

ABSTRACT

COOK, RACHEL LOUISE. Long-term Effects of Forest Plantations on Soil Carbon in Brazil. (Under the direction of Dr. Jose Luiz Stape and Dr. H. Lee Allen).

Human activity is increasing atmospheric carbon dioxide at an unprecedented rate. Deforestation and forest degradation, primarily in the tropics, account for the second largest contribution of carbon to the atmosphere following the burning of fossil fuels. One method to reduce greenhouse gas emissions includes reforestation. Forest establishment increases aboveground carbon sequestration, but effects on belowground carbon are poorly understood. Current evidence supports soil carbon gains from reforestation of cropland, but changes in soil carbon following conversion of pasture to broadleaf or conifer plantation tree species are less clear. In Brazil, approximately 6.5 million hectares have been converted to forest plantations, primarily in the Southeast. There is poor understanding concerning tree species' effects (such as *Eucalyptus* and *Pinus*) and the long-term consequences of short-rotation plantation management on soil carbon stocks. To address these issues, our objectives were to: 1) quantify total soil organic carbon stocks (SOC_T) in pasture, conifer, and broadleaf plantations to a 45 cm depth following up to 34 years of pasture reforestation; 2) quantify mineral soil carbon turnover from 0-45 cm with stable carbon isotopes following reforestation of C4 pasture with C3 forests; and 3) quantify the stocks of soil carbon and their changes following approximately two decades of short- (6-7 year) rotation *Eucalyptus* silviculture in Eastern Brazil.

For our first objective, we sampled the organic horizon and three depths at 15-cm intervals up to 45 cm, referred to as SOC_T , to compare ten Pasture-Broadleaf and ten Broadleaf-Conifer paired plots (ages 6 to 34 years). Results showed no difference in SOC_T between Pasture-Broadleaf plots (36.0 and 36.8 Mg C ha⁻¹, respectively), and only a 6% increase in SOC_T in Broadleaf compared to Conifers (38.3 and 36.0 Mg C ha⁻¹, respectively). There was no significant trend with age or total stemwood biomass and SOC_T , showing SOC_T to be fairly stable. For our second objective, we used the inherent differences in isotopic discrimination in C4-grass and C3-tree photosynthetic pathways to estimate changes

in the proportion of stable carbon isotopes in soil organic carbon. Pasture carbon (SOC₄) decreased at the same rate (0.21 Mg C ha⁻¹ yr⁻¹) that forest carbon (SOC₃) accumulated in the top 0-15 cm. There was a slight decrease in SOC₄ in the 15-30 cm and 30-45 cm soil layers (-0.17 Mg C ha⁻¹ yr⁻¹ and -0.083 Mg C ha⁻¹ yr⁻¹, respectively). Soil carbon turnover showed that SOC₃ becomes predominant over SOC₄ in the 0-15 cm layer following 9.7 years of reforestation, and 8 years in the 15-30 cm layer. The 30-45 cm layer remained dominated by SOC₃ from the original forest cover. Finally, to meet our third objective, we repeated a sampling of soils in 306 *Eucalyptus* plantations across three regions along the Eastern Coast of Brazil from 0-15 and 15-30 cm to quantify soil carbon stocks and changes following two decades of continuous forestry operations. We found that soil carbon stocks in the original sampling period (1984-1993), 2001, and 2010, as well as change over time depended on region, and to some extent: mean annual temperature, wet and dry seasonal precipitation, initial soil carbon content, and soil clay content. The study of soil carbon changes over time is important to understand the role of land-use change in the global carbon cycle and its potential for climate change mitigation. These studies provide a robust analysis of soil carbon changes following reforestation with different tree species in a homogeneous environment, and a single tree species across a heterogeneous landscape.

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Long-term Effects of Forest Plantations on Soil Carbon in Brazil

by
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DEDICATION

To my family for a lifetime of endless support and encouragement.

BIOGRAPHY

Rachel Louise Cook grew up on a cattle farm in southern Illinois before leaving home to attend high school at the Illinois Math and Science Academy. It was there that Rachel discovered her love for biology and research science, participating in heron rookery observation, working in the school greenhouse, and starting a botanical survey in Iowa.

Rachel enrolled at Saint Louis University in August 2001 where she majored in Biology and minored in Anthropology. She gained research experience in the lab and greenhouse, infecting soybeans with parasitic nematodes, extracting DNA, and running electrophoresis gels and western blots. For three summer field seasons, she traveled to Portal, Arizona to camp in the desert and collect data on ant populations. She studied abroad in Madrid, Spain for a semester and studied Cave Biology in the Ozarks of Missouri and Primate Ecology in Nicaragua.

Rachel went on to pursue a Master of Science at NC State University researching phytoremediation of petroleum contaminants. Her time at NCSU inspired greater interest in environmental sustainability, bioenergy, and permaculture and led her to continue on to a PhD program focusing on soil carbon sequestration in forest plantations in Brazil. Through this international research experience, she achieved one of her lifetime goals, learning Portuguese. During her PhD program, Rachel enriched her experience through participation in two intensive short courses: one in Porto Seguro, Brazil concerning Ecophysiology and Process-Based Modeling, and a Soil Science Institute in Fort Collins at Colorado State University. Dan Binkley and Jane Higgins were kind enough to host Rachel (and her broken foot) for six weeks in Fort Collins for the CSU Summer Soils Institute and one month of summer research.

Rachel plans to continue research in forest and soil systems, remediation, and restoration to work towards the goal of environmental sustainability both on the global and local scales. In her spare time she also likes to go road biking, garden, raise chickens and rabbits, play the ukulele, and teach hand spinning.

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INTRODUCTION

The rise in atmospheric CO₂ has been found to be the major driver of global climate change due to human activity such as burning fossil fuels and deforestation. Atmospheric carbon dioxide concentrations have increased from 280 ppm prior the Industrial Era (approximately 1750) to current levels of 380 ppm, and are expected to increase to 535 to 983 ppm by 2100 based on current trends (IPCC, 2007). In order to offset some of these emissions, there has been interest in establishing forests both for above- and belowground carbon sequestration (FAO, 2011; Lal, 2004).

The Role of Soil Organic Carbon

The organic carbon stored globally in soils accounts for more than three times that currently sequestered in plant biomass, and can act as a source or sink of carbon to the atmosphere depending on land use practices (Dixon *et al.*, 1994; Jobbágy & Jackson, 2000). From 1850-2005, it has been estimated that changes in land use and management have released approximated 160 Pg C into the atmosphere, with 60% of this total being released from tropical regions, mostly due to deforestation (Houghton, 2003; Richter and Houghton, 2011; FAO, 2011). Worldwide, forests act as a sink for atmospheric carbon, sequestering approximately 2.3 Pg C per year from 1990 to 2007 (Pan *et al.*, 2011), but gross sources from land-use change equal approximately 4.3 Pg C per year (Richter and Houghton, 2011). Soil organic carbon may provide an additional major sink for atmospheric carbon to help mitigate climate change.

Soil organic carbon makes up approximately half of the non-living portion of organic matter (Post *et al.*, 2001). Increasing the quantity of soil organic carbon also enhances soil quality by increasing cation exchange capacity, water-holding capacity and infiltration, soil structure, pH-buffering capacity, aggregation, and microbial diversity (Lal, 2004). These functions can be especially important in soils with low native fertility or in coarse-textured soils with low water-holding capacity (Zinn *et al.*, 2002).

Land-Use Change and Brazil

Tropical systems have received much attention concerning land-use change and carbon emissions due to high rates of deforestation and forest degradation (FAO, 2011). In fact, deforestation accounts for more than half of greenhouse gas emissions from Brazil (Cerri *et al.*, 2010). Reforestation can play a significant role in recovering degraded landscapes and sequestering soil carbon (Silver *et al.*, 2000). While soil carbon gains following reforestation of cropland have been documented (Guo and Gifford, 2002; Laganière *et al.*, 2010), there is a need for a better understanding of soil carbon change following forest plantation establishment on pasture.

Brazil provides an interesting landscape to address changes in soil carbon following land-use change. European colonization in the 1860s led to rapid deforestation of the Atlantic Forest nearest the coast. During the 1930s and 1940s, exploitation of more inland Atlantic forest continued and spread to the more western Savannah-like vegetation (Cerrado) from the 1950s to 1980s. From the 1990s to present, most deforestation pressure is located primarily in the Amazon and the Cerrado (Cerri *et al.*, 2010).

Deforestation and demands from a growing population in Southeastern Brazil has led to reforestation and development of plantation forestry. As a result of plantation policies initiated in the 1960s, by the 1980s Brazil became a major exporter of pulp and paper. Currently, essentially all pulp and paper products come from plantations (Sedjo, 1999). One common land-use sequence in this region consisted of: clearing of native Atlantic Forest for pasture or annual crops (later generally converted to pasture as production declined), and finally reforestation with *Eucalyptus* or *Pinus* species mainly for cellulose, wood products, firewood, or charcoal production (Turnbull, 2000). Plantation forests now cover approximately 6.5 million hectares in Brazil (ABRAF, 2011). The scale of this conversion from pasture to plantations necessitates, and provides an opportunity for, a better understanding of soil carbon changes following land-use conversion.

Factors Driving Soil Carbon Change

Changes in land use from pasture to forest can affect soil organic carbon stocks by creating different quantities and quality of plant inputs into the soil system. Altered environmental conditions also affect decomposition rates by influencing soil microclimate through changing sunlight penetration, soil moisture, and rates of evaporation.

Carbon inputs in soil can come from two major sources: aboveground litterfall or root turnover. Traditionally, it was thought that the litter layer was the main source of organic carbon for the mineral soils as it decomposes and is mixed into the mineral layer by soil biotic activity (Schmidt *et al.*, 2011). However, based on rates of decomposition, only a small quantity of the forest floor ever gets incorporated into mineral soils. Soil organic carbon accrual has been shown to be correlated with fine root growth, but not necessarily with leaf detrital input (Russell *et al.*, 2004). Therefore, it is more likely that most mineral soil carbon originates from plant roots.

There are clear differences between root systems of pasture and forest species. Trees are known to have deeper rooting systems than herbaceous plants as well as larger rooting biomass (Jackson *et al.*, 1996; Canadell *et al.*, 1996). Trees typically have higher fine root C:N ratio (70:1) and more lignin, making litter more difficult for microbes to degrade, whereas grass fine roots (C:N ratio of 44:1) should be more easily decomposable (Guo *et al.*, 2007). Though it has been thought that soil organic carbon (SOC) would increase with more lignified plant tissue input, a decline in SOC was shown with increasing lignin content in fine roots (Russell *et al.*, 2004). Decomposition of carbon inputs can be influenced by priming, or the ability of microbes to decompose new plant materials, but the significance of this effect is still controversial (Fontaine *et al.*, 2003).

Differences in soil carbon inputs and decomposability may explain why different tree species have a significant effect on soil carbon. Broadleaf plantations have been shown to increase soil carbon stocks to a greater extent than conifers (Guo and Gifford, 2002; Laganière *et al.*, 2010). However, this comparison between broadleaf and conifers in meta-analyses can be skewed due to the higher occurrence of conifers in higher latitudes

(Laganière *et al.*, 2010). Therefore, an accurate comparison of vegetation should be within the same climate.

Climate can have variable effects on soil carbon storage since rainfall and temperature affect both inputs from plant production and outputs from microbial decomposition. Soil carbon tends to remain constant or decrease following reforestation of pasture in sites where rainfall is less than 1250 mm per year, but can lose 10-15% where rainfall is between 1200-1500 mm. When annual rainfall exceeds 1500 mm, 25% of soil carbon can be lost to the atmosphere (Guo and Gifford, 2002). This correlation between rainfall and soil carbon sequestration has been linked to soil nitrogen losses from leaching (Kirschbaum *et al.*, 2008).

Soil nitrogen plays a key role in plant productivity and is tightly coupled with decomposition or stabilization of soil carbon (Maquere *et al.*, 2008). Sources of nitrogen in forest plantations primarily include fertilization, wet and dry atmospheric deposition, and biological N-fixation where leguminous plant species are present. Nitrogen can be lost from the soil system through volatilization from burning, leaching, erosion, and tree harvesting (Laclau *et al.*, 2005; Laclau *et al.*, 2010). The complex interactions of soil nitrogen and carbon cycling will require much future research to understand biogeochemical functioning of forests.

Soil properties such as clay content and mineralogy can play a significant role in soil carbon change over time (Don *et al.*, 2010; Laganière *et al.*, 2010). Soil mineralogy, particularly iron and aluminum oxide content, is well correlated with soil carbon, but is secondary to textural controls (Zinn *et al.*, 2007). Chemical, physical, or biological protection in the mineral soil can prevent microbial decomposition of soil organic carbon. Specific mechanisms include selective preservation, interactions with surfaces and metal ions, and spatial inaccessibility (Six *et al.*, 2002; von Lutzow *et al.*, 2007).

Other factors affecting soil carbon change over time include management practices. When pre-planting disturbance is great, including extensive heavy machinery traffic or burning, this can cause greater losses in soil carbon during plantation establishment (Laganière *et al.*, 2010). Management practices, such as longer rotations, retaining forest

litter, fertilizer application, and reducing fire intensity may lessen impacts on soils and help retain soil organic carbon over time (Paul *et al.*, 2002).

Stable Carbon Isotopes

Stable carbon isotope techniques can be utilized to gain a greater understanding of the dynamics of soil carbon following land-use change. Naturally occurring ^{13}C isotopes are incorporated from the atmosphere into plant tissue at different rates based on the photosynthetic pathway of the plant (Balesdent *et al.*, 1987). Most tropical pasture grasses use a C4-photosynthetic pathway, which incorporates more ^{13}C into plant tissue than trees, which use a C3-photosynthetic pathway, and discriminate against ^{13}C to a larger degree (Ehleringer *et al.*, 2000). Since soil organic carbon maintains essentially the same $\delta^{13}\text{C}$ signature as the plant inputs, soil carbon turnover can be estimated when C4 pastures are converted to C3 trees (Bernoux *et al.*, 1998). The history of land-use change in Brazil, from original C3 Atlantic Forest, to C4 pasture, back to C3 tree plantations, and the high plant productivity and rapid carbon cycling in soils provides a valuable setting for investigating soil carbon dynamics associated with land use change.

Soil Carbon Sampling

Sampling methodology plays a significant role in carbon monitoring. Soils in the landscape are inherently heterogeneous due to variation in parent material, landscape position, vegetation history, and previous land uses. Many studies may confound treatment effects with this inherent variation in the landscape without careful consideration when selecting paired sites and chronosequences. There is a great need for careful, repeated soil sampling (Richter *et al.*, 2007). Depending on the system in question and the percent change in soil carbon, three to 20 years may be needed to determine soil carbon stocks with an acceptable confidence (Post *et al.*, 2001; Smith, 2004).

Additionally, typical soil sampling depths average approximately 30 cm for soil organic carbon studies (Laganière *et al.*, 2010). Only recently has more attention been paid to understanding deep soil carbon and the effects of land-use change. Deep rooting activity

may actually reactivate the decomposition of previously stable, buried carbon. Therefore, future investigation considering deeper soil profiles may be critical to understand the roles soils play in the global carbon cycle (Fontaine *et al.*, 2007).

Aims and Objectives

The purpose of the three following studies is to examine the effects on soil carbon stocks following reforestation in tropical regions with plantation tree species. Our objectives were to: 1) determine the impact of species effect on soil carbon stocks to a 45 cm depth following reforestation of pasture to conifer and broadleaf plantations, 2) quantify soil carbon turnover to 45 cm using stable carbon isotopes and a chronosequence of C3 forest plantations that replaced C4 tropical pasture grasses over a period of 34 years, 3) quantify soil carbon stocks and changes from 0-30 cm soil depth over time following the repeated soil sampling of 306 operational *Eucalyptus* plantations across the Brazilian Eastern Coastal Region and assess soil and climate conditions that may be the driving factors. Understanding the consequences of vegetation effects and long-term silviculture on soil organic carbon can help to elucidate the role that soils may play in mitigating climate change, and indicate sustainability of plantation forestry in Brazil.

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Chapter 1 – Total soil carbon stocks following conversion of tropical pasture to broadleaf and conifer plantations

Abstract

Forest establishment on agricultural land has been proposed as a means to increase soil carbon sequestration. Changes in soil organic carbon following forest establishment on pastures have been shown to depend on tree species, among other environmental factors. We sampled a 34-year chronosequence of conifer and broadleaf plantations to quantify soil carbon stocks following reforestation of cattle pasture in a sandy Oxisol (Typic Hapludox) in southeastern Brazil. Adjacent, paired plots consisted of ten Pasture-Broadleaf (P-B) and ten Broadleaf-Conifer (B-C) plantations. Pasture species consisted of primarily *Brachiaria decumbens*. Broadleaf plantations were primarily *Eucalyptus*, but also included one plot of *Liquidambar styraciflua*, one of *Gmelina arborea*, and one of *Dalbergia nigra* (a native leguminous tree species). Conifer stands were made up of *Pinus* species. Average aboveground productivity of stemwood for broadleaf and conifer plantations (\pm standard error) was 19.4 (\pm 2.0) and 11.4 (\pm 1.0) Mg dry matter ha⁻¹ yr⁻¹, respectively. Total soil organic carbon (SOC_T), which included the organic horizon and the top 45 cm of mineral soil, in P-B paired plots averaged (\pm standard error) 36.0 \pm 1.7 Mg C ha⁻¹ in pastures and 36.8 \pm 1.9 Mg C ha⁻¹ in broadleaf plantations. For B-C paired vegetation plots, broadleaf plantations averaged (\pm standard error) 38.3 \pm 1.9 Mg C ha⁻¹ and conifer plantations averaged 36.0 \pm 1.6 Mg C ha⁻¹. Our results show no significant difference in SOC_T between broadleaf plantations and pasture paired plots, but a small increase (6%) in SOC_T in broadleaf plantations compared to conifers. Results from the chronosequence approach (stand ages from 6-34 years) showed no significant relationship between age of forest stand and SOC_T. Our results provide evidence that conifer and broadleaf plantations maintain similar levels of total soil organic carbon to pasture land-use up to 34 years following land conversion.

Introduction

Plants sequester atmospheric carbon in living biomass and provide inputs for soil organic matter. Increasing soil organic matter content, and therefore soil organic carbon, can both improve soil quality and potentially contribute to mitigating climate change (Schoenholtz *et al.*, 2000; Pan *et al.*, 2011). Soils have the capacity to store a great quantity of carbon, accounting for more carbon than is found in the atmosphere and living plant biomass combined (Jobbágy & Jackson, 2000). Forest soils in particular are estimated to contain more than 70% of carbon contained in soils worldwide (Batjes, 1996; Jobbágy and Jackson, 2000; Six *et al.*, 2002).

Soil organic carbon can provide a stable sink for atmospheric carbon due to chemical, physical, or biological protection from microbial decomposition through occlusion in micropores and association with silt or clay particles (Six *et al.*, 2002). Recalcitrant soil organic carbon can have residence times ranging from 2,000-8,000 years (Martel and Paul, 1974; Fontaine *et al.*, 2007). When conditions allow for the accumulation of carbon in soils from roots and plant litter, soil quality improves with increased cation exchange capacity (CEC), aggregation, water-holding capacity, infiltration, microbial diversity, and pH-buffering capacity (Lal, 2004). Therefore, soil organic carbon has been hypothesized as a useful indicator for the sustainability of land use practices, in that increasing soil organic carbon indicates improvement in soil quality (Schoenholtz *et al.*, 2000). Increasing soil organic carbon content is particularly important in soils where inherent fertility has been depleted through natural weathering processes or erosion, to provide additional CEC, water holding capacity, and nutrient capital (Maquere *et al.*, 2008; Zinn *et al.*, 2002).

Soil carbon can be lost rapidly in tropical ecosystems following land-use change (Detwiler, 1986). Conversion of forests to agricultural land depletes soil carbon concentrations by 20-50% (Post and Mann, 1990; Guo and Gifford, 2002; Laganière *et al.*, 2010). To reverse this trend, reforestation or afforestation of former agricultural or pastures has been suggested as a means to sequester atmospheric carbon in soils (Lal, 2005). It is still unclear under what conditions tree plantations will increase soil carbon stocks compared to previous land uses (Guo and Gifford, 2002). Due to the variability and complexity among

factors affecting soil organic carbon and sampling inadequacies, especially following land-use change, our ability to predict the magnitude and direction of change in soil carbon following reforestation or afforestation has thus far been limited.

Experimental studies and meta-analyses have shown emerging trends relating changes in soil carbon to previous land use, tree species, and soil properties, pre-planting disturbance, and climate (Post and Kwon, 2000; Guo and Gifford, 2002; Laganière *et al.*, 2010). Plantation forest soils tend to accumulate about 25% more soil carbon when established on croplands compared to pasture or grasslands (Guo and Gifford, 2002; Laganière *et al.*, 2010). Clay soils (>33% clay content) can store approximately 25% more soil carbon than sandy soils, most likely due to physical protection (Blanco-Canqui and Lal, 2004). Minimizing pre-planting disturbance, such as the use of heavy machinery, can improve soil carbon stock gains from 4% to 19% (Laganière *et al.*, 2010).

Climate factors have a variable influence on soil carbon sequestration both from effects on plant productivity and carbon allocation but also microbial decomposition. Tropical and subtropical forest plantations, with warm, humid climates, can have higher soil carbon inputs than other biomes due to high plant productivity (Lugo and Brown, 1993), though these same climate conditions also increase decomposition of organic matter and thus can reduce potential for soil carbon storage (Lal, 2005).

Several studies have shown that tree species can have a significant effect on accumulation of soil carbon following forest establishment on former agricultural lands in the tropics (Zinn *et al.*, 2002; Russell *et al.*, 2004; Laganière *et al.*, 2010). Tree species can have different inputs into soil, both in terms of biomass production and nutrient content. Nitrogen-fixing trees, for example, have been shown to sequester more carbon than non-nitrogen fixing trees (Resh *et al.*, 2002). Since tree species appears to be an important factor in determining soil carbon shifts, we focus on commercial broadleaf and conifer plantation species. The field site for this in Brazil provides an ideal location to test tree species effects in a low carbon, sandy Oxisol with high plant productivity, assuming that if any change is taking place, the sampling should be able to capture it.

Commercial plantation tree species are of particular importance due to increasing intensity and area of production. Worldwide, commercial broadleaf plantations of *Eucalyptus* currently cover over 20 million hectares (ha). Brazil is the largest producer of *Eucalyptus* in the world, with *Eucalyptus* plantations extending across 4.75 million ha (Iglesias-Trabado and Wilstermann, 2008). Pine, the second most abundant industrial forest plantation species in Brazil, covers 1.76 million ha (ABRAF, 2011). In the 1980s, plantation forests in Brazil began to be established on pastures (primarily *Brachiaria*) that were used for cattle production for decades. Most pastures were established following brief agricultural land-use after initial deforestation. Impacts from the expansion of tropical plantation forestry on soil organic carbon, and therefore on soil quality and long-term sustainability, need further examination to better inform management decisions, methodologies in carbon accounting, and climate change policy.

Trends from meta-analyses indicated that reforestation with *Eucalyptus* plantations maintains soil organic carbon stocks compared to pasture while pines may decrease stocks in the mineral soil (Guo and Gifford, 2002; Paul *et al.*, 2002; Berthrong *et al.*, 2009). However, when the organic horizon is included in total soil organic carbon, both *Eucalyptus* and *Pinus* plantations were shown to increase soil carbon stocks by approximately 12% over former agricultural land (Laganière *et al.*, 2010). Many studies only measure mineral soil and exclude the organic horizon (O-horizon) as part of the soil profile, which inherently underestimates total soil organic carbon (SOC_T) stocks. The organic layer can contain a significant part of the carbon in soil profile following forest establishment, in one case accounting for 96% of carbon accumulation in a soil profile following establishment of *Pinus taeda* on former agricultural fields (Richter *et al.*, 1999). Across many studies, the contribution of *Pinus* plantation organic layer averages 57% of carbon in the sampled soil profile (to approximately 30 cm), while *Eucalyptus* typically has a smaller, but still significant portion of organic layer, accounting for 16% of SOC_T (Laganière *et al.*, 2010).

Another carbon stock rarely considered in soil profile consists of roots, which typically provide a longer lived, less decomposable reserve than aboveground litter. Pasture grasses have been shown to have 36% greater mass of fine roots than plantation pines in the

top 15 cm (Guo *et al.*, 2007). This thick, fibrous root system in upper soil layers may account for why pastures tend not to lose soil carbon compared to forests following land-use conversion (Don *et al.*, 2010). The structure of roots differs between forest and pastures, in that tree root biomass is made up primarily of coarse roots and has a higher fine root C:N ratio (70:1), compared to grasses (44:1) (Guo *et al.*, 2007). Additionally, tree roots are known to extend deeper in the soil profile than herbaceous plants and have greater rooting biomass (Canadell *et al.*, 1996; Jackson *et al.*, 1996). *Eucalyptus* roots have been shown to mirror plant height in their rooting depth in absence of physical or chemical barriers (Christina *et al.*, 2011), whereas *Brachiaria* root biomass decreases drastically below 30 cm (Paciullo *et al.*, 2010).

For this reason, sampling depth may play a role in determining changes in soil carbon stocks following land-use change. In the meta-analysis by Laganière *et al.* (2010), the average sampling depth to test changes in soil organic carbon following afforestation of pastures was 34 cm. Since trees have much greater rooting depths than herbaceous plants, deeper soil sampling may be needed to capture a larger percentage of change occurring in the soil profile. However, in a study by Bashkin and Binkley (1998), there was no significant difference in soil organic carbon under sugar cane and *Eucalyptus* below 55 cm. In southeastern Brazil, Maquere *et al.* (2008) showed that carbon concentrations decreases until depths below 40 cm, and that most changes actually occur in the forest floor and upper soil horizons following reforestation of pasture. Appropriate sampling depths will depend on the system being sampled.

A variety of sampling techniques have been used in soil carbon studies following land-use change. Paired-plot designs, using adjacent vegetation types on similar soils and landforms, help ensure that differences between plots are due to differences in vegetation treatment and not due to environmental factors. Additionally, sampling paired plots across a variety of ages of stands allows for the use of a chronosequence approach (Richards *et al.*, 2007). Through space for time substitution, the patterns occurring over real time following land-use conversion can be addressed. This approach is useful when no major

environmental, topographical, or historical treatment differences exist across different ages of sites.

