

BELOWGROUND IMPACT OF NAPIER AND GUINEA GRASSES GROWN FOR  
BIOFUEL FEEDSTOCK PRODUCTION

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YUDAI SUMIYOSHI

Thesis Committee:  
Susan E. Crow, Chairperson  
Creighton M. Litton  
Jonathan L. Deenik

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## LIST OF ABBREVIATIONS

ADF.....	acid detergent fiber
AIC.....	Akaike Information Criterion
C.....	carbon
C <sub>R</sub> .....	root carbon
C <sub>S</sub> .....	soil carbon
F <sub>A</sub> .....	aboveground litterfall
F <sub>S</sub> .....	soil CO <sub>2</sub> efflux
GG.....	guinea grass
IPCC.....	Intergovernmental Panel on Climate Change
<i>k</i> .....	decay constant
NDF.....	neutral detergent fiber
NFC.....	non-fiber carbohydrates
NG.....	napier grass
PxD.....	pearl millet and dwarf napier hybrid
RMSEA.....	mean square error of approximation
SRMR.....	standardized root mean square residual
TBCF.....	total belowground carbon flux
TLI.....	Tucker Lewis Index

## CHAPTER 1. INTRODUCTION

### Global Perspective

Global interest in development of renewable energy options largely stems from growing concerns about climate change, energy security, and environmental sustainability. Biofuel production from plant feedstocks is one candidate among many available for the development of diverse renewable energy programs. Plants use solar energy to transform CO<sub>2</sub> into sugars that eventually become plant biomass. Upon use of this biomass as an energy source, the CO<sub>2</sub> released into the atmosphere would be limited to the amount recently assimilated to the biomass from the atmosphere. As such, these energy production systems are considered renewable. Despite its premise, biofuel production involves other sources of CO<sub>2</sub> emissions through the use of fossil fuels in the production and processing of the biomass. However, use of sustainable management and production practices during the cultivation of feedstocks may sequester carbon (C) in soil under favorable conditions, thereby providing an offset to some or all of the CO<sub>2</sub> emissions associated with biofuel feedstock production. Therefore, sustainability of a biofuel production system is dependent on efficient biomass production and energy conversion technologies, as well as soil C sequestration capability.

### Biofuels in Hawai'i

Being the most isolated archipelago in the Earth, concerns about climate change and energy security are particularly poignant in Hawai'i. Climate change can cause sea level rise (IPCC, 2007) and changes in precipitation pattern (Diaz, 2005, Oki, 2004), both of which may threaten endemic species and human life. In terms of energy security, Hawai'i relied on fossil fuels for 75 % of electricity and 97 % of transportation fuels in 2010 (Hawaii, 2010). Therefore, the development of a renewable biofuel production system is needed for sustainability of Hawai'i's future.

In response to the issues of climate change and energy security, Hawai'i created a partnership with the United States Department of Energy called the Hawai'i Clean Energy Initiative, which aimed at providing 70% of Hawai'i's energy from renewable sources by 2030 (HB2421, state of Hawaii). Corn grain and soybean oil are currently dominant feedstocks for biofuel production in the continental U.S. However, globally

escalating demands for both food and energy have raised concerns about the potential for food based biofuels to be sustainable, abundant, and environmentally beneficial energy sources. Utilization of perennial grasses as biofuel feedstocks has been purported to mitigate negative consequences due mainly to their higher yield and lower requirements of fertilizer and pesticides (Liebig *et al.*, 2008). In Hawai'i, the use of perennial C4 grasses such as sugarcane, energy cane, and sterile hybrids have been identified as the best potential candidates due to high biomass yielding potential.

However, to date most assessments on these species has focused on aboveground yields and agronomic requirements (Keffer *et al.*, 2009, Kinoshita, 1995) and little or no attention has been given to the environmental consequences of their growth (Osgood, 2010). In order to assess the sustainability of the biofuel production system, a life cycle assessment is needed to quantify inputs and outputs of the materials and energy in production, distribution, utilization, and disposal of a biofuel production. In this study, napier and guinea grasses were selected as two priority candidate crops for intensive assessment with a focus on the role of potential soil C sequestration within the life cycle analysis.

### Candidate Crops

Characteristics such as fast growth, lower inputs, and high belowground biomass make perennial C4 grasses like switchgrass (*Panicum virgatum*) and *Miscanthus* potential candidates for future biofuel production in the continental U.S. (Brandão *et al.*, 2011, Parrish & Fike, 2005). In Hawai'i, prolific yield potential of napier (*Pennisetum purpureum*) and guinea grasses (*Megathyrsus maximus*) in a tropical environment make them stronger candidates as a feedstock for biofuel production. Yields of irrigated and unirrigated napier grass on soils suitable for sugar production in Hawai'i were estimated to be 49 and 40 Mg of biomass ha<sup>-1</sup> yr<sup>-1</sup>, respectively (Kinoshita, 1995). Although no trial has been done on guinea grass in Hawai'i prior to this study, yield of guinea grass reported in Mexico reaches up to 24 Mg of dry matter ha<sup>-1</sup> yr<sup>-1</sup> (Reynoso *et al.*, 2009). Perennial C4 grasses like napier and guinea grasses can be harvested by ratooning, which leaves the lower part of the plant and living roots and soil undisturbed, thereby

potentially contributing substantially to the sustainability of the entire production system via soil C sequestration, erosion control, and improved soil fertility.

Aside from their high yield potential for biofuel, perennial herbaceous grasses like napier and guinea grasses have the potential to sequester a large amount of C in soil compared to annual crops due to their physiology and potential for conservation agricultural practices associated with no-tillage cultivation. These species produce extensive root system both in the surface root zone, primarily through fine roots and in the deep soil profile, penetrating to depths of up to 4.5 m (Khanal et al., 2010). Total root biomass of more than 3000 and 1600 g m<sup>-2</sup> in 0-60 cm depth has been reported for napier and guinea grasses respectively in Gangtok, India with mid altitude subtropical climate (Singh, 1999a, Singh, 1999b). Compared to annual crops such as corn and soybeans, napier and guinea grasses are perennial and can be ratooned for as many as 4 years without any reduction in production (Samson et al., 2005). Absence of tillage in turn results in less aeration, lower decomposition rates and increased soil C sequestration (Clifton-Brown *et al.*, 2007).

### Soil C Sequestration

Whether the quantity of soil C is changing or at equilibrium, it is the sum of continuous fluxes of input and output of C (Six & Jastrow, 2002). Soil C pool will increase if the input is greater than output. The input can differ by soil fertility and climatic conditions while output through decomposition of soil C can be suppressed due to reduced tillage or increased chemical recalcitrance of soil C.

Processes associated with accumulation and turnover of soil C, such as decomposition and physical protection of organic matter in soil aggregates, appear to be remarkably similar across wide range of ecosystems and climate (Grandy & Neff, 2008). Following initial litter decomposition, only a small portion of plant residue enters the soil to become stabilized through chemical recalcitrance, aggregate protection, and organomineral interactions (von Lutzow et al., 2006) or lost over time through further decay. The quantity of crop residue input applied to soil has been shown to positively correlate with equilibrium soil C pool within several cropping systems (Larson *et al.*, 1972, Rasmussen *et al.*, 1980). A recent meta-analysis revealed that differences in soil C

pool between till and no-till soils were due to C input differences (Virto *et al.*, 2012). However, in other cases, input quantity itself has no impact on soil C pool (Al-Kaisi & Grote, 2007, Sanderson, 2008), suggesting an effect of other factors such as input quality (i.e., whether the residue decomposes quickly or slowly). Therefore, both the quantity and quality of crop residue inputs must be considered simultaneously to determine drivers of soil C sequestration in a given system.

Traditionally, residue inputs with lower chemical quality and therefore lower decomposability were thought to contribute to accumulation of soil C more than residue with higher chemical quality, hence the chemical stabilization of C in soil due to the complex structure of molecules (Jastrow & Miller, 1996). In accordance with the theory, Grandy and Neff (2008) reported that the majority of recent, plant-derived soil C was comprised of lignin-related recalcitrant compounds. Further, ample evidence suggests that plant tissue decomposition rate, or decay constant ( $k$ ), is related to its tissue chemical characteristics such as the C:N and the lignin:N ratios (Johnson *et al.*, 2007, Silver & Miya, 2001). Keeping abiotic factors and the microbial community constant, quality of the residue is determined the rate of decomposition (Berg *et al.*, 2000).

Indeed, the amount of soil C pool under switchgrass has been shown to positively relate to the root C:N ratio, the ratio often used as a predictor of root decomposition (Ma *et al.*, 2000).

Appreciation for the complexity of the influence C input quality has on soil C stabilization is growing, yet conclusive evidence for the impact of the input quality on soil C pools is elusive (Kleber & Johnson, 2010, Schmidt *et al.*, 2011). Recent evidence suggests that plant tissue chemical recalcitrance may not play as much of a role as once thought in soil C stabilization (Kiem & Kogel-Knabner, 2003, Marschner *et al.*, 2008). Compounds such as sugars which were traditionally considered labile may stay in soil longer than more complex compounds like lignin by microbial transformation and physical protection by aggregation (Bol *et al.*, 2009). These findings suggest that factors such as efficiency of residue to get incorporated into soil aggregates may play a role that is as important or greater than chemical recalcitrance in soil C accumulation.

To my knowledge, there is no available published literature examining the belowground input quantity and quality of napier or guinea grass accessions and their

impact on soil C pool, making selecting the right accession and predicting benefits of biofuel production difficult. Better understanding of (1) differences between species and across accessions in quantity and quality of C input, and (2) relationships between the quantity and quality factors of belowground C inputs and how these factors influence soil C is imperative in selection of the best suited variety for the biofuel production in Hawai'i.

### Objectives

Objectives of this study therefore were twofold. The first objective was to discern differences between species and accessions in the quantity and quality of C input after three cycles of ratooning to determine which accession of grass is best suited for potential soil C sequestration in Hawai'i. In order to quantify total amount of belowground C input, mass balance approach called total belowground C flux (TBCF) was used. Although a common assumption of the TBCF approach is that soil C pools are nearly at steady state (Giardina & Ryan, 2002), this may not be true in the system experiencing abrupt change from fallow grassland to intensively managed high yielding grass system like in this study. Therefore, changes in both soil and root C overtime were also quantified over the measurement period.

The second objective was to develop a conceptual model of causal relationships among quantity and quality variables of C fluxes and pools in soil using structural equation modeling (SEM). Structural equation modeling is a multivariate statistical method which aims to disentangle the effect of the multiple explanatory variables into hypothesized causal pathways (Grace, 2006, Tabachnick & Fidell, 2007). Several recent ecological studies have used SEM to investigate the causal relationships among multicollinear predicting variables and their effect on soil CO<sub>2</sub> efflux (F<sub>s</sub>) (Geng *et al.*, 2012, Matias *et al.*, 2012), soil C storage (Brahim *et al.*, 2011, Jonsson & Wardle, 2010), and soil microbial community (Eisenhauer *et al.*, 2012). The method can be either (1) *a priori* confirmation of statistical adequacy of proposed model, or (2) *a posteriori* modeling building from exploratory data analysis (Petraitis *et al.*, 1996). This study utilized both approaches to model soil C dynamics. The variables in the models were selected after the data collection, while the initial model was formulated using theory

from previous literature. Nevertheless, the purpose of SEM in this study was to delineate patterns of direct and indirect effects of explanatory variables by formulating causal model based on both results of this study and prior knowledge of soil C accumulation. Understanding of factors affecting soil C accumulation will guide selection and breeding of improved accessions for soil C sequestration.

### Hypotheses

In this study, the following three hypotheses related to quantity of biomass production, root chemistry and decomposition, and soil C dynamics were tested to address the objectives of the study.

**Hypothesis 1: Quantities of pools and fluxes of soil C vary across accessions and between species due to positive relationship to their aboveground yield.** Both napier and guinea grass accessions examined in the study were chosen in order to encompass a wide range of aboveground yields based on results from the first ratoon harvest in March 2010. Since belowground studies are often labor intensive, any relationships between aboveground yield and belowground fluxes that could then be used to predict belowground C processes will be useful for selection of suited accessions for maximizing both biomass production and soil C sequestration. Although the shoot to root ratio varies with environmental conditions and growth stages (Wilson, 1988), the ratio has been shown to remain relatively constant across accessions within species and between closely related grass species in both greenhouse (Crush *et al.*, 2005) and field experiment (Bolinder *et al.*, 2002). Total belowground C flux, the total amount of plant C allocated to belowground is tightly linked to aboveground net primary productivity in forest environment (Litton *et al.*, 2007). Lastly, aboveground biomass has been either directly related to  $F_S$  (Matias *et al.*, 2012) or indirectly associated with  $F_S$  through its association with belowground biomass (Geng *et al.*, 2012). Therefore, fluxes of C (TBCF and  $F_S$ ) and pools of C (soil and root biomass C) were expected to positively associated with aboveground yield and vary across accessions and species.

**Hypothesis 2: The root decay constant ( $k$ ) varies across accessions and between species due to a negative relationship between  $k$  and root lignin concentration.** A negative relationship between root lignin concentration and  $k$  has been

observed in prior studies (Johnson *et al.*, 2007, Melillo *et al.*, 1982, Zhang *et al.*, 2008). Also, initial root chemistry data collected prior to the study suggest variability in root lignin concentrations between napier and guinea grass accessions (Sumiyoshi Y, preliminary data). Therefore, root  $k$  of the studied grasses was expected to vary according to root chemical characteristics. Furthermore, visual observation of napier and guinea grass roots from the preliminary root samples suggested thicker root diameter with more fine root hairs in napier grass accessions compared to guinea grass accessions (Sumiyoshi Y, unpublished data). Even within the same species, accessions differed in root diameter and amount of root hairs, which suggested differences in their decomposability. Since a recent study suggested the importance of tissue architectural differences on decomposability (Lindedam *et al.*, 2009), root morphological differences between napier and guinea grass accessions may add variability in  $k$ . Nonetheless, the roots of napier and guinea accessions with greater root lignin concentration were expected to decay slower than those with lower root lignin concentrations. Therefore, accessions with greater amount of root lignin will result in slower decomposition.

**Hypothesis 3: Quantities of pools and fluxes and quality of root biomass interact to influence the soil C pool after three ratoon cycles.** Since ratooning of grasses removes most aboveground biomass without incorporating residue into soil, the primary input of C to soil in the system is senescent leaves on the soil surface and root turnover in the soil of which root production and turnover is expected to be much greater. A positive relationship between the amount of both above and belowground C fluxes and the amount of soil C pool after three ratoon cycles was expected. The influence of C:N was shown to positively relate to soil C pool (Ma *et al.*, 2000). Therefore, a negative relationship between root  $k$  and the amount of soil C pool was expected. If a relationship between quantity and quality of C inputs to soil C exists, then the grass accessions with the greatest amount of root biomass C pool and the lowest root  $k$  would be expected to accumulate the most soil C after three ratoon cycles.

## CHAPTER 2. MATERIALS AND METHODS

### Study Site

The study was conducted in the napier and guinea grass accession trial plots established on October 2009 at the University of Hawai‘i Experiment Station in Waimanalo, Hawai‘i (21°N, 157°W). The Waimanalo Station is located at alluvial fans at 30 m elevation and has a mean annual temperature of 24.6°C (Ikawa, 1985). The mean annual precipitation is 938 mm, most of which concentrated between November and April (Service, 1972). The soil is a silty clay with smectitic and halloysitic mineralogy (Waialua series, Very-fine, mixed, superactive, isohyperthermic Pachic Haplustolls) (Ikawa, 1985).

The field plot design was a randomized complete block design with 4 replicates and 8 grass accessions as treatments. Accessions studied were 3 napier and 4 guinea accessions and 1 pearl millet and dwarf napier cross hybrid (PxD) (Table 1). They were selected based on yield data from the past planting to encompass wide range of yield potential.

Table 1. Names and origins of napier and guinea grass accessions.

Accession Name	Species	Origin	Codes
‘Local’	napier	Australia	NG1
‘Purple’		Australia	NG2
‘Merkeron’		Tifton, Georgia (31°N, 83°W) (Burton, 1989)	NG3
‘OG03’	guinea	Laie (21°N, 157°W), Oahu	GG1
‘OG05’		Pūpūkea (21°N, 158°W), Oahu	GG2
‘K06’		Anahola (22°N, 159°W), Kauai	GG3
‘MG04’		Makawao (20°N, 156°W), Maui	GG4
pearl millet and dwarf napier hybrid	hybrid	Mealani Research Station (20°N, 155°W), Hawaii Island	PxD

Each grass plot consisted of four rows of grass planted in 2 x 3 m area on November 2009, with a non-planted buffer of 0.6 m between plots. Inter-rows and buffers were covered with mats to suppress weed growth. Plants were planted from stem cuttings, similar to sugarcane. Plants were irrigated three days a week from 9:00 to 14:00 with drip irrigation for entire duration of the study at about 700 gallons irrigation day<sup>-1</sup>.

Aboveground yield

Grasses were ratooned every 8 months at maturity, which occurred on March, and November 2010, and July 2011. Irrigation tubes were reinstalled after ratooning in November 2010. At each ratooning, grasses were cut at 10 cm aboveground using a chainsaw and all aboveground biomass were removed from the plot. Fertilization occurred when grasses were first planted on November 2009, and after second ratooning in November 2010, with granular triple 16 formulation at the rate of 53 kg N ha<sup>-1</sup>, 23 kg phosphorus ha<sup>-1</sup>, and 44 kg potassium ha<sup>-1</sup>. Additionally, micronutrients were applied so that they are not limiting the grass growth. Aboveground biomass yields from three ratooning were combined, multiplied by C concentrations of leaf materials and annualized as g C m<sup>-2</sup> yr<sup>-1</sup>.

