ecosystem functions such as net primary productivity, litter and root input, and root distribution (Schimel et al., 1995; Connin et al., 1997; Jackson et al., 2000). Similar to the effect of afforestation on C sequestration (Arevalo et al., 2009 and 2011; Cantarello et al., 2011), previous studies found that silvopastures have the potential to accumulate more SOC than tree-less pastures (Schimel et al., 1995; Montagnini and Nair, 2004; Nair et al., 2007; Haile et al., 2008; Fernández-Núñez et al., 2010; Casals et al., 2014).
In south Florida, bahiagrass is the predominant forage species used in grazing systems because of its persistence and adaptability to a wide range of management conditions (Chambliss and Sollenberger, 1991). Previous studies showed that the bahiagrass production increased in response to N fertilization (Chambliss and Sollenberger, 1991; Obour et al., 2009; Silveira et al., 2013).

**Soil Microbial Community**

**Ecosystem C and the Soil Microbial Community**

The soil microbial community plays an important role on SOC sequestration because they affect a number of processes controlling SOC dynamics, such as decomposition, respiration, and physical stabilization (Ingram, 2008). Soil respiration accounts for 77 Pg C yr\(^{-1}\). (Raich and Potter, 1995) and it represents the largest flux of CO\(_2\) emissions from terrestrial ecosystems to the atmosphere (Schlesinger and Andrews, 2000). Soil respiration represents the sum of CO\(_2\) release from soil microorganism and root respiration (Lundegårdh, 1927).

Soil microorganisms decompose soil organic matter as a source of energy to sustain growth and reproduction (Sylvia et al., 2005). To decompose soil organic matter, soil microorganisms produce extracellular enzymes to catalysis the degradation of chemical compounds, such as β-glucosidase for cellulose and peroxidases for lignin (Breznak and Brune, 1994). Soil microorganisms use plant residues and dead soil microbes as substrates to obtain nutrients and energy, such as the cellulose, chitin and chitosan from fungal cells and N-acetyl glucosamine and N-acetylmuramic acid in peptidoglycans from bacterial cells (Sylvia et al., 2005).

However, microbial biomass C (MBC) is not necessarily positively related to soil respiration (Wang et al., 2003). To understand the role of microorganisms on C
emissions, it is important to study the response of microbial structure. Among soil decomposers, nearly 80 to 90% of the biomass and respiration come from fungi and bacteria (Chapin III et al., 2002). In environments with low nutrient availability, fungi can absorb nutrients via hyphae, thus, they are more competitive than bacteria in utilizing and decomposing C substrates. Anderson and Domsch (1975) studied the contribution of bacterial and fungal respiration and reported that fungi had greater respiration than bacteria in both agricultural and forest soils. Similar results were reported by Anderson et al. (1975) who also suggested a greater contribution of fungi compared with bacteria in soil respiration.

**Response of the Soil Microbial Community to Land Use**

Grassland management and land use changes can affect microbial community biomass and structure. For example, it was observed previously that microbial biomass was greater in grasslands than cultivated lands (Steenwerth et al., 2002). They also found that different land use types and management inputs resulted in distinct soil microbial groups. Steenwerth et al. (2006) using canonical correspondence analysis to compare microbial community structures in perennial grasslands and found that microbial community composition was related to land use type.

Microbial community biomass and composition response to land use changes are often related to changes in vegetation composition (McKinley et al., 2005) and adoption of management strategies that may affect the amounts and quality of C inputs. Perkins and Nowak (2013) demonstrated different proportions of fungi, gram negative bacteria and actinomycetes due to change in plant composition.

Different land use management intensification and strategies, such as the adoption of fertilizer and different grazing intensities, can also affect microbial
community. For example, Denef et al. (2009) studied temperate grasslands and demonstrated that N fertilization promoted an abundant microbial community by increasing gram positive bacteria while fungi abundance was reduced. Grazing activity can also influence microbial community. Microbial biomass C and N were reported to increase under grazing in subtropical pastures compared to non-grazed systems (Wang et al., 2006). Abril and Bucher (1999) demonstrated that cellulolytic and nitrifier groups were affected by grazing intensity, particularly in the dry season. These authors also showed that ammonifier and free-living N-fixing groups decrease in overgrazed ecosystems (Abril and Bucher, 1999).

The impact of land use on the microbial community is complex and several factors including vegetation conversion and management utilization should be considered. Millard and Singh (2010) studied microbial community diversity response to grassland vegetation and found that proper management practices, such as diverse vegetation composition and fertilization resulted in a shift in microbial community from fungi to bacteria dominated. However, they also indicated that other studies showed that heterogeneity of soil properties and microbial structures resulted in different soil microbial community responses. Jangid et al. (2011) observed shifts in microbial communities along two successional gradients in response to plant species. These authors also reported that microbial community composition was a more sensitive indicator of land use history than vegetation type and soil properties. Similar results were observed by Stark et al. (2008) studying the impacts of lupin amendment on soil microbial community. These authors also suggested that soil microbial biomass and activity can be affected by land use management history.
Regulators of Soil Microbial Enzyme Activity

The production and activity of microbial enzymes in soils is regulated by various factors including composition of the microbial community, temperature, soil moisture, and organic matter inputs. Among these factors, the organic matter inputs (both quantity and quality) are related to the C supply and nutrient inputs and thus influence composition of the microbial community and presence of enzyme substrate. Thus they play an important role in affecting enzyme activities with subsequent effects on availability of labile C and nutrients (Weintraub et al., 2012).

Studies suggest that different types of enzymes will likely respond differently to nutrient addition. For example, Shackle et al. (2000) found that β-glucosidase activity increased with cellulose addition while sulphatase activity decreased and phosphatase activity was not changed. It has been also suggested that phosphorus additions can stimulate alkaline phosphatase activity but may have limited effect on other enzymes, such as arylsulfatase, β-d-glucosidase, protease, and phenol oxidase (Wright and Reddy, 2001). Research has also shown that some enzymes respond differently to complex nutrient additions compared with single nutrients (Allison and Vitousek, 2005). Moreover, response of enzyme activity to different regulators can vary widely.

Among different enzymes, β-glucosidase is considered important and commonly studied as it is related to C cycling. Previous studies observed that β-glucosidase activity was correlated with C mineralization (Allison and Vitousek, 2005). β-Glucosidase activity determines the abundance of glucose to supply energy to microbes, which is produced by degradation of cellobiose catalyzed by β-glucosidase (Turner et al., 2002). The activity of β-glucosidase is important also because it is a good indicator of change in soil environmental conditions caused by land use management.
(Bandick and Dick, 1999; Acosta-Martinez et al., 2008; Stott et al., 2010). Previous studies showed that the activity of β-glicosidase varied depending on vegetation type. For example, β-glucosidase activity was found to be greater in pasture compared with forest and crop land (Acosta-Martinez et al., 2007). Management practices that can promote soil C and N accumulation are also expected to promote enzyme activity. Weintraub et al. (2012) found that enzyme activity was correlated to total soil C, and litter additions to the soil increased β-glucosidase activity. Similarly, Allison and Vitousek (2005) and Shackle et al. (2000) also found that the addition of C or C and N increased β–glucosidase activity.

**Phospholipid Fatty Acid and Its Response to Land Use Change**

Different types of lipids such as phospholipids, glycolipids, lipopolysaccharides and lipoproteins are associated with soil microorganisms. These lipids sometimes have fatty acids which can be used to group microorganisms. To isolate the fatty acids, lipids should be extracted first. Among different lipids, the phospholipid is most commonly used as an indicative of different soil microorganism groups. Phospholipid can be obtained by a series of extraction using water-chloroform-methanol and analyzed by gas chromatography. Both microbial biomass and community structure can be determined using phospholipid fatty acid (PLFA) analysis (Frostegård and Bååth, 1996). PLFA analysis can also be used as indicators of microbial response to land use change (Inglett et al., 2011; Wilkinson et al., 2002; Steenwerth et al. 2002; Steenwerth et al. 2006). For example, McKinley et al. (2005) studying ecosystem conversion from farmland to prairie observed greater increase in proportion of bacteria compared to fungal. In a mixed- grass prairie, Ingram et al (2008) used PLFA analysis to investigate the impacts of grazing management on microbial community response. Similarly,
Steenwerth (2006) also observed changes in microbial community structure as revealed by PLFA analysis.

Corroborating previous studies, Denef et al. (2009) reported that total abundance of PLFA in grassland increased with the use of N fertilizer (Denef et al., 2009). However, the impact of N additions on specific microbial groups has not been fully understood. While some studies showed that N fertilizer can inhibit the abundance of arbuscular mycorrhizal fungal PLFAs (C16:1w5) (Denef et al., 2009), others found N additions associated with an increase in fungal activity (Rousk and Bååth, 2007). The impacts of N fertilizer on bacterial are even more complicated. The study of Denef et al. (2009) found that relative abundance of biomarker PLFAs for gram-positive bacteria (such as a15:0, i16:0 and i17:0) and gram-negative bacteria (17:0cy) increased while other gram-negative bacteria (18:1w7) decreased with N fertilization.

Soil microbial community structure determines the soil C process and responses to land use change. However, it is not well understood that how will the soil microbial community structure be affected by long-term grazing land intensification in subtropical ecosystems.
CHAPTER 3
LONG-TERM IMPACTS OF LAND USE CHANGE ON ECOSYSTEM C STOCKS AND DISTRIBUTION IN SUBTROPICAL GRAZING LANDS

Introduction

Land use change is one of the most important anthropogenic activities affecting the global C cycle (Houghton, 1995; Houghton et al., 1999; Watson, 2000). Estimates suggest that between 1850 and 2000, about 156 Pg C was released to the atmosphere in response to land use changes (Houghton, 2003). Although land use change is currently recognized as a considerable source of the atmospheric greenhouse gas CO₂, land-use practices can also act as a mitigation strategy (Houghton, 2003). A number of studies have shown that C sequestration in terrestrial ecosystems can be promoted by adopting optimal land management strategies (Schimel, 2000; Silver et al., 2000; Conant et al. 2001; Arevalo et al., 2009; Arevalo et al., 2011; Cantarello et al. 2011). For instance, while deforestation has negative impacts on SOC storage (Eswaran et al., 1993), afforestation can promote SOC sequestration (Arevalo et al., 2009 and 2011; Cantarello et al., 2011).

Grazing land intensification typically involves the introduction of productive plant species, increased use of fertilizer, and greater stocking rate. Although some practices such as overgrazing and improper soil fertilization management are expected to detrimentally affect SOC stocks (Giardina et al., 2004, Mack et al., 2004; Su et al., 2005; Han et al., 2008), strategies that result in greater C input and/or lesser decomposition rate can promote C accumulation in soil (Batjes, 1999; Conant et al., 2001; Braz et al., 2013). Numerous studies in temperate and tropical regions showed that N additions can promote above- and below-ground plant production (Johnson et al.,...
1994; Johnson et al., 2001), and subsequently improve SOC sequestration (Malhi et al., 1991 and 1997; Van Cleve and Moore, 1978; Johnson and Curtis, 2001). Similarly, a significant body of literature has demonstrated that grazing management can have major impacts on important ecosystem processes such as nutrient cycling and storage (Ingram et al., 2008). For example, grazing intensity can influence litter and root deposition and decomposition rates (Shariff et al., 1994), SOC and N stocks (Frank et al., 1995), and characteristics (Naeth et al., 1991; Schuman et al., 1999). Adoption of silvopasture, an agroforestry management practice that integrates trees with forage and livestock production, can promote SOC sequestration compared with non-forested grasslands (Haile et al., 2008; Casals et al., 2014).

Despite the high-profile public debate about the impacts of land use intensification on ecosystem C balance and climate change (Houghton et al., 1999; Schuman et al., 1999; Reeder and Schuman, 2002; Rees et al., 2004; Bonino 2006; Arevalo et al., 2009, 2011; Cantarello et al., 2011), our understanding is limited of how grazing land management can be manipulated to promote long-term ecosystem C sequestration. Most previous studies on the impacts of grazing land intensification on ecosystem C responses were focused on a single management factor. However, from economic and practical perspectives, grazing land intensification often involves a combination of multiple management practices aimed at increasing productivity; therefore, the interpretation of previous studies focused on single management practices is often limited. Moreover, considering the large area occupied by grazing lands in the USA (~ 30% of the land surface) (Follett et al., 2001), it is critical to understand how grazing land strategies affect SOC and related ecosystem responses.
Addressing this knowledge gap is particularly important in the southeast USA where SOC plays a major role in grassland sustainability. Because grassland soils contain appreciable amounts of C and the majority (~90%) of ecosystem C (Burke et al., 1997), relatively small changes in SOC stored in grassland soils can have significant impacts on the global C cycle (Parton, 1995). This study takes advantage of long-term experiments in which three different management intensities where established in adjacent fields in a subtropical environment. In this study, grazing land intensification was defined as increased use of N fertilizer, greater stocking rate, and introduction of a productive trees or perennial grass. The specific objective of this study was to quantify and characterize ecosystem C stocks and distribution in the above- and belowground biomass, litter, and soil pools under the three land-use types.

Methods

Study Area

The experimental sites were located at University of Florida, IFAS, Range Cattle Research and Education Center (27°23’N, 81°57’W), in Ona, Florida. The topography was relatively flat (slope of 0 to 2%) and the predominant soil series was Ona fine sand (sandy, siliceous, hyperthermic Typic Alaquods) and Smyrna sand (sandy, siliceous, hyperthermic Aeric Alaquod). Mean annual precipitation was ~1650 mm and average annual maximum/minimum temperatures were 28.2/16.7°C (Sellers, 2009). In this study, three field-replicated (n = 2) grazing land ecosystems with different vegetation type and intensification level were studied: native rangeland, pine (Pinus elliotti Engelm.)-bahiagrass (Paspalum notatum Flueggé) silvopasture, and bahiagrass pasture, herein referred as sown pasture. Since each replicated experimental unit was
located adjacent to each other, they had the same soil type and climate condition. All sites were established and consistently managed for more than 20 years. More details about the experimental sites are described in Adewopo et al. (2014). Briefly, native rangelands represented the system which is considered here under the lowest management intensity. The management practices applied in the native rangeland consisted of periodic burning (every 3 to 4 years) and low grazing intensity (~ 125 animal days ha\(^{-1}\) yr\(^{-1}\)). Silvopastures consisted of slash pine trees and Pensacola bahiagrass with greater management intensification than native rangeland. Nitrogen fertilization history consisted of application of 67 kg N ha\(^{-1}\) yr\(^{-1}\) in 1998, 1999, 2001, 2003 to 2007, and 2010 and average stocking rates of 207 animal days ha\(^{-1}\) yr\(^{-1}\). Sown pastures represent the typical beef cattle production system in Florida and consisted of rotationally stocked pastures (stocking rate of 360 animal days ha\(^{-1}\) yr\(^{-1}\)) receiving 67 kg N ha\(^{-1}\) yr\(^{-1}\) since 1991 and dolomitic limestone (1 Mg ha\(^{-1}\)) in 2001 and 2008.

In each experimental unit, five equidistant quadrats (20 m × 20 m) were established along transects (~75 m apart) within each experimental unit. The tree biomass determination for silvopasture and the root, litter and soil sampling for three land use types were conducted in these quadrats.

**Aboveground Biomass**

Aboveground biomass was determined in June and July 2012 (summer) and January 2013 (winter). Because the vegetation species composition varied among the ecological units, different procedures were used to determine above-ground biomass associated with each grazing land ecosystem.

In the sown pastures, summer and winter aboveground biomass determination occurred 5 days after cattle were removed from the pasture. Aboveground biomass was
measured using the double sampling procedure as described by Dubeux et al. (2006d). Briefly, 40 disk plate settling heights (0.25 m² aluminum disk) were recorded across each experimental unit. Disk plate settling height was calibrated by correlating 23 disk readings with the total herbage harvested below the disk. The coefficients of determination ($r^2$) ranged from 0.75 to 0.94. The average of 40 disk readings in each experimental unit was used to estimate above-ground biomass associated with the sown pastures.

