

THE POTENTIAL CARBON SEQUESTRATION OF *EUCALYPTUS*  
*GRANDIS* IN CONJUNCTION WITH ITS USE AS A BIOFUEL  
FEEDSTOCK

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE  
UNIVERSITY OF HAWAI‘I AT MĀNOA IN PARTIAL FULFILLMENT  
OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE IN NATURAL RESOURCES AND  
ENVIRONMENTAL MANAGEMENT

DECEMBER 2012

By

Mataia Reeves

Committee:

Susan Crow, Chairperson

Christian Giardina

J.B. Friday

Scott Turn

Keywords: Carbon, Biofuel, *Eucalyptus grandis*, Sequestration, Soil

## ACKNOWLEDGEMENTS

I would like to acknowledge Susan Crow, for her patience, support and superior mentorship throughout this process. I would also like to thank the members of my committee, Christian Giardina, J.B. Friday and Scott Turn for their contributions, time and assistance. I would like to express my gratitude to the Center for Bioenergy Research and Development for funding sources that made this study possible. Thank you to Yudai Sumiyoshi, Mark Miller, Mariko Panzella, Heather Kikkawa, and Bryan Patterson for their assistance in the lab and the field. I would also like to acknowledge David Beilman for the use of his lab and equipment. The members of Forest Solutions, Nicholas Koch, Jim Thain, Willie Rice, as well as Tom and Kim deserve my gratitude for their hospitality and help during my field season.

## ABSTRACT

Biomass conversion to biofuel is a renewable energy option being considered as an alternative to fossil fuel combustion because of its potential to offset greenhouse gas emissions through carbon (C) sequestration into soil. This study examined the C stocks and dynamics of a *Eucalyptus grandis* plantation located in Hawaii. The study had two objectives: 1) To assess the C resources in the plantation, including live biomass C and soil C stock, and 2) To determine the potential for soil C sequestration, by measuring changes in both total soil C stock and C pool sizes. Overall soil C stock in the *E. grandis* plantation was significantly higher than that of the pasture, however, the distribution of C with depth and among C pools with different rates of turnover varied. These results demonstrate that soil has the capacity to provide a long-term carbon offset within a renewable biofuel production system.

## TABLE OF CONTENTS

Acknowledgements.....	ii
Abstract.....	iii
List of Tables.....	iv
List of Figures.....	v
Chapter 1. Background and Introduction.....	1
Biofuel and carbon accounting.....	1
The need for biofuel research in Hawai‘i.....	2
<i>Eucalyptus grandis</i> as a biofuel feedstock.....	2
Soil carbon.....	3
Soil Fractionation.....	3
Objectives.....	4
Hypotheses.....	5
Chapter 2. The Potential Carbon Sequestration of <i>Eucalyptus grandis</i> in Conjunction with its Use as a Biofuel Feedstock.....	6
Abstract.....	6
Introduction.....	6
Methods.....	9
Soil and site description.....	9
Above and below ground biomass and carbon.....	9
Soil carbon stock.....	9
Soil fractionation.....	10
Statistical analyses.....	11
Results.....	11
<i>E. grandis</i> above and below ground biomass carbon, total soil C.....	11
Pasture vs. <i>E. grandis</i> soil C by depth.....	12
Relative distribution of soil C among pools.....	12
Soil C stock by fraction.....	13
Percent change.....	14
Discussion.....	15
Current C resource within the plantation.....	15
Total carbon accumulation following land use change.....	15
Quantifying changes in distribution of C among soil pools.....	16
Conclusion.....	17
Appendix 1. Tables.....	19
Appendix 2. Figures.....	20
Appendix 3. Sampling design and semivariogram.....	29
Appendix 4. Mass balance, soil fractionation.....	30
References.....	34

## LIST OF TABLES

Table 1. Plot pair labels, elevation (m), (MAT), (°C), (MAP), (mm), soil series, and taxonomic description of soil series.....	17
Table 2. Mean soil C by depth in <i>E. grandis</i> and pasture.....	18

## LIST OF FIGURES

Figure 1. Site map of study area located on the Island of Hawaii.....	20
Figure 2. Optimized soil fractionation method .....	21
Figure 3. Above and below ground biomass carbon, soil C in <i>E. grandis</i> plantation.....	22
Figure 4. Soil carbon by to 1m depth in pasture and <i>E. grandis</i> .....	23
Figure 5. Mean soil carbon by depth in pasture and <i>E. grandis</i> .....	24
Figure 6. Soil carbon density by kriging.....	25
Figure 7. Carbon recovered in each fraction as percent of total carbon.....	26
Figure 8. Carbon stock by fraction at three depths.....	27

## CHAPTER 1. BACKGROUND AND INTRODUCTION

### **Biofuel and Carbon Accounting**

As global climate change progresses, the search for ways to offset greenhouse gas enrichment of the atmosphere is intensifying. Alternative energy sources are being examined and considered as replacements for fossil fuel for this reason. The conversion of biomass to biofuel is one of these alternatives, as its use may help to mitigate climate change (Mitikka, 2004). The advantages of biofuel production are that it is renewable and its production may emit less net CO<sub>2</sub> and other greenhouse gases than fossil fuel does. However, in order to determine if the latter is indeed the case, investigation into the entire biofuel system must occur. Carbon (C) accounting is a useful tool that can be used to assess the potential of biofuel as an alternative energy source. All C inputs and outputs are quantified and recorded, and this account can then be compared with other energy sources, or within types (feedstocks, management regimes) of biofuel systems.

The C balance forms an integral part of a life-cycle analysis (LCA), an analysis performed in order to understand the entire system and its inputs and outputs. The U.S. Environmental Protection Agency (EPA, 2011) defines a LCA as “a technique to assess the environmental aspects and potential impacts associated with a product, process, or service, by: compiling an inventory of relevant energy and material inputs and environmental releases; evaluating the potential environmental impacts associated with identified inputs and releases; [and] interpreting the results to help you make a more informed decision”. Decisions about what type of feedstock to grow, how and when to harvest, and what type of conversion process should be used can all be informed by a LCA.

Since the growth of a biofuel feedstock removes CO<sub>2</sub> from the atmosphere, its use can reduce greenhouse gas emissions when compared to fossil fuels (Searchinger et al., 2008; Cherubini et al., 2009). For this reason, biofuel production is often considered a C neutral process, as the release of CO<sub>2</sub> during the conversion to energy by combustion is offset by the initial growth process (Cherubini et al., 2009). However, land use change is often associated with biofuel growth, and can be responsible for a very large part of the C debt in the system (Sheehan 2009; Searchinger et al., 2008; Fargione et al., 2008). A large portion of this C debt can occur due to disturbance to the soil C pool, through tilling and/or harvest.

Globally, soil contains more C than above ground vegetation and the atmosphere combined (Lal, 2008, Schlesinger, 1997.) Thus, any disturbance to this C pool can have proportionally large effects on the C balance of the entire system (Cherubini et al, 2009). For example, disturbance from tilling can disrupt protection mechanisms such as aggregation and cause increased loss of soil C from the system (Post and Kwon, 2000). Therefore, management practices, including tilling and harvesting methods, must be examined when assessing the advantages and disadvantages of a biofuel system. Some studies have found that afforestation with *Eucalyptus* increased soil C sequestration, especially when combined with minimum tillage (Lima, et al., 2006). Martens, et al. (2003) observed a mean gain of 29% in soil carbon with afforestation of pasture land, likely due to improved aggregation in soil not disturbed by tillage, because aggregation protects soil C from decomposition by making it physically unavailable to microbial

decomposers. Thus, land use change can lead to positive or negative changes to the soil C pool, dependent upon management practices.

### **The Need for Biofuel Research in Hawai‘i**

The State of Hawai‘i and the Hawaiian Electric Company (HECO) are both very interested in biofuel research and use. The Hawai‘i Clean Energy Initiative (HCEI) states a goal of 40% of the state’s energy to come from renewables by 2030 (Hawaii Clean Energy Initiative, 2012). This doubles the amount of renewables currently required by the Renewable Portfolio Standard Law (HECO Future Energy Agreement, 2008). Biofuels will most likely be a large part of the renewable energy mix. In 2009, HECO completed construction of the 110 MW Campbell Industrial Park Generating Station, which will be fueled entirely by biodiesel. Furthermore, HECO plans to convert “existing fossil fuel generators to renewable biofuels, ultimately using crops grown locally and in a sustainable manner (HECO Future Energy Agreement, 2008). HECO is currently “seeking long-term contracts for biofuels from feedstocks grown in Hawai‘i” (HECO, Biofuel Activities as of May 2012).

### ***Eucalyptus grandis* as a Biofuel Feedstock**

There are many choices available for biofuel feedstock, ranging from algae to grasses to hardwoods. This study focuses on the use of *Eucalyptus grandis* as the biofuel feedstock. *E. grandis* is being considered for use as a biofuel feedstock due to its high yield, good stem form, high wood density, and its resistance to pests and disease (Rockwood et al., 2008; Simmons et al., 2008). *E. grandis* is also attractive for use as a biofuel as it can be grown and harvested in short rotation, has low lignin content, and is adapted to growth in the tropics (Hinchee et al., 2009). On the island of Hawai‘i, there are approximately 230,000 acres suitable for growing *E. grandis* as a biofuel feedstock (Whitesell, et al, 1992). Of these, ranch land and abandoned land are considered suitable sites for *E. grandis* plantations (Whitesell et al., 1992; Poteet, 2006). The site for this study is a monoculture of *E. grandis* located on the Island of Hawai‘i. The land was previously used for pasture, and is currently surrounded by same. As such, this site allows for investigation of the effects of afforestation on the C balance with a land use change from pasture to *E. grandis*.

This study is primarily concerned with examining the effects of afforestation on the soil C stock and dynamics, however, above and below ground biomass is also quantified to provide an inventory of C stock in the *E. grandis* plantation and demonstrate the magnitude of the soil C resource particularly at these sites. To quantify above and below ground biomass, allometric equations were used. Studies on *E. grandis* have been conducted in Hawai‘i (Kaye et al., 2000; Dudley & Fownes, 1991; Schubert et al., 1988), and as these studies are often site and species specific, the equations built by Schubert et al. were determined to be the best choice for the estimation of above ground biomass in this study. Below ground biomass is estimated as a percentage of above ground biomass (Saint-Andre et al., 2005, Misra et al., 1998, Greenhouse Challenge Vegetation Sinks Workbook in the Commonwealth of Australia, 1999).

## Soil Carbon

The effect of land use change on the soil C stock can be large and may tip the balance of sustainability for the entire biofuel system. Thus, including quantification of this stock in the potential C sequestration of the plantation is imperative. The soils present at this study site inherently contain a large amount of C, due to their mineralogy. They are ash-derived Andisols containing amorphous minerals and hydrated oxides such as allophane, imogilite and ferrihydrite which have large surface area and variable charge leading to a high capacity of these soils to bond with organic matter (Torn et al., 1997). The overall C stock of the soil is determined by the balance between C entering the soil from above and below ground biomass contributions and C exiting the soil due to decomposition and C mineralization (Kögel-Knabner et al., 2008).

The mechanisms that lead to soil C sequestration are those that protect the C from exiting the soil through decomposition and/or mineralization, such as protection within soil aggregates, chemical bonding between organic C and minerals, and inherent chemical resistance due to molecular composition of certain substances (Sollins et al., 1996). Aggregation protects soil C by making it inaccessible to microbial decomposers (Sollins et al., 1996), and if these aggregates are not physically disturbed, their presence leads to lasting stabilization of soil C (Six et al., 2000b). Organo-mineral bonding occurs when soil organic matter sorbs strongly to mineral surfaces (Kleber et al., 2007), which leads to a decrease in biodegradability of this soil C pool and thus a longer turnover time. Iron oxide surfaces and amorphous minerals, such as those present in the soils investigated here, are important sorbents for organic matter (Kogel-Knabner, 2008, Kramer et al., 2012). The third mechanism for soil C protection, that of inherent chemical resistance, refers to the molecular structure of substances such as aliphatic compounds and lignin-derived aromatic carbons that are resistant to decay by their very nature (Mikutta et al., 2006). Recently, the role of recalcitrance in soil organic matter stabilization has been minimized in favor of the more physical protection mechanisms of aggregate stability and organo-mineral interactions (Kleber, 2010, Schmidt et al., 2011).

## Soil Fractionation

In order to elucidate these protection mechanisms, a comparison of three common soil fractionation methods was conducted with the goal of creating a fractionation method optimal for the type of soils present at this study site. The resulting fractions from these three methods were analyzed for C content, and isotope analysis was performed and used to calculate mean residence time, an indication of the average time a C molecule has resided in the soil. In this way, the soil fractions were related to turn-over time and C sequestration potential.

The Sollins method is a density cut method in which soil is sequentially separated into fractions with a high density solution (Sollins et al., 2006). The more labile, newly introduced C is found in the lightest fraction, while the more stable, older and longer-lasting C is found in the denser organo-mineral fractions (Sollins et al., 2006). This method was found to be useful in separating between the lightest, short-term material and the denser, mineral-driven and more recalcitrant fractions, but it did not directly explore the role of aggregation in soil C stabilization. The Golchin method was therefore performed on these soils (Golchin et al., 1994). This method uses ultra-sonication to

break up soil aggregates, after separating out the lightest fraction. The ultra-sonication frees the C that is physically protected by the aggregation process, the occluded fraction. The separation between the light fraction and the occluded fraction was found to be informative, as the light fraction contained much newer C than the occluded fraction (<1 year vs. hundreds of years). The Six method of soil fractionation, which separates the soil into macro and micro aggregates, was also tested for incorporation into the new optimized method (Six et al., 2002a). This method resulted in large losses of C during the fractionation procedure but did indicate that there may be more C present in macro aggregates in the *E. grandis* plantation soil than in the pasture soil.

The comparison of these three methods led to the creation of an optimized fractionation method (Crow and Reeves *in preparation*). This method separates the soil into five fractions: the light fraction (<1.8 g/mL), the occluded light fraction (< 1.8 g/mL after ultrasonic disruption with  $\approx 150$  J/mL), and three sequential density fractions (1.8 – 2.0 g/mL, 2.0 – 2.4 g/mL, and >2.4 g/mL). The light fraction represents newly incorporated organic matter with turnover times on the annual to decadal scale, while the occluded light fraction is that fraction that is protected within aggregates, and has a turnover time on the centennial scale. The sequential density fractions have turnover times that increase along with density from the centennial to millennial scale. These turnover times were validated by  $^{14}\text{C}$  based mean residence time (MRT) using a one-pool steady-state model. MRT in the light fraction was 3-5 years, while that of the occluded was 200-300 years. The 1.8-2.0 g/mL fraction MRT was 100-200 years, increased to 400-700 years in the 2.0-2.4 g/mL fraction, and was 800-1500 years in the >2.4 g/mL fraction. An initial comparison of land uses: native forest, pasture and *E. grandis*, showed that the optimized method successfully isolated C pools with different C sequestration potential and was sensitive to changes in soil C pools due to land use change.