The purpose of this study was to quantify total soil organic carbon stocks (SOC_T) following reforestation of tropical pasture to conifer or broadleaf plantation forests, primarily *Eucalyptus* and *Pinus* genera, located in southeastern Brazil to a 45 cm depth. A larger quantity of soil organic carbon under a vegetation type would indicate greater potential for belowground sequestration of atmospheric carbon. The objectives of this study were to 1) determine soil carbon concentration and bulk density to estimate mineral soil carbon (SOC_M), 2) quantify carbon content of the organic horizon to determine total soil organic carbon (SOC_T) of each plot, 3) determine if paired Pasture-Broadleaf or Broadleaf-Conifer vegetation treatments have different soil carbon stocks, 4) evaluate trends over time in SOC_T in each soil layer (O-horizon, 0-15 cm, 15-30 cm, and 30-45 cm) based on the forest plantation chronosequence. Determining the extent to which conversion of pasture to conifers or broadleaf forest plantations influences soil organic carbon will aid in understanding the role of land-use change in climate change mitigation and the carbon cycle.

Methods

Site Description

All soil samples for this project were collected at the Anhembi Experimental Research Station, which lies in the center of the state of Sao Paulo, Brazil, and has been owned by the University of Sao Paulo (USP) since 1972 (**Figure 1.1**). Native seasonal semi-deciduous Atlantic Forest was cleared between 1950 and 1955, most likely burned, and planted for several years in cotton agriculture, then replaced with *Bracharia decumbens* and managed for cattle pasture, like much of the surrounding landscape. Under the management of the University of Sao Paulo, the 663.5 ha field site has been gradually converted from pasture to broadleaf and conifer forest plantations consisting mainly of *Eucalyptus* and *Pinus* stands (**Figure 1.2**). A private forest reserve, Barreiro Rico, located 6 km away on similar

soils and landform provides an example of undisturbed mature Atlantic Forest (Ferez, 2010; Assumpção *et al.*, 1982; Oliveira *et al.*, 1999).

The field site is located at latitude 22°40'S and longitude 48°10' W and lies approximately 230 km northwest of Sao Paulo city at an elevation of 455 m. The topography is flat with, at most, 2% slope. The climate is humid subtropical (Cwa) in the Köppen system, with hot, rainy summers and colder, dry winters. The mean annual temperature is 21.0°C, with the coldest month having an average of 17.1°C and the hottest month averaging 23.7°C. Extreme minimum temperatures reach 5°C and maximum temperatures can climb to 34°C. Annual mean rainfall averages 1350 mm, with a dry season between the months of April and September (Hijmans *et al.*, 2005; Campoe, 2008).

Soils at this site are classified as Typic Dystrophic Red-yellow Latosols according to the Brazilian taxonomic system or sandy Oxisol (Typic Hapludox) in U.S. soil taxonomy. These soils have low base cation saturation, and cation exchange capacity is typically <170 mmol_c kg⁻¹. The pH (in CaCl₂) of these soils is low, usually <4.3. They are well drained and colors range from 7.5YR 4/6 to 5YR 5/8 along the profile (Andrade *et al.*, 2010). Additional information concerning physical and chemical attributes of soil profiles can be found in Appendix 1. The site is located on the Sedimentary Basin of Paraná, São Bento Group (Triassic to Lower Cretaceous), and is represented by the Pirambóia and Botucatu Formation, which is dominated by sandstones, conglomerates, siltstones, and mudstones (IPT, 1981).

Forest Plantation Establishment

Forest plantation establishment follows a standard protocol consisting of: i) Initial leaf-cutting ant control with sulfluramid baits once per month before site preparation; ii) One harrowing and two orthogonal disking operations after burning the pasture (up until 1989) or over the grass (after 1989); iii) Marking the planting rows with a ripper to assure correct distance between rows (in general three meters); iv) Manual planting of the seedlings (two meter spacing) and watering if necessary, i.e. in periods of low rainfall; v) Fertilization at planting totaling 150 kg ha⁻¹ of triple super phosphate (42% P₂O₅) applied in a 10 cm-deep hole 10 cm from the seedling, plus 200 kg/ha of NPK 10:20:10 applied at a 20 cm distance

around the plant two months after planting, totaling 20 kg N/ha, 40 kg P/ha and 17 kg K/ha; v) Controlling competing vegetation with manual weeding in the row and mechanized chopping between rows until canopy closure at around year 2; and vi) Annual leaf-cutting and controls during the dry season (April to September).

Most of the Anhembi forest stands consist of valuable genetic materials for seed production and are managed for long rotations. There are an average of two 40% thinnings from below during the first 30 years, on average at age 16 and 25. **Table 1.1** details stand species, spacing, year of planting, and age of thinning. Thinning increases light in the understory, therefore some competing vegetation still persists in some stands. This protocol was applied across all species. The 4- to 6-month-old seedlings were produced at the University of Sao Paulo nurseries in Piracicaba or Itatinga, 100 km from Anhembi, in a 300 cm³ subsoil plastic-bag container (until 1990) or 50 cm³ plastic tube container (after 1990).

Experimental Design

Paired plots, in conjunction with a chronosequence design, allow for greater validation of inherent assumptions of homogeneity. Paired vegetation types, which were located approximately two meters apart, consisted of ten Pasture-Broadleaf plantation pairs (P-B) and ten Broadleaf-Conifer plantation pairs (B-C) (**Figure 1.3** and **Figure 1.4**). Of the ten P-B paired plots, the broadleaf plantations consisted of seven *Eucalyptus* stands, one *Liquidambar styraciflua* stand, one *Gmelina arborea* stand, and one *Dalbergia nigra* stand (a native leguminous tree species). A paired plot of reference, native, unmanaged, mature forest with an adjacent pasture (Ref-P) within the Anhembi field station serves as a comparison. The B-C paired plots consist entirely of *Eucalyptus* or *Corymbia* for broadleaf plantations and tropical *Pinus* genera for conifer plantations.

Stands ranging in age from 6-34 years were used to create a chronosequence under the assumption that sites only differ in age with no systematic bias caused by confounding biotic or abiotic factors. The homogeneity of our site in topography, soil characteristics (i.e. clay content), silvicultural treatments, and the proximity of paired vegetation plots created an

ideal situation for detecting changes in soil carbon stocks following reforestation using a chronosequence design.

Soil Sample Collection

To determine total soil carbon stocks, we collected mineral soil, organic horizon (O-horizon), and bulk density samples from April to July 2009. Mineral and O-horizon soil samples were collected along three transects for each pair of plots. Transects were located 14 m apart and were sampled every 7 m, beginning at the edge of the stand (**Figure 1.5**). This created a total of nine sampling locations per vegetation plot. Mineral soils were collected with a hand auger at depths from 0-15 cm, 15-30 cm, and 30-45 cm. At each of the sampling locations, five subsamples were composited and homogenized in the field for a total of 27 mineral soil samples per vegetation plot (3 points x 3 transects x 3 depths).

The O-horizon was collected within a 25 cm x 25 cm wooden frame in the same spatial sampling pattern as mineral soils (five combined samples for each point, i.e. 5 x 625 cm²) to create nine samples per vegetation plot. One O-horizon sample from each plot was oven-dried offsite for correction of water loss and then combusted in muffle furnace for loss on ignition to determine correction factors for ash content.

Bulk density samples were collected at the center of each plot at each depth with a volumetric ring measuring 5 cm in diameter and height (98 cm³). These samples were oven dried and weighed at ESALQ-USP in Piracicaba, Brazil. No stone content correction was necessary for bulk density.

Soil Processing and Analysis

All soil samples were air dried, pulverized, and passed through a 2 mm sieve. Organic matter that did not pass through, such as coarse roots, was excluded. To quantify soil texture for each sampled plot at a finer resolution, a composite of nine sub-samples were analyzed for each 15 cm depth by hydrometer at the Laboratory of Applied Ecology at ESALQ-USP for sand, silt, and clay fractions. All mineral soil samples are archived at North Carolina State University Forest Productivity Laboratory.

To achieve low variation in dry combustion analysis, samples required homogenization due to significant differences in soil carbon content between soil particle sizes (data not shown). Using a roller-mill method, approximately 2.5 g of each soil sample was placed inside a two-ounce square glass bottle with six 304-stainless steel rods (9.5 mm diameter, 44.5 mm length) and securely placed inside of a standard, rubber-lined rock tumbler to rotate for 24 hours (Arnold and Schepers, 2004). This generally produced a fine powder able to pass through a #60 mesh (0.250 mm) sieve, though some samples required additional pulverizing by mortar and pestle for remaining particles. This method produced a homogenous final product with low coefficient variation (< 5%) in duplicate samples.

Soil total carbon and nitrogen were determined by dry combustion and infrared detection using a CD Instruments NC2100 elemental analyzer (CE Elantech, Inc., Lakewood, New Jersey). For quality assurance, NIST (National Institute of Standards and Technology) certified reference material, well characterized in-house soil standards, and sample duplicates (5% of analyzed samples) were monitored for precision and accuracy of each analysis to stay within 10% coefficient of variation. Soils were tested for inorganic soil carbon (carbonates) with acid fumigation (Harris *et al.*, 2001). None were found, so all measured carbon in this study is assumed to be organic in origin. This was expected due to the acidic nature of these soils.

Mineral soil organic carbon (SOC_M) stocks (Mg C ha⁻¹) for each sampled depth were calculated by multiplying the concentration of carbon by the bulk density and depth of the soil layer:

$$\text{SOC}_M = C * \text{BD} * L$$

where C is the concentration of carbon in % of weight, BD is the bulk density (g cm⁻³) of each layer in each plot, and L is the depth of the soil layer (15 cm). The SOC_M stocks were calculated by summing the carbon stocks for each layer (0-15, 15-30, and 30-45 cm). Carbon values for the organic horizon were calculated as 50% of the mass of the organic matter following corrections for ash and water content and extrapolated to a hectare basis. Total soil

organic carbon (SOC_T) stocks (Mg C ha⁻¹) were calculated as the sum of carbon from the mineral soil and organic horizon for each plot:

$$\text{SOC}_T = \text{SOC}_M + \text{SOC}_{\text{Org}}$$

where SOC_M is mineral soil carbon content (Mg C ha⁻¹ to 45 cm) and SOC_{Org} is the O-horizon carbon content (Mg C ha⁻¹).

Forest Plantation Inventory

For each forest plantation stand, a circular inventory plot of 616 m² was established in January of 2012. Plots 32e and 28c were inventoried in July 2009 and 2010, respectively, due to harvesting in 2010 and 2011. All trees within each inventory plot were measured for diameter at breast height (DBH, 1.30 m above ground level). Heights were measured for 20% of inventory trees plus those with dominant heights. For each plot, a plot-level hypsometric equation was developed to estimate the height of the remaining trees. The bole biomass of each *Eucalyptus* tree was calculated according to the equation developed by Doi et al. (2010) in this region. For pine, the biomass equation was developed by Bizon (2005) at sites 200 km south of Anhembi. The *Eucalyptus* equation was used for the other hardwood plots since their DBH, height, and wood density were comparable to the *Eucalyptus* genera, though this may introduce some small error into the estimation of biomass for these hardwoods. The summation of the biomass of all trees in the inventory plot was expanded to a hectare basis. Biomass removed from thinnings was estimated based on the dominance curve developed by Doi et al. (2010) and added to the estimate of total aboveground stemwood biomass for each stand, in Mg ha⁻¹. Biomass estimates were multiplied by 0.5 to covert dry matter to carbon, and then divided by the age of the stand to obtain the wood net primary productivity (WNPP, in Mg C ha⁻¹ yr⁻¹) for each forest stand associated with the soil samples. Coarse root carbon was estimated for conifers by multiplying stemwood biomass by 0.27 (Bizon, 2005), and for broadleaf species by multiplying stemwood by 0.20 (Stape *et al.*, 2004).

Statistics

In this randomized block design, vegetation is the main treatment in an analysis of variance (ANOVA). Paired plots were considered the blocking factor to further distinguish vegetation effects from any site variability. Separate ANOVAs were run with a general linear model for P-B and B-C paired plots. For our dependent variables, soil carbon was analyzed as the total soil profile to 45 cm (SOC_T). Two independent class variables consisted of vegetation and blocks. Continuous independent covariates originally included total soil nitrogen and clay content. Clay content was dropped from the model as a covariate due to a lack of significance, which fit our assumptions of soil texture homogeneity. We used a bivariate model for forest stand age and SOC_T to determine changes in SOC_T following reforestation. Comparisons for differences between vegetation treatments in ANOVA were analyzed using pair-wise comparisons protected Least Significant Difference (LSD) with a significance level of $P < 0.05$. All statistics were calculated using the statistical packages SAS v 9.2 and JMP v 9.0 (SAS Institute, Cary, NC).

Results & Discussion

Site and Stand Attributes

The Anhembi Experimental Research Station provided an ideal location for testing vegetation effects on soil organic carbon stocks. Site preparation, planting methods, fertilization, topography, and climate were all constant across the site. Clay content did not differ significantly among vegetation treatments in the sampled plots, ranging from 14-27% to a 45 cm depth with a mean (\pm standard error) of 19.8% (± 0.5). These conditions support the validity of using these forest plots in a chronosequence.

Total aboveground stemwood biomass presents a way to investigate the amount of carbon flux from the atmosphere to each forest stand. The distribution of total aboveground stemwood biomass and age of forest stands is shown in **Figure 1.6**. Total aboveground biomass and WNPP can be found in **Table 1.2**. Broadleaf vegetation type showed a greater overall WNPP than conifer plantations (19.4 ± 2.0 and 11.4 ± 1.0 Mg dry matter $\text{ha}^{-1} \text{yr}^{-1}$,

respectively). SOC_T made up 13% of broadleaf stemwood carbon and 16% of conifer stemwood carbon (**Figure 1.7**). Neither WNPP nor aboveground biomass of stemwood were significantly correlated with SOC_T (data not shown). Variation in WNPP and aboveground biomass is most likely due to inherent genetic differences among species.

Rezende *et al.* (1999) estimated aboveground NPP of *Brachiaria* pasture grasses in southern Bahia to be approximately 30 Mg dry matter $\text{ha}^{-1} \text{yr}^{-1}$, which due to even distribution of rainfall throughout the year in southern Bahia, is likely much greater than pasture productivity at Anhembi. Based on differences in forest productivity between these two regions, it is likely on site pasture productivity would fall into the same range as WNPP (2.5 to 31 Mg dry matter $\text{ha}^{-1} \text{yr}^{-1}$).

Total Soil Organic Carbon

Values for SOC_T will generally be reported in a sequence from the native Reference forest and pasture (Ref-P) to Pasture-Broadleaf (P-B) to Broadleaf-Conifer (B-C) paired plots as a logical comparison based on land-use change. In the Ref-P paired plots, unmanaged reference forest had the greatest amount of SOC_T compared to any other plot at the field site with 55.6 Mg C ha^{-1} while the pasture contained 39.5 Mg C ha^{-1} (**Table 1.3**). SOC_T for the P-B paired plots, pasture averaged 36.0 ± 1.7 Mg C ha^{-1} and broadleaf plantations averaged 36.8 ± 1.9 Mg C ha^{-1} , showing no statistically significant difference in the general linear model. For B-C paired vegetation plots, SOC_T averaged (\pm standard error) 38.3 ± 1.9 Mg C ha^{-1} for broadleaf plantations and 36.0 ± 1.6 Mg C ha^{-1} for conifer plantations. There was, however, a difference in SOC_T in the B-C paired plots, showing 6% greater SOC_T in broadleaf than in the conifer plantations, which was due to greater average soil carbon concentration (and not bulk density) in the broadleaf stands. Details of bulk density, SOC_T , soil nitrogen stocks, and clay content per depth can be found in **Table 1.4**, **Table 1.5**, and **Table 1.6**. The general linear model showed SOC_T was positively correlated with total soil nitrogen stocks in the mineral soil in the B-C plots, but not in the P-B plots (**Table 1.7**).

To estimate soil carbon change over time in the two forest types, the difference between forest and pasture soil carbon stocks was divided by the mean age of the stand types

(Broadleaf = 22 yrs, Conifer = 28 yrs) and there can be 95% confidence that the change in SOC_T falls in the interval of -0.13 to $0.36 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ for conversion to broadleaf plantations, and -0.26 to $0.50 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ for conversion to pine. These results are comparable to a recent meta-analysis, where a 3% change in SOC_T following afforestation of pastures was not significantly different from zero (Laganeire *et al.*, 2010).

Using the chronosequence approach, the SOC_T of all forest stands to 45 cm was analyzed as a function of time and found no significant change (**Figure 1.8**). This result was similar to another study that showed no relationship between forest stand age and soil carbon accrual (Wellock *et al.* 2011). In B-C paired plots, a bivariate model of age and SOC_T was significant ($P=0.03$). However, the estimate ($-0.35 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$) indicated a negative relationship with SOC_T over time. This loss was driven by soil carbon decreases in the lower two layers (data not shown). This decrease over time may be due to deeper rooting profiles and long-lived roots of tree species compared to pastures with higher rates of fine root turnover (Jackson *et al.*, 1996; Canadell *et al.*, 1996; Guo *et al.*, 2007).

The age of plantations has been thought to be an important factor in soil organic carbon stocks following forest establishment. Changes in soil carbon following afforestation have been observed to decrease during the five years following forest establishment and recovering pre-agricultural soil carbon levels after approximately 30 years (Paul *et al.* 2002). This observed pattern is thought to occur due to increased amounts of carbon inputs as the stand matures, changes to the soil microclimate, and enhanced organic matter protection (Bouwman and Leemans, 1995; Six *et al.*, 2002; Del Galdo *et al.*, 2003). The stands in this study did not appear to follow this pattern.

Neither the forest plantations nor the pasture appear to match the SOC_T present in the mature reference forest, which contained $55.6 \text{ Mg C ha}^{-1}$. To compare our reference forest with other studies, we found $42.7 \text{ Mg C ha}^{-1}$ in the top 30 cm of mineral soils. In the native, undisturbed, mature seasonal semi-deciduous forest located 6 km from our field site, Ferez (2009) found $\text{SOC}_T 35.5 \pm 10.9 \text{ Mg C ha}^{-1}$ in the top 30 cm of mineral soil and $4.0 \pm 1.4 \text{ Mg C ha}^{-1}$ in the O-horizon. Bernoux *et al.* (2002) estimated $41.7 \text{ Mg C ha}^{-1}$ in the same type of

mature forest in the top 30 cm. The reference forest appears to be a fairly representative sample of expected numbers for this forest type.

Exclusion or inclusion of the organic horizon has been shown to change the general trends in total soil carbon accumulation following forest establishment (Paul *et al.*, 2002; Laganière *et al.*, 2010). The O-horizons in all of our sites were relatively small, averaging < 5 Mg C ha⁻¹. We found no difference in vegetation types in terms of the O-horizon. These results were similar to a study by Zinn *et al.* (2002), that found no difference between *Eucalyptus* and Cerrado litter on a sandy Entisol, but different from their results found on a loamy Oxisol where *Pinus* had greater litter quantity than *Eucalyptus*.

The C:N ratios in the mineral soil to a 45 cm depth were not significantly different in vegetation types, for B-C plots averaging (\pm standard error) 13.4 ± 0.1 for broadleaf and 13.7 ± 0.1 for conifer plantations. In P-B plots, C:N ratios also did not differ significantly with 14.8 ± 0.1 for broadleaf plantations and 14.6 ± 0.1 for pasture. These results are interesting in that plantations are often considered to have a lower quality (higher C:N ratio) of litter and therefore increase the C:N ratio in the soil (Berthrong *et al.*, 2009). However, we saw little difference in C:N ratios across vegetation types. There was a significant depth-dependent relationship in the B-C plots, with C:N ratios decreasing with depth from 14.7, to 13.2, to 12.8 from 0-15 cm down the profile. However, there was no depth-dependent relationship in the P-B plots.

The balance of carbon gains and losses in the soil is the result of many factors contributing to soil carbon dynamics. Inconsistency in the effects of species on soil carbon and nitrogen is often attributed to factors such as erosion, atmospheric deposition, above- and belowground litter inputs, and rainfall (Guo and Gifford, 2002; Kirshbaum *et al.*, 2008; Wei *et al.*, 2010). While inherent soil properties, such as high clay content, can lead to higher soil carbon, variation in this trend can be linked to length of time since clearing, the type of land use, and the soil texture. Key drivers typically thought to control change in soil carbon following reforestation are type of forest established, previous land use, climate, clay content, and pre-planting disturbance (Guo and Gifford, 2002; Paul *et al.*, 2002; Laganière *et al.*, 2010). Of these factors, our experiment essentially controlled for previous land use, climate,

clay content, and pre-planting disturbance, and even so, found no major differences due to type of forest established.

Standard methodology for analyzing soil organic carbon may play a role in results following land-use change. Some authors suggest that soil carbon stocks can be higher in pastures due to greater fibrous, fine root production and sloughing, and lower carbon decomposition and turnover (Richards *et al.*, 2007). However, it may be that this trend is due to grinding and sieving techniques as opposed to a real loss of soil carbon. One must consider how the various components of soil carbon are measured, which can vary greatly in methodology. Most studies measure the < 2 mm fraction only (as in this study), which can have implications for changes in soil carbon after reforestation. Sieving removes litter, larger roots, and some fungal hyphae, reducing soil carbon estimates for forests while more fine root material may pass through a 2 mm sieve in soils sampled from under pastures (Paul *et al.*, 2002). Fine roots in the first meter of soil can be five times greater under pastures than under adjacent afforested sites (Rhoades *et al.*, 2000). These considerations should be taken into account when comparing soil carbon results among various studies and within the same study comparing vegetation systems with different rooting systems, and may indicate a need for further research into soil preparation for carbon analysis.

Conclusions

While different tree species have been shown to be an important factor in changing soil carbon stocks following afforestation in some situations, we found little difference in total soil organic carbon beneath paired pasture and broadleaf plantations and only 6% greater SOC_T value in broadleaf compared to conifer plantations. Additionally, forest stands did not appear to increase total soil organic carbon over time as measured through a chronosequence approach. Total soil nitrogen appeared to be a positively associated with SOC_T in B-C paired plots, but not in P-B plots.

Soil organic carbon is important for sustainable land-use, particularly in the tropics, and for possible climate change mitigation. Changes in soil organic carbon dynamics can

create either a source or sink for atmospheric carbon depending on management, but the controlling factors are still somewhat difficult to elucidate. Our results indicate that for storing soil organic carbon, broadleaf plantations are essentially equivalent to the pastures, but increase SOC_T slightly in comparison to the conifer plantations sampled in this study.

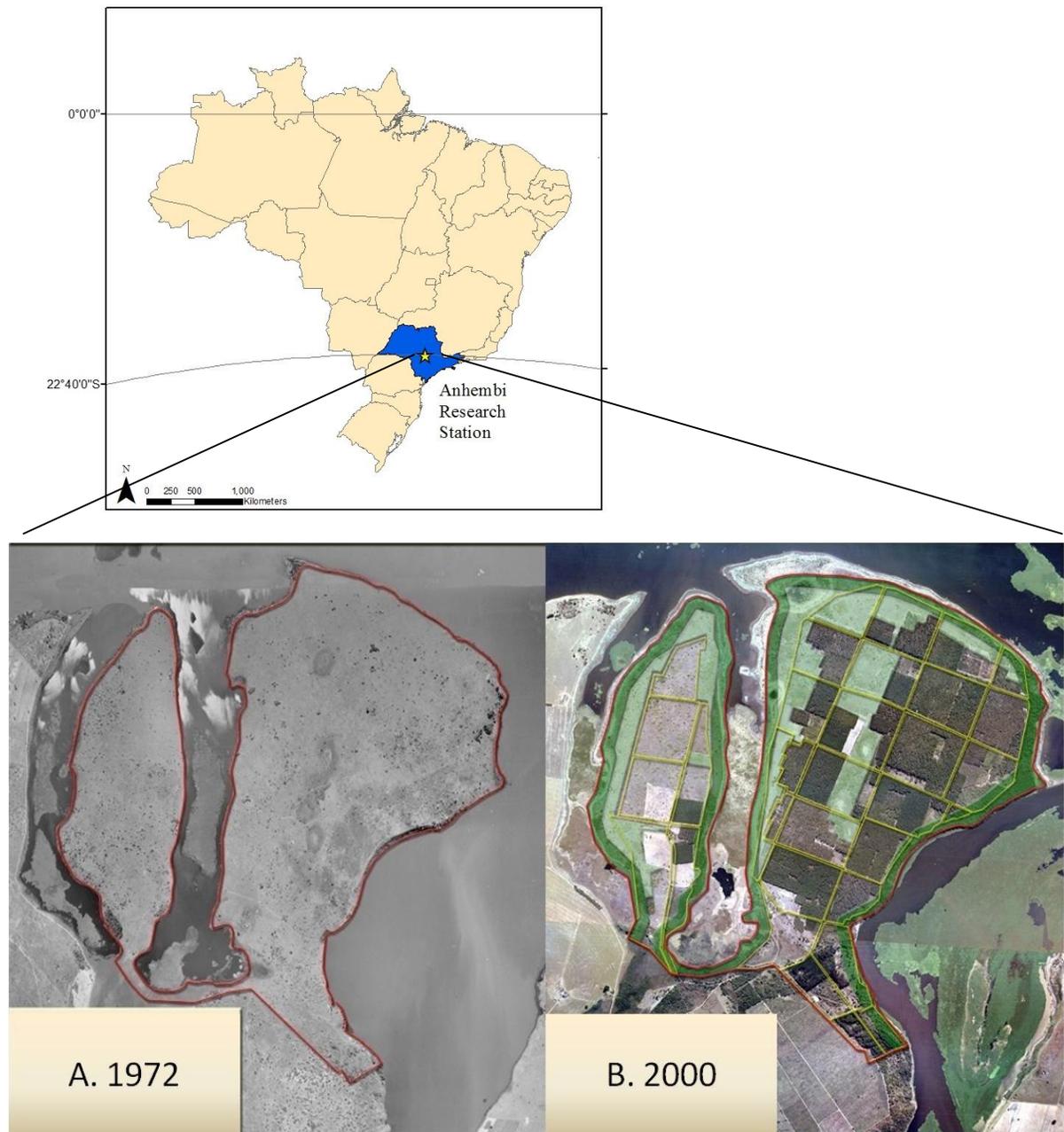


Figure 1.1 Anhembi Research Station, located in Sao Paulo state in southeastern Brazil (above). Aerial photos (below) shows predominance of pasture in A) 1972, followed by B) reforestation of the majority of the field site as of 2000.