#### Belowground C fluxes and pools

Monthly F<sub>S</sub> rates were measured between 9:00-18:00 from each plot using a LI-6400XT portable photosynthesis system attached with a soil respiration chamber (LI-COR Inc., Lincoln, NE, USA) from August 2010 to July 2011. The target value was set close to the ambient CO<sub>2</sub> concentration (370~390 μmol CO<sub>2</sub> depending on wind speed), and the ΔCO<sub>2</sub> value was set as ±5 μmol CO<sub>2</sub>. Air inside the soil respiration chamber was adjusted to 5 μmol CO<sub>2</sub> below the ambient CO<sub>2</sub> concentration, and F<sub>S</sub> was measured until CO<sub>2</sub> concentration reached 5 μmol CO<sub>2</sub> above ambient concentration. In each plot, F<sub>S</sub> was measured on five 10 cm diameter polyvinyl chloride collars inserted 2 cm to soil (reduced to 3 collars plot<sup>-1</sup> after November 2010 based on lack of within plot variation in F<sub>S</sub>). Living vegetation inside the collars was removed by clipping. Soil temperature at 10 cm depth was measured adjacent to each collar with a temperature probe. Volumetric soil moisture was measured at 5 cm depth using an impedance probe (Hydra Soil Moisture Probe, Stevens Water Monitoring Systems Inc., Beaverton, OR, USA), calibrated to the study site.

Annual cumulative  $F_S$  ( $\text{g C m}^{-2} \text{ yr}^{-1}$ ) was calculated using linear interpolation between monthly measurements (Litton *et al.*, 2008). Mathematically, the interpolation can be expressed as:

$$F_S = \frac{360[\sum_1^i (D_i - D_{i-1}) \left(\frac{F_i + F_{i+1}}{2}\right)]}{(D_i - D_1)} \quad [\text{Eq. 1}]$$

where  $F_i$  is monthly soil  $\text{CO}_2$  efflux rates ( $\text{g C m}^{-2} \text{ d}^{-1}$ ) and  $D_i$  is date of the efflux measurement, starting at 1 as July 2010 and ending as  $i$  in August 2011. Daily  $F_i$  values were not corrected for diel variability because (1) 16 hours of flux measurements taken every 2 hours from 11:00 am to 3:00 am the next day from 10 collars in one replicate of NG1 showed no statistical differences between measurements overtime (Tukey multiple comparison with 95 % confidence), (2) no correlation between instantaneous efflux rates with soil temperature during the 18 hour measurement was observed ( $r=-0.030$ ;  $p=0.704$ ;  $n=160$ ), (3) relatively small range of soil temperature were observed (ranged between 21 to 25 °C throughout measurement period), (4) day to day and time to time fluctuations in soil moisture within each sampling month expressed as coefficient of variation (CV) was on average  $< 6 \pm 4 \%$ , and (5) minimal diel patterns in  $F_S$  have been observed in tropical dry forest (Litton *et al.*, 2008) and tropical wet forest in Hawai'i (Giardina & Ryan, 2002, Litton *et al.*, 2011).

For aboveground litterfall ( $F_A$ ), two perforated aluminum pans (16 cm x 26 cm with 2 cm ribs on four sides) were placed on the inter-rows in each plot to collect senescent leaves and other detritus and the two values were averaged within a plot. Litterfall measurements were made monthly for five times from February to July 2011. Assumptions were made that the litterfall rate during the study period remained constant from August 2010 to July 2011. Collected litter residues were oven dried at 75 °C to constant mass and weighed. Carbon concentrations of the ratooning aboveground biomass were used as surrogates for C concentrations in litterfall. The average monthly litterfall rate from the five measurements was multiplied by 12 to determine annual cumulative  $F_A$  ( $\text{g C m}^{-2} \text{ yr}^{-1}$ ).

Soil samples for soil C ( $C_S$ ) and root C ( $C_R$ ) were collected at two sampling periods: (1) after the first ratooning on April 2010 and (2) after 3<sup>rd</sup> ratooning on August

2011. On April 2010, two cored (5 cm diameter) samples for  $C_R$  and two augered (6 cm diameter) samples for  $C_S$  were collected at two depths (0-15 and 15-30 cm) from each plot. For August 2011, four cored (5 cm diameter) samples (two for  $C_R$  and two for  $C_S$ ) were collected at the same depth increments. Additionally, two baseline plots with four replicates each were constructed at adjacent field in (1) bare soil where had been tilled and never been planted and (2) fallow grass area where it was not ploughed for 20 years. Two controls were not part of randomized complete block design and therefore were not directly compared to grass plots. However they were used as reference for the soil C pools prior to the grass cultivation.

In both years, collected soil samples for  $C_S$  were air dried at 25 °C for a week, sieved to 2 mm, subsampled, oven dried at 75 °C, ground with a ball mill (Retsch MM200 mixer mill, Retsch GmbH, Hann, Germany) to pass through 250 µm and C concentrations was determined using an elemental analyzer (Costech ECS 4010 CHNSO Analyzer, Costech Analytical Technologies Inc., Valencia, CA, USA). For  $C_R$ , Cored soil samples were split into 4 parts, inserted into 250 ml nalgen bottles and shaken for 16 hours with 100 ml of 10 % sodium hexametaphosphate. Dispersed soils were wet sieved through 0.5 mm sieve, and collected roots were washed with deionized water, ground to pass through 250 µm using UDY cyclone mill (Tecator, Inc., Boulder, CO) and root C concentrations were determined following procedure described as before. No separation of live and dead roots was conducted.

The soil C pool was expressed using the equivalent mass of soil method (Ellert & Bettany, 1995, Gifford & Roderick, 2003), which negates the issue of core compaction during sampling. Equivalent mass of soil ( $\text{g C m}^{-2}$ ) at target depth  $t$  was calculated for each plot as:

$$C_S(t) = C_S(Z_a) + \frac{C_S(Z_b) - C_S(Z_a)}{M_S(Z_b) - M_S(Z_a)} (M_S(t) - M_S(Z_a)) \quad [\text{Eq. 2}]$$

where  $M_S(Z_a)$  and  $M_S(Z_b)$  are mass of soil of first and second increments at depth  $Z_a$  and  $Z_b$ ,  $C_S(Z_a)$  and  $C_S(Z_b)$  are mass of C of both depth increments, and  $M_S(t)$  is target mass of soil that all samples are compared against. This linear interpolation allows comparison of soil C pools without the need of accurate core volume. The target soil mass was selected as  $300 \text{ kg m}^{-2}$  as it was the approximate mass of soil samples collected to roughly 30 cm

depth. The soil C pools were expressed as  $\text{g C m}^{-2}$  in  $300 \text{ kg of soil m}^{-2}$  (approximately equivalent to 30 cm depth). The root biomass in soil cores were expressed in terms of C by multiplying root C concentration and mass of each root biomass extracted from wet sieving and expressed as  $\text{g m}^{-2}$  to 30 cm depth. Average values of two cores for  $C_S$  and  $C_R$  were calculated and used to represent each plot. Annualized changes in  $C_S$  ( $\Delta C_S$ ) and  $C_R$  ( $\Delta C_R$ ) during two sampling period (16 months) were calculated as differences between 2010 and 2011 pools and annualized to per year.

Additionally, belowground C input of grass accessions was estimated as TBCF using the mass balance approach. This mass balance approach quantifies the sum of all pertinent C inputs that plants send belowground including belowground net primary productivity, root respiration, root exudation, and C flow to symbionts, most of which are extremely difficult to measure independently (Giardina & Ryan, 2002). Use of this method for quantifying autotrophic impulse of C belowground outside the forest ecosystems has been limited (Adair *et al.*, 2009, Ford *et al.*, 2012), and this study is the first attempt to quantify TBCF in napier and guinea grass grown in Hawai'i. However, as long as all components of C fluxes pertinent to the system is measured, the mass balance approach should be applied in any environment. The total amount of C input to belowground as TBCF can be calculated as:

$$\text{TBCF} = F_S - F_A + \Delta(C_S + C_R + C_L) \quad [\text{Eq. 3}]$$

where TBCF is the total amount of C flux that plant send belowground (Giardina & Ryan, 2002),  $F_S$  is annualized soil surface  $\text{CO}_2$  efflux ( $\text{g C m}^{-2} \text{ yr}^{-1}$ ),  $F_A$  is aboveground litter fall ( $\text{g C m}^{-2} \text{ yr}^{-1}$ ),  $C_S$ ,  $C_R$  and  $C_L$  are soil, root, and litter layer C pools ( $\text{g C m}^{-2}$ ), respectively. This approach assumes there are negligible amounts of C lost through erosion and leaching. Soil erosion in the study site was very limited due to a flat topography and limited water availability. Leaching loss of C was also assumed to be negligible, as it has been shown to account for  $< 2 \%$  of  $F_S$  (Kindler *et al.*, 2011). It was also assumed that changes in soil and root biomass C pools below 30 cm were negligible during the measurement period, based on observation of roots during trenching. Changes in litter layer C were not quantified for the study because (1) changes in litter layer were heavily influenced during harvest, and (2) all aboveground biomass, including the litter

layer, is harvested in an ideal biofuel production system, and (3) in forest ecosystem changes in litter layer account for 2.1 % of TBCF (Giardina & Ryan, 2002).

#### Belowground input quality

Grass stalks with roots were excavated from soil pits (30 cm x 30 cm, 15 cm in depth) from each plot, washed with deionized water, air dried at 30 °C for one week. About 20 grams of sub-sample was analyzed for neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, and acid unhydrolyzable compounds (contains lignin and other recalcitrant compounds but hereafter referred to as lignin) using standard reagents (Van Soest, 1963) on a Fiber Analyser (Ankom, Macedon, NY, USA) at the Agricultural Diagnostic Service Center within University of Hawai'i at Mānoa. The quantities of non-fiber carbohydrates (NFC) such as organic acids, sugars, and starch was determined as  $100 - \text{NDF}$  and hemicellulose was calculated as  $\text{NDF} - \text{ADF}$  using results of the fiber analysis (Hall, 2003). The lignin value estimated using the sequential digestion method is a coarse estimate of lignin since polyphenolic and other unsaturated substances such as tannins and suberin may be included in the value (Van Soest & Wine, 1968). Carbon and N of these samples were determined following the procedure described previously for  $C_S$  and  $C_R$ .

Additionally, a common area root decay experiment was conducted during December 2010 and August 2011 to determine root decomposition constant ( $k$ ) using the litter bag method (Ostertag, 2001). For each replicate of each accession (4 replicates x 8 accessions = 32 experimental units), 5 subsamples (0.5 g each) of air dried root materials at 2–3 cm length were placed in 5 cm x 5 cm nylon mesh bags (0.132 mm mesh). The burial site was established in a 1.5 x 7 m around bare ground adjacent to the accession plots. Each set of 5 bags was randomly buried in 32 lines of 5 bags separated by 5 cm borders. Each bag was buried at a 45 ° angle to the surface and inserted 3.5 cm into the soil. The experiment was initiated in December 2010, and bags from each replicate set ( $n=5$ ) were collected randomly at 1, 2, 3, 5, and 8 months after deployment. The site was covered with a weed mat and irrigated three times a week in order to simulate conditions similar to the accession plots. Collected bags were rinsed with deionized water to wash off soil, dried to a constant mass at 75 °C, and residue C and N were determined

following the procedure described above. For each experimental unit, a first order single pool exponential function was applied for the root C decline over time to characterize decomposition (Wider & Lang, 1982);

$$L_t = e^{-kt} \quad [\text{Eq. 4}]$$

where  $L_t$  represents the proportion of original mass at time  $t$  (year), and  $k$  represents the decay constant ( $t^{-1}$ ). Both first order single and double pool exponential functions were considered, but a single pool exponential function was best suited for the data based on  $r^2$ . The fit of single pool functions as  $r^2$  were measured for all experimental units and mean  $r^2$  was 0.90 with a range of 0.69 to 0.99. No data points were removed to improve  $r^2$  values.

### Statistical Analyses

Analysis of Variance was performed to test the effect of accessions on belowground C quantity and quality using the software R 2.15 (R\_Development\_Core\_Team, 2012). Prior to analysis, homogeneity of variances for block and accession effects were tested using Levene's test on all response variables. Graphical assessment of normality and outliers revealed no severe non-normality or outliers in any of the variables. A wrong accession was planted in one of the replicates of GG2 and therefore was omitted from the data analysis, giving GG2 only 3 replicates. Unorthogonality created by the missing observation on sum of squares of accession effect was negligible and therefore type II sum of squares was used to calculate F values. When effect of accession on response variables was significant at  $p \leq 0.05$ , Tukey multiple comparisons were performed to detect accessions with significant differences. In order to determine significant differences across species, two preplanned custom contrasts comparing napier+PxD vs guinea, and napier vs PxD were tested using multcomp package in R (Hothorn *et al.*, 2008). Since PxD is half siblings, it was originally included as napier in first contrast, and then differences between napier and PxD were tested. This way two custom contrasts are independent of each other, negating the risk of inflating type I experiment-wise error rate (Seltman, 2012).

Structural equation modeling was also performed using “sem” package in R to investigate how belowground C quantity and quality affect soil C storage (Fox *et al.*, 2012). This method tests hypothetical causal links among variables in both unique and shared contributions by developing a series of regression equations (i.e. structural equations) that represent each potential combination of the causal links. Variables are connected with one way arrows indicating the flow of causal relationships. Regression coefficients are then parameterized simultaneously using maximum likelihood method (Grace, 2006) for each arrow. The variance and covariance matrix from the parameterized coefficients was tested against the matrix from the data to determine overall fit of the hypothesized models to the data.

All plots were treated as independent and thus all 32 plots were included in the analysis. Given the small sample size (n=32) only observed variables and their relationships were considered. Also, variables were limited to six (root lignin concentration, aboveground yield,  $k$ ,  $F_S$ ,  $C_R$  2010, and  $C_S$  2011) in order to minimize estimated parameters (Grace, 2006).

Since SEM relies on variance and covariance matrix of the variables, assessment of univariate and multivariate distribution of variables for outliers, linearity, normality is crucial for subsequent inferences (Tabachnick & Fidell, 2007). Univariate outlier is extreme value on one variable, while multivariate outlier is an unusual combination of scores on two or more variables. The linearity refers to a straight line relationship between two variables. Univariate normality has to do with symmetry and peakedness of a distribution of one variable. Multivariate normality refers to the assumption that the variability in scores for one continuous variable is roughly the same at all values of another continuous variable. All univariate and bivariate outliers, linearity, and normality were assessed graphically with bivariate scatterplots using Minitab 16 (Minitab Inc., State College, PA). Additionally, residuals from all structural equations pertinent to hypothesized model were assessed for outliers and normality to assess multivariate normality and outliers.

The acceptability of final model was first determined by  $\chi^2$  tests ( $p > 0.05$ ). Non-significant  $\chi^2$  indicates that variance and covariance matrix of the hypothesized model is no different from observed data. Model fit were further ensured by various fit indicators

such as low root mean square error of approximation (RMSEA) ( $<0.05$ ), high Tucker Lewis Index (TLI) ( $>0.95$ ), standardized root mean square residual (SRMR) ( $<0.06$ ), and low Akaike Information Criterion (AIC) (Hu & Bentler, 1999). The modification indices ( $>5$ ) were considered when the paths had theoretical support and estimated parameters could be interpreted (Grace, 2006, Jöreskog & Sörbom, 1989). Additionally, test of indirect effect through intermediate variable, called mediation, was done using delta method (Sobel, 1982).

## CHAPTER 3. RESULTS

### Aboveground yield

Aboveground yield ranged from 931 to 1805 g C m<sup>-2</sup> yr<sup>-1</sup> (Table 2). Although accession effect was only marginally significant (p=0.07), average of napier accessions and PxD was significantly greater than that of guinea accessions (p=0.026) (Fig. 1). Comparison of napier accessions and PxD was not significant (p=0.358), suggesting they were similar in yield.

Table 2. Means (and SE) of pools (g m<sup>-2</sup>) and fluxes (g m<sup>-2</sup> yr<sup>-1</sup>) of carbon for grass accessions (n=4 except for GG2 with n=3).

Species		Yield	C <sub>S</sub>		C <sub>R</sub>		ΔC <sub>S</sub>	ΔC <sub>R</sub>	F <sub>S</sub>	F <sub>A</sub>	TBCF
			2010	2011	2010	2011					
napier	NG1	1805 (300)	5156 (168)	5534 (221)	50 <sup>a</sup> (6)	154 (10)	284 <sup>ab</sup> (120)	79 (11)	1784 (216)	229 (59)	1917 (223)
	NG2	1351 (87)	4908 (20)	5424 (64)	39 <sup>ab</sup> (8)	106 (11)	387 <sup>a</sup> (37)	50 (14)	1325 (114)	215 (17)	1546 (99)
	NG3	931 (277)	5062 (143)	5409 (110)	34 <sup>ab</sup> (2)	110 (16)	260 <sup>ab</sup> (45)	57 (13)	1788 (107)	213 (19)	1892 (163)
hybrid	PxD	1590	5393	5483	39 <sup>ab</sup>	91	68 <sup>b</sup>	39	1536	128	1516
guinea	GG1	1007 (170)	5273 (187)	5391 (123)	40 <sup>ab</sup> (7)	111 (14)	88 <sup>b</sup> (62)	54 (8)	1489 (173)	120 (8)	1510 (166)
	GG2	1135 (250)	5144 (184)	5327 (148)	41 <sup>ab</sup> (5)	96 (15)	137 <sup>b</sup> (49)	41 (8)	1565 (235)	145 (45)	1599 (332)
	GG3	983 (198)	5002 (253)	5262 (115)	21 <sup>b</sup> (2)	83 (25)	195 <sup>ab</sup> (123)	47 (18)	1555 (182)	170 (35)	1627 (144)
	GG4	1085 (168)	5334 (300)	5617 (133)	35 <sup>ab</sup> (5)	85 (29)	213 <sup>ab</sup> (159)	37 (23)	1518 (105)	110 (15)	1658 (233)
		(230)	(258)	(221)	(6)	(14)	(70)	(14)	(195)	(6)	(157)

Means with different alphabetical letters were significantly different by Tukey multiple comparisons at 95 % confidence. Yield, aboveground yield (g m<sup>-2</sup> yr<sup>-1</sup>); F<sub>S</sub>, soil CO<sub>2</sub> efflux (g m<sup>-2</sup> yr<sup>-1</sup>); F<sub>A</sub>, litter fall (g m<sup>-2</sup> yr<sup>-1</sup>); C<sub>S</sub>, soil C pool (g m<sup>-2</sup>); C<sub>R</sub>, root carbon (g m<sup>-2</sup>); ΔC<sub>S</sub>, differences in soil C pools (g m<sup>-2</sup> yr<sup>-1</sup>); ΔC<sub>R</sub>, differences in root C pools (g m<sup>-2</sup> yr<sup>-1</sup>); TBCF, total belowground carbon flux (g m<sup>-2</sup> yr<sup>-1</sup>). Soil C pools were expressed in terms of equivalent mass of 300 g m<sup>-2</sup> which was approximately equal to 0-30 cm depth.

