



THE UNIVERSITY OF NEW ENGLAND

School of Environmental and Rural Science

**Vetiver Grass in Australia and Ethiopia: Soil Organic
Carbon Storage potential and Mechanisms for Carbon
Sequestration**

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CERTIFICATION OF DISSERTATION

I certify that the ideas, experimental work, results, analyses, software and conclusions reported in this dissertation are entirely my own effort, except where otherwise acknowledged. I also certify that the work is original and has not been previously submitted for any other award, except where otherwise acknowledged.



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Date

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PREFACE

This thesis is written in thesis by publication (journal article) format and parts of the literature review and references might be repeated in succeeding chapters. Formatting of the review and each experimental chapter follows the editorial styles of the relevant journals. Formatting of introduction and the synthesis/Conclusion chapters follows the editorial style of Soil Research.



Please be advised that this thesis contains chapters which have been either published or submitted for publication.

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ABBREVIATIONS

ANOVA	Analysis of Variance
ASC	Australian Soil Classification
BD	Bulk Density
C ₃	Plants that follow a C ₃ photosynthetic path ways
C ₄	Plants that follow a C ₄ photosynthetic path ways
CIAT	Tropical Pastures Program of the Centro Internacional de Agricultura Tropical
CO ₂	Carbon Dioxide
CSIRO	Commonwealth Scientific and Industrial Research Organization
EIAR	Ethiopian Institute of Agricultural Research
EWNRA	Ethio wetlands and Natural Resources Association
FDC	Fractions calibration database
H ₃ PO ₄	Orthophosphoric acid
HOC	Humus Organic Carbon
IRMS	Isotope Ratio Mass Spectrometer
LECO	Laboratory Equipment Corporation elemental analyser
Mg ha ⁻¹	Mega Gram per Hectare
MIR	Mid-infrared spectroscopy
NMR	Nuclear magnetic resonance spectroscopy
PCA	Principal component analysis
PLSR	Partial least squar regression
POC	Particulate organic carbon
POM	Particulate organic matter
ROC	Resistant organic carbon
SCaRP	Soil carbon research program
SE	South East
SOC	Soil organic carbon
SW	South West
TN	Total nitrogen
TOC	Total organic carbon
USDA	United States Department of Agriculture
WRB	World reference base
WB	World Bank
δ ¹³ C	Delta thirteen carbon (isotopic ratio- ¹³ C/ ¹² C)

ABSTRACT

Globally, soil organic carbon (SOC) has declined as a result of human induced disturbance with negative effects on production and productivity. Maintaining SOC has the combined effect of contributing to climate change mitigation efforts and agro-ecosystem functioning in addition to its potential for sustaining soil health. A primary source that can contribute to soil carbon (C) sequestration is plant biomass, and an important component of this is the biomass found below-ground. SOC sequestration using plant species with high photosynthetic efficiency, deep roots and high biomass production therefore has considerable potential for soil carbon storage. Perennial tropical grasses, particularly those with deep root systems, are therefore likely to contribute significantly to SOC and the introduction of perennial tropical grasses could potentially contribute large quantities of C through the soil profile and facilitate SOC sequestration. A range of tropical pasture species have been investigated for their SOC storage potential, but vetiver grass, given its extensive use globally and its large biomass production, has considerable, as yet unquantified, potential for long term C storage. The main aim of this research was to examine the SOC quantity, nature and distribution in soils under vetiver. Specifically, the work 1) examined SOC content, stock and profile distribution under vetiver; 2) determined the quantity of SOC attributable to vetiver (C_4 -C) compared with soil dominated by previous C_3 carbon; 3) examined the above- and below-ground vetiver biomass production and the relative rate of decomposition, and 4) determined the allocation of soil C under vetiver to its component fractions (POC, HOC and ROC) differentiated on the basis of particle size and chemical composition.

A series of research questions were examined under this PhD research work: *In chapter 3* undisturbed soil core samples were collected to 1.0 m soil depth from Gunnedah, Australia to determine the soil carbon content and depth distribution down the soil profile under vetiver compared with native and tropical pastures and cropland soil. The result showed a larger TOC stock under vetiver (123 Mg ha^{-1}) compared with tropical pasture (93 Mg ha^{-1}) and cropping soils (78 Mg ha^{-1}) while vetiver and native pastures (111 Mg ha^{-1}) showed no significant difference in TOC stocks. For all plant types, a decrease in SOC content was observed with increasing soil depth but a larger stock of C was found under vetiver at almost all depths through the soil profile compared with cropping soils, but on an annual basis, not much more than other tropical grasses. Soils under vetiver had higher (less negative) $\delta^{13}\text{C}$ compared with native, tropical pastures and cropping soils. This was particularly true in the surface soil layers but persisted to some degree through the whole soil profile. Both litter and roots probably contributed to the additional C stock by vetiver (43.5%) and results indicated a significant C turnover through the whole soil profile resulting in a modest net accumulation of soil C.

In *chapter 4* the impact of vetiver grass on carbon sequestration and its SOC input and the quantity of SOC attributable to vetiver (C_4 carbon) compared with soil dominated by pre-existing (C_3) Carbon determined. Undisturbed soil core samples were collected to 1.0 m soil depth from Southwest Ethiopia. The result showed a larger TOC stock under vetiver (mean 262 Mg C ha⁻¹) compared with coffee (mean 178 Mg C ha⁻¹), particularly, at the surface soil layers and decline was observed with increasing soil depth between plant types. Low $\delta^{13}C$ (more negative) values were recorded at the soil surface layers increasing with increasing soil depth for both vetiver and coffee. However, the $\delta^{13}C$ values were significantly higher (less negative) under vetiver in comparison with coffee, particularly at the surface soil layers which suggests a continuous new C addition and a significant C turnover in the soil system.

In *chapter 5* vetiver plant material was therefore grown under a glasshouse condition for biomass production assessment and subsequently incubated to determine the relative decomposition rate between the above- and below-ground vetiver biomass in different soil types. Vetiver showed a high biomass production (268 Mg ha⁻¹ of fresh and 120.2 Mg ha⁻¹ of dry biomass) potential and the shoot to root biomass ratio was determined to be 1.49 and 1.28, for the fresh and dry biomass, respectively.

In *chapter 6* the amount of allocation of soil carbon to particulate, humus and resistant fractions differentiated based on particle size and chemical composition. The stocks of soil C fractions indicated significant variations which changes from the labile POM to the HOM across site and vegetation types. Hence, the dominant C fraction was HOC (58%) for vetiver and all vegetation types. The ratio of POC to HOC stocks was also very low indicating the lesser vulnerability of C because of the high proportion of HOC component fraction given its less labile nature which could help the carbon stay in the soil for longer time and changes quite slowly.

Despite the continuous new C addition under vetiver the significant soil C turnover could be due to the more rapid decomposition of the root material than the shoot which could have been impacted by the lower C:N ratio of the root compared with the shoot. Hence, promoting the use of vetiver, particularly due to its potential to produce a large biomass, is a promising strategy to enhance soil C storage. Hence, growing vetiver has the potential for high rate of C accumulation because this grass is building up the more stable HOC fraction which is less vulnerable to change and to use this in the C accounting program can be feasible. This study investigated that vetiver due to its fast growth, large biomass production (both above- and below-ground) potential and extensive use has considerable potential for C sequestration, particularly on C depleted soils. *In conclusion*, in this work it has been demonstrated that vetiver grass has an important role in storing large TOC stock, has the potential to add new carbon despite high rates of turnover; produce high biomass and have high root to shoot decomposition which might be a reason for high turnover rates and larger organic carbon

accumulation in the more resistant (hemic organic carbon fraction) carbon pool throughout the 1.0 m soil profile and has considerable potential for both restoration of soil health and for storing additional soil carbon to offset greenhouse gas emissions.

Key words: Australia, biomass, carbon fractions, *Coffea Arabica*, decomposition, Ethiopia, native pastures, soil carbon, tropical grass, vetiver

PUBLICATIONS ARISING FROM THIS THESIS

The contents of some of the chapters of this research thesis have been submitted to journals and are awaiting publication or have been presented in national and international conferences.

Journal Articles

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Tessema, B., Wilson, B., Daniel, H., Baldock, J., Adimassu, Z. and Kristianson, P. (2018). Soil Carbon Storage Potential and Depth Distribution under Vetiver (*Chrysopogon zizanioides*) Grass in SW Ethiopia and its Implications. (Submitted to Soil Use and Management Journal).

Tessema, B., Wilson, B., Daniel, H., Baldock, J., and Kristianson, P. (2018). Functional Links between SOC, Biomass and Decomposition of Vetiver (*Chrysopogon zizanioides*) grass in Soils of Different Texture. (To be submitted to Soil Use and Management Journal or similar).

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Conference Proceedings and Presentations

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Tessema, B., Wilson, B., Daniel, H., Baldock, J., and Adimassu, Z. (2016). Soil Carbon Change in Different Pasture Types in SE Australia. Proceedings of the 10th International Rangeland Congress: *The Future Management of Grazing and Wild Lands in a High Tech World*, 16-22 July 2016. Saskatoon, SK, Canada

Tessema, B., Wilson, B., and Daniel, H. (2014). Soil carbon storage under the tropical grass Vetiver (*Vetiveria zizanioides*; *Chrysopogon zizanioides*) in Australia. Poster presentation. Proceedings of the Soil Science Australia National Soil Science Conference. 23-27 November, 2014, Melbourne, Australia.

CHAPTER 1: INTRODUCTION

General Introduction

Vetiver grass (*Vetiveria zizanioides*; *Chrysopogon zizanioides*) is a fast growing C₄ perennial tropical grass of the Poaceae family (Gaspard et al., 2007; Abate & Simane, 2010). It can grow up to 1–2 m tall above ground and can produce a large and complex root system penetrating deep into the soil profile (Lavania, 2003; Gaspard et al., 2007). Unlike most other grasses which spread and stabilize soil horizontally, all types of vetiver roots penetrate the soil vertically (Lavania, 2003; Lavania & Lavania, 2009). Lavania and Lavania (2009) stated that the roots of vetiver are fast growing as a bunch and can extend as much as 3 cm per day or up to 2 m in just six months into the deeper soil layers. Vetiver tolerates intensive harvest, grazing or mowing and does not spread as a weed (Hon et al., 2008). In a study of three cultivars of vetiver under saline irrigation, Tomar and Minhas (2004) and Singh and Dagar (2009), found that vetiver produced 72.6 -78.7 Mg ha⁻¹ shoot dry biomass and 1.12 to 1.71 Mg ha⁻¹ root biomass over three years in a sandy-loam soil.

Vetiver is widely distributed in tropical and sub-tropical regions of the world, principally in India, its place of origin, but is also now common in Southeast Asia, tropical Africa, South Africa, and Central and South America (Greenfield, 1988; Lavania & Lavania, 2009; Abate & Simane, 2010). Vetiver also has a range of species (*Chrysopogon zizanioides*, *Chrysopogon filipes*) native to different regions and countries such as Africa and Australia (Veldkamp, 1999; Lavania, 2003; Lavania & Lavania, 2009; Abate & Simane, 2010). Vetiver grass is adapted to a wide range of climatic conditions from dry to wet and soil types from sandy to clay rich, acidic or alkaline (Lavania & Lavania, 2009; Abate & Simane, 2010). It is known to grow under a wide temperature range (-15°C to > 55°C), soil pH < 3; > pH 10, annual rainfall (< 300 mm to > 5,000 mm), is tolerant to salinity, prolonged water logging and is resistant to pests and diseases (Grimshaw, 2008). It can tolerate intensive harvest, grazing or mowing and it does not spread as a weed (Adams et al., 2008). A native grass of India, vetiver was initially valued for its aromatic oil (Lavania & Lavania, 2009), but has become a multipurpose grass used extensively for livestock fodder, roof thatching, handicrafts, perfumes, medicine, insect control and as an ornamental planting in gardens (Greenfield, 1988; Grimshaw & Helfer, 1995; Truong, 2000; Truong et al., 2001; Percy & Truong, 2003; Truong et al., 2004; Abate & Simane, 2010; Singh et al., 2011).