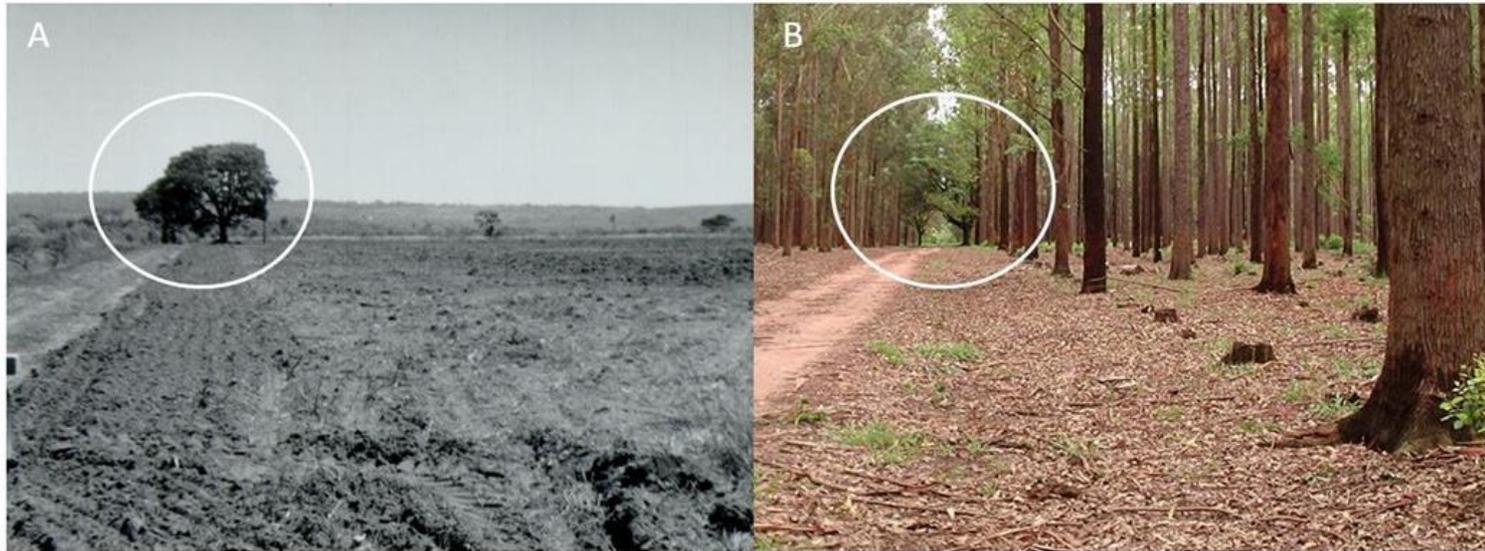


Figure 1.2 Historic photo of Anhembi Experimental Research Station (A) in 1974 and (B) in 2004 after reforestation of pastures. Circled trees are a consistent frame of reference.

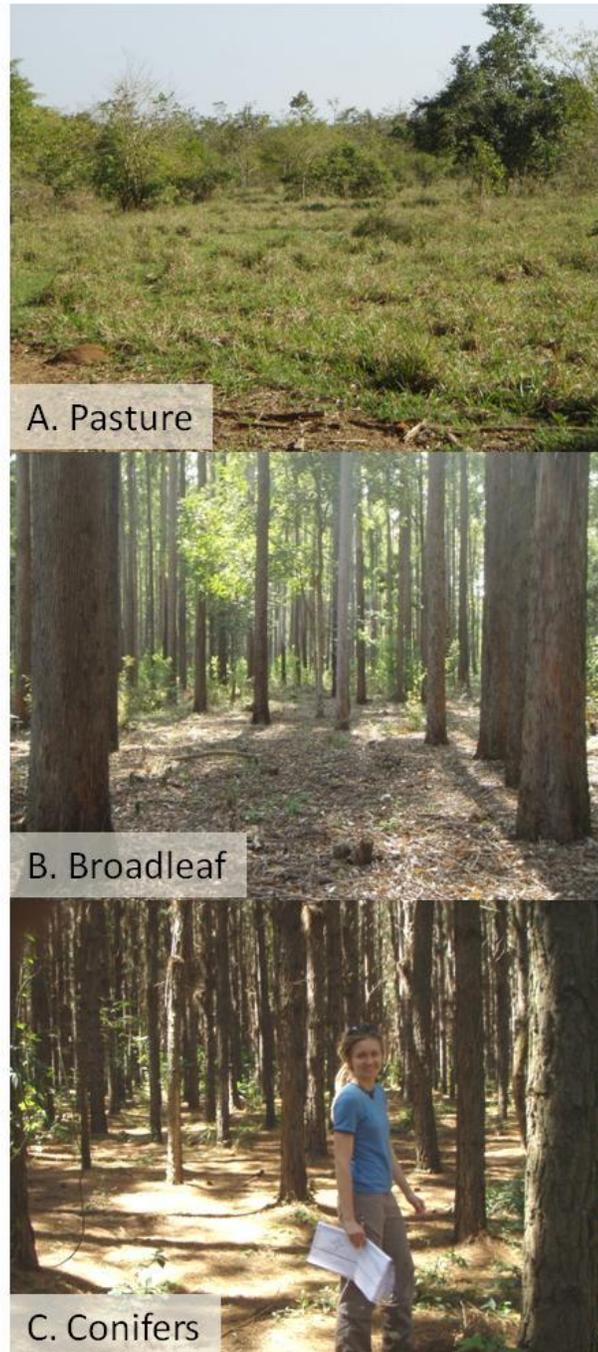


Figure 1.3 Sampled plots consisted of A) Pastures, B) Broadleaf plantations, and C) Conifer plantations.

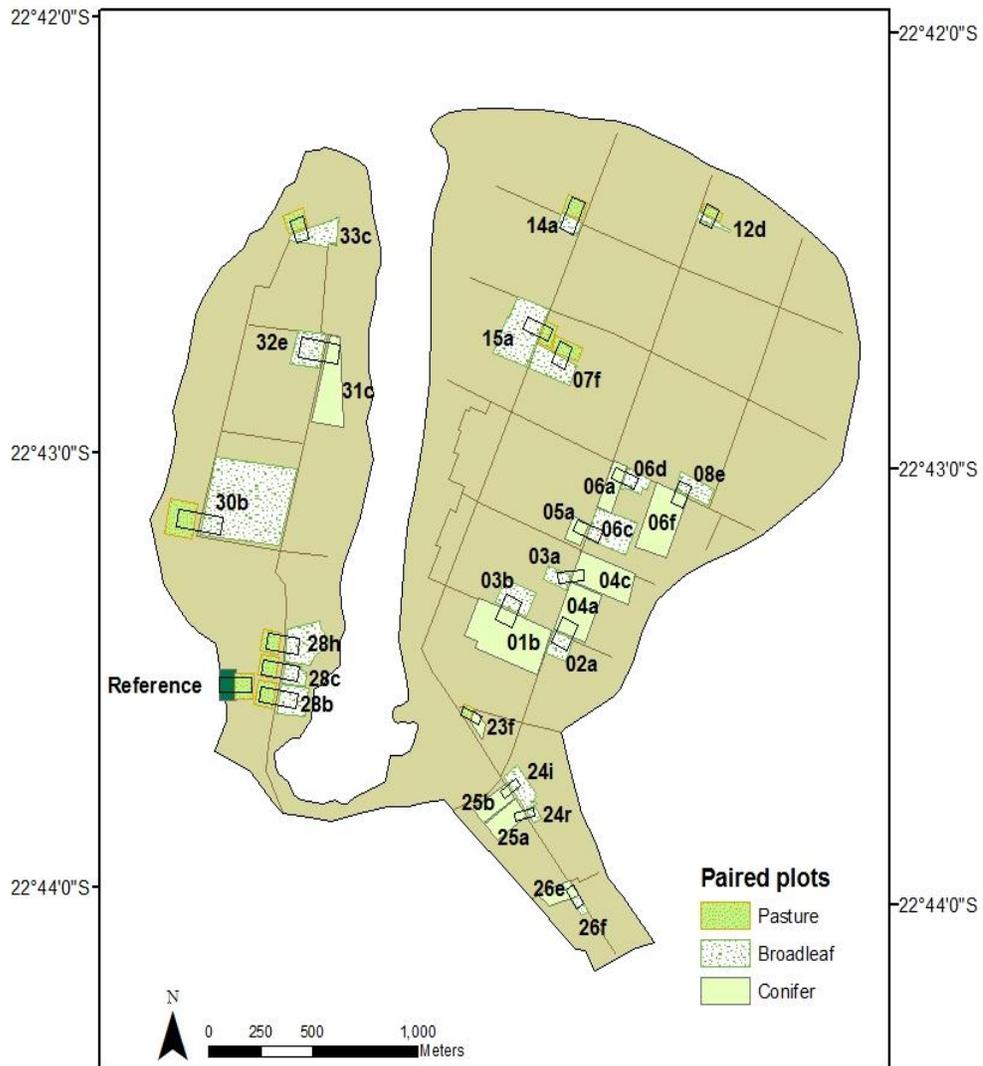


Figure 1.4 Paired plots for chronosequence soil sampling. Sampling plots consist of 10 Pasture-Broadleaf pairs and 10 Broadleaf-Conifer paired sites. One paired Reference plot of unmanaged, mature stand with pasture serves as a comparison.

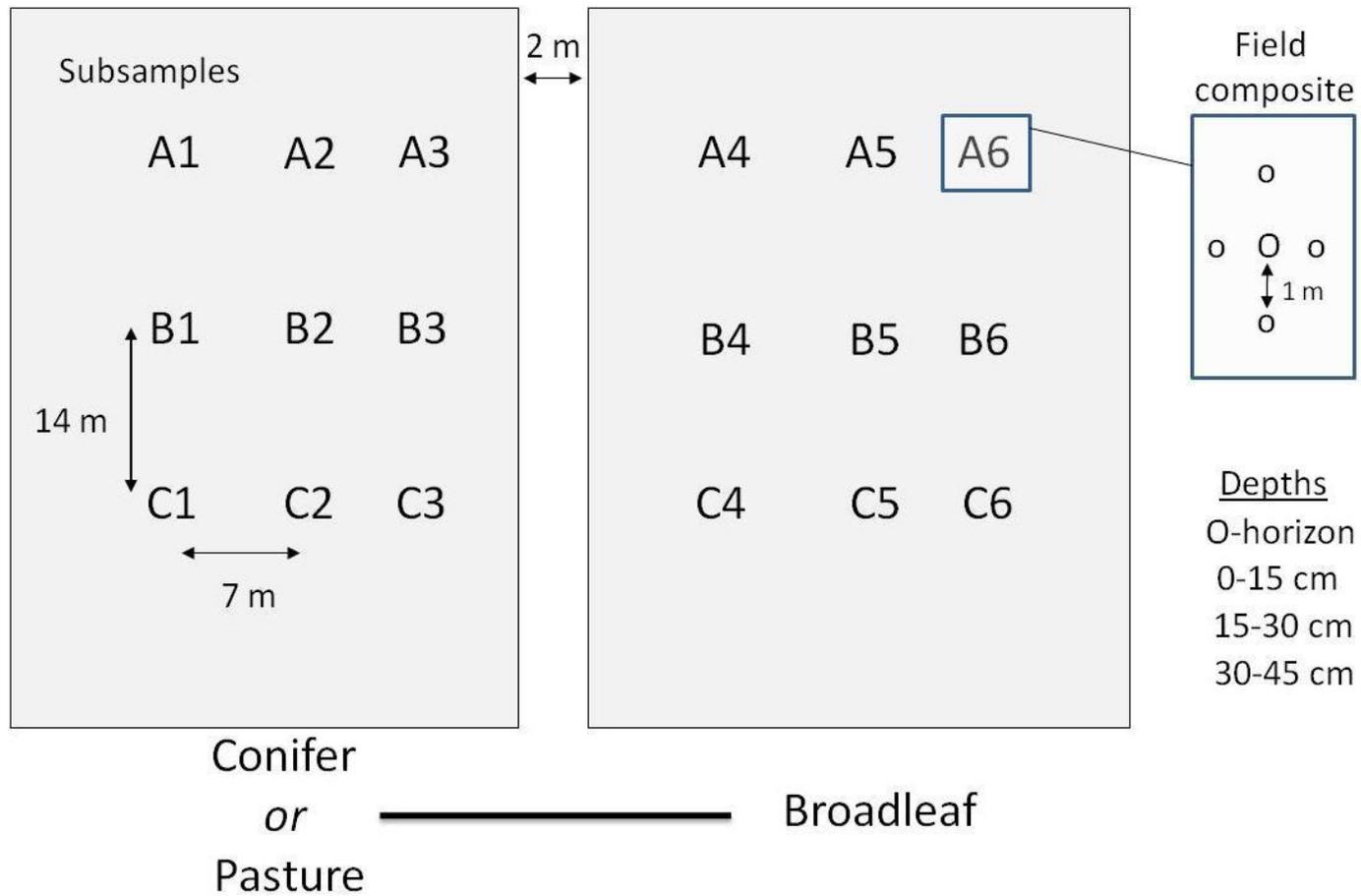


Figure 1.5 Sampling design for collection of the organic horizon (O-horizon) and mineral soil at three depths (0-15 cm, 15-30 cm, 30-45 cm). Each subsample (A1-C6) consisted of five composited points in the field for each depth.

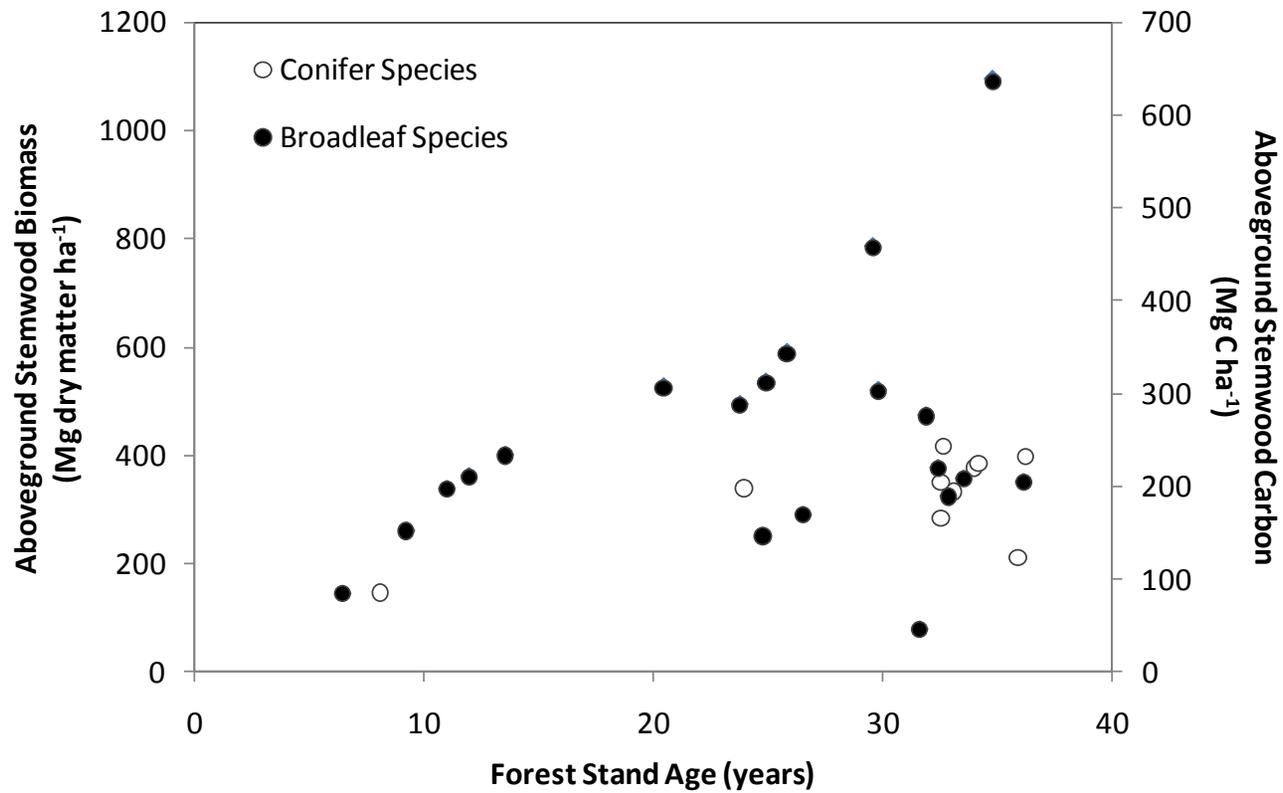


Figure 1.6 Relationship between forest stand age (years) and total stemwood aboveground biomass on the left axis (Mg dry matter ha⁻¹) and total stemwood carbon on the right axis (Mg C ha⁻¹).

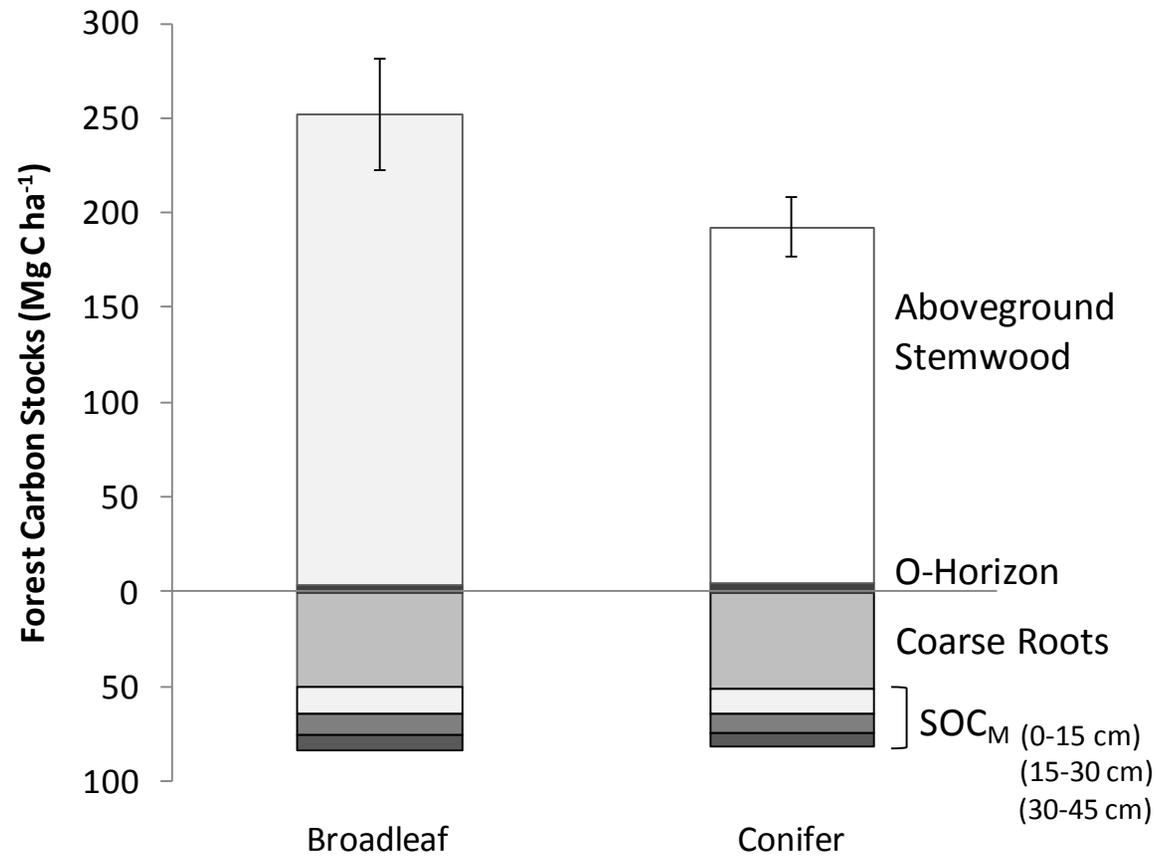


Figure 1.7 Mean forest carbon stocks for Broadleaf and Conifer plantations include aboveground stemwood carbon (branches and leaves not included), organic horizon (O-horizon), coarse roots, and mineral soil carbon (SOC_M) for each depth (Mg C ha⁻¹). SOC_T (mineral soil + O-horizon) makes up 13% of broadleaf stemwood carbon and 16% of conifer stemwood carbon.

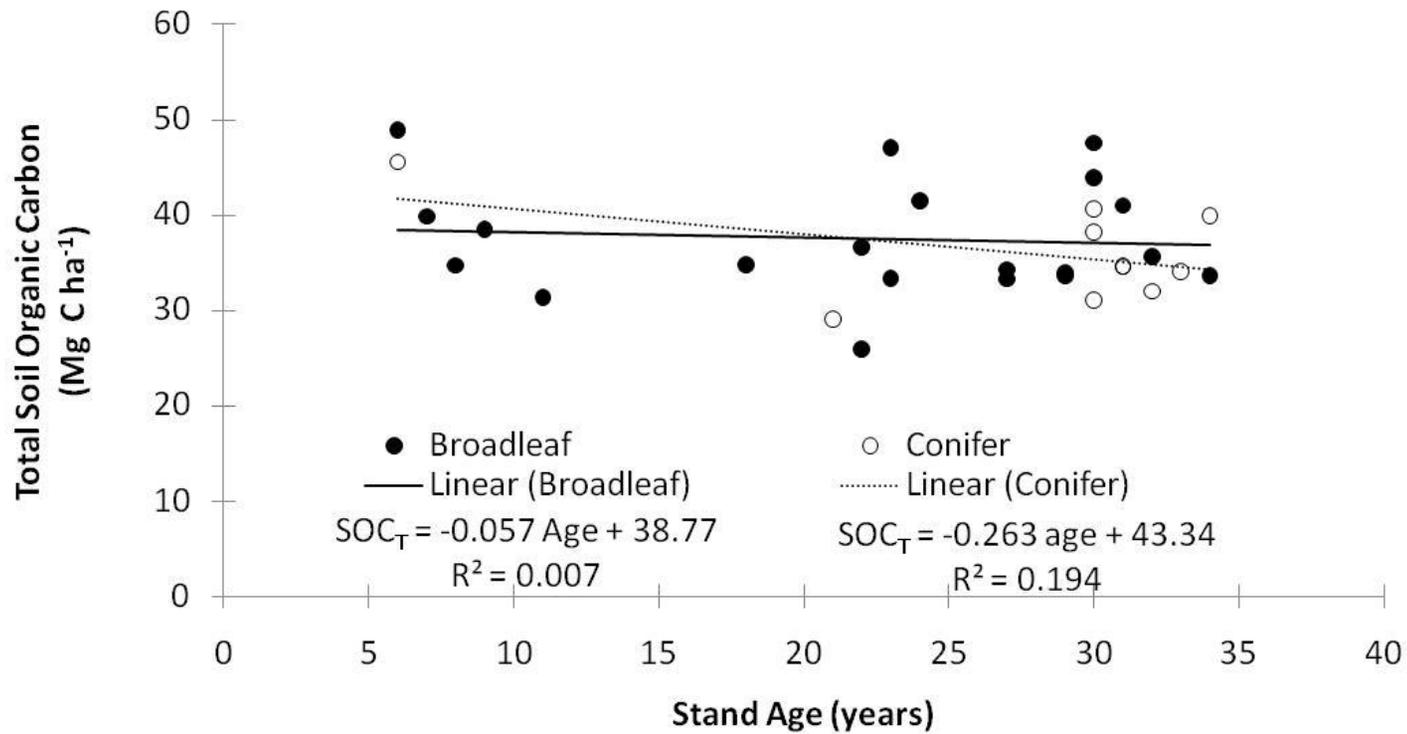


Figure 1.8 The chronosequence of broadleaf and conifer stands shows no significant relationship between age and total soil organic carbon (SOC_T) ($P = 0.37$).

Table 1.1 Forest stand establishment details for Pasture-Broadleaf (P-B) stands and Broadleaf-Conifer (B-C) plantations.