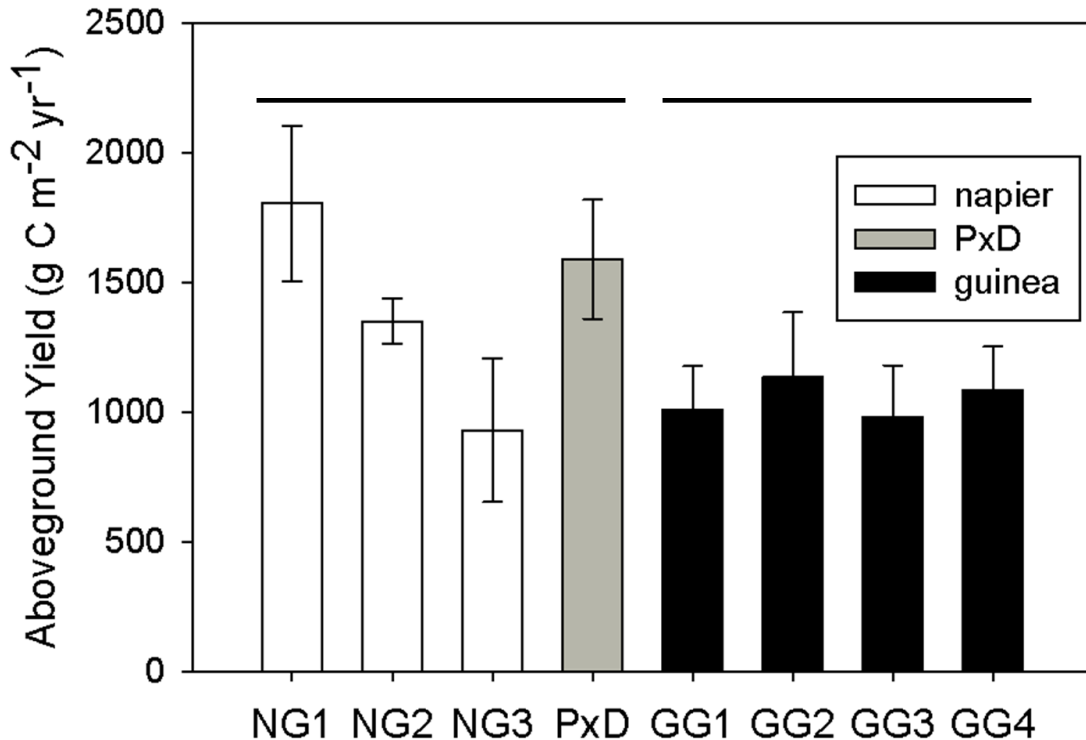


Figure 1. Means ( $\pm$  SE) of aboveground yield from three ratooning which occurred on March, and November 2010, and July 2011. White vertical bars indicate napier accessions, gray vertical bar indicates PxD, and black vertical bars indicate guinea accessions. Species with unconnected horizontal bars indicate significant species differences at  $p \leq 0.05$ .

#### Belowground C fluxes and pools

Instantaneous flux rates measured over the course of the study varied within grass accessions, likely as a result of ratooning, fertilization and increase in soil moisture (Fig. 2). The ratooning on November 2010 and fertilization after the ratoon had mixed effects on instantaneous  $F_S$  rates. Accessions except GG1 and NG3 decreased or maintained  $F_S$  rates after the ratooning. Leading up to the ratoon,  $F_S$  rates were on average approximately  $3 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ . After ratooning,  $F_S$  rates on average increased to approximately  $5 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ . The irrigation tubes were repaired right after the ratooning in November 2010. Before the repairing the soil moisture was very close to permanent wilting point of the soil (15 bar soil gravimetric moisture =  $27.5 \% \times \text{BD} = 32.45 \%$ ) (Ikawa, 1985). After the repair it reached to around 45 %, which was about 75 % of porosity. Therefore, the increase in overall  $F_S$  was most likely caused by the increase in the soil moisture after the repair of irrigation tubes. Flux rates of napier

accessions were more variable than those of guinea accessions. When  $F_S$  was annualized, differences were not significant for accessions ( $p=0.128$ ) or species ( $p=0.374$  for guinea vs napier+PxD and  $p=0.465$  for napier vs PxD).

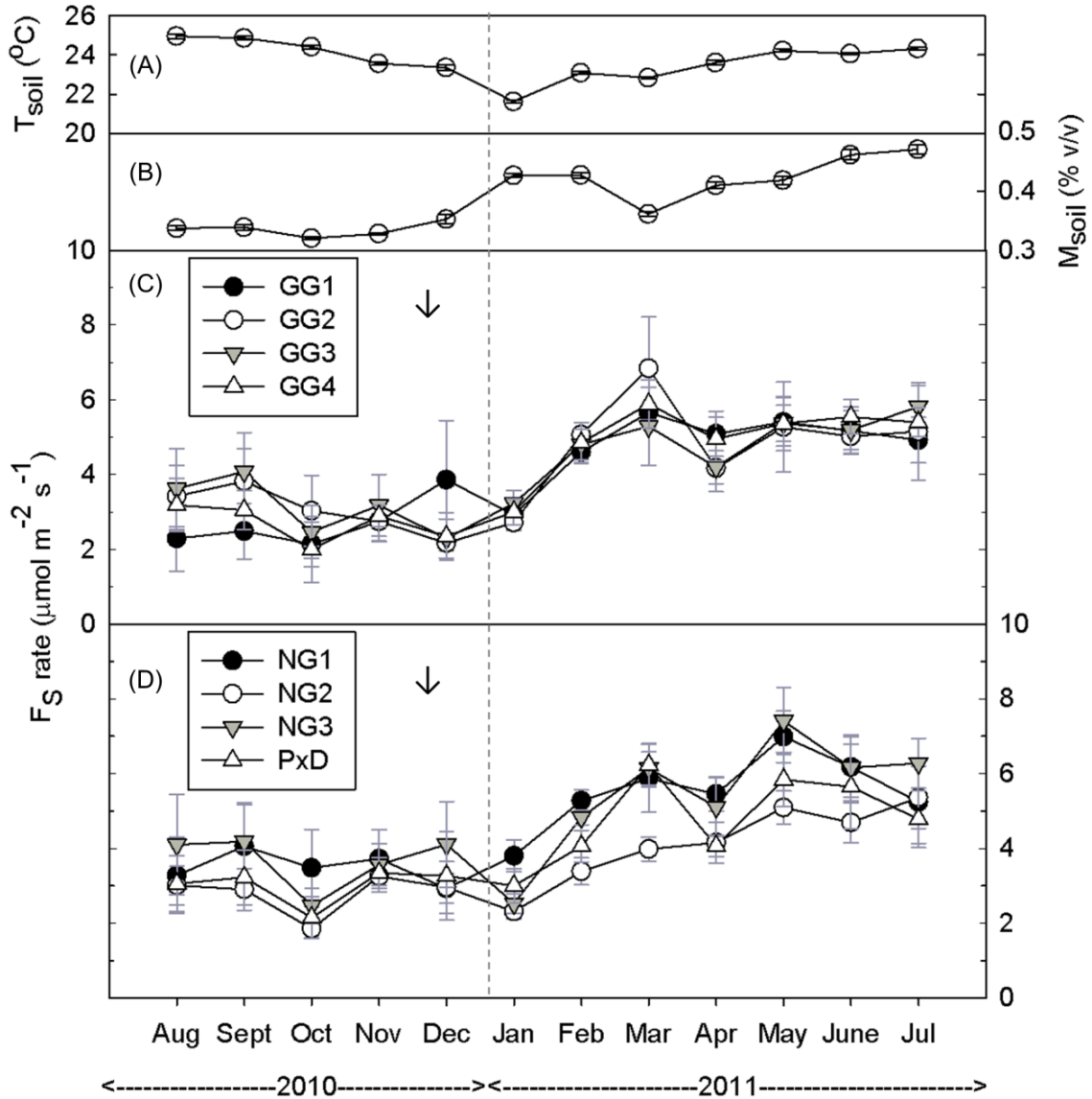


Figure 2. One year time course of accession means ( $\pm$  SE) for monthly (A) soil temperature, (B) volumetric soil moisture, (C) soil surface  $\text{CO}_2$  flux ( $F_S$ ) for guinea grass accessions, (D) and for napier grass accessions and PxD. Arrows indicate date of ratoon harvest and subsequent fertilizer application and the dotted vertical line in each panel delineates 2010 and 2011 measurements.

Annualized litter fall ranged from 110 to 193  $\text{g C m}^{-2} \text{ yr}^{-1}$ . Average  $F_A$  of napier grasses accessions and PxD was significantly ( $p=0.002$ ) greater  $F_A$  than that of guinea

grass accessions (Fig. 3). Although marginal, significant differences were observed between average of napier accessions and PxD ( $p=0.003$ ).

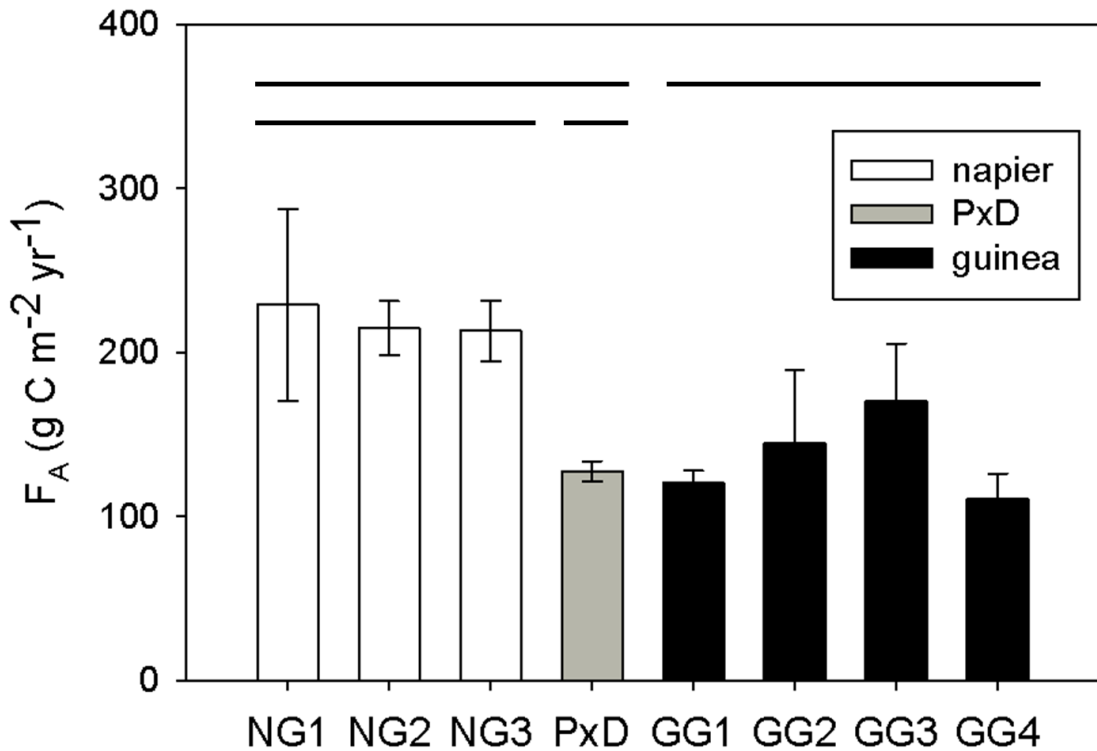


Figure 3. Means ( $\pm$  SE) of aboveground litterfall ( $F_A$ ). White vertical bars indicate napier accessions, gray vertical bar indicates PxD, and black vertical bars indicate guinea accessions. Species with unconnected horizontal bars indicate significant species differences at  $p \leq 0.05$ .

Soil C pools ranged from 4908 to 5617 g C m<sup>-2</sup> in 2010. Soil C in PxD was significantly ( $p=0.024$ ) greater than the average of napier accessions in 2010. However, significant differences were not observed in 2011 as grass accessions caught up to the level of PxD. Although accession effect on soil C pools for both years were not significant, the values were consistently greater in 2011 than in 2010, resulting in positive  $\Delta C_S$  for all grass accessions (Fig. 4). Tukey comparisons determined that NG2 had significantly greater  $\Delta C_S$  compared to GG1, GG2, and PxD. Adjusted for overall alpha of 0.05, NG2 had  $\Delta C_S$  statistically significantly greater than zero. Significant ( $p$ =negative dependence) was observed between  $\Delta C_S$  and  $C_S$  2010, suggesting slowing down of accumulation of soil C as it reaches to its limit (Fig. 5).

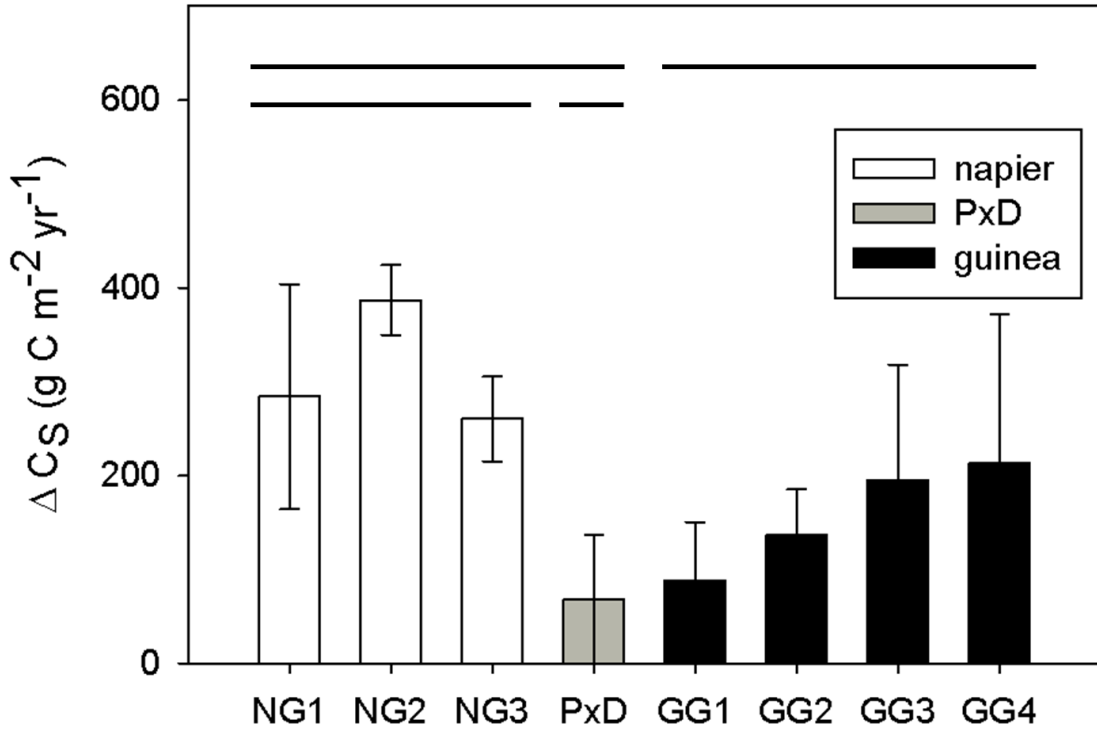


Figure 4. Means ( $\pm$  SE) of changes in soil C pool from 2010 to 2011 ( $\Delta C_S$ ). White vertical bars indicate napier accessions, gray vertical bar indicates PxD, and black vertical bars indicate guinea accessions. Species with unconnected horizontal bars indicate significant species differences at  $p \leq 0.05$ .

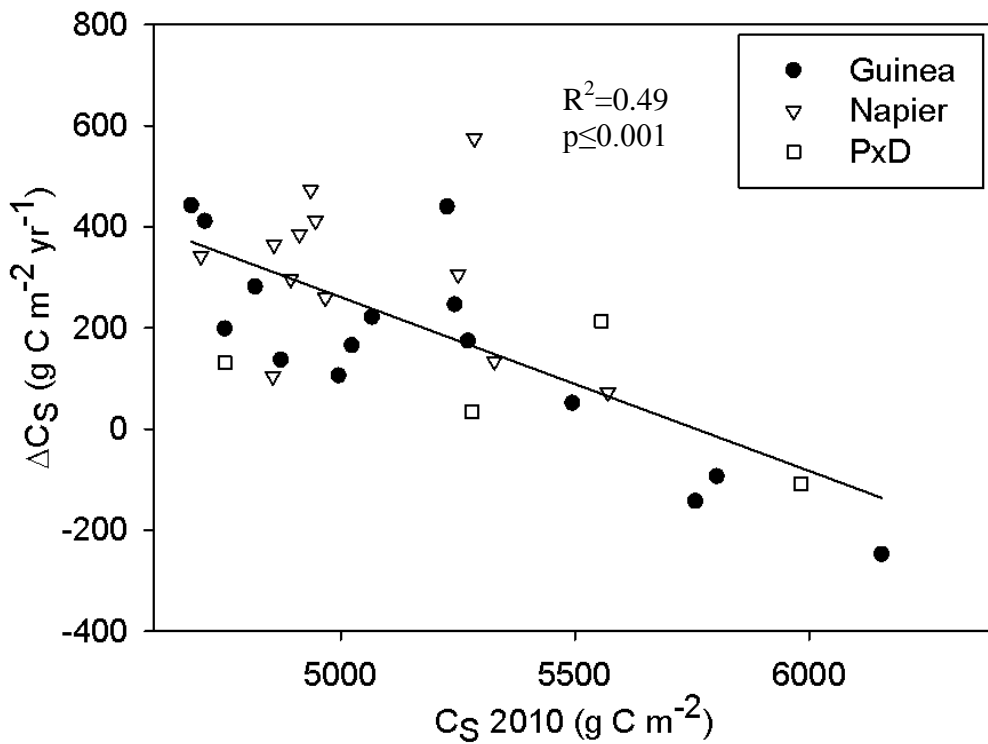


Figure 5. Changes in soil C pools from 2010 to 2011 in relation to soil C pools at 2010.