Aboveground biomass determination in the silvopasture and native rangeland ecosystems used a combination of direct and indirect measuring procedures. In the silvopasture, aboveground biomass included both grass and tree biomass were determined 4 weeks after grazing activity. Grass biomass was estimated as described for the sown pastures, while tree biomass was determined in the sampling quadrats (20 m × 20 m) using the diameter at breast height procedure (~10 to 40 trees from each of the five quadrats per experimental unit). The allometric equation $\ln (\text{aboveground biomass}) = -2.5563158 + 2.5209397 \times \ln (\text{diameter at breast height})$ described by Gonzalez-Beneke et al. (2010) was used to calculate tree biomass. In the native rangeland, a double sampling procedure combining indirect estimation of ground cover and direct biomass determination (clipping and weighing) was used (Ebrahimi et al., 2008). Vegetation abundance of saw palmetto (*Serenoa repens* Bartr.) and the other shrub and grass species was determined by estimating the percentage ground cover (Küchler and Zonneveld, 1988). In total, vegetation abundance were estimated in twelve 2 m × 2 m areas along diagonal transect in each experimental unit. Above ground biomass was destructively sampled by clipping four reading areas (2 m × 2 m) within
each experimental unit for a total of 8 sampling (four sampling per replicate x two replicates) to represent the range of aboveground biomass in the native rangeland. Shrub and grass samples were separated and dried at 55°C until constant weight. A regression equation was developed to relate the vegetation cover percentage of palmetto and other grasses with the aboveground biomass \( r^2 = 0.7 \). The average of 12 readings in each experimental unit was used to estimate above-ground biomass associated with the native rangeland.

The aboveground biomass C in each land use type were calculated as 50% of aboveground biomass. Total ecosystem C stock was calculated as the sum of aboveground biomass C, litter C mass, root C mass, and SOC.

**Belowground Biomass**

Root biomass was determined by randomly collecting three soil cores (4.8 cm diameter) from each of the 5 sampling quadrats along transect. Fifteen soil cores were collected from each experimental unit for root biomass determination (5 quadrats x 3 cores = 15 samples per replicated unit). In July 2012, sampling depths were 0 to 10, 10 to 20, 20 to 30, 30 to 60, and 60 to 90 cm, while in February 2013, root samples were collected from the 0 to 10 and 10 to 20 cm depth intervals. Samples were gently washed with deionized water and root separation was performed using sieves (250 μm sieve mesh). After separation, root samples were oven-dried at 65°C until constant weight. Dry root subsamples were ground (0.425 mm screen) and a subset was combusted at 550°C for 5 hours to determine ash concentration (Baskin and Baskin, 1974). A second subset was used to determine total C and N concentrations using a Flash EA 1112 Series elemental analyzer (Thermo Fisher Scientific Inc., Waltham, Massachusetts). Root C and N mass were calculated based on ash-free root weight. The neutral
detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin concentration of root samples were run in an ANKOM fiber analyzer (ANKOM Technology, 2005, 2013a, 2013b).

**Litter Biomass**

Litter (dead plant material above soil surface) biomass was measured in summer (June, 2012) and winter (February, 2013). In each sampling quadrat, two 1 m × 1 m areas were randomly selected for litter biomass determination. A total of 10 samples (2 samples × 5 quadrats = 10) were collected per experimental unit. Samples were oven-dried at 65°C to a constant weight, ground through a 1 mm screen sieve and analyzed for ash, total C and N concentrations and fiber. Root samples were combusted at 550°C for 5 hours to determine ash concentration (Baskin and Baskin, 1974). Total C and N concentrations of litter samples were determined using a Flash EA 1112 Series elemental analyzer (Thermo Fisher Scientific Inc., Waltham, Massachusetts). Litter C mass and N mass were calculated based on an ash-free litter weight. The NDF, ADF and acid detergent lignin concentration was run in an ANKOM fiber analyzer (ANKOM Technology, 2005, 2013a, 2013b).

**SOC**

Four randomly located soil cores (diameter of 2.2 cm) were collected from each quadrat and composited within depth. In June/July 2012, soil depth intervals were 0 to 10, 10 to 20, 20 to 30, 30 to 60, and 60 to 90 cm, while in January/February 2013, soil samples were collected from the 0 to 10 and 10 to 20 cm depths. One additional undisturbed soil core was collected from a randomly selected location in each quadrat for soil depth interval bulk density determination. Soil samples were air-dried and sieved through a 2 mm screen. For total SOC and N determinations, soil samples were
pulverized with ceramic beads and concentrations were determined using a Flash EA 1112 Series elemental analyzer (Thermo Fisher Scientific Inc., Waltham, Massachusetts). For bulk density determination, soil samples collected at each depth interval were dried at 105°C until constant weight.

**Statistic Analysis**

The effect of land use on ecosystem C pools were analyzed using SAS PROC MIXED procedure (SAS 9.4, 2013) with land use type, season and their interactions as fixed effects and replicates were considered random effects. Measurements in each experimental unit were considered one replicate. The effect of land use on root C and N mass were analyzed within each depth (0 to 10, 10 to 20, 20 to 30, 30 to 60 and 60 to 90 cm). The effect of land use on SOC and soil N stocks were analyzed on each depth accumulation (0 to 10, 0 to 20, 0 to 30, 0 to 60 and 0 to 90cm). Mean comparisons ecosystems were made using Fisher's Least Significant Difference (LSD) test by PDIFF test of the LSMEANS procedure in SAS. If the F-test $P$ values were $\leq 0.05$, treatments and their interactions were considered significant.

**Results**

**Aboveground Biomass**

Land use affected total above-ground biomass but no seasonal effect was observed. Silvopasture showed greater aboveground biomass (118 Mg ha$^{-1}$) than native rangeland (8 Mg ha$^{-1}$) and sown pasture (4 Mg ha$^{-1}$) (Table 3-1). This occurred because of pine tree biomass in silvopastures, which accounted for approximately 98% (116 Mg ha$^{-1}$) of total aboveground biomass in this ecosystem. The grass component in the silvopasture represented ~ 2% (2 Mg ha$^{-1}$) of aboveground ecosystem biomass. Because of the large variability associated with measurement of aboveground biomass,
no differences were observed between native rangeland and sown pasture. However, sown pasture had greater aboveground biomass (4.3 Mg ha$^{-1}$) than the grass component in native rangeland and silvopasture (2.2 and 2.7 Mg ha$^{-1}$, respectively).

**Litter C and N Composition**

Litter C mass was affected by land use ($P < 0.0001$, Table 3-2). No season effect was found on litter C mass across the three land uses. Greater litter C mass was observed in the silvopasture (2.4 Mg C ha$^{-1}$) than in the native rangeland and sown pasture (0.2 and 0.3 Mg C ha$^{-1}$, respectively).

There was an interaction between land use and season on litter N mass ($P < 0.0001$). During summer sampling, greater litter N mass was observed in the silvopasture compared with native rangeland and sown pasture (40 kg N ha$^{-1}$ vs. 4 and 6 kg N ha$^{-1}$, respectively). However, during the winter sampling, litter N mass was least for native rangeland (1 kg N ha$^{-1}$), intermediate for sown pasture (12 kg N ha$^{-1}$) and greatest for silvopasture (24 kg N ha$^{-1}$). Litter N decreased from 40 kg N ha$^{-1}$ (summer) to 24 kg N ha$^{-1}$ (winter) in silvopasture, but no seasonal effect was observed in the other ecosystems.

There was no season x land use interaction for litter C:N ratio. The greatest litter C:N ratio was observed in silvopasture (~77 averaged across summer and winter), while bahiagrass showed the smallest C:N ratio (36). Averaged across three management ecosystems, C:N ratio increased from summer to winter (53 vs. 68).

Litter ADF concentration decreased with grazing land intensification (Figure 3-1B). On average, native rangeland showed the greatest litter ADF concentration (571 g kg$^{-1}$), followed by silvopasture (524 g kg$^{-1}$) and sown pasture (381 g kg$^{-1}$). Litter NDF concentration (Figure 3-1 A) was similar in the native rangeland and sown pasture (777
and 761 g kg\(^{-1}\), respectively), but was smaller in silvopasture (664 g kg\(^{-1}\)). In contrast, the greatest lignin concentration (202 g kg\(^{-1}\)) was observed in silvopasture litter followed by native rangeland and sown pasture (126 and 28 g kg\(^{-1}\), respectively, Figure 3-1C).

**Belowground Biomass**

Land use affected root C and N biomass (\(P < 0.001\)) (Table 3-3). Greater root C biomass (14 C Mg ha\(^{-1}\), 0 to 90 cm depth) was observed in the native rangeland compared with sown pasture and silvopasture (9 and 6 Mg C ha\(^{-1}\), respectively). This result shows that land conversion decreased root C biomass. Major differences were observed in root C biomass among land use types in the 0 to 60 cm depth interval, where native rangeland exhibited greater root biomass than the other ecosystems. Silvopasture exhibited the smallest root C biomass at 0 to 10, 20 to 30 and 30 to 60 cm depths but no difference was found between sown pasture and silvopasture at the 10 to 20 cm depth. There were no differences in root C biomass among the three ecosystems at the greatest depth range from 60 to 90 cm.

Data from summer sampling event showed that the majority of the root C mass in the silvopasture and sown pasture (~85 and 76%, respectively) occurred in the top 20 cm stratum, while in the native rangeland ~ 62% of total root C biomass was present in this layer.

The greatest total root N biomass (0 to 90 cm depth) was observed in the sown pasture (0.30 Mg N ha\(^{-1}\)) compared with native rangeland (0.20 Mg N ha\(^{-1}\)) and silvopasture (0.16 Mg N ha\(^{-1}\)) (Table 3-3). No difference was detected in root N biomass among land uses below 60 cm depth.

Seasonal differences in root C and N biomass were observed but the interaction between season and land use was not significant. Regardless of the ecosystem, root C
biomass (0 to 20 cm) was generally greater in the summer (8.8, 6.9, and 5.1 Mg C ha\(^{-1}\) for native rangeland, sown pasture, and silvopasture, respectively) compared with winter (8.0, 5.5, and 2.1 Mg C ha\(^{-1}\)) \((P = 0.0011)\). Similarly, there was a slight reduction \((P = 0.0039)\) in root N biomass (0 to 20 cm depth) observed in winter sampling (0.13, 0.21, and 0.07 Mg N ha\(^{-1}\) for native rangeland, sown pasture, and silvopasture, respectively) compared with summer sampling (0.15, 0.23 and 0.14 Mg N ha\(^{-1}\), respectively).

Root NDF, ADF, and lignin concentrations decreased with grazing land intensification (Figure 3-2). Native rangeland showed the greatest root NDF and ADF concentrations (828 and 728 g kg\(^{-1}\), respectively) among the three grazing land ecosystems. Sown pasture and silvopasture had similar root ADF and NDF (746 and 518 g kg\(^{-1}\) for sown pasture and 728 and 578 g kg\(^{-1}\) for silvopasture, respectively). Similarly, the greatest root lignin concentration was observed in native rangeland (233 g kg\(^{-1}\)), followed by silvopasture (150 g kg\(^{-1}\)) and sown pasture (82 g kg\(^{-1}\)).

**SOC and N Stocks**

Grazing land intensification promoted SOC and soil N accumulation at the 0 to 90 cm depth (Figure 3-3). Native rangeland exhibited the smallest SOC stock (76 Mg C ha\(^{-1}\)) compared with silvopasture and sown pasture (100 and 110 Mg C ha\(^{-1}\), respectively). No differences were observed in SOC between silvopasture and sown pasture.

Similar to SOC, soil N stocks (0 to 90 cm depth) increased in response to grazing land intensification (Figure 3-3). Soil N stocks increased from 3.3 Mg N ha\(^{-1}\) in the native rangeland to 5.2 and 5.3 Mg N ha\(^{-1}\) in the silvopasture and sown pasture, respectively. No differences in soil N stocks were observed between silvopasture and sown pasture.
Data from summer sampling showed that vertical distribution of SOC in the soil profile was not influenced by management intensification. On average, about 51% of the SOC (39, 53 and 54 Mg C ha\(^{-1}\) for native rangeland, sown pasture and silvopasture, respectively) and 61% of soil N stocks (2.0, 3.3 and 3.3 Mg N ha\(^{-1}\), respectively) were found in the surface 20 cm stratum. Soil C:N ratio was greater in subsurface soil compared with surface soil (~16 to 27 for 10 to 90 cm depth vs. 15 to 22 for 0 to 10 cm depth). Data from summer and winter sampling showed that stratification ratio of SOC (the ratio of SOC at 0 to 10 cm to SOC at 10 to 20 cm depth) decreased as management intensification increased (2.5 for native rangeland, 1.9 and 1.7 for silvopasture and sown pasture). No seasonal effect was found on stratification ratio of SOC.

**Total Ecosystem C Stocks and Distribution Among The Various Pools**

Silvopasture had the greatest ecosystem C stock (168 Mg C ha\(^{-1}\)) compared with sown pasture (121 Mg C ha\(^{-1}\)) and native rangeland (94 Mg C ha\(^{-1}\)) (Table 3-4). There were no differences between native rangeland and sown pasture.

The greater ecosystem C stock in silvopasture was attributed to the aboveground biomass pool which was about 15 and 28 times that in native rangeland and sown pasture, respectively. Above-ground C represented about 34% of total ecosystem C stocks in the silvopasture, while in the native rangeland and sown pasture, it accounted for 4 and 2% of overall ecosystem C, respectively (Table 3-4).

Despite the smaller aboveground biomass C, sown pasture showed similar ability to store SOC as the silvopasture system (110 and 101 Mg C ha\(^{-1}\), respectively). The SOC accounted for 90% of total ecosystem C stocks in sown pasture, while it represented ~ 61% in silvopasture. Sown pasture also showed greater root C mass than
silvopasture (9 vs. 6 Mg C ha\(^{-1}\); 7.5 vs. 3.5% of total ecosystem C, respectively), which suggested markedly different C allocation patterns among these ecosystems.

Similar to sown pasture, native rangeland accumulated a large proportion of ecosystem C belowground (96%). Of that total, SOC accounted for ~81% and root biomass C accounted for 15% (14 Mg C ha\(^{-1}\)). This compares with 7.5% contribution of root biomass C in sown pasture (9 C Mg ha\(^{-1}\)) and 3.5% in silvopasture (6 Mg C ha\(^{-1}\)). Despite native rangeland accumulating more C in plant biomass (above and below ground), SOC stock was smaller for rangeland than for sown pasture.

**Discussion**

**Aboveground Biomass**

Differences in aboveground biomass associated with the three land uses were mainly due to the predominant vegetation species composition. Our results showed the greatest aboveground biomass was associated with silvopasture. This occurred because of the high (98%) contribution of the tree biomass to above-ground biomass in this ecosystem. Although sown pastures showed the least above ground biomass among the three ecosystems, aboveground biomass associated with the grass component was greater in the sown pasture compared with silvopasture and native rangeland. The relatively smaller bahiagrass biomass in silvopasture compared with sown pasture was due to lower N fertilization levels (Sumner et al., 1991; Johnson et al., 2001) and competition with trees for resources (primarily light, nutrients, and water availability). The slash pine canopies can absorb some photon flux and only part of the incident photosynthetically active radiation (PAR) can penetrate through and arrive at the plants below (Gholz et al., 1991).
In native rangeland, the vegetation was dominated by low-quality species including saw-palmetto and grasses like wiregrass (*Aristida stricta* Michx.) (Moore, 1974). The biomass of saw palmetto consists of fronds and the rachis (Gholz, 1999), which accounted for ~64% (5 Mg ha^{-1}) of above ground biomass of native rangeland in our study. Despite the presence of native grass species in the native rangeland, biomass and quality of the plant material was relatively low as described previously (Kalmbacher, 1983). Unlike the native rangeland, bahiagrass in the sown pasture is highly productive and responds well to N fertilization (Chambliss and Sollenberger, 1991). In our study, greater biomass associated with bahiagrass in sown pasture compared with native grass species in the rangeland contributed to greater SOC sequestration.