Paired Plot	Plot ID	Species	Planting Year	Spacing (m)	Thinning ages (yrs)	Stocking (trees ha ⁻¹)	Age at Inventory (yrs)
P-B	07f	<i>Eucalyptus saligna</i>	1998	3x2	8	1056	13.5
	12d	<i>Eucalyptus urophylla</i>	1991	3x2	9, 18	325	20.5
	14a	<i>Corymbia maculata</i>	1982	3x2	12, 16	325	29.8
	15a	<i>Corymbia maculata</i>	1987	3x2	11, 16	325	24.9
	23f	<i>Dalbergia nigra</i>	1987	3x2		942	24.8
	28b	<i>Gmelina arborea</i>	1979	3x2	23, 31	309	32.9
	28c	<i>Liquidambar styraciflua</i>	1986	3x2	16	1072	23.8
	28h	<i>Eucalyptus urophylla</i>	2000	3x2	2	633	12.0
	30b	<i>Eucalyptus grandis</i>	2001	3x2	9	1267	11.0
	33c	<i>Eucalyptus grandis</i>	2002	3x2	7	568	9.2
B-C	01b	<i>Pinus oocarpa</i>	1979	3x2	19, 25, 30	179	32.6
	03b	<i>Eucalyptus tereticornis</i>	1980	3x2	16, 20	747	31.6
	04a	<i>Pinus kesiya</i>	1976	4x4	22, 33	114	35.9
	02a	<i>Corymbia citriodora</i>	1975	3x2	19, 23, 34	179	36.2
	04c	<i>Pinus kesiya</i>	1979	3x2	19, 30	406	32.7
	03a	<i>Eucalyptus camaldulensis</i>	1978	3x2	20, 31	666	33.6
	05a	<i>Pinus caribaea</i> var <i>hondurensis</i>	1975	3x2	23, 24	179	36.2
	06c	<i>Eucalyptus pellita</i>	1985	3x2	12, 25	227	26.6
	06a	<i>Pinus caribaea</i> var <i>caribaea</i>	1978	3x2	20, 31	309	33.1
	06d	<i>Eucalyptus cloeziana</i>	1986	3x2	11, 24	227	25.8
	06f	<i>Pinus caribaea</i> var <i>caribaea</i>	1988	3x2	12, 21	455	24.0
	08e	<i>Eucalyptus urophylla</i>	1980	3x2	16, 19	227	31.9
	25a	<i>Pinus oocarpa</i>	1977	3x2	23, 32	244	34.2
	24r	<i>Eucalyptus pyrocarpa</i>	1982	3x2	18	276	29.6
	25b	<i>Pinus caribaea</i> var <i>caribaea</i>	1978	3x2	22, 31	292	34.0
	24i	<i>Eucalyptus grandis</i>	1977	3x2	23	877	34.8
	26e	<i>Pinus pseudostrobus</i>	1979	3x2	19, 24	162	32.6
26f	<i>Corymbia totelliana</i>	1979	3x2	19, 23	617	32.4	
31c	<i>Pinus tecunumanii</i>	2003	3x2	7	1315	8.1	
32e	<i>Eucalyptus camaldulensis</i>	2003	3x2		1657	6.4	

Table 1.2 Aboveground stemwood carbon (Mg C ha⁻¹) and WNPP (Mg C ha⁻¹ yr⁻¹) for broadleaf and conifer plantations. (P-B = Pasture-Broadleaf; B-C = Conifer-Broadleaf)

Paired Plot	Vegetation	Plot ID	Age at Inventory (yrs)	Aboveground	
				Stemwood (Mg C ha ⁻¹)	WNPP (Mg C ha ⁻¹ yr ⁻¹)
P-B	Broadleaf	07f	13.5	201	14.8
		12d	20.5	264	12.9
		14a	29.8	261	8.7
		15a	24.9	268	10.8
		23f	24.8	126	5.1
		28b	32.9	163	4.9
		28c	23.8	248	10.4
		28h	12.0	181	15.2
		30b	11.0	169	15.4
		33c	9.2	131	14.2
B-C	Conifer	01b	32.6	142	4.4
	Broadleaf	03b	31.6	40	1.3
	Conifer	04a	35.9	105	2.9
	Broadleaf	02a	36.2	176	4.9
	Conifer	04c	32.7	208	6.4
	Broadleaf	03a	33.6	179	5.3
	Conifer	05a	36.2	199	5.5
	Broadleaf	06c	26.6	145	5.5
	Conifer	06a	33.1	166	5.0
	Broadleaf	06d	25.8	296	11.5
	Conifer	06f	24.0	170	7.1
	Broadleaf	08e	31.9	237	7.4
	Conifer	25a	34.2	193	5.6
	Broadleaf	24r	29.6	394	13.3
	Conifer	25b	34.0	189	5.5
	Broadleaf	24i	34.8	548	15.7
	Conifer	26e	32.6	175	5.4
	Broadleaf	26f	32.4	189	5.8
Conifer	31c	8.1	73	9.0	
Broadleaf	32e	6.4	73	11.3	

Table 1.3 Mean soil organic carbon stocks (\pm standard error) for each pair of vegetation plots at each sampling depth. Pasture-Broadleaf pairs (n=10) and Broadleaf-Conifer pairs (n=10).

Vegetation pairs	O-horizon (Mg C ha ⁻¹)	Mineral soil (Mg C ha ⁻¹)						Total (Mg C ha ⁻¹)
		0-15 cm		15-30 cm		30-45 cm		
Natural Stand	1.9	26.6		16.1		11.0		55.6
Pasture	1.6	14.6		13.4		9.9		39.5
Pasture	2.5 (0.41)	12.4 (0.57)		11.9 (0.81)		9.1 (0.48)		36.0 (1.73)
Broadleaf	1.8 (0.21)	13.5 (0.84)		12.2 (0.81)		9.4 (0.47)		36.8 (1.87)
Broadleaf	5.2 (0.85)	14.2 (0.78)		10.5 (0.66)		8.4 (0.46)		38.3 (1.93)
Conifer	4.9 (0.90)	13.0 (0.67)		10.5 (0.56)		8.0 (0.36)		36.0 (1.60)

Table 1.4 Plots were analyzed for bulk density (Mg m^{-3}) at one central point per plot. Total soil carbon stocks results are reported for each sampling depth (O-horizon, 0-15, 15-30, and 30-45 cm). Standard deviation (stdev) represents within-plot variation (n=9).

Paired Plot	Species	Bulk Density (Mg m^{-3})			Carbon stocks per depth (Mg C ha^{-1})					
		0-15 cm	15-30 cm	30-45 cm	O-Horizon	0-15 cm (stdev)	15-30 cm (stdev)	30-45 cm (stdev)		
01b03b	broadleaf	1.06	1.24	1.42	3.1	12.4 (1.3)	9.4 (1.9)	9.0 (0.6)		
01b03b	conifer	1.12	1.34	1.33	2.7	12.1 (0.7)	8.6 (0.2)	7.7 (0.5)		
04a02a	broadleaf	1.17	1.41	1.18	3.3	12.7 (1.4)	10.5 (1.3)	7.2 (0.4)		
04a02a	conifer	1.10	1.44	1.37	5.1	11.4 (1.9)	9.8 (1.9)	7.8 (1.6)		
04c03a	broadleaf	1.28	1.41	1.33	2.9	18.4 (4.0)	11.7 (2.9)	8.0 (2.9)		
04c03a	conifer	1.30	1.54	1.28	3.2	15.5 (2.7)	12.3 (2.5)	9.6 (2.8)		
05a06c	broadleaf	1.33	1.44	1.40	7.8	13.5 (0.7)	10.9 (0.7)	9.4 (0.3)		
05a06c	conifer	1.27	1.42	1.30	4.7	15.8 (1.4)	10.9 (0.7)	8.7 (0.3)		
06a06d	broadleaf	1.20	1.34	1.28	3.9	11.6 (0.3)	9.8 (0.3)	8.1 (0.4)		
06a06d	conifer	1.47	1.54	1.41	2.1	13.6 (1.3)	10.6 (2.8)	8.3 (0.7)		
06f08e	broadleaf	1.39	1.41	1.32	3.6	12.3 (1.1)	9.6 (0.7)	8.2 (1.9)		
06f08e	conifer	1.25	1.53	1.54	2.8	9.3 (0.7)	9.0 (0.5)	8.0 (0.7)		
25a24r	broadleaf	1.26	1.40	1.37	6.3	13.0 (1.5)	7.8 (1.4)	6.2 (1.1)		
25a24r	conifer	1.42	1.60	1.49	3.4	12.2 (3.0)	9.5 (1.2)	6.9 (0.5)		
25b24i	broadleaf	1.26	1.55	1.36	5.5	13.7 (2.2)	9.4 (1.6)	7.1 (1.3)		
25b24i	conifer	1.46	1.63	1.60	7.3	12.0 (1.9)	8.3 (1.2)	7.1 (0.5)		
26e26f	broadleaf	1.36	1.37	1.43	11.5	17.3 (2.9)	9.8 (1.6)	9.0 (1.3)		
26e26f	conifer	1.37	1.56	1.40	11.4	12.7 (1.8)	8.1 (1.6)	6.1 (1.6)		
31c32e	broadleaf	1.40	1.51	1.36	4.8	17.2 (2.4)	15.6 (2.3)	11.4 (1.6)		
31c32e	conifer	1.48	1.43	1.21	6.6	15.8 (2.1)	13.5 (1.6)	9.7 (1.4)		
07f	broadleaf	1.24	1.48	1.36	2.1	10.7 (1.2)	10.4 (1.5)	8.2 (0.8)		
07f	pasture	1.42	1.43	1.24	2.6	10.9 (0.9)	9.2 (1.0)	6.8 (0.6)		
12d	broadleaf	1.09	1.23	1.24	2.0	12.4 (1.2)	11.2 (1.4)	9.2 (1.3)		

Table 1.4 Continued.

12d	pasture	1.07	1.51	1.38	1.5	10.3	(2.0)	12.3	(1.3)	9.2	(0.5)
14a	broadleaf	1.27	1.40	1.40	1.5	13.7	(1.6)	10.8	(1.4)	8.3	(0.6)
14a	pasture	1.21	1.39	1.30	1.7	12.2	(1.0)	10.6	(0.8)	7.8	(1.2)
15a	broadleaf	1.14	1.24	1.36	0.9	10.4	(2.1)	6.9	(2.8)	7.7	(1.5)
15a	pasture	1.35	1.24	1.34	2.3	10.5	(1.6)	7.3	(1.3)	6.9	(1.0)
23f	broadleaf	1.35	1.39	1.30	2.9	14.3	(2.4)	11.2	(2.0)	8.3	(0.9)
23f	pasture	1.15	1.42	1.32	5.1	12.1	(1.0)	11.4	(1.7)	9.0	(1.0)
28b	broadleaf	1.39	1.48	1.27	1.1	18.2	(3.5)	15.1	(1.3)	9.5	(1.3)
28b	pasture	1.18	1.57	1.42	0.6	12.3	(2.1)	13.5	(1.3)	10.9	(1.9)
28c	broadleaf	1.13	1.39	1.19	1.7	16.1	(2.7)	16.5	(3.3)	12.8	(3.8)
28c	pasture	1.16	1.36	1.23	1.4	15.1	(0.5)	14.6	(1.6)	10.4	(2.0)
28h	broadleaf	1.22	1.43	1.39	2.7	11.4	(1.4)	14.1	(1.9)	10.4	(0.5)
28h	pasture	1.07	1.38	1.17	3.2	15.8	(2.1)	15.6	(0.6)	9.5	(0.8)
30b	broadleaf	1.24	1.38	1.34	2.1	11.4	(0.5)	11.4	(2.1)	9.8	(0.1)
30b	pasture	1.26	1.39	1.35	3.3	12.2	(1.2)	10.8	(1.2)	9.8	(0.7)
33c	broadleaf	1.35	1.36	1.23	1.2	16.0	(0.5)	13.3	(2.8)	9.4	(1.2)
33c	pasture	1.11	1.44	1.45	3.5	12.9	(1.3)	13.7	(1.2)	11.0	(0.7)
Ref	broadleaf	1.45	1.31	1.26	1.9	26.6	(6.7)	16.1	(2.8)	11.0	(2.2)
Ref	pasture	1.22	1.45	1.26	1.6	14.6	(2.5)	13.4	(2.3)	9.9	(1.4)

Table 1.5 Paired plots were analyzed soil nitrogen stocks (Mg N ha⁻¹) at each mineral soil sampling depth (0-15, 15-30, and 30-45 cm). Standard deviation (stdev) represents the within-plot variation (n=9).

Plot	Species	Nitrogen stocks per depth (Mg N ha ⁻¹)						C:N Ratio
		0-15 cm	(stdev)	15-30 cm	(stdev)	30-45 cm	(stdev)	0-45 cm
01b03b	broadleaf	0.81	(<0.1)	0.67	(0.1)	0.70	(0.1)	14.2
01b03b	conifer	0.73	(<0.1)	0.62	(0.1)	0.57	(0.1)	14.8
04a02a	broadleaf	0.96	(0.1)	0.88	(0.1)	0.64	(0.0)	12.2
04a02a	conifer	0.87	(0.1)	0.84	(0.1)	0.71	(0.1)	12.0
04c03a	broadleaf	1.44	(0.3)	0.95	(0.2)	0.64	(0.2)	12.5
04c03a	conifer	1.08	(0.1)	1.03	(0.2)	0.79	(0.3)	12.9
05a06c	broadleaf	0.99	(0.0)	0.87	(0.1)	0.78	(0.0)	12.8
05a06c	conifer	1.05	(0.1)	0.82	(0.1)	0.70	(0.1)	13.7
06a06d	broadleaf	0.80	(0.1)	0.76	(0.0)	0.63	(0.0)	13.4
06a06d	conifer	0.96	(0.1)	0.85	(0.3)	0.67	(0.0)	13.1
06f08e	broadleaf	0.92	(0.1)	0.81	(0.0)	0.71	(0.1)	12.4
06f08e	conifer	0.69	(0.0)	0.77	(0.0)	0.72	(0.0)	12.1
25a24r	broadleaf	0.72	(0.1)	0.48	(0.1)	0.37	(0.1)	17.1
25a24r	conifer	0.62	(0.1)	0.53	(0.0)	0.40	(0.1)	18.4
25b24i	broadleaf	0.91	(0.2)	0.67	(0.1)	0.53	(0.1)	14.4
25b24i	conifer	0.73	(0.1)	0.57	(0.1)	0.50	(0.1)	15.2
26e26f	broadleaf	1.02	(0.1)	0.63	(0.1)	0.60	(0.1)	16.1
26e26f	conifer	0.76	(0.1)	0.54	(0.1)	0.41	(0.1)	15.7
31c32e	broadleaf	1.61	(0.2)	1.54	(0.2)	1.22	(0.1)	10.1
31c32e	conifer	1.57	(0.2)	1.42	(0.1)	1.06	(0.1)	9.6
07f	broadleaf	0.69	(0.1)	0.71	(0.1)	0.57	(0.1)	15.0
07f	pasture	0.77	(0.1)	0.66	(0.1)	0.48	(0.0)	14.0

Table 1.5 Continued.

12d	broadleaf	0.78	(0.1)	0.73	(0.1)	0.61	(0.1)	15.4
12d	pasture	0.69	(0.1)	0.84	(0.1)	0.62	(0.0)	14.8
14a	broadleaf	0.79	(0.1)	0.63	(0.1)	0.47	(0.0)	17.3
14a	pasture	0.77	(0.1)	0.64	(0.1)	0.46	(0.1)	16.3
15a	broadleaf	0.56	(0.1)	0.37	(0.2)	0.41	(0.1)	18.7
15a	pasture	0.63	(0.1)	0.43	(0.1)	0.37	(0.1)	17.2
23f	broadleaf	1.07	(0.2)	0.84	(0.1)	0.63	(0.0)	13.3
23f	pasture	0.86	(0.1)	0.80	(0.1)	0.63	(0.1)	14.2
28b	broadleaf	1.18	(0.2)	0.93	(0.1)	0.55	(0.1)	16.1
28b	pasture	0.77	(0.1)	0.81	(0.1)	0.63	(0.1)	16.5
28c	broadleaf	1.12	(0.2)	1.19	(0.2)	0.93	(0.3)	14.0
28c	pasture	1.11	(0.1)	1.09	(0.1)	0.81	(0.2)	13.3
28h	broadleaf	0.84	(0.1)	1.05	(0.2)	0.76	(0.0)	13.5
28h	pasture	1.07	(0.2)	1.05	(0.0)	0.66	(0.1)	14.7
30b	broadleaf	0.91	(0.0)	0.96	(0.2)	0.82	(0.0)	12.1
30b	pasture	0.96	(0.1)	0.88	(0.1)	0.80	(0.1)	12.4
33c	broadleaf	1.24	(0.1)	1.05	(0.2)	0.74	(0.0)	12.8
33c	pasture	1.03	(0.1)	1.09	(0.1)	0.94	(0.1)	12.3
Ref	broadleaf	2.02	(0.5)	1.23	(0.2)	0.86	(0.2)	13.0
Ref	pasture	1.05	(0.2)	1.03	(0.2)	0.79	(0.1)	13.2

Table 1.6 All plots were analyzed for soil texture with nine composite samples per mineral soil depth (0-15, 15-30, and 30-45 cm).

Plot	Species	Clay content (%)			Silt content (%)		
		0-15 cm	15-30 cm	30-45 cm	0-15 cm	15-30 cm	30-45 cm
01b03b	broadleaf	14	18	18	6	6	7
01b03b	conifer	14	18	20	6	6	6
04a02a	broadleaf	18	18	24	6	9	7
04a02a	conifer	18	20	22	6	6	8
04c03a	broadleaf	12	14	16	11	10	10
04c03a	conifer	16	18	26	11	12	12
05a06c	broadleaf	14	18	20	6	5	7
05a06c	conifer	14	18	20	6	6	7
06a06d	broadleaf	18	22	24	9	8	10
06a06d	conifer	20	24	24	6	7	8
06f08e	broadleaf	16	20	22	5	5	5
06f08e	conifer	12	14	16	3	2	3
25a24r	broadleaf	14	18	22	5	7	7
25a24r	conifer	16	18	20	3	5	6
25b24i	broadleaf	12	14	16	4	6	4
25b24i	conifer	12	14	16	4	6	7
26e26f	broadleaf	19	19	18	3	3	4
26e26f	conifer	16	20	22	3	3	4
31c32e	broadleaf	18	22	24	6	7	8
31c32e	conifer	18	20	22	4	5	7
07f	broadleaf	18	20	22	5	8	7
07f	pasture	16	18	22	5	6	5
12d	broadleaf	22	24	26	7	7	7
12d	pasture	18	20	24	7	7	5

Table 1.6 Continued.

14a	broadleaf	20	22	22	5	6	7
14a	pasture	18	20	22	5	5	6
15a	broadleaf	20	22	24	6	7	7
15a	pasture	18	22	24	6	6	6
23f	broadleaf	18	20	20	3	5	7
23f	pasture	14	18	20	6	6	6
28b	broadleaf	16	20	22	10	10	10
28b	pasture	16	18	20	9	9	9
28c	broadleaf	23	25	29	8	9	9
28c	pasture	21	25	29	9	8	8
28h	broadleaf	22	26	28	7	8	7
28h	pasture	24	30	32	9	10	12
30b	broadleaf	22	20	26	3	6	6
30b	pasture	18	24	20	5	5	6
33c	broadleaf	20	22	22	7	7	10
33c	pasture	18	20	18	7	8	11
Ref	broadleaf	16	18	20	8	7	8
Ref	pasture	14	18	20	9	9	8

Table 1.7 Results of general linear models for Pasture-Broadleaf (P-B) and Broadleaf-Conifer (B-C) paired plots developed to determine the significance of vegetation effects on total soil organic carbon (SOC_T) stocks after forest establishment. (SON = soil organic nitrogen)

Model	Factor	df	F-value	P-value	R²
P-B SOC _T	Veg	1	0.46	0.5167	0.91
	Block	9	9.53	0.0013	
	Model	19	8.60	0.0017	
	SON	1	6.73	0.0319	
	Veg	1	5.46	0.0477	
B-C SOC _T	Block	9	12.04	0.0009	0.96
	Model	19	18.51	0.0002	

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APPENDIX

Appendix 1. Soil profile description for Typic Dystrophic Red-yellow Latosols and soil nutrient details for a soil profile located within the Anhembi Experimental Research Station.

Morphological Characteristics and Analysis (Andrade *et al.*, 2010):

Deep to very deep soil. In the horizons below 80 cm, angular block structure was observed with strong microaggregates, which is typical of Latosols. Along the profile, color varied from 7.5YR 4/6 to 5YR 5/8. The texture is mostly sandy loam (sand content between 61-76%). The soil is dystrophic along the profile, has a low CEC (< 17 cmol/kg), and is acidic (pH < 4.3). The low clay content means there is a low water-holding capacity and the structure and high sand content creates good drainage and resistance to compaction.

Therefore, the risk of erosion of this soil is low. However, if the structure is destroyed, the risk of erosion increases due to the sandy soil texture.

Profile: Typical Dystrophic Red-yellow Latosol

- A** 0-10 cm: Dark reddish brown (5 YR 3/2, humid; 5 YR 4/1 dry); medium texture; small, moderate subangular blocky, granular, slightly hard, friable, not plastic, slightly sticky, clear transition.
- AB** 10-35 cm: Strong brown (7.5 YR 4/6, humid; 7.5 YR 5/6, dry); few small mottles, yellowish red (5 YR 5/8), medium texture; medium, moderate, angular blocks, slightly hard, very friable, not plastic, slightly sticky, flat and clear transition.
- BA** 35-80 cm: Yellowish red (5 YR 4/6, humid; 7.5 YR 5/8, dry) and yellow-red (7.5 YR 6/6, humid; 10 YR 7/6, dry); medium texture; medium and large, weak, angular blocks that crumble into strong microaggregates; slightly hard, very friable; slightly plastic, slightly sticky; fuzzy/wavy transition.
- Bw** 80-175 cm mottled yellowish red (5 YR 5/8, humid) and yellowish-brown (10 YR 5/4, humid); medium texture; medium and large, weak, angular blocks that crumble into strong microaggregates; slightly hard, very friable; slightly plastic, slightly sticky; fuzzy/wavy transition.

Roots: small and medium roots common in A horizon; medium roots common and small roots rare in AB; small and medium roots rare in BA and Bw

GPS (UTM): 22K 0789749 / 7486326

Chemical Attributes											
Horizon	Depth	P	C	pH	K	Ca	Mg	H+Al	SB	T	V
	cm	mg kg ⁻¹	g kg ⁻¹	CaCl ₂	cmol _c kg ⁻¹						%
A	0-10	8	17.6	4.5	0.30	1.61	0.73	3.7	2.6	6.3	42
AB	10-35	2	5.4	4.0	0.07	0.26	0.13	3.3	0.5	3.8	12
BA	35-80	2	3.4	4.1	0.03	0.45	0.09	2.8	0.6	3.4	17
Bw	80-175	1	2.7	4.0	0.01	0.28	0.05	3.0	0.3	3.3	10
C	175	2	2.0	4.1	0.02	0.15	0.06	2.5	0.2	2.7	9

Physical Attributes								
Horizon	Depth	Clay	Silt	Sand			Clay Activity	Texture Class
				Total	Medium	Fine		
	cm	%						cmol _c kg ⁻¹ clay
A	0-10	16	13	71	58	13	39	Sandy loam
AB	10-35	20	9	71	57	14	19	Sandy clay loam
BA	35-80	24	10	66	14	52	14	Sandy clay loam
Bw	80-175	24	10	66	15	51	14	Sandy clay loam
C	175	22	10	68	15	53	12	Sandy clay loam

Chapter 2 – Soil carbon dynamics following reforestation of tropical pastures

Abstract

Stable carbon isotopes can be used to differentiate between plant carbon sources in the mineral soil. Differing photosynthetic pathways of C₄ grasses and C₃ trees create unique stable carbon isotopic signatures that can be used in evaluating soil organic carbon dynamics following reforestation. Based on isotopic signatures in one Reference unmanaged forest and pasture pair (Ref-P), seven paired Pasture-Broadleaf (P-B) plantations, and ten Broadleaf-Conifer (B-C) plantations, we estimated turnover of soil organic carbon (SOC) derived from pasture (SOC₄) and from planted forests (SOC₃) at three depths from 0-45 cm. Decomposition rates and mean residence times of SOC were estimated from a chronosequence approach based on stands ranging in age from 6-34 years. Results from this approach show that the age of conversion from C₄-pasture dominated soils following reforestation was depth-dependent. For all plantations (averaging 16.8 Mg dry matter ha⁻¹ yr⁻¹ wood NPP or WNPP) the estimated age when soils became dominated by C₃-forest carbon (for both conifers and broadleaf plantations) was 9.7 years at 0-15 cm and 8 years at 15-30 cm. Soils at a 30-45 cm depth remained dominated by SOC₃ from the original forest. Turnover rates showed that in the 0-15 cm depth, the rate of forest SOC₃ accumulation (0.21 Mg C ha⁻¹ yr⁻¹) matched the rate of SOC₄ disappearance (-0.21 Mg C ha⁻¹ yr⁻¹). The slight decrease in mineral soil organic carbon over time in the 15-30 cm and 30-45 cm soil layers is due to a loss of SOC₄ (-0.17 Mg C ha⁻¹ yr⁻¹ and -0.083 Mg C ha⁻¹ yr⁻¹, respectively) while SOC₃ rate of change stayed constant. These results clearly illustrate slower rates of pasture derived soil carbon decomposition with increasing depth. Mean residence times (MRT) for C₄ pasture carbon increased significantly with depth with (mean ± standard error) 30 ± 4.5, 33 ± 4.7, and 73 ± 18 years for 0-15 cm, 15-30 cm, and 30-45 cm, respectively. MRT was not dependent on forest vegetation type (conifer or broadleaf).

Introduction

Land-use change accounts for approximately 34% of total anthropogenic carbon dioxide emissions (Richter and Houghton, 2011). To balance this flux of CO₂ to the atmosphere, there has been much interest in understanding how soils can sequester more atmospheric carbon with improved land-use practices. Reforestation or afforestation has garnered interest for its potential to restore higher levels of soil carbon, but the lack of a clear understanding of soil organic carbon turnover following land-use change has made it difficult to make clear recommendations.

Conversion of forests to agricultural practices has been shown to reduce levels of soil organic carbon (Detwiler, 1986). However, changes in soil organic carbon following reforestation of productive pastures has been shown to be insignificant (Laganière *et al.*, 2010, Wellock *et al.*, 2011). This lack of total change, however, can be due to balanced rates of decomposition of soil organic carbon derived from the prior land cover and accumulation of soil carbon from new vegetation (Bashkin and Binkley, 1998). To differentiate between plant sources of soil organic carbon following land-use change, we can take advantage of different photosynthetic pathways between C₄ and C₃ plants, which take up naturally abundant ¹³C isotopes to different degrees (Balesdent *et al.*, 1987). Tropical grasses (C₄ plants) generally have δ¹³C values of -10‰ to -15‰, while trees (C₃ plants) range in δ¹³C values between -21‰ to -30‰ (Ehleringer *et al.*, 2000). We can use this information to understand soil carbon dynamics and calculate soil carbon turnover by quantifying fractions of soil carbon following land-use change since soil organic carbon maintains essentially the same signature as the plant inputs (Bernoux *et al.*, 1998).

Relative contributions of C₃ trees or C₄ grasses to soil organic carbon can be quantified using a mass balance of stable isotopes approach (Del Galdo *et al.*, 2003; Marin-Spiotta *et al.*, 2009). Turnover rates from C₄-grass-derived soil carbon to C₃-tree-derived carbon following reforestation can be calculated from the change in ¹³C ratios by using many stands of variable ages, known as a chronosequence approach (Bernoux *et al.*, 1998; Richards *et al.*, 2007). By sampling stands with a range of ages, we can estimate the overall change in carbon following land-use change by substituting space for time. This is a

common approach when long-term soil data are lacking, and relies on the assumption of no major biotic or abiotic differences among stands other than age. A homogenous soil type, topography, and a paired-plot sampling design (that groups adjacent vegetation types) can help assure these assumptions are valid.