Root C pools ranged from 21 to 50 g C m<sup>-2</sup> in 2010. Accession effect was marginally significant (p=0.054) and ad hoc Tukey comparisons determined that NG1 was significantly greater than GG3. However, the significant differences were not observed in 2011 as increases in root biomass pool from 2010 to 2011 were highly variable across replicates within accessions, as CVs as high as 120 % of its mean in GG4 was observed (Table 2). Due to the large variability, comparisons of  $\Delta C_R$  across accessions and species were not significant.

Total belowground C flux (sum of  $F_S$ ,  $\Delta C_S$ ,  $\Delta C_R$ , minus  $F_A$ ) was calculated using measured fluxes. However, significant variability in both  $\Delta C_S$  and  $F_A$  canceled each other out, resulting in no significant differences across accessions (p=0.115). Although no significant differences were observed between guinea and napier accessions and PxD, napier accessions were marginally (p=0.061) greater in TBCF compared to PxD (Fig. 6). Aboveground yield and TBCF was weakly, but positively correlated with TBCF (r=0.433, p=0.015; n=31). Significant block effect (p $\leq$ 0.05) was observed in  $F_S$ ,  $F_A$ ,  $\Delta C_S$  and TBCF.

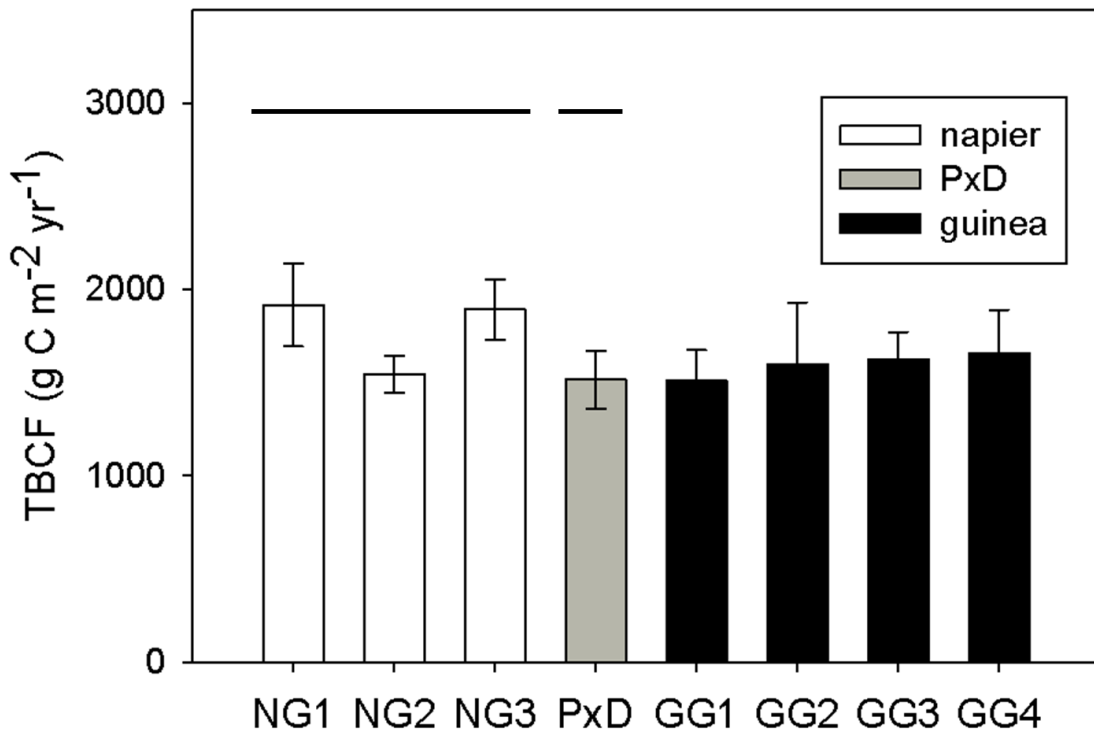


Figure 6. Means ( $\pm$  SE) of total belowground C flux (TBCF). White vertical bars indicate napier accessions, gray vertical bar indicates PxD, and black vertical bars indicate guinea accessions. Species with unconnected horizontal bars indicate significant species differences at p $\leq$ 0.05.

### Belowground input quality

Root chemical characteristics varied across accessions and species after accounting for block effect (Table 3). The mean concentration of root NFC, which contains soluble sugars and amino acids, varied significantly ( $p=0.008$ ) from 21.25 to 26.84 % across accessions. Tukey comparisons determined that root NFC concentrations were significantly ( $p\leq 0.05$ ) greater for NG1 and NG3 than GG3. Averaged over species, napier and PxD together had significant ( $p\leq 0.0001$ ) greater root NFC concentrations than guinea grass, while napier and PxD were not significantly ( $p=0.195$ ) different. Root hemicellulose varied marginally ( $p\leq 0.061$ ) across accessions, and significant differences were observed between the mean of three napier accessions and PxD. No accession or species effect was observed in root cellulose concentration.

Table 3. Means (and SE) of chemical composition (%) and decay constant ( $\text{yr}^{-1}$ ) of root biomass for grass accessions ( $n=4$  except for GG2 with  $n=3$ ).

Species		NFC	HC	CEL	Lignin	C	N	C:N	Lignin:N	<i>k</i>
		%								
napier	NG1	26.84 <sup>a</sup>	21.69	32.91	18.56 <sup>bc</sup>	42.53 <sup>bc</sup>	0.65	68	30 <sup>ab</sup>	1.64
		(1.55)	(1.05)	(0.7)	(0.28)	(0.2)	(0.08)	(9)	(4)	(0.13)
	NG2	25.20 <sup>ab</sup>	22.54	33.6	18.67 <sup>bc</sup>	42.60 <sup>bc</sup>	0.71	63	28 <sup>ab</sup>	1.64
		(0.785)	(0.89)	(0.34)	(0.47)	(0.43)	(0.08)	(8)	(4)	(0.13)
	NG3	26.83 <sup>a</sup>	21.09	32.63	19.45 <sup>abc</sup>	42.45 <sup>bc</sup>	0.73	60	28 <sup>ab</sup>	1.38
		(2.75)	(1.40)	(0.44)	(0.99)	(0.62)	(0.08)	(7)	(4)	(0.1)
hybrid	PxD	24.76 <sup>ab</sup>	23.91	33.49	17.85 <sup>c</sup>	42.79 <sup>bc</sup>	0.77	57	24 <sup>b</sup>	1.69
		(0.57)	(1.15)	(0.8)	(0.67)	(0.47)	(0.09)	(6)	(3)	(0.3)
guinea	GG1	23.45 <sup>ab</sup>	21.75	34.27	20.53 <sup>ab</sup>	45.27 <sup>a</sup>	0.59	80	37 <sup>a</sup>	1.23
		(1.77)	(0.52)	(0.67)	(0.8)	(0.16)	(0.06)	(11)	(6)	(0.18)
	GG2†	23.47 <sup>ab</sup>	21.55	34.05	20.92 <sup>a</sup>	44.81 <sup>abc</sup>	0.55	82	39 <sup>ab</sup>	0.95
		(1.19)	(0.51)	(1.04)	(0.88)	(0.96)	(0.03)	(4)	(4)	(0.21)
	GG3	21.25 <sup>b</sup>	24.62	33.17	20.96 <sup>a</sup>	43.74 <sup>abc</sup>	0.69	66	32 <sup>ab</sup>	1.14
		(0.87)	(0.43)	(0.41)	(0.83)	(0.5)	(0.08)	(9)	(5)	(0.12)
	GG4	22.67 <sup>ab</sup>	23.28	33.22	20.84 <sup>a</sup>	44.77 <sup>ab</sup>	0.7	69	33 <sup>ab</sup>	1.38
		(1.39)	(0.48)	(0.9)	(0.87)	(0.31)	(0.11)	(11)	(6)	(0.22)

\*Means with different alphabetical letters were significantly different by Tukey multiple comparisons at 95 % confidence. C, root carbon concentration; N, root nitrogen concentration; NFC, non-fiber carbohydrates; HC, hemicellulose; CEL, cellulose; *k*, root decay constant.

Root lignin concentration ranged narrowly, yet significantly ( $p \leq 0.0001$ ) from 17.85 to 20.96 across accessions, with GG2, GG3, and GG4 significantly greater than NG1, NG2, and PxD. Preplanned comparisons of species determined a significant and distinct pattern of root lignin concentrations in order of  $PxD < \text{napier grass} < \text{guinea grass}$ . Root C concentrations ranged significantly ( $p = 0.001$ ) across accessions, and guinea accessions on average had significantly greater root C compared to the average of napier accessions and PxD. Tukey comparisons determined that GG1 had significantly ( $p \leq 0.05$ ) greater C concentrations compared to any of napier accessions and PxD. Root N concentrations varied only marginally ( $p = 0.085$ ) across accessions. However, napier and PxD as a group had significantly ( $p = 0.024$ ) greater root N concentrations, while napier and PxD were not significantly ( $p = 0.166$ ) different from each other.

In addition to measured root characteristics, ratios of C:N and lignin:N were calculated. The root C:N ratios ranged widely from 57 to 82 and it was significantly ( $p = 0.038$ ) different across accessions. Napier accessions and PxD together had significantly ( $p = 0.004$ ) lower C:N ratio compared to guinea accessions on average. Root lignin:N ratios were less variable across accessions compared to C:N ratios, but varied significantly ( $p = 0.032$ ) from 24 to 39 across accessions. Tukey comparisons determined that GG1 had significantly ( $p \leq 0.05$ ) greater lignin:N compared to PxD. Similar to C:N ratios, lignin:N ratios were significantly ( $p = 0.001$ ) less for napier and PxD compared to guinea accessions.

Decay constant,  $k$ , ranged from 0.95 to 1.69 ( $\text{yr}^{-1}$ ), although only marginally significant ( $p = 0.091$ ) effect of accessions was observed. However, on average roots of napier accessions and PxD decayed significantly ( $p = 0.004$ ) faster constant compared to guinea accessions, while napier accessions and PxD were similar in decay constant. Overall, napier and PxD accessions were similar to each other in most root characteristics except hemicellulose and lignin concentrations, while guinea accessions were consistently different from napier and PxD. Block effect was significant on concentrations of NFC, cellulose, lignin, and N, and ratios of C:N and lignin:N.

Pearson correlation analysis determined that when all accessions were treated as independent,  $k$  was significantly correlated with the all root components except cellulose and hemicellulose (Table 4). High negative and significant correlations with  $k$  were

observed in root C and lignin concentration, C:N, and lignin:N. The  $k$  was also significantly and positively correlated with root N and NFC concentrations. No significant relationships were observed between  $k$  and hemicellulose or cellulose.

Table 4. Pearson correlation coefficients ( $r$ ) across the concentration of compositional factors and decay constant calculated from litterbag experiment ( $n=31$ ).

<b>Root composition factors</b>	<b>r</b>	<b>p</b>
C	-0.390	0.030
<b>N*</b>	<b>0.497</b>	<b>0.004</b>
NFC	0.483	0.006
Hemicellulose	0.021	0.911
Cellulose	-0.286	0.118
<b>Lignin*</b>	<b>-0.709</b>	<b>&lt;0.001</b>
C:N	-0.520	0.003
Lignin:N	-0.597	<0.001

\*Bolded factors indicate largest  $r$  for positive and negative correlations.

The results of the correlation analysis were used to identify factors controlling root decomposition for the initial development of the SEM. Root lignin concentration had highest  $r$  value and lowest  $P$  value, suggesting that it was best predictor of  $k$ . Multiple regression analysis suggested that adding N to  $k = \beta_0$  lignin model did not significantly improve  $r^2$  values of the original model since lignin and N were highly collinear ( $r = -0.739$ ;  $P \leq 0.0001$ ;  $n=31$ ). Moreover, including both variables as ratio such as Lignin:N into the SEM was statistically undesirable since it implies non independence of slopes of two effects and thus dilute the true effect of two terms. For these reasons, and limited sample size, root lignin concentration was used as sole predictor of root decay in the SEM.

#### SEM: Quantity and quality of belowground C input in relation to $C_S$

In the initial results-driven model, root lignin and aboveground yield were used as exogenous variables (independent variable) and  $C_R$  2010,  $F_S$ ,  $k$ , and  $C_S$  2011 were used as endogenous variables (dependent variable) (Fig. 7). Lack of a line connecting variables indicates no hypothesized direct effect between two variables.  $C_S$  2011 was used as opposed to  $\Delta C_S$  because grasses were planted before sampling  $C_S$  2010 and thus  $\Delta C_S$  ( $C_S$  2011 -  $C_S$  2010) represents latter half of soil C accumulation. Graphical assessment of

bivariate relationships found no outliers, kurtosis or severe deviation from linearity and multivariate normality.

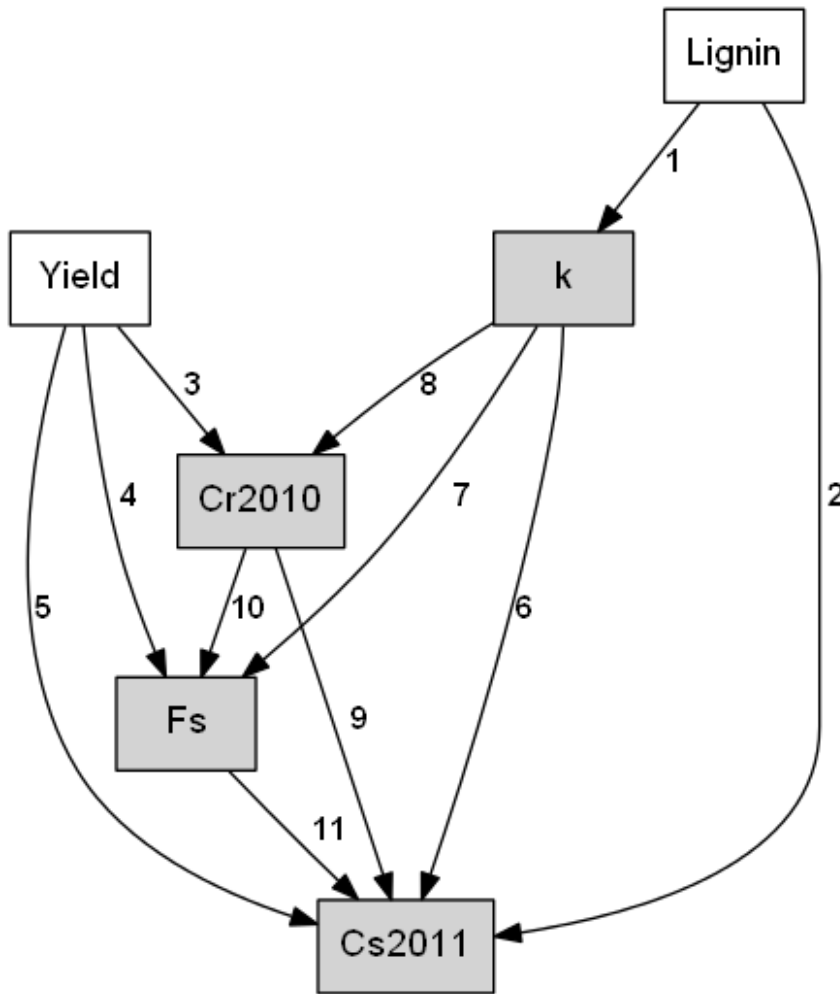


Figure 7. Conceptual model of hypothesized relationships between soil C after 20 month of grass growth and quantity and quality of grass soil C inputs and outputs. Path 1 represents effect of root lignin concentration on  $k$  expected from previous literature (Prescott, 2010). Path 2 represents direct effect of root lignin concentration on  $C_S$  2011 not accounted for by other endogenous variables. Path 3 represents relationship between aboveground yield and  $C_R$  at beginning of the study on April 2010, which tested if shoot to root ratio remains constant (Bolinder *et al.*, 2002, Crush *et al.*, 2005). Path 4 represents direct effect of aboveground yield to  $F_S$  not accounted for by root biomass (Raich & Tufekcioglu, 2000). Path 5 represents direct effect of yield to  $C_S$  2011 that was not accounted for by amount of root biomass or soil  $CO_2$  efflux. Path 6 represents influence of  $k$  onto  $C_S$  2011 not accounted for by amount of  $C_R$  2010 and  $F_S$  or any other variables. Path 7 represents influence of  $k$  on  $F_S$ . Path 8 represents effect of  $k$  on amount of root biomass pool. Path 9 represents direct effect of amount of root biomass which was potential input to soil C pool. Path 10 represents relationship between  $C_R$  2010 to  $F_S$  (Craine *et al.*, 2001). Path 11 represents effect of loss of soil C as  $F_S$  to  $C_S$  2011.