Due to its fast growth, tolerance of a range of environmental conditions and ease of establishment, vetiver is also considered to be an effective solution to environmental degradation and is widely used for conservation, rehabilitation including soil and water conservation, steep slope stabilization, remediation of contaminated and saline soils, land rehabilitation, stream bank stabilization and

wastewater treatment (Truong, 2000; Chen et al., 2004a; Gaspard et al., 2007; Abate & Simane, 2010). Vetiver has been called a “living nail” or “living contour bank” due to its effectiveness in soil conservation and use in road and water infrastructure (Abate & Simane, 2010). Vetiver is now being used extensively in Ethiopia as a biological soil and water conservation measure, for steep slope stabilization and wetland rehabilitation (Awoke, 2002; Hailu, 2009; Kebede & Yaekob, 2009; Abate & Simane, 2010; Terefe, 2011; Awoke, 2013). There is also potential for its future use as a biofuel source, and in conservation and carbon sequestration (Truong, 2000).

Clifton-Brown et al. (2007), suggested that perennial grasses have significant potential to accumulate carbon and have advantages over trees for biomass and carbon accumulation due to their more rapid establishment and potential for annual harvest. Tropical perennial grasses are especially useful in this regard. It has also been proposed that perennial grasses translocate large quantities of carbon to the root system as a reserve for spring growth and can therefore be introduced as a viable option to facilitate soil carbon sequestration (Zimmermann et al., 2012). Several studies relating to above- and below-ground carbon storage have focused on a range of perennial tropical grasses (Gaspard et al., 2007; Altenor et al., 2009; Lavania & Lavania, 2009; Singh et al., 2011; Singh et al., 2013). For example, *Miscanthus*, is a species with a similar growth habit to vetiver, including a deep root system and high biomass production. This grass has been shown to accumulate significant quantities of additional soil carbon down to 1.0 m soil depth (Schneckenberger & Kuzyakov, 2007; Zimmermann et al., 2012). Schneckenberger and Kuzyakov (2007) showed a 3.0 and 2.4 g (kg soil)⁻¹ new carbon on a sandy and loamy soils, respectively for 0-10 cm layer of the soil profile. In addition Poehlau and Don (2013), observed a mean C₄ carbon sequestration rate of 0.78 - 0.19 Mg ha⁻¹ yr⁻¹ under *Miscanthus*, which increased with mean annual temperature. A study conducted by the International Center for Tropical Agriculture (CIAT) on *Andropogon guyanus* (gamba grass/Rhodesian bluegrass/tambuki grass), a closely related grass to vetiver, with a root system penetrating up to 1m in tropical soils, demonstrated a capacity to store up to 53 tonnes of CO₂ equivalent as soil organic matter ha⁻¹ yr⁻¹ (Vietmeyer, 1997; Truong, 2000).

Vetiver, due to its fast growing nature and the deep root system, has been recommended as a potential candidate for carbon sequestration (Lavania & Lavania, 2009). Vetiver root penetration into subsoil is a potential mechanism of facilitating long term carbon storage deep in the soil, reducing the chance of decomposition and carbon loss. However, few field level estimates of soil carbon sequestration potential of vetiver appear to be available (Singh et al., 2011). Some studies have compared vetiver to other grasses of a lesser performance in terms of growth rate and biomass carbon accumulation (Gaspard et al., 2007). For example, Tomar and Minhas (2004), demonstrated a high dry matter production potential of vetiver (99.9 t ha⁻¹ dry weight basis) under saline irrigation compared with lemon grass (*Cymbopogon citratus*) and palmarosa grass (*Cymbopogon martinii*). Vietmeyer (1997),

estimated the rate of carbon storage by a single vetiver plant to be as much as 2.0 kg year⁻¹ assuming vetiver roots penetrated up to 5m in tropical soils. Similarly Lavania and Lavania (2009), assuming 50% dry matter of the vetiver root as carbon, estimated a potential addition of 1.0 kg m⁻² year⁻¹ of soil carbon. Singh et al. (2011), indicated the potential significance of carbon sequestration by vetiver grass in India and suggested that carbon sequestration (in biomass) could be as much as 20 Mg C ha⁻¹. These workers also recommended that the grass could be a sustainable option to contribute to climate change mitigation at a small farmer- or larger-scales. However, empirical studies of the contribution of vetiver to soil carbon are needed to more directly determine such potential for impacting on climate change mitigation.

Although it has not been used extensively in Australia, the value of vetiver has been demonstrated in Victoria where, due to its salt tolerance, it has been used to lower saline water tables (Truong et al., 2004). A range of perennial tropical grasses such as Kikuyu (*Pennisetum clandestinum*) and Rhodes (*Chloris gayana*) grass have been studied in Australia for their potential for soil carbon storage and concluded that the SOC accumulated was dominated by SOC derived from these grasses (Sanderman et al., 2013a; Sanderman et al., 2013b). Vetiver, as a fast growing, large biomass producing plant could be a potential candidate for carbon sequestration, however evidence on the quantity and mechanisms of SOC storage needs to be investigated.

Aims and Objectives

While there are many reports highlighting the potential of perennial grasses to increase SOC, further research is needed to experimentally quantify the effects of land management practices using vetiver on SOC storage potential, including sampling of deeper soil horizons (e.g. down to 1.0 m). The considerable variation in SOC concentrations under pastures indicates that many other factors may influence carbon storage, e.g. soil type, elevation. Further research is therefore needed to elucidate the impact of these factors on SOC concentrations in soils at a landscape scale. Of particular importance is the need to determine the rate of turnover and cycling of added carbon and the extent to which it is retained in the soil system. The major objectives of this project were therefore to examine the carbon quantity, nature and distribution in soils under vetiver. The specific aims of this research work were to:

1. Measure and estimate soil carbon content and depth distribution down the soil profile under vetiver compared with native and tropical pastures and other crops
2. Quantify the impact of vetiver grass on carbon sequestration and its soil organic carbon input and determine the quantity of SOC attributable to vetiver (C₄ carbon) compared with soil dominated by previous (C₃) carbon,

3. Quantify vetiver above- and below-ground biomass and the relative rate of carbon evolved from the decomposition of both above- and below-ground biomass in different soil types, and
4. Quantify the allocation of soil carbon to particulate, humus and resistant fractions differentiated on the basis of particle size and chemical composition.

Thesis Outline

Chapter 1: Introduction, scope and aim of the research project

This chapter provides a brief overview and scope of the study. It identifies the research gaps and presented the thesis aims and key research questions addressed in this thesis.

Chapter 2: Soil Carbon Storage Potential of Tropical Pastures: A Review

This chapter presents a review of literature on the important principles and studies related to the current study, assessing the works undertaken in the area and identifying the relevant research gaps.

*Chapter 3: Soil Carbon Storage and Distribution under Vetiver (*Chrysopogon zizanioides*) using Stable Isotope Analysis*

This chapter addresses the amount and depth distribution of carbon stored under vetiver compared with native and other tropical pastures in Australia. The chapter analyses the soil carbon storage potential of vetiver compared with native pasture and other tropical pastures and the new carbon added by vetiver in Australia.

*Chapter 4: Soil Carbon Storage Potential and Depth Distribution under Vetiver (*Chrysopogon zizanioides*) Grass in SW Ethiopia and its Implications*

This chapter addresses the amount and depth distribution of soil carbon stored under vetiver compared with other crops in Ethiopia. The chapter investigates the soil carbon storage potential of vetiver grass and the depth distribution compared with coffee in Ethiopia.

*Chapter 5: Functional Links between SOC, Biomass and Decomposition of Vetiver (*Chrysopogon zizanioides*) grass in Soils of Different Texture*

This chapter addresses the relative proportions of vetiver root and shoot biomass and the relative decomposition (the rapidity of decomposition) potential and how this differs with different soil texture. This chapter also evaluates the above- and below-ground biomass production potential of vetiver grass under a glasshouse conditions using a range of contrasting soil types.

Chapter 6: Predicted Contents of Soil Carbon Fractions under Vetiver Grass in Australia and Ethiopia

This chapter addresses the quantifies the dominant carbon fractions added in the soil system under vetiver, mixed native pastures and coffee in Australia and Ethiopia. The chapter documents the quantity, vertical distribution and turnover of each fraction, and the mechanism of carbon addition by vetiver.

Chapter 7: Synthesis

The final chapter summarizes how the empirical research links together and collectively addresses the gaps in the literature regarding the effect of vetiver on soil carbon dynamics. The chapter also provides recommendations for further research work.

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CHAPTER 2: Soil Carbon Storage Potential of Tropical Grasses: A Review

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Abstract

Environmental degradation and climate change are key current threats to world agriculture and food security and human-induced changes have been significant drivers of this global environmental change. An important component is land degradation which results in a diminished soil organic carbon stock with concomitant loss of soil condition and function. Land management to improve soil organic matter content, condition and productivity is therefore a key strategy to safeguard agricultural production, food supply and environmental quality. Soil organic carbon sequestration through the use of plant species with high photosynthetic efficiency, deep roots and high biomass production is one important strategy to achieve this. Tropical pastures have particular potential in this regard and have been used extensively for land rehabilitation. Tropical pastures are adapted to a wide range of environmental conditions and have advantages over trees for biomass and carbon accumulation due to their rapid establishment, suitability for annual harvest, continual and rapid growth rates. Tropical pastures also have the potential for organic carbon storage in subsoil horizons due to their deep root systems and have potential use as biomass energy crops which could further promote their use as a climate change mitigation option. Here we aimed to review current knowledge regarding the soil organic carbon storage potential of tropical grasses worldwide and identified knowledge gaps and current research needs for the use of tropical grasses in agricultural production system.

Key words: Soil carbon, Tropical grass, vetiver grass, climate change mitigation

Introduction

Environmental degradation and climate change are key current threats to world agriculture and food security (Rosenzweig & Hillel, 1998; Fischer et al., 2002; Lal, 2004a; Nelson et al., 2009; Hamza & Iyela, 2012). Human-induced changes to land cover have been significant drivers of this global environmental change, of which, soil degradation resulting from land conversion, agricultural intensification, soil disturbance and increased erosion have been key factors (Lambin et al., 2001; Lal, 2004b; Girmay & Singh, 2012; Meshesha et al., 2014). An important component of this land degradation globally has been a diminished soil organic carbon (SOC) stock with concomitant loss of soil condition and function, compromising food production and agricultural sustainability (Lambin et al., 2003; Pielke Sr et al., 2003; Chapin III et al., 2010). Land and soil management to increase soil organic matter content, soil condition and productivity is therefore a key need globally to safeguard agricultural production, food supply and environmental quality.

Organic carbon in soils globally is estimated to be between 1500 and 1600 Gt (Eswaran et al., 1993; Jobbágy & Jackson, 2000) to 1.0 m depth which represents a significant component of the global carbon cycle, storing more carbon than is contained in vegetation and the atmosphere combined (Batjes, 1998; Houghton, 2005a, 2005b). It has been estimated that worldwide, soils have lost between 42 and 78 Gt of their original SOC as a result of management pressures (Lal & Follett, 2009). With this carbon depletion, however, comes a significant opportunity, since soils are believed to have the capacity to store an additional 0.4–1.2 Gt Cyear⁻¹ with the introduction of more judicious land management practices (Lal, 2003; Freibauer et al., 2004; Lal, 2004b, 2004a; Lal et al., 2004; Rabbi et al., 2014). As such, soils globally have considerable potential to offset greenhouse gas (GHG) emissions and SOC storage has been widely promoted as an important strategy to help meet national and international emissions reduction targets (Smith et al., 2014). Additional SOC storage might therefore have the dual benefit of contributing to our response to climate change globally whilst helping to restore soil condition and function to promote sustainable land management, improved production and productivity (Lal, 2003; Freibauer et al., 2004; Lal, 2004b, 2004a; Rabbi et al., 2013b).

Methodologies and management practices that reduce SOC loss or promote the storage of additional soil carbon are being actively investigated globally. It has been widely reported

that cultivation accelerates organic matter decomposition by exposing sites within soil aggregates that were previously protected (Kimble et al., 1998; Grandy & Robertson, 2006; Janik et al., 2007; Gallo et al., 2009; Schuman et al., 2009; McKenzie & Mason, 2010) while soil erosion, vegetation clearing and removal of crop residue are also known to result in long-term soil carbon loss (Lemma et al., 2007; Shiferaw et al., 2013). However, there are management practices which seem to either arrest SOC loss (e.g. minimum tillage) or to promote carbon storage such as afforestation, pasture conversion, grazing management, cover crops, water harvesting, erosion control and the use of soil amendments including biochar (Oladele & Braimoh, 2011). Not all of these are practical in production landscapes globally and not all will be equally effective in the management of SOC. The effectiveness of various management practices is therefore being explored to facilitate optimum carbon storage that can be integrated with agricultural production systems.