The purpose of this study was to quantify C4-pasture- and C3-forest-derived carbon following reforestation of tropical pasture with conifer and broadleaf plantations. The specific objectives were to 1) calculate the percentage of pasture- and forest-derived carbon for each mineral soil depth based on $\delta^{13}\text{C}$ signatures, 2) determine SOC turnover rates for C4 and C3 carbon pools at each soil depth, and 3) estimate mean residence time of pasture-derived carbon at three soil depths following reforestation. This study provides a robust analysis of soil organic carbon dynamics following reforestation of tropical pastures with conifer and broadleaf plantations.

Methods

Site Characterization

Soil samples were collected at the Anhembi Experimental Research Station, located in the center of the state of Sao Paulo, Brazil. Across this 663.5 ha field site, paired-plots considered for this analysis consisted of one unmanaged, native reference forest with adjacent pasture (Ref-P), seven Pasture-Broadleaf (P-B) plantations, ten Broadleaf-Conifer (B-C) plantations, and (**Figure 2.1**). All broadleaf plantations fall under the genera *Eucalyptus* and *Corymbia*, while conifer plantations all consist of *Pinus*. Sampling depths were located at 0-15, 15-30, and 30-45 cm. Records for silvicultural treatments across forest stands show consistent site preparation and minimal fertilization, creating ideal conditions for testing vegetation effects over time. Additional site information and sampling design are detailed in Chapter 1.

Stable Carbon Isotopes

To determine stable carbon isotope ratios, a composite of all soil samples for each plot ($n = 9$) for each of the three depths was analyzed by continuous flow isotope ratio mass

spectrometry (CF-IRMS) (PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer, Sercon Ltd., Cheshire, UK) at the University of California Davis Stable Isotope Facility. The standard notation for carbon isotope composition is expressed as δ values relative to the Pee Dee Belemnite standard (Ehleringer *et al.*, 2000):

$$\delta^{13}\text{C} (\text{‰}) = [(R_{\text{sample}} - R_{\text{standard}}) - 1] \times 1000$$

where R is the molar ratio of ^{13}C to ^{12}C (heavy to light isotope) of the sample or standard (PDB $R_{\text{standard}} = 0.112$) (Bernoux *et al.*, 1998).

Different photosynthetic pathways in trees (C3) and tropical grasses (C4) create recognizable isotopic carbon signatures in their plant biomass and their corresponding soil organic matter. The mass balance of stable isotopes approach can use ^{13}C values to estimate the percentage of pasture (%SOC₄) and tree (%SOC₃) derived soil carbon by the equations (Bashkin and Binkley, 1998):

$$\% \text{SOC}_4 = (\delta_s - \delta_o) / (\delta_L - \delta_o) \times 100$$

$$\% \text{SOC}_3 = 100 - \% \text{SOC}_4$$

where δ_s is the $\delta^{13}\text{C}$ of the soil sample, δ_o is the $\delta^{13}\text{C}$ of reference forest soil, and δ_L is the $\delta^{13}\text{C}$ of pasture litter ($-15.69 \pm 0.32\text{‰}$, $n = 7$). Residual carbon from the C3 native forest vegetation that preceded pasture conversion are taken into account through this calculation.

Percentages of C4 and C3 carbon were multiplied by the quantity of mineral soil organic carbon (SOC_M) for each 15 cm soil layer to estimate soil carbon derived from C3 trees and C4 grasses (methods and values for SOC_M are shown in Chapter 1). Any increase in C3-tree carbon must be from current tree stands, though total C3-carbon represents a mixture of old and new C3-tree carbon. To estimate soil carbon turnover, results were analyzed with an exponential decay function assuming a single soil carbon pool to estimate

the rate of loss of pasture-derived carbon (Bernoux *et al.*, 1998; Del Galdo *et al.*, 2003; Richards *et al.*, 2007):

$$k = -\frac{\ln\left(\frac{C_t}{C_0}\right)}{t}$$

where C_0 and C_t are the amounts of C4 soil carbon initially (in the pasture) and at time t (in the forest), respectively. Since B-C plots were not directly paired with an adjacent pasture, the mean pasture values of SOC₄ from all seven P-B pasture plots for each depth were used for C_0 to determine turnover (**Table 2.1**). The rate of loss, k , represents the loss of C4 soil carbon (Del Galdo *et al.*, 2003; Richards *et al.*, 2007). Mean residence time (MRT) of pasture-derived C4 soil carbon following reforestation is calculated as $1/k$.

Statistics

In this randomized block design, vegetation is the main treatment of experimental interest in an analysis of variance (ANOVA). Paired plots were considered the blocking factor to increase precision in the model. ANOVAs were run with a general linear model for B-C and P-B paired plots. Our dependent variables, $\delta^{13}\text{C}$ and MRT, were modeled with independent class variables consisting of vegetation, blocks, and depth. MRT required log transformation prior to analysis due to unequal variance. Since vegetation was consistently non-significant in the models, and age and depth were significantly correlated with changes in $\delta^{13}\text{C}$, we used a bivariate linear model to calculate turnover time based on age and SOC₃/SOC₄ stocks (Mg C ha^{-1}) of. Multiple comparisons in ANOVA were analyzed using Tukey's honestly significant difference (HSD) with a significance level of $P < 0.05$. Statistics were calculated using the statistical packages SAS v 9.2 and JMP v 9.0 (SAS Institute, Cary, NC).

Results and Discussion

Litter $\delta^{13}\text{C}$ Values

Values for $\delta^{13}\text{C}$ for broadleaf plantation litter collected from the soil surface averaged (\pm standard error) $-28.85 \pm 0.07\text{‰}$, while conifers averaged $-27.94 \pm 0.14\text{‰}$, and pastures averaged $-15.69 \pm 0.32\text{‰}$, with seven replicates per vegetation type. These values fall within the range of typical C3 tree litter and C4 pasture litter (Ehleringer *et al.*, 2000). Root litter values can vary slightly from aboveground inputs due to secondary fractionation and biochemical composition of plant tissue, but may only differ by 1‰ (Billings and Richter, 2006; Lima *et al.*, 2006), which still leaves a clear difference between pasture and forest litter values.

Soil $\delta^{13}\text{C}$ Values

The $\delta^{13}\text{C}$ values of the mineral soil differed significantly between pasture and tree vegetation type. However, conifer and broadleaf $\delta^{13}\text{C}$ mineral soil signatures were not statistically different in the general linear model (Trouve *et al.*, 1994). Average mineral soil $\delta^{13}\text{C}$ values for pasture averaged -19‰ in the top 15 cm and became more negative (to -21‰) with depth. Conifer and broadleaf plantation mineral soil averaged -24‰ in the topmost soil layer and did not vary significantly with depth.

To more closely examine within-plot variability, analysis of all sub-samples in one paired plot ($n = 54$; 9 points \times 3 depths \times 2 vegetation types) for $\delta^{13}\text{C}$ showed remarkably little variability at each depth for each vegetation type (**Figure 2.2**). There is a clear trend for $\delta^{13}\text{C}$ to become more negative (closer to a forest $\delta^{13}\text{C}$ signature) as depth increases in pasture, but there was no significant difference in $\delta^{13}\text{C}$ values as depth increases in the forest soil. This small amount of variation at each depth for each vegetation validated the methodology of compositing sub-samples per depth for analysis across vegetation types.

The $\delta^{13}\text{C}$ values for soils in the Reference pasture site provided a good example of residual C3 carbon remaining predominant in the deeper soil layers. Calculating %SOC₃ and % SOC₄ from $\delta^{13}\text{C}$ showed 53% forest-derived carbon remaining in 30-45 cm soil layer

(**Figure 2.3**). The adjacent paired pasture values reflect favorably with the mean pasture values across all seven paired P-B sites, and show a stronger signal from the C₄-pasture grass input in the upper two layers (**Table 2.1**). This could be attributed to the fact that the Reference pasture had little encroachment by woody plants and less tree leaf litter falling from nearby forest stands compared to other pastures.

Reference forest $\delta^{13}\text{C}$ values remain consistent along the soil profile (**Table 2.1**) and are the least $\delta^{13}\text{C}$ enriched of all the plots (**Figure 2.4**). Comparison of $\delta^{13}\text{C}$ values in the P-B plots also showed a significant interaction between vegetation type and depth. Pasture sites contain significantly more %SOC₄ and amount of SOC₄ in the upper two layers (0-15 and 15-30 cm). Tukey's multiple comparison showed that the deepest soil layer (30-45 cm) was not significantly different from any of the paired broadleaf forest soil $\delta^{13}\text{C}$ values. The main effect of vegetation type or a depth x vegetation interaction were not significant for $\delta^{13}\text{C}$ values in B-C plantation pairs, but age and soil depth (along with the blocking factor) were significant in the model. These results are reasonable considering the similar values $\delta^{13}\text{C}$ for conifer and broadleaf litter input.

SOC₃ and SOC₄ Turnover

The chronosequence approach allows the use of stands of different ages to mimic changes over real time. This method works well in field situations where environmental factors, site resources, and land-use history are essentially constant, such as this field site. Forest stand age may be less appropriate for comparisons among sites in different regions with highly different productivities. In that case, total aboveground biomass may be a surrogate with highly different site resources or stand management. However, in our case, the chronosequence approach and forest stand age proved to be a valuable tool for estimating soil carbon turnover.

Initial examination of changes in mineral soil organic carbon showed no significant relationship with increasing forest stand age (**Figure 2.5**). However, the use of stable carbon isotopes allowed us to separate accumulation and decomposition of pasture and forest derived carbon. While there may be no total change in mineral soil organic carbon, the

differentiation between carbon types (SOC₃ versus SOC₄) allowed us to delve more deeply into the dynamics of soil carbon gains and losses.

The age of conversion from a C4- to C3-dominated soil profile following reforestation was calculated based on rates of change in %SOC₃ and %SOC₄ in the forest plots for each soil depth (**Figure 2.6**). Values for P-B and B-C plots can be found in **Table 2.2** and **Table 2.3**. The intersection of linear regressions for %SOC₃ and %SOC₄ shows the point at which soils become dominated by forest-derived carbon following reforestation. In the 0-15 cm layer, the estimated age of conversion to a C3-dominated soil was 9.7 years. In the second soil layer (15-30 cm), the soil became C3-dominated following 8 years of reforestation. Lack of intersection in the deepest profile (30-45 cm) reinforces the observation in the Reference forest-pasture pair that deeper soils never became dominated by C4-grass carbon, and have consistently been C3 dominated from original forest cover. The shorter time period in the 15-30 cm layer is likely a reflection of the fact that this soil layer never received as much C4 carbon as the top-most layer. The 0-15 cm layer in forest stands has likely continued to receive a small amount of C4 input from some grassy understory growth.

Accumulation of C3 and decomposition of C4 soil stocks were calculated based on %SOC₃/%SOC₄ and mineral soil carbon (SOC_M) stocks. Results for SOC_M can be found in Chapter 1, **Table 1.4**. Changes in SOC₃ and SOC₄ over time showed that in the 0-15 cm depth, the rate of forest SOC₃ accumulation (0.21 Mg C ha⁻¹ yr⁻¹) matched the rate of SOC₄ disappearance (-0.21 Mg C ha⁻¹ yr⁻¹) (**Figure 2.7**). The slight decrease in total soil organic carbon over time in the 15-30 cm and 30-45 cm soil layers is due to a loss of SOC₄ (-0.17 Mg C ha⁻¹ yr⁻¹ and -0.083 Mg C ha⁻¹ yr⁻¹, respectively) while SOC₃ stayed essentially constant. These results clearly illustrate slower rates of decomposition of C4-pasture-derived soil carbon with increasing depth. The depth dependence and constant state of SOC₃ are consistent with other observations of minimal incorporation of new forest carbon in deeper soil layers following decades of forest establishment on agricultural land (Billings and Richter, 2006).

The results from this analysis contribute a great deal of information to understanding the soil carbon dynamics of pasture- and forest-derived carbon following reforestation. Depth is consistently a significant factor while the difference between conifer or broadleaf vegetation matters little to decomposition rates of pasture carbon. The lack of significant increase in C3-forest carbon in the bottom two layers (15-30 and 30-45 cm) may be due to longer-lived coarse roots of trees at these depths being a greater sink for carbon than soils, or simply, that there is a balance of carbon inputs with microbial decomposition of new and old C3 carbon.

Mean Residence Time

Mean residence times (MRT) for C4-pasture carbon following reforestation increased significantly with depth, but was not dependent on forest conifer versus broadleaf vegetation type (**Figure 2.8**). Average values (\pm standard error) per depth were 30 ± 4.5 , 33 ± 4.7 , and 73 ± 18 years for 0-15 cm, 15-30 cm, and 30-45 cm, respectively. These ages fall into the range of mean residence times calculated for forest to pasture conversion in the Brazilian Amazon, showing between 1.25 years in the active pool and 443 years in the slow pool (Lisboa *et al.*, 2009). There were four out of 81 decay rates that were not detectable (negative values) due higher SOC₄ values in the reforested plots compared to the pasture. This may have been due to continued C4 input from a grassy understory while the forest stand was young.

The SOC₄ MRT was negatively correlated with the age of forest plantations at the 0-15 cm depth but not below. This effect is less likely due to actual longer turnover times in younger forest plantations, and more a result of continued input of C4 from the understory before canopy closure. As forests age, and there is less contribution from a grassy understory in the upper layers, the MRT appears to decline more. Our results show higher mean residence times for deeper soil layers which agrees with other studies (Fontaine *et al.*, 2007). One issue concerning MRT calculations is that it uses pasture carbon as initial C and forest C4 carbon as C at time t . Using the pasture as time zero is only completely valid if there is no

additional C4 carbon accumulating in the pasture following reforestation. Therefore, in this case, our values for C_0 may be slightly overestimated.

Conclusion

Stable carbon isotopic signatures allow the deconstruction of soil organic carbon dynamics following reforestation of C4-pasture with C3-forest systems. Total soil organic carbon results may indicate that very little change is occurring, when in fact there are very different rates of decomposition and accumulation occurring at each soil depth.

The chronosequence approach and number of paired-plot replicates allowed the calculation of C4 turnover following reforestation with broadleaf and conifer plantations. While forest vegetation type had similar effects on turnover rates, the soil depth remained a significant factor. Overall, it was found that deeper soil profiles (30-45 cm) remained dominated by old forest C3-carbon, even after more than 37 years of pasture establishment, which supported sampling to a 45 cm depth. For all plantations, soil organic carbon became dominated by C3-forest carbon at the 0-15 cm layer after 9.7 years of forest establishment and 8 years in the 15-30 cm layer. Rate of soil carbon turnover in the 0-15 cm profile showed rates of accumulation of SOC_3 were mirrored by the same rate of loss of SOC_4 ($0.21 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ and $-0.21 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$, respectively). The two deeper soil profiles showed SOC_3 has stayed constant while SOC_4 has been lost over time at a rate of $-0.17 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ for the 15-30 cm depth and $-0.083 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ for the 30-45 cm depth. Additionally, mean residence times follow the same pattern of more stable carbon with increasing depth with 30, 33, and 73 years for 0-15 cm, 15-30 cm, and 30-45 cm, respectively.

Implications for the conversion of pasture to forest carbon will prove promising if the stability of C3-forest soil carbon is greater than C4-pasture carbon. Soil organic carbon that is more resistant to decomposition would create a more promising sink for atmospheric carbon. Gaining a more thorough understanding of soil organic carbon dynamics following land-use change will be crucial to future climate change modeling scenarios and to predict our ability to manage systems to sequester carbon in more stable soil pools.

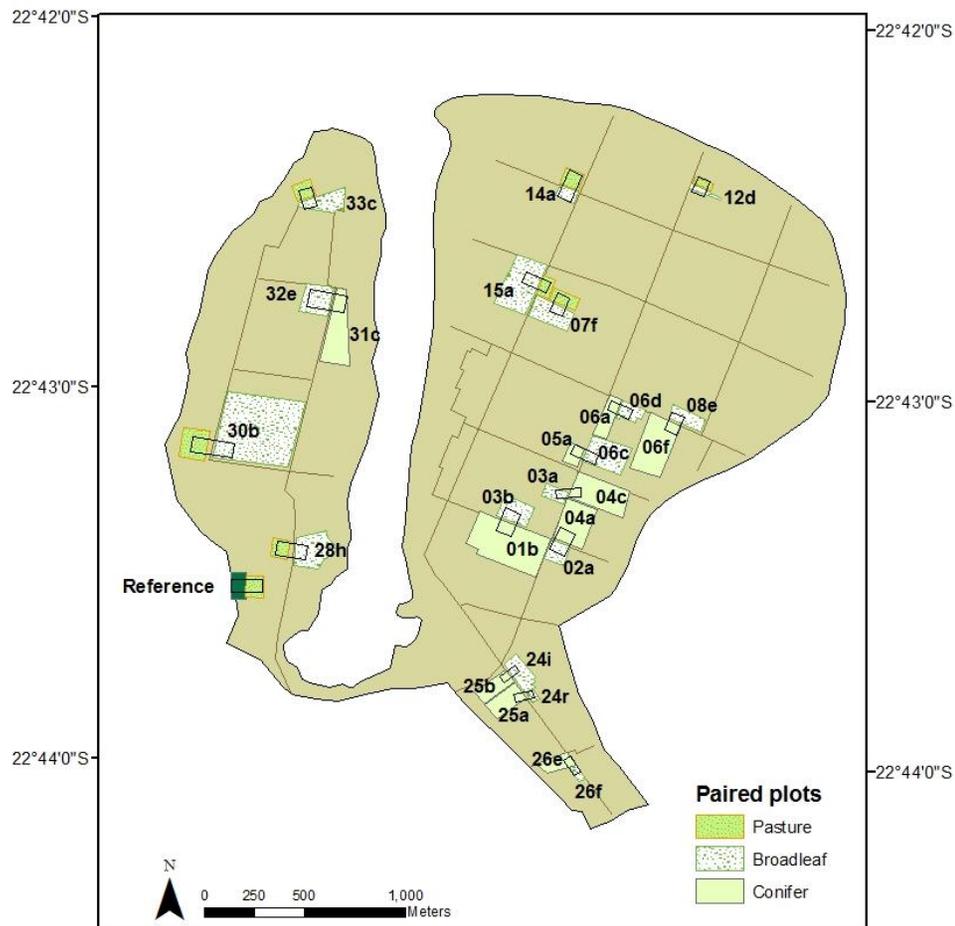


Figure 2.1 Map of plots at Anhembi Experimental Research Station analyzed for stable carbon isotopes.

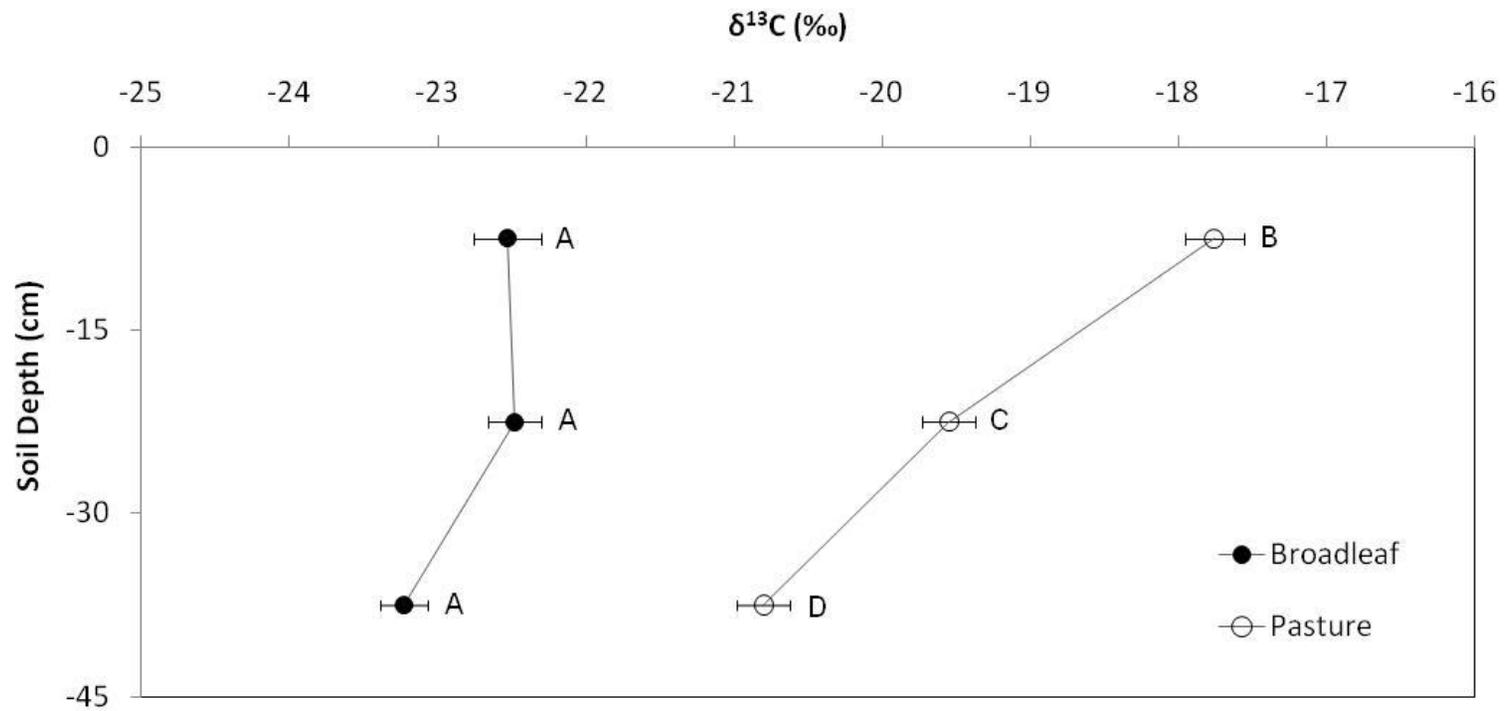


Figure 2.2 One paired Pasture-Broadleaf plot (plot 14a, age 27) was analyzed for within-plot variability. Points are located at the midpoint of sampling depth. Each point represents the mean of nine samples at each depth in each vegetation type (54 samples total). Error bars represent standard error of the mean. Significantly different values are represented by different letters according to Tukey's HSD at the 0.05 level.

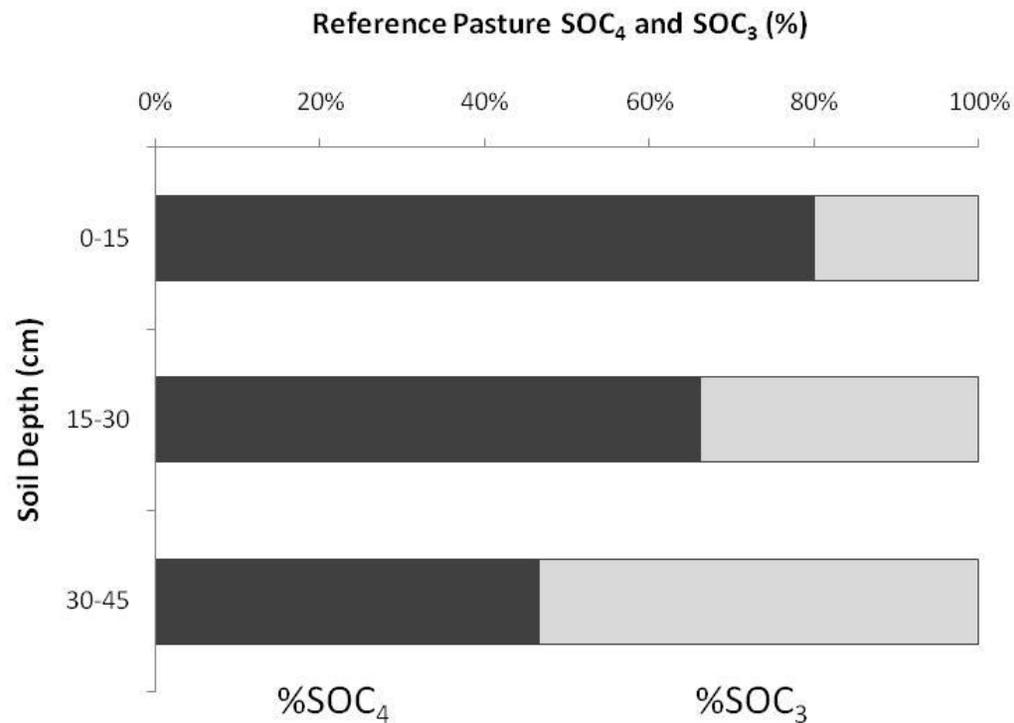


Figure 2.3 The pasture associated with the native unmanaged reference forest shows that pasture-derived carbon (SOC₄, dark gray) is dominant in the upper two soil layers (80% and 66% of mineral soil carbon in 0-15 and 15-30 cm), but forest carbon (SOC₃, light gray) from the original Atlantic Forest remains predominant (53%) in the lower soil profile even after approximately 37 years of pasture establishment.