**Litter Composition**

Greater litter C and N mass in the silvopasture was consistent with the greater aboveground biomass present in this ecosystem. This response occurred because of the high input of litter mass from pine needles and branches. Lack of difference in litter C mass between native rangeland and sown pasture was likely caused by the large variability associated with this variable. The relatively small litter mass in native rangeland is consistent with the characteristics of the vegetation present in this ecosystem. Saw palmetto, the predominant vegetation in the native rangeland, has limited ability to accumulate aboveground litter because the dead foliage often stays attached to the plant (Gholz et al., 1999). Although silvopasture exhibited the greatest litter C mass, the slash pine needles typically have high lignin, low N and P concentrations, and slow decomposition and mineralization rates (Gholz et al., 1985). Therefore, the role of above-ground litter on SOC accumulation in silvopasture systems
is expected to be limited at the same time period. Fiber analysis showed that silvopasture litter contained the greatest lignin concentration among three land uses and greater ADF than sown pasture. In contrast, it was observed that silvopasture had the smallest NDF concentration among three land uses. High concentrations of lignin and ADF are considered indicators of poor litter quality (Hammel, 1997), while NDF is more easily decomposed than ADF and lignin as it contains hemicellulose (Van Soest, 1985). These results suggested that litter in sown pasture may be more susceptible to decomposition due to its chemical composition.

In addition to vegetation type, grazing intensity can also affect litter accumulation and composition (Naeth et al., 1991; Liu et al., 2011a). Previous studies under conditions similar to those of the current study showed that pasture management intensification (defined as greater N fertilizer and stocking rate) increased litter quality (lower C:N ratio and greater litter N concentration) (Dubeux et al., 2006c; Liu et al., 2011a). Similarly, our results also showed that litter C:N ratio decreased as intensification increased. It is expected that greater litter N concentration and low litter C:N ratio in sown pasture will likely result in greater decomposition rates (Lupwayi and Haque, 1999; Ross et al., 2002) compared with the other ecosystems. Dubeux et al. (2006b) found that greater management intensity of bahiagrass pastures can enhance litter turnover and nutrient release. High quality litter can contribute to soil organic matter formation via microbial processes (Cotrofu et al. 2013) so it is more likely that litter C from the sown pasture is incorporated into the SOC pool than litter C from the other two ecosystems.
Root Biomass

Root turnover represents a major C source in soil (Tate et al., 1993). Our data are consistent with other grassland studies that reported ~ 60% of root biomass occurred in the top 15 cm (Weaver et al., 1935; Gill et al., 1999). Approximately 62 to 85% of root biomass C was observed in the 0 to 20 cm soil depth. Our results also suggested that grazing land conversion and changes in vegetation composition (native vs. silvopasture and sown pasture) resulted in lower C allocation in the root biomass pool.

Despite the relatively greater root C accumulation in the native rangeland, root of saw palmetto, the predominant species, had high NDF, ADF, and lignin concentrations. It is anticipated that these roots from native rangeland are more recalcitrant and difficult to decompose compared with sown pasture and silvopasture systems. Also, the high above- and belowground productivity of bahiagrass may facilitate the litter and root residues to be incorporated into SOC.

SOC

Data suggested that grazing land intensification through the adoption of more productive plant species, increased stocking rate, and greater fertilizer input promoted SOC and soil N stocks. SOC stocks (0 to 90 cm depth) increased by 32 and 45% due to conversion from native rangeland to silvopasture and sown pasture, respectively. Similarly, soil N stocks increased, on average, by ~59% in response to intensification.

These results are consistent with previous studies that observed conversion from C₃-dominated native vegetation to highly productive C₄ grass-based vegetation and improved grazing management increased forage production and SOC accumulation (Fisher et al., 1994, Conant et al., 2001). Grazing activity and methods can also have
impacts on SOC accumulation. Schuman et al. (1999) studying the impacts of grazing management on a mixed-grass rangeland observed that SOC (0 to 30 cm depth) increased in response to grazing activity compared to non-grazed ecosystems. Similarly, previous studies also reported that adoption of improved grazing methods such as rotational stocking can improve forage utilization (Gammon, 1978), and subsequently promote SOC accumulation (Wood and Blackburn, 1984; Conant et al., 2003; Wright et al., 2004). Our earlier results found that grazing land intensification can promote the C concentration in silt and clay fractions, which can contribute to protect C in soil (Silveira et al., 2014).

Numerous studies have documented increases in SOC after conversion of native and managed ecosystems into silvopasture systems (Sharrow and Ismail, 2004; Kirby and Potvin, 2007; Nair et al., 2009; Subhrajit et al., 2010). The tree component in silvopasture contributes to increase of SOC by promoting C inputs and transfer to the soil (Montagnini and Nair, 2004). Earlier studies in Florida Spodosols showed that silvopasture has greater ability to store C in soil compared with bahiagrass pastures (Nair et al., 2007; Fernández-Núñez et al., 2010). In the current study, however, SOC stocks in silvopasture and sown pasture were similar. Despite the presence of trees in the silvopasture, greater N fertilizer inputs resulted in greater grass production in the sown pastures which may have contributed to similar net SOC accumulation rates in both ecosystems.

Vertical distribution of SOC within the soil profile followed a similar pattern as root biomass C. On average, ~ 51% of total SOC stocks at the 0 to 90 cm depth was found in the top 20 cm depth, while ~ 62 to 85% of root biomass occurred at this soil depth.
Similar results were reported by Gill et al. (1999), who showed that a higher percentage of root biomass (∼57%) was present in the top 15 cm stratum compared with SOC stocks (23% of total SOC). This result illustrates the complexity associated with the processes controlling C accumulation and stabilization, particularly in subsurface soils where C tended to accumulate in more recalcitrant forms (Rumpel and Kögel-Knabner, 2011). In contrast to the data presented by Jobbágy and Jackson (2000), which showed SOC vertical distribution was influenced by vegetation (shrub, grasslands and forest), in this current study no differences in SOC vertical distribution among three grazing land ecosystems were observed. However, SOC stratification ratio (0 to 10 cm: 10 to 20 cm) in the native rangeland was greater than in the more intensively managed ecosystems. This response occurred because SOC stocks at the 10 to 20 cm depth were smaller in the native rangeland than that in the silvopasture and sown pasture (Figure 3-3).

**Total Ecosystem C Stocks and Distribution Among The Various Pools**

Total ecosystem C was ∼ 39 to 79% greater in the silvopasture compared with sown pasture and native rangeland. These data demonstrated the potential for tree-based grazing land ecosystems to sequestrate significant amounts of C. This is in agreement with other studies that have also documented the relatively high C sequestration potential of agroforestry ecosystems (Udawatta and Jose, 2012), particularly associated with the woody biomass and soil components (Schoeneberger, 2009). Silvopastures are also suggested to utilize resources (light, nutrient, water) more efficiently than treeless systems (Sharrow and Ismail, 2004; Thevathasan and Gordon, 2004; Udawatta and Jose, 2012). Data reported by Sharrow and Ismail (2004) indicated that 11 years after establishment, silvopasture systems accumulated 520 kg ha⁻¹ year⁻¹.
more C than grass-based pastures. Similar results were reported by Arevalo et al. (2009) studying agriculture, hybrid poplar, native aspen, and grassland ecosystems. These later authors found total ecosystem C was greater in tree-based ecosystems compared with grass-dominated biomes. Reports in Florida further suggested that slash pine plantation remained effective at storing ecosystem C 34 years after establishment (Gholz and Fisher, 1982). In the current study, aboveground biomass in silvopasture was an important component of total ecosystem C. While aboveground biomass C in silvopasture represented ~ 34% of total ecosystem C, ~ 4 and 2% of total ecosystem C was associated with above-ground biomass in native rangeland and sown pasture, respectively. Our data corroborate previous studies that suggested that a greater proportion of ecosystem C in grass-dominated ecosystems is associated with SOC (Taylor and Lloyd, 1992; Houghton and Hackler, 2000; Liu et al., 2011). Although native rangeland had greater ability to accumulate C in plant biomass, it had smaller SOC stocks than sown pasture. Thus, plant biomass may not necessarily be related to ecosystem ability to store C in the soil. Other factors such as chemical composition of plant material as well as microbial activity can also affect the amount and characteristics of C inputs and subsequent SOC accumulation.

**Summary and Conclusions**

Conversion of native rangeland into more intensively managed silvopasture and sown pasture systems favored ecosystem and SOC accumulation; however, C allocation among above- and below-ground pools varied among the three ecosystems. Incorporation of trees into grass-based grazing land ecosystems resulted in greater C allocation in aboveground biomass while the majority of ecosystem C was associated with SOC in native rangeland (shrub-dominated) and sown pastures (grass-dominated).
Data suggest that introduction of more productive plant species such as conversion of C3-dominated native vegetation into C4 grasses and adoption of proper grazing and fertilization management strategies can be beneficial for enhancing C sequestration in terrestrial ecosystems.
Table 3-1. Aboveground biomass (Mg ha\(^{-1}\)) as affected by land use type.

<table>
<thead>
<tr>
<th>Land use type</th>
<th>Grass component</th>
<th>Shrub/tree component</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native rangeland</td>
<td>2.7 ± 0.1 b</td>
<td>4.9 ± 0.5b</td>
<td>7.7 ± 0.4 b†</td>
</tr>
<tr>
<td>Silvopasture</td>
<td>2.2 ± 0.1 c</td>
<td>116 ± 17a</td>
<td>118 ± 17 a</td>
</tr>
<tr>
<td>Sown pasture</td>
<td>4.3 ± 0.1 a</td>
<td>-----------</td>
<td>4.3 ± 0.1 b</td>
</tr>
</tbody>
</table>

† Data are means across summer and winter sampling events ± standard error. Means followed by the same letter within a column are not different (\(P > 0.05\)) based on the LSD test.
Table 3-2. Litter C mass, N mass and C: N ratio as affected by season and land use type.

<table>
<thead>
<tr>
<th>Land use type</th>
<th>Summer</th>
<th>Winter</th>
<th>Mean</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>litter C mass (Mg C ha(^{-1}))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native rangeland</td>
<td>0.2 ± 0.0 †</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.0 b</td>
<td>-----</td>
</tr>
<tr>
<td>Silvopasture</td>
<td>2.2 ± 0.3</td>
<td>2.6 ± 0.3</td>
<td>2.4 ± 0.2 a</td>
<td>-----</td>
</tr>
<tr>
<td>Sown pasture</td>
<td>0.4 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.3 ± 0.1 b</td>
<td>-----</td>
</tr>
<tr>
<td>Mean</td>
<td>0.9 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>0.6731§</td>
<td></td>
</tr>
<tr>
<td></td>
<td>litter N mass (kg N ha(^{-1}))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native rangeland</td>
<td>4.3 ± 1.1 b</td>
<td>1.4 ± 0.6 c</td>
<td>2.9 ± 0.7</td>
<td>0.3370</td>
</tr>
<tr>
<td>Silvopasture</td>
<td>40.4 ± 3.8 a</td>
<td>23.5 ± 2.1 a</td>
<td>32.0 ± 2.9</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Sown pasture</td>
<td>6.3 ± 0.8 b</td>
<td>12.1 ± 2.3 b</td>
<td>9.2 ± 1.4</td>
<td>0.0541</td>
</tr>
<tr>
<td>Mean</td>
<td>17.0 ± 3.3</td>
<td>12.4 ± 2.0</td>
<td></td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td>C:N ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native rangeland</td>
<td>55 ± 5.3</td>
<td>81 ± 6.9</td>
<td>68 ± 5.2 b</td>
<td>-----</td>
</tr>
<tr>
<td>Silvopasture</td>
<td>71 ± 3.4</td>
<td>82 ± 3.4</td>
<td>77 ± 2.6 a</td>
<td>-----</td>
</tr>
<tr>
<td>Sown pasture</td>
<td>32 ± 1.4</td>
<td>41 ± 2.0</td>
<td>36 ± 1.6 c</td>
<td>-----</td>
</tr>
<tr>
<td>Mean</td>
<td>52 ± 3.6</td>
<td>68 ± 4.4</td>
<td>&lt;.0001</td>
<td></td>
</tr>
</tbody>
</table>

†Data are means ± standard errors. Land use means within a season or average of two seasons and response variable followed by the same letter are not different (P > 0.05) based on the LSD test. §Data from summer and winter sampling events were not statistically different if P value higher than 0.05.
### Table 3-3. Root C and N mass as affected by land use type

<table>
<thead>
<tr>
<th>Land use type</th>
<th>Soil depth (cm)</th>
<th>Root C mass (Mg C ha(^{-1}))</th>
<th>Root N mass (Mg N ha(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-10</td>
<td>10-20</td>
<td>20-30</td>
</tr>
<tr>
<td>Native rangeland</td>
<td>5.3±0.3(^{†})a</td>
<td>3.1±0.2a</td>
<td>2.1 ± 0.2a</td>
</tr>
<tr>
<td></td>
<td>(39%)</td>
<td>(23%)</td>
<td>(15%)</td>
</tr>
<tr>
<td>Silvopasture</td>
<td>2.9±0.4b</td>
<td>0.7±0.2b</td>
<td>0.3 ± 0.0c</td>
</tr>
<tr>
<td></td>
<td>(67%)</td>
<td>(18%)</td>
<td>(4%)</td>
</tr>
<tr>
<td>Sown pasture</td>
<td>5.1±0.3a</td>
<td>1.1±0.1b</td>
<td>0.7 ± 0.1b</td>
</tr>
<tr>
<td></td>
<td>(62%)</td>
<td>(14%)</td>
<td>(8%)</td>
</tr>
<tr>
<td>Native rangeland</td>
<td>0.11±0.01b</td>
<td>0.03±0.002a</td>
<td>0.02 ± 0.002a</td>
</tr>
<tr>
<td></td>
<td>(53%)</td>
<td>(19%)</td>
<td>(11%)</td>
</tr>
<tr>
<td>Silvopasture</td>
<td>0.10±0.011b</td>
<td>0.01±0.003b</td>
<td>0.01 ± 0.001b</td>
</tr>
<tr>
<td></td>
<td>(75%)</td>
<td>(12%)</td>
<td>(4%)</td>
</tr>
<tr>
<td>Sown pasture</td>
<td>0.19±0.01a</td>
<td>0.03±0.002a</td>
<td>0.02 ± 0.003a</td>
</tr>
<tr>
<td></td>
<td>(66%)</td>
<td>(13%)</td>
<td>(6%)</td>
</tr>
</tbody>
</table>

\(^{†}\)Data represent means ± standard error. Means in parenthesis are % of total root biomass C. Root C and N mass at 0- to 20-cm depth are shown as average of data from summer and winter sampling events. Means followed by the same letter within a soil depth and response variable are not different (\(P > 0.05\)) based on the LSD.
Table 3-4. Ecosystem C stocks and distribution among the various above- and below-ground pools as affected by grazing land intensification.