Soil C content does not only differ in which functional pools it occurs in, but is known to differ with depth in the soil profile. Different types of vegetation have dissimilar rooting depths, leading to changes in the distribution of root exudate soil C along with depth (Joggaby and Jackson, 2000; Powlson et al. 2011). There may also be different mechanisms leading to the stabilization and longevity of soil C between surface and subsoil (Salome et al., 2010). Soil C in the subsoil is known to have a longer turnover time, due to protection within soil aggregates and/or chemical recalcitrance as well as decreased microbial activity (Lorenz and Lal, 2005). For these reasons, soil fractionation was performed on surface soil, mid-depth (50 cm), and deeper soil (1m).

### **Objectives:**

The project had two objectives: 1) to quantify current C resources present in the *E. grandis* plots: above ground biomass C in the trees, below ground root C, and soil C; and 2) address the question of how soil C dynamics are affected by afforestation with *E. grandis*, by comparing C content and transfers between C pools in samples taken from the *E. grandis* plantation as well as from adjacent pastureland.

**Hypotheses:**

Hypothesis 1: Soil C stock from 0 – 1 m will be greater than above-ground biomass C in the *E. grandis* trees

Hypothesis 2a: Soil C stock in the top 1m of soil under *E. grandis* will be greater than in the adjacent pasture land. However, the pasture will have more soil C (due to root biomass) within the 0-30 cm depth, due to differences in rooting depth and root density.

Hypothesis 2b: The accumulation of C in the surface soil of the pasture will be found in the labile soil C pool; and a majority of the soil C in the *E. grandis* cores will be found in the physically protected and organo-mineral C pools.

## CHAPTER 2. THE POTENTIAL CARBON SEQUESTRATION OF *EUCALYPTUS GRANDIS* IN CONJUNCTION WITH ITS USE AS A BIOFUEL FEEDSTOCK

### **Abstract**

Woody biomass conversion to biofuel is a renewable energy option being considered as an alternative to fossil fuel combustion because its use decreases dependence on oil imports and improves energy security, it has a high efficiency of conversion, and it has the potential to offset greenhouse gas emissions through carbon (C) sequestration into soil through land use conversion into sustainably managed plantations. This study examined the C stocks and dynamics of a *Eucalyptus grandis* plantation established on land previously under pasture for nearly a century located on the Island of Hawaii. The study had two objectives: 1) To assess the C resources in the plantation, including live biomass C (both above and below ground) and soil C stock, and 2) To determine the potential for soil C sequestration, and therefore greenhouse gas offsets, by measuring changes in both total soil C stock and C pool sizes with conversion of pasture to managed plantation. Mean soil C stock to 1 m depth in the *E. grandis* plantation was  $593 \pm 16.3$  ton/ha, which was much greater than the amount of C found in the above and below ground biomass ( $57.22 \pm 4.22$  ton/ha and  $10.07 \pm 0.74$  ton/ha, respectively). Overall soil C stock in the *E. grandis* plantation was significantly higher than that of the pasture, ( $516.8 \pm 21.1$ ), however, the distribution of C with depth and among C pools with different rates of turnover varied. The total C stock was not different between pasture and *E. grandis* in the surface soil (0-18cm), however there was significantly greater C within the most actively cycled (i.e., with the least C sequestration potential) pool in the pasture. In the mid-depth soil (18-72 cm), there was significantly greater total C stock and C within mineral-associated and stable aggregate protected pools with mean residence time of 500+ years (i.e., with high potential for C sequestration) in the *E. grandis*. These results demonstrate that these soils have the capacity to provide a long-term carbon offset within a renewable biofuel production system.

### **Introduction**

As global climate change progresses and we search for ways to offset greenhouse gas enrichment of the atmosphere, alternative energy sources are being examined and considered as replacements for fossil fuel. The conversion of biomass to biofuel is one of these alternatives, as its use may help to mitigate climate change (Mitikka, 2004). The advantages of biofuel production are that it is renewable, and its production may emit less net CO<sub>2</sub> and other greenhouse gases than fossil fuel does. However, in order to determine if the latter is indeed the case, investigation into the entire biofuel system must occur. Carbon (C) accounting is a useful tool that can be used to assess the potential of biofuel as an alternative energy source. All C inputs and outputs are quantified and recorded,

and this account can then be compared with other energy sources, or within types (feedstocks, management regimes) of biofuel systems.

Since the growth of a biofuel feedstock removes CO<sub>2</sub> from the atmosphere, its use can reduce greenhouse gas emissions when compared to fossil fuels (Searchinger et al., 2008; Cherubini et al., 2009). For this reason, biofuel production is often considered a C neutral process, as the release of CO<sub>2</sub> during the conversion to energy by combustion is offset by the initial growth process (Cherubini et al., 2009). However, the growth of biomass for biofuel production is often associated with land use change. Land use change can be difficult to quantify and can be responsible for causing an overall C debt in the system (Sheehan 2009; Searchinger et al., 2008; Fargione et al., 2008, Plevin et al., 2010). A large portion of this C debt can occur due to disturbance to the soil C pool, through tilling and site preparation.

Globally, soil contains more C than above ground vegetation and the atmosphere combined (Lal, 2008, Schlesinger, 1997.) Thus, any change to this C pool can have proportionally large effects on the C balance of the entire system (Cherubini et al, 2009). For example, disturbance from tilling can disrupt protection mechanisms such as aggregation and cause increased loss of soil C from the system (Post and Kwon, 2000). On the other hand, low-till and no-till practices can cause an increase in soil C (West and Post, 2002, Six et al., 2002b). Therefore, management practices, including tilling methods must be examined when assessing the advantages and disadvantages of a biofuel system.

The choice of feedstock is also an important consideration. This study focused on the use of *Eucalyptus grandis* as the biofuel feedstock. *E. grandis* is being considered for use as a biofuel feedstock due to its high yield, good stem form, high wood density, and its resistance to pests and disease (Rockwood et al., 2008; Simmons et al., 2008). *E. grandis* is also attractive for use as a biofuel as it can be grown and harvested in short rotation, has low lignin content, and is adapted to growth in the tropics (Hinchee et al., 2009). The site for this study was a monoculture of *E. grandis* located on the Island of Hawai'i. The land was previously used for pasture, and currently is surrounded by the same. As such, this site allowed for investigation of the effects of afforestation on the C balance with a land use change from pasture to *E. grandis*.

The soil C pool was expected to be sensitive to land use change, due to the fact that rooting depth and density differs greatly between *E. grandis* and pasture grasses. In general, trees have deeper roots than grasses, which changes the distribution of root C inputs along the soil profile. *Eucalyptus* species have been found to have roots at least as deep as 10 m (Westman and Rogers, 1977, Robinson et al., 2006), whereas Kikuyu grass (*Cenchrus clandestinus*) which dominates the pasture land has a much shallower rooting depth of 2 m (Nie et al., 2007). Although both *E. grandis* and Kikuyu grass roots are generally denser in the surface soil vs. the subsurface, grass roots tend to be much denser in the top 10 cm than tree roots (Jackson et al., 1996). This density difference can also be inferred from the root to shoot ratio of *E. grandis* (0.20) vs. *C. clandestinus* (0.85), demonstrating that *C. clandestinus* has a much denser rooting pattern than *E. grandis*, at least at the surface (Schooler et al., 2010, Misra et al., 1998, Saint-Andre et al., 2005). Therefore, direct soil C inputs from root exudates as well as root mortality and eventual incorporation into the soil likely differ in quantity and by depth between the *E. grandis* and pasture.

The soils present at this site inherently contain a large amount of C, due to their mineralogy. They are ash-derived Andisols containing amorphous minerals and hydrated oxides such as allophane, imogilite and ferrihydrite; which have large surface area and variable charge leading to a high capacity of these soils to bond with organic matter (Torn et al., 1997). The overall C stock of the soil is determined by the balance between C entering the soil from above and below ground biomass contributions and C exiting the soil due to decomposition and C mineralization (Kögel-Knabner et al., 2008).

The mechanisms that lead to soil C sequestration are those that protect C from exiting the soil through decomposition and/or mineralization, such as protection within soil aggregates, chemical bonding between organic C and minerals, and inherent chemical resistance due to molecular composition of certain substances (Sollins et al., 1996). Aggregation protects soil C by making it inaccessible to microbial decomposers (Sollins et al., 1996), and if these aggregates are not physically disturbed, their presence leads to lasting stabilization of soil C (Six et al., 2000b). Organo-mineral bonding occurs when soil organic matter sorbs strongly to mineral surfaces (Kleber et al., 2007), which leads to a decrease in biodegradability of this soil C pool, and thus a longer turnover time. Iron oxide surfaces and amorphous minerals, such as those present in the soils investigated here, are important sorbents for organic matter (Kögel-Knabner, 2008, Kramer et al., 2012). The third mechanism for soil C protection, that of inherent chemical resistance, refers to the molecular structure of substances such as aliphatic compounds and lignin-derived aromatic carbons that are resistant to decay by their very nature (Mikutta et al., 2006). Recently, the role of recalcitrance in soil organic matter stabilization has been minimized in favor of the more physical protection mechanisms of aggregate stability and organo-mineral interactions (Kleber, 2010, Schmidt et al., 2011).

Soil C content is known to differ with depth in the soil profile. As mentioned, different types of vegetation have dissimilar rooting depths, leading to changes in the distribution of root exudate soil C along with depth (Joggaby and Jackson, 2000; Powlson et al. 2011). There may also be different mechanisms leading to the stabilization and longevity of soil C between surface and subsoil (Salome et al., 2010). Soil C in the subsoil is known to have a longer turnover time, due to protection within soil aggregates and/or chemical recalcitrance as well as decreased microbial activity (Lorenz and Lal, 2005). For these reasons, soil fractionation was performed on surface soil, mid-depth (50 cm), and deeper soil (1m).

In addition to differences in rooting depth and density, there exists a difference in quantity and quality of both above ground and root litter inputs between the *E. grandis* and the pasture. The *E. grandis* has a higher quantity of litter inputs into the soil C pool than does the pasture, as the *E. grandis* forest floor is undisturbed, thus all above ground litter is eventually incorporated into the soil, whereas in the pasture there is very little litter input, due to grazing. There is also a difference in the quality of above ground and root litter. Kikuyu grass has a lignin content of approximately 6% (Marais, 2001, Vasquez et al., 2011), significantly less than the lignin content of *E. grandis*, which is approximately 30% (Chaves Arantes et al., 2011, Rodrigues et al., 1998, Morais et al., 1991). The lignin content affects the organo mineral binding process, as dissolved organic matter derived from litter leachate high in lignin chemically binds to short range ordered minerals (Kramer et al., 2012). The andic soils at this site have a high content of amorphous, short range ordered minerals, thus it is expected that this specific organo-

mineral binding process will be particularly important in these soils. As *E. grandis* roots and litter contain a much higher percentage of lignin than do the roots and litter of Kikuyu grass, and litterfall rates are greater in *E. grandis*, it is expected that there will be an increase in overall soil C, as well as an increase in the more stable fractions of the *E. grandis* compared to the pasture.

This study had two objectives: 1) to quantify current C resources present in the *E. grandis* plots: above ground biomass C in the trees, below ground root C, and soil C; and 2) address the question of how soil C dynamics are affected by afforestation with *E. grandis*, by comparing C content and transfers between C pools in samples taken from the *E. grandis* plantation as well as from adjacent pastureland. It was hypothesized that the soil C stock in the first meter of soil *E. grandis* plantation would be greater than that of above ground biomass present in the trees themselves. Furthermore, this C stock will be greater than that in the adjacent pasture land soil. However, the pasture will have more soil C (due to root biomass) within the 0-30 cm depth, due to differences in rooting depth and root density. Finally, it was hypothesized that the majority of C in the surface soil of the pasture will be found in the labile soil C pool; while that of the *E. grandis* soil will be found in the physically protected and organo-mineral C pools.

## Methods

### Soil and Site Description

The study area is located on the Hamakua coast in the northern part of the Island of Hawaii, along an elevational gradient of approximately 600 m to 1300 m (Fig. 1). The study area receives a mean annual rainfall amount of 1400 mm to 3175 mm, and the mean annual temperature ranges from approximately 14 °C to 20 °C (Soil Survey Staff, 2011). As elevation increases, precipitation and temperature decrease throughout the site. Soils are volcanic ash derived from lava flows that occurred 64,000 to 300,000 years ago, and are either Hydrudands or Hapludands (Table 1). Six of the twelve study sites are located in an *Eucalyptus grandis* plantation of approximately 3000 hectares, (19°58' N 155°30' W to 20°02' 155° 27' W), which was converted from pasture land and planted with *E. grandis* from 2001 to 2004. The plantation was ripped and ridged with a disk plow to 18-24" depth, and planted with seedlings obtained from seeds from South Africa (Nicholas Koch, personal communication, January 2010). The six plots in the *E. grandis* plantation have been continuously monitored since establishment and measurements such as diameter at breast height (DBH), and tree height have been taken annually for every tree in each of the 400 m<sup>2</sup> plots.

Six pasture plots of equal size were established in adjacent pasture land in 2011, at approximately equal elevation and in the same soil series as the *E. grandis* plots (Table 1). Local topography was also taken into account during the establishment of the pasture plots, so that the pasture plots were selected in areas that were relatively flat or gently sloping, to most closely match the *E. grandis* plots. The pastureland is dominated by *Cenchrus clandestinus*, a common perennial C<sub>4</sub> grass that is naturalized in Hawaii (Motooka et al., 2003). Grasses from the genus *Digitaria* are also present at the sites. The pasture land is currently being grazed. Above ground biomass of grazed Kikuyu grass is likely approximately 2 ton/ha (Blackmore and Vitousek, 2000).

In order to make a valid comparison of soil C stock and dynamics between the soil under *E. grandis* and pasture, the paired sites were constrained by the following:

- Past land use was the same before the establishment of *E. grandis*. Both the pasture and the *E. grandis* plantation are located on land that was converted from native forest dominated by *Metrosideros polymorpha* and *Acacia Koa* 100+ years ago.
- The soil series is the same for each of the pairs, thus parent material, mineralogy, regional topography and weathering are the also the same.
- The elevation is similar in each of the pairs, as is the precipitation and temperature.
- Local topography is very similar, as all plots are located on flat to gentle slopes.
- *E. grandis* was planted in the particular area due to the availability of property for lease from the landowner, and not for reasons such as a difference in soil fertility or climate.