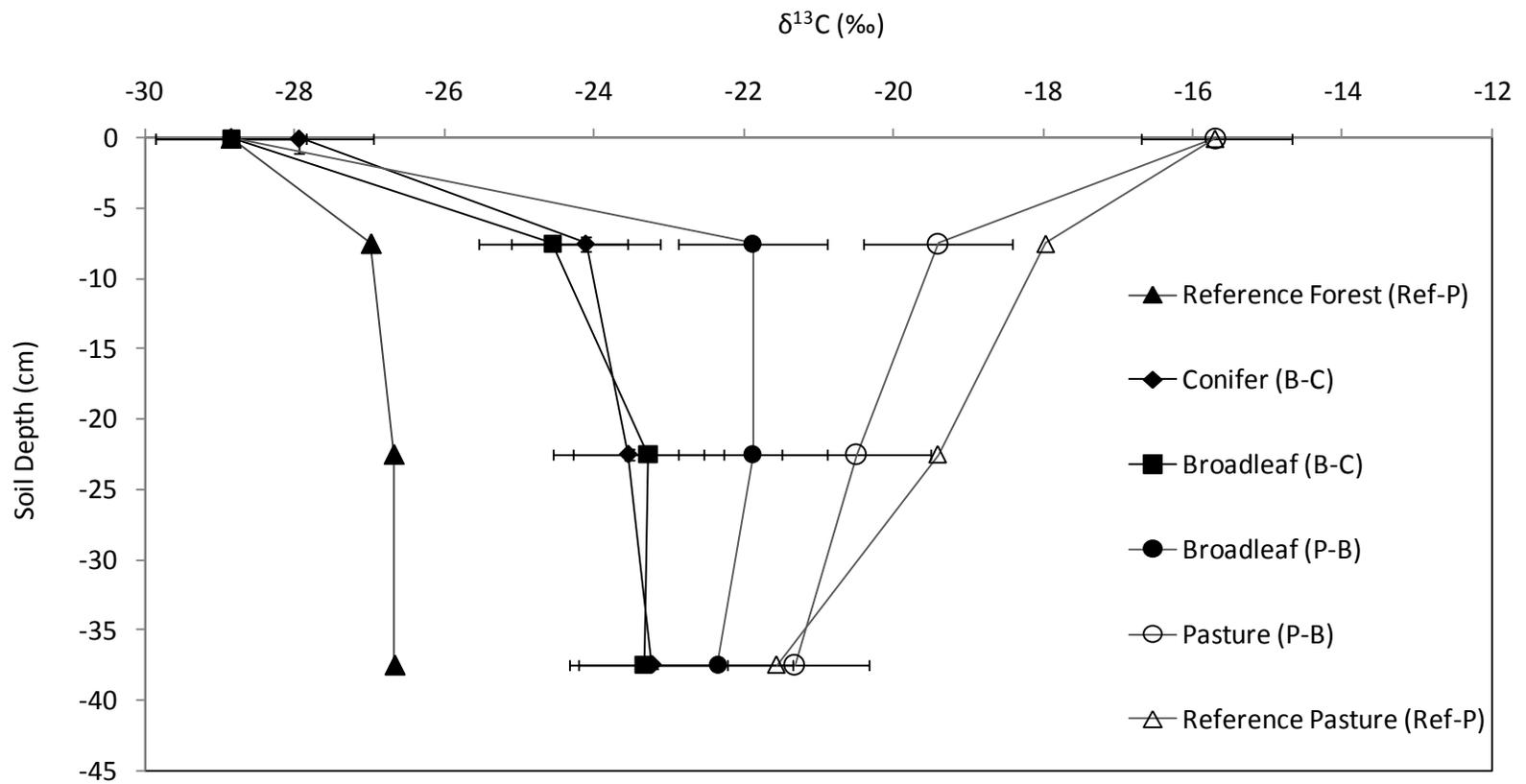


Figure 2.4 Values for $\delta^{13}\text{C}$ of paired native reference forest and pasture (Ref-P) ($n = 1$), Broadleaf-Conifer (B-C, $n = 10$) and Pasture-Broadleaf (P-B, $n = 7$) plantations. Values at soil depth of zero represent litter values ($n = 7$ for each litter type). Error bars represent standard error of the mean.

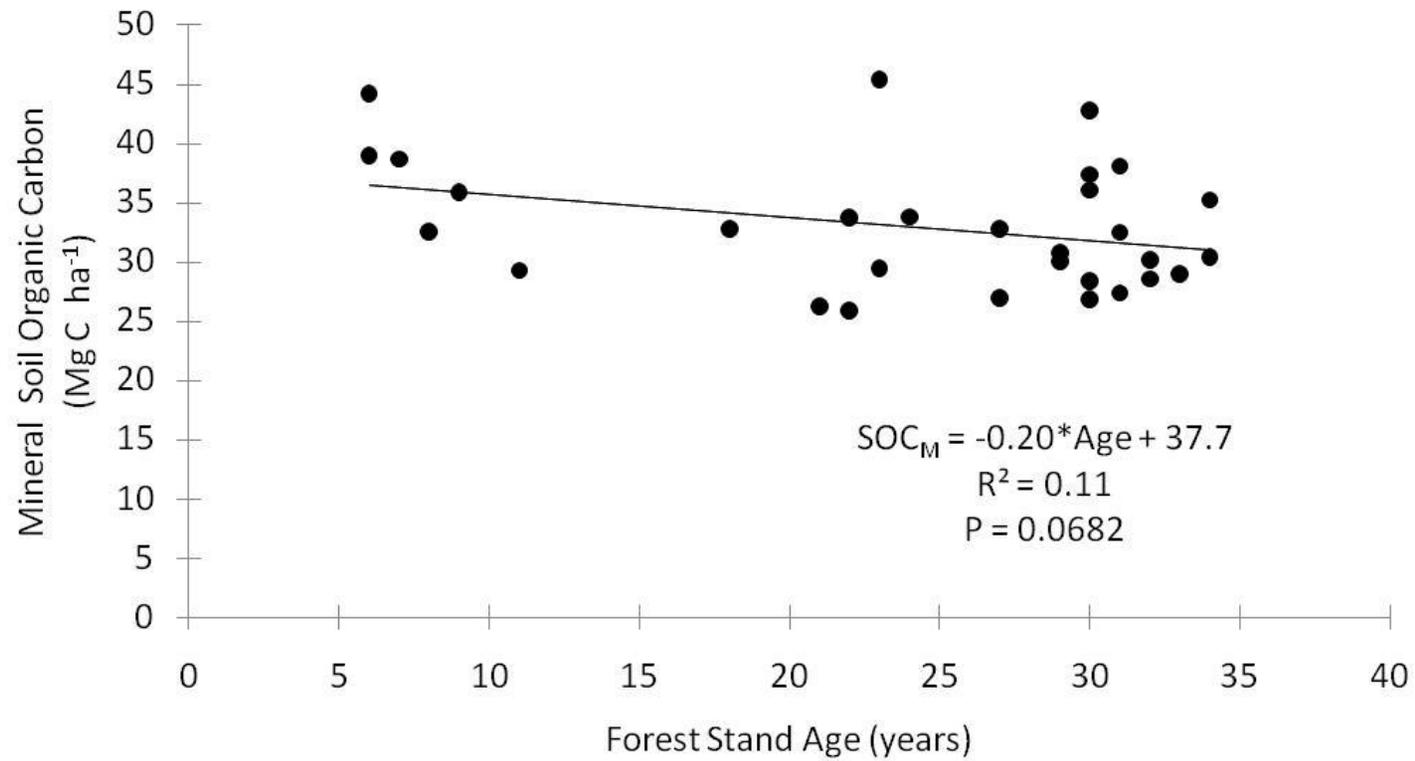


Figure 2.5 The chronosequence of all forest stands shows no significant change in mineral soil organic carbon (SOC_M) over time in the 0-45 cm soil profile.

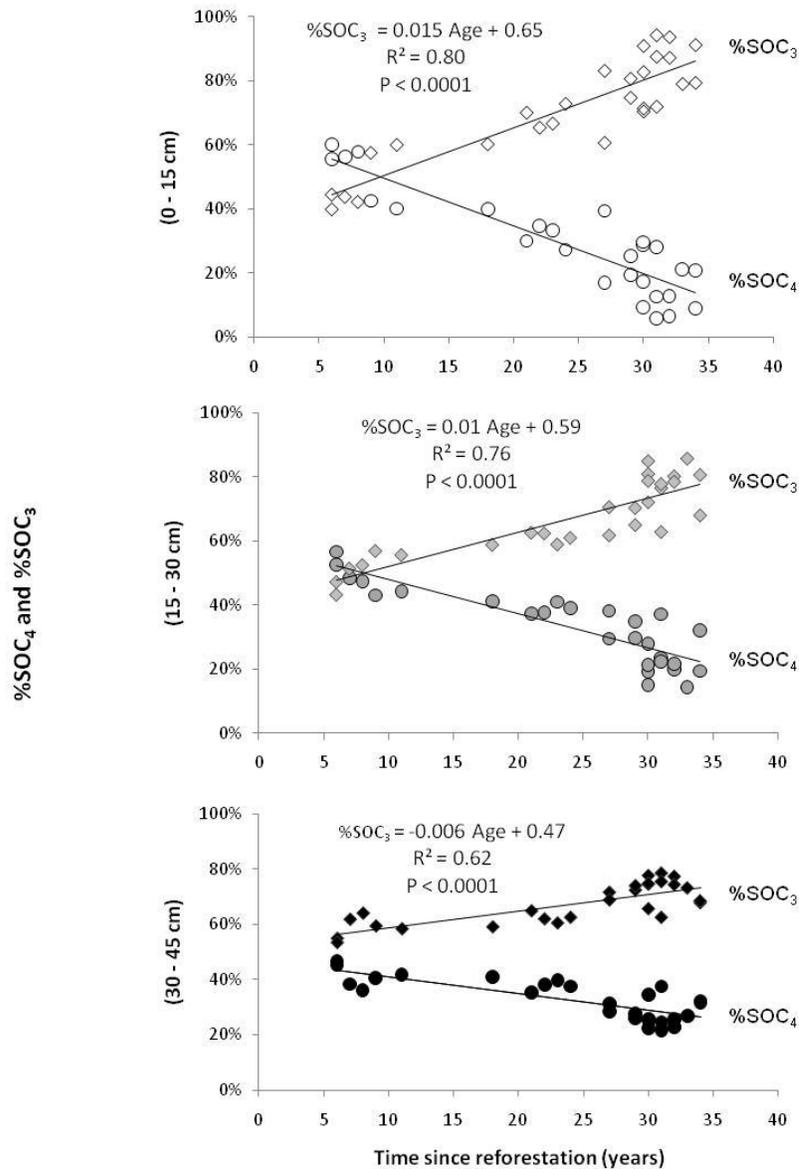


Figure 2.6 The chronosequence approach shows a significant depth dependence of %SOC₃ (forest-derived carbon) and %SOC₄ (pasture-derived carbon) over time following reforestation. Rate of decrease is not dependent on forest vegetation type. Points represent both conifer and broadleaf plantations and each forest plot is represented by two points for each depth. Lines intersect at 9.7 years in the 0-15 cm layer and at 8 years in the 15-30 cm layer. Lack of intersection in the 30-45 cm layer illustrates that deeper soil layers were continuously dominated by residual C3 carbon from native forest.

SOC in Forest Plantation (Mg C ha⁻¹)

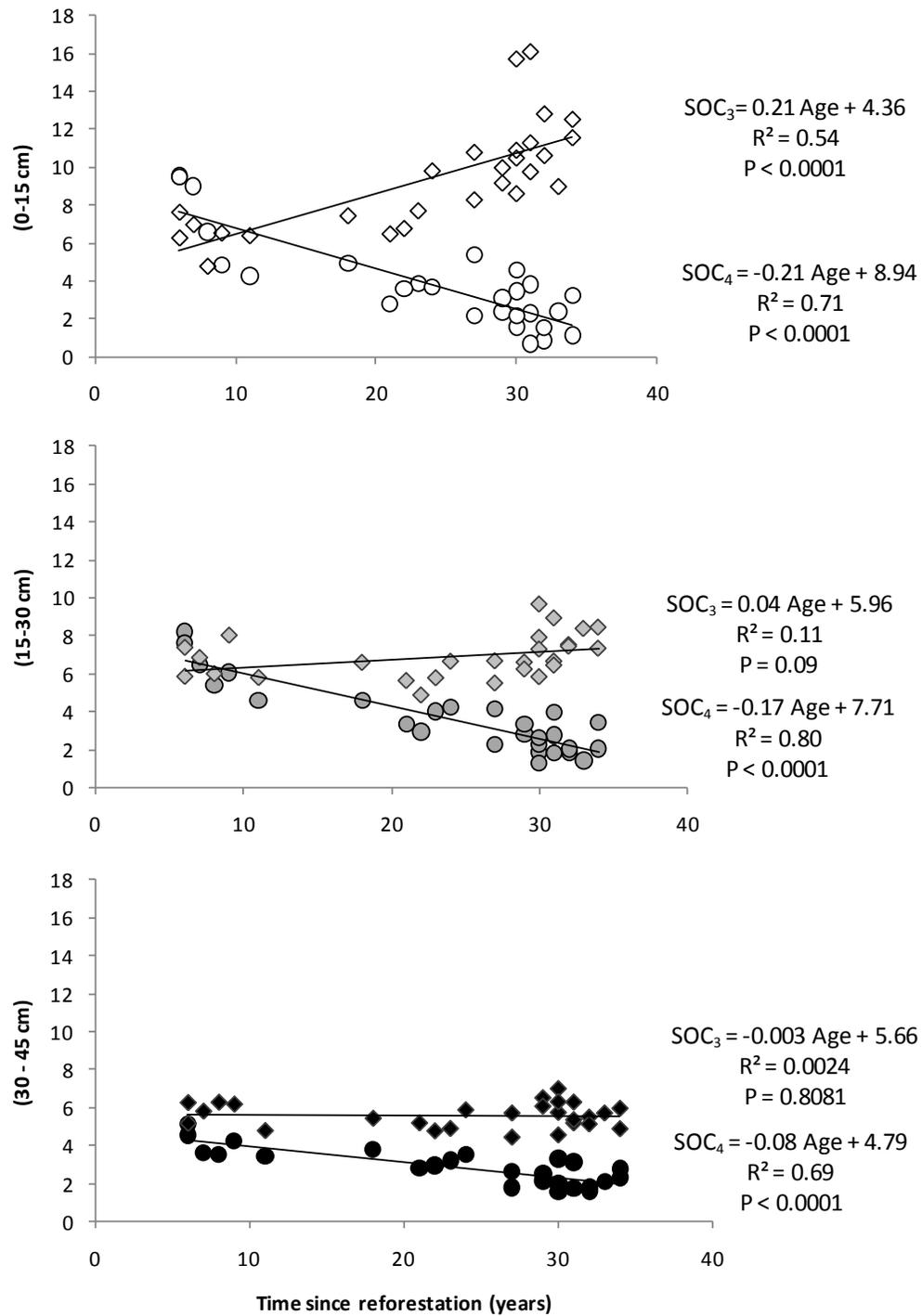


Figure 2.7 Soil organic carbon (SOC) rates of change (Mg C ha⁻¹ yr⁻¹) derived from pasture (SOC₄) and from forests (SOC₃) depend on soil depth.

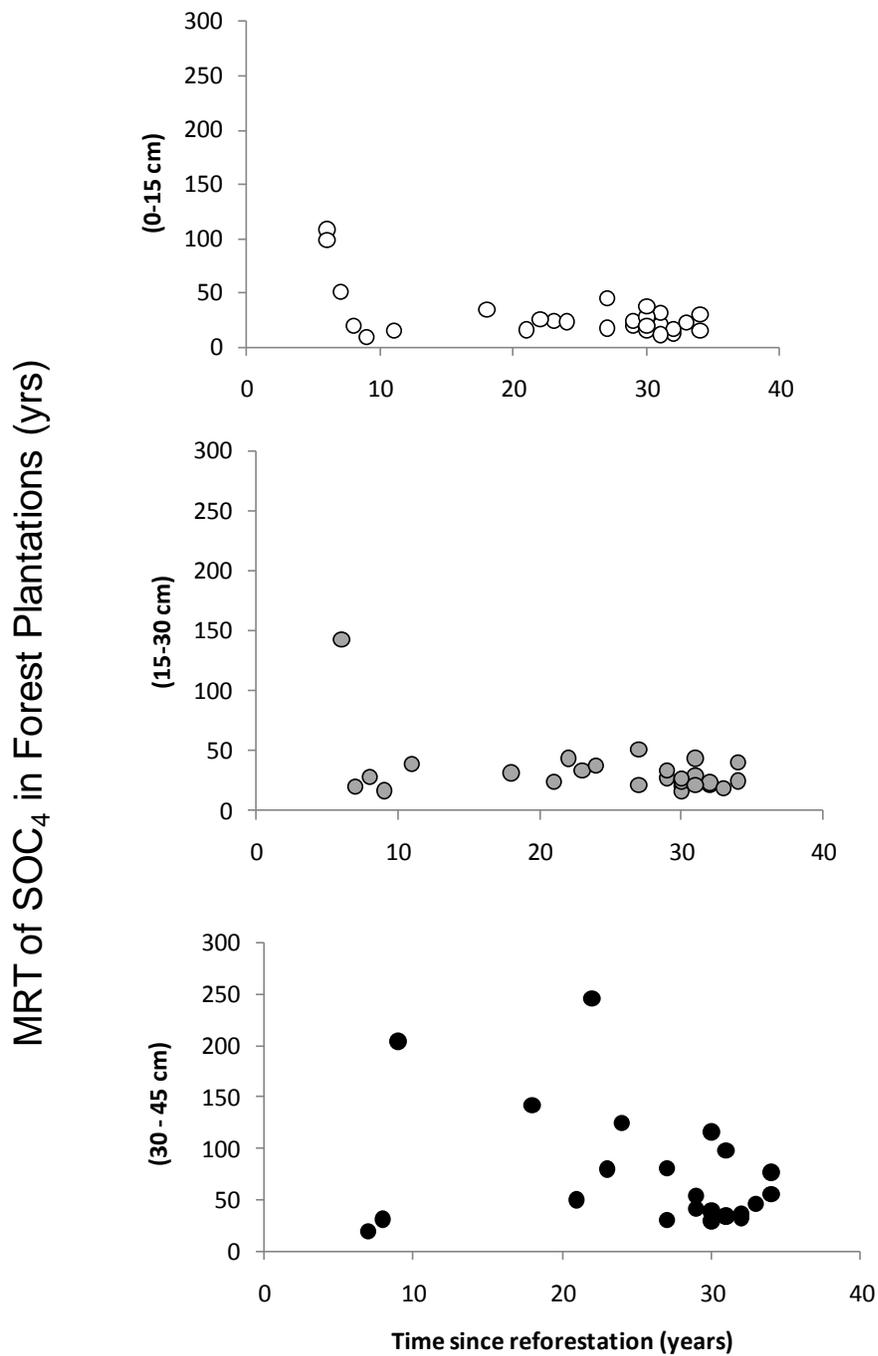


Figure 2.8 Mean residence time (years) shows greater turnover time deeper in the soil profile. Values range from 9 to 246 years of soil carbon turnover and are not dependent on Conifer or Broadleaf vegetation type.

Table 2.1 Stable carbon isotope values for the Reference forest with paired pasture show that pasture-derived C3 carbon remains predominant in the 30-45 cm sampling depth.

Depth (cm)	Reference Forest	Reference Pasture			Mean of Pastures (n = 7)		
	$\delta^{13}\text{C}$ (‰)	$\delta^{13}\text{C}$ (‰)	%SOC ₄	%SOC ₃	$\delta^{13}\text{C}$ (‰)	SOC ₄ (Mg C ha ⁻¹)	SOC ₃ (Mg C ha ⁻¹)
0-15	-26.98	-17.95	80	20	-19.41	10.1	2.5
15-30	-26.68	-19.40	66	34	-20.49	8.0	4.1
30-45	-26.66	-21.56	47	53	-21.32	4.3	4.9

Table 2.2 Composite $\delta^{13}\text{C}$ (‰) values for Pasture-Broadleaf paired plots per depth and percentage SOC₄ and SOC₃.

Plot	Age (yrs)	Depth (cm)	Pasture			Broadleaf Forest			MRT (yrs)
			$\delta^{13}\text{C}$ (‰)	%SOC ₄	%SOC ₃	$\delta^{13}\text{C}$ (‰)	%SOC ₄	%SOC ₃	
33c	7	0-15	-19.36	67	33	-20.63	56	44	51
		15-30	-20.24	60	40	-21.35	48	52	20
		30-45	-21.19	51	49	-22.46	38	62	20
30b	8	0-15	-19.60	65	35	-20.45	58	42	20
		15-30	-20.95	53	47	-21.47	47	53	29
		30-45	-22.45	40	60	-22.71	36	64	31
28h	9	0-15	-17.67	83	17	-22.18	43	57	9
		15-30	-19.16	69	31	-21.95	43	57	17
		30-45	-20.09	61	39	-22.20	41	59	204
07f	11	0-15	-20.74	55	45	-22.46	40	60	15
		15-30	-21.44	49	51	-21.81	44	56	39
		30-45	-22.06	44	56	-22.09	42	58	n.d.
12d	18	0-15	-18.18	78	22	-22.48	40	60	35
		15-30	-19.36	68	32	-22.16	41	59	32
		30-45	-20.11	61	39	-22.16	41	59	142
15a	22	0-15	-20.90	54	46	-23.07	35	65	26
		15-30	-21.81	46	54	-22.55	38	62	44
		30-45	-22.05	44	56	-22.48	38	62	245
14a	27	0-15	-17.76	82	18	-22.53	39	61	46
		15-30	-19.55	66	34	-22.48	38	62	51
		30-45	-20.8	55	45	-23.23	31	69	81

Table 2.3 Composite $\delta^{13}\text{C}$ values for Broadleaf-Conifer (B-C) paired plots per depth, percentage SOC₄, SOC₃, and Mean Residence Time (MRT) in years. Pasture has residual C3 from prior land conversion from native forest.

Plot	Broadleaf age (yr)	Conifer age (yr)	Depth (cm)	Broadleaf Forest				Conifer Forest			
				$\delta^{13}\text{C}$ (‰)	%SOC ₄	%SOC ₃	MRT (yrs)	$\delta^{13}\text{C}$ (‰)	%SOC ₄	%SOC ₃	MRT (yrs)
31c32e	6	6	0-15	-20.7	56	44	109	-20.2	60	40	98
			15-30	-20.9	53	47	n.d.	-20.5	57	43	143
			30-45	-21.7	45	55	n.d.	-21.5	47	53	n.d.
06a06d	23	31	0-15	-23.2	33	67	24	-23.8	28	72	32
			15-30	-22.2	41	59	34	-22.6	37	63	44
			30-45	-22.3	40	60	80	-22.5	38	62	98
05a06c	24	34	0-15	-23.9	27	73	24	-24.6	21	79	30
			15-30	-22.4	39	61	38	-23.2	32	68	41
			30-45	-22.5	38	62	125	-23.2	32	68	77
25a24r	27	32	0-15	-25.1	17	83	18	-25.5	13	87	17
			15-30	-23.4	29	71	22	-24.3	22	78	24
			30-45	-23.5	28	72	30	-23.8	26	74	36
01b03b	29	30	0-15	-24.8	19	81	20	-23.7	29	71	28
			15-30	-23.4	30	70	28	-25.0	15	85	17
			30-45	-23.6	28	72	54	-23.9	26	74	39
06f08e	29	21	0-15	-24.1	25	75	25	-23.6	30	70	16
			15-30	-22.8	35	65	34	-22.6	37	63	24
			30-45	-23.8	26	74	42	-22.8	35	65	50
26e26f	30	30	0-15	-25.9	9	91	16	-25.0	17	83	20
			15-30	-24.6	19	81	21	-23.6	28	72	24
			30-45	-24.2	22	78	40	-23.9	25	75	29

Table 2.3 Continued.

04c03a	31	30	0-15	-25.6	13	87	21	-23.6	30	70	38
			15-30	-24.1	24	76	29	-24.3	21	79	27
			30-45	-24.3	22	78	34	-22.9	34	66	116
25b24i	32	31	0-15	-26.3	6	94	13	-26.3	6	94	12
			15-30	-24.5	20	80	22	-24.2	22	78	21
			30-45	-24.2	23	77	33	-24.0	25	75	35
04a02a	34	33	0-15	-26.0	9	91	16	-24.6	21	79	23
			15-30	-24.5	19	81	25	-25.1	14	86	19
			30-45	-23.1	32	68	56	-23.7	27	73	46

*No detect (n.d). are due to negative mean residence time from higher SOC₄ value in the forest than the adjacent pasture.

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Chapter 3 - *Eucalyptus* forest plantations effects on soil organic carbon across Eastern Brazil

Abstract

Managing vegetation to sequester atmospheric carbon in soil organic matter has drawn attention both for the possibilities to improve soil quality and mitigate climate change. Concurrently, growing demand for wood products has caused a dramatic increase in the intensity of *Eucalyptus* production and the total area planted to over 20 million hectares worldwide, with largely unknown consequences for soil carbon sequestration and long-term sustainability of forest production. Thus far, impacts of short-rotation (6- to 8-year harvest cycle) *Eucalyptus* plantations on soil organic carbon have been variable and poorly understood. To quantify soil carbon stocks and changes over time and across a geographic gradient, we sampled 306 operational *Eucalyptus* plantations for the third consecutive collection in a 20-year period. Changes in soil carbon stocks varied among regions from the original sampling period (1984-1993) to 2010. From tropical to subtropical: Region 1 (Bahia state) stayed the same ($-0.07 \pm 0.08 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$), Region 2 (Espirito Santo state) showed a decrease in soil carbon stocks over time ($-0.80 \pm 0.10 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$), and Region 3 (Sao Paulo state) increased soil carbon stocks ($0.27 \pm 0.06 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$). Soil carbon stocks were also dependent on temperature and precipitation. For each time period, rates of soil carbon accumulation or loss were significantly affected by region, initial soil carbon concentration, and precipitation. Soil carbon concentration followed similar trends to soil carbon stock, except in Sao Paulo where stocks increased mainly due to increases in bulk density. Inherent spatial heterogeneity of soils requires repeated sampling across the landscape to be confident in soil organic carbon changes over time. Our study is currently the most robust examination of effects of *Eucalyptus* plantation on soil organic carbon in the literature to date.

Introduction

Land-use change is a major concern, especially in the tropics where deforestation generates 6 to 39% of global anthropogenic CO₂ emissions (IPCC, 2007). As a growing world population demands more forest resources, plantations will have to expand or become more productive on the same amount of land. *Eucalyptus* plantations now cover approximately 20 million hectares (ha) worldwide (Iglesias-Trabado and Wilstermann, 2008) and 4.75 million ha in Brazil (ABRAF, 2011), supplying mainly pulpwood, charcoal, and firewood, but are also expanding into the solid wood products market. Considering the magnitude of this expanding land use, it will become increasingly important to examine the sustainability of *Eucalyptus* silviculture in terms of long-term productivity.

Ensuring future productivity of plantations is important to land-holding forestry companies. They are invested in maintaining productivity on their current land base to maintain the supply of pulpwood to their production chain. In the tropics, where soils are typically weathered from the warm, humid climate, and are susceptible to degradation, soil organic matter could aid in maintaining site productivity and therefore the sustainability of their operations (Zinn *et al.* 2002). Soil orders often found in the tropics, such as Oxisols, Ultisols, and Entisols, as classified under U.S. soil taxonomy, are dominated by low-activity, kaolinitic clays, are often low in cation exchange capacity (CEC), have poor base saturation, and are acidic and have high aluminum levels. Soil organic carbon serves several important functions, such as: 1) providing a source and sink for plant nutrients and CEC, 2) promoting soil aggregation and tilth, 3) increasing infiltration and lowering runoff, 4) increasing microbial diversity, 5) providing soil strength for soil aggregates, and 6) buffering pH with weak acid groups (Lal, 2004).