An approach that has attracted particular attention is the use of perennial grass species within the production system, which appear to significantly increase SOC across a range of environments and this is particularly true where these perennial grasses replace cropping systems (Davidson & Ackerman, 1993; Paustian et al., 1997; Conant et al., 2001; Young et al., 2005). Pastures are varied in terms of their geographical distribution and species composition comprising native and exotic, annual and perennial grasses, legumes, herbs and shrubs (Lesslie et al., 2006). They are the primary resource for many farm industries and are the basis for the production of meat, wool, milk and fodder Schuman et al. (2002) estimated the SOC under grazing lands of the world to be 10–30% of the total global SOC stock. While, Janssens et al. (2005) estimated the overall C sink in grassland soils of most European countries to average approximately $60\text{g C m}^{-2}\text{year}^{-1}$.

Tropical perennial grass species have been particularly promoted due to the high biomass and carbon accumulation resulting from their excellent photosynthetic efficiency, rapid establishment, fast growth, deep root systems and potential annual harvest (Clifton-Brown et al., 2007; McKenzie & Mason, 2010; Schwenke et al., 2014) and Parton et al. (1993) suggested that tropical grasses have significant potential as a carbon sink. However, there is a research need to fully quantify their capacity to store additional soil carbon relative to other management systems and hence, their potential for GHG abatement and soil condition recovery (Tubiello et al., 2007; Lavania & Lavania, 2009; Chan & McCoy, 2010).

Here we aim to review current knowledge with regard to the biomass production and SOC storage potential of pastures compared with other land management systems worldwide with a particular focus on tropical pastures. We identify knowledge gaps and current research needs to fully explore the potential of these tropical pasture species for carbon storage.

Cropland Conversion to Perennial Pastures

Cropland conversion to pasture is believed to have considerable potential to store significant quantities of additional SOC (Schuman et al., 2002; Freibauer et al., 2004; Derner & Schuman, 2007). For example, Conant et al. (2001) and Conant (2012), reviewed studies worldwide and concluded that cropland conversion to grasslands can create a significant carbon sink, with a mean 5% annual increase in SOC. In the mid-western United States, agricultural land conversion to perennial grassland showed a constant rate of $62\text{g C m}^{-2}\text{ year}^{-1}$ SOC accumulation over 40 years in the top 10 cm (McLauchlan et al., 2006). Similarly, Abberton et al. (2010) reported that, in temperate regions, most grasslands can be considered soil carbon sinks of up to $40\text{g C m}^{-2}\text{ year}^{-1}$ following cropland conversion. Post and Kwon (2000), further estimated that land use change from cropping to grassland could result in an increase of $33.2\text{g soil C m}^{-2}\text{ year}^{-1}$ in the USA. A meta-analysis in temperate grasslands also showed that at the 0–30 cm soil depth over 20 years SOC sequestration reached $44\text{g C m}^{-2}\text{ year}^{-1}$ which is half of the rate ($95\text{g C m}^{-2}\text{ year}^{-1}$) at which SOC is lost over a 20 year period following permanent grassland conversion to an annual crop (Soussana et al., 2004). These estimates suggest that SOC recovery is possible but is usually slower than initial loss. Research in the south eastern United States also suggested up to $100\text{g C m}^{-2}\text{ year}^{-1}$ could be sequestered in soil following conversion of cropland into optimally grazed pastures (where the available pasture matches the animal needs). These increases have been attributed to the fast growth habit of pastures, negligible erosion and the minimal disturbance to soil compared to cropping (McKenzie & Mason, 2010). Hence, these studies demonstrate the potential increase of SOC as a result of cropland conversion to grasslands.

In Australia, conversion of crop and annual pasture lands to perennial pasture has been widely promoted for improved soil condition and fertility in existing farming systems (McKenzie & Mason, 2010; Sanderman et al., 2013b). An improved pasture, usually grasses in combination with legume systems, could be regarded as an attractive option for CO₂ mitigation because of their soil carbon sequestration potential (Amézquita et al., 2010; Chan

& McCoy, 2010). Cropland conversion to perennial pasture might therefore, be an important strategic option to improve soil health and SOC storage. However, significant knowledge gaps remain regarding the quantity of carbon that might be stored under cropland conversion to different pasture types under different management practices and locations, the distribution of this carbon through the whole soil profile, rates of turnover of carbon thus added and ultimately the stability and longevity of this new carbon.

Soil Organic Carbon Storage under Tropical Pastures

Tropical pastures grow widely in tropical regions of the world and many of them have adapted to grow under a variety of environmental conditions. Many of these pasture species have a distinctive carbon fixing (photosynthesis) pathway and are referred to as C₄ plants (Chan & McCoy, 2010). **Most** plant species have the more primitive C₃ pathway, described by the Calvin Cycle (Ludlow et al., 1985) but an additional C₄ pathway evolved in species in the wet and dry tropics. C₄ pastures are those that have the photosynthetic processes divided between mesophyll and bundle sheath cells that are anatomically and biochemically separate, while C₃ pastures are those which use only the Calvin cycle photosynthesis pathway for fixing CO₂ which takes place inside the chloroplast in mesophyll cells (Ludlow, 1985; Wang et al., 2012).

In terms of photosynthetic efficiency, C₄ grasses are approximately 50% more efficient than C₃ plants as a result of this distinctive carbon fixation mechanism (Kajala et al., 2011). Wang et al. (2012), indicated that more efficient use of light and CO₂ in C₄ plants results in an increase in both biomass production and CO₂ fixation. Hence, as a result of their high photosynthetic efficiency and productivity, tropical C₄ grasses might be expected to have larger potential for SOC sequestration compared with temperate and annual pastures (Neal et al., 2013). Most tropical pastures are important perennials and provide a permanent soil cover and thus prevent soil surface erosion (Peters et al., 2012), which is of particular importance in the prevention of SOC loss by erosion. Greenfield (1988) hypothesized that, with appropriate management practices, tropical grasses could have a significant potential as a soil carbon sink. It is only recently that work to examine the SOC storage potential of these tropical grasses has been undertaken (Fisher et al., 2007) and our knowledge of perennial tropical species growth, interaction with the soil, potential quantities and mechanisms of carbon storage remains incomplete.

It has been speculated that carbon storage in sub-soils might be an important mechanism leading to increased SOC storage in soils (Lavania & Lavania, 2009; SIDA, 2010b) and it is known that tropical grasses translocate large quantities of carbon to their root systems (Lavania & Lavania, 2009). This suggests an effective translocation to deeper soil layers which might be an important mechanism for carbon storage under this vegetation type (Kuzuyakov & Domanski, 2000; Peters et al., 2012; Zimmermann et al., 2012). The deep rootedness of tropical pastures might therefore, potentially play an important role in transporting carbon to deeper soil layers and therefore facilitate SOC storage. Indeed, Fisher et al. (1994), estimated that the introduction of deep rooted African grass pastures in Colombia might account for the sequestration of 100–507 Mt soil carbon year⁻¹ if their study sites were indeed representative of similar pastures throughout South America. These studies indicate the potential benefits of introducing deep rooted tropical perennial grasses for SOC storage but also the need for further carbon inventory.

A number of studies have considered the soil carbon storage potential of tropical pastures by comparison with other management systems. An empirical, five year study of tropical ecosystems in South America by Amézquita et al. (2010), demonstrated that although tropical pastures were second only to native forest in the quantity of SOC stored, organic carbon in the soils of these pasture systems represented a higher proportion (95–98%) of the total ecosystem carbon (above and below ground) than comparable native tropical forest systems (61%) and silvo–pastoral systems (90%). Desjardins et al. (2004), reported that where tropical forest was converted to tropical pasture in Brazilian Amazonia, a slight increase in SOC content occurred in both sandy and clay soils. Post and Kwon (2000), described the similarity of the average rates of SOC accumulation in forest and grasslands of 33.8 and 33.2g C m⁻² y⁻¹, respectively through time following management but above ground carbon is lost. In Australia, Chan and McCoy (2010) also identified the potential of introduced perennial pasture (Kikuyu) to store a mean of 73 Mg C ha⁻¹ in soil which was similar to soils under native trees (77 Mg ha⁻¹). Under some circumstances, tropical pastures have been reported to have a greater capacity to store SOC compared with trees or forest. Guo et al. (2008), reported 15–20% larger soil C stocks under native pasture dominated by Kangaroo grass (*Themeda triandra* Forssk) compared with an exotic, 16 year old pine plantation to 1.0 m of the soil profile, These findings seem to be convincing, although some caution must be attached to many such results given that they are for soil but discount above-ground biomass

and are rarely reported on an equivalent mass basis (Sanderman et al., 2013a). There is therefore growing evidence that tropical pastures might have the capacity to store SOC that is at least equivalent to that of forest systems in terms of rate and quantity of accumulation. However, the quantity and rate of carbon accumulation would appear to be moderated by environmental conditions and both preceding and ongoing management practices. Consideration and knowledge of the behavior and potential carbon storage of particular tropical grass species has much to add to this debate.

Some specific tropical grass species have been highlighted as having potential for improving soil carbon storage although their efficiency in this regard is determined by a range of environmental and management factors (Neill et al., 1997; Fearnside & Barbosa, 1998; Amézquita et al., 2004; Amézquita et al., 2010). Fearnside and Barbosa (1998), suggested that management practices could determine whether tropical pasture soils could be net sinks or sources of carbon. This study by Fearnside and Barbosa (1998), in Brazilian Amazonia, showed that under “typical” (without inputs or other practices) and “ideal” (with variety of appropriate practices) management, tropical pasture soils were a net carbon source releasing an average of 12 Mg C ha⁻¹ following deforestation. Here, evidence relating to the additional SOC and values of the most prominent tropical grasses are reviewed.

***Andropogon guyanus* (gamba/ tambuki grass, Rhodesian bluegrass)**

Andropogon guyanus (gamba grass, Rhodesian bluegrass, tambuki grass) is a deep rooted African grass which has been identified as a species with great potential for both soil and biomass carbon sequestration. A study, conducted by CIAT, in Colombia showed that *Andropogon guyanus* could store an additional SOC of up to 53 Mg ha⁻¹ yr⁻¹ of CO₂ equivalent (Mishra et al., 1997; Truong, 2000; Chomchalow et al., 2001) to 1.0 m depth in the soil. Fisher et al. (1994), also demonstrated the capacity for this species to store up to a total SOC of 237 Mg ha⁻¹ of additional SOC to 1.0 m under a 6 year old *Andropogon guyanus* pasture where nearly half of the SOC stored was found in the 0.4–1.0 m deep soil layer (Table 1). Hence, *Andropogon guyanus* is one of a range of tropical perennial grass species that can be used widely in Africa and in areas where it is introduced for different purposes and is identified as having large biomass production, extensive root systems and therefore potential for net addition of carbon storage.

Lemongrass (*Cymbopogon citratus*) and Palmarosa (*Cymbopogon martinii*)

Lemongrass (*Cymbopogon citratus*) and Palmarosa (*Cymbopogon martinii*) are tropical grasses from the same genus and known for large quantities of biomass production which have promising potential for carbon storage. Singh and Dagar (2009), indicated that lemongrass and Palmarosa had the potential to produce 27.7 and 30.0 Mg ha⁻¹ yr⁻¹ fresh biomass and 10.4 and 24.3 Mg ha⁻¹ yr⁻¹ dry biomass, respectively. Singh et al. (2011), further found that lemongrass could store up to 5.38 Mg C ha⁻¹ yr⁻¹ in biomass, with additional SOC storage of 3.08 Mg C ha⁻¹ yr⁻¹ where pasture replaced cropping. While *Palmarosa* could store up to 6.14 Mg C ha⁻¹ in total biomass and an associated increase in SOC of 2.79 Mg ha⁻¹ (Singh et al., 2013). Therefore, both lemongrass and palmarosa are economically important perennial grasses due to their medicinal and aromatic nature and have shown substantial potential to store significant quantities of additional soil carbon following cropland conversion (Schuman et al., 2002; Freibauer et al., 2004; Derner & Schuman, 2007).