### **Above and Below Ground Biomass and Carbon**

Above ground (ABG) biomass and C were computed from the year 2011 measurements of tree height and DBH. The following equation was used to calculate ABG biomass:  $Y_{\text{total}} = 0.069413 * \text{DBH}^{2.1472} * \text{H}^{0.3129}$ , where Y is total dry weight biomass in kg, DBH is diameter at breast height (1.3 m) in cm, and H is height in meters (Schubert et al., 1988). This equation was developed in Hawaii from destructive harvest of *E. grandis* on the islands of Hawaii and Kauai. To convert to C, the above ground biomass is then multiplied by the C density of the stem of the tree, which was measured by elemental analysis and found to be 51.12% (Turn et al., 2005). Below ground C, or root biomass C, was calculated as 20% of ABG biomass C, which is a value that has been found for *E. grandis* in other studies (Saint-Andre et al., 2005; Misra et al., 1998; Giardina and Ryan, 2002).

### **Soil C Stock**

Soil cores were taken from five points in each of the plots, the four corners and the center. This sampling design was informed by intensive sampling of one of the plots, from which it was determined that the samples were behaving independently at a distance of 10m (Appendix 1). Thus, 5 cores per plot were taken to  $\approx$  1m in 15-18 cm increments with a slide hammer corer of diameter 4.39 cm. The soil samples were split in half vertically and stored frozen. One half of the core was used for computing C stock, while the other half was used for soil fractionation. Bulk density was calculated from the mass of the entire core. Soils were oven-dried to 105°C, sub-sampled, homogenized, ground to pass through a 250  $\mu\text{m}$  sieve, and analyzed for C content in by elemental analysis (Costech Inc., CHN Analyzer). Soil C stock was computed in the following manner:

$$\text{Bulk density (g/cm}^3\text{)} * \text{Depth of core (cm)} * \%C \text{ (g C/g soil)}$$

### **Soil Fractionation**

An optimized fractionation method was created specifically for the soils present in this study (Fig. 2). This method separates the soil into five fractions: the light fraction (<1.8 g/mL), the occluded light fraction (< 1.8 g/mL after ultrasonic disruption with  $\approx$ 150 J/mL), and three sequential density fractions (1.8 – 2.0 g/mL, 2.0 – 2.4 g/mL, and >2.4 g/mL). The light fraction represents newly incorporated organic matter with turnover times on the annual to decadal scale, while the occluded light fraction is that fraction that is protected within aggregates, and has a turnover time on the centennial scale. The

sequential density fractions have turnover times that increase along with density from the centennial to millennial scale.

Three of the depth increments were chosen for soil fractionation: the surface (top 15-18 cm), mid-depth (capturing the 50cm depth) and the deepest core (capturing the 1m depth). From each plot, cores 1-5 of the corresponding depths were composited and then subsampled for fractionation. Moisture content was determined for each of the samples.

30 g of field moist soil (<2mm) was placed in a 250 mL centrifuge bottle, and 100 mL of 1.8 g/cm<sup>3</sup> sodium polytungstate (SPT) was added. Samples were then gently inverted five times by hand and centrifuged at 2800 rpm for 10 minutes. Floating material was then aspirated, rinsed with deionized water by vacuum filtration, and dried to 65°C. The density of the remaining supernatant was measured to insure that the density of the solution was within 0.02 g/cm<sup>3</sup> of the target density. After the light fraction was removed by aspiration, the soil was transferred to a beaker and 75-100 mL of 1.8 g/cm<sup>3</sup> SPT was added to bring the soil and solution to a 100 mL volume. The solution was then subjected to ultrasonication with a Fisher Scientific FB-505 Sonic Dismembrator with a power output of 500 Watts and frequency of 20 kHz at a target energy of 15,000 J/mL in order to break up aggregates. After sonication, the soil solution was transferred back into centrifuge bottles and centrifuged at 2800 rpm for ten minutes. The occluded fraction was then aspirated, rinsed and dried. The density was then raised to 2.0 g/cm<sup>3</sup>, and then to 2.4 g/cm<sup>3</sup>, with the floating material removed, rinsed, and dried at each step. The recovered material from each fraction was then homogenized, ground to pass through a 250 µm sieve, and analyzed for C content by elemental analysis (Costech Inc., CHN Analyzer).

Fractionation results were calculated as follows:

Multiplying the dry weight of each fraction as well as the entire sample (g) by % C (g C/g soil), divided by 100, resulting in g C in each fraction and in the bulk soil sample. The light fraction (< 1.8 g/mL) and the occluded fraction (< 1.8 g/mL after sonication) are then reported as a proportion of C expected by dividing the amount recovered in each fraction by the amount of C expected in the bulk sample. The mineral fractions (1.8 – 2.0 g/mL, 2.0 – 2.4 g/mL, and > 2.4 g/mL) are reported as a proportion of C recovered by the following:

- 1) The sum of the mineral fractions (g C recovered) was determined.
- 2) Relative distributions of each mineral fraction were computed by dividing the g C recovered in each fraction by the sum of the mineral fractions.
- 3) The g of C expected in the mineral fractions was determined by taking the difference of the expected total g C minus the g C recovered in the light and occluded fractions.
- 4) The relative distributions of each of the mineral fractions were then multiplied by the g of C expected in the mineral fractions from step (3).
- 5) The proportion of C recovered in the mineral fractions is computed by dividing the results of step (4) by the g C expected in the sample.

The calculations for the mineral fractions were performed in this manner due to the assumption that the mineral fractions had absorbed SPT during the fractionation

procedure, and despite multiple rinses, added to the mass (g soil) recovered in each of the fractions, resulting in a C loss/gain ranging from 42% loss to 31% gain. The SPT itself was tested for C, and was found to contain a negligible 0.005% C. The light and occluded fractions were reported on a basis of C expected, because it was assumed that they did not gain mass from SPT absorption, as this would have been observable. To correct for the mass gain in the mineral fractions, the relative distributions were used, assuming that SPT absorption was equally distributed among the three mineral fractions.

### **Statistical Analysis**

Geographic Information Systems software (Esri, 2012) was used to interpolate soil C stock values across and between the plots by kriging. Paired Student's t-tests were performed in Minitab (Minitab, Inc., 2012), to determine significant differences ( $\alpha = 0.10$ ) between C stock by land use, C stock present at different depths by land use, and C stock in fractions by land use. P values  $< 0.10$  are reported as significant, given the known heterogeneity of these systems.

## **Results**

### ***E. grandis* Above and Below Ground Biomass Carbon, Total Soil C**

Mean above ground (ABG) biomass of the *E. grandis* was  $111.94 \pm 8.27$  ton/ha (mean  $\pm$  1 standard error). Maximum ABG biomass of 134 ton/ha occurred in Plot 3 at a mid-elevation of 888 m, while minimum ABG biomass of 80 ton/ha was found at the highest elevation site (1319 m) in Plot 6. Mean BLG biomass was  $22.39 \pm 1.65$  ton/ha, and as BLG was calculated as a proportion of ABG, it followed the same elevational patterns. Mean ABG biomass C was  $57.22 \pm 4.22$  ton/ha, while mean BLG biomass C was  $10.07 \pm 0.74$  ton/ha (Fig. 3). Mean *E. grandis* soil C to 1 m was  $593 \pm 16.3$  ton/ha, almost ten times higher than that of ABG and BLG biomass C combined, as was hypothesized (Fig.3). This highlights both the large capacity of these soils to store C, as well as the importance the soil C pool plays in the overall C balance of the entire system.

Plots planted with *E. grandis* contained more soil C overall than plots in pasture, as was hypothesized (Fig.4a). In four of the six plots, *E. grandis* contained more soil C than did pasture (Fig. 4a). The mean pasture soil C stock to 1 m depth was  $516.8 \pm 21.1$ , significantly less than the mean *E. grandis* soil C stock of  $593 \pm 16.3$ , (Student's paired T-test, T-value = -3.13, p-value = 0.004; Fig. 4b).

### **Pasture vs. *E. grandis* Soil C by Depth**

Soil C decreased with depth in both the pasture and the *E. grandis*, with the steepest decline seen in the pasture from 18-36 cm depth (Fig. 5). This steep decline is most likely due to the majority of grass roots being present at the 0-18 cm depth. By depth increment, *E. grandis* plots contained more soil C on average than pasture plots, except in the surface core (Fig. 5). Again, this is most likely due to the root density of grasses being high at the surface. Soil C was significantly higher in the *E. grandis* mid-depth cores: 18-36 cm, 36-54 cm, and 54-72 cm (Table 2). Soil C density to 1m in the *E. grandis* plantation and the adjacent pasture plots was interpolated by kriging, displaying estimations of soil C at unsampled locations, based on measured values from the sampled plots (Fig. 6a-6f). The significant differences in soil C density become more and more evident as depth increases with *E. grandis* containing more soil C than the pasture in a majority of the pairs (Fig. 6a-6f).

Soil C was more evenly distributed by depth in the *E. grandis* than the pasture. In the *E. grandis*, 22% of the total C to 1m depth was found in the surface core, 20% in the 18-36 cm core, 18% in the 36-54 cm core, 16% in the 54-72 cm core, 13% in the 72-90 cm core, and 12% in the 90-100 cm core. In contrast, for the pasture, 27% of the total soil C to 1 m depth was found in the 18-36 cm core, decreasing to 16% in the 18-36 cm and 36-54 cm cores, 15% in the 54-72 cm core, 14% in the 72-90 cm core, and 13% in the 90-100 cm core.

### Relative Distribution of Soil C Among Pools

In the top 18 cm, the light fraction of the pasture contained significantly more C by proportion than that of the *E. grandis* ( $23.9 \pm 10.6\%$  vs.  $13.3 \pm 5.9\%$ ; Paired Student's T-test, T-value = 2.23, P-value = 0.076; Fig. 7a, 7b). The occluded fraction contained similar amounts of C by proportion, with the pasture containing  $8.24 \pm 1.96\%$ , and *E. grandis*  $9.47 \pm 5.16\%$ . In the 1.8-2.0 g/mL fraction, *E. grandis* soil C by proportion was  $28.23 \pm 6.21\%$ , significantly higher than the pasture at  $16.31 \pm 3.2\%$  (Paired Student's T-test, T-value = -2.08, P-value = 0.092). The 2.0-2.4 g/mL fraction contained similar proportions of soil C in the pasture ( $46.4 \pm 1.2\%$ ) and the *E. grandis* ( $45.5 \pm 1.25\%$ ). The proportion of the heaviest fraction soil C was also similar for the pasture ( $5.13 \pm 2.61\%$ ) and the *E. grandis* plantation ( $3.47 \pm 1.02\%$ ).

At mid-depth, the mean proportion of soil C in the light fraction was higher for the *E. grandis* plantation ( $15.7 \pm 7.11\%$ ) compared with the pasture ( $9.97 \pm 6.64\%$ ; Fig. 7c, 7d). The proportion of the occluded fraction of the *E. grandis* was significantly higher ( $4.53 \pm 0.81\%$ ) than in the pasture ( $2.90 \pm 1.21\%$ ; Paired Student's T-test, T-value = -2.65, P-value = 0.045). There was proportionally more soil C in the 1.8-2.0 g/mL fraction of the pasture ( $20.4 \pm 11.9\%$ ) than in the *E. grandis* ( $13.5 \pm 3.90\%$ ), though not significantly so. The heavier fractions contained similar proportions of soil C in the pasture (2.0-2.4 g/mL:  $64.3 \pm 14.8\%$ , >2.4 g/mL:  $2.42 \pm 1.07\%$ ) and *E. grandis* (2.0-2.4 g/mL:  $64.1 \pm 10.5\%$ , >2.4 g/mL:  $2.21 \pm 0.36\%$ ).

In the deepest core, no significant differences were found in the proportion of C by fraction between pasture and *E. grandis* (Fig. 7e, 7f). The light fraction of the pasture contained  $3.97 \pm 1.18\%$  of the total C by proportion, while the light fraction of the *E. grandis* contained  $4.02 \pm 1.10\%$ . The total C by proportion found in the occluded fraction of the pasture was  $2.02 \pm 0.79\%$ , similar to the  $2.84 \pm 1.18\%$  found in *E. grandis*. The pasture contained  $8.67 \pm 4.27\%$  of total C by proportion in the 1.8-2.0 g/mL fraction,  $79.46 \pm 4.02\%$  in the 2.0-2.4 g/mL fraction, and  $5.89 \pm 2.14\%$  in the >2.4 g/mL fraction. In *E. grandis*,  $10.32 \pm 2.99\%$  was found in the 1.8-2.0 g/mL fraction,  $76.27 \pm 3.5\%$  in the 2.0-2.4 g/mL fraction, and  $6.55 \pm 3.22\%$  in the >2.4 g/mL fraction. Thus, the distribution of soil C by proportion of total changed by depth, with more soil C found in the organic fractions in the surface and mid-depth cores of both the pasture and *E. grandis*, while at 1m depth, the majority of soil C was found in the mineral fractions.

### Soil C Stock by Fraction

In the 0-18 cm depth, there was slightly more soil C overall found in the pasture than in the *E. grandis* ( $139.07 \pm 6.08$  ton/ha vs.  $133.26 \pm 4.70$  ton/ha). While overall soil C was approximately equal, the distribution of soil C was significantly different in the light fraction, as well as in the 1.8-2.0 g/mL fraction (Fig. 8a, 8b). In the light fraction, the pasture contained significantly more soil C ( $34.2 \pm 14.9$  ton/ha) than did the *E.*

*grandis* ( $16.8 \pm 7.0$  ton/ha), as was hypothesized (T-value = 2.18, P-value = 0.081). Soil C present in the occluded fraction was quite similar between the two land uses, with the pasture containing  $11.31 \pm 2.52$  ton/ha and the *E. grandis* containing  $10.98 \pm 5.26$  ton/ha. In the 1.8-2.0 g/mL fraction, there was significantly more soil C present in the *E. grandis* ( $36.80 \pm 8.42$  ton/ha) than in the pasture ( $24.09 \pm 7.06$  ton/ha), (T-value = -2.18, P-value = 0.087). In the heavier two fractions, the pasture contained  $63.1 \pm 15.6$  ton/ha in the 2.0-2.4 g/mL fraction, and  $6.35 \pm 3.11$  ton/ha in the >2.4 g/mL fraction. In *E. grandis*,  $60.3 \pm 16.3$  ton/ha was found in the 2.0-2.4 g/mL fraction, and in the >2.4 g/mL fraction there was  $4.35 \pm 1.23$  ton/ha. Both the pasture and the *E. grandis* contained a majority of the soil C in the 2.0-2.4 g/mL fraction at this depth.