Maintaining or increasing quantities of soil organic carbon indicate that organic matter is being replenished at the same or greater rate than it is being decomposed or eroded. Soil carbon changes following forest establishment are often associated with land-use history and soil properties (de Koning *et al.*, 2003; Don *et al.*, 2010; Laganière *et al.*, 2010). However, few studies have examined the long-term effects of short-rotation *Eucalyptus* silviculture on soil carbon sequestration across a broad geographic gradient.

Long-term soil experiments are traditionally difficult to initiate and sustain over time due to the organization and collaboration required between researchers and land-owners, and the short-term nature of funding and land tenure (Richter *et al.*, 2007). However, this method is more robust than paired or chronosequence studies, both of which, in comparison, tend to overestimate the changes in soil carbon stocks (Laganière *et al.*, 2010). This research will continue a long-term soil experiment across the Brazilian landscape to evaluate the effects of short-rotation *Eucalyptus* plantations on soil organic carbon dynamics on a tropical to subtropical gradient. A third repeated sampling of 306 plots across the states of Bahia, Espirito Santo, and Sao Paulo on operational plantations owned by Copener, Fibria, and Suzano, respectively was carried out in 2010.

The objectives of this study were to 1) quantify mineral soil carbon stocks and change over time following short (6- to 8-year)-rotation *Eucalyptus* plantations across 306 sites in Brazil; and 2) determine the significance of mean annual temperature, wet and dry seasonal precipitation, soil clay content, and initial carbon content in influencing soil carbon stocks and changes over time. This research far exceeds any other published field study in temporal and spatial scale concerning effects of *Eucalyptus* forest establishment on soil carbon dynamics in the tropics.

Methods

The most direct method for measuring change in soil carbon over time, called retrospective study design or the repeated inventory approach, is to re-sample the same location and ensure the soil samples are collected, processed, and analyzed according to consistent protocols (Paul *et al.*, 2002). This research project was designed for sufficient spatial and temporal scale analysis to detect significant differences in soil carbon stocks and changes in soil organic carbon over time based on the repeated sampling and soil archives (Richter *et al.*, 1999).

Site Descriptions

Field sites were located in three regions in Brazil ranging from tropical to subtropical climate: Region 1 (Bahia state), Region 2 (Espírito Santo), and Region 3 (Sao Paulo). There were 110 sites established in each region for a total of 330 original sampling points. Since the original sampling period some sites are no longer in operational *Eucalyptus* silviculture due to loss of ownership or to being set aside as natural reserves. As of 2010, soil carbon stocks were analyzed for 107 sites in Region 1, 98 sites in Region 2, and 101 sites in Region 3, for a total of 306 sites.

All sites are located between the latitudes of 11° 33' 9" S and 24° 12' 40" S and range in elevation from 8 to 1000 m above sea level (**Figure 3.1**). Based on the FAO Worldclim database, mean annual temperature and annual rainfall for each region are summarized in **Table 3.1** (Hijmans *et al.*, 2005). Three soil orders, Entisols, Oxisols, and Ultisols, according to the U.S. taxonomic system, are distributed across the three regions (**Table 3.2**).

Natural ecosystems for these regions are Atlantic Forest. *Eucalyptus* plantations were converted from agricultural land-uses (pasture or cropland) that typically had declined in productivity several decades after initial deforestation, in part likely due to soil erosion. Most *Eucalyptus* plantations were established in the mid-1980s and have been in continuous production since planting. Prior to the year 2000, intensive site preparation, including burning, harrowing/bedding, and little fertilization were common practice. As silvicultural practices have evolved, within the last ten years, minimum cultivation has become the predominant management in Brazil with more harvest residues such as branches and bark left on site and elimination of burning. Productivity has increased with silvicultural advancements, such as fertilization, and improved tree genetics. The major species used in these plantations are *Eucalyptus grandis x urophylla* hybrids in Regions 1 and 2, and *Eucalyptus grandis* in Region 3.

Experimental Design and Soil Sampling

Soils were first collected from 1984-1993 by horizon from one soil pit in each of the selected operational *Eucalyptus* stands during soils surveys (Region 1: Krejci 1985/91;

Region 2: EMBRAPA, 2000; Region 3: Rizzo 1984/86). Each site was re-sampled in 2001 within 5-20 m of the original soil pit (Stape *et al.*, 2002). Consistent protocols were followed for 2001 and June-August 2010 sampling. For soil carbon concentration, nine samples were collected at two depths (0-15 cm and 15-30 cm) and composited in the field (**Figure 3.2**). Bulk density was collected with volumetric rings at three sampling points from within the 0-15 cm and 15-30 cm depths and composited in the field.

Some markers from 2001 were discovered in the field in 2010, though planting and harvesting activities often made the stone monuments difficult or impossible to find. Sampling locations have been marked on maps within each company with distances from reference points which aid in accurate re-sampling. Points have also been marked with GPS to allow for more precise future sampling and mapping.

Laboratory Analysis

Soils collected for carbon and nitrogen analysis were oven-dried, ground, and passed through a 2 mm sieve to remove rocks, coarse vegetation, and roots. Bulk density samples were dried at 60°C overnight and weighed. To prepare homogenous soils with low variation for carbon and nitrogen analysis, approximately 2.5 grams of each sample were placed in two-ounce, square glass bottles with six 304-stainless steel metal rods (9.5 mm diameter, 44.5 mm length). Samples were placed securely in a standard, rubber-lined, rock tumbler and rotated for 24 hours (Arnold and Schepers, 2004). Any particles that did not pass through a #60 (0.250 mm) sieve were ground by mortar and pestle.

Total soil carbon was determined by dry combustion and infrared detection using a CD Instruments NC2100 elemental analyzer (CE Elantech, Inc., Lakewood, New Jersey). Well characterized in-house soil standards, a NIST certified soil standard, and sample duplicates were monitored to stay within 10% coefficient of variation in each analysis for quality assurance. At least 5% of samples were run as duplicates in every batch of analysis. Because dry combustion measures both organic and inorganic soil carbon, soils were tested by acid fumigation to verify no carbonates were present (Harris *et al.*, 2001). Carbonates were not expected to be present considering the weathered, acidic nature of the soils.

All soil samples from 2001 and 2010 are archived in Brazil by the company associated with each region in Brazil (Region 1: Copener; Region 2: Fibria; Region 2: Suzano). Approximately 30 samples in Region 2 were archived from the original soil survey for correlations between initial Walkley-Black analysis and current dry combustion analysis.

Calculations and Statistical Analysis

For both soil sampling depths at each location, mineral soil organic carbon (SOC_M) stocks (Mg C ha⁻¹) were calculated as:

$$\text{SOC}_M = C * \text{BD} * L$$

where C is the concentration of carbon (% per weight), BD is the bulk density (g cm⁻³) of each plot, and L is the depth of the soil layer (15 cm). For soil carbon from 0-30 cm, soil carbon stocks from both layers (0-15 cm and 15-30 cm) were summed. Bulk density was calculated by dividing the dry mass by the volume of the cylinder and by the number of cylinders (n=3).

A general linear model was used after verifying linearity of scatter plots. In our multivariate models, dependent variables consisted of soil carbon stocks at each time point (original, 2001, and 2010) and the changes over time (original to 2001, 2001 to 2010, original to 2010). Continuous independent variables consist of soil clay content, mean annual temperature, dry and wet seasonal precipitation, and initial carbon concentration. Class variables consist of soil order and region. Annual precipitation was divided into wet and dry seasonal rainfall after a principal component analysis of the variables indicated clustering of wet and dry months. Significant *P*-values were set at the 0.05 level. Statistics were analyzed using SAS v 9.2 and JMP v 9.0 (SAS Institute, Cary, NC).

Results

Soil Carbon Stocks, Changes, and Bulk Density

Analysis of periodic sampling of soil carbon stocks and changes over time in continuous-rotation *Eucalyptus* plantations in Brazil indicates variable responses across regions. Soil carbon stocks (mean \pm standard error) in the original sampling were highest in Region 2 (41 ± 1.9 Mg C ha⁻¹), followed by Region 1 (33 ± 1.9 Mg C ha⁻¹) and Region 3 (20 ± 0.9 Mg C ha⁻¹). Region 2 soil carbon stocks decreased by 35% to 26 Mg C ha⁻¹ in 2001, but did not decrease significantly (6%) to 25 Mg C ha⁻¹ in 2010. Region 1 saw no significant decrease (2%) to 32 Mg C ha⁻¹ in 2001 and (4%) 31 Mg C ha⁻¹ in 2010. Finally, Region 3 increased by 3% to 21 Mg C ha in 2001 and by 29% to 27 Mg C ha⁻¹ in 2010.

There were significant positive site-to-site correlations in soil carbon stocks between time periods across all sites: original to 2001 ($r = 0.44$, $P = 0.0001$), original to 2010 ($r = 0.16$, $P = 0.0038$), and 2001 to 2010 ($r = 0.63$, $P = 0.0001$). The strongest correlation between 2001 and 2010 likely reflects the consistent collection and analysis protocols in the two later time periods.

Across all sites, from the original sampling to 2001 soil carbon stocks decreased by $0.44 (\pm 0.08)$ Mg C ha⁻¹ yr⁻¹, but increased from 2001 to 2010 by $0.16 (\pm 0.08)$ Mg C ha⁻¹ yr⁻¹ (**Figure 3.3**). Across the entire time period, from original sampling to 2010, soil carbon stocks decreased by $0.20 (\pm 0.06)$ Mg C ha⁻¹ yr⁻¹. However, these main effects are less important since results varied depending on regions. From the original sampling to 2010 Region 1 stayed the same (-0.07 ± 0.08 Mg C ha⁻¹ yr⁻¹), Region 2 showed a decrease in soil carbon stocks over time (-0.80 ± 0.10 Mg C ha⁻¹ yr⁻¹), while and Region 3 increased (0.27 ± 0.06 Mg C ha⁻¹ yr⁻¹) (**Figure 3.4**). In the first time period, changes were driven by decreases in soil carbon concentration in Region 2 (**Figure 3.5**), but increases in the second time period were driven by increases in bulk density in Region 3 (**Figure 3.6**). Because bulk density was not analyzed in the original sampling period, values from 2001 were applied to the original carbon concentrations to calculate soil carbon stocks. This essentially makes any change from the original to 2001 sampling due to change in soil carbon concentration.

Bulk density increased significantly in Region 1 (+5%) and Region 3 (+30%), but decreased in Region 2 (-6%) between 2001 and 2010 (**Table 3.3**). Despite the large increase in bulk density in Region 3, the average is still less than the average bulk densities of the other two regions. The cause of the large increase in bulk density in Region 3 is still under consideration.

Variability decreased significantly within each region from the first sampling period to the second sampling period (**Figure 3.7**). According to a two-sided F-test, the variances of changes in soil carbon stocks decreased in the second time period for each region (Region 1: $P = 0.0005$; Region 2: $P < 0.0001$; Region 3: $P = 0.0006$), as can be seen by the narrowing of the spread of the histograms. The shifts towards soil carbon accumulation can also be seen.

Significance of Soil and Climate Variables

Soil carbon stocks were significantly associated with region, soil order, percent clay, initial soil carbon concentration, mean annual temperature, and seasonal precipitation, but to varying degrees depending on the time period (**Table 3.4**). Mean annual temperature is the only significant independent variable found in all three time periods. However, temperature is positively associated with soil carbon stocks in the original time period, but negatively associated with 2001 and 2010 soil carbon stocks. Soil carbon changes over time consistently are associated with region, initial soil carbon concentration, and dry season precipitation.

Discussion

Understanding long-term soil carbon dynamics in *Eucalyptus* plantations is critical to developing sustainable land management practices. However, predicting soil organic carbon changes has proven difficult due to highly variable responses. We found variable responses in mineral soil organic carbon across regions that depended on climate and soil factors. We also found a decrease in variability in changes in soil carbon stocks over time in each region. In other studies, soil organic carbon has been previously shown to increase in a 30-year

Eucalyptus chronosequence planted on a degraded pasture on a clayey Oxisol in southeastern Brazil (Lima *et al.*, 2006) and in short- and long-term rotations of *Eucalyptus* compared with *Cerrado* and pastures (Maquere *et al.*, 2008). However, in central Brazil no significant difference was found between native *cerrado* and *Eucalyptus* soil carbon concentrations in loamy and clayey soils, or in a loamy Oxisol (Neufeld *et al.*, 2002; Zinn *et al.*, 2002; Zinn *et al.* 2007). Other studies have shown afforestation of native *cerrado* with *Eucalyptus* plantations lost soil organic carbon in the top 0-60 cm (9 Mg C ha⁻¹ or -17%) in an Entisol (Zinn *et al.*, 2002). In *Eucalyptus* plantations in Jari (Amazon), there was an 87% soil carbon loss, compared to native forests (Beldini *et al.*, 2010). The conflicting results in the literature concerning the effects of *Eucalyptus* plantations may be due to the fact that studies cross a wide variety of climate and soil conditions, soil preparation and management, stand age, and land-use history (Zinn *et al.*, 2002).

It has generally been assumed that forest plantations should improve soil carbon sequestration due to high aboveground productivity, recalcitrant carbon inputs, and deeper rooting depth (Lal, 2005; Don *et al.*, 2010; Laganière *et al.*, 2010). However, both the magnitude and direction of shifts in soil carbon have been associated with depth of soil sampling, past land-use change, climate, tree species, stand age, management practices, and soil texture (Paul *et al.*, 2002; Jandl *et al.*, 2007; Laganière *et al.*, 2010). Our results show that even when species, soil sampling depth, and silvicultural management are held essentially constant, soil carbon dynamics still vary across regions and depend on factors such as initial carbon concentration, precipitation, temperature, and clay content.

Both soil carbon stocks and changes were positively associated with clay content in our results. Clay content has been previously shown to have a linear relationship with soil carbon content (Feller and Beare, 1997). Confounding effects such as landscape position, drainage, NPP, and initial soil carbon content may have contributed to past difficulties in showing that fine textured soils improve soil carbon accumulation following afforestation (Paul *et al.*, 2002; Laganière *et al.*, 2010). According to a meta-analysis by Laganière *et al.* (2010) of 33 of the most recent papers concerning afforestation of agricultural land, higher clay content (>33%) did positively correlate with greater accumulation of soil carbon. In

fact, soils with higher clay content were able to accumulate approximately 25% more carbon compared to coarser textured soils.

Our results showed a significant negative relationship between initial soil carbon and soil carbon stocks, meaning that sites that started with more carbon lost more carbon. Initial soil carbon content has been previously shown to be correlated with change in soil carbon following land-use change (Mann, 1986; Lugo and Brown, 1993). A meta-analysis of afforestation, found that in the first five years in the top 10 cm, soil carbon was lost by 3.46% per year compared to initial soil carbon during the first five years following forest establishment, but recovered pre-planting levels at approximately age 30 (Paul *et al.*, 2002). However, the *Eucalyptus* plantations in our study are harvested every 6-8 years, so it is difficult to predict if there would be a similar pattern in soil carbon.

Contrary to Post and Kwon (2000), we found an inverse relationship between soil carbon accumulation rates and mean annual temperature, but soil carbon stocks did increase with increasing temperature. Laganière *et al.* (2010) found that restoration of soil carbon following afforestation of crop and pasture varied with climatic zone. While high temperatures and rainfall contribute to high net primary productivity and accumulation of carbon in plant biomass in tropical zones, these same climatic conditions also stimulate higher rates of decomposition, reducing soil carbon stocks (Lal, 2005).

Seasonality of rainfall may also prove important as we saw significant association with either wet or dry seasonal precipitation. Dry season precipitation was always positively correlated to soil carbon stocks across all three sampling periods, whereas wet season precipitation was positively correlated with changes in soil carbon over each of the time periods. Overall, for most land use type changes, higher soil moisture and precipitation mean higher changes in soil carbon. Therefore soils in humid regions may be more vulnerable than dry regions to land-use change (Don *et al.*, 2010).

Our best model relating soil carbon change (from the original sampling to 2010) to region, clay content, initial carbon concentration, mean annual temperature, and mean annual precipitation explained 79% of variation. This model explained more variation than models in other studies that combined similar factors to explain soil carbon response to afforestation.

In a review by Don *et al.* (2010), their best explanation of variation related soil carbon change to land-use change, mean annual temperature, mean annual precipitation, and clay mineral type. This model explained 55% of the variability, which still left almost half unexplained.

Pre-planting disturbance can have a significant effect on soil carbon accumulations. Laganière *et al.* (2010) found that minimizing disturbance could increase soil carbon stocks by 15%. Soil carbon dynamics, however, have been found to be independent of disturbance type (fire, harvest, agricultural abandonment, or plantation) (Yang *et al.*, 2011). Mechanical soil preparation can increase spatial variation in soil carbon, which increases the difficulty in detecting changes (Paul *et al.*, 2002).

Another factor that may contribute to unexplained variation would be historic land use, which has been shown to be a dominant factor in controlling changes in soil organic carbon (Paul *et al.*, 2002). According to Laganière *et al.* (2010), afforestation increased soil carbon stocks by 26% for croplands, but forest plantations have little to no effect on soil carbon compared to pasture or grasslands since these land uses are typically less disturbed. Changes in soil carbon following land use change may be affected by a priming effect, or the ability of microbes to decompose new plant materials, though the significance of this effect is still controversial (Fontaine *et al.*, 2003).

The increase in bulk density in the Region 3 could be due to several factors. There may have been a systematic sampling procedure difference between 2001 and 2010. Three plots with the greatest change were re-sampled in 2012 and values were found to be similar to 2010 bulk density results (data not shown). However, the bulk density values are larger than expected for this area, particularly in sites with higher clay content. While machinery traffic has increased over the last decade, this same intensification has occurred in all three regions without similar impacts. Seasonal differences have been shown in bulk density in Region 3, with rainy season bulk density having values by up to 15% greater than in the dry season (Paciullo *et al.*, 2010). While instruments also can have a significant impact on bulk density (Folegatti *et al.*, 2001), we used the same type of bulk density sampler in Region 1 as Region 3 and saw a 5% and 30% increase respectively. This highlights the challenges in

landscape scale estimation of soil carbon stocks due to variability of bulk density measurements. For this reason, it is important in long-term soil surveys to carefully document field sampling procedures to maximize confidence in changes over time.

Overall, the subtle shifts in soil carbon stocks towards accumulation and the decrease in variability across all regions indicates that improvements in forest management are either maintaining or beginning to improve soil carbon stocks following continuous *Eucalyptus* production.

Conclusions

Soil organic carbon can be used as a measure of soil quality, and to develop a better understanding of the sustainability of intensive *Eucalyptus* production across a broad geographic gradient. The long-term nature and wide distribution of this work helps to fill some of the gaps in our understanding of soil carbon dynamics in the tropics following conversion to *Eucalyptus* plantations.

Results from the *Eucalyptus* sites show that even with the same species and silvicultural treatments, soil carbon stocks and changes over time vary across regions. Multivariate modeling shows some important factors controlling both soil carbon stocks and changes over time, including initial carbon concentration, clay content, mean annual temperature, and precipitation. Soil and climate factors can have different influences on soil carbon stocks versus change over time. Dry season rainfall (when systems are most water limited) may be related to increasing carbon stocks due to plant productivity. Sites with greater amounts of initial soil carbon and higher mean annual temperature typically lost more soil carbon over time. At present, results suggest soil carbon stocks continue to change in response to the past 20 years of *Eucalyptus* cultivation.

Improving carbon sequestration in intensively managed forests addresses environmental stewardship by protecting soil quality and can help to meet increasing needs for renewable resources and ecosystem services by sustaining forest productivity. Accurate estimation of soil carbon stocks and prediction of soil carbon changes are important both for

land-use planning and management and for addressing carbon emissions and carbon sequestration on the national and global scale. These issues must be answered regionally before carbon policy can encourage practices for soil carbon storage.

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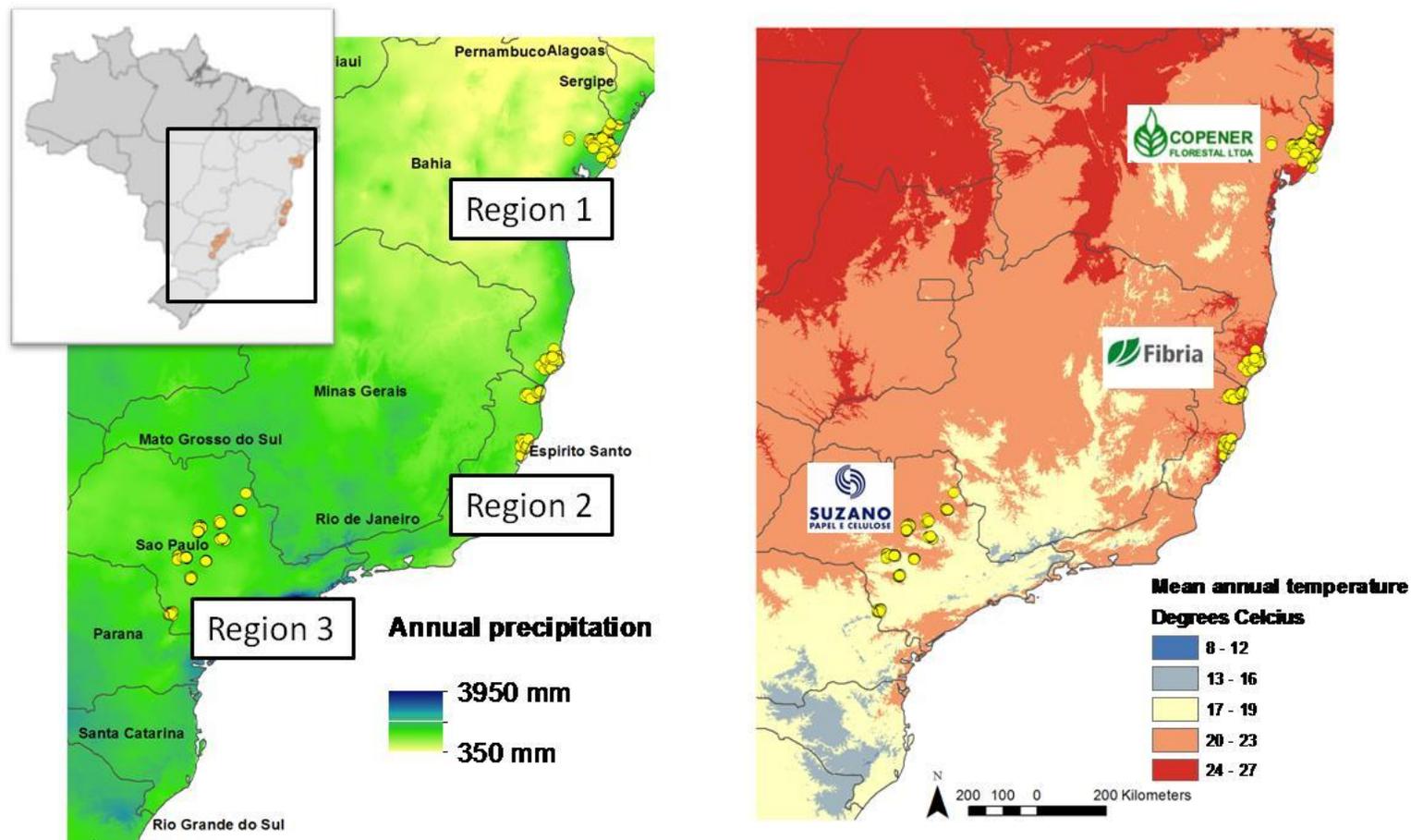


Figure 3.1 The 306 field sites are located in the states of Bahia (Region 1), Espírito Santo (Region 2), and São Paulo (Region 3) in Brazil, ranging from tropical to subtropical climates. Climate variables include precipitation (left) and mean annual temperature (right). The sites in Regions 1, 2, and 3 correspond to the companies Copener, Fibria, and Suzano respectively.

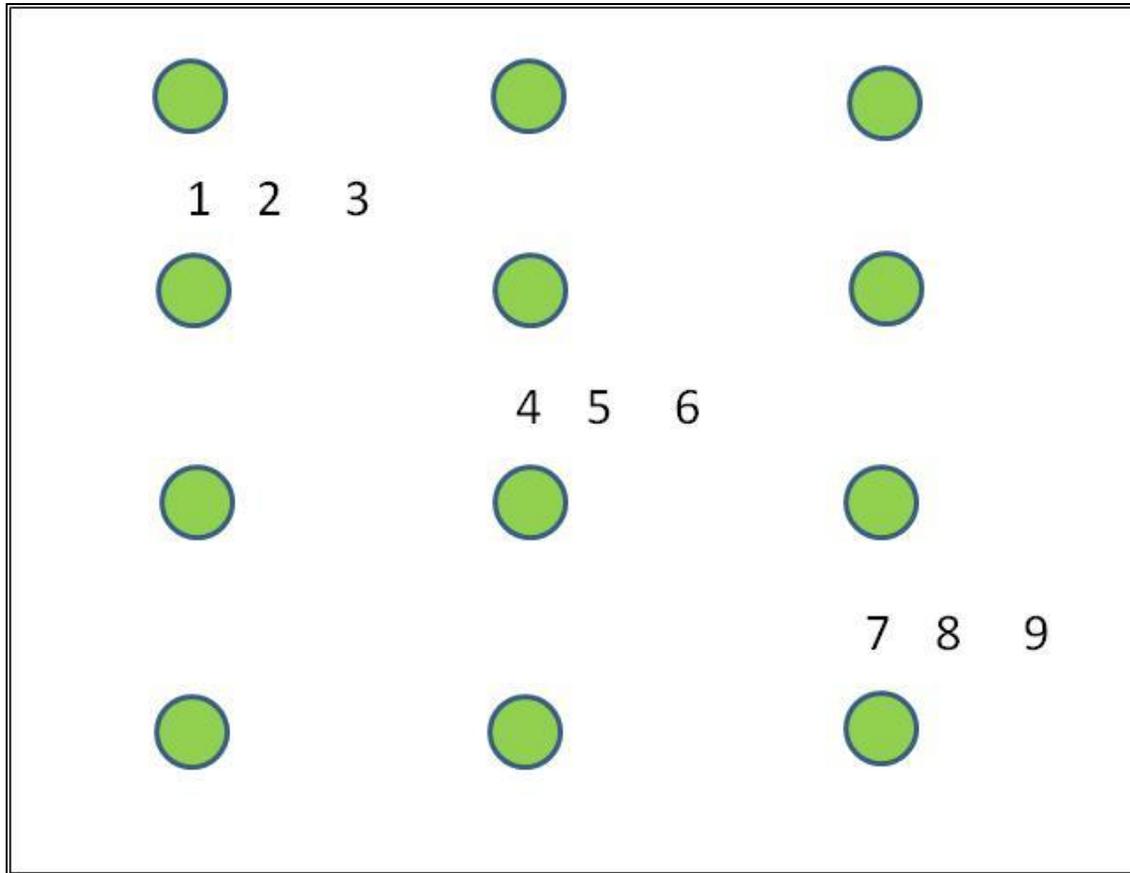


Figure 3.2 Sampling scheme for each plot. Circles represent trees in planting rows. Points 1-9 were composited in the field at a 0-15 cm and a 15-30 cm depth for carbon concentration. Bulk density was collected at the same depths at points 4, 5, and 6, combined for an average.