Kikuyu (*Pennisetum clandestinum*)

Kikuyu (*Pennisetum clandestinum*) is a tropical (C₄) grass native to East Africa which can produce large biomass and has a weedy nature (i.e. readily spreads to other areas where it is not preferred). Currently, it is widely distributed in Africa, Tropical and Temperate Asia, Australasia, Pacific, North America, and South America having been introduced as a productive pasture species. The root structure of kikuyu makes it efficient in extracting soil nutrients and water and minimizing soil erosion. Fulkerson and Slack (1993), found a dry biomass of 3–5 Mg ha⁻¹ yr⁻¹ while Neal et al. (2009) reported up to 8.4 Mg ha⁻¹ yr⁻¹ dry matter production potential of Kikuyu grass in Australia (about 4x that of Fulkerson and Slack, 1993). Neal et al. (2009), also indicated Kikuyu planting in Australia had shown also a total increase of 7g C kg⁻¹ of SOC over three years of establishment which was approximately 2.6 t C ha⁻¹ year⁻¹ at the 0–0.1m soil profile (Neal et al., 2013). In Western and South Australia Kikuyu pasture systems showed 0.90 ± 0.25 and 0.26 ± 0.13 Mg C ha⁻¹ year⁻¹ of SOC sequestration rate, respectively (Sanderman et al., 2013a; Sanderman et al., 2013b). Chan and McCoy (2010) similarly found a SOC stock of 67.2 Mg ha⁻¹ at 0–0.2 m depth from a permanent Kikuyu field in NSW across a range of soil types which was a 23 Mg C ha⁻¹ higher than an equivalent native pasture for over 15 years period. Hence, kikuyu has significant potential and has been demonstrated to be useful in a range of environments.

However, it is not attractive due to its known weedy nature, which can cause invasion of agricultural and native systems. In addition, its biomass production potential is not as high as other perennial grass species and it is only a grazing species.

Miscanthus (*Miscanthus giganteus*)

Miscanthus (*M. giganteus*), a C₄ perennial tropical grass with a deep root system and high biomass production, has received considerable attention as a potential bioenergy crop over the last 25 years particularly in Europe, although relatively few commercial plantations exist globally (Robertson et al., 2015). The low input requirements of *Miscanthus* make it particularly attractive as a bioenergy crop (Robertson et al., 2013; Robertson et al., 2015). Due to the high above- and below-ground biomass production, *Miscanthus* is also believed to have considerable potential for SOC (Clifton-Brown et al., 2007; Dondini et al., 2009; Robertson et al., 2013; Robertson, 2014; Robertson et al., 2015). This species has shown a significant amount of additional SOC stored down to 1.0 m soil depth (Schneckenberger & Kuzyakov, 2007; Zimmermann et al., 2012). Using $\delta^{13}\text{C}$ analysis, Schneckenberger and Kuzyakov (2007) found as much as 3 g and 2.4 g kg⁻¹ additional SOC under *Miscanthus* in the upper 1.0 m of sandy and loamy soils, respectively. Clifton-Brown et al. (2007), estimated total carbon mitigation potential of *Miscanthus* after 15 years of planting ranging from 5.2–7.2 Mg C ha⁻¹ yr⁻¹ in both soil and biomass in southern Ireland, Europe. This study by Clifton-Brown et al. (2007), also estimated that *Miscanthus* could mitigate 4.0–5.3 Mg C ha⁻¹ yr⁻¹ in emissions if the harvested biomass of *Miscanthus* was combusted as an alternative fuel source to coal. More recently, Poeplau and Don (2013), observed a mean new carbon sequestration rate of 0.78 ± 0.19 Mg ha⁻¹ yr⁻¹ under *Miscanthus*, which increased with mean annual temperature. Dondini et al. (2009), also reported a significantly higher total SOC stored under a 14 year *Miscanthus* plantation (131.3 Mg C ha⁻¹) compared to arable cropland (105.8 Mg C ha⁻¹) and highlighted its CO₂ mitigation potential. Hence, studies have identified the high carbon sequestration potential of C₄ *Miscanthus* through large carbon inputs and *Miscanthus* therefore has considerable SOC storage potential. The focus on this species has been driven largely by its biofuel potential with soil carbon storage representing an added potential value. This does emphasise the possibility of multiple benefits being derived from this and similar tropical grass species and that the use of these grasses might be framed within the management of species within integrated management systems.

Vetiver (*Chrysopogon zizanioides*)

Vetiver (*Chrysopogon zizanioides*) is a fast-growing perennial tropical grass of the Poacea family which is a close relative of *Andropogon guyanus*. Originally native to India, it is now distributed widely throughout the tropical and sub-tropical regions of the world (Greenfield, 1988; Lavania & Lavania, 2009; Abate & Simane, 2010) and is adapted to a wide range of climates and environments (Lavania & Lavania, 2009; Abate & Simane, 2010; Awoke, 2013). Although vetiver has a range of varieties internationally including *C. zizanioides*, *Lawsonii* (India) and *C. filipes*, *C. elongata* (Australia, Indonesia), *C. zizanioides* (hereafter referred to as Vetiver) has attracted most attention due to its tolerance of a wide range of conditions including salinity, prolonged waterlogging and most common pests and diseases (Grimshaw, 2008). It can grow to 1–2.0 m of a shoot height and 3–4.0 m across using a complex root system that penetrates deep into the soil profile (Lavania & Lavania, 2009; Abate & Simane, 2010), it has also been shown to grow well even where soil nitrogen supply is limited (Mondyagu et al., 2012), tolerate intensive harvest, grazing or mowing and does not spread as a weed (Hon et al., 2008) which is a major advantage contrasting with other species such as kikuyu grass. Vetiver also typically has high survival rates following sowing, on a saline soil had a 93–98% survival rate which was significantly better than lemongrass, citronella and palmarosa (Tomar & Minhas, 2004; Alberti et al., 2010).

Vetiver was initially valued for its aromatic oil in India (Lavania & Lavania, 2009). However, it has now become a multipurpose grass used extensively internationally in soil and water conservation, steep slope and stream bank stabilization, mine site and contaminated and saline land rehabilitation, wastewater treatment, fodder for livestock, roof thatching, handcraft, perfumes, medicines, pest control and also as an ornamental grass (Greenfield, 1988; Grimshaw & Helfer, 1995; Lavania, 2000; Truong, 2000; Truong et al., 2001; Grimshaw, 2002, 2003; Percy & Truong, 2003; Chen et al., 2004b; Truong et al., 2004; Lavania & Lavania, 2009; Abate & Simane, 2010; SIDA, 2010b; Singh et al., 2011; Awoke, 2013). *Vetiver* is salt tolerant and can grow in both acidic and alkaline soils, which is superior to most other grasses. Vetiver has been used with success in Victoria, Australia to lower saline water tables (Truong et al., 2004; Awoke, 2013). Overall, due to its fast growing nature, ease and low cost of establishment, low maintenance and tolerance of a range of environmental conditions, *Vetiver* is widely used to address environmental degradation and

for conservation and rehabilitation across Asia, Africa and Latin America (Gaspard et al., 2007; Abate & Simane, 2010).

The fast-growing nature of tropical pastures can be related to their prolific shoot and root production. In a study of three different cultivars of *Vetiver* grown in a sandy-loam soil under saline irrigation, Singh and Dagar (2009), reported that vetiver produced between 24.2–26.2 Mg ha⁻¹ yr⁻¹ shoot biomass which is comparable with the result by Singh et al. (2013) of 28.6 Mg ha⁻¹ yr⁻¹. Singh et al. (2013), further compared yearly *Vetiver* shoot biomass production with lemongrass (10.5 Mg ha⁻¹ yr⁻¹) and palmarosa (11.1 Mg ha⁻¹ yr⁻¹) highlighting *Vetiver*'s superior productivity over the two grasses. Tomar and Minhas (2004) similarly, studied the relative performance of *Vetiver* against lemongrass and citronella palmarosa on a saline-loam soil and the dry weight biomass of *Vetiver* was 45.5 Mg ha⁻¹ yr⁻¹, compared to palmarosa (14.55 Mg ha⁻¹ yr⁻¹) and lemongrass (8.05 Mg ha⁻¹ yr⁻¹), while citronella did not survive on this saline soil.

Vetiver can also produce a large, complex and massive root system penetrating deep into the soil profile (Lavania, 2003; Chairaj & Roongtanakiat, 2004; Gaspard et al., 2007). According to Lavania and Lavania (2009), vetiver roots are fast growing as a bunch penetrating the soil system vertically and can extend as much as 2.0 m depth in just six months into deeper soil layers. Results from Singh and Dagar (2009), also showed root biomass production of vetiver between 1.12–1.71 Mg ha⁻¹ after three years of plantation. Similarly Singh et al. (2011), reported the vetiver dry root biomass production of 1.56 Mg ha⁻¹ yr⁻¹ which was comparable to lemongrass (1.57 Mg ha⁻¹ yr⁻¹) but higher than palmarosa (0.65 Mg ha⁻¹ yr⁻¹). Hence, these studies have demonstrated that vetiver produce much larger above- and below-ground biomass than other grasses within short period of time and the roots can also penetrate into deeper soil profiles.

Root biomass production is an important plant component that can contribute to soil carbon sequestration. However, in less disturbed or no-till soils generate lower temperatures that decrease decomposition rates and can result in low root penetration due to high bulk density which lower carbon inputs at depth. A strong fibrous root system, penetrating deep into the soil profile and growing vertically rather than horizontally (presumably avoiding competition with other plants), is therefore desirable to maximize soil carbon sequestration. *Vetiver* root systems might therefore potentially facilitate long term deep carbon storage and reduce the

chance of decomposition and carbon loss (Lavania & Lavania, 2009). Further findings of Grimshaw (2008) and Lavania and Lavania (2009) support this, whereby *Vetiver* roots were found to contribute significantly more to additional SOC storage than those other grass species. *Vetiver* therefore seems superior compared with range of other species, as yet, poorly quantified potential for carbon storage. Hence, the extent of SOC sequestration potential of *vetiver* grass and other potential tropical grass species still needs further research.

Due to its rapid and large biomass production, an extensive and fast growing root system, *vetiver* would seem to have the capacity to rapidly store or contribute large quantities of carbon in addition to its other varied uses (Mondyagu et al., 2012; Singh et al., 2013). It has also been identified as one of the most promising plants among the deep-rooted, tropical perennial grasses that could contribute to SOC storage and thus climate change mitigation (Truong, 2000; Gaspard et al., 2007; Lavania & Lavania, 2009; Abate & Simane, 2010; SIDA, 2010b). Awoke (2013) took this recommendation further, by highlighting the potential of *Vetiver* grass for both above- and below-ground C sequestration by planting strategically on river banks, highways slope embankments and also in crop fields. However, few studies of carbon storage have been focused on *Vetiver* with most attention being focused on a range of other temperate and perennial tropical grasses.

Most of studies relating to tropical grasses to date have focused on the actual biomass production potential. However, there are only few studies which have considered the actual net accumulation of carbon stored in the soil under tropical grasses which is an indication to focus research efforts (Table 1). Therefore, there is a need for controlled studies to determine not only biomass and inputs but also the net effect of tropical perennial grasses in terms of carbon storage and the mechanisms, stability and longevity of the carbon stored such as the rate of new carbon turnover and carbon cycling of the newly added carbon and the extent to which it is retained in the soil system.