In the mid-depth cores, Student's paired T-test shows that *E. grandis* contained significantly more soil C overall than pasture ( $106.04 \pm 4.86$  ton/ha vs.  $83.98 \pm 5.05$  ton/ha; T-value = 2.88, P-value = 0.007; Fig.8c, 8d). In the pasture,  $10.53 \pm 7.71$  ton/ha was found in the light fraction, and  $2.83 \pm 1.48$  ton/ha was found in the occluded fraction. The *E. grandis* contained slightly more soil C in the light fraction at  $15.36 \pm 6.0$  ton/ha, as well as in the occluded fraction,  $4.64 \pm 0.63$  ton/ha. The 1.8-2.0 g/mL fraction of the pasture had  $20.0 \pm 12.6$  ton/ha of soil C, while the same fraction contained  $14.7 \pm 4.6$  ton/ha of soil C in the *E. grandis*. There was significantly more soil C stock in the 2.0-2.4 g/mL of the *E. grandis* ( $69.0 \pm 11.9$  ton/ha) than in the pasture ( $48.4 \pm 9.6$  ton/ha; T-value = -2.98, P-value = 0.031). The >2.4 g/mL fraction contained similar amounts of soil C, with *E. grandis* containing  $2.38 \pm 0.47$  ton/ha and pasture containing  $2.26 \pm 1.28$  ton/ha. Thus, there were differences found in both the overall C stock as well as the distribution of this stock between fractions at this depth.

In the deepest cores, both the C stock and the distribution among fractions were similar between pasture and *E. grandis* (Fig. 8e, 8f). Overall C stock was  $59.83 \pm 4.04$  ton/ha in the pasture, and  $63.87 \pm 6.08$  in *E. grandis*. In the light fraction of the pasture, there was  $2.24 \pm 0.73$  ton/ha of soil C present, and  $2.37 \pm 0.72$  ton/ha in the *E. grandis*. The occluded fraction contained  $1.25 \pm 0.59$  ton/ha in the pasture, and  $1.72 \pm 0.78$  in the *E. grandis*. The 1.8-2.0 g/mL fraction in the pasture had  $5.17 \pm 2.60$  ton/ha of soil C, while *E. grandis* contained  $5.94 \pm 1.70$  ton/ha. The soil C in these three fractions decreased considerably with depth, resulting in the majority of soil C being found in the 2.0-2.4 g/mL fraction in both pasture ( $43.29 \pm 2.97$  ton/ha) and *E. grandis* ( $42.43 \pm 1.64$  ton/ha). The heaviest fraction contained  $2.95 \pm 0.99$  ton/ha in the pasture, and  $3.57 \pm 1.79$  ton/ha in *E. grandis*.

### **Percent Change**

Overall, the land use change from pasture to *E. grandis* at this site resulted in a 14.7% gain in soil C (ton/ha) to 1m depth. In the top 18 cm, pasture contained 50.9% more soil C in the light, most easily decomposed labile fraction, while the *E. grandis* contained 52.8% more soil C in the 1.8-2.0 g/mL fraction. In terms of C sequestration, the light fraction is expected to turnover in < 5 years, while the 1.8-2.0 g/mL fraction will likely reside in the soil for over 100 years, suggesting that *E. grandis* not only contains more soil C in general, but that the soil C present in the *E. grandis* is preferentially stored in a longer term C pool. Further evidence of this is seen at mid-depth (36-54 cm),

wherein *E. grandis* contained 42.6% more soil C in the 2.0-2.4 g/mL fraction, which is expected to reside in the soil for 500+ years.

## Discussion

### Current C Resource within the Plantation

In the studied plantation, soil C stock in the top 1m in the *E. grandis* plantation was almost ten times greater than that present in the above and below ground biomass combined. Many studies consider only the C fixed into biomass through photosynthesis in the assessment of a renewable fuel production system, potentially ignoring a large C pool that could provide an overall C offset for the system (Perez-Garcia, 2005, Ragauskas, 2006). The large amount of soil C found in this study relative to the amount of biomass C highlights the importance of including soil C stock in any biofuel study, as even small changes to this large pool will affect the C budget of the entire system.

### Total Carbon Accumulation Following Land Use Change

As hypothesized, this study found that afforestation with *E. grandis* increased total soil C. Mean soil C to 1 m increased by approximately 15%, or by 76 ton/ha in 10 years. In contrast, Bashkin and Binkley, 1998, found that afforestation with *E. saligna* after sugar cane cultivation at a site also located on the northwestern part of the Island of Hawaii did not increase total soil C to 55cm depth after 10-15 years. They did find an increase of 11.5 ton/ha in soil C in the surface layer due to afforestation with *E. saligna*, however, it was offset by losses of sugar cane soil C in the subsurface soil. The different findings between the current study and that of Bashkin and Binkley, 1998, are likely explained by different effects of sugar cane cultivation vs. pasture as past land use, as the afforestation was by a very similar species of *Eucalyptus* for a similar time period. It may be that the 60+ years of sugar cane cultivation resulted in a loss of soil structure, limiting the soil's ability to retain C when afforestation did occur. Sugar cane cultivation and fertilization could potentially have adversely affected both physical properties of the soil, due to tillage and physical disturbance, as well as chemical properties of the soil due to frequent fertilization, resulting in a decrease in the potential C storage by the mechanisms of aggregation and organo-mineral bonding. In accordance with this study, a review of 30+ studies by Don, Schumacher and Freibauer reports an average gain in soil C of 12.4 ton/ha (to an average depth of 35 cm, average of 28 years), translating to an 18% increase with land use change from grassland to secondary forest in 28 years, or just under a 2% increase per year. As land use change often exerts a negative effect on the C balance of a biofuel production system, finding an increase in soil C with afforestation in this study was promising.

By depth, pasture contained slightly more soil C in the top 18 cm than did *E. grandis*, as was hypothesized. From 18-72 cm, there was significantly more soil C present in *E. grandis*. This may be explained by the differences in rooting depth and density of Kikuyu grass vs. *E. grandis*. Kikuyu grass has shallower roots and a higher root density at the surface, explaining the increase in soil C in the top 18 cm. In contrast, *E. grandis* roots are deeper, and their density does not decline as sharply with depth as do Kikuyu roots, so root exudate and dead root biomass C is potentially input more evenly along the soil profile from 18–72 cm. Alternatively, the increase in total soil C with depth could be due to transport of dissolved organic matter from surface layers to deeper

mineral horizons and sorption to mineral surfaces. There exists a difference in the chemistry of the dissolved organic matter between the two vegetation types, due to the difference in lignin content, as discussed below.

The lack of difference in total C stock at the lowest depth suggests that the differences detected at the more shallow depths captured the entire snapshot of change facilitated by the conversion of pasture to *E. grandis*.

Often, the effects of afforestation on soil C dynamics are investigated only in the surface soil (Paul et al., 2002, Binkley and Resh, 1999, Lima et al., 2006). This study found that 78% of the total soil C to 1m in the *E. grandis* was found below 18 cm, and 58% was found below 36 cm. These results suggest that attention should be paid not just to surface soil C dynamics, but also to changes that are occurring deeper in the soil profile, particularly if mean residence time increases with depth, as has been observed in other studies (Lal, 2004, Paul et al., 1995).

### **Quantifying Changes in Distribution of C among Soil Pools**

Soil under *E. grandis* accumulated C in fractions associated with longer turnover times, i.e., the physically protected and organo-mineral fractions. Gains in these fractions have more value for long-term C storage and greenhouse gas offsets, than do gains in the easily decomposed light fraction. In the surface core, there was more C found in the light fraction of the pasture soil than the *E. grandis*, and similar amounts found in the occluded fractions. In contrast, Lima et al., (2006) found a linear increase in soil C in the light and occluded fractions of the top 20cm of soil with land use change from pasture to *Eucalyptus*. In the present study, it was also found that there was more soil C present in the surface soil of *E. grandis* in the 1.8-2.0 g/mL fraction than that same fraction in the pasture, suggesting that soil C is being preferentially sequestered into a fraction associated with a longer turnover time in the *E. grandis* soil. Additionally, at mid-depth, *E. grandis* contained significantly more soil C in the 2.0-2.4 g/mL fraction than did the pasture, a fraction associated with a turnover time of 500+ years, again indicating that the soil C present in *E. grandis* is sequestered for a longer time period. Although the soil fractionation process is not directly comparable, Del Galdo et al. (2003) also found that afforestation caused a higher proportion of soil C in the surface 30 cm to be sequestered into the more stable microaggregate silt and clay fractions, in accordance with this study.

The differences between root depth and density do not fully explain the increase in overall soil C in the *E. grandis* vs. the pasture, nor the increase in soil C found in fractions associated with longer turnover times in the *E. grandis*. As these sites were constrained by climate and soil series, the large difference is the change in vegetation for the past decade. This change undoubtedly affects root biomass as well as litter quantity and quality. The *E. grandis* has considerably more soil C inputs from litter than the pasture, as the *E. grandis* forest floor is undisturbed, allowing all litter to eventually be incorporated into the soil, whereas the pasture is grazed grassland with very few litter inputs into the soil. It has been found that root litter contributes a larger portion of total soil C inputs in grasslands than in forests (Kogel-Knabner, 2002).

There is a difference in the chemistry of the dissolved organic C in the pasture and the *E. grandis* that could explain differences in SOC storage and quality. Kikuyu grass has a lignin content of approximately 6% (Marais, 2001, Vasquez et al., 2011), whereas *E. grandis* has a much higher lignin content of approximately 30% (Chaves Arantes et al., 2011, Rodrigues et al., 1998, Morais et al., 1991). Kramer et al., 2012,

found that in andic soils, such as the soils present in this study, dissolved organic matter composed of aromatic acids chemically binds to short range ordered minerals, and it is this specific organo-mineral binding process that is primarily responsible for long-lasting C storage. The origin of this dissolved organic matter are compounds high in lignin from litter leachate (Kramer et al., 2012). As *E. grandis* contains a much higher percentage of lignin than does Kikuyu grass, and litterfall rates are greater in *E. grandis*, it follows that the overall increase in soil C, and particularly the increase in the more stable fractions, is very likely driven by this process.

The results of the fractionation may help to explain which portion of the gains in soil C in the *E. grandis* are due to differences in rooting depth and density or differences in litter quantity and quality. In the top 18 cm, more light fraction C was found in the pasture than in the *E. grandis*. As the light fraction is comprised mainly of slightly decomposed roots, this gain is likely due to root biomass being higher at the surface in the pasture than in the *E. grandis*. However, the gain of soil C the 1.8-2.0 g/mL fraction of the surface soil in the *E. grandis* is possibly due to litter leachate high in lignin content being preferentially sorbed to the mineral portions of the soil. It is very likely that the significant gain in soil C present in the 2.0-2.4 g/mL fraction of the *E. grandis* at mid-depth is due to that sorption process, and not explained directly by differences in root density or depth. However, the slight increase in the light and occluded fraction of the *E. grandis* at mid-depth is likely due to the greater rooting depth of *E. grandis*, as accumulations in these fractions are from newly incorporated organic matter, such as root exudates and mortality.

The *E. grandis* may have gained more soil C than was measured by this study. Tillage is suspected to cause a loss in soil C, due to disturbance to physical structure of aggregates and loss of organic matter (Six et al., 2002b, Rhoton et al., 2002) Since the *E. grandis* plantation was ripped and ridged before planting, it is probable that soil C was lost during this process, and was subsequently regained in the past decade of undisturbed growth. Since *E. grandis* can regrow by coppicing after harvest, tillage would not be necessary in the second rotation. Future studies of the amount of total soil C and the amount present in fractions after harvest and after a second rotation would certainly add to the body of knowledge concerning the effect on soil C of growing *E. grandis* as a biofuel feedstock.

Rockwood et al. (2008) suggested that the use of *Eucalyptus* as a biofuel is probable in the tropics, particularly when *Eucalyptus* is already growing on land not being used for agriculture and where its use would not cause “environmental impact.” To assess environmental impact of a biofuel system, the effects on the soil C pool need to be taken into account. Some studies have attempted to do so; however, they are often limited by a lack of data, which was the case in a study by Langholtz et al., (2009). Other studies simply acknowledge that land use change and associated loss of soil C are important aspects to consider in the production of biofuel, but do not attempt to quantify this step in the production system (Simmons et al., 2008). The results from this study agree with the conclusions of Fargione et al., (2008): for biofuel production to reach the goal of emissions reduction when compared to that of fossil fuels, the biofuel production system with the least effect on the soil C pool should be chosen.

## Conclusion

The results from this study suggest that the C offset from soil sequestration could be quite significant and could tip the balance in the overall assessment of the entire biofuel production system. Future management decisions should consider this potential offset. The method of harvesting chosen should be one that preserves the soil structure, and does not induce loss of soil C. For example, leaving residue on the soil surface during harvest has been found to increase soil C, while residue removal resulted in a decrease in soil C (Johnson and Curtis, 2001, Chen and Xu, 2005). Another potential benefit of growing *E. grandis* for biofuel is that the next rotation can be grown by coppicing, as opposed to tilling and re-planting, resulting in the preservation of the present soil C stock and possibly additional accumulation into the soil C pool.

This study demonstrated that a land use change from pasture to *E. grandis* resulted in a large overall gain of soil C, with much of this gain occurring in the longest lived soil C fractions. At the surface depth, there was an accumulation of 12 ton/ha in the 1.8-2.0 g/mL fraction of the *E. grandis* soil compared to the same fraction in the pasture soil. This gain is more than the mean BLG (root) biomass of 10 ton/ha found in the plantation. Fine root turnover time likely ranges from 3-18 years (Gaudinski, et al., 2001), while the 1.8-2.0 g/mL fraction has a turnover time of 100-200 years, demonstrating that soil C dynamics are at least as, and possibly more important than, BLG biomass in terms of C sequestration, and should be quantified in any C accounting model or life cycle analysis. At mid-depth, *E. grandis* contained 21 ton/ha more soil C in the 2.0-2.4 g/mL fraction than did pasture. In a plantation of 3000 ha, this gain is equal to 63,000 tons of C, or an additional 568 ha of ABG biomass, and unlike the ABG, which will be harvested, it will remain as a C offset to the system for hundreds of years. Alternatively, the gain in the 2.0-2.4 g/mL fraction in the *E. grandis* could be thought of as a gain approximately equal to the C emissions produced by the burning of 7,800 gallons of gasoline (U.S. EPA, 2012). It is apparent that the inclusion of the soil C stock in the overall C balance will provide a C offset to the entire biofuel production system.

## APPENDIX 1. TABLES

Table 1. Plot pair labels, elevation (m), mean annual temperature (MAT), (°C), mean annual precipitation (MAP), (mm), soil series, and taxonomic description of soil series.

Plot #	Elevation (m)	MAT (C)	MAP (mm)	Soil Series	Taxonomic Description	<i>E. grandis</i> planting date (year)
Euc 6/Pas 6	1319/1333	14	1650	Punohu	Medial, ferrihydritic, isothermic Hydric Hapludands	2002
Euc 5/Pas 5	1239/1246	14	1650	Punohu	See above	2001
Euc 4/Pas 4	1102/1092	14	1905	Maile	Hydrous, ferrihydritic, isomesic Acrudoxic Hydrudands	2001
Euc 3/Pas 3	888/986	19	3175	Border of Maile and Honokaa	See above and below	2001
Euc 2/Pas 2	877/823	19	3175	Honokaa	Hydrous, ferrihydritic, isothermic Acrudoxic Hydrudands	2004
Euc 1/Pas 1	609/655	19	3175	Honokaa	See above	2004

Table 2. Mean soil C by depth in *E. grandis* and pasture. Significant differences ( $P < 0.05$ ) were found in the 18-36 cm, 36-54 cm, and 54-72 cm cores (shown in bold).