<table>
<thead>
<tr>
<th>Land use type</th>
<th>Ecosystem C pools (Mg C ha(^{-1}))</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total ecosystem C</td>
<td>Aboveground biomass C</td>
<td>Litter C</td>
<td>Root C</td>
<td>SOC</td>
</tr>
<tr>
<td>Native rangeland</td>
<td>94(^{\dagger}) b</td>
<td>3.8 b</td>
<td>0.2 b</td>
<td>14 a</td>
<td>76 b</td>
</tr>
<tr>
<td></td>
<td>(4.1%)</td>
<td>(0.2%)</td>
<td>(15%)</td>
<td>(80.7%)</td>
<td></td>
</tr>
<tr>
<td>Silvopasture</td>
<td>168 a</td>
<td>59 a</td>
<td>2.4 a</td>
<td>6.0 c</td>
<td>101 a</td>
</tr>
<tr>
<td></td>
<td>(34.5%)</td>
<td>(1.4%)</td>
<td>(3.5%)</td>
<td>(60.6%)</td>
<td></td>
</tr>
<tr>
<td>Sown pasture</td>
<td>121 b</td>
<td>2.1 b</td>
<td>0.3 b</td>
<td>9.1 b</td>
<td>110 a</td>
</tr>
<tr>
<td></td>
<td>(1.7%)</td>
<td>(0.3%)</td>
<td>(7.5%)</td>
<td>(90.5%)</td>
<td></td>
</tr>
</tbody>
</table>

\(^{\dagger}\) Data are means of two seasons. Means in parenthesis are % of total ecosystem C. Means followed by the same letter within pool and response variable are not different (\(P > 0.05\)) based on the LSD test.
Figure 3-1. Litter neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin concentrations as affected by land use type. A) Litter NDF. B) Litter ADF. C) Litter lignin. Values followed by the same letter are not statistically different ($P > 0.05$) based on the LSD test.
Figure 3-2. Root neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin concentrations as affected by land use type. A) Root NDF. B) Root ADF. C) Root lignin. Values followed by the same letter are not different ($P > 0.05$) among management ecosystems based on the LSD test.
Figure 3-3. Soil organic C and N stocks accumulation (0-100 cm depth) as affected by land use type. Bars represent 1 standard error. Data at 0- to 10-cm and 0- to 20-cm depth are shown as average of summer and winter sampling events. Means within a soil depth followed by an asterisk are different ($P \leq 0.05$) based on the LSD test.
CHAPTER 4
CHANGES IN SOIL ORGANIC CARBON POOLS IN RESPONSE TO GRAZING LAND INTENSIFICATION IN SUBTROPICAL ECOSYSTEMS

Introduction

Soil organic carbon (SOC) is an important terrestrial carbon (C) pool and plays an integral role in global C cycling. Despite the wide range of SOC accumulation in different soil types and environmental conditions, it is estimated that ~ 1500 Pg of C is found in the upper 100 cm of soil (Eswaran et al., 1995; Batjes, 1996). This estimate represents ~ 1.5 and 1 times more C in soils than that present in the vegetation and atmosphere, respectively (Post et al., 1990; Houghton, 1995; Hillel and Rosenzweig, 2009). Although less vulnerable than C stored in above-ground vegetation, SOC can be affected by a variety of biotic and abiotic factors. Among the human-induced factors affecting SOC, land use management represents an important aspect that can have either positive or negative impacts on SOC stocks. The impact of land-use management on SOC stocks have been documented extensively across different natural and managed ecosystems (Conant et al., 2001; Franzluebbers and Stuedemann, 2002; Lal, 2005; Shrestha and Stahl, 2008).

Estimates showed that grazing lands occupy ~ 3.6 billion hectares which can offset about 20% of the annual C dioxide (CO₂) emitted from land use changes (Follett and Reed, 2010). In the USA, grazing lands also play a key role in climate change mitigation as they occupy a large area (~634 million acres) (USDA-NRCS, 1997) and have potential to sequestrate 13 to 70 Mt SOC year⁻¹ (Lal et al., 2007).

However, grazing land management can affect the equilibrium between C inputs and losses with subsequent effects on SOC dynamics. Positive effects of grazing land management are often associated with management practices that promote C inputs
(fertilization, introduction of productive species, use of legumes) and/or reduce physical disturbance and SOC decomposition (proper grazing management, erosion control) (Fearnside and Barbosa, 1998; Abril and Bucher, 1999; Conant et al., 2001; Conant et al., 2003). Introduction of more productive species and integration of trees into grass-based production systems can also promote SOC sequestration (Schimel et al., 1995; Montagnini and Nair, 2004). Numerous reports demonstrated that silvopasture systems, for instance, can alter ecosystem characteristics, such as net primary productivity (Schimel et al., 1995), root distribution (Jackson et al., 2000), litter input (Connin et al., 1997), C cycling (Gill and Burke, 1999) and nutrient retention (Nair et al., 2007; Bambo et al., 2009).

The impacts of grazing land intensification (e.g., conversion of extensively managed native ecosystem into more intensively managed pasture systems) on SOC in subtropical ecosystems are not fully understood. Since SOC decomposition is typically more pronounced in humid subtropical ecosystems, enhancing SOC stocks in areas subjected to high precipitation can be a major challenge. In the USA Coastal Plain region, soils are characterized by coarse texture and relatively low SOC concentrations compared with other regions. The warm and moist climate conditions associated with the lack of clay minerals to protect SOC against microbial mineralization favor fast SOC turnover in these soils. While short-term studies are useful indicators of early changes in SOC as affected by grazing land management (Silveira et al., 2013), questions still remain as to the long-term effects of grazing land intensification on SOC stocks. Soil organic C is a key component for sustaining production as it affects nutrient supply to plants, soil erosion control, soil physical and chemical properties (e.g., aggregation,
cation exchange) and water holding capacity (Miller and Donahue 1990). Therefore, in addition to the positive effect on global C cycle, identifying management strategies that promote SOC accumulation can also enhance grazing land sustainability.

Characterizing SOC pools and changes that occur in response to grazing land intensification is a key to ascertain long-term C sequestration in grazing land ecosystems. Because increasing SOC stocks is not necessarily synonymous with increased SOC stability, it is important to characterize the various SOC pools and understand how they respond to management. Particulate organic C (POC) and light-free C (LF) fraction have been extensively used as indicators of labile C (Cambardella and Elliott 1992; Franzluebbers and Arshad, 1997; Wander and Bollero, 1999; Chan, 2001; Franzluebbers and Stuedemann, 2002; Garten, 2002). Research has shown that intensively-managed grazing systems can increase C associated with the POC pool compared with extensively grazed systems (Conant et al., 2003). Franzluebbers and Stuedemann (2002) found that both POC and C associated with the soil mineral fraction were greater in pastures receiving high nitrogen (N) fertilization levels compared with low N fertilization levels. Dubeux et al. (2006a) concluded that increasing management intensification (defined as greater stocking rate and N fertilization levels) increased C concentration in the LF fraction with no effect detected in the bulk soil.

Numerous studies have also used size/density separation technique to characterize changes in SOC in response to land use management (Six et al., 1998; Helfrich et al., 2006). Six et al. (1998) observed that C associated with the intra-aggregate POM (iPOM) fraction was affected by tillage management but no effects were observed on LF. Mineral-associated C (mineral-C), often recognized as a stable C
pool, in some circumstances (i.e., coarse-textured soils) can also be susceptible to land use management such as N fertilization (Cusack et al., 2011) and grazing management (Silveira et al., 2013).

Despite the vast body of literature addressing the potential impacts of land-use management on SOC size/density fractions, limited research has been conducted in coarse-textured soils in subtropical regions. Soil organic C responses to grazing land intensification in coarse-textured Coastal Plain soils are expected to be different than those in temperate regions dominated by fine texture. This study was designed to evaluate the long-term impacts (> 20 yr) of grazing land intensification on SOC stocks and distribution among the various size/density pools.

**Material and Methods**

**Site Description and Treatments**

The study was conducted at the University of Florida, Range Cattle Research and Education Center in south-central Florida (27°23’N, 81°57’W). Mean annual precipitation of this area is ~ 1650 mm. Average maximum/minimum temperatures are 28 and 17°C, respectively. Field-replicated (n = 2) experimental sites consisted three grazing land ecosystems: extensively managed native rangeland and more intensively managed silvopasture and sown pasture. Management intensity (herein defined as N level, net primary productivity, and grazing intensity) represents commonly used practices in beef cow-calf operations in the region. The native rangelands consisted of the least disturbed site, thus, it was considered as a baseline for the purpose of this study.

Each field replicated ecological unit was ~ 6 ha in area and all sites were located under the same soil type, topography, and climatic conditions. The major soil order was
Spodosol and the main soil series were Ona fine sand (sandy, siliceous, hyperthermic Typic Alaquods) and Smyrna sand (sandy, siliceous, hyperthermic Aeric Alaquod). All experimental sites were established and consistently maintained for over 20 years. More detailed information about the experimental sites is presented in Adewopo et al. (2014). Briefly, native rangelands consisted primarily of pine-palmetto flatwoods (Kalmbacher et al., 1984) and the predominant vegetation structure consisted of scattered slash pine (*Pinus elliotti* Engelm.), saw palmetto (*Serenoa repens* Bartr.) and pineland threeawn (*Aristida stricta* Michx.). Native rangelands have never received commercial fertilizer, but are subjected to periodic burning (every 3 to 4 years) and occasional grazing activities (~125 animal days ha$^{-1}$ yr$^{-1}$). Pine-bahiagrass silvopasture was established in 1991 on an 11-yr-old Pensacola bahiagrass (*Paspalum notatum* Flügge) stand (Kalmbacher and Ezenwa, 2005). In the year of 1998, 1999, 2001, 2003 to 2007, and 2010, N fertilizer was applied in silvopasture at 67 kg ha$^{-1}$ yr$^{-1}$. From March to September, rotational stocking was conducted as 2 weeks of grazing followed by 5 weeks of resting period. Stocking rates were 207 animal days ha$^{-1}$ yr$^{-1}$. Sown pastures consisted of a 31-yr-old bahiagrass stand that has received annual application of ~ 67 kg N ha$^{-1}$ since 1991. Sown pastures have been rotationally stocked year-round (1 week grazing period followed by 1 week of resting period). Stocking rates are typical of the beef cow-calf production system in the region (360 animal days ha$^{-1}$ yr$^{-1}$).

**Soil Sampling and Analyses**

**Soil C and N stocks**

Five quadrats (20 m × 20 m), spaced ~75 m apart, were established along a diagonal transect within each field-replicated experimental unit. Four randomly located soil cores (diameter of 2.2 cm) were taken within each quadrat in June/July 2012.
(summer) and January/February 2013 (winter) at soil depths of 0 to 10 and 10 to 20 cm and composited within a depth for C and N determination. One random soil core was sampled in each quadrat for bulk density determination. Soil samples were air-dried, and subsequently sieved through a 2-mm screen to remove large roots and rocks. A subsample of soil was pulverized using ceramic beads for total SOC and N determination in a Flash EA 1112 Series elemental analyzer (Thermo Fisher Scientific Inc., Waltham, Massachusetts). Soil samples collected for bulk density determination were oven-dried at 105°C for 48 hours and bulk density was calculated by dividing the soil dry weight by the core volume (g soil cm⁻³).

**Particulate Organic C and N and Mineral Associated C and N Fractions**

Particulate organic C and N (POC and PON) and mineral associated C and N (mineral-C and mineral–N) determinations were determined on the soil samples collected in summer and winter using the procedure described by Cambardella and Elliott (1992). Briefly, the procedure consisted of equilibrating 10 g of air-dried soil with 30 mL of 5 g L⁻¹ Na₄P₂O₇ solution. The suspension was shaken in a reciprocal shaker (180 osc minute⁻¹) for 15 hours, and then transferred to a 53-μm screen sieve and washed with distilled water until leachate was clear. The material that passed through the sieve (< 53 μm) was regarded as the mineral fraction while the material retained on the sieve (> 53 μm) was considered the POC fraction. Both fractions were transferred to appropriate containers and dried at 55°C until constant weight. Samples were then ground for C and N determination using the same method as described for soil samples.

**Soil Aggregate Analysis**

Soil aggregate size separation was performed in the samples collected in the summer following the wet sieving method described by Six et al. (1998). Briefly,
aggregates were separated by wet-sieving air-dried samples through a series of sieves (250 and 53 μm). Separated soil fractions included macroaggregates (250-2000 μm), microaggregates (53-250 μm) and mineral associated fraction (silt+clay fraction) (<53 μm), and they were oven-dried at 55°C, and weighed. The macroaggregates were then suspended in 20 mL of 1.85 g cm⁻³ sodium polytungstate solution to isolate the LF C, fine intra-aggregate particulate organic matter (FiPOM) (53-250 μm) and coarse intra-aggregate particulate organic matter (CiPOM) (>250 μm). The same procedure was applied to microaggregates to divide samples into two density fractions: LF and microaggregate iPOM (53-250 μm).

**Statistical Methods**

Data were analyzed using SAS PROC MIXED procedure (SAS 9.4, 2013). The SOC and soil N stocks and the SOC associated with different soil fractions were analyzed within each soil depth. Land use types, season and their interactions were considered as fixed effects and replicates were considered random effects. If treatment effects could not be detected for any soil depth, then these data were analyzed across two soil depths with land use type, season, soil depth and their interactions as fixed effects (Table 4-3, Table 4-4). Measurements in each experimental unit were considered one replicate. The PDIFF test of the LSMEANS procedure was used to compare means. Treatments and their interactions were considered significant if F-test P values were ≤ 0.05.

**Results**

**SOC and N Stocks**

Both SOC and soil N stocks (0 to 10 cm and 10 to 20 cm) increased in response to grazing land intensification (Table 4-1). Native rangeland exhibited the smallest SOC
and soil N stocks (37 and 1.7 Mg ha\(-1\) at the 0 to 20 cm depth, respectively) compared with silvopasture (55 and 3.1 Mg ha\(-1\), respectively) and sown pasture (54 and 3.1 Mg ha\(-1\), respectively). No differences were observed in SOC and soil N stocks between the silvopasture and sown pasture systems. Soil SOC and soil N stocks decreased with increasing soil depth and no season effect (summer vs. winter sampling) was observed.

The ratio of SOC and soil N decreased as management intensification increased. At 0 to 20 cm depth, native rangeland exhibited the greatest soil C:N ratio (21 and 26 for summer and winter, respectively) compared with silvopasture (16 and 19 for two seasons, respectively) and sown pasture (17 and 18 for two seasons, respectively). In native rangeland, soil C:N ratio was generally greater for winter compared with summer.

POC, PON, Mineral-C and Mineral-N

Grazing land intensification promoted C accumulation in both POC and mineral-C fractions (Table 4-2). At the 0 to 20 cm depth, C associated with the POC and mineral fraction in the native rangeland (17 and 14 Mg ha\(-1\), respectively) was ~ 1.3 to 1.6 times smaller than the sown pasture (28 and 22 Mg ha\(-1\), respectively) and silvopasture (23 and 29 Mg ha\(-1\), respectively). A much greater proportion of total C was associated with the POC fraction in the native rangeland and sown pasture (average of 55 and 56% of total C at the 0 to 20 cm depth, respectively) compared with the silvopasture (44% of total C). Conversely, mineral-C accounted for ~ 46 to 73% of total C in the silvopasture (0 to 10 and 10 to 20 cm depths, respectively). Particulate organic C stocks and the proportion relative to total SOC generally decreased with soil depth while there was a trend that mineral-C ($P= 0.0787$) and the proportion of total C increased with soil depth (Table 4-2).
A seasonal effect (summer vs. winter sampling) was observed on POC and mineral-C; however, the interaction between land use and season was not significant. Averaged across grazing land ecosystems, POC at the 0 to 20 cm depth was smaller in winter (20 Mg ha\(^{-1}\)) compared with summer (25 Mg ha\(^{-1}\)), while mineral-C was greater in winter (25 Mg ha\(^{-1}\)) than summer (19 Mg ha\(^{-1}\)).

Similar to POC and mineral-C, grazing land intensification promoted accumulation of N in the PON and mineral-associated fractions (Table 4-2). The greatest PON concentration (0 to 20 cm depth) was observed in the sown pasture (1.6 Mg ha\(^{-1}\)), followed by silvopasture (1.2 Mg ha\(^{-1}\)) and native rangeland (0.8 Mg ha\(^{-1}\)). At 0 to 20 cm depth, silvopasture exhibited the greatest N stocks associated with the mineral fraction (1.9 Mg ha\(^{-1}\)), followed by sown pasture (1.4 Mg ha\(^{-1}\)), and native rangeland (0.9 Mg ha\(^{-1}\)). The land use type × season interaction on mineral-N was significant at 0 to 10 cm depth. At this soil depth, silvopasture exhibited the greatest mineral-N concentration while the difference between native rangeland and sown pasture was not found in summer (data not shown). However, in both of data from winter sampling (0 to 10 cm) and 10 to 20 cm across two season, the order of mineral-N concentration was silvopasture > sown pasture > native rangeland, consisting with the data at 0 to 20 cm depth.