The hypothesized initial model fitted data well. Ad hoc model modification was not considered as the value of the highest modification index was less than 5 (Jöreskog & Sörbom, 1989). However, non-significant paths from lignin and yield to  $C_S$  2011 were removed because (1) they were initially uncertain paths and (2) the probability associated with these paths ( $p=0.824$  and  $0.671$  for lignin to  $C_S$  2011 and yield to  $C_S$  2011, respectively) suggested limited chance that such an effect was present in the study. Paths from  $k$  to  $C_R$  2010,  $C_R$  2010 to  $F_S$ , and  $C_R$  2010 to  $C_S$  2011 were not significant ( $P \leq 0.10$ ), but these paths were retained in the model because such effects may still be present in future studies. As a result of these adjustments to the conceptual model, the final model fit the data well with  $\chi^2 = 3.15$ ,  $df = 6$ ,  $p = 0.79$ ,  $RMSEA = 0$ ,  $TLI = 1.18$ , and  $SRMR = 0.05$  (Fig. 8).

Some paths were expected while others were unexpected from previous literature and theory. Root lignin concentration had highly significant ( $p \leq 0.001$ ) negative effect on  $k$ , as expected from correlation analysis of root chemical compositions (Table 4). The standardized path coefficient of  $-0.680$  in lignin to  $k$  path implies that as lignin decreases by one standard deviation, it is predicted that  $k$  increases by 68 % of its standard deviation (Table 5; Fig. 8). Expressed in terms of an unstandardized coefficient, one unit decrease in lignin concentration would result in an increase of  $0.159$  in  $k$ . Expressed in terms of relevant ranges, as lignin concentrations increase its effective range from  $15.88$  to  $22.53$ ,  $k$  was predicted to decrease 52.8 % of its effective range from  $0.565$  to  $2.569$ . Almost half (46 %) of the variation in  $k$  was accounted for by root lignin concentrations (Fig. 8).

As expected, aboveground yield had significant and direct positive effect on  $C_R$  2010 ( $p=0.004$ ) and  $F_S$  ( $p=0.03$ ) (Table 5), explaining 24 and 23 % of variation in each variable, respectively (Fig. 8). Aboveground yield did not have direct effect on  $C_S$  2011, and therefore whatever effect the yield had on  $C_S$  2011 was through  $F_S$  and  $C_R$  2010. Soil  $CO_2$  efflux had marginally significant negative effect on  $C_S$  2011, as it represents gaseous loss of C from soil (Fig. 8).

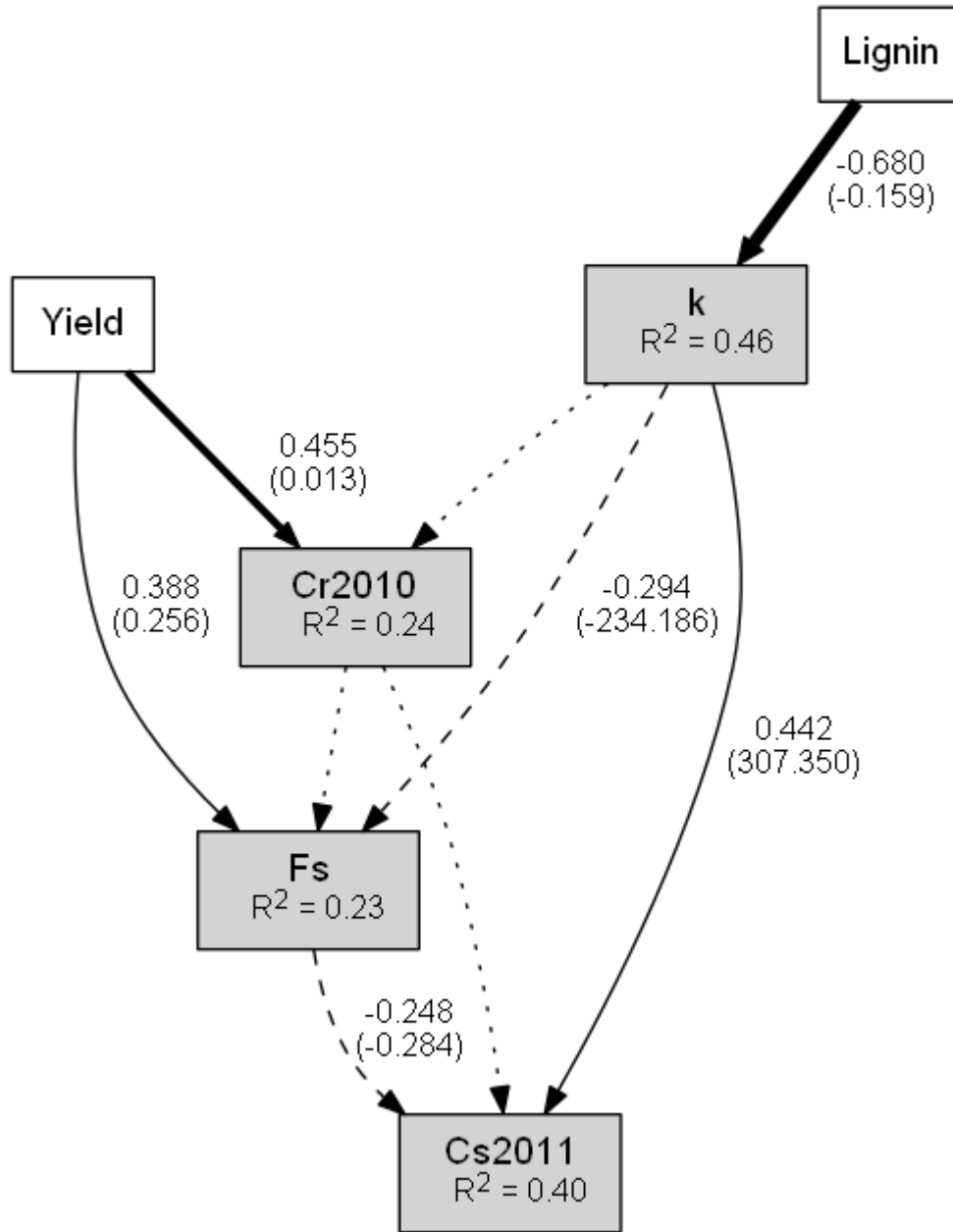


Figure 8. Best fitting model resulted from structural equation modeling (SEM). Values on arrows are standardized coefficients and values on parentheses are unstandardized coefficients. Only values with significant paths were reported. The solid arrows indicate statistically significant paths at  $p \leq 0.05$ . The bold arrows indicate paths at  $p \leq 0.01$  (thick bold arrows indicate  $p \leq 0.001$ ). Dashed arrows indicate marginally significant paths. Dotted arrows indicate non-significant paths. Model fitted data well ( $\chi^2 = 3.15$ ,  $df = 6$ ,  $p = 0.79$ ,  $RMSEA = 0$ ,  $TLI = 1.18$ , and  $SRMR = 0.05$ ).

Table 5. Summary of direct & indirect effects in the structural equation modeling (n=32).

Direct effects							
Paths		Unstd. coeff.	SE	Z	P	Std. coeff.	Std. relevan t
<i>k</i>	<b>Lignin -&gt; <i>k</i></b>	<b>-0.159</b>	<b>0.031</b>	<b>-5.158</b>	<b>&lt;0.001</b>	<b>-0.680</b>	<b>-0.528</b>
$C_R$ 2010	<b>yield -&gt; <math>C_R</math> 2010</b>	<b>0.013</b>	<b>0.005</b>	<b>2.911</b>	<b>0.004</b>	<b>0.455</b>	<b>0.358</b>
	<i>k</i> -> $C_R$ 2010	6.464	5.487	1.178	0.239	0.184	0.200
$F_S$	<b>yield -&gt; <math>F_S</math></b>	<b>0.256</b>	<b>0.118</b>	<b>2.176</b>	<b>0.030</b>	<b>0.388</b>	<b>0.409</b>
	$C_R$ 2010 -> $F_S$	-1.178	4.12	-0.286	0.775	-0.052	-0.068
	<i>k</i> -> $F_S$	-234.186	128.64	-1.82	0.069	-0.294	-0.421
$C_S$ 2011	<b><i>k</i> -&gt; <math>C_S</math> 2011</b>	<b>307.350</b>	<b>103.95</b>	<b>2.957</b>	<b>0.031</b>	<b>0.442</b>	<b>0.548</b>
	$C_R$ 2010 -> $C_S$ 2011	3.291	2.831	1.162	0.245	0.166	0.190
	$F_S$ -> $C_S$ 2011	-0.248	0.129	-1.926	0.054	-0.284	-0.246
Indirect effects							
$C_R$ 2010	Lignin -> <i>k</i> -> $C_R$ 2010	-1.031	0.897	-1.148	0.251	-	-
$F_S$	Lignin -> <i>k</i> -> $F_S$	37.338	21.750	1.717	0.086	-	-
	yield -> $C_R$ 2010 -> $F_S$	-0.016	0.055	-0.285	0.776	-	-
$C_S$ 2011	<b>Lignin -&gt; <i>k</i> -&gt; <math>C_S</math> 2011</b>	<b>-49.004</b>	<b>19.104</b>	<b>-2.565</b>	<b>0.010</b>	-	-
	<i>k</i> -> $C_R$ 2010 -> $C_S$ 2011	21.271	25.708	0.827	0.408	-	-
	<i>k</i> -> $F_S$ -> $C_S$ 2011	57.987	43.825	1.323	0.186	-	-
	yield -> $C_R$ 2010 -> $C_S$ 2011	0.043	0.040	1.080	0.280	-	-
	yield -> $F_S$ -> $C_S$ 2011	-0.063	0.044	-1.442	0.149	-	-
	$C_R$ 2010 -> $F_S$ -> $C_S$ 2011	0.292	1.031	0.283	0.777	-	-

Significant effects ( $p < 0.05$ ) are given in bold.

SE of indirect effects were estimated using delta method.

Lignin, root lignin concentration (%); *k*, root decay constant ( $\text{yr}^{-1}$ );  $F_S$ , annualized soil  $\text{CO}_2$  efflux ( $\text{g C m}^{-2} \text{ yr}^{-1}$ );  $C_S$ , soil C pool ( $\text{g C m}^{-2}$ );  $C_R$ , root C pool ( $\text{g C m}^{-2}$ ).

Contrary to expectation,  $C_R$  2010 was a poor predictor of both  $F_S$  and  $C_S$  2011 ( $p = 0.775$  and  $0.245$ , respectively). Due to the weak effects of  $C_R$  2010 on  $F_S$  or  $C_S$  2011, indirect effect of yield on  $C_S$  2011 through  $F_S$  and  $C_R$  2010 were not significant ( $p = 0.149$  and  $p = 0.280$ , respectively).

Root  $k$  was the only variable in the model with significant direct effect on  $C_S$  2011 (Fig.8). Together with  $F_S$ ,  $k$  explained 40 % of variation in  $C_S$  2011. However,  $k$  had a positive effect on  $C_S$  2011 as opposed to the negative effect that was originally expected. Moreover, a direct effect of lignin was not observed in the model, while the indirect negative effect of lignin on  $C_S$  2011 through  $k$  was determined to be significant ( $P \leq 0.05$ ) using delta method (Table 5). Therefore effect of lignin on  $C_S$  2011 was negatively mediated through intervening variable  $k$ . Greater root lignin concentration would result in lesser amount of soil C pool, which was contrary to initial expectation.

Furthermore, root  $k$  had a marginally significant ( $p=0.069$ ) negative effect on  $F_S$  (Table 5). Contrary to initial expectations, root lignin indirectly affected  $F_S$  positively at marginal significance (Table 5). Greater root lignin concentration would result in greater amount of  $F_S$ , which was again contrary to initial expectation. Since the variables used in the hypothesized model were selected after the data collection, the SEM was not strictly confirmatory. Despite the fact that causal relationships were based on previous literature, the final model requires additional validation. Nonetheless, the final model indicated that the indirect effect of belowground input quality was a more important determinant of the soil C pool than the indirect effect of aboveground biomass yield.

## CHAPTER 4. DISCUSSION

### Belowground input quantity: fluxes and pools

As hypothesized in H1, aboveground yield was positively and significantly related to  $F_S$ ,  $C_R$  2010 and TBCF. Positive correlations between  $F_S$  and aboveground net primary productivity (Raich & Tufekcioglu, 2000) and leaf area index (Litton *et al.*, 2008), which are analogous to yield data in our agronomic setting, were previously reported and support these results.  $F_S$  from the grass accessions ranged from 1325 to 1788 g C in  $\text{CO}_2 \text{ m}^{-2} \text{ yr}^{-1}$ , which was slightly above reported values of 1020 (Tufekcioglu *et al.*, 2001), 1260 (Dornbush & Raich, 2006), and 1347 g C  $\text{m}^{-2} \text{ yr}^{-1}$  (Bremer *et al.*, 1998) for switchgrass in temperate environments and within the range observed in tropical forest environment with fountain grass (*Pennisetum setaceum*) (Litton *et al.*, 2008). However, the variability in aboveground yield did not directly translate into significant variability across accessions in  $F_S$ . This may be attributed to the fact that  $F_S$  is a mixture of root and microbial respiration, and therefore will be influenced by other factors as well, such as amount of exudation, and the microbial community and their activity.

Contrary to expectations in H1, both  $C_S$  2010 and 2011 were not related to aboveground yield nor did they vary significantly across accessions. This may be attributed to the relatively short duration of the study, and the fact that it was fallow grassland for 20 years prior to tillage in preparation for this trial. Residual C within the soil likely dominated any small differences in total C pool due to grass accession during the short duration of the study. However, comparison of the  $C_S$  2010 data with samples collected from adjacent fallow areas revealed that the C pool from the adjacent baseline area was greater than that from accessions plots in 2010 (5423 vs 5174 g  $\text{m}^{-2}$  for baseline and accession plots, respectively) but not in 2011 (5509 vs 5442 g  $\text{m}^{-2}$  for baseline and accession plots, respectively). These results suggest a dynamic system responding rapidly to the decay of roots following initial tillage in preparation for the trial with a decrease in soil C pool once new root inputs from the planted feedstock grasses begin to accumulate soil C.

The differences in  $C_S$  2010 and 2011 was negatively associated with  $C_S$  2010, suggested a reduction in accumulation of soil C, likely due to differences in baseline soil

C pools. As a result, much less variability was observed in  $C_S$  2011 across accessions and species. Nonetheless, only 4 out of 31 plots observed a decrease in soil C pool from April 2010 to August 2011. Ample evidence suggests that the microbial community reacts to changes in C inputs as a result of land use change and in some cases is stimulated to decompose more old soil C, causing a priming effect (Kuzyakov, 2002). Therefore, it was encouraging to observe no decrease in soil C pool, despite the acute land use change from unmanaged fallow grassland to an intensively managed high yielding grass system.

A simple life-cycle analysis revealed that the soil C accumulation observed in this study would offset most of the total energy input involved in the production of the studied biomass feedstocks. At the given fertilizer application rate, the net emission resulting from production and use of fertilizer was calculated as  $11 \text{ g C m}^{-2} \text{ yr}^{-1}$  using SimaPro PhD (PRé Consultants, Amersfoort, The Netherlands). The total amount of gasoline needed for ratooning grasses was estimated at  $\sim 150 \text{ gallons ha}^{-1} \text{ yr}^{-1}$ , which would result in emissions of  $37 \text{ g C m}^{-2} \text{ yr}^{-1}$  (density of gasoline =  $0.75 \text{ kg/L}$ ; gasoline is 87 % C). Assuming biomass will be converted to energy on site, total C emissions from biomass production was estimated at  $48 \text{ g C m}^{-2} \text{ yr}^{-1}$ . In turn, the increase of  $C_S$  from 2010 to 2011 ranged from 68 to  $387 \text{ g C m}^{-2} \text{ yr}^{-1}$ . Therefore, all eight accessions studied here would more than offset C emissions associated with feedstock production. However, recent research suggests a finite capacity of soil to accumulate C, which is based largely on the amounts and properties of silt and clay particles in the soil (Six *et al.*, 2002, Stewart *et al.*, 2009). Therefore, the true potential offset of C emission through soil C accumulation in a grass feedstock production system should be determined over longer temporal scales.

Significant variation in  $C_R$  2010 across accessions was not observed in 2011, suggesting that maximum root density was established by 2011. Measurements were limited to 30 cm depth due to the presence of a clay-rich plow layer and, thus, roots may be growing deeper as it saturated at the top. Visual assessment conducted to determine root growth in the deeper soil horizon revealed that most roots were still concentrated in the top soil with very few roots observed beyond 30 cm depth. Aboveground yield though was related to both 2010 and 2011 root C pools, showing coupling of above- and belowground production. Root biomass C at the end of the study in 2011 ranged from 83

to  $154 \text{ g C m}^{-2}$  to 30 cm depth, which was an order of magnitude smaller than root biomass observed in an Indian soil (Singh, 1999a). The study was only available literature of root biomass of napier and guinea grasses. Therefore it was difficult to discern the cause of this difference because of confounding factors such as plant age, soil type, nutrient availability, and climate.

Consistent with the observed pattern between TBCF and aboveground net primary productivity in forest ecosystems (Litton *et al.*, 2007), TBCF was positively related to aboveground yield in this study which was the first to use this approach in an agricultural system. The results suggest that coupling of above- and belowground C fluxes may be a universal process in terrestrial ecosystems beyond the forest environment. Considering components of TBCF, variability across accessions and species were all likely due to the variability in  $\Delta C_S$ . This usually was not the case for forest environments where steady state of soil C pools is often assumed (e.g. (Keith *et al.*, 1997, Maier *et al.*, 2004, Michael G. Ryan *et al.*, 1996). The range of TBCF was between 1507 and 1954  $\text{g C m}^{-2} \text{ yr}^{-1}$ , which was nearly twice as much as values reported in a nutrient poor sandy soil grassland in Minnesota (Adair *et al.*, 2009) and close to values for a *Eucalyptus* forest in Hawai'i (Ryan *et al.*, 2004). As the soil C pool reaches equilibrium, the TBCF values may become lower than observed in this study.