Table 1: Total soil carbon stored under different tropical grasses ($Mg\ ha^{-1}\ yr^{-1}$)

Grass type	Year since planted	Sample depth (cm)	Mg C $ha^{-1}\ yr^{-1}$	Mg C ha^{-1}	Country/ region	Source
African grass	-	-	8.67	-	Latin America	(Fisher et al., 2007)
<i>Andropogon guyanus</i>		0-100	14.45	-	Latin America	(Mishra et al., 1997; Fisher et al., 2007)
<i>Brachiaria dictyoneura</i>	3.5	-	8.57	30	Latin America	(Fisher et al., 1994)
Lemongrass	-	0-30	3.08	-	India	(Singh et al., 2013)
"	-	0-30	5.38	-	India	(Singh et al., 2011)
Palmarosa	-	0-30	2.79	-	India	(Singh et al., 2013)
"	-	0-30	6.14	-	India	(Singh et al., 2011)
Kikuyu	3	0-10	2.6	34 g kg^{-1}	Australia	(Neal et al., 2013)
"	15	0-20	-	67.2	Australia	(Chan & McCoy, 2010)
"	-	0-30	0.9	-	West Australia	(Sanderman et al., 2013a; Sanderman et al., 2013b)
"	-	0-30	0.26	-	South Australia	(Sanderman et al., 2013a; Sanderman et al., 2013b)
Miscanthus	10	0-80	0.78	-	Europe	(Poepflau & Don, 2013)
"	2.5	0-30	0.73	1.82	UK	(Zimmermann et al., 2012)
"	2.5	0-30	0.87	2.17	UK	(Zimmermann et al., 2012)
Vetiver	5	0-30	5.54	-	India	(Singh et al., 2011; Singh et al., 2013)

Factors Affecting Carbon Sequestration of Tropical Pastures

Tropical pastures grow continually year round and are adapted to a wide range of soil and climate conditions because of the close interaction between climate factors and soil properties (Reichle et al., 1999; McKenzie & Mason, 2010), suggested that in addition to soil type, management and site history, determining the direction and magnitude of change in soil carbon stock could be important factors. Chan and McCoy (2010) similarly, indicated the higher effectiveness of tropical pastures in increasing SOC storage under appropriate management. Wilson and Lonergan (2013), also demonstrated in Australia that native and improved pastures in this environment had the same SOC quantity and that historical and contemporary management practice is a key factor influencing net SOC. The management of tropical pastures is therefore a critical determinant of whether the soils under this land use will represent a source or a sink of atmospheric carbon (Fearnside & Barbosa, 1998). Poor pasture management such as over grazing, frequent burning and conversion to cultivated agricultural land could result in degradation and low productivity which can reverse the carbon sequestration potential of tropical pastures leading to carbon loss by erosion and oxidation (Scurlock & Hall, 1998). Hence, the effects of tropical pastures on soil carbon are likely to vary as a result of environmental and management factors. For example Dalal et al. (2013b), demonstrated historical management as a key driver of SOC stock particularly in the surface soil layers. Therefore, there is a need for controlled studies that measure soil carbon with some certainty of the effects of both environmental and management factors.

Clay soil types in general play a greater role to slow the rate of decomposition than sandy soils. Similarly, a water saturated soil might have lower rates of organic matter breakdown because of a lack of oxygen for soil organisms compared to soils exposed to the atmosphere. Therefore, soil improvement and adding essential inputs are important to increase the rate of organic carbon addition and pasture production (McKenzie & Mason, 2010). In addition, McKenzie and Mason (2010) indicated that deep soil profiles with fertile subsoil allow deep root penetration into subsoil which is much cooler (less likely to promote decomposition) than the topsoil. Hence, maximizing the carbon input by increasing the net primary production through nutrient addition, increased nutrient and water use efficiency and minimizing the rate of organic matter decomposition after deposition in soil are important factors which can help to increase the amount of carbon sequestered from the atmosphere (Reichle et al., 1999).

Carbon accumulation in pasture lands can also be determined by the length of time the land remains under pasture (Neill et al., 1997). Hence, regardless of technologies or mechanisms, the length of time must also be taken into account when considering long-term carbon storage. Bouman et al. (1999), stated that, due to various economic and biophysical dimensions, sustainability of tropical pastures can also be affected by the pasture type, age and management which in turn can affect the carbon accumulation. Hence, McDermot and Elavarthi (2014), recommended that best management practices, site specific policies and using technological options can be good opportunities to consider to bring positive effect on soil carbon accumulation by using tropical grasses.

Therefore, factors such as input versus outputs, climatic conditions, soil type and properties, land use control, management practices are the factors affecting SOC storage. Whenever there is a vegetation cover change from C₃ to C₄ plants, the ratio of stable carbon isotopes ($\delta^{13}\text{C}$) can be used to track changes in SOC between the C₃ and C₄ plants and the quantity of “new” carbon added (Ehleringer et al., 2000; Schneckenberger & Kuzyakov, 2007; Dalal et al., 2013b). Stable C isotopes ($\delta^{13}\text{C}$) used to discriminate between C₃ and C₄ plants carbon inputs as a result of changes in the vegetation cover (Morgun et al., 2011). C₃ type plants are characterised by the range of $\delta^{13}\text{C}$ values approximately from -22 to -33 ‰ and the C₄ plants have a range from -10 to -18 ‰. Hence, the use of stable C isotopes offers a useful quantitative technique to allow the estimation of organic carbon storage and turnover in soils, even when TOC changes are of limited magnitude (Dalal et al., 2011b). In addition the use of density/size fractions of carbon can help to interpret carbon changes and the allocation of SOC to its component fractions which can provide an indication of the vulnerability of organic carbon stocks to change (Baldock et al., 2013a).

Opportunities and Economic benefits of Tropical Pastures

Biomass energy is currently receiving considerable attention in response to climate change and ever increasing global energy demand (Hoogwijk et al., 2005; Sims et al., 2006; Berndes & Hansson, 2007; de Wit et al., 2014) and tropical pastures would appear to have potential for the production of biofuels. For example, Clifton-Brown et al. (2007), suggested that *Miscanthus* which is known for its high biomass production has value as a potential biofuel and the area over which it grows could therefore be expanded significantly throughout Europe. *Miscanthus* is grown in many European countries such as Austria, Denmark, France,

Germany, Hungary, Poland, Switzerland and the United Kingdom. In France for instance, *Miscanthus* cultivation has increased since the first plantation in 2006 (NovaBiom, 2015). The EU Biofuel directive promotes the expansion of biofuels and *Miscanthus* particularly as a biomass energy source (Edwards et al., 2010). Hence, growing biomass energy crops (mainly tropical perennial grasses) specially in Europe is becoming common and expanding to consider for potential soil carbon storage and this needs to be explored further even in other parts of the world and using other different potential grasses.

Economic analysis suggests that soil carbon sequestration is amongst the most beneficial and cost effective options available for reducing net greenhouse gas emission, particularly over the next 30 years until alternative energy sources are developed and become economically feasible (Rice, 2006). In terms of carbon capture and storage, and this combined with bio-energy creation, the Intergovernmental Panel on Climate Change - IPCC (2014), directly relate economic growth with climate change mitigation strategies, especially for developing countries. Although this has its challenges, there are projects that have been implementing clean development mechanisms as part of the Kyoto response towards climate change mitigation (CDM) (Ringius, 2002; Olsen, 2007; Olsen & Fenhann, 2008). From an economic and environmental perspective, Amézquita et al., (2004); Abberton et al. (2010), have drawn attention to the capacity of tropical pastures to rehabilitate degraded lands and the associated potential for carbon sequestration as an attractive option from both an economic and environmental perspective and in this regard tropical pastures clearly have particular value.

Conclusion

Tropical pastures are potential candidates to contribute to climate change mitigation efforts through additional SOC storage due to their high biomass production, fast growth rates, and deep root systems. Using tropical grasses has also a low cost of implementation to rehabilitate degraded lands and improve soil productivity through increasing SOC. The existing literature on tropical grasses potential for soil carbon sequestration provide positive evidence and encourage further investigations. Therefore:

- Cropland conversion to tropical perennial pastures might be of an important strategic option to improve soil health and soil carbon storage. However, this needs further investigation.

- A range of tropical perennial grasses were assessed and that some are suited to specific situations, but vetiver seems to be an all-round winner and would seem to have considerable as yet not quantified potential for carbon sequestration and the extent of SOC contribution from tropical grass species still remains unknown
- Carbon inventory in deeper soil profiles under tropical perennial grasses also needs to be undertaken
- The rate of soil carbon turnover and cycling of the new carbon added and the extent to which it is retained in the soil system needs to be clear
- Best management practices, site specific policies and technological options which can reverse and have positive effect on soil carbon storage are essential to be considered

The work presented in this thesis will therefore use experimental research to quantify the effects of planting vetiver grass (i.e. a widely distributed perennial tropical grasses) on SOC storage potential by sampling deeper soil horizons (e.g. down to 1.0 m) with the main objective of examining the quantity, nature, fractions and distribution of SOC compared with other plant types.

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CHAPTER 3: Soil Carbon Storage and Distribution under Vetiver (*Chrysopogon zizanioides*) using Stable Isotope ($\delta^{13}\text{C}$) Analysis

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Abstract

Cropland conversion to pasture has a demonstrated capacity to store additional soil carbon and perennial tropical pastures appear to have particular value in this regard. Due to its extensive use globally and large biomass production, Vetiver grass is currently attracting particular attention for its potential for long-term carbon storage. Using stable C isotope ratio ($\delta^{13}\text{C}$) analysis, we examined the quantity and distribution of soil carbon under vetiver compared with tropical pasture, native pastures and cropping soils at Gunnedah, Australia to 1m depth. TOC under vetiver (123 Mg ha^{-1}) was significantly higher than tropical pasture (93 Mg ha^{-1}) and cropping soils (78 Mg ha^{-1}) but statistically similar to soils under native pastures (111 Mg ha^{-1}). For all plant types, a decrease in organic carbon concentration was observed with increasing soil depth but a larger stock of carbon was found under vetiver at almost all depths through the 1.0 m soil profile. Soils under vetiver had significantly higher $\delta^{13}\text{C}$ values compared with tropical, native pastures and cropping soils, particularly in the surface soil layers. Both litter and roots were probably contributing to the additional carbon stock by vetiver (43.5%) and results indicated a significant carbon turnover through the whole soil profile even where the net accumulation of carbon was modest ($\sim 2\%$ per annum). Vetiver, due to its fast growth and large biomass, has considerable potential for carbon sequestration, particularly on carbon depleted soils. There is a need to continue advancing knowledge on the potential of tropical perennial grasses such as vetiver to influence land management decisions.

Key words: Australia, turnover, native pasture, tropical pasture

Introduction

Much research internationally is currently focused on the need to remove carbon dioxide from the earth's atmosphere and to sequester and store additional carbon in terrestrial sinks as a means of mitigating climate change. Soils are the largest terrestrial carbon reservoir and soil carbon sequestration is considered to be a potentially efficient and effective means of storing additional carbon to offset greenhouse gas emissions (Lal, 2011; Lal et al., 2012; McDermot & Elavarthi, 2014). Globally, land management options that can effectively store additional carbon are being actively investigated. Approaches that have been proposed for carbon storage include; minimum tillage, afforestation, grazing management, cover crops, water harvesting and the use of soil amendments including biochar (Grandy & Robertson, 2006; Janik et al., 2007; Gallo et al., 2009; Schuman et al., 2009; McKenzie & Mason, 2010; Oladele & Braimoh, 2011). However, there is particularly strong evidence that carbon sequestration and stabilisation might be achieved by introducing or re-establishing perennial grasses to replace other more intensive agricultural systems thus increasing surface herbage mass, below-ground root growth and therefore carbon addition (Fisher et al., 1994; Batjes, 1998; Conant et al., 2001; Sanderman et al., 2013a; Sanderman et al., 2013b). Hence, cropland conversion to grassland has been proposed as a means to sequester significant quantities of atmospheric carbon (Conant et al., 2001; Conant, 2012).

Tropical perennial grass species have received particular attention recently with regard to their carbon sequestration potential (Hansen et al., 2004a; Poeplau & Don, 2013), due to their rapid establishment, large biomass and large, complex rooting systems. The wide use of tropical perennial grasses throughout the tropics and beyond could therefore have considerable potential to store significant quantities of additional carbon both in biomass and soils (Sanderman et al., 2013b). The vertical distribution of soil carbon is an important

feature of the soil organic carbon (SOC) pool, where plant type, climate and plant biomass production might be determining factors controlling C inputs to soil profiles with depth (Ehleringer et al., 2000; Jobbágy & Jackson, 2000). Tropical perennial pastures are known to have root systems that penetrate deep into the soil and can therefore potentially facilitate storage of additional carbon in deep soil profiles where it might be protected and less susceptible to decomposition (Batjes, 1998; Lavania & Lavania, 2009; SIDA, 2010b).

Vetiver (*Chrysopogon zizanioides*) is a fast growing, C₄ perennial tropical grass that grows up to 1–2m above ground and can produce a large, complex and massive root system penetrating deep into the soil profile. It is widely distributed in tropical and sub-tropical regions through planting (Lavania, 2003; Gaspard et al., 2007; Abate & Simane, 2010). Due to its fast growth, tolerance of a range of environmental conditions and ease of establishment, vetiver is considered to be an effective solution to environmental degradation and is widely used for conservation and rehabilitation including soil and water conservation, slope stabilization, remediation of contaminated and saline lands, stream bank stabilization and wastewater treatment (Truong, 2000; Gaspard et al., 2007; Lavania & Lavania, 2009; Abate & Simane, 2010). However, vetiver might also have a value in adding to SOC stocks.