Depth (cm)	Pasture Mean Soil C (ton/ha)	<i>E. grandis</i> Mean Soil C (ton/ha)	T-Value	P-Value
0-18	139.07 ± 6.08	133.26 ± 4.70	-0.960	0.344
<b>18-36</b>	83.71 ± 4.23	117.79 ± 4.17	6.260	<b>0.000</b>
<b>36-54</b>	83.98 ± 5.05	106.04 ± 4.86	2.880	<b>0.007</b>
<b>54-72</b>	80.05 ± 4.98	95.24 ± 4.27	2.870	<b>0.008</b>
72-90	69.98 ± 4.92	76.44 ± 4.38	0.950	0.348
90-100	59.83 ± 4.04	63.87 ± 6.08	0.630	0.536

## APPENDIX 2. FIGURES

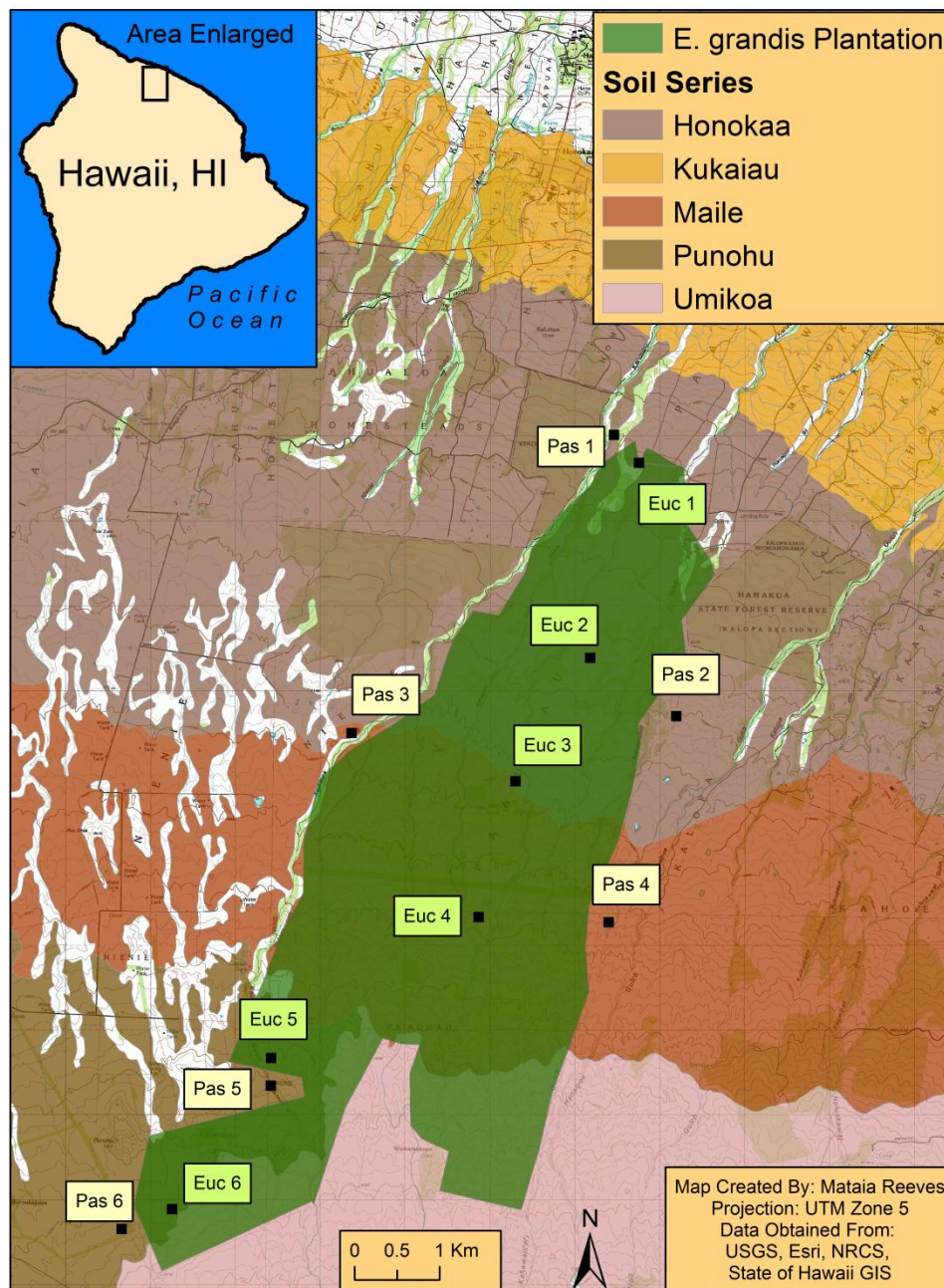


Fig. 1. Site map of study area located on the Island of Hawaii, displaying paired plots: Euc denotes *E. grandis* plots, and Pas denotes pasture plots. Green boundary encloses the *E. grandis* plantation. Soil series are displayed in colors described in legend.

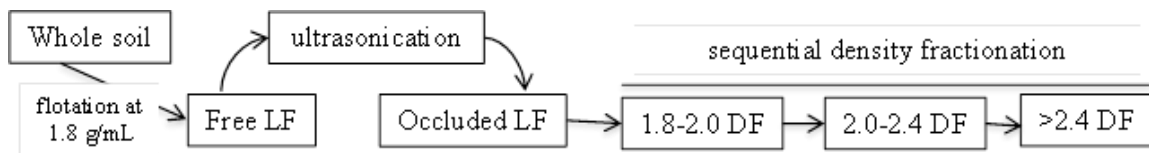


Fig. 2. Optimized soil fractionation method, specific for the soils present in this study. This method separates the soil into five fractions: the light fraction ( $<1.8$  g/mL), the occluded light fraction ( $<1.8$  g/mL after ultrasonic disruption with  $\approx 150$  J/mL), and three sequential density fractions (1.8–2.0 g/mL, 2.0–2.4 g/mL, and  $>2.4$  g/mL).

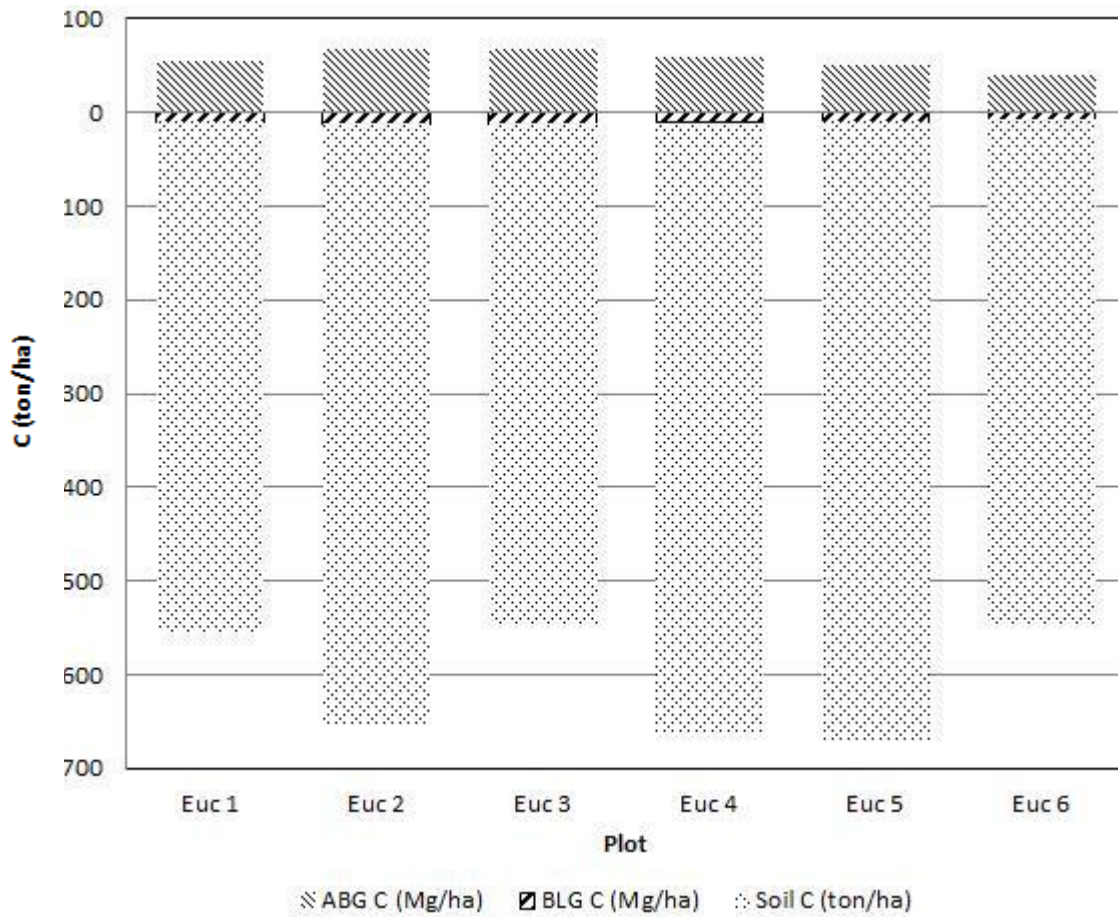


Fig. 3. Above ground C (ton/ha), below ground C (ton/ha), and soil C (ton/ha) in the *E. grandis* plantation. Each bar represents one of the *E. grandis* plots.

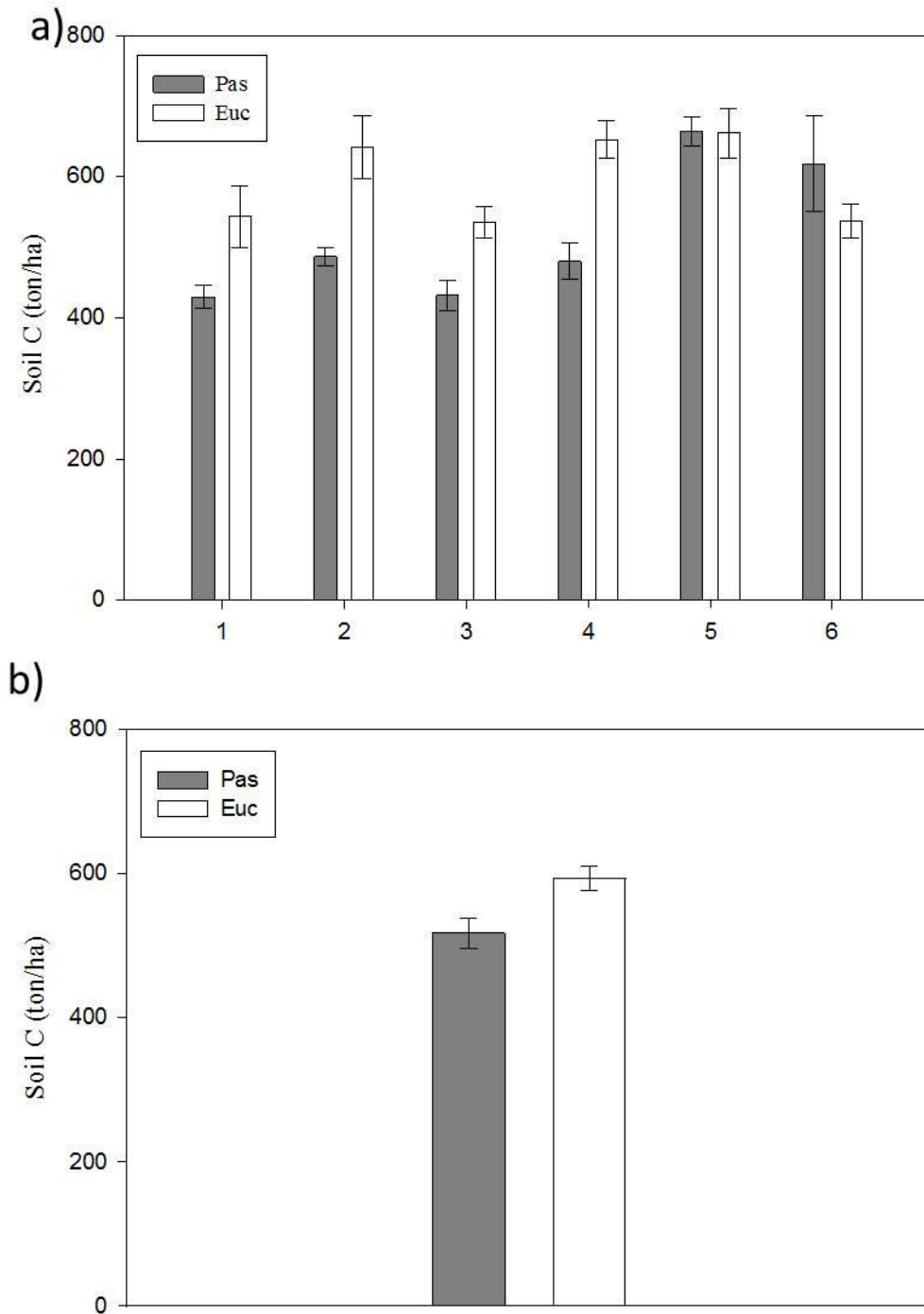


Fig. 4. Soil C (ton/ha) to 1m depth. (a) Soil C (ton/ha) to 1m depth in the *E. grandis* and pasture pairs. (b) Mean soil C (ton/ha) to 1m depth in pasture and *E. grandis*, different letters represent significant differences ( $P \leq 0.05$ ). Error bars display one standard error.