Many studies found positive correlations between  $F_S$  and root biomass of grass species (Craine *et al.*, 2001, Kucera & Kirkham, 1971, Maher *et al.*, 2010). The root respiration is major component of  $F_S$  besides exudation, and microbial activity in many environments including grassland (Raich & Schlesinger, 1992). Contrary to these findings, the path between  $C_R$  2010 and  $F_S$  in the SEM model was not significant (Table 5; Fig. 8). This may be attributed to the fact that  $C_R$  measured in the study was mixture of live and dead roots. Studies showed that recently produced photosynthate may contribute largely to the variability in  $F_S$  in both forest and grassland (Bremer *et al.*, 1998, Craine *et al.*, 1999, Hogberg *et al.*, 2001). Plant activity in aboveground biomass expressed in terms of yield may have been influencing amount of  $F_S$  independent of  $C_R$ , since the relationship between aboveground yield and  $F_S$  was statistically significant in this study.

### Belowground input quality

The root chemical characteristics such as root N and lignin concentrations observed in this study were in similar range of values reported from temperate C4 grasses (Vivanco & Austin, 2006). The root lignin concentration was remarkably similar across accessions within species (Table 3), which suggests that there exist greater intrinsic species differences than intraspecific variability. For most measures of root chemical properties, except hemicellulose and lignin, napier grass accessions and PxD were more similar to each other than to guinea accessions (Table 3). This may be attributed to the fact that PxD is a half sibling of napier grass.

As hypothesized in H2, root decay was strongly related to root lignin concentrations, confirming a recent review which identified lignin as the most reliable predictor of decomposition rate (Prescott, 2010). Root lignin, or more formally known as acid unhydrolyzable residue in recognition that more than just structural lignin remains in the residual fraction, has a complex molecular structure and thus breaks down slowly compared to cellulose and hemicellulose (Chapin *et al.*, 2002). However, only half ( $r^2=0.50$ ) of the variation in root decay was explained by lignin concentration. Other factors such as differences in root tissue three dimensional architecture (Lindedam *et al.*, 2009) and root diameter (Scheu & Schauermaun, 1994) may also be influencing root decomposition.

Lignin was most important, but other factors such as N and NFC were also related to  $k$ , consistent with previous literature (Johnson *et al.*, 2007, Silver & Miya, 2001). Root  $k$  was positively related to root N concentrations likely due to microbial immobilization of N during decomposition of root mass into their cells as they consume C (Tisdale *et al.*, 1985). Root decomposition was also related to NFC since they are relatively easily decomposable energy sources for microbes due to their molecular size. Despite the presence of both labile and recalcitrant components in root biomass, single pool exponential function was best fit for the mass loss over time in the study. This indicated that root decomposition occurred at a continuous pace and was not partitioned into two pools of (1) a rapidly decomposing or active pool and (2) a slowly decomposing recalcitrant pool. This may be attributed to the fact that only the early stage of decomposition was observed in the relatively short 8 month period. However, these

results agreed with the data from comparisons of different environments for long term decomposition that concluded that slow second stage decomposition does not occur in the tropics (Harmon et al., 2009).

Decay constants were different at marginal significance across accessions, ranging from 0.95 to 1.69 yr<sup>-1</sup>, which was fairly close to the range observed for temperate grass roots (Vivanco & Austin, 2006). This result was consistent with the recent review of litter decomposition which concluded that manipulating single chemical characteristic would not contribute to drastic differences in decomposition rate (Prescott, 2010). However, on a species basis, accessions of napier grass and PxD had statistically greater *k* compared to guinea grass accessions. The clear separation between napier and guinea grasses was likely due to the significantly greater lignin concentrations and lower soluble compounds for napier grass accessions and PxD compared to guinea grass accessions (Table 3). The significant differences in root decomposition across accessions and species despite the same water and fertilizer application and decomposition conditions suggests that genetic differences were one of major influences on the root decomposition.

Differences in mesh size of the litterbag play a large role in interpreting ecological meaning of decomposition studies. Mesh size used in the study was 0.132 mm, which was smaller than more typical 1-2 mm mesh size (Silver & Miya, 2001) to minimize loss of mass through the mesh. The mesh size used in this study excluded most macro and mesofauna. When effect of mesh sizes were compared on the soil surface, residue decomposition was drastically different in 0.15 and 2 mm mesh sizes due to exclusion of macro-arthropods (Yang & Chen, 2009). However, when comparisons were made with buried samples, many studies find no effect of mesh size on root decomposition (Camire *et al.*, 1991, McLaugherty *et al.*, 1982, McLaugherty *et al.*, 1984, Scheu & Schauer mann, 1994, Seastedt, 1988). Differences in effect of mesh size between buried and surface bags may be due to the fact that microbes are principle decomposers of residues within soil. Therefore, although the exclusion of macrofauna was possible, *k* measured in this study was a good index of root decomposition.

### Carbon input and its quality in relation to Cs

The results highlighted that in this system root quality was more important than input quantities for soil C storage. Contrary to what was hypothesized in H3, grasses with more decomposable root residues, lower lignin, and greater labile compounds resulted in higher soil C pool after 21 months of grass growth. Mass loss from the litterbag can be divided into three ways, (1) respiration, (2) fragmentation, (3) leaching as dissolved organic carbon (DOC) (Swift *et al.*, 1979), and (4) assimilated to microbial biomass (Bol *et al.*, 2009). While respiration by microbes mineralize organic matter into CO<sub>2</sub>, the latter three can be considered as direct input into soil C pool. The soil (Waialua series) in this study is dominated by smectites (2:1 layered silicates) which has limited capacity to adsorb negatively charged organic acids (Hue, 1991). Therefore DOC is not likely to be the predominant mechanism for stabilization of C. The results may be interpreted that root  $k$  represents how fast roots were turning into fragments, and microbial biomass rather than lost from soil as CO<sub>2</sub> or leached to deeper soil as DOC. The results can be further supported by a marginally significant and negative relationship between  $k$  and  $F_s$ . If the loss from litter bag was mostly due to microbial respiration, the relationship should have been positive. The fact that soil with faster decaying roots emitted less CO<sub>2</sub> supports the idea that  $k$  actually represents the rate of root C input to soil rather than loss of root C as CO<sub>2</sub> to the atmosphere.

The likely mechanism for soil C accumulation was through incorporation of fragments and microbial biomass C from roots into micro aggregates in absence of tillage (Six *et al.*, 2000). Soils can be fractionated into different functional fractions based on size, density and chemical separation techniques (Golchin *et al.*, 1994, Six *et al.*, 2002, Sollins *et al.*, 2009). Impacts of quantity and quality may be more apparent in more sensitive fractions such as particulate organic matter or light fractions and occluded light fractions, both of which may be influenced by plant properties (O'Brien *et al.*, 2010) and management practices (Six *et al.*, 2002). Therefore a logical next step for this study is to explore impacts of belowground input quantity and quality on soil fractions.

The results presented here agree with similar study conducted in forest environment which showed that lower lignin concentration of litterfall resulted in more soil C accumulation due to greater inputs through fragments (Huang *et al.*, 2011).

Together, the results from this study appear to agree with the recent understanding that microbial transformation of plant residue and physical protection within aggregates is a more important driver of soil C stabilization than chemical recalcitrance per se (Bol *et al.*, 2009, Kleber *et al.*, 2011, Schmidt *et al.*, 2011). Furthermore, recent reviews indicate that there is not enough evidence that selective preservation of recalcitrant compounds, such as lignin, serve as a major stabilization mechanism for soil C (Denef *et al.*, 2009, Marschner *et al.*, 2008). Indeed, there is evidence that labile compounds such as sugars and amino acids may actually be preserved in soil through physical protection via aggregations and contributing to soil C pool (Grandy & Neff, 2008). Empirically, Smith *et al.* (2007) observed that labile organic matter that was initially utilized rapidly persisted longer as soil C. Together with the results obtained in this study, this development of new understanding about soil C stabilization emphasizes the idea that initial chemical composition of C in input may not be as important as microbial transformation and physical protection processes in soil.

The results may also solve the dilemma between biomass to biofuel conversion efficiency and soil C sequestration. High lignin concentration in aboveground biomass is undesirable for recovery of glucose in pretreatment (Dien *et al.*, 2006) and subsequent cellulosic fermentation (Vogel & Jung, 2001). Therefore, reduced lignin concentration in aboveground biomass has been an important goal for improving energy conversion efficiency during biofuel production. On the other hand, genetically engineer lignin concentration of leaves and roots to slow microbial decomposition and extend the residence time of plant residues in soil to sequester C has been recently suggested (Jansson *et al.*, 2010). Since the results of this study support the idea that breeding accessions with reduced lignin concentrations in plant biomass may not hinder capacity to sequester soil C, breeders can concentrate their effort to develop optimal genotypes for energy conversion. In terms of soil C sequestration, investment in sustainable agricultural practices such as ratooning is more important aspect to considered, rather than genetically engineering grasses to have more lignin concentration.

Although quantity and quality of belowground C explained close to 40 % of the variability in soil C pool, the majority (60%) of this variability remains unexplained. This suggests either (1) some other explanatory variables missing in the model or (2) variables

must be measured in different methods. Aboveground litterfall was omitted from the SEM model since it was extrapolated to annual value from 5 monthly measurements. Moreover, inclusion of  $F_A$  in the model did not improve the  $r^2$  for  $C_S$  2011 and thus was not included in the model. The soil microbial community composition and enzymatic activity are another crucial variables missing from the model, as they have impact on soil C dynamics by priming and stabilizing organic matter in more protected aggregates for further stabilization (Grandy & Neff, 2008, von Lutzow *et al.*, 2006). The ratio of fungi to bacteria may be important factor for predicting soil C dynamics as fungi may contribute more to soil C stabilization through formation of aggregates in hyphal networks (Six *et al.*, 2006). Use of stable isotope like  $^{13}C$  to fractionate sources of carbon is another promising method (Werth & Kuzyakov, 2010), although in the current study previous land use was  $C_4$  grasses which inhibited from using this method. The root biomass measured in this study was not separated into live and dead roots which was limited as a representation of belowground C input. These factors together may explain the variability in soil C pool more clearly and therefore should be explored further in future.

Since the all 32 plots were treated as independent, the study had limited capability to determine cause and effect of quantity and quality to soil C pool. Moreover, since SEM conducted in this study was not strictly confirmatory, the final model presented here is still a tentative model until additional data validates it (Petraitis *et al.*, 1996).

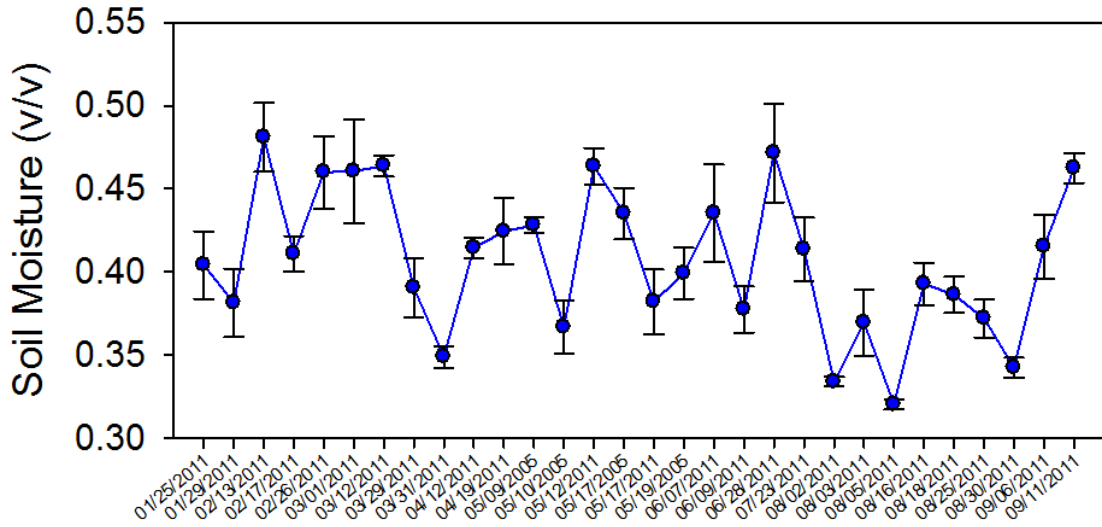
Furthermore, the study was relatively short term (less than 2 years) and thus equilibrium soil C pool may increase more than original pool when the grasses are planted longer. It has been suggested that soil C pool takes several decades to reach new equilibrium after land use change (Dalal & Mayer, 1986, Hermle *et al.*, 2008, West & Post, 2002). Therefore, a longer term study is warranted to observe the true impact of napier, guinea, and PxD grasses on soil C pool. The results were also limited to top 0-30 cm in soil and changes below the depth was assumed to be negligible. This assumption may result in an inability to adequately measure impacts on how grasses affect soil C pools (Harrison *et al.*, 2011), and future studies should consider dynamics of soil C at deeper depths.

## CHAPTER 5. CONCLUSION

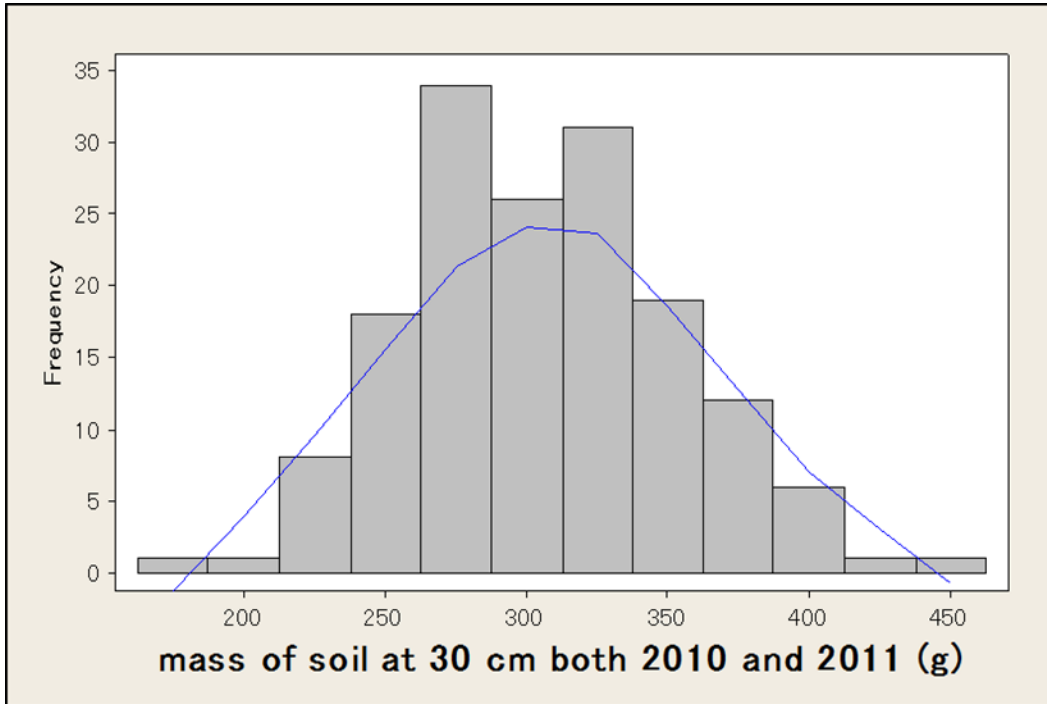
Ability of land management to sequester or restore soil C pool depends on climatic and antecedent management. Some even suggest that soil C stabilization is an ecosystem property rather than soil or plant (Schmidt *et al.*, 2011). In accordance with the recent buildup of evidences for new paradigm of soil C stabilization and accumulation (Kleber & Johnson, 2010), this study showed that plant factors such as belowground C quantity and quality were important but in nontraditional way. The results emphasized the importance of microbial transformation of plant residue inputs. Furthermore, in this relatively short term study significant increase in soil C was observed in one accessions of napier grass, and all 8 accessions studied have managed to offset energy input required to grow their biomass. It was also encouraging to observe no sign of a priming effect despite the fact that the site was converted from fallow grassland to intensively managed high yielding grass system.

However, applicability of the results beyond climate and soil type is unknown, as every soil has different capacity to hold C in soil, and microbial community is able to shift to adjust increase in C inputs (Stewart *et al.*, 2009). Therefore it may be premature at this point to lay too much hope in the ability to breed better accessions with quantity and quality characteristics resulting in soil C sequestration. Also, land use change such as turning abandoned agricultural land into biofuel production in Hawai'i should be examined at each case to assess its impacts on soil C pool. Long term soil C sequestration is different from temporary accumulation of soil C. Therefore, how the results from this relatively short term study (~1.5 year) will translate to C sequestration in the long-term is still yet to be determined.