Particulate organic N and mineral-N were also influenced by soil depth and season. During winter there were lesser PON concentrations (0.9 Mg ha\(^{-1}\)) compared with summer (1.4 Mg ha\(^{-1}\)) at 0 to 20 cm depth. Particulate N stocks and the relative proportion of total soil N associated with the PON fraction decreased with soil depth. The N concentrations associated with the mineral fraction also decreased with soil depth.
depth but increased in winter (1.6 Mg ha⁻¹) compared with summer sampling (1.2 Mg ha⁻¹).

**Soil Aggregates**

Soil mass distribution in aggregate size fractions was not affected by grazing land management. At the 0 to 10 cm depth, the greatest proportion of soil mass was recovered in the 250 to 150 μm fraction (average of 38 g 100 g⁻¹ soil), followed by the 150 to 53 and 2000 to 250 μm fractions (average of 35 and 25 g 100 g⁻¹ soil, respectively). The smallest soil mass was associated with the < 53 μm fraction (average of 2.2 g 100 g⁻¹ soil). At the 10 to 20 cm depth, the greatest soil mass was recovered in 150 to 53 μm aggregate size fraction (44 g 100 g⁻¹ soil), and decreased in the following order: 250 to 150 μm size fraction (36 g 100 g⁻¹ soil), and 2000 to 250 μm size fraction (18 g 100 g⁻¹ soil), and < 53 μm size fraction (3.5 g 100 g⁻¹ soil).

The C concentration associated with the aggregate size fractions followed a different pattern as compared to soil mass. Despite the relatively small soil mass recovered in the < 53 μm aggregate size class, the greatest C concentration was observed in this fraction. At the 0 to 10 cm, average C concentration in < 53 μm fraction (92 g kg⁻¹ aggregate) was 61% greater than that in the 2000 to 250 μm fraction (57 g kg⁻¹ aggregate), 4 times greater than 250 to 150 μm fraction (18 g kg⁻¹ aggregate), and 7 times greater than 150 to 53 μm fraction (11 g kg⁻¹ aggregate). At the 10 to 20 cm depth, C concentration in the <53 μm fraction (87 g kg⁻¹ aggregate) was ~ 2, 7 and 9 times greater than that in 2000 to 250 μm (27 g kg⁻¹ aggregate), 250 to 150 μm (11g kg⁻¹ aggregate) and 150 to 53 μm fractions (9 g kg⁻¹ aggregate), respectively.

Management intensification affected C distribution among the various aggregate size classes (Figure 4-1). In general, C concentration associated with the
macroaggregate, microaggregate and mineral associated aggregate size fractions increased as grazing land intensification increased (Figure 4-1 A, B). For instance, at the 0 to 10 cm depth, C concentration associated with the 2000 to 250 μm fraction was ~ 1.2 times greater in sown pasture (70 g kg\(^{-1}\) aggregate) and silvopasture (68 g kg\(^{-1}\) aggregate) than the native rangeland (32 g kg\(^{-1}\) aggregate). Similar trend was observed at 10 to 20 cm depth. The C concentration associated with the fine particle size (< 53 μm fraction) was also affected by grazing land management. At the 0 to 10 cm depth, silvopasture showed 20% greater C concentration (102 g kg\(^{-1}\) aggregate) than native rangeland (85 g kg\(^{-1}\) aggregate) in this fraction, while at the 10 to 20 cm depth, sown pasture (94 g kg\(^{-1}\) aggregate) and silvopasture (103 g kg\(^{-1}\) aggregate) showed ~ 50 and 63% greater C concentration than native rangeland (63 g kg\(^{-1}\) aggregate). Soil organic C mass (g C kg\(^{-1}\) soil) associated with macroaggregate and microaggregate were also promoted by grazing land intensification (Figure 4-1 C, D). However, SOC mass associated with the mineral associated fraction was not affected by grazing land intensification.

The relative distribution of C mass associated with each aggregate size fraction (% of total C in soil) was not affected by management intensification. On average, ~ 50% of total soil C present in the 0 to 10 cm was found in the 2000 to 250 μm fraction, while ~ 26, 15 and 8% of total C was associated with the 250 to 150 μm, 150 to 53, and < 53 μm fractions, respectively.

**Size/Density Fraction**

The C associated with macroaggregate CiPOM and FiPOM was not affected by grazing land management. However, management intensification increased mass of C associated with microaggregate iPOM (Figure 4-2). At the 0 to 10 cm depth, the
greatest microaggregate iPOM C was found in sown pasture (1.3 g kg\(^{-1}\) soil), followed by silvopasture (1.0 g kg\(^{-1}\) soil), and native rangeland (0.7 g kg\(^{-1}\) soil). No land use effect on macro- and microaggregate iPOM was observed at the 10 to 20 cm depth.

Grazing land management showed no effect on macroaggregate LF C fraction, however, microaggregate LF C was affected by grazing land type (Table 4-3). The greatest sum of LF C (both macro- and microaggregates) was found in sown pasture (24.7 and 7.8 g kg\(^{-1}\) soil for 0 to 10 and 10 to 20 cm depths, respectively) compared with silvopasture (13.7 and 6.7 g kg\(^{-1}\) soil for 0 to 10 cm and 10 to 20 cm depths, respectively) and native rangeland (12.9 and 2.9 g kg\(^{-1}\) soil for 0 to 10 cm and 10 to 20 cm depths, respectively). Similarly, land use management increased mass of C associated with LF from microaggregates across the two depths (Table 4-3). The mass of C associated with this fraction in sown pasture (9.9 and 5.2 g kg\(^{-1}\) soil for 0 to 10 cm and 10 to 20 cm depths, respectively) and silvopasture (8.6 and 4.8 g kg\(^{-1}\) soil for 0 to 10 cm and 10 to 20 cm depths, respectively) was greater than native rangeland (5.5 and 1.8 g kg\(^{-1}\) soil for 0 to 10 cm and 10 to 20 cm depths, respectively). Light-free C in both macroaggregate and microaggregate fractions decreased as soil depth increased.

A greater proportion of microaggregate-C was associated with the LF fraction in the sown and silvopasture (78 and 76% of total microaggregate-C) compared with the native rangeland (70% of total microaggregate-C) (Figure 4-3A). A similar trend was found in 10 to 20 cm depth, where the proportions were 71, 64, and 42% for sown pasture, silvopasture, and native rangeland.

Mineral-associated C fraction (mSOC) was not affected by grazing land intensification. On average, the sum of mSOC present in both macro- and
microaggregates were 5, 4 and 6 g kg\(^{-1}\) soil at 0 to 10 cm and 6, 5, 5 g kg\(^{-1}\) soil at 10 to 20 cm for native rangeland, sown pasture and silvopasture, respectively. However, the proportion of microaggregate mSOC of total microaggregate C decreased with grazing land management (Figure 4-3B). Native rangeland had greater mSOC proportion (21\%) in microaggregates than sown pasture (12\%) and silvopasture (15\%) at 0 to 10 cm depth. A similar trend was found at the 10 to 20 cm depth, where the proportions were 46\% for native rangeland and 21 and 27\% for sown pasture and silvopasture, respectively.

The proportion of total C associated with LF of total SOC was smaller at 10 to 20 cm depth than 0 to 10 cm depth (47 vs. 68\%, respectively) (Table 4-4). Sown pasture tended to have a greater proportion of LF relative to total SOC than native rangeland (69\% vs. 46\%, P=0.0798). Conversely, the proportion of mSOC relative to total SOC increased with soil depth (22\% at 0 to 10 cm vs. 46\% at 10 to 20 cm). Greater proportion of mSOC was observed in native rangeland than in sown pasture (43 vs. 24\%).

**Discussion**

**SOC and Soil N**

Ecosystem management practices such as vegetation composition, fertilizer, grazing intensity and strategy can influence SOC and N dynamics (Conant et al., 2001; Schuman et al., 2002; Wright et al., 2004; Rees et al., 2005; Derner and Schuman, 2007; Follett and Reed, 2010). Our results are in agreement with previous reports that demonstrated that grazing land management intensification promotes SOC and N accumulation. Our data show that conversion of native rangeland to more intensively-managed sown pastures resulted in an increase of 49\% in SOC stocks. This result is in
agreement with Conant et al. (2001) who reported an average of 31% (range of 4 to 154%) increase in SOC as a result of intensification of management including fertilization, improved grazing management and introduction of plant species with greater production potential. In the current study, the mean annual SOC increase during the 31-years following conversion of native rangeland into sown pasture was ~ 0.57 Mg ha\(^{-1}\) yr\(^{-1}\). These data are within the SOC sequestration range of 0.1 to 3.0 Mg ha\(^{-1}\) yr\(^{-1}\) Conant (2001) and similar to the mean SOC sequestration rates for the southeastern USA (0.41 Mg ha\(^{-1}\) yr\(^{-1}\)) (Conant et al., 2003). Compared to the meta-analysis performed by Conant et al. (2001), our results are slightly greater than the mean rate of SOC increase caused by fertilization (0.3 Mg ha\(^{-1}\) yr\(^{-1}\)), improved grazing (0.35 Mg ha\(^{-1}\) yr\(^{-1}\)), and conversion from native to pasture (0.35 Mg ha\(^{-1}\) yr\(^{-1}\)).

In the current study, SOC and N accumulation in sown pasture was primarily due to the conversion of C3 native species into more productive C4 grasses, and adoption of grazing and fertilizer management practices that resulted in greater ecosystem productivity (Adewopo et al., 2014). Conversion of native vegetation to fast growing warm-season grasslands for livestock, combining with proper management can promote SOC (Fisher et al., 1994; Conant et al., 2001) and N (Wright et al., 2004) sequestration.

Several studies found that grazing can increase SOC stocks compared with non-grazed ecosystems (Manley et al., 1995; Schuman et al., 1999; Reeder and Schuman, 2002; Derner and Schuman, 2007; Franzluebbers and Stuedemann, 2009), especially when well managed (Redder et al., 2004; Wright et al., 2004; Ingram et al., 2008) and where nutrient cycling and forage growth are promoted (Sharif et al., 1994; Schuman et al., 2002). Grazing can promote plant residue decomposition and incorporation into the
soil through trampling (Naeth et al., 1999), stimulate root exudation (Dyer and Bokhari, 1976) and transfer of plant C and N into soil (Schuman et al., 1999). However, overgrazing can reduce root-derived C inputs (Holland and Detling, 1990) due to less C allocation to roots (Detling et al., 1979) and reduced root biomass (Schuster, 1964), subsequently resulting in significant C and N losses, soil disruption and erosion (Ball and Ryden, 1984; Wright et al., 2004). In our study, although sown pasture and silvopasture were subjected to greater grazing intensity, these systems resulted in greater SOC accumulation compared with the native rangeland. Our results are consistent with Conant et al. (2003) who reported a 22% increase in SOC in intensively-managed pastures compared with extensively-managed systems in the southeastern United States.

In addition, the use of N fertilizer and more productive species also promoted SOC accumulation in the sown and silvopasture systems. Fertilization with N has shown positive effects on SOC in variety of ecosystems (Malhi et al., 1991; Nyborg et al., 1994; Malhi et al., 1997; Reeder et al., 1998; Schuman et al., 2002; Derner and Schuman, 2007). In grazing lands, N increases forage production (Samuel and Hart, 1998) and C inputs, and subsequently promotes SOC accumulation (Malhi et al., 1991). It was also observed that N addition increased herbage nutritive value (Dubeux et al., 2006d) and litter deposition and quality (Dubeux et al., 2006b and c, Liu et al. 2001a and b).

The introduction of trees into grass-dominated pasture also promoted SOC accumulation compared to native rangeland. This was likely due to changes in root distribution and organic matter inputs and allocation (Haile et al., 2008; Jackson et al., 2000; Haile et al., 2010). Similar results have been reported previously in several
environmentals (Sharrow and Ismail, 2004; Kirby and Potvin, 2007; Nair et al., 2009; Subhrajit et al., 2010). Haile et al. (2010) studied silvopasture systems in Florida in an environment similar to that of the current study and observed that a large proportion of the stable SOC fraction derived from slash pine, which suggested that silvopasture may enhance recalcitrant forms of SOC compared with bahiagrass pasture. In a previous study, Haile et al. (2008) reported greater SOC stocks in silvopasture compared with bahiagrass pasture. Similarly, Casals et al. (2014) studied silvopasture in tropical regions and found that both SOC and N stocks were greater in silvopasture than in open grassland. They suggested that SOC accumulation in silvopasture was due to the higher plant and root residue inputs and lower SOC decomposition rates. However, in the current study there were no differences in SOC and N stocks between sown pasture and silvopasture. Despite the different vegetation cover, grazing intensity, and nutrient inputs, both systems resulted in similar SOC and N accumulation. Although less N fertilizer was applied to the silvopasture, lower stocking rates were used which may have balanced C inputs and outputs. In addition, greater above-ground litter production (derived primarily from pine trees) and ability to absorb and store nutrients in the below-ground pools (Buresh and Tian, 1997; Nair et al., 1999; Nair et al., 2007) favored SOC accumulation in silvopasture systems.

**POC, PON, Mineral-C, Mineral-N**

Although grazing land intensification promoted C accumulation in both POC and mineral-associated fractions, a much greater proportion of C was associated with the POC pool in the sown pasture compared with the other ecosystems. Conversely, silvopasture promoted greater C accumulation in the mineral-associated fraction. Moreover, differences between sown pasture and silvopasture were detected in POC
and PON, but not in total SOC. The reason for this response is that POC and PON are much more sensitive indicators of changes in SOC and N caused by land management than is SOC (Cambardellar and Elliott, 1992; Wander and Bollero, 1999; Franzluebbers et al., 2000; Chan, 2001; Silveira et al., 2013).

The increases in POC stocks in response to management intensification observed in this current study are in agreement with Franzluebbers and Stuedemann (2002). In a study on pastures in southern Piedmont USA, they observed an increase in POC in response to fertilization. They also suggested that grazing can increase POC, particularly at surface soil depths, because plant residue incorporation is promoted by cattle trampling. Conant et al. (2003) studied the impacts of grazing land management intensification on SOC dynamics and found that POC concentrations were generally greater in intensively managed pastures compared with sites subjected to extensive grazing or hay removal. The greater proportion of POC relative to total SOC at shallow soil depths observed in the current study is consistent with previous studies (Franzluebbers and Stuedemann, 2002; Conant et al., 2003).

Despite the mineral-associated C being generally recognized as a relatively stable fraction (Hassink and Whitmore, 1997; Six et al., 2002), this C pool can be influenced by land use change (Neff, 2002; Conant et al., 2003; Cusack et al., 2011). Our results showed greater SOC associated with the mineral fraction in the silvopasture compared with the other ecosystem, likely due to the presence of the tree component in the ecosystem. Haile et al. (2010) studying silvopastures in Florida Spodosol found that a greater proportion of SOC associated with mineral associated fraction soil was derived from slash pine trees, particularly at deep soil depths. Previous studies
indicated that tree-derived SOC has lower decomposition rates than grass-derived SOC (Wynn and Bird, 2007), therefore, it is expected to preferentially accumulate in more stable SOC pools such as the mineral-associated fraction (Anderson and Paul, 1984). The increased mineral-associated C fraction in the silvopasture suggested that the tree component SOC may promote C accumulation in more stable forms as compared with grass-dominated sown pasture.