Few field level studies of the soil carbon sequestration potential of vetiver are available but Viemeyer (1997) estimated the rate of soil carbon storage by a single vetiver plant to be as much as 2 kg year⁻¹ assuming vetiver roots penetrate to 5m in tropical soils. Lavania and Lavania (2009), also estimated 10 Mg ha⁻¹ year⁻¹ of soil carbon could be added under vetiver plantation, assuming (50%) of the dry root biomass as carbon. In addition Singh et al. (2011), indicated the potential significance of soil carbon sequestration by vetiver could be as much as 20 Mg ha⁻¹ in India and these workers recommended that vetiver could be a sustainable option to contribute to climate change mitigation and could potentially also benefit in net

return for farmers from the global carbon market and farm productivity (Jürgens et al., 2006; Brown et al., 2011; Gao et al., 2016). However, no accurate field measurements have been made to quantify the carbon storage potential of vetiver by comparison with other management systems.

Where a change in vegetation cover from C₃ to C₄ plants takes place, the ratio of stable carbon isotopes ($\delta^{13}\text{C}$) can be used to track changes in SOC and therefore the quantity of new carbon added (Ehleringer et al., 2000; Schneckenberger & Kuzyakov, 2007; Dalal et al., 2013a). This offers a useful quantitative technique to allow the estimation of organic carbon storage and turnover in soils, even when TOC changes are of limited magnitude such as in Australia (Dalal et al., 2011b).

The present study aimed to quantify soil organic carbon, quantity and distribution to 1.0 m depth in the soil profile under a 22 year old, experimental vetiver plot at Gunnedah Research Centre, NSW, SE Australia. We aimed to determine: i) the capacity of vetiver and other grasses to accumulate soil carbon by comparison with a reference (C₃) cropping soil, ii) the depth distribution of this carbon and iii) the source and potential turnover of the carbon using stable C isotope analysis. Our hypothesis therefore, was that vetiver can store more soil carbon compared to cropping, native and tropical plant types due to its large and fast-growing biomass (root and shoot).

Materials and Methods

Experimental design

Gunnedah Research Centre (GRC) is located in northwestern New South Wales (Figure 1) in SE Australia. The site is located at 31.03 °S and 150.27 °E in a landscape dominated by ridges of Carboniferous-Permian sandstones and conglomerates, Permo-Triassic and Tertiary basalts surrounded by long to very long foot slopes and extensive plains of colluvial and alluvial sediments derived from these materials. Soils are moderately deep to deep Ferrosols according to Australian Soil Classification (Isbell, 2016), which is USDA equivalent Oxisols (USDA, 1999) and WRB equivalent Ferralsols (FAO, 2014) on upper foot slopes with deep to very deep black soils (Vertosol-ASC, Vertisol-USDA and WRB) on lower slopes. Annual rainfall at the GRC is 638 mm (Australia summer dominant) and the average maximum and minimum temperatures are 24.6 °C and 12.2 °C. The vegetation of the area is dominated by open woodland and grassland vegetation. The land use in and around the area is predominantly grazing on steeper slopes and cultivated for cropping on deeper soils with pastures used in a rotation system.

We used a nested plot experimental design for soil sampling from four plant types: vetiver grass, tropical pastures, native pastures and cropping. The sites on which these pastures had been established was previously cultivated (wheat/chickpea) until 1992. In this experiment, we used vetiver which had been established in strips on a previously cropped soil and an adjacent mixed (C₃ and C₄) native pasture mainly composed of Queensland blue grass (*Dicanthium sericeum*), slender bamboo grass (*Austrostipa verticillata*), wallaby grass (*Austrodanthonia spp.*) and windmill grass (*Chloris spp.*). Both the vetiver and native pastures were established at the same time (1993). The site was then grazed pasture until a tropical mixed pasture of Rhodes (*Chloris gayana*), Bambatsi panic (*Panicum spp.*), purple

pigeon (*Setaria incrassate*) was established in 2005 on one side of the same paddock replacing the native pasture at this location. Soil samples were collected from each of the three plant types in 2014. We also used a reference soil profile from adjacent cropping soils that have been continuously cropped throughout the period to determine soil characteristics in the absence of any of the pasture types. Here we assume no change in the cropping soil.

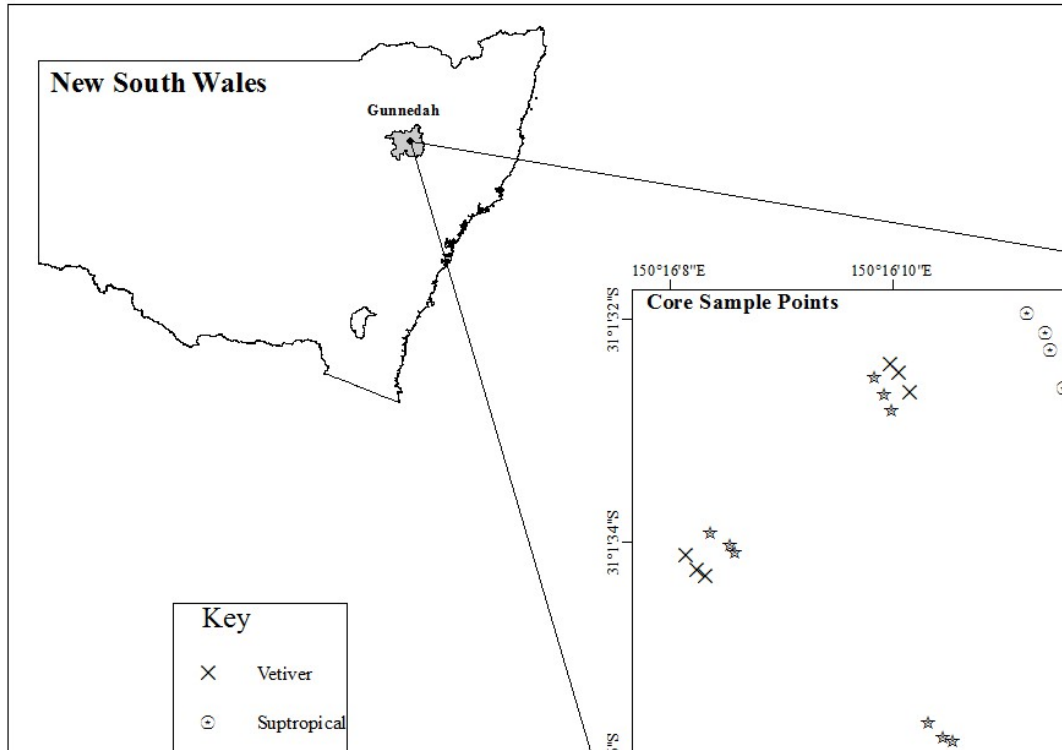


Figure 1: Study area (Gunnedah Research Centre-shaded area) where soil core samples were collected from 1m soil depth under vetiver, native and tropical pastures at Gunnedah Research Centre, NSW, Australia.

Soil sampling

Undisturbed soil samples were collected using a hydraulic core sampler with a 50 mm internal diameter steel core to 1.0 m depth. Three plots were selected in each plant type and the reference cropping field, where three replicate core samples were taken from each plot. The distance between replicate core samples within each plot was 4.0 m. The core sampling followed the same pattern for all plant types and the cropping field. Each individual soil core

was divided into 7 depth increments (0-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.4, 0.4-0.5, 0.5-0.7 & 0.7-1.0 m) resulting in a total of 63 samples for each of the plant types and 21 samples from the reference cropping site, which is a total of 210 soil samples were collected overall.

Sample preparation and analysis

Each individual soil sample was oven dried (40°C) until a constant mass was reached. All samples were then crushed to pass through a <2mm sieve and a subsample of 20g further crushed using a ball mill to pass through a <0.2mm sieve for analyses. Bulk density of each sample was calculated using the mass of the <2mm fraction corrected for 105°C moisture and gravel (>2mm) content (Equation 1). Each soil was tested for the presence of carbonates (using 1M HCl) and, where necessary, acid pre-treatment (using 2% orthophosphoric acid H₃PO₄) was undertaken to remove these prior to further analysis. A total of 41 samples showed positive reaction to the acid test and were pre-treated and these samples were predominantly found in the deeper parts of the soil profiles examined.

$$BD_{corrected} = \frac{m_{<2mm}}{(V_{total} - V_{gravel})} \quad \text{Equation 1}$$

Where: *BD* is bulk density, *m* is mass, *v* is volume

Soil Organic Carbon (SOC) and Total Organic Nitrogen (TON) concentration and natural abundance of ¹³C of all samples were analysed using Sercon 20-22 continuous flow isotope ratio mass spectrometer (IRMS) connected to a ANCA-GSL sample preparation unit. For each sample, the ratio of ¹³C to ¹²C was determined against a known Pee Dee Belemnite standard at the UNE Environmental Analysis Laboratory (Equation 2). Isotope ratios were expressed using the *delta* notation (δ) of units per mil or parts per thousand (‰) using the following calculation (Dalal et al., 2011a).

$$\delta^{13}\text{C} (\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \quad \text{Equation 2}$$

Where R is the molar ratio of the heavy to light isotopes ($^{13}\text{C}/^{12}\text{C}$) of the sample or standard (Ehleringer et al., 2000; Dalal et al., 2011a).

Data processing and analysis

Total carbon (Mg ha^{-1}) was calculated as the product of SOC (%), bulk density and sample depth. Bulk densities were then corrected (Equation 1) for differences between plant types using a standard mass of soil determined from oven dry equivalent soil mass. Values of C stock were then expressed on an equivalent soil mass basis after (Sanderman et al., 2009). Statistical analysis was undertaken to detect differences between plant types and soil depth increments. Statistical differences ($P < 0.05$) were tested in a two-way analysis of variance (ANOVA) using Tukey's HSD as a post-hoc analysis between treatments (plant type and depth as the key explanatory factors). Hence, SOC (%), TOC (Mg ha^{-1}), $\delta^{13}\text{C}$ (‰) were determined using a non-linear least square regression (NLS) procedure based on an exponential decay model (Equation 3).

$$y = a \times e^{-bx} \quad \text{Equation 3}$$

where y = carbon, x = soil depth, a = y-intercept and b = decay constant (> 0).

Data analysis was completed using the RStudio statistical software (Version 00.99.879). Total carbon stock is dependent upon mass of soil sampled. In order to plot an appropriate statistical model for TOC (Mg ha^{-1}) to determine change with depth down the whole soil profile, we therefore used 10 cm increment equivalents for each depth. Due to the larger sample depth increment in deeper soils, a corrected value representing a 10 cm increment at these depths was calculated for these samples in order to plot a continuous curve of TOC with depth.

The distinctive $\delta^{13}C$ values of the various plant types examined enabled an estimation of the additional (or replacement) carbon that had been contributed by the various plant types by comparison with the reference (C_3) cropping soil. The $\delta^{13}C$ values determined from the sites studied were therefore used to calculate the proportion of total carbon contributed by the C_4 pastures (vetiver and tropical) compared with cropping field using the formula (Equation 4). Due to the mixed C_3 and C_4 plants in the native pasture, it was not possible to reliably calculate additional carbon in this way. Isotopic $\delta^{13}C$ signatures of (-12.73 ‰) and (-14.70 ‰) were determined for vetiver and tropical samples, respectively and a reference cropland (-27.36 ‰) soil against which to assess the new carbon input by the C_4 vegetation for different depth increments.

$$C_4 - C = \frac{C_x - C_3}{C_4 - C_3} \times 100$$

Equation 4

where, $C_4 - C$ is the C_4 plant (e.g. vetiver) derived carbon, C_x is measured $\delta^{13}C$, $C_3 - \delta^{13}C$ of the reference C_3 crop soil i.e. cropping soil. C_4 is the $\delta^{13}C$ of the C_4 grass biomass.

Results

SOC and TON Concentration

Analysis of variance indicated that plant type ($P < 0.05$) and depth ($P < 0.001$) were both significant factors influencing the concentration of carbon (Figure 2a). The difference between plant types was indicated by a higher concentration of carbon under vetiver ($P < 0.05$) especially in the top 30 cm compared with tropical pasture, native pastures and the cropping soil. The concentration of carbon did not differ significantly between the tropical pasture, native pasture and cropping soils at any soil depth. Carbon concentration therefore varied in the order vetiver > native pasture = tropical pasture = cropping. Plant type ($P < 0.05$) and depth ($P < 0.001$) were also both significant factors influencing soil nitrogen concentration (Figure 2b). The decline in carbon and nitrogen concentration with increasing soil depth was consistent between plant types.