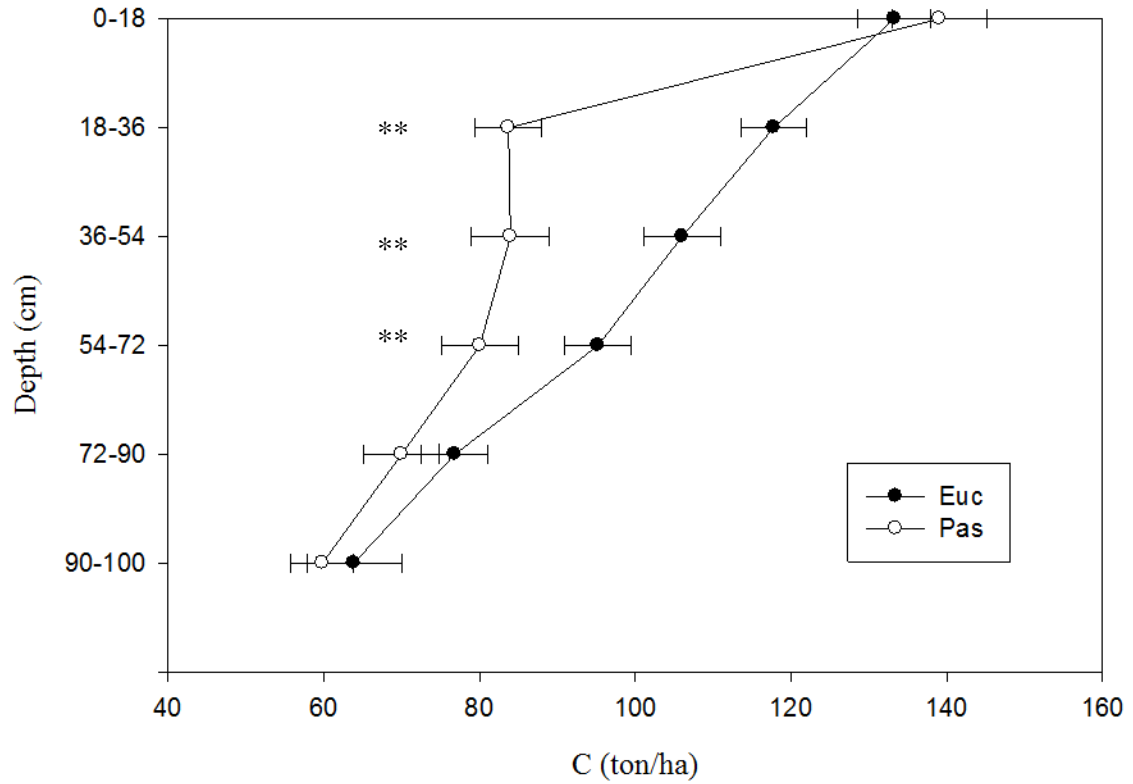
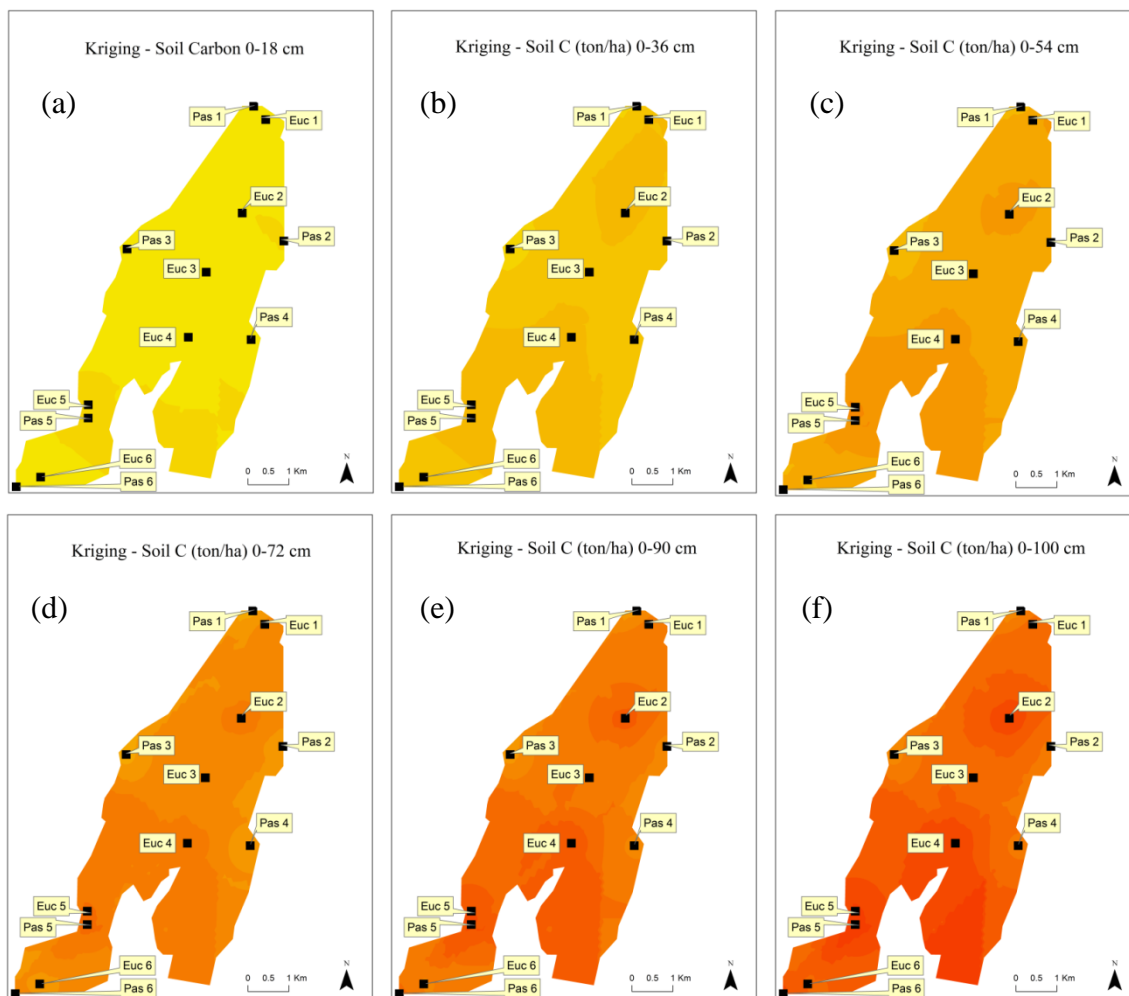


Fig. 5. Mean soil C (ton/ha) by depth (cm) to 1m in *E. grandis* and pasture. \*\*denote significant differences ( $P < 0.05$ ).



### Soil C (ton/ha)

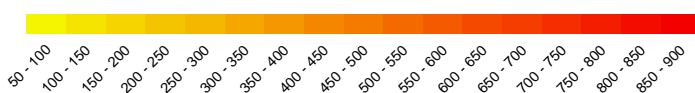


Fig. 6. Soil C (ton/ha) density by kriging in the *E. grandis* and adjacent pasture plots, displaying estimations of soil C at unsampled locations within and between the sampled locations. Soil C density is displayed by increasing depth in the series of figures: (a) 18 cm (b) 36 cm (c) 54 cm (d) 72 cm (e) 90 cm (f) 1m. (g) Map showing area for kriging (a-f), dark line denotes *E. grandis* plantation boundary.



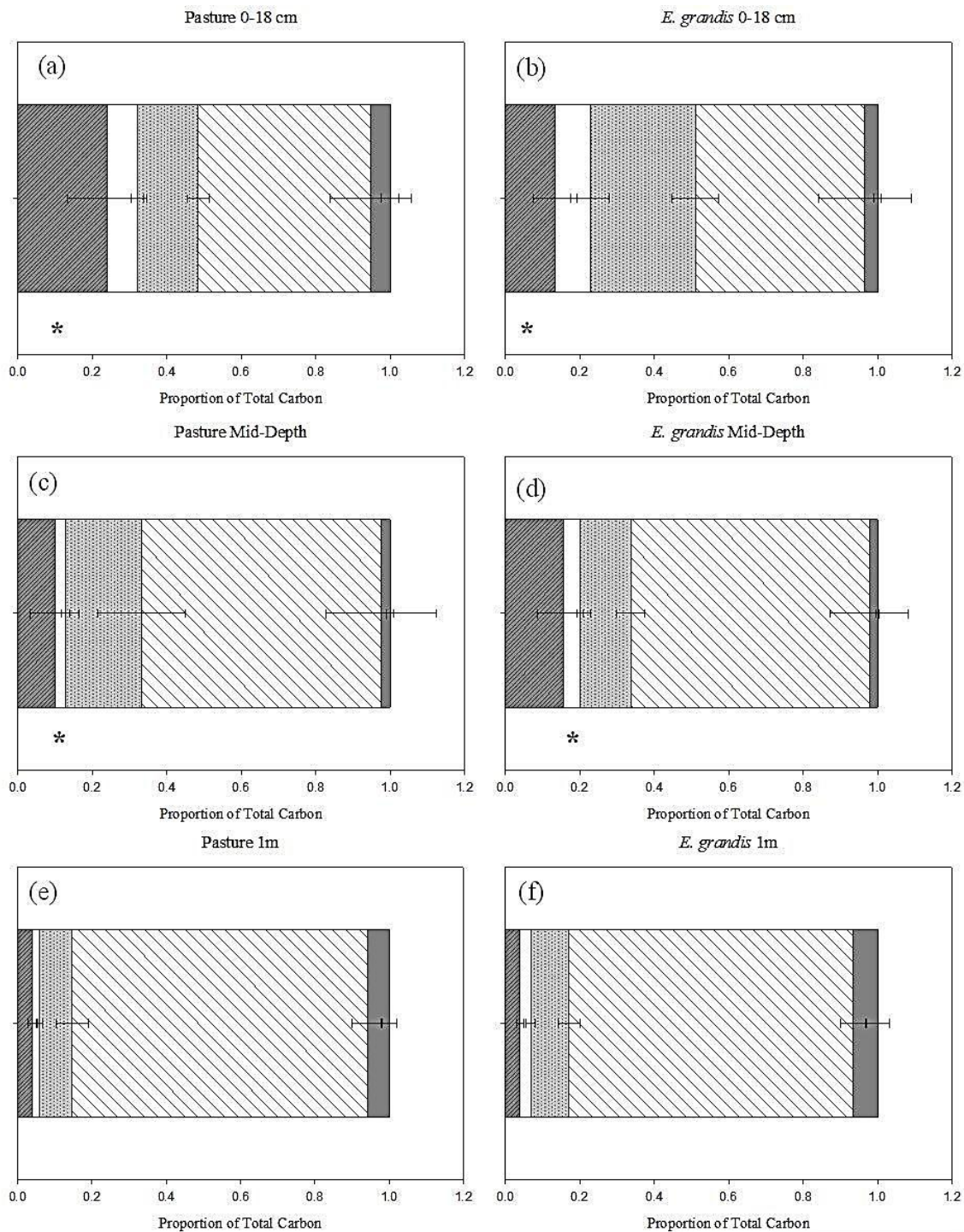
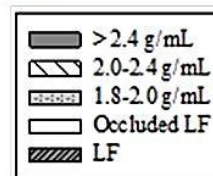


Fig. 7. C recovered in each fraction as % of total C in pasture and *E. grandis* soils at 3 depths. (a) Pasture 0-18 cm (b) *E. grandis* 0-18 cm (c) Pasture 36-54 cm (d) *E. grandis* 36-54 cm (e) Pasture 1m (f) *E. grandis* 1m. \* denotes significant differences (P < 0.10).



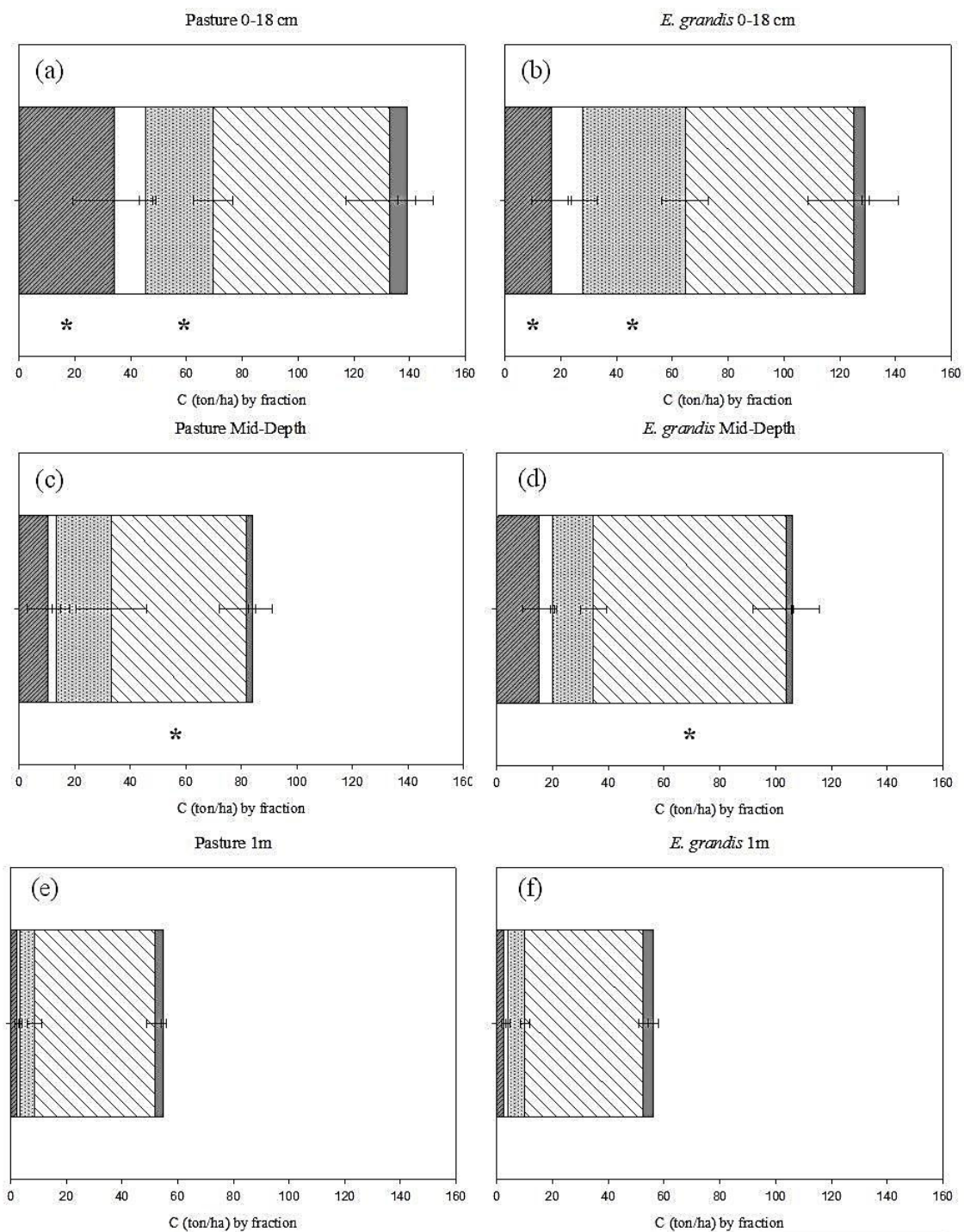
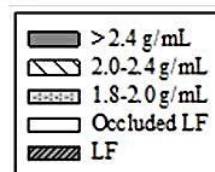


Fig. 8. C stock (ton/ha) by fraction in pasture and *E. grandis* at 3 depths. (a) Pasture 0-18 cm (b) *E. grandis* 0-18 cm (c) Pasture 36-54 cm (d) *E. grandis* 36-54 cm (e) Pasture 1m (f) *E. grandis* 1m. \*denotes significant differences ( $P < 0.10$ ).