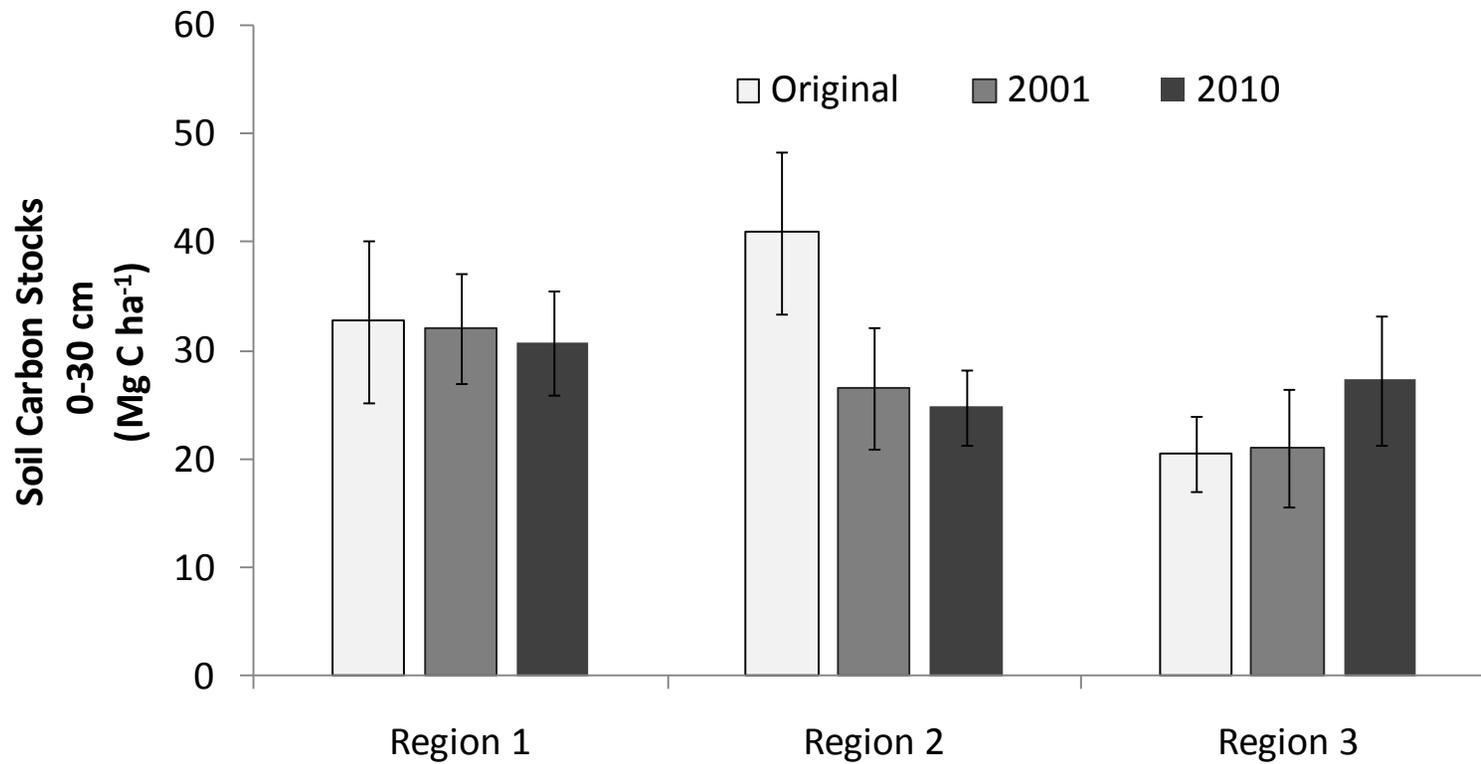


Figure 3.3 Soil carbon stocks from 0 to 30 cm sampling depth under short-rotation *Eucalyptus* silviculture show different trends over time depending on region sampled. Error bars represent 95% confidence intervals. Original sampling dates range from 1984-1993.

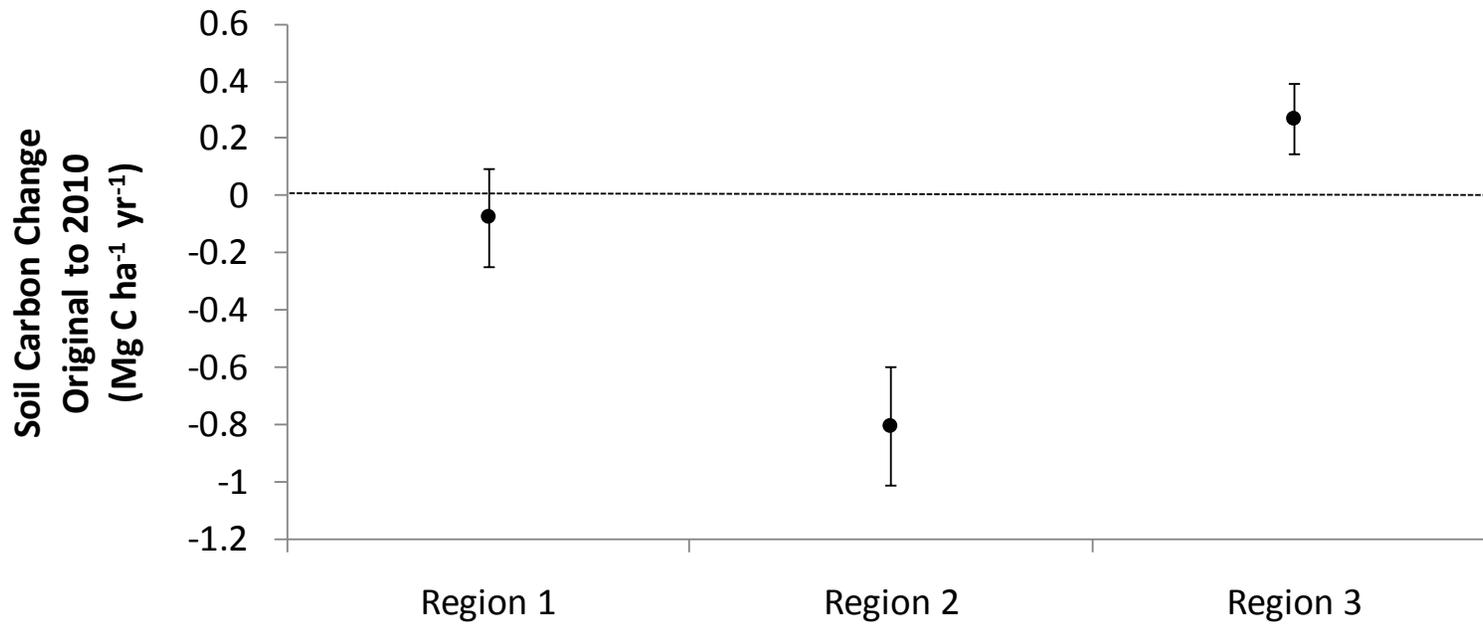


Figure 3.4 Annualized soil carbon change from original sampling period (1984-1993) to 2010. Error bars represent 95% confidence intervals. Mean soil carbon change for Region 2 ($-0.80 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$) and Region 3 ($0.27 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$) are significantly different from zero.

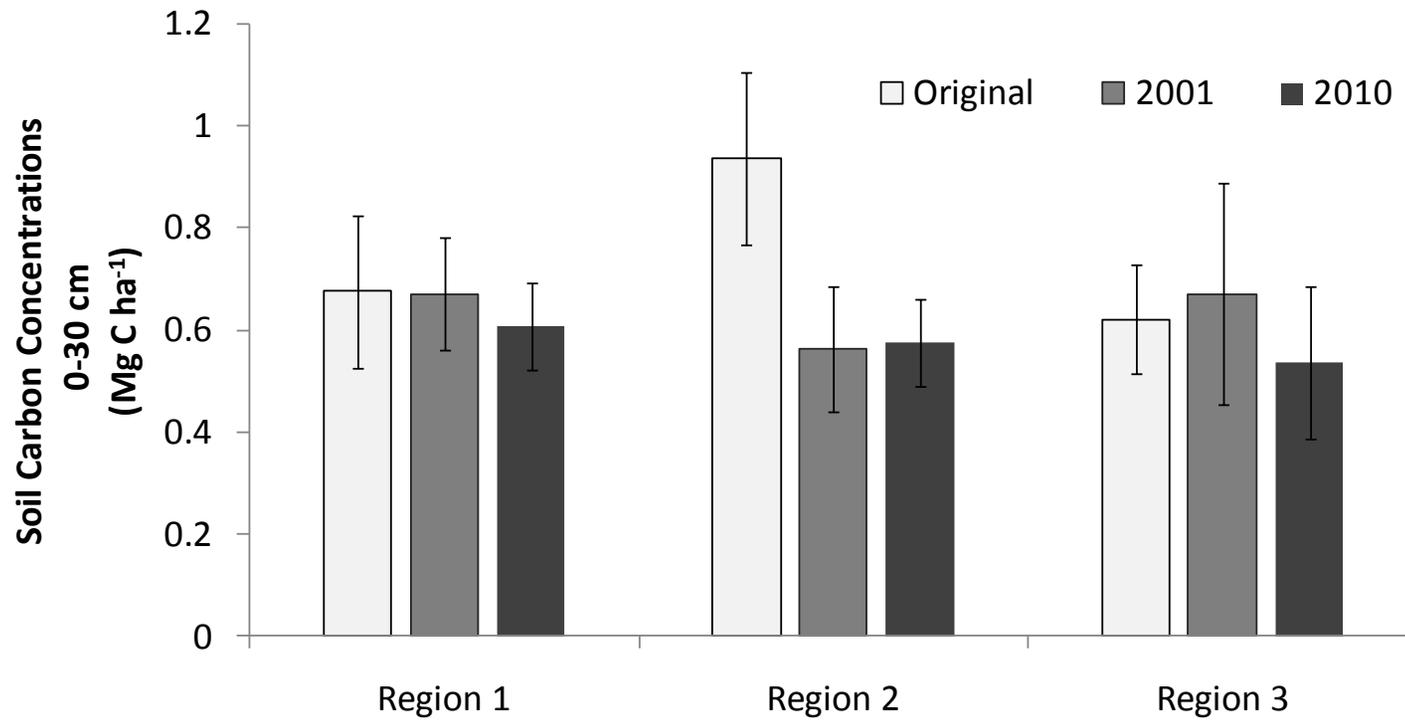


Figure 3.5 Mean soil organic carbon concentrations (%) for each region at each sampling period: original (1984-1993), 2001, and 2010. Error bars represent 95% confidence intervals.

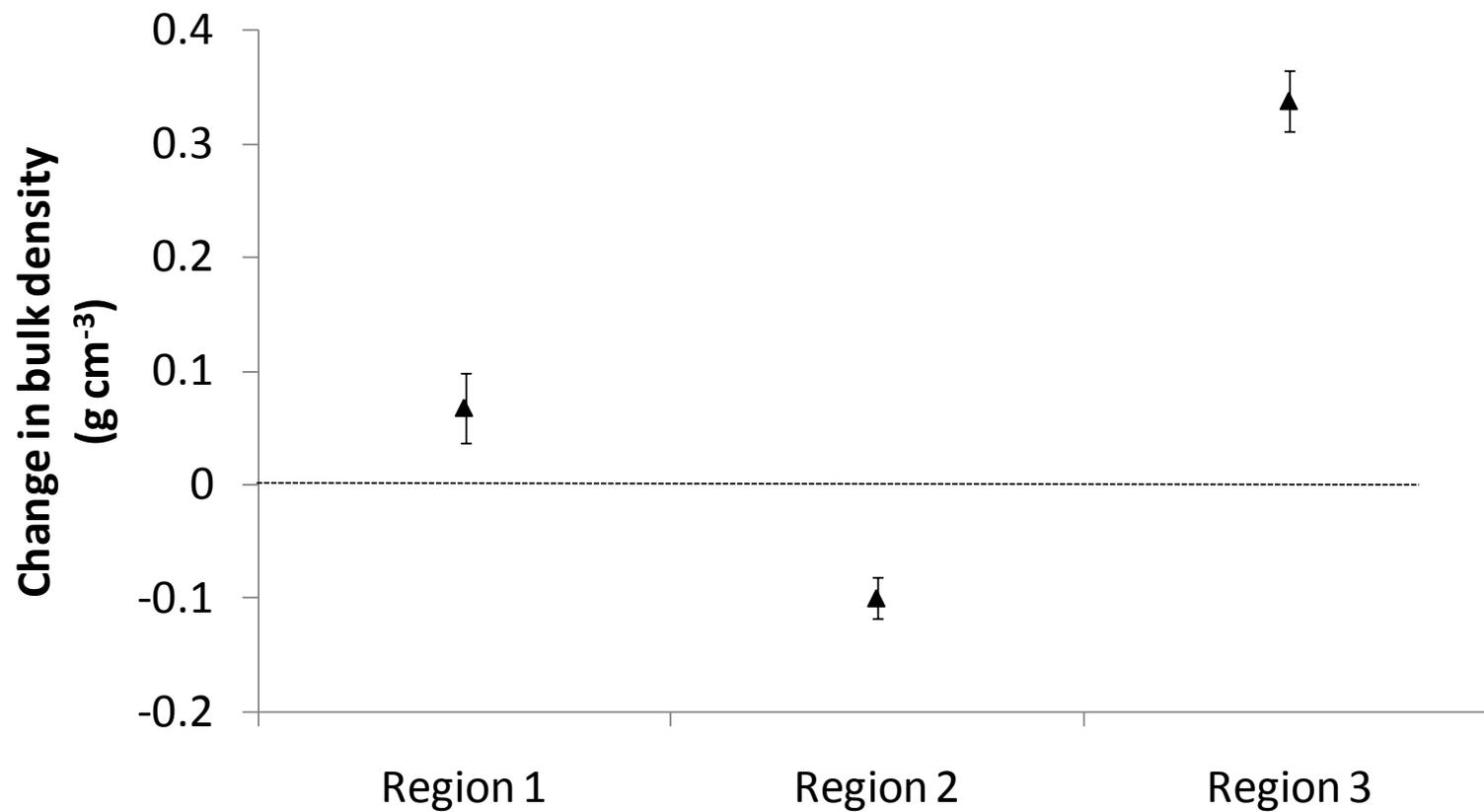


Figure 3.6 Changes in bulk density (g cm^{-3}) across the three regions from 2001 to 2010 show Region 2 decreased (-6.3%) while Region 1 and Region 3 increase (4.6% and 30%, respectively). Error bars represent 95% confidence intervals.

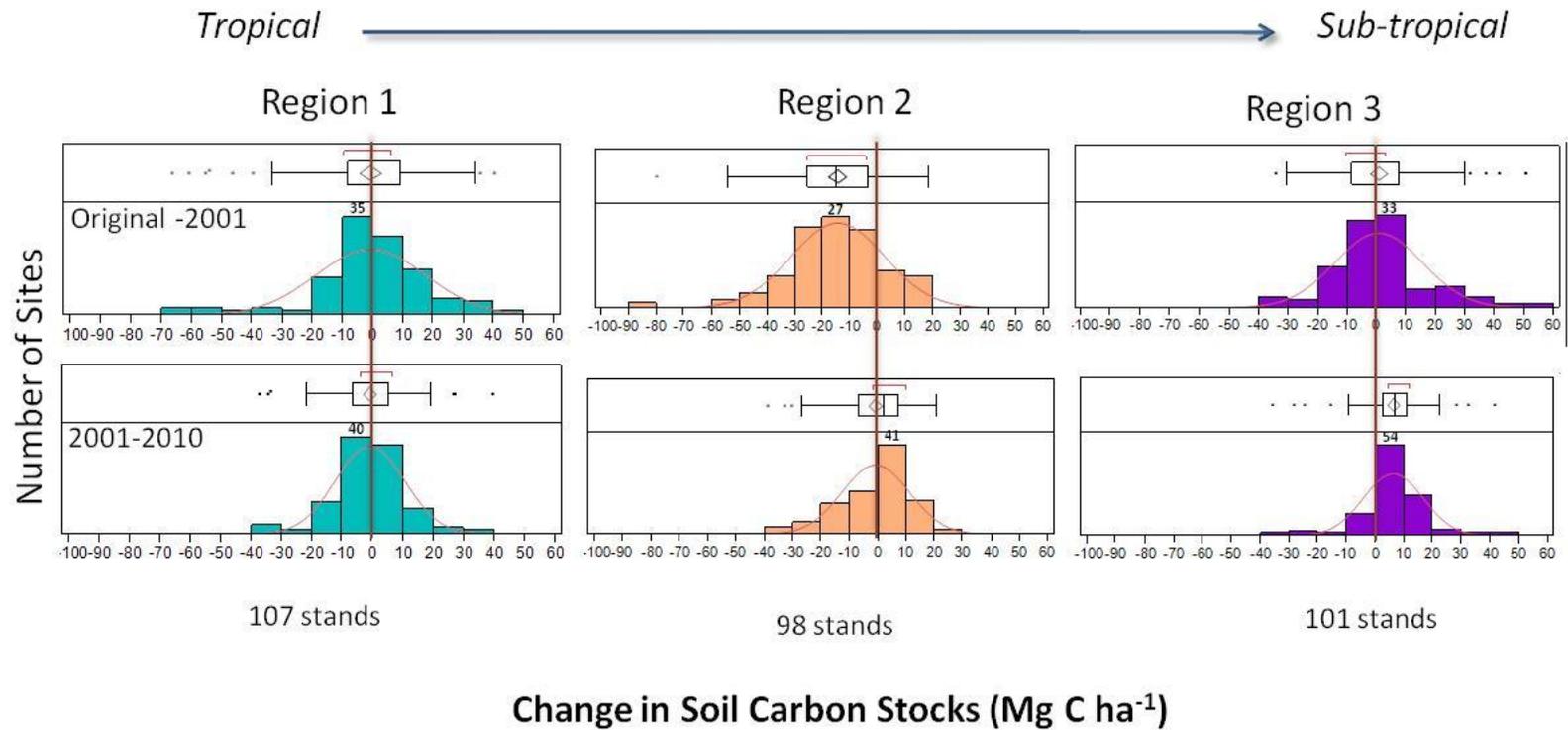


Figure 3.7 Histograms for each soil sampling region (from tropical to sub-tropical *Eucalyptus* plantations) show the number of sites with the quantity of change in soil carbon stocks (Mg C ha^{-1}) for each site. The first row represents the first time period from the original soil sampling (1984-1993) to 2001 and the second row represents the second time period from 2001 to 2010. Vertical lines at zero indicate no change. The number of sites in the greatest histogram bin are superimposed on the figures. Variability in change in soil carbon stocks was reduced significantly from the first time period to the second time period.

Table 3.1 Summary of latitude, mean annual temperature (MAT), and mean wet and dry season precipitation for each study region.

Region	Latitude	MAT	Precipitation (mm)	
			Dry Season	Wet Season
Region 1	12° S	25°C	503	1054
Region 2	19° S	24°C	504	878
Region 3	23° S	20°C	318	1044

Table 3.2 Distribution of three soil orders found in the original 330 study sites in Brazil.

Region	Number of Sites = 330		
	Entisol	Oxisol	Ultisol
Region 1	13	4	93
Region 2	0	9	101
Region 3	45	51	14

Table 3.3 Soil bulk density for 2001 and 2010. While Region 3 saw the largest increase, the mean bulk density for 2010 is still less than the other two regions.

Region	Bulk Density (g cm⁻³)		Change
	2001	2010	
Region 1	1.61	1.68	4.60%
Region 2	1.59	1.49	-6.30%
Region 3	1.13	1.47	30%

Table 3.4 General linear models for soil organic carbon (SOC) stocks and changes. Degrees of freedom equal 275. (Initial C = soil carbon concentration (%) in the original sampling; Clay = clay content (%))

Independent variables	Significant dependent variables	F-value	P-value	Explained variance (%)
SOC stocks (original)	region + soil order + (45.2) initial C + (2.1) tmean + (-0.02) dry months ppt	1321.48	<0.0001	98
SOC stocks (2001)	region + (0.58) clay + (6.9) initial C + (-5.2) tmean + (0.02) wet months ppt	37.29	<0.0001	56
SOC stocks (2010)	(0.54) clay + (-3.7) tmean + (0.05) dry month ppt + (0.02) wet month ppt	34.86	<0.0001	54
SOC change (original-2001)	region + (0.05) clay + (-2.9) initial C + (-0.43) tmean + (0.002) dry months ppt + (0.001) wet month ppt	69.26	<0.0001	70
SOC change (2001-2010)	region + (-0.5) initial C + (0.006) dry months ppt	11.29	<0.0001	28
SOC change (original-2010)	region + (0.03) clay + (-2.0) initial C + (-0.2) tmean + (0.003) dry months ppt + (0.0009) wet month ppt	126.2	<0.0001	79

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Final Conclusions

Soil organic matter provides an important component of fertility in highly weathered soils commonly found in the tropics and plays an integral role in the global carbon cycle. The purpose of this study was to determine the effects of plantation forestry on soil organic carbon stocks, changes, and turnover, due to species effect (conifer or broadleaf) and long-term continuous *Eucalyptus* silviculture. To accomplish this, we determined total soil organic carbon to 45 cm in pasture, broadleaf, and conifer plantations in the Anhembi Experimental Field Station, located in the state of Sao Paulo, Brazil. Secondly, to quantify soil carbon turnover, we used stable carbon isotopes to differentiate rates of loss and accumulation of pasture- and forest-derived soil carbon along a 34-year chronosequence. Finally, we quantified soil carbon stocks and changes following more than 20 years of short-rotation *Eucalyptus* silviculture in 306 sites across three regions in Brazil for the third consecutive collection and correlated stocks and changes with soil and climate factors. These studies, which may represent the largest scale analysis of *Eucalyptus* plantation soil organic stocks to date, present a robust experimental design to determine vegetation effects on soil carbon stocks.

Overall we saw little evidence of a species effect on total soil organic carbon following reforestation of pasture to broadleaf plantations, and only a 6% increase in SOC_T in broadleaf plantations over conifers. While there may be little effect of reforestation on total soil carbon, the dynamics based on stable carbon isotopes showed a far more interesting picture of carbon gains and losses over time. We found equal rates of C4-forest soil carbon accrual to C3-pasture carbon decline in the top 0-15 cm layer. Slight decreases in mineral soil carbon in the 15-30 cm and 30-45 cm layers were driven by losses in C4-pasture-derived carbon, while forest carbon remained stable. Additionally, the percent changes in forest and pasture carbon showed that the 0-15 cm soil became forest-carbon dominated after 9.7 years of reforestation, whereas it only took 8 years in the 15-30 cm soil layer. This may be due to continued inputs from C4-grasses in the forest understory, or due to the fact that the 15-30 cm layer was never as thoroughly dominated by forest carbon, and therefore took less time to

lose the pasture carbon signature; or some combination of the two. The 30-45 cm layer remained dominated by C3-forest carbon from the original native forest throughout. The change in soil carbon from pasture to forest carbon may be important in terms of soil carbon stability or recalcitrance. Incubation studies or fractionation of archived soil samples could be the next step in determining the consequences of soil carbon turnover for long-term stability. The remaining C3-carbon signature in the deeper soil layers and the calculated mean residence times support the idea that deeper soil layers contain a more stable carbon reserve. Our results from the stable carbon isotopes concerning pasture and forest carbon may be useful for future soil carbon modeling.

The rapid increase in *Eucalyptus* production raises questions as to the role plantation forestry has in soil carbon change on a large scale. The long-term, landscape-scale quantification of soil carbon stocks and changes following continuous *Eucalyptus* silviculture showed changes were dependent on region, showing a decrease, no change, or an increase in soil carbon stocks over time. Stocks and changes depended on clay content, initial soil carbon, mean annual temperature, and wet and dry season precipitation to varying degrees. These results indicate that *Eucalyptus* silviculture can have variable results on soil carbon stocks over time. Changes in stocks can also be driven by changes in either soil carbon concentration or bulk density. This work provided a continuation of a long-term soil study across a geographic gradient in Brazil, which will be essential to gaining a better understanding of the role of land-use change in the global carbon cycle and provides an indication of sustainable forest production.

Variability is inherent in the nature of soil studies due to heterogeneous landscape and climate conditions. Plantation forests span an interesting gap between agricultural and forest systems. Because these systems are much less complex, and more environmental variables are controlled than in a natural forest system, they provide an opportunity to study soil carbon and nutrient cycling across the broad landscape by controlling species and management regimes. High rates of plant productivity and rates of decomposition in tropical regions additionally create an opportunity to study biological processes on the scale of years or decades that would take decades or centuries in temperate or boreal ecosystems.

Continuation of this work and other long-term carbon monitoring studies will be crucial to gain a better understanding of how we can manage forest systems to enhance soil carbon storage and where best to focus our efforts. Large-scale studies, both in time and space, will continue to be required to gain a better understanding of how land-use change affects soil carbon storage and therefore the global carbon cycle.