**SOC Distribution Among The Various Aggregate Classes**

Macroaggregates (2000 to 250 μm fraction) accumulated the greatest C mass compared with other soil aggregate fractions. On average across three land use ecosystems, this fraction accounted for ~ 50% of total SOC at the 0 to 10 cm depth. Similar responses were reported by Sarkhot et al. (2007) who indicated that ~ 50% of the total SOC was associated with macroaggregate fraction in a Florida Spodosol. These authors also concluded that this fraction was more sensitive to management intensification compared with the fine aggregate classes. Corroborating these results, Conant et al. (2004) observed that the majority of the total SOC was associated with the macroaggregate fraction in pasture and forest ecosystems in the southeastern USA. Tonucci et al. (2011) also found that macroaggregate had the greatest C content among all aggregate size fractions in silvopasture systems. In our study, SOC concentration in macroaggregates in sown pasture and silvopasture was ~ twice as great as native rangeland. These results are in agreement with the general assumption that C associated with the coarse fraction is more labile (Tiessen and Stewart, 1983) and easily decomposed (Hassink, 1995; Six et al., 2002) than the finer fractions and, therefore, more sensitive to ecosystem management (Elliott and Coleman, 1988; Six et al., 2004). This response may be also due to the fact that binding agents present in
macroaggregates are primarily temporary, such as roots exudates and hyphae, which are easily influenced by management (Tisdall and Oades, 1982).

Despite the greater soil mass recovered in the microaggregate class (average of ~ 40 g kg\(^{-1}\) soil), the greatest SOC concentration was associated with the mineral-associated fraction. Despite the finer size aggregates are more resistant to change in ecosystem management than macroaggregates (Elliott, 1986; Beare et al., 1994; Hassink, 1995; Six et al., 2002; Conant et al., 2004), long-term grazing land intensification affected SOC associated with microaggregates and mineral-associated fractions. Results showed that grazing land intensification promoted SOC mass associated with microaggregates. Also, there was a trend for SOC mass (g C kg\(^{-1}\) soil) at the 0 to 10 cm depth associated with mineral fractions to be affected grazing land management \((P = 0.0875)\). Although no treatment effect on SOC mass (g C kg\(^{-1}\) soil) was observed in the mineral fraction, C concentration in this fraction increased in response to grazing land intensification. Enrichment of C in this fraction has been reported in various studies under similar environmental and soil type conditions (Dubeux et al., 2006a; Silveira et al., 2013). Silveira et al. (2013) reported that C concentration in <53 μm fraction was a sensitive indicator of soil response to ecosystem management because the mineral fraction was dominated by quartz and kaolinite (Harris and Carlisle, 1987) and had low ability to protect SOC compounds. Results of X-ray analysis revealed that this fraction was dominated by quartz (data not presented). Change in C concentration in the mineral associated fraction may also occur because the binding agents in this fraction are temporary or transient and, consequently, can be sensitive to management (Tisdall and Oades, 1982). Unlike earlier studies that showed
increased management intensity promoted the decomposition of C in clay and silt fraction (Dubeux et al., 2006a; Silveira et al., 2013), we observed an increase in SOC concentration associated with the mineral fraction as management intensification increased. These inconsistent results may due to different time scales between these experiments, which suggested that long-term grazing land intensification have potential to accumulate SOC associated with finer size fractions. For instance, in the silvopasture the increases in mineral-C concentration relative to the native rangeland ranged from 20 and 63% for the 0 to 10 and 10 to 20 cm depths, respectively. In sown pasture, significant increases (~50%) in mineral-C concentrations were observed in the 10 to 20 cm depth only. The relatively greater ability of silvopasture to sequester C in mineral-associated pools has been attributed to the contribution of slash tree inputs, particularly at deeper soil depths (Haile et al., 2010).

LF and iPOM

Our results showed an increase in C associated with microaggregate iPOM as management intensification increased. No effects of grazing land intensification were observed on macroaggregate CiPOM and FiPOM. This result suggested that SOC associated with iPOM was likely protected in microaggregates within macroaggregates (Six et al., 1998).

Data indicated that LF C and its proportion relative to total SOC increased with increased management intensity. Light-free fraction is highly correlated with residue input (Gregorich and Jazen, 1996; Six et al., 1998) and has been widely recognized as a sensitive indicator of land use management (Janzen et al., 1992). Our previous data on litter deposition and composition as affected by grazing land management (data not presented here) are consistent with the increases in LF C observed in the current study.
Corroborating our finding, previous studies in similar environmental conditions also observed an increase in litter deposition and quality (Dubeux, 2006b and c) in response to intensification, and a subsequent increase in C accumulation in the LF fraction (Dubeux et al., 2006a). Positive relationships between LF C and C and N mineralization have been demonstrated in the literature (Sierra, 1996). Therefore, the greater C accumulation in LF in the more intensively managed systems suggests that grazing land intensification can promote C accumulation in more easily mineralizable C forms, particularly in the sown pasture.

A greater proportion of microaggregate SOC was associated with the mSOC in native rangeland, while microaggregates LF C was greater in sown pasture and silvopasture. The change in distribution of SOC among the various microaggregate components was caused by the increase of LF C in sown pasture and silvopasture. We also found that the proportion of total mSOC of total SOC was greater in native rangeland than sown pasture while the proportion of total LF C had a trend to be increased in sown pasture. Compared to LF C, mSOC is a relatively stable C and cannot be easily changed. Six et al. (1998) studied the influence of cultivation on soil aggregation and they also found than the mSOC was rarely affected by treatment.

**Summary and Conclusions**

Long-term grazing land intensification (herein defined as change in vegetation composition, grazing management, and N fertilizer inputs) promoted SOC in subtropical ecosystems. Both sown pasture and silvopasture showed greater SOC, POC, mineral-C than native rangeland. Despite the comparable SOC stocks, a greater proportion of SOC in sown pasture was associated with the POC pool, while in the silvopasture mineral-C was more predominant. These results showed than sown pasture
accumulated more C in labile forms while silvopasture tended to sequester more C in stable state. Management intensification also affected SOC distribution into aggregate sizes fractions. Carbon associated with macroaggregate, LF C and microaggregate iPOM increased in response to management intensification. Results indicated that grazing land intensification can promote SOC accumulation, particularly in more easily mineralizable forms.
Table 4-1. SOC and N stocks and C:N ratio as affected by grazing land intensification

<table>
<thead>
<tr>
<th></th>
<th>SOC (Mg C ha(^{-1}))</th>
<th>Soil N (Mg N ha(^{-1}))</th>
<th>C:N ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Summer</td>
<td>Winter</td>
<td>Mean</td>
</tr>
<tr>
<td>0-10 cm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native rangeland</td>
<td>26.8(^\dagger)</td>
<td>23.4</td>
<td>25.1 b</td>
</tr>
<tr>
<td>Silvopasture</td>
<td>33.2</td>
<td>38.4</td>
<td>35.8 a</td>
</tr>
<tr>
<td>Sown pasture</td>
<td>34.0</td>
<td>34.1</td>
<td>34.0 a</td>
</tr>
<tr>
<td>10-20 cm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native rangeland</td>
<td>11.8</td>
<td>11.2</td>
<td>11.5 b</td>
</tr>
<tr>
<td>Silvopasture</td>
<td>20.4</td>
<td>18.2</td>
<td>19.3 a</td>
</tr>
<tr>
<td>Sown pasture</td>
<td>20.6</td>
<td>20.1</td>
<td>20.4 a</td>
</tr>
<tr>
<td>0-20 cm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native rangeland</td>
<td>38.6</td>
<td>34.6</td>
<td>36.6 b</td>
</tr>
<tr>
<td>Silvopasture</td>
<td>53.6</td>
<td>56.7</td>
<td>55.1 a</td>
</tr>
<tr>
<td>Sown pasture</td>
<td>54.6</td>
<td>54.2</td>
<td>54.4 a</td>
</tr>
</tbody>
</table>

\(^\dagger\)Values represent the average of ten measurements. Averages followed by the same lower case letter within soil depth or the same capital letter within each land use ecosystem are not statistically different \((P > 0.05)\) according to LSD test. Asterisk (*) means seasonal difference were significant within land use type \((P \leq 0.05)\).
Table 4-2. Particulate organic C (POC) and N (PON) and mineral associated-C and -N (Mineral-C and Mineral-N) as affected by grazing land intensification

<table>
<thead>
<tr>
<th>Grazing land ecosystem</th>
<th>Soil depth</th>
<th>POC (Mg ha(^{-1}))</th>
<th>Mineral-C (Mg ha(^{-1}))</th>
<th>PON (Mg ha(^{-1}))</th>
<th>Mineral-N (Mg ha(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-10 cm</td>
<td>10-20 cm</td>
<td>0-20 cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native rangeland</td>
<td>14.0±1.0c (63%)</td>
<td>3.4±0.3c (35%)</td>
<td>17.3±1c (55%)</td>
<td>8.3±0.7c (37%)</td>
<td>0.61±0.06c (53%)</td>
</tr>
<tr>
<td>Silvopasture</td>
<td>17.6±1.3b (54%)</td>
<td>5.0±0.4b (27%)</td>
<td>22.6±1b (44%)</td>
<td>15.3±1.1a (46%)</td>
<td>0.95±0.08b (49%)</td>
</tr>
<tr>
<td>Sown pasture</td>
<td>20.7±1.2a (65%)</td>
<td>6.8±0.6a (40%)</td>
<td>27.6±1a (56%)</td>
<td>22.6±1.6b (44%)</td>
<td>1.27±0.07a (62%)</td>
</tr>
</tbody>
</table>

†Values represent the average across summer and winter sampling ± standard error. Values in parentheses are the percentage of total SOC or soil N. Averages followed by the same letter within soil depth are not statistically different (\(P > 0.05\)) according to LSD test.
Table 4-3. Grazing land intensification effects on LF fraction C (g kg\(^{-1}\) soil) in macroaggregate, microaggregate and the sum of LF C from both fractions.

<table>
<thead>
<tr>
<th>Soil depth(cm)</th>
<th>Native rangeland</th>
<th>Silvopasture</th>
<th>Sown pasture</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Macroaggregate LF C (g C kg(^{-1}) soil)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-10 cm</td>
<td>7.4±5.7</td>
<td>5.0±2.6</td>
<td>14.8±0.1</td>
<td>9.1±2.5 A</td>
</tr>
<tr>
<td>10-20 cm</td>
<td>1.1±0.2</td>
<td>1.8±0.3</td>
<td>2.6±0.3</td>
<td>1.9±0.3 B</td>
</tr>
<tr>
<td>Mean</td>
<td>4.3±3.0 a</td>
<td>3.4±1.4 a</td>
<td>8.7±3.5 a</td>
<td></td>
</tr>
</tbody>
</table>

|                | Microaggregate LF C (g C kg\(^{-1}\) soil) | | | |
| 0-10 cm        | 5.5±1.0          | 8.6±0.4      | 9.9±1.2      | 8.0±0.9 A |
| 10-20 cm       | 1.8±0.5          | 4.8±0.3      | 5.2±0.1      | 3.9±0.7 B |
| Mean           | 3.7±1.2 b        | 6.7±1.1 a    | 7.5±1.4 a    |           |

|                | Total LF C (g C kg\(^{-1}\) soil) | | | |
| 0-10 cm        | 12.9±4.8         | 13.7±2.3     | 24.7±1.1     | 17.1±2.8 A |
| 10-20 cm       | 2.9±0.7          | 6.7±0.0      | 7.8±0.4      | 5.8±1.0 B  |
| Mean           | 7.9±3.5 b        | 10.2±2.2 b   | 16.2±4.9 a   |           |

Mean values followed by different lower case letter are not statistically different among three ecosystems \((P > 0.05)\). Mean values followed by different upper case letter are not statistically different between two depths \((P > 0.05)\). Bars represent 1 standard error.
Table 4-4. Effect of grazing land intensification on proportion of total C associated with LF, CiPOM, FiPOM, and mSOC (% of total SOC).

| Soil depth | Native rangeland | Grazing land ecosystem | | | |
|---|---|---|---|---|
| | | Sown pasture | Silvopasture | Mean |
| LF C:SOC (%) | | | | |
| 0-10 cm | 61 | 81 | 62 | 68A |
| 10-20 cm | 31 | 57 | 52 | 47B |
| Mean | 46a | 69a | 57a |
| CiPOM C:SOC (%) | | | | |
| 0-10 cm | 3 | 0 | 2 | 1.5A |
| 10-20 cm | 1 | 1 | 0 | 0.7A |
| Mean | 2a | 0.5a | 0.8a |
| FiPOM C:SOC (%) | | | | |
| 0-10 cm | 10 | 6 | 8 | 8A |
| 10-20 cm | 8 | 7 | 6 | 7A |
| Mean | 9a | 6a | 7a |
| mSOC C:SOC (%) | | | | |
| 0-10 cm | 27 | 13 | 28 | 22B |
| 10-20 cm | 60 | 35 | 42 | 46A |
| Mean | 43a | 24b | 35ab |

Mean values followed by different lower case letter are statistically different ($P > 0.05$) among land uses according to LSD test. Mean values followed by different capital letter statistically different ($P > 0.05$) between two depths according to LSD test.
Figure 4-1. Grazing land intensification effects on soil C concentration associated with aggregate size classes. A) and B) Soil C concentration in aggregates (g C kg\(^{-1}\) aggregate). C) and D) Soil C concentration in soil (g C kg\(^{-1}\) soil). Values followed by different letter within size fraction are statistically different (\(P > 0.05\)) according to LSD test. Bars represent 1 standard error.
Figure 4-2. Grazing land intensification effect on intra-aggregate particulate organic matter (iPOM). CiPOM and FiPOM = coarse (2000-250 μm) and fine (250-53 μm) intra-aggregate particulate organic matter in macroaggregates, respectively. Microaggregate iPOM = fine (250-53 μm) intra-aggregate particulate organic matter in microaggregates. Bars represent 1 standard error. Values followed by different letter within each fraction are statistically different ($P > 0.05$) according to LSD test.
Figure 4-3. Land use effects on proportion of microaggregate LF C and microaggregate mSOC of total microaggregate C. A) Proportion of microaggregate LF C. B) Proportion of microaggregate mSOC. Values presented as mean ± standard error with different letter within each depth are statistically different ($P > 0.05$) according to LSD test.
CHAPTER 5
SOIL MICROBIAL COMMUNITY RESPONSES TO LAND USE INTENSIFICATION IN SUBTROPICAL GRAZING LAND ECOSYSTEMS

Introduction

Biogeochemical processes in soil regulating organic matter decomposition and carbon (C) stabilization are affected by soil microbial community composition and activity (Ingram, 2008). The impacts of land use management on soil microbial community responses have been documented in a variety of ecosystem types and climatic conditions (McKinley et al., 2005; Bradley et al., 2006; Wang et al., 2006; Rousk, and Bååth, 2007; Stark et al., 2008; Denef et al., 2009). Shifts in vegetation community in response to land use conversion as well as management intensity have been shown to have major effects on soil microbial community abundance, composition and activity. McKinley et al. (2005) found that the conversion of farmland into prairie increased total microbial biomass with a greater proportion of bacteria relative to fungi. Research has also demonstrated that different grass species can change the relative abundance of fungi, actinomycetes, and gram negative bacteria (Perkins and Nowak, 2013). It was suggested in previous studies that vegetation composition affects the amounts and quality of C inputs with consequent effects on soil microbial community structure (Batten et al., 2006), especially in the rhizosphere (Kowalchuk et al., 2002). Plant species composition can also affect labile C inputs such as root exudates, dead root cells, and fine root turnover (Shamoot et al., 1968).

Soil microbial community can also be affected by land use management practices such as application of nitrogen (N) fertilizer (Denef et al., 2009) and grazing activity (Ingram, 2008). Microbial community responses to land use intensification are often associated with changes in the quality and quantity of C substrates. Previous
studies showed overgrazing can deplete high quality C substrate and, consequently, reduce microbial biomass and activity (Ingram, 2008). Root exudates may also respond to grazing intensity, which in turn, can affect the functional diversity and activity of soil microbial communities (Bardgett et al., 1998; Hamilton and Frank, 2001). There is a paucity of information on important questions regarding how microbial community (biomass and structure) and microbial-mediated C decomposition processes change in response to increasing intensification in grazing land ecosystems, particularly in subtropical regions.