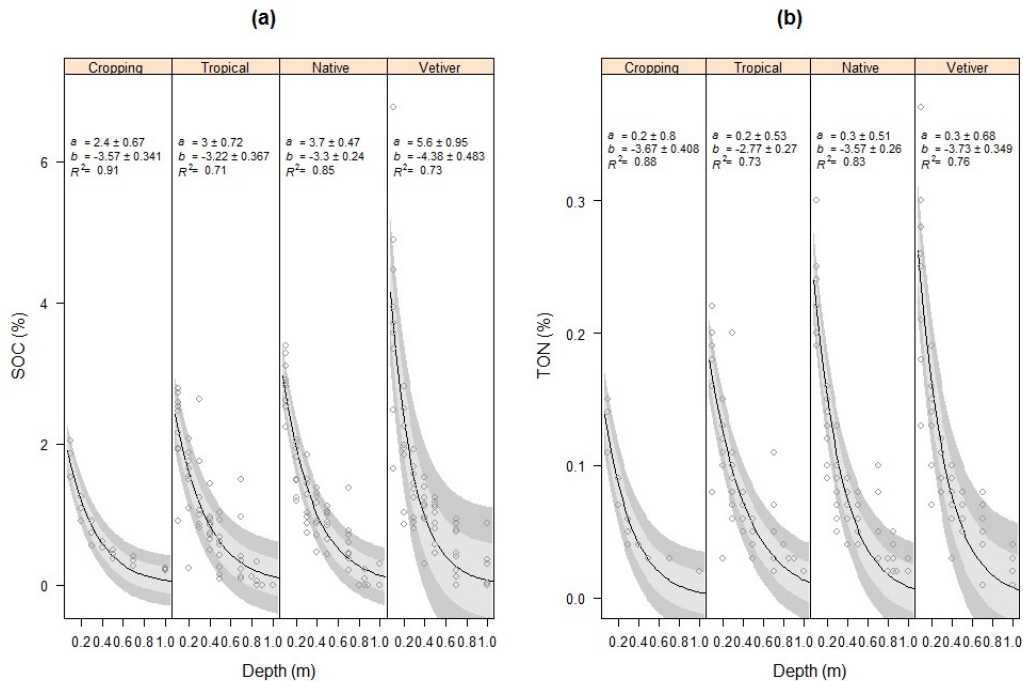


Figure 2: SOC (a) and TON (%) (b) and profile distribution in soil cores (1.0 m depth) under vetiver, tropical, native pastures and a cropping soil in Gunnedah Research Centre. Plots show the raw data (o), a fitted exponential model (—) and confidence bands (1 and 2 SE). Values of the intercept (a), the slope (b) and Coefficient of determination (R²).

TOC Stock

Analysis of variance indicated a main effect of both plant type ($P < 0.001$) and depth ($P < 0.05$) on TOC stock (Figure 3). The difference between plant types was indicated by a larger carbon stock under vetiver and native pastures compared with tropical pasture ($P < 0.001$) and the cropping soil ($P < 0.001$) where vetiver = native pasture > tropical pasture > cropping. TOC stored in soils under vetiver, native, tropical pastures and the cropping soil to 1.0 m was 123 ± 35.2 , 111 ± 21.7 , 93 ± 16.9 and 78 ± 7.3 Mg C ha⁻¹, respectively. For all plant types, a decrease in TOC was observed with increasing soil depth and this decrease consistently followed an exponential trend which was statistically similar between plant types.

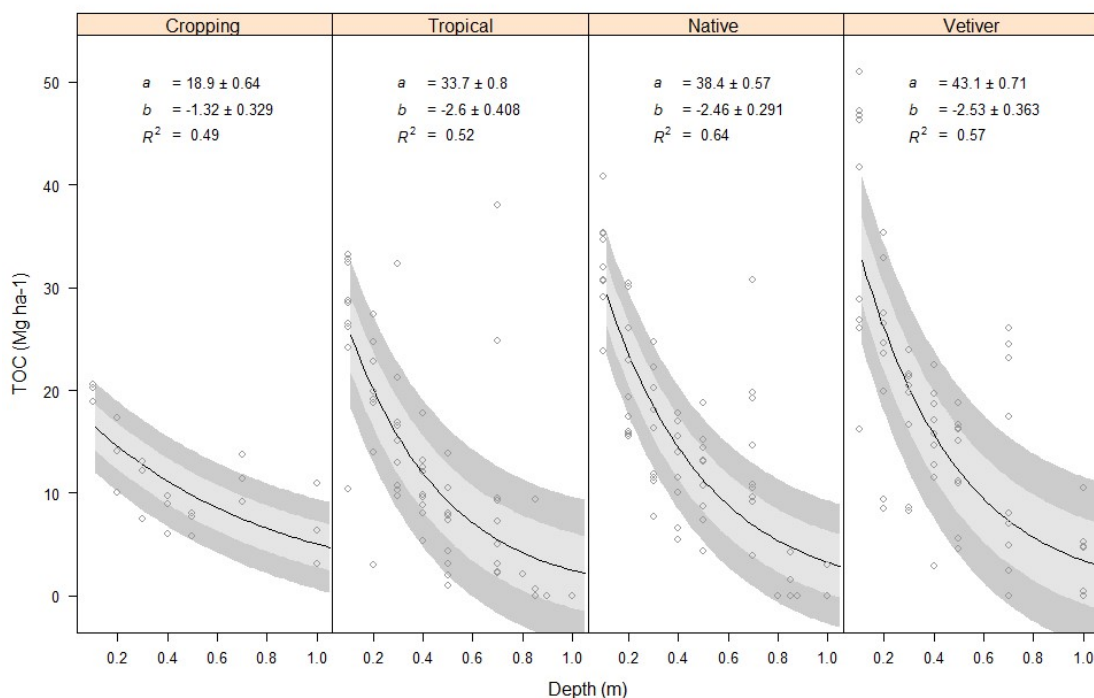


Figure 3: Total TOC (Mg ha⁻¹) in soil cores (1.0 m deep) under vetiver, tropical, native pastures and cropping soil at Gunnedah Research Centre. Plots show the raw data (o), a fitted exponential model (—) and confidence bands (1 and 2 SE). Values of the intercept (a), the slope (b) and Coefficient of determination (R²).

Stable Carbon Isotopes ($\delta^{13}\text{C}$)

Significant differences were observed in $\delta^{13}\text{C}$ values between plant types ($P < 0.001$) and depth ($P < 0.01$) (Figure 4). The cropping paddock had lower (more negative) $\delta^{13}\text{C}$ values than the tropical, native and vetiver paddocks at the surface increasing slightly (less negative) in deeper soil layers. Soils under native and tropical grasses had higher (less negative) $\delta^{13}\text{C}$ values compared with the cropping soil again with increasing (less negative) $\delta^{13}\text{C}$ values with increasing soil depth. For vetiver, $\delta^{13}\text{C}$ values were higher (less negative) than for all other plant types (cropping soil and tropical and native pastures) through the whole soil profile, particularly in the surface soil layers, declining (becoming more negative) slightly but significantly with increasing soil depth.

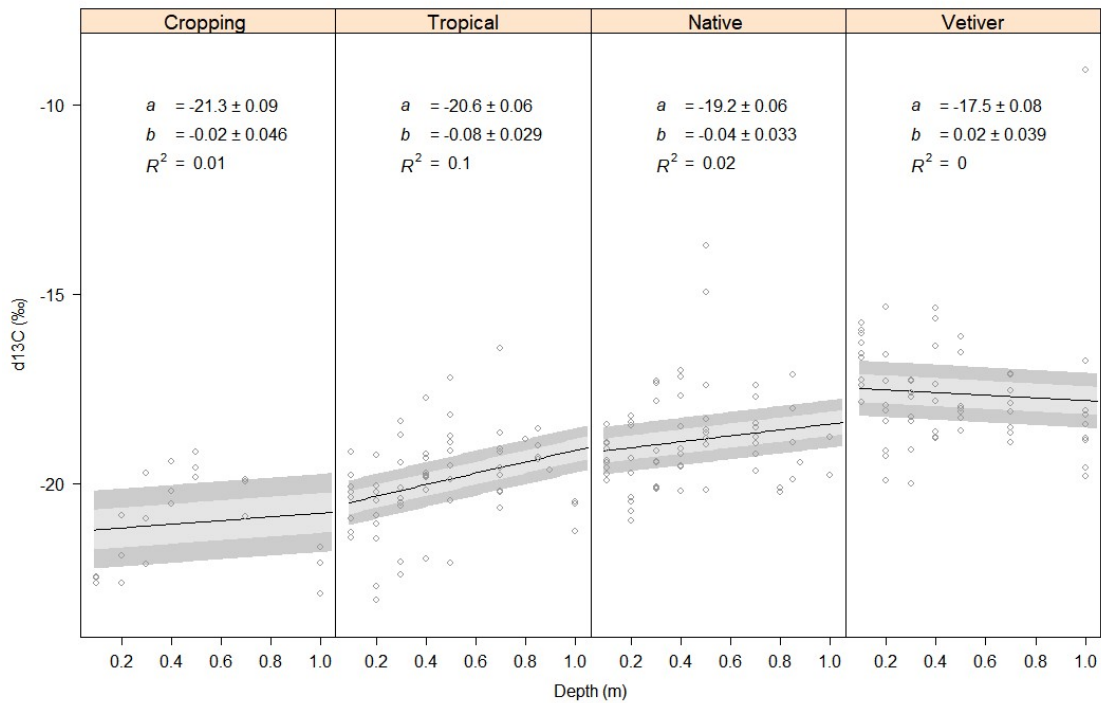


Figure 4: Carbon isotope ratios ($\delta^{13}\text{C}$) in soil cores (1.0 m deep) under vetiver (C_4), tropical (C_4), native (mixed C_3 and C_4) pastures and an adjacent cropping field in Gunnedah Research Centre. Plots show the raw data ($^{\circ}$), a fitted linear regression model ($-$) and confidence bands (1 and 2 SE). Values of the intercept (a), the slope (b) and Coefficient of determination (R^2).

New Carbon input by Vetiver

Using the cropping paddock as a reference site, which retains a C₃ crop $\delta^{13}\text{C}$ value from –19.54 to –22.52) at different depths in the soil profile, significant differences were observed in the new C₄ carbon contribution by both vetiver and tropical C₄ grasses (Figure 5). By comparison with the cropping soil, the mean total carbon input by vetiver through the period that it had been growing was 43.5 % across all depths while, under the tropical pastures it was 18.6 %. The carbon addition by vetiver was higher than by tropical pastures at all soil depths. The age (i.e. time of establishment) of each plant type (vetiver = 22 years; tropical = 9 years) was used to calculate an average net addition of new SOC of 1.98 % per annum and 2.07 % per annum by vetiver and tropical pasture, respectively.

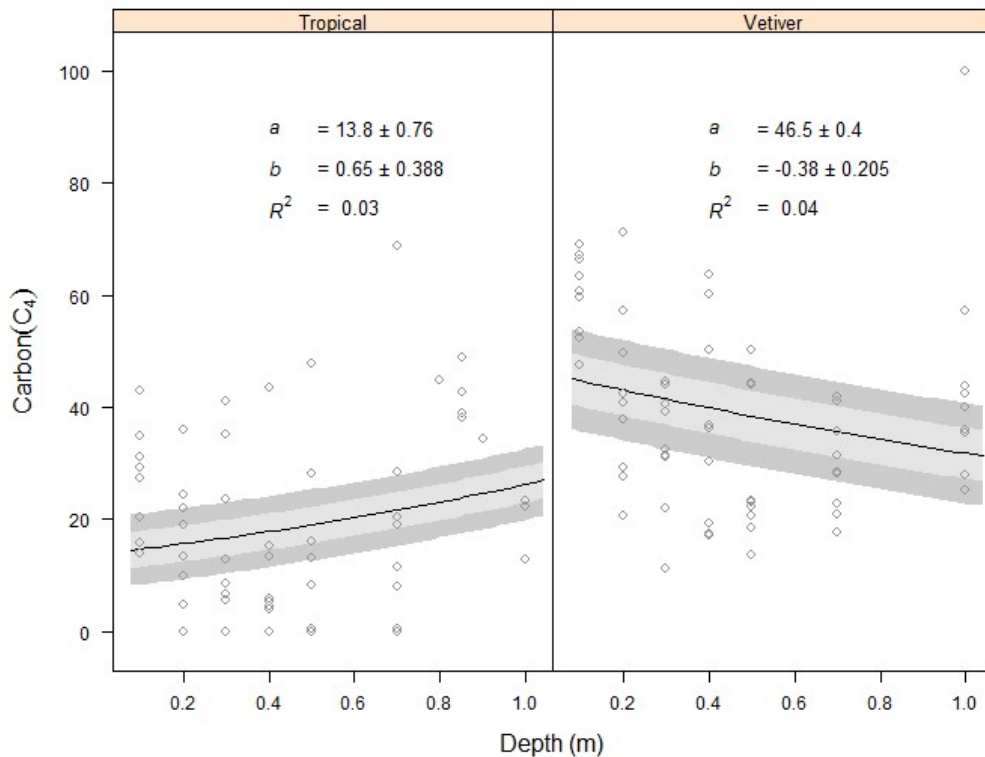


Figure 5: Estimated/calculated C input in soil cores (1.0 m deep) under vetiver, tropical and native pastures in Gunnedah Research Centre. Plots show the raw data (o), a fitted linear regression (–) and confidence bands (1 and 2 SE). Values of the intercept (a), the slope (b) and Coefficient of determination (R²).