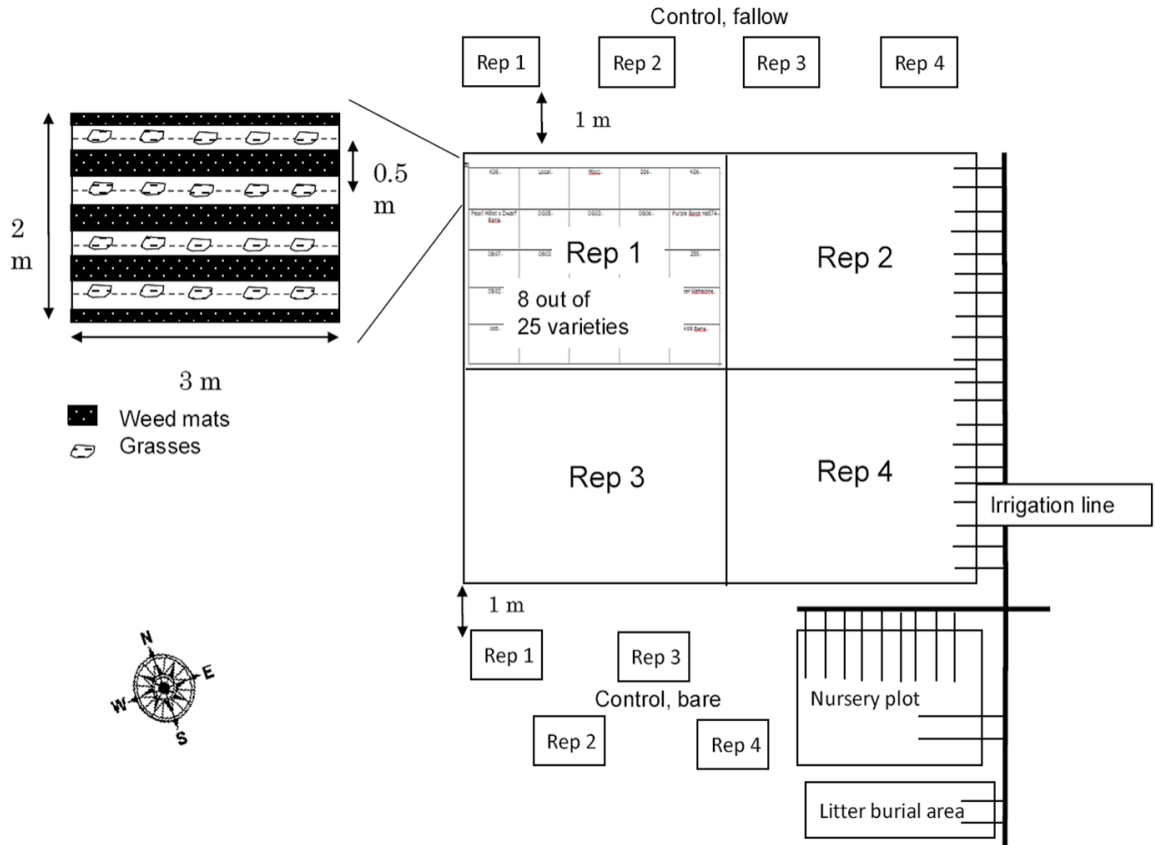
## APPENDIX



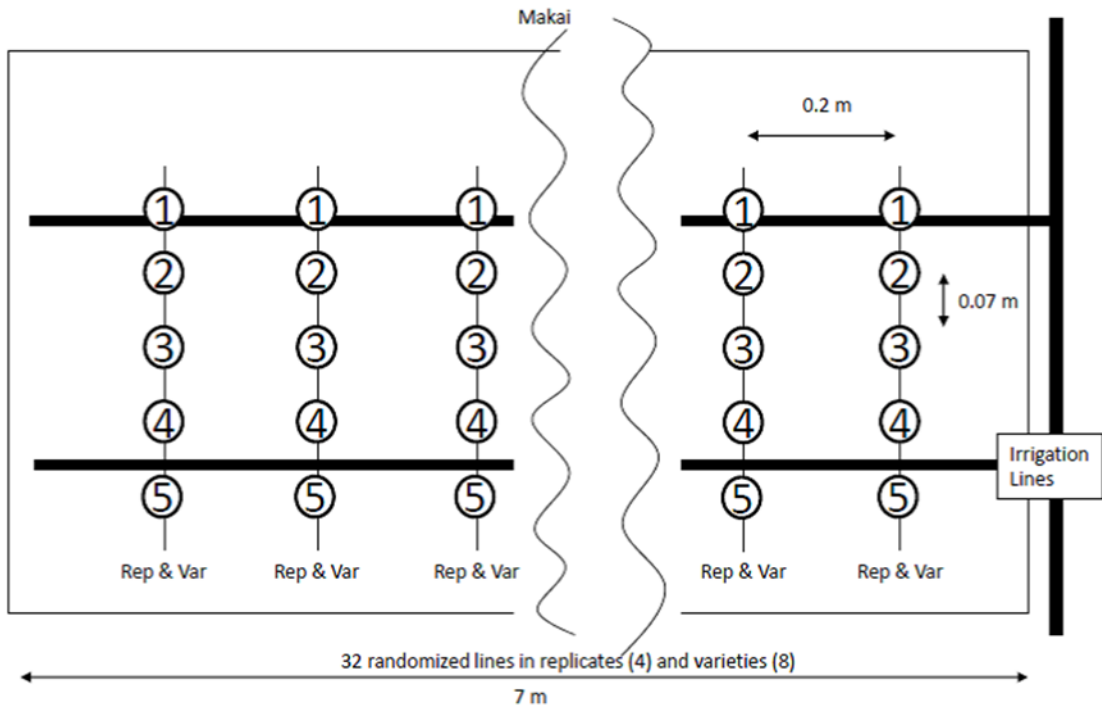
**Appendix A.** Means and standard error of volumetric soil moisture (% v/v) of litterbag decay experiment plot (n=4).



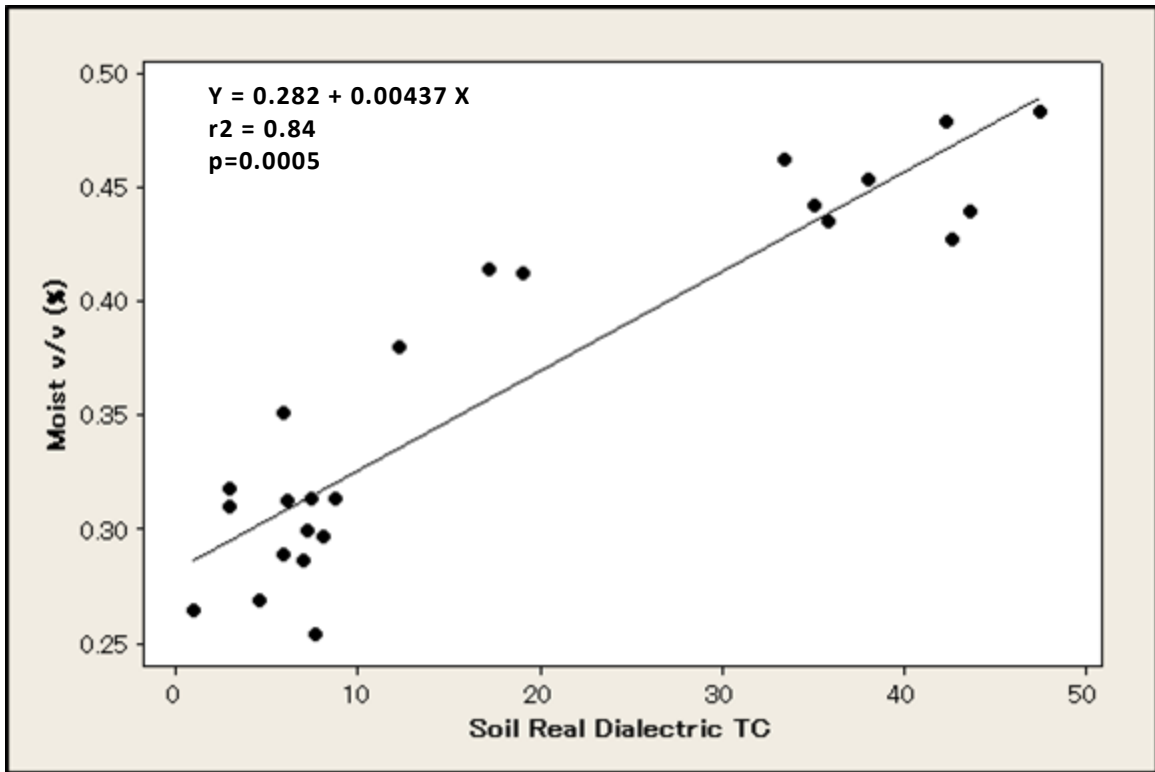
**Appendix B.** Histogram of mass of soil collected to 30 cm depth from Waimanalo in both 2010 and 2011. Arbitrary mean of 300 g was used to approximate 30 cm depth using equivalent mass method.



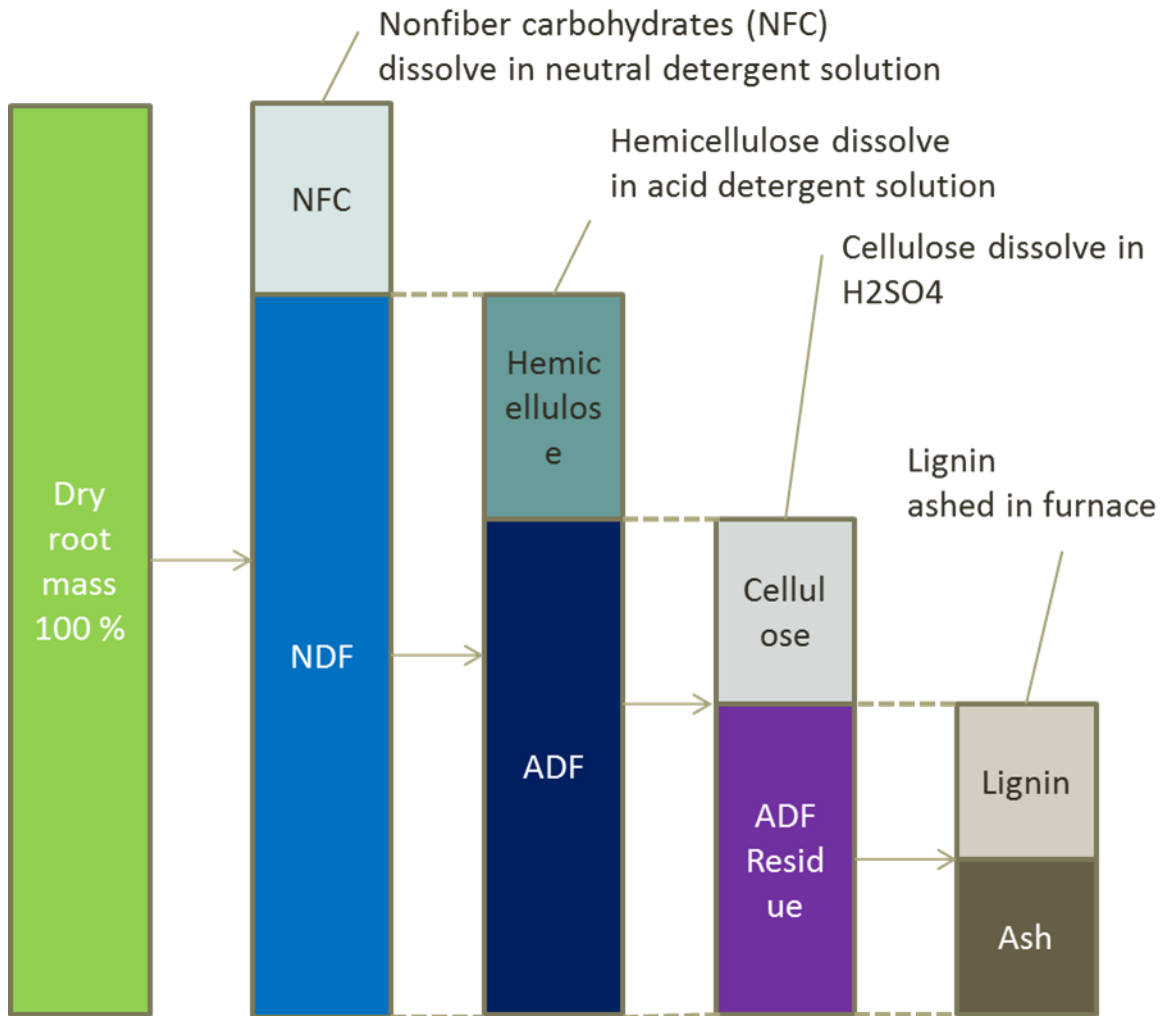
**Appendix C.** Map of the field plots in Waimanalo Research Center



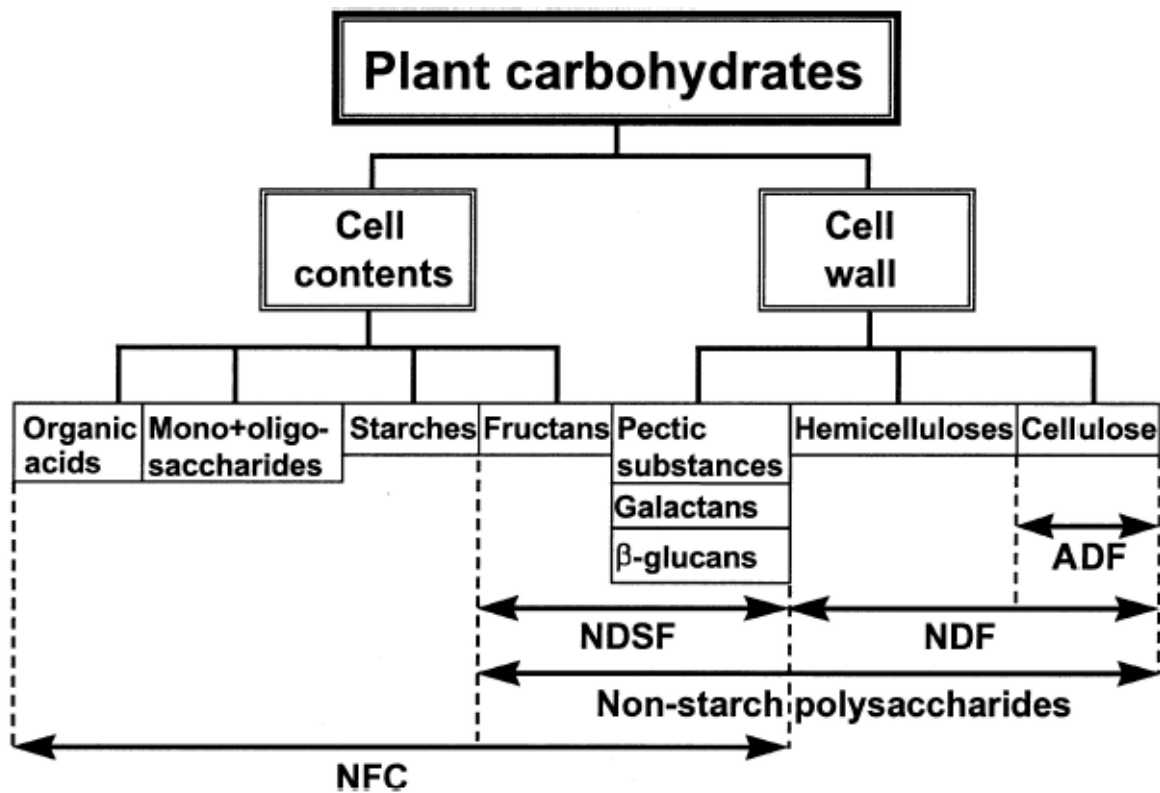
**Appendix D.** Plot design of litterbag decay experiment.



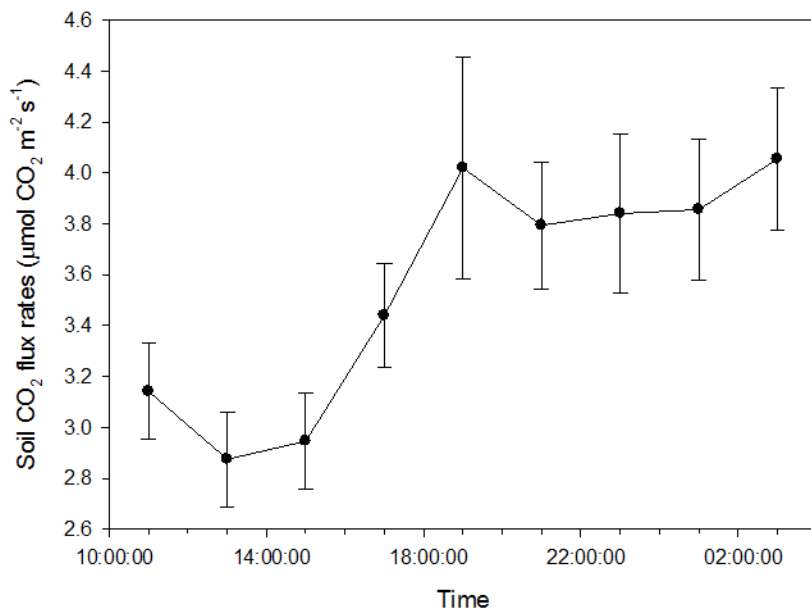
**Appendix E.** Soil moisture probe calibration equation using scatterplot between soil real dielectric measured from probe to volumetric soil moisture obtained by drying volumetric core.



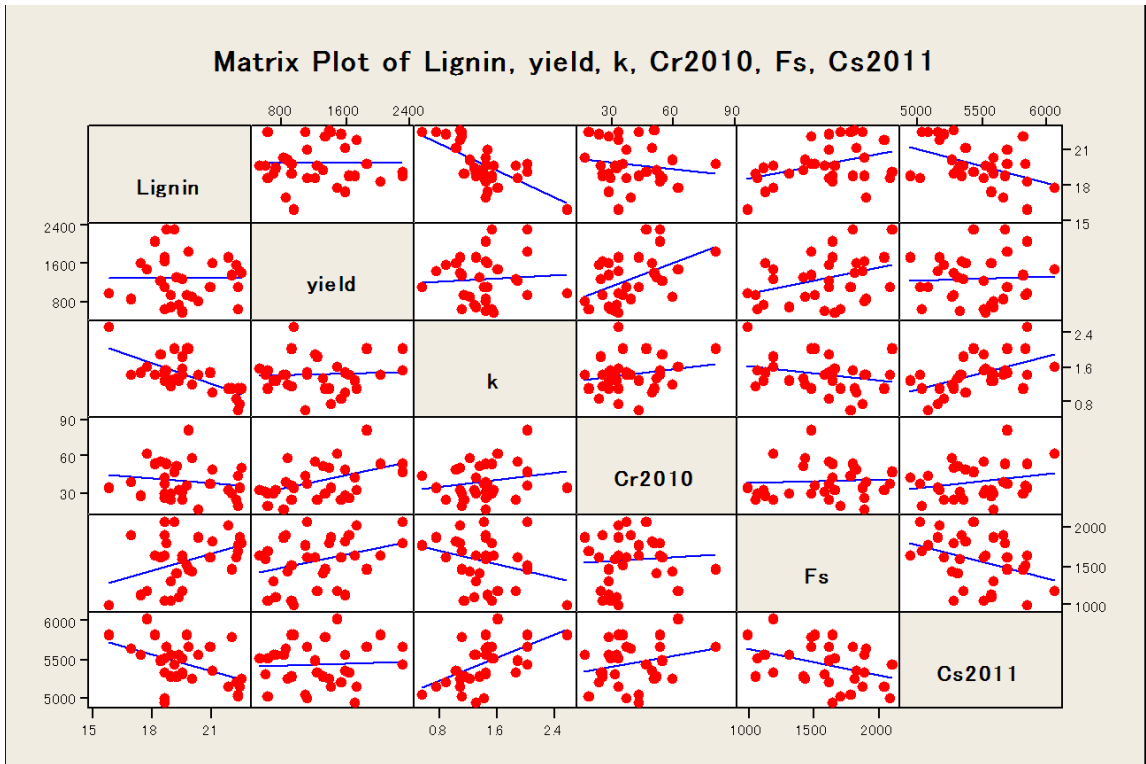
**Appendix F.** Schematic of fiber analysis conducted on root biomass (Van Soest, 1963).