Phospholipid fatty acid (PLFA) analysis is a commonly used procedure to evaluate biomass and structure of soil microbial community (Frostegård and Bååth, 1996). PLFA analysis is widely known as a reproducible, sensitive, and rapid method although studies also suggested that this method can poorly detect some specific microbial groups, which may represent a major limitation of this procedure (Frostegård et al., 2011). This technique is often utilized to evaluate the impact of land uses on microbial community composition and diversity in different ecosystems and environments (Yao et al., 2000; Harris, 2003; Steenwerth et al., 2006; Denef et al. 2009; Inglett et al., 2011). Lovell et al. (1995) used PLFA analysis to evaluate changes in microbial biomass after repeated N fertilization. These authors observed no differences in microbial biomass as affected by short-term (1 yr) N application but significant changes were observed after 10 yr. Because microbial responses may not be detectable in the short-term (Bradley et al., 2006), it is important to consider the duration and intensity of soil disturbance on microbial community responses.
The changes in organic matter inputs caused by different land uses will have impacts on enzyme activities. Thus, enzyme activity is a sensitive indicator of microbial responses to different management practices (Dick, 1994). One of the most important enzymes related to soil C cycling is β-glucosidase. It plays a central role in the soil C cycle as it can catalyze cellulose degradation by cleaving cellobiose to glucose, which is an important energy source for soil microbes (Turner et al., 2002). Many previous studies observed changes in β–glucosidase activity under different land-use practices (Bandick and Dick, 1999; Acosta-Martinez et al., 2008; Stott et al., 2010).

The objective of this study was to investigate microbial biomass, activity and community structure responses to long-term (> 20 yr) land use intensification in subtropical grazing lands subjected to different management intensities.

**Methods**

**Study Area**

The experiment was located in Ona, Florida (27°23'N, 81°57'W) and consisted of three field replicated grazing land ecosystems: native rangeland, silvopasture, and sown pasture subjected to different intensification levels. Management intensity (defined here as the introduction of productive plant species, greater N input, and greater stocking rate) was low in native rangeland and relatively high in silvopasture and sown pasture. The sites were consistently managed for more than 20 years. The climate is characterized by mean minimum and maximum temperature of 17 to 28° C, respectively. Mean annual precipitation is ~ 1650 mm. The topography is flat with slope < 2%. Main soil series included Ona fine sand (sandy, siliceous, hyperthermic Typic Alaquods,) and Smyrna sand (sandy, siliceous, hyperthermic Aeric Alaquods).
Native rangeland was subjected to burning every 3 to 4 years, and it has no previous history of fertilizer application. The predominant vegetation in the rangeland consists of shrubs and perennial grasses, including saw palmetto (\textit{Serenoa repens Bartr.}), chalky bluestem (\textit{Andropogon capillipes Nash}), broomsedge bluestem (\textit{Andropogon virginicus L.}), creeping bluestem (\textit{Schizachyrium stoloniferum Nash}), wiregrass (\textit{Aristida stricta Michx.}), and forbs including red root (\textit{Lachnanthes caroliniana Lam.}) and golden rod (\textit{Solidago fistulosa Mill.}) (Kalmbacher et al., 1984). Silvopasture consisted of a 12-yr-old stand of slash pine (\textit{Pinus elliotti} Engelm) trees planted into a Pensacola bahiagrass (\textit{Paspalum notatum} Flueggé) pasture. Silvopasture sites received 67 kg N ha\(^{-1}\) per year in 1998, 1999, 2001, 2003, 2007 and 2010. Sown pastures were a 31-yr-old stand of bahiagrass rotationally stocked with a 7-day grazing period followed by a 7 days resting period and receiving annual N application of 67 kg ha\(^{-1}\) since 1991.

Grazing intensity was 125, 207, and 360 animal days ha\(^{-1}\) yr\(^{-1}\) for native rangeland, silvopasture, and sown pasture, respectively. More detailed information about the experimental sites are presented in Adewopo et al. (2014).

**Soil Sampling and Microbial Biomass Determination**

In each experimental unit, five 20 m × 20 m quadrats were established ~ 75 m apart along a diagonal transect. Eight random soil cores (2 cm x 20 cm) were collected (0 to 10 and 10 to 20 cm depths) from each sampling quadrat and composited within soil depth. Soil sampling occurred in the summer (September, 2012) and was repeated in the winter (January/February, 2013). Immediately after collection, soil samples were placed in plastic bags and stored in a cooler with ice until being transported to the lab. Coarse roots and rocks were removed and soil moisture concentration was determined
by oven-drying a sub-sample at 105°C for 48 hours. Samples were divided into two subsamples and stored separately at either 4°C or -20°C.

Microbial biomass C and N (MBC and MBN) concentrations were estimated using the chloroform fumigation-extraction method (Vance et al., 1987). Briefly, one set of subsamples (2.5 g soil each) stored at 4°C were equilibrated with 25 ml of 0.5-M K₂SO₄, shaken for 1 hour on a longitudinal shaker, and vacuum-filtered through #41 Whatman filter paper. The second set was fumigated with ethanol-free chloroform (24 h; 25°C) before extraction. Total organic C (TOC) in the extracts was measured in a Shimadzu TOC-5000A analyzer (Columbia, Maryland). Microbial biomass C concentration was calculated by subtracting the extractable TOC in non-fumigated soils from extractable TOC in chloroform-treated soil. A set of subsamples of the extracts were digested with sulfuric acid and copper sulfate mixture (EPA method 351.2, 1993) and analyzed for NH₄-N concentration using a Technicon AutoAnalyzer (Seal Analytical, Mequon, Wisconsin) for microbial biomass N determination as described for MBC.

**Microbial Activity**

Potential C mineralization rate was estimated using the laboratory incubation method (Zibilske, 1994). Approximately 5 g of fresh, moist soil was placed into a 150-ml flask, and incubated in the dark at 25°C for 10 days. Soil moisture concentration was maintained at 60% water-filled pore space to maximize aerobic microbial activity (herein determined by CO₂ production). The CO₂ concentration in the headspace was measured on days 1, 2, 3, 4, 7, 8, 9, and 10. At each sampling date, a 500-mL gas sample was collected from the headspace and injected into a PerkinElmer Clarus 400 gas chromatograph (GC) (Waltham, Massachusetts). The gas chromatograph was equipped with a Poropak N (Supelco, Bellefonte, Pennsylvania) column and
thermal-conductivity detector (TCD) for CO₂ analysis. A linear regression between CO₂ concentration and incubation time was used to calculate the potential C mineralization rate.

Potentially mineralizable N was estimated using the anaerobic incubation procedure (White and Reddy, 2000). Briefly, 2.5 g of soil received 5 ml of distilled de-ionized (DDI) water and was incubated under anaerobic conditions at 40°C for 10 days. After the incubation, 20 mL of 2-M KCl solution was added to the samples and the slurry was shaken on a longitudinal shaker for 1 hour. Suspension was transferred to centrifuge tubes and centrifuged for 10 minutes at 6000 rpm. The solution was then filtered through a Whatman #41 filter paper and analyzed for NH₄-N concentrations. Another set of subsamples (2.5 g soil) was reacted with 25 mL of 2-M KCl solution to obtain extractable NH₄ prior to incubation. All extracts were analyzed for NH₄-N on an AQ₂ discrete analyzer (SEAL Analytical, Inc., Mequon, Wisconsin). Potentially mineralizable N was calculated as the difference in NH₄-N concentration before and after incubation.

β-Glucosidase Enzyme Activity

Activity of β-glucosidase enzyme was measured using a modified fluorometric procedure previously described by Waldrop et al. (2004) and German et al. (2012). Briefly, 2 g of field-moist soil was exposed to 98 ml of sodium acetate buffer (pH 5.0) and homogenized to remove aggregations. The suspension was incubated at 20°C with 100μL of methylumbelliferyl (MUF) substrate solution for 4 hours. Formation of the fluorescent product MUF during this period was measured at excitation/emission wavelengths of 360/460 in a fluorometer (Biotek, Winooski, Vermont). Before the
enzyme assays were measured in the fluorometer, 10 μL of 2-Μ NaOH were added to each sample to adjust pH to 10.

Determination of buffer pH and concentration of enzyme substrates followed the procedure described in German et al. (2011). Also, quenching of the fluorescent product by the soil matrix was measured by making quenching curves. β-Glucosidase activity was reported as nmol MUF released g⁻¹ soil h⁻¹, and β–glucosidase specific activity was also analyzed by normalizing the β–glucosidase activity by MBC (mol kg⁻¹ MBC h⁻¹).

**Microbial Community Structure**

Phospholipid fatty acid analyses were conducted on the frozen soil samples. Total lipids were extracted from frozen-dried soil (equivalent to 4.0 g of dry soil) using a modified chloroform - methanol extraction procedure described by Bligh and Dyer (1959). After extraction, PLFA was then separated from other lipids using solid phase extraction and then identified using an Agilent 7820A Gas Chromatograph (Santa Clara, California). The peaks were identified using bacterial standards and identification software from the microbial identification system (Microbial ID, Inc., Newark, Delaware). We analyzed the total PLFA and also PLFA in groups (Findlay and Dobbs, 1993; Kourtev et al., 2003; Bradley et al., 2006; Steenwerth et al., 2006; Ingram et al., 2008; Denef et al., 2009; Inglett et al., 2011; Jangid et al., 2011). For example, the fatty acids of bacterial origin included gram positive biomarkers (a 13:0, i13:0, a14:0, i14:0, a15:0, i15:0, i15:1, i16:0, a17:0 and i17:0), gram negative biomarkers (10:0 3OH, 12:0, 12:0 2OH, 12:0 3OH, 14:0, 15:0, i15:0 3OH, 15:0 3OH, 16:1 2OH, 16:1w7c, 17:0, cy17:0, 17:1w7c, cy19:0) and general bacterial biomarkers (16:0 2OH, 16:0 nOH, 18:0 2OH, 11,12cy 19:0). Fungal original PLFA included 18:1w9c, 18:2w6c, 18:3w6c and 16:1w5c. Also, the ratio of fungal/bacterial and bacterial gram positive/gram negative were
analyzed. The PLFA nomenclature is recognized as A/BωC, where “A” stands for the total number of C atoms in the fatty acid, “B” identifies the number of double bonds from the aliphatic (ω) end of the molecule and “C” is the C atom from the aliphatic end before the double bond. They may be followed by “c” or “t” which mean cis or trans configuration. And the prefix such as “i” or “a” are iso or anteiso branching. Additionally, “me” identifies mid-chain branching and “cy” shows cyclopropyl fatty acids.

**Statistical Methods**

Data were analyzed using SAS PROC MIXED procedure (SAS 9.4, 2013). The impacts of land use types on soil microbial biomass, activities, community composition and enzyme activity were analyzed within each soil depth. Land use types, season, and their interactions were considered as fixed effects and replicates were considered random effects. The PDIF test of the LSMEANS procedure was used to compare means. Treatments and their interactions were considered significant if F-test P values were ≤ 0.05.

**Results**

**MBC and MBN Concentration**

No seasonal effect (winter vs. summer sampling) was observed on MBC, thus data represent the average across seasons. Microbial biomass C concentration was greater in sown pasture at the 0 to 10 cm soil depth (334 mg kg⁻¹) compared to silvopasture and native rangeland (193 and 232 mg kg⁻¹, respectively) but no effect was observed in the 10 to 20 cm depth (Figure 5-1A).

Microbial biomass N also affected by land use types at the 0 to 10 cm depth (Figure 5-1B). Averaged across summer and winter sampling, bahiagrass showed greater MBN concentrations than silvopasture at 0 to 10 cm (34 vs. 24 mg kg⁻¹,
respectively). Although no interaction between land use management and season was detected, there was a seasonal effect on MBN at the 0 to 10 cm depth, and MBN was smaller in winter (24 mg kg\(^{-1}\)) than in summer (33 mg kg\(^{-1}\)). Similar to MBC, MBN concentrations decreased with soil depth.

**Microbial Activity**

Microbial activity (measured as potentially mineralizable C and N) was promoted in sown pasture (Figure 5-2A and B). At the 0 to 10 cm depth, potentially mineralizable C was greater in sown pastures (1.2 mg CO\(_2\)-C kg\(^{-1}\) d\(^{-1}\)) than the other land uses (0.5 and 0.6 mg CO\(_2\)-C kg\(^{-1}\) d\(^{-1}\) for native rangeland and silvopasture, respectively); however, no treatment effect was observed in the 10 to 20 cm depth. Potentially mineralizable C decreased with soil depth and no seasonal effect was observed.

Management intensification also increased PMN concentration. At 0 to 10 cm depth, the greatest PMN was associated with sown pasture (4.4 mg kg\(^{-1}\) d\(^{-1}\)) followed by silvopasture (2.9 mg kg\(^{-1}\) d\(^{-1}\)) and native rangeland (1.9 mg kg\(^{-1}\) d\(^{-1}\)). At the 10 to 20 cm depth, sown pasture and silvopasture had greater PMN concentration than native rangeland (1 and 0.9 mg kg\(^{-1}\) d\(^{-1}\) vs. 0.7 mg kg\(^{-1}\) d\(^{-1}\)). PMN concentrations decreased with soil depth. On average, PMN was greater in winter (1 mg kg\(^{-1}\) d\(^{-1}\)) than in summer (0.8 mg kg\(^{-1}\) d\(^{-1}\)) at 10 to 20 cm depth.

**β-Glucosidase Activity**

Grazing land management intensification affected β-glucosidase activity and there was land use x season interaction (Figure 5-3A). At the 0 to 10 cm depth, the highest β-glucosidase activity was observed in sown pasture in summer (203 nmol g\(^{-1}\) soil hr\(^{-1}\)) followed by silvopasture and native rangeland (101 and 72 nmol g\(^{-1}\) soil hr\(^{-1}\), respectively). At 0 to 10 cm depth in winter, silvopasture and sown pastures showed
comparable β-glucosidase activities and both were greater than native rangeland (89 and 98 nmol g\(^{-1}\) soil h\(^{-1}\) vs. 44 nmol g\(^{-1}\) soil h\(^{-1}\), respectively). At 10 to 20 cm depth, silvopasture and sown pasture also had greater β-glucosidase activities compared with native rangeland in both seasons.

Greater β-glucosidase occurred in summer than winter in sown pastures at both depths (203 vs. 98 nmol g\(^{-1}\) soil h\(^{-1}\) for 0 to 10 cm, and 37 vs. 23 nmol g\(^{-1}\) soil h\(^{-1}\) for 10 to 20 cm), in native rangeland (72 vs. 44 nmol g\(^{-1}\) soil h\(^{-1}\) for 0 to 10 cm, and 22 vs. 12 nmol g\(^{-1}\) soil h\(^{-1}\) for 10 to 20 cm) and in silvopasture at 10 to 20 cm depth (49 vs. 22 nmol g\(^{-1}\) soil h\(^{-1}\)). For all three ecosystems and both seasons, β-glucosidase activity was greater at the 0 to 10 cm depth than the 10 to 20 cm depth.

We also analyzed the response of specific β-glucosidase activity (per kg MBC) to land use management intensification (Figure 5-3B). Similar to enzyme activity, sown pastures showed the greatest specific β-glucosidase activity in summer at 0 to 10 cm depth, followed by silvopasture and native rangeland (0.7, 0.5 and 0.3 mol kg\(^{-1}\) MBC h\(^{-1}\), respectively). In contrast, in winter at 0 to 10 cm depth, silvopasture had greater specific activity than sown pasture and native rangeland (0.5 vs. 0.3 and 0.2 mol kg MBC\(^{-1}\) h\(^{-1}\), respectively). In summer at 10 to 20 cm depth, specific β-glucosidase activity was greatest in silvopasture (0.6 mol kg\(^{-1}\) MBC h\(^{-1}\)) and there was a trend that greater in sown pasture than native rangeland (0.3 vs. 0.2 mol kg\(^{-1}\) MBC h\(^{-1}\), \(P = 0.063\)).