### APPENDIX 3. SAMPLING DESIGN AND SEMIVARIOGRAM

To inform sampling design, a large number of surface (0-30 cm) soil cores were taken in the lowest elevation *E. grandis* plot, Euc 1. This was done to discover in-plot variability and spatial dependence of soil C stock in the surface soil, where the greatest variability was expected. Forty-nine of a total of 54 samples were taken on a grid design, every three meters. The other five samples were taken at random points within the plot (Russell Yost, 2011, personal communication). These samples were dried at 105 °C, ground and homogenized, sub-sampled, and analyzed for C content. In order to assess spatial dependence, and sampling distance for the one meter cores, a semivariogram was constructed from this data (Fig. A-1) (R, 2011). The semivariogram indicated that at distances over 10 m, the samples are behaving independently. Thus, it was determined that five-one meter deep soil cores (one at each corner and one in the middle of the plot) would capture a majority of the spatial variability.

#### APPENDIX 4. MASS BALANCE, SOIL FRACTIONATION

Mass balance was computed from the 65°C dry weights in each fraction. The sum of these was recorded as total recovered for each sample. Moist soil weight of the entire sample was multiplied by a moisture factor (dry/moist) to compute 65° C dry weight equivalent. Percent recovery is the result of the total recovered divided by the dry weight equivalent of the beginning sample mass.

Mass balance percent recovery ranged from 94-234% for the pasture, and from 102–272% for *E. grandis*. These high mass recoveries were likely due to SPT binding to mineral surfaces of the mineral fractions (1.8-2.0 g/mL, 2.0-2.4 g/mL, and >2.4 g/mL) after sonication, despite multiple rinses with deionized water. For this reason, mass balance is presented as a proportion of mass recovered in each fraction.

Figures A-2a and A-2b show a comparison of mass recovered in each fraction in the 0-18 cm depth. It was found that the pasture contained  $17.7 \pm 11\%$  of total mass in the light fraction, while *E. grandis* contained less at  $9.6 \pm 5.3\%$ . In the pasture,  $5.41 \pm 1.99\%$  of the mass was found in the occluded fraction vs.  $7.42 \pm 5.05\%$  in the occluded fraction of the *E. grandis*, indicating that *E. grandis* contained slightly more organic matter by mass in the aggregate protected fraction than did the pasture. In the mineral fractions, the pasture soil was composed of  $14.8 \pm 3.73\%$  in the 1.8-2.0 g/mL fraction,  $54.1 \pm 12.2\%$  in the 2.0-2.4 g/mL fraction, and  $7.97 \pm 3.11\%$  in the >2.4 g/mL fraction. The mineral fractions of *E. grandis* soil were distributed as follows:  $24.59 \pm 6.59\%$  in the 1.8-2.0 g/mL fraction,  $52.3 \pm 6.59\%$  in the 2.0-2.4 g/mL fraction, and  $6.12 \pm 1.64\%$  in the >2.4 g/mL fraction. Both soils contained a majority of the mass in the 2.0-2.4 g/mL fraction. The increase observed in the 1.8-2.0 g/mL fraction of *E. grandis* (9.79%) is likely due to a transfer from the light fraction pool to a more minerally protected pool, as the mass present in the 2.0-2.4 g/mL and the >2.4 g/mL pool is approximately equal in pasture and *E. grandis*.

In the mid-depth cores, both the pasture and *E. grandis* contained less light and occluded fraction by mass compared to the surface depth (Fig.A-2c, A-2d). This would be expected because there are less direct inputs to soil from fresh plant material available for incorporation into the soil at mid-depth vs. the surface. Interestingly, the *E. grandis* contained more mass in the light fraction,  $6.91 \pm 3.40\%$  than did the pasture,  $4.50 \pm 3.50\%$ . It is probable that this is due to a difference in rooting density between the two vegetation types, although that was not measured in this study. The mass recovered in the occluded fraction was similar in *E. grandis* ( $1.48 \pm 0.42\%$ ) and pasture ( $1.11 \pm 0.60\%$ ). In the 1.8-2.0 fraction, the pasture contained more mass by proportion ( $17.8 \pm 12.0\%$ ) than did the *E. grandis* ( $10.1 \pm 3.7\%$ ). The majority of the mass for both pasture ( $73.1 \pm 13.6\%$ ) and *E. grandis* ( $75.8 \pm 9.2\%$ ) was found in the 2.0-2.4 g/mL fraction, as it was in the surface cores. The >2.4 g/mL fraction of the pasture contained  $3.56 \pm 1.05\%$  of the mass recovered, while in the *E. grandis*, this fraction comprised  $5.66 \pm 2.52\%$ .

For the deepest core, that capturing the 1m depth, the mass recovered in the light, occluded and 1.8-2.0 fractions decreased compared to the mid-depth and surface cores (Fig.A-2e,A-2f). In the pasture,  $1.72 \pm 0.97\%$  was contained in the light fraction,  $0.48 \pm 0.25\%$  in the occluded and  $5.34 \pm 2.69\%$  in the 1.8-2.0 g/mL fraction. For *E. grandis*,  $1.30 \pm 0.44\%$  was found in the light fraction,  $0.97 \pm 0.41\%$  in the occluded and  $7.69 \pm$

3.06% in the 1.8-2.0 g/mL fraction. A majority of the mass was again found in the 2.0-2.4 g/mL fraction for both the pasture ( $84.1 \pm 3.47\%$ ) and the *E. grandis* ( $81.51 \pm 3.54\%$ ), as with the other depths. The proportion of the mass found in the >2.4 g/mL fraction was highest at this depth for both pasture ( $8.36 \pm 2.65\%$ ) and *E. grandis* ( $8.53 \pm 3.13$ ). The decrease in the mass recovered in the organic fractions coupled with the increase in the heavier, more passive mineral fractions in both the pasture and the *E. grandis* suggests that the 1m depth core captured sub-surface soil dynamics, where root exudates and fresh inputs were not having a significant influence.

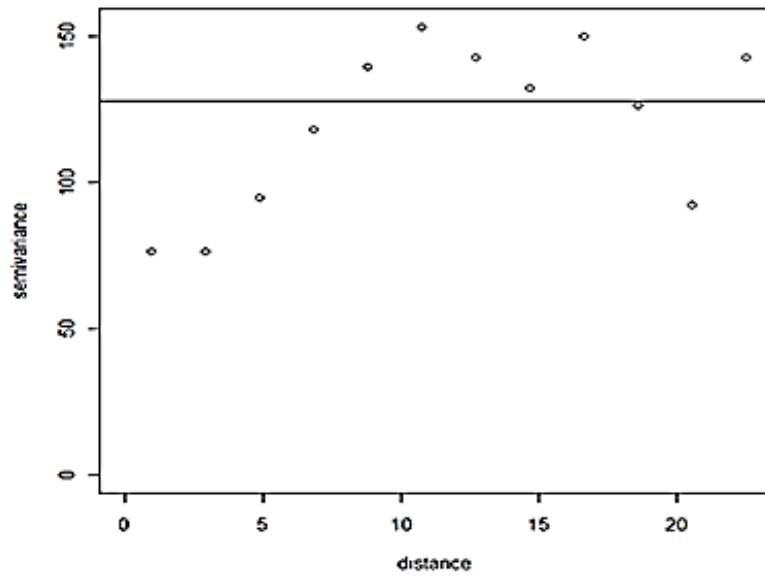


Fig. A-1. Semivariogram constructed from 54 surface samples in plot Euc 1. The range at which the semivariance reaches the sill (10m) portrays sample independence.

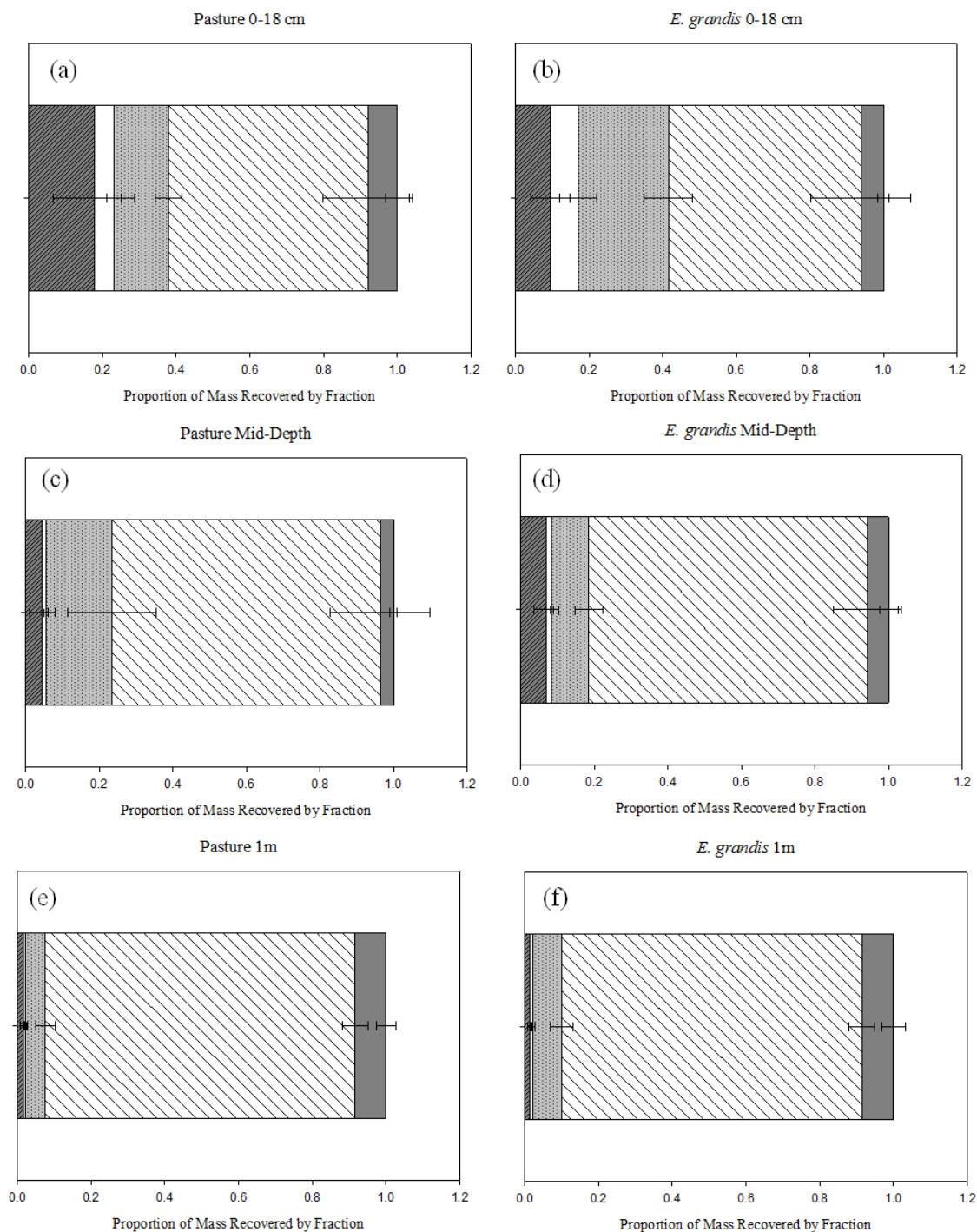
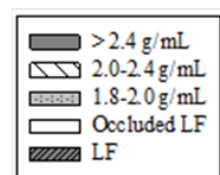


Fig. A-2. Proportion of mass recovered in each fraction as % of total mass recovered in pasture and *E. grandis* soils at 3 depths. (a) Pasture 0-18 cm (b) *E. grandis* 0-18 cm (c) Pasture 36-54 cm (d) *E. grandis* 36-54 cm (e) Pasture 1m (f) *E. grandis* 1m.



## REFERENCES

- Bashkin, M.A., & Binkley, D. (1998). Changes in soil carbon following afforestation in Hawaii. *Ecology*, 79(3), 828-833.
- Blackmore, M. & Vitousek, P.M. (2000). Cattle Grazing, Forest Loss, and Fuel Loading in a Dry Forest Ecosystem at Pu'u Wa'aWa'a Ranch, Hawai'i. *Biotropica*, 32(4), 625-632.
- Chaves Arantes, M.D., Trugilho, P.F., Tarcísio Lima, J., Oliveira Carneiro, A.C., Alves, E., & Guerreiro, M.C. (2011). Longitudinal and radial variation of extractives and total lignin contents in a clone of *Eucalyptus grandis* W.Hill ex Maiden x *Eucalyptus urophylla* S. T. Blake. *Cerne*, 17, 283-291.
- Chen, C.R., & Xu, Z.H. (2005). Soil carbon and nitrogen pools and microbial properties in a 6-year-old slash pine plantation of subtropical Australia: impacts of harvest residue management. *Forest Ecology and Management*, 206 (1-3), 237-247.
- Cherubini, F., Bird, N.D., Cowie, A., Jungmeir, G., Schlamadinger, B., & Woess-Gallash, S. (2009). Energy- and greenhouse gas-based LCA of biofuel and bioenergy systems: Key issues, ranges and recommendations. *Resources, Conservation and Recycling* 53, 434-447.
- Commonwealth of Australia, 1999, Greenhouse Challenge Vegetation Sinks Workbook, Quantifying Carbon Sequestration in Vegetation Management Projects, Version 1.0, Canberra.
- Del Galdo, I., Six, J., Peressotti, A., & Cotrufo, M.F. (2003). Assessing the impact of land-use change on soil C sequestration in agricultural soils by means of organic matter fractionation and stable C isotopes. *Global Change Biology*, 9, 1204-1213.
- Don, A., Schumacher, J. & Freibauer, A. (2011) Impact of tropical land-use change on soil organic carbon stocks – a meta-analysis. *Global Change Biology*, 17, 1658-1670.
- Dudley, N.S. & Fownes, J.H. (1991). Preliminary biomass equations for eight species of fast-growing tropical trees. *Journal of Tropical Forest Science*, 5(1) 68-73.
- ESRI 2011. ArcGIS Desktop: Release 10. Redlands, CA: Environmental Systems Research Institute. [Software]
- Fargione, J., Hill, J., Tilman, D., Polasky, S., & Hawthorne, P. (2008). Land clearing and the biofuel carbon debt. *Science*, 319, 1235-1238.
- Gaudinski, J.B., Trumbore, S.E., Davidson, E.A., Cook, A.C, Markewitz, D., & Richter, D.D. (2001). The age of fine-root carbon in three forests of the eastern United States measured by radiocarbon. *Oecologia*, 129, 420-429.