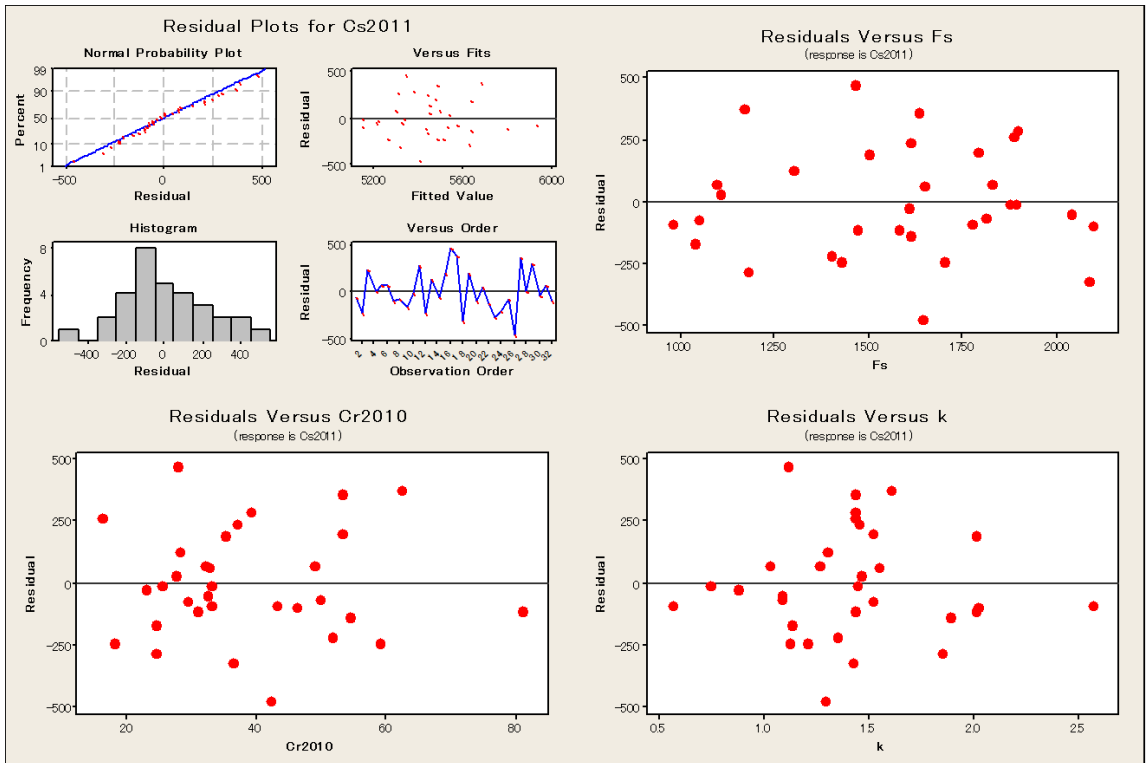
**Appendix G.** Schematic of plant biomass constituents (Obtained from Hall, 2003)



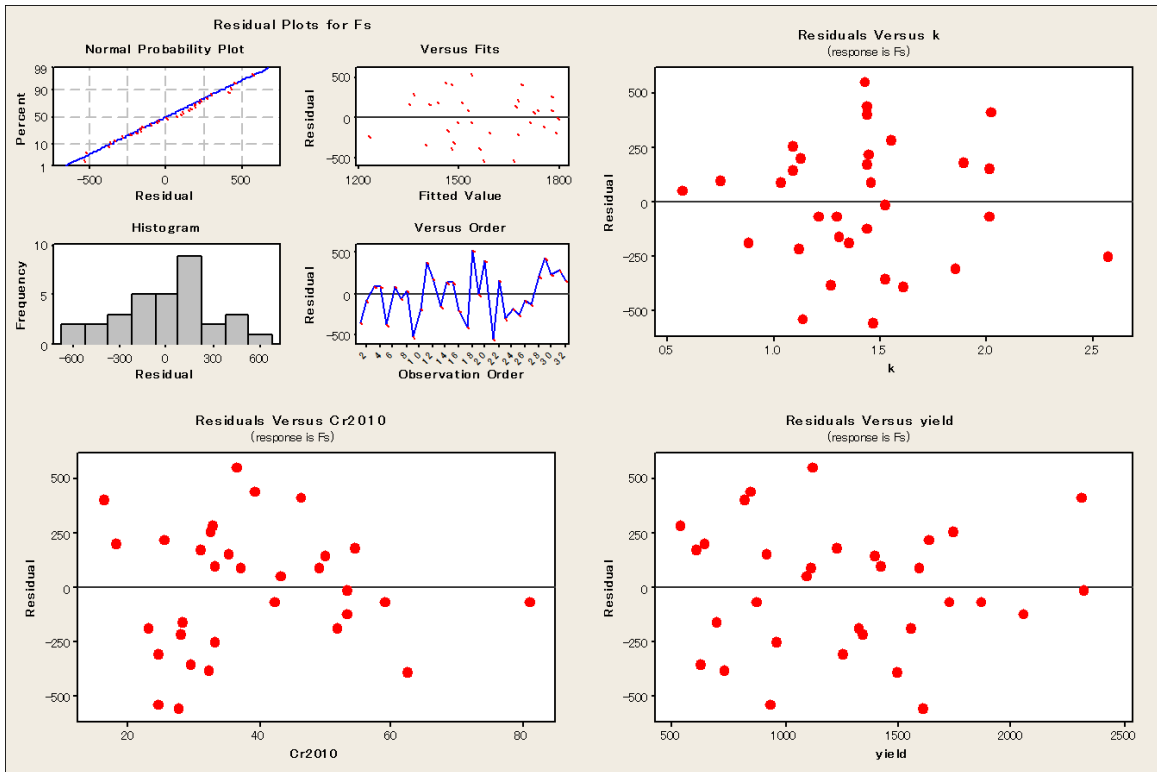
**Appendix H.** Mean soil CO<sub>2</sub> efflux rates and standard error over 16 hours from 11:00 am to 3 am next day to observe diel cycle (n=10).



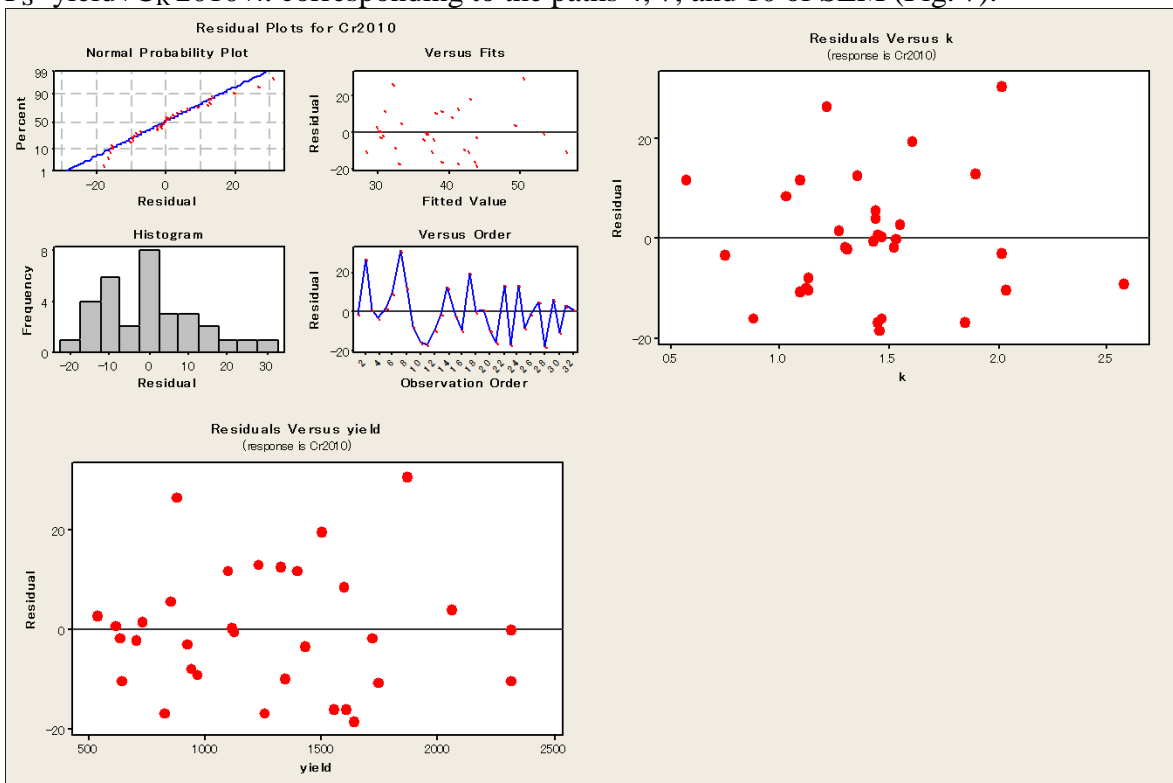
**Appendix I.** Matrix plot of variables involved in SEM to observe multivariate normality, kurtosis, and linearity in bivariate relationships.



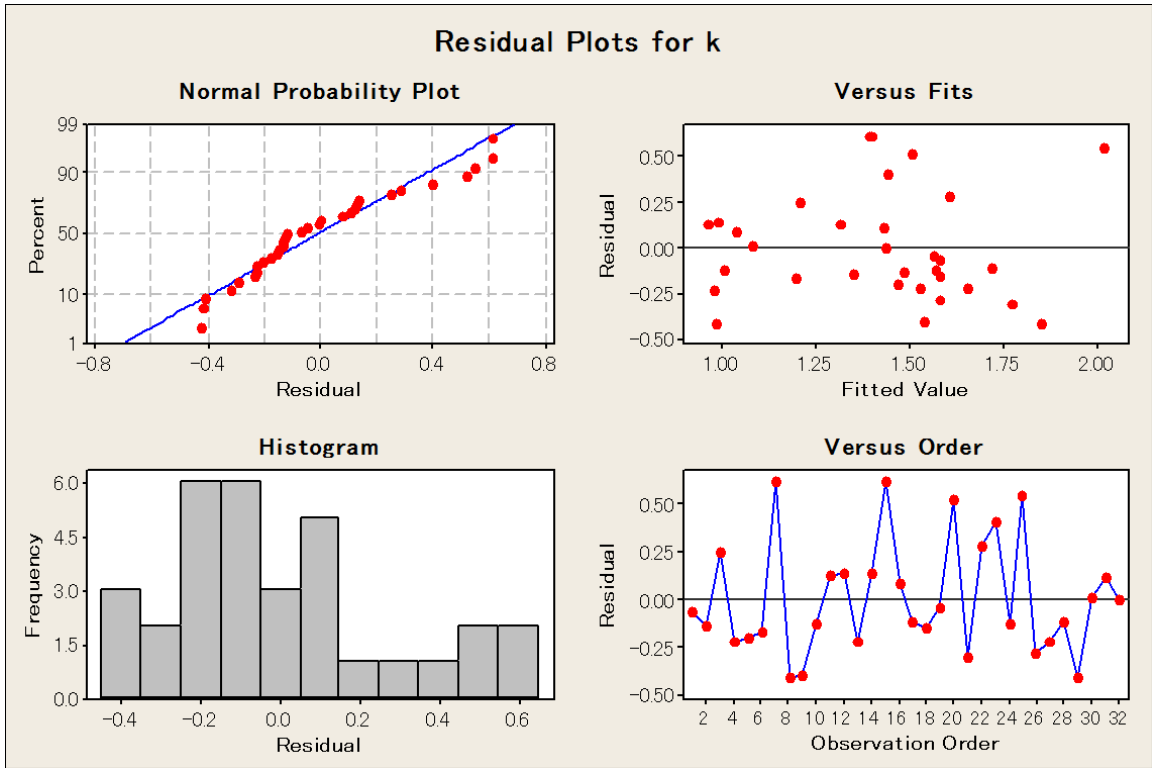
**Appendix J.** Normality of residuals and linearity of relationships ascertained for model  $C_S 2011 = F_S + C_R 2010 + k$  corresponding to the paths 6, 9, and 11 of SEM (Fig. 7).



**Appendix K.** Normality of residuals and linearity of relationships ascertained for model  $F_S = yield + C_R 2010 + k$  corresponding to the paths 4, 7, and 10 of SEM (Fig. 7).



**Appendix L.** Normality of residuals and linearity of relationships ascertained for model  $C_R 2010 = yield + k$  corresponding to the paths 3, and 8 of SEM (Fig. 7).



**Appendix M.** Normality of residuals ascertained for model  $k=\text{lignin}$  corresponding to the paths 1 of SEM (Fig. 7).

**Appendix N.** ANOVA F and P values of the random effect of blocks (as replicates), fixed effect of accessions and custom contrasts of species on (A) pools and (B) fluxes.

			(A) Pools			
	year		Block	Accession	GG vs NG+PxD	NG vs PxD
df			3	7	1	1
C <sub>S</sub>	2010	F	<i>14.17</i>	1.93	1.2	<b>5.98</b>
		P	<i>&lt;.0001</i>	0.118	0.287	<b>0.024</b>
	2011	F	<i>8.03</i>	0.97	0.37	0.05
		P	<i>0.001</i>	0.477	0.547	0.829
C <sub>R</sub>	2010	F	1.24	2.43	2.62	0.16
		P	0.323	0.056	0.122	0.693
	2011	F	1.59	1.8	3.17	2.67
		P	0.222	0.144	0.09	0.118

			(B) Fluxes			
			Block	Accession	GG vs NG+PxD	NG vs PxD
Yield	F		1.45	<b>2.29</b>	<b>5.77</b>	0.89
	P		0.259	<b>0.07</b>	<b>0.026</b>	0.358
F <sub>S</sub>	F		<i>10.21</i>	1.88	0.83	0.56
	P		<i>&lt;.0001</i>	0.128	0.374	0.465
F <sub>A</sub>	F		<i>5.58</i>	<b>4.07</b>	<b>11.99</b>	<b>11.62</b>
	P		<i>0.006</i>	<b>0.006</b>	<b>0.002</b>	<b>0.003</b>
ΔC <sub>S</sub>	F		<i>8.66</i>	<b>2.86</b>	<b>5.16</b>	<b>9.94</b>
	P		<i>0.001</i>	<b>0.031</b>	<b>0.034</b>	<b>0.005</b>
ΔC <sub>R</sub>	F		0.92	0.84	1.28	1.77
	P		0.449	0.564	0.271	0.198
TBCF	F		<i>12.49</i>	1.95	2.57	3.93
	P		<i>&lt;.0001</i>	0.115	0.125	0.061

Significant effects (p<0.05) are given in bold; Significant random effects of block are given in italics. Yield, aboveground yield; F<sub>S</sub>, soil CO<sub>2</sub> efflux; F<sub>A</sub>, litter fall; C<sub>S</sub>, soil C pool; C<sub>R</sub>, root carbon; ΔC<sub>S</sub>, differences in soil C pools; ΔC<sub>R</sub>, differences in root C pools; TBCF, total belowground carbon flux.

**Appendix O.** ANOVA F and P values of the random effect of blocks (as replicates), fixed effect of accessions, and custom contrasts of species on chemical composition of root biomass.

Root fibers					
		Block	Accession	GG vs NG+PxD	NG vs PxD
	df	3	7	1	1
NFC	F	<i>11.08</i>	<b>3.87</b>	<b>18.75</b>	1.79
	P	<i>&lt;.0001</i>	<b>0.008</b>	<b>&lt;.0001</b>	0.195
Hemicellulose	F	2.13	2.38	0.49	<b>5.01</b>
	P	0.128	0.061	0.492	<b>0.037</b>
Cellulose	F	<i>4.21</i>	0.91	1.56	0.48
	P	<i>0.018</i>	0.522	0.226	0.498
Lignin	F	<i>17.48</i>	<b>8.66</b>	<b>52.4</b>	<b>4.75</b>
	P	<i>&lt;.0001</i>	<b>&lt;.0001</b>	<b>&lt;.0001</b>	<b>0.042</b>

C & N, ratios and decay constant

		Block	Accession	GG vs NG+PxD	NG vs PxD
C	F	0.6	<b>5.74</b>	<b>34.39</b>	0.23
	P	0.62	<b>0.001</b>	<b>&lt;.0001</b>	0.639
N	F	17.3	2.15	<b>5.92</b>	2.07
	P	<.0001	0.085	<b>0.024</b>	0.166
C:N	F	15.99	<b>2.71</b>	<b>10.91</b>	1.31
	P	<.0001	<b>0.038</b>	<b>0.004</b>	0.265
Lignin:N	F	17.71	<b>2.83</b>	<b>14.92</b>	2.02
	P	<.0001	<b>0.032</b>	<b>0.001</b>	0.171
<i>k</i>	F	2.28	2.11	<b>10.85</b>	0.49
	P	0.111	0.091	<b>0.004</b>	0.493

Significant fixed effects ( $p < 0.05$ ) are given in bold; Significant random effects of block are given in italics. C, root carbon concentration; N, root nitrogen concentration; NFC, non-fiber carbohydrates; *k*, decay constant.

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