Unlike β-glucosidase activity, specific β-glucosidase activity decreased with soil depth only in bahiagrass at summer sampling and silvopasture at winter sampling. Seasonal effect (summer vs. winter) was found in sown pasture at both depths (0.7 vs. 0.3 mol kg\(^{-1}\) MBC h\(^{-1}\) for 0 to 10 cm and 0.3 vs. 0.2 mol kg\(^{-1}\) MBC h\(^{-1}\) for 10 to 20 cm) and
in silvopasture at 10 to 20 cm depth (0.6 vs. 0.2 mol kg\(^{-1}\) MBC h\(^{-1}\)) while not in native rangeland.

**Phospholipid Fatty Acid Analysis**

Grazing land intensification promoted the total PLFA biomass in sown pasture (Figure 5-4). The greatest PLFA biomass was observed in sown pasture (222 µmol kg\(^{-1}\) soil) at 0 to 10 cm depth in summer, followed by native rangeland and silvopasture (179 and 116 µmol kg\(^{-1}\) soil, respectively). The land use type × season interaction was only significant at 0 to 10 cm depth. Despite the difference was not significant in winter at 0 to 10 cm depth, sown pasture exhibited greater PLFA biomass than native rangeland and silvopasture at the 10 to 20 cm depth, across two seasons (average of 115 vs. 82 and 89 µmol kg\(^{-1}\) soil for sown pasture, native rangeland and silvopasture, respectively). Total PLFA biomass decreased with soil depth. Seasonal difference was observed at 0 to 10 cm in sown pasture (222 vs. 131 µmol kg\(^{-1}\) soil for summer and winter sampling, respectively) and silvopasture (116 vs. 156 µmol kg\(^{-1}\) soil for summer and winter sampling, respectively).

Results showed that the relative abundance of bacteria (mol % of bacteria of total PLFA biomass) increased while fungi abundance decreased with grazing land intensification and was the greatest in silvopasture at 0 to 10 cm depth (Figure 5-5). Bacteria group relative abundance was the smallest in native rangeland, which accounted for 32 and 30 % of total PLFA biomass at 0 to 10 and 10 to 20 cm depth, followed by sown pasture (40 and 42% for two depths, respectively) and the greatest in silvopasture (51 and 51%). Similar trend was also detected in gram-negative bacteria. The greatest abundance of gram-negative bacteria was observed in silvopasture (36 and 33% for 0 to 10 and 10 to 20 depth, respectively), followed by sown pasture (23 and
26% for two depths, respectively) and native rangeland (19 and 17% for two depth, respectively). At 10 to 20 cm depth, sown pasture exhibited the greatest gram-positive bacteria relative abundance (12 vs. 9 and 9% for sown pasture, native rangeland and silvopasture, respectively).

A slightly difference was found in bacteria relative abundance between summer and winter sampling at 0 to 10 cm depth (39 vs. 43% respectively, \( P = 0.0082 \)). Seasonal difference was also found in gram- negative bacteria (averaged of 25 vs. 28 % for summer and winter sampling, \( P = 0.0221 \)) and in gram- positive bacteria relative abundance (9 vs. 10%, \( P = 0.0335 \)).

Unlike bacteria groups, the relative abundance of fungi decreased as grazing land intensification increased. The greatest fungi abundance was observed in the native rangeland (28 and 29% for 0 to 10 and 10 to 20 cm depth), compared to silvopasture (9 and 9% for two depths) and sown pasture (16 and 17% for two depths). Silvopasture also exhibited the smallest fungi relative abundance. No seasonal effect was observed in fungi relative abundance.

The ratio of gram-positive: gram-negative bacteria was also affected by grazing land intensification (Figure 5-6). At 0 to 10 cm depth, silvopasture had the lowest ratio (0.25 for silvopasture vs. 0.48 and 0.52 for native rangeland and sown pasture, respectively) because of its greater gram negative relative abundance. The ratio was also higher in native rangeland at 10 to 20 cm depth (0.53) compared the silvopasture (0.35).

Differently, the ratio of bacteria: fungi was the highest in silvopasture (~6), followed by sown pasture (~2) and the smallest in native rangeland (~1) for both depths.
Discussion

Microbial Biomass and Activity

Management intensification increased MBC, MBN, potentially mineralizable C and N, and β-glucosidase activity, particularly in the top 10 cm soil depth. These results are consistent with previous reports of a positive effect of land use intensification on microbial biomass and activity (Mawdsley and Bardgett, 1997; Wang et al. 2006; Mandal et al. 2007). More intensively-managed systems (here defined as greater N inputs and grazing intensity) can promote C accumulation in labile forms that are easily utilized by soil microbes. Labile C fractions are often unprotected by soil colloids, therefore, they can be quickly utilized by the soil microbial community (Dalal and Mayer, 1986). Previous studies demonstrated that particulate organic C and N increased as grazing land intensification increased (Adewopo et al., 2014). Increased particulate organic matter and N are also expected to promote N mineralization and turnover (Six et al., 2002), and therefore enhance microbial biomass and activity.

Positive impact of N fertilization on microbial communities has been reported in the literature. Mandal et al. (2007) observed an increase in MBC and MBN in response to a long-term (34 year) history of N fertilization to cropland systems. The increase in microbial biomass in sown pasture observed in the current study was likely due to greater N availability compared with the other ecosystems. Moreover, N addition can also increase litter deposition (Foster and Gross, 1998; Dubeux et al., 2006c) and turnover (Dubeux et al., 2006b), which promotes nutrient cycling and microbial activity. Similar to N additions, grazing promotes microbial activity as compared with non-grazed systems. Under similar environmental conditions as the current study, Wang et al. (2006) observed greater MBC and N in grazed bahiagrass compared with pastures not
subjected to grazing for 6 years. Grazing often improves litter quality (Barger et al., 2004), decomposition and soil N mineralization (Shariff et al., 1994). However, high stocking rate can also decrease litter deposition and C inputs (Rezend et al., 1999; Naeth et al., 1991) with subsequent negative impacts on microbial-mediated processes. Grazing can also break down litter particles into smaller pieces and promote physical contact with soil, resulting in faster C decomposition (Naeth et al., 1991). Furthermore, grazing activity can increase root decomposition or exudation which, in turn, may increase microbial biomass (Mawdsley and Bardgett, 1997).

**β-Glucosidase Enzyme Activity**

Results showed that β-glucosidase activity was increased by grazing land intensification. The greater β-glucosidase activity in silvopasture and sown pasture compared with native rangeland suggest that β-glucosidase activity can be used as an indicator of changes in land use management, which has also been shown previously (Knight and Dick, 2004). The β-glucosidase activity in sown pasture was, on average, ~0.5 to 1.8 times greater than that in native rangeland. Silvopasture had lower β-glucosidase activity than sown pasture, but it was 1.4 to 2 times of that native rangeland. Our results showed that sown pasture and silvopasture had greater SOC associated with LF fraction than native rangeland. Thus it is possible that grazing land intensification supplies more substrate for β-glucosidase.

The positive effect of grazing land intensification on β-glucosidase activity has been related to increases in C deposition and turnover rate (Neill et al., 1997). Acosta-Martinez et al. (2007) studying the response of β-glucosidase activity to land use change found that pastures showed greater activity than forest due to grazing and excreta deposition. In our study, sown pasture had greater enzyme activity than
silvopasture only at the 0 to 10 cm depth in summer. Our findings suggest that sown pasture had a greater accumulation of labile organic C in summer while silvopasture had a relatively consistent amount of available substrate for microorganisms in soil through a year.

Our data showed a general decline of β-glucosidase activity from summer to winter sampling. This change in β-glicosidase activity may due to the decline in available substrate for soil microbes with less soil organic matter inputs in winter. The β-glicosidase activity declined with soil depth due to the lower soil microbe biomass deeper in the profile.

It has been shown that specific enzyme activity is more closely related to microbial communities than enzyme activity, particularly gram-negative bacteria (Waldrop et al., 2000). In our study, specific enzyme activity response to management intensification was consistent with enzyme activity at the 0 to 10 cm depth for summer sampling, which was greatest in sown pastures, followed by silvopasture and native rangeland. Conversely, silvopasture exhibit greater specific β-glucosidase activity than native rangeland and sown pastures at 0 to 10 cm depth for winter sampling and 10 to 20 cm depth for summer sampling. It is possible that the specific β-glicosidase activity was inhibited in sown pasture and native rangeland by less available substrate or changed microbial community structure in winter and at lower depth.

**PLFA**

In this study, 77 different PLFA biomarkers were detected and used to calculate total PLFA biomass. Our PLFA data were consistent with soil MBC and indicated that microbial biomass was promoted in sown pasture. We also detected greater relative
abundance of gram-positive and gram-native bacteria and inhibited fungi as grazing land intensification increased.

A previous study found that conversion from farmland to prairie could increase total PLFA biomass (McKinley et al., 2005). However, it was suggested that land use history is a more important factor to affect soil microbial community (Stark et al., 2008; Jangid et al., 2011). Grazing management can affect the microbial community. Our results were consistent with previous study which found an increase of total PLFA biomass under proper grazing intensities (Ingram et al., 2008). Despite no difference was detected in the abundance of soil microbial groups from the study of Ingram et al. (2008), they found a changed structure of microbial community.

In our study, the increase of total PLFA biomass and bacteria relative abundance and the decrease of fungi relative abundance in silvopasture and sown pasture were also likely related to N fertilization. The increase of bacteria:fungi ratio due to the use of N fertilization was also found by Frey et al. (2004) and Bardley et al. (2006). Previous studies also observed greater total PLFA biomass, relative abundance of gram-positive bacteria or smaller relative abundance of fungi and gram negative bacteria in grasslands receiving N fertilization (Peacock et al., 2001; Bradley et al., 2006; Denef et al., 2009; Högberg et al., 2007). In our study, sown pasture had the greatest N input, which might be the reason for it to have smaller relative abundance of gram-negative bacteria than silvopasture. In our study, the increased PLFA biomass was consistent with greater labile soil C in sown pasture. It has been also suggested that greater N availability or cycling rates will increase bacterial abundance and decrease fungal percentage in soil microbial community (Högberg et al., 2003; Bradley et al., 2006). In
our study, we found greater potentially mineralizable N in silvopasture and sown pasture, suggesting greater available N and N cycling which could promote the change in soil microbial community. It was indicated in other study that less disturbed terrestrial ecosystems with lower N input and management intensification tend to be dominated by fungal groups (Bardgett and McAlister, 1999). Native rangeland in our study had the smallest disturbance since was under the lowest grazing land intensification, which may be the reason for native rangeland had the greater fungal relative abundance than sown pasture and silvopasture.

**Summary and Conclusions**

Soil microbial biomass and activity were promoted by long-term grazing land intensification. Sown pasture exhibited the greatest MBC, MBN, PMC and PMN among three land use types. The conversion of native rangeland to more intensively managed silvopasture and sown pasture also increased β-glucosidase enzyme activity. Results from this study suggested that grass-dominated grazing lands subjected to more intensive management have the potential to increase labile C input and subsequently promote microbial biomass and activity. Data also demonstrated that extensively managed native rangeland has a higher relative abundance of fungal while more intensively managed silvopasture and sown pasture had more bacteria in microbial community.
Figure 5-1. Soil MBC and MBN concentrations as affected by grazing land intensification. A) MBC. B) MBN. Bars represent means across summer and winter sampling ± standard error. MBC and MBN within soil depth followed the same letter are not different ($P > 0.05$) based on the LSD test.
Figure 5-2. Potentially mineralizable C (PMC) and N (PMN) as affected by grazing land intensification. A) PMC. B) PMN. Bars represent means across summer and winter sampling ± standard error. Means within soil depth followed the same letter are not different ($P > 0.05$) based on the LSD test.
Figure 5-3. β-Glucosidase activities and specific β-glucosidase activities as affected by grazing land intensification. A) β-Glucosidase activities. B) β-Glucosidase specific activities. Bars represent means ± standard error. Enzyme activities followed the same letter within each season and soil depth were not different based on the LSD test (P > 0.05)
Figure 5-4. PLFA biomass as affected by grazing land intensification. Data represent the 0 to 10 cm depth. Bars represent means ± standard error. PLFA followed the same letter within each season and soil depth were not different based on the LSD test (P>0.05).
Figure 5. Gram positive and negative bacteria, bacterial and fungi as affected by grazing land intensification. Bars represent means ± standard error. PLFA followed the same letter within each soil depth were not different based on the LSD test (P>0.05).
Figure 5-6. The ratio of gram-positive: gram negative bacteria and bacteria and fungi as affected by grazing land intensification. Bars represent means ± standard error. PLFA followed the same letter within soil depth were not different based on the LSD test (P>0.05).
CHAPTER 6
CONCLUSIONS

Long-term grazing land intensification (herein defined as change in vegetation composition, grazing management, and N fertilizer inputs) can favor ecosystem and SOC accumulation. However, C allocation through the various ecosystem components can be altered by grazing land intensification. The incorporation of trees into grass-based grazing land ecosystems resulted in greater C allocation in aboveground biomass. Conversely, the majority of ecosystem C is associated with SOC in native rangeland (shrub-dominated) and sown pastures (grass-dominated).

Conversion of native rangeland into more intensively managed silvopasture and sown pasture systems also promoted SOC fractions in subtropical ecosystems. Both sown pasture and silvopasture had greater SOC, POC, mineral-C than native rangeland. Despite the comparable SOC stocks, a greater proportion of SOC in sown pasture was associated with the POC pool, while in the silvopasture mineral-C was more predominant. Management intensification affected SOC distribution into aggregate sizes fractions. Carbon concentration increased with management intensification in all aggregate sizes and mass of C was increased in macroaggregates and microaggregates. Carbon associated with LF C and iPOM from microaggregates was also enhanced in sown pasture and silvopasture. The sum of C associated with LF from macroaggregates and microaggregates was the greatest in sown pasture. These results of different fractionation methods showed that sown pasture accumulated more labile C in soil while silvopasture had more SOC in stable state. We also found that C associated with silt+ clay fractions was sensitive to ecosystem management in subtropical sandy soils despite of its low contribution to soil mass.
Consistent with the results from ecosystem C and SOC, our data showed that microbial biomass and activities were also promoted by grazing land intensification, especially in sown pasture. The relative abundance of bacterial groups was promoted while the fungal groups were inhibited with grazing land intensification. Our study suggested that introduction of more productive plant species and adoption of more intensive grazing and fertilization management can be beneficial for enhancing C sequestration in terrestrial ecosystems. Data also showed that long-term management intensification had a positive effect on SOC in subtropical grazing lands and management methods affected the pattern of distribution into different C pools and microbial communities and activities. Further studies examining the characteristics and stability of C associated with increased level of intensification are warranted.
LIST OF REFERENCES


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BIOGRAPHICAL SKETCH

Sutie Xu was born in China. She majored in plant science and technology as an undergraduate student at Shandong Agricultural University. Her major was aimed for students hoping to develop a broad background in agriculture, plant protection and horticulture.

In 2007, Sutie received her bachelor’s degree and started her master’s program in ecology at the Institute of Botany, Chinese Academy of Sciences. To better understand the environmental change, she conducted her study at the Inner Mongolia Grassland Ecosystem Research Station (Chinese Academy of Sciences). The main focus of Sutie’s master’s research program was biodiversity and ecosystem functioning, which was based on a project carried out on an area of typical grassland in Inner Mongolia. In particular, she studied the responses of below-ground ecosystem function (soil C, N and P processes) to variation of plant functional groups. Her work focused on soil C, N and P, the soil microbial properties, the potential SOC mineralization and N transformation, etc.

In 2011, Sutie entered the Soil and Water Science Department of the University of Florida for her PhD program. Advised by Dr. Maria Silveira, Sutie conducted her study at Ona, Florida at the Range Cattle Research and Education Center (RCREC). Her PhD program focused on investigating the effects of long term land use change on ecosystem C in subtropical grazing lands. During her PhD period, Sutie received a graduate student research grant awarded by Southern Region USDA Program on Sustainable Agriculture Research and Education (SARE). She was also recognized as the third place winner in a graduate student poster competition at American Society of Agronomy Southern Branch Annual Meeting, in 2013. After graduation, Sutie plans to
pursue her research career at Michigan State University as a post-doctoral fellow and her work will focus on soil C cycling in grazing land.