- Giardina, C.P., & Ryan, M.G. (2002). Total Belowground Carbon Allocation in a Fast-growing *Eucalyptus* Plantation Estimated Using a Carbon Balance Approach. *Ecosystems*, 5, 487-499.
- Golchin, A., Oades, J.M., Skjemstad, J.O., & Clarke, P. (1994). Study of free and occluded particulate organic matter in soils by solid state  $^{13}\text{C}$  CP/MAS NMR spectroscopy and scanning electron microscopy. *Australian Journal of Soil Research*, 32, 285-309.
- Hawaiian Electric Company. (2008) *Future Energy Agreement*. Honolulu, HI. Retrieved from:  
[http://www.heco.com/vcmcontent/StaticFiles/pdf/STATE\\_HECO\\_Energy\\_Future\\_Agreement\\_10\\_20\\_08.pdf](http://www.heco.com/vcmcontent/StaticFiles/pdf/STATE_HECO_Energy_Future_Agreement_10_20_08.pdf)
- Hawaiian Electric Company. (2012) *Latest Clean Energy News, Biofuel Activities as of May 2012*. Honolulu, HI. Retrieved from: <http://www.heco.com/portal/site/heco>
- Hawaii Clean Energy Initiative. (2012). *Hawaii Clean Energy Initiative*. Honolulu, HI. Retrieved from: <http://www.hawaiiicleanenergyinitiative.org/>
- Hinchee, M., Rottmann, W., Mullinax, L., Zhang, C., Chang, S., Cunningham, M., Pearson, L., & Nehra, N. (2009). Short-rotation woody crops for bioenergy and biofuels applications. *In Vitro Cellular and Developmental Biology*, 45:6, 619–629.
- Jackson, R.B., Canadell, J., Ehlenringer, J.R., Mooney, H.A., Sala, O.E., & Schulze, E.D. (1996). A global analysis of root distributions for terrestrial biomes. *Oecologia*, 108, 389-411.
- Jobbagy, E.G., & Jackson, R.B. (2000). The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications*, 10(2), 423-436.
- Johnson, D.W., & Curtis, P.S. (2001) Effects of forest management on soil C and N storage: meta-analysis. *Forest Ecology and Management*, 140, 227-238.
- Kaye, J.P., Resh, S.C., Kaye, M.W. & Chimner, R.A. (2000). Nutrient and carbon dynamics in a replacement series of *Eucalyptus* and *Albizia* trees. *Ecology*, 81:12, 3267-3273.
- Kleber, M., Sollins, P. & Sutton, R. (2007). A conceptual model of organo-mineral interactions in soils: self-assembly of organic molecular fragments into zonal structures on mineral surfaces. *Biogeochemistry*, 85, 9-24
- Kleber, M. (2010). What is recalcitrant soil organic matter? *Environmental Chemistry*, 7(4), 320-332.

Kögel-Knabner, I., Guggenberger, G., Kleber, M., Kandeler, E., Kalbitz, K., Scheu, S., Eusterhues, K. & Leinweber, P. (2008). Organo-mineral associations in temperate soils: Integrating biology, mineralogy, and organic matter chemistry. *Journal of Plant Nutrition and Soil Science*, 171, 61–82.

Kramer, M., Sanderman, J., Chadwick, O., Chorover, J., & Vitousek, P.M. (2012). Long-term carbon storage through retention of dissolved aromatic acids by reactive particles in soil. *Global Change Biology*, 18, 2594–2605.

Lal, R. (2004). Soil carbon sequestration to mitigate climate change. *Geoderma*, 123, 1-22.

Langholtz, M., Carter, D.R., Rockwood, D.L., & Alavalapati, J.R.R. (2009). The influence of CO<sub>2</sub> mitigation incentives on profitability of eucalyptus production on clay settling areas in Florida. *Biomass and Bioenergy*, 33, 785-792.

Lima, A.M.N., Silva, I.R., Neves, J.C.L., Novais, R.F., Barros, N.F., Mendonca, E.S., Smyth, T.J., Moreira, M.S., & Leite, F.P. (2006). Soil organic carbon dynamics following afforestation of degraded pastures with eucalyptus in southeastern Brazil. *Forest Ecology and Management*, 235, (1-3), 219-231.

Lorenz, K. and R. Lal. (2005). The depth distribution of soil organic carbon in relation to land use and management and the potential of carbon sequestration in subsoil horizons. *Advances in Agronomy*, 88, 35-66.

Marais, J.P. (2001). Factors affecting the nutritive value of Kikuyu grass (*Pennisetum clandestinum*) – A review. *Tropical Grasslands*, 35, 65-84.

Martens, D.A., Reedy, T.E., & Lewis, D.T. (2003). Soil organic carbon content and composition of 130-year crop, pasture and forest land-use managements. *Agronomy -- Faculty Publications*. Paper 124. Retrieved from: <http://digitalcommons.unl.edu/agronomyfacpub/124>

Mikutta, R., Kleber, M., Torn, M.S., & Jahn, R. (2006). Stabilization of soil organic matter: association with minerals or chemical recalcitrance? *Biogeochemistry*, 77, 25–56.

Minitab Statistical Software (2012). (Version 16) [Software]. Available from <http://www.mintab.com>.

Misra, R.K., Turnbull, C.R.A., Cromer, R.N., Gibbons, A.K., & LaSala, A.V. (1998). Below- and above-ground growth of *Eucalyptus nitens* in a young plantation, I. Biomass. *Forest Ecology and Management*, 106, 283–293.

Mittika, M. (2004). Global biomass fuel resources. *Biomass and Bioenergy*, 27, 613–620.

- Motooka, P., Castro, L., Nelson, D., Nagai, G., & Ching, L. (2003) Weeds of Hawaii's pastures and natural areas. CTAHR Publications and Information Office, Honolulu, HI
- Morais, S.A.L., Nascimento, E.A. & Pilo-Veloso, D. (1991). Studies of *Eucalyptus grandis*. Part I: Estimation of lignin and polyphenols content in *Eucalyptus grandis* by infrared spectroscopy. *Journal of Brazilian Chemical Society*, 2, 129-132.
- Nie, Z.N., Miller, S., Moore, G.A., Hackney, B.F., Boschma, S.P., Reed, K.F.M., Mitchell, M., Albertsen, T.O., Clark, S., Craig, A.D., Kearney, G., Li, G.D., & Dear, B.S. (2008). Field evaluation of perennial grasses and herbs in southern Australia. 2. Persistence, root characteristics and summer activity. *Australian Journal of Experimental Agriculture*, 48, 424-435.
- Paul, E.A., Follett, R.F., Leavitt, S.W., Halvorson, A., Peterson, G.A., & Lyon, D.J. (1995). Radiocarbon dating for determination of soil organic matter pool sizes and dynamics. *Soil Science Society of America Journal*, 61(4), 1058-1067.
- Paul, K.I., Polglase, P.J., Nyakuengama, J.G., & Khanna, P.K. (2002). Change in soil carbon following afforestation. *Forest Ecology and Management* 168, 241-257.
- Perez-Garcia, J., Lippke, B., Comnick, J. & Manriquez, C. (2005). An assessment of carbon pools, storage and wood products substitution using life-cycle analysis results. *Wood and Fiber Science*, 37, 140-148.
- Plevin, R.J., O'hare, M., Jones, A.D., Torn, M.S. & Gibbs, H.K. (2010). Greenhouse gas emission from biofuels' indirect land use change are uncertain but may be much greater than previously estimated. *Environmental Science and Technology*, 44, 8015-8021.
- Post, W.M. & Kwon, K.C. (2000). Soil Carbon Sequestration and Land-Use Change: Processes and Potential. *Global Change Biology*, 6, 317-328.
- Poteet, M. (2006). Biodiesel crop implementation in Hawaii. The State of Hawaii Department of Agriculture, Hawaii Agriculture Research Center. Retrieved from [https://www.eere-pmc.energy.gov/states/Hawaii\\_Docs/biodiesel\\_report-revised.pdf](https://www.eere-pmc.energy.gov/states/Hawaii_Docs/biodiesel_report-revised.pdf)
- Powlson, D.S., Whitmore, A.P., & Goulding, W.T. (2011). Soil carbon sequestration to mitigate climate change: a critical re-examination to identify the true and the false. *European Journal of Soil Science*, 62, 42-55.
- Ragauskas, A.J., Williams, C.K., Davison, B.H., Britovsek, G., Cairney, J., Ecker, C.A., Frederick, W.J., Hallett, J.P., Leak, D.J., Liotta, C.L., Meilenez, J.R., Murphy, R., Templer, R., & Tschaplinski, T. (2006). The path forward for biofuels and biomaterials. *Science*, 311, 484-489.

Rhoton, F.E., Shipitalo, M.J., & Lindbo, D.L. (2002). Runoff and soil loss from Midwestern and Southeastern silt loam soils as affected by tillage practice and soil organic matter content. *Soil and Tillage Research*, 66, 1-11.

Robinson, N., Harper, R.J., & Smettem, K.R.J. (2006). Soil water depletion by *Eucalyptus* spp. Integrated into dryland agricultural systems. *Plant Soil*, 286, 141-151.

Rockwood, D.L., Rudie, A.W., Ralph, S.A., Zhu, J.Y. & Winandy, J.E. (2008). Energy Product Options for *Eucalyptus* Species Grown as Short Rotation Woody Crops. *International Journal of Molecular Sciences*, 9, 1361-1378.

Rodrigues, J., Faix, O., & Pereira, H. (1998). Determination of lignin content of *Eucalyptus globulus* wood using FTIR spectroscopy. *Holzforschung*, 52, 46-50.

Saint-Andre', L., M'Boub, A.T., Mabiala, M., Jourdan, C., Roupsard, O., Deleporte, P., Hamela, O., & Nouvellon. (2005). Age-related equations for above- and below-ground biomass of a *Eucalyptus* hybrid in Congo. *Forest Ecology and Management*, 205, 199–214.

Salome, C., Nunan, N., Pouteau, V., Lerch, T.Z., & Chenu, C. (2010). Carbon dynamics in topsoil and in subsoil may be controlled by different regulatory mechanisms. *Global Change Biology*, 16, 416-426.

Schlesinger, W.H. (1997) *Biogeochemistry: An Analysis of Global Change*, (2<sup>nd</sup> ed.) San Diego, CA: Academic Press

Schmidt, M.W., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssen, I.A., Kleber, M., Kogel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., & Trumbore, S.E. (2011). Persistence of soil organic matter as an ecosystem property. *Nature*, 478, 49-56.

Schooler, S.S., Cook, T., Prichard, G., & Yeates, A.G. (2010). Disturbance mediated competition: the interacting roles of inundation regime and mechanical and herbicidal control in determining native and invasive plant abundance. *Biological Invasions* 12(9), 3289-3298.

Schubert, T.H., Strand, R.F., Cole, T.G., & McDuffie, K.E. (1988). Equations for Predicting Biomass of Six Introduced Subtropical Tree Species, Island of Hawaii. United States Department of Agriculture, Forest Service Pacific Southwest Forest and Range Experiment Station, Research Note PSW-401.

Searchinger, T., Heimlich, R., Houghton, R.A., Dong, F., Elobeid, A., Fabiosa, J., Tokgoz, S., Hayes, D., & Yu, T. (2008). Use of U.S. croplands for biofuels increases greenhouse gases through emissions from land use change. *Science*, 319:5867, 1238-1240.

- Sheehan, J. (2009). Biofuels and the conundrum of sustainability. *Current Opinion in Biotechnology*, 20:318–324.
- Simmons, B.A., Loque, D., & Blanch, H.W. (2008) Next-generation biomass feedstocks for biofuel production. *Genome Biology*, 9:12, 242-242.6.
- Six, J., Callewaert, P., Lenders, S., De Gryze, S., Morris, S.J., Gregorich, E.G., Paul, E. A. & Paustian, K. (2002a). Measuring and understanding carbon storage in afforested soils by physical fractionation. *Soil Science Society of America Journal* 66, 1981–1987.
- Six, J., Elliott, E.T., & Paustian, K. (2000a). Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. *Soil Biology and Biogeochemistry*, 32, 2099-2103.
- Six, J., Feller, C., Denef, K., Ogle, S.M., de Moraes Sa, J.C., & Albrecht, A. (2002b). Soil organic matter, biota and aggregation in temperate and tropical soils – Effects of no-tillage. *Agronomie*, 22 (7-8), 755-775.
- Six, J., Paustian, K., Elliott, E.T. & Combrink, C. (2000b) Soil structure and organic matter I. Distribution of aggregate-size classes and aggregate-associated carbon. *Soil Science Society of America Journal*, 64(2), 681-689.
- Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Soil Series Classification Database. Available at: <http://soils.usda.gov/technical/classification/scfile/index.html>.
- Sollins, P., Swanston, C., Kleber, M., Filley, T., Kramer, M., Crow, S., Caldwell, B, Lajtha, K., & Bowden, R. (2006). Organic C and N stabilization in a forest soil: evidence from sequential density fractionation. *Soil Biology and Biochemistry*, 38(11), 3313-3324.
- Sollins, P., Homann, P., & Caldwell, B.A. (1996) Stabilization and destabilization of soil organic matter: mechanisms and controls. *Geoderma*, 74, 65-105.
- Torn, M.S., Trumbore, S.E., Chadwick, O.A., Vitousek, P.M., & Hendricks, D.M. (1997). Mineral control of soil organic carbon storage and turnover. *Nature* 389, 170-173.
- Turn, S.Q., Keffer, V., Dakka, S., Evans, D., & Wang, W. (2005). Preliminary Gasification Testing of Eucalyptus Fuels. University of Hawaii, Hawaii Natural Energy Institute, School of Ocean and Earth Sciences and Technology. Retrieved from: <http://hawaii.gov/dbedt/info/energy/publications/biomass-gasification-2005.pdf>
- United States Environmental Protection Agency. (2011). Life cycle assessment research. Retrieved from: <http://www.epa.gov/nrmrl/lcaccess/>

United States Environmental Protection Agency. (2012). Greenhouse gas equivalencies calculator. Retrieved from: <http://www.epa.gov/cleanenergy/energy-resources/calculator.html#results>

Vasquez, A.F.L., Rey, G.A.O., & Rodriguez, F.A.R. (2011). Obtaining of reducing sugars from Kikuyu grass (*Pennisetum clandestinum*). *Avances Investigación en Ingeniería*, 13, 98-101.

West, T.O. & Post, W.M. (2002) Soil organic carbon sequestration rates by tillage and crop rotation, a global data analysis. *Soil Science Society of America Journal*, 66(6), 1930-1946.

Westman, W.E., & Rogers, R.W. (1977). Biomass and Structure of a Subtropical Eucalypt Forest, North Stradbroke Island. *Australian Journal of Botany*, 25, 171-191.

Whitesell, C. D., DeBell, D.S., Schubert, T.H., Strand, R.F., & Crabb, T.B. (1992). Short-rotation management of Eucalyptus: guidelines for plantations in Hawaii. U.S. Department of Agriculture, Pacific Southwest Research Station. General Technical Reports: PSW-GTR-137.