Discussion

SOC concentration and TOC stock

Previous studies have demonstrated that conversion of cropping land to perennial pasture can result in an accumulation of additional carbon through the soil profile (Conant et al., 2001; McLauchlan et al., 2006; Alberti et al., 2010). In the current study, the vetiver pasture had accumulated a higher organic carbon concentration through the whole soil profile compared with the cropping soil but no significant difference existed between tropical (9 years) or native pasture (22 years) and the cropping soil. Hence, vetiver is superior and more effective at increasing soil carbon concentration compared with other grass types and a cultivated soil at the GRC.

When soil carbon values were expressed as TOC stock, accounting for differences in bulk density, all three pasture types had significantly larger organic carbon stock than the reference cropping soil. The values for SOC concentration and TOC stocks were different due to the differences in bulk density used in the TOC stock. The statistically similar organic carbon stock between vetiver and native pasture suggests that these two pasture types, established at the same time (22 years prior to sampling) and with the same antecedent soil conditions, were equally effective at storing additional soil organic carbon over this time. The tropical pasture was more recently established at the GRC than the other pasture types, having been established only 9 years prior to sampling. This accounts for its organic carbon stock being intermediate between cropping and the other pasture types. When TOC stock relative to the cropping soil was expressed on an annual basis, since time of establishment, annual accumulation rates of vetiver, native and tropical pastures were similar (123, 111 and 93 Mg ha⁻¹, respectively). These annual accumulation rates compare favourably with estimates elsewhere. For example, Poeplau and Don (2013) estimated 0.78 Mg ha⁻¹ for

Miscanthus in Europe. The lack of differences in the soil carbon stock between plant types could also have been influenced by soil and environmental factors and farming practices (e.g. tillage, biomass removal, altered hydrology from irrigation, nutrient inputs from fertiliser).

Our results therefore confirm that conversion of cropping to a range of perennial pastures does indeed result in accumulation of additional carbon but that, on an annual basis, vetiver performs in a similar way to the other pastures studied at the GRC and indeed other pastures examined internationally.

$\delta^{13}\text{C}$ and New Carbon (C_4 -carbon) input by Vetiver

In addition, vetiver had a higher (less negative) $\delta^{13}\text{C}$ value through the whole soil profile compared with the other vegetation types, demonstrating that more C_3 derived carbon has been replaced by C_4 derived carbon under vetiver. Almost universally, $\delta^{13}\text{C}$ signatures are reported to decrease with increasing soil depth (Ehleringer et al., 2000; Badeck et al., 2005; Schwendenmann & Pendall, 2006). In this study, the $\delta^{13}\text{C}$ signature for vetiver decreased with increasing soil depth. However, the lower $\delta^{13}\text{C}$ (more negative) values under native, tropical and cropping soils increased with increasing soil depth likely due to increasing organic carbon decomposition, litter decomposition and humus formation with depth agrees with the general report (Ehleringer et al., 2000; Chen et al., 2005; Ma et al., 2009). C_4 plants affect $\delta^{13}\text{C}$ signature of the plant material which suggests a progressive shift toward the C_4 plant carbon (Ehleringer et al., 2000; Schneckenberger & Kuzyakov, 2007; Dalal et al., 2013a). This change in the $\delta^{13}\text{C}$ values could have been attributed to a gradual shift in relative contributions of the C_4 plant components which is in agreement with similar other studies (Ehleringer et al., 2000).

Our result indicated that the shift influencing the $\delta^{13}C$ value, is an indication of addition of C_4 carbon as explained by other workers (Schneckenberger & Kuzyakov, 2007; Dalal et al., 2011b; Zimmermann et al., 2012; Zatta et al., 2013). Hence, the new carbon added by vetiver (53 Mg C ha⁻¹) and tropical pasture (17 Mg C ha⁻¹) through the whole soil profiles could suggest the contribution either through roots or other processes such as microbial activities (Ehleringer et al., 2000) and litter inputs (Schneckenberger & Kuzyakov, 2007). Even though the tropical pasture was established later (9 years), it accumulated new SOC at an equivalent rate (~2% annually) as vetiver. The rate of carbon sequestration for vetiver (2.05 Mg C ha⁻¹ yr⁻¹) and for tropical (1.5 Mg C ha⁻¹ yr⁻¹) pasture is higher than the mean C_4 -carbon sequestration rate reported for *Miscanthus* (0.78 ± 0.19 Mg C ha⁻¹ year⁻¹) (Poeplau & Don, 2013). Hence, the new carbon added by vetiver (C_4) replaced the loss of older carbon stored by the preceding C_3 crops, likely due to its long and extended root system and earlier establishment than tropical pastures.

Despite the significant turnover of carbon under vetiver even where the net accumulation of carbon was limited, the new carbon added by vetiver demonstrated both an increase in concentration and total stock. However, it was not large under tropical pastures because of later age of establishment which might have resulted in the expected differences of increase. However, when expressed on an annual basis, they are about equivalent. We therefore, assume that the better performance of vetiver in the new carbon (C_4 -C) addition can have a promising effect in terms of replacing the C_3 carbon depleted soil carbon even in the deeper soil profiles specially if planted on degraded soils.

Conclusion

Our study indicates that vetiver increases the SOC concentration and the total carbon stock (Mg ha^{-1}) compared with native, other tropical pastures and cropping. But on an annual basis, both vetiver and tropical pastures were similar. The $\delta^{13}\text{C}$ value also showed a significant carbon turnover through the whole soil profile, even where net carbon addition was modest. We therefore believe that vetiver due to its wider adaptability and extensive use has considerable potential for carbon sequestration, particularly on carbon depleted soils. Hence, there is a need to continue advancing knowledge on the potential of tropical perennial pasture species such as vetiver to influence land management decisions using perennial grasses and hence contribute to climate change mitigation action and restore degraded lands in tropical and sub-tropical regions of the world.

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CHAPTER 4: Soil Carbon Storage Potential and Depth Distribution under Vetiver (*Chrysopogon zizanioides*) Grass in Southwest Ethiopia and its Implications

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Summary

Globally, soil organic carbon has declined as a result of human induced disturbance with negative effects on production and productivity. Maintaining SOC has a combined effect of sustaining soil health and agro-ecosystem functioning in addition to its potential contribution to climate change mitigation. The introduction of perennial tropical grasses can potentially add significant biomass carbon and translocates large quantities of carbon to the root mass to facilitate additional SOC storage. This study aimed to evaluate the viability of using vetiver grass for soil carbon sequestration, given its wide distribution and extensive use in Africa and elsewhere internationally. Using soil organic carbon concentration (SOC) and total organic carbon stock (TOC) combined with stable isotope ratio ($\delta^{13}C$) analysis, we examined the quantity and distribution of soil carbon under vetiver compared with coffee to 1.0 m soil depth in a series of sites in Ethiopia. Larger mean SOC and TOC stocks were found through the whole profile under vetiver (262 Mg/ha) compared with coffee (178 Mg/ha), particularly in the surface layers. A decline in SOC and TOC was observed with increasing soil depth and a high $\delta^{13}C$ values (less negative) were recorded at the soil surface layers increasing with increasing depth for both plant types. The $\delta^{13}C$ values were however, significantly higher for vetiver through the whole soil profile, indicating the replacement of C_3 carbon by C_4 carbon, particularly in the surface soil layers. Our results suggest a flow of new carbon into the soils under vetiver but significant carbon turnover in the soil system resulting in only a modest increase in carbon storage overall.

Key words: $\delta^{13}C$, Coffee, *Coffea arabica*, Jimma, Anno, Metu

Introduction

Globally, soil organic carbon (SOC) has declined as a result of soil disturbance associated with land clearing, agricultural development and soil erosion with negative effects on soil productivity. However, this soil degradation can be reversed to some degree by replenishing and storing more soil carbon that in turn promotes soil health (Freibauer et al., 2004; Lal, 2004a; Rabbi et al., 2013a). Maintaining SOC has a combined effect of sustaining soil health, agro-ecosystem function, productivity and potentially contributes to climate change mitigation efforts (Sommer & Bossio, 2014). Activities such as soil tillage accelerate soil organic matter decomposition by exposing sites within soil aggregates that were previously protected (McKenzie & Mason, 2010) and soil erosion, vegetation clearing and removal of crop residues result in long-term SOC loss (Shiferaw et al., 2013). Some management practices, however, promote carbon storage and reduce soil carbon loss or add new carbon. Among the alternative management practices being explored to facilitate carbon storage integrated with agricultural production, is the use of tropical perennial grass species (Glover et al., 2008).

Many Ethiopian soils are SOC depleted as a result of environmental and land degradation (WB, 2008; Abebe et al., 2012; Shiferaw et al., 2013; Shiferaw et al., 2015; Solomon et al., 2015). The causes of this degradation are complex and diverse and include the rapidly growing population, reliance on subsistence agriculture, unsustainable farming practices, high dependence on biomass for household energy and poor livestock management including expansion of livestock population beyond the carrying capacity of the land (Lambin et al., 2003; Shiferaw et al., 2013; Shiferaw et al., 2015; Solomon et al., 2015). Ethiopian soils are therefore carbon depleted but are believed to have great potential for storing additional carbon if appropriate land management practices and carbon input measures are undertaken. According to Shiferaw et al. (2013), sustainable land management which favours rehabilitation of degraded lands and employing best management practices has been taken up as a priority by the Ethiopian Government. This not only reflects the goals of the growth and transformation plan for Ethiopia, but will also enhance carbon storage in Ethiopian soils (SIDA, 2010a; Brown et al., 2011; Solomon et al., 2015; Rimhanen et al., 2016).

Internationally, cropland conversion to grassland has been proposed as a means of sequestering significant quantities of atmospheric carbon (Conant et al., 2001; Conant, 2012) and the introduction of perennial grasses to agricultural production systems has been proposed as a viable option to facilitate additional soil carbon storage (Freibauer et al., 2004). Tropical perennial grasses have particular potential for SOC storage since they have large biomass production and are believed to translocate large quantities of carbon to their expansive root systems (Zimmermann et al., 2012), which potentially adds carbon deeper in the soil profile. Clifton-Brown et al. (2007), demonstrated that tropical perennial grasses such as *Miscanthus giganteus* produce large below ground biomass and have the potential to accumulate large quantities of carbon due to their rapid establishment and potential for annual harvest. *Miscanthus* has received considerable attention with regard to its value as a bioenergy crop and the soil carbon storage associated with its growth in Europe (Robertson et al., 2013; Robertson et al., 2015) and significant addition of new carbon has been demonstrated under this species down to 1.0 m soil depth i.e. as much as 3 g and 2.4 g C kg soil⁻¹ new carbon on sandy and loamy soils over 9 and 12 years, respectively (Schneckenberger & Kuzyakov, 2007; Zimmermann et al., 2012). Dondini et al. (2009), also reported in Ireland a higher total soil carbon stored over 14 years of planting under *Miscanthus* (131.3 Mg C ha⁻¹) compared with arable cropland (105.8 Mg C ha⁻¹) to a 0.6m soil depth further highlighting the CO₂ mitigation potential of this species.

A tropical grass with similar characteristics to *Miscanthus* is vetiver. Vetiver (*Chrysopogon zizanioides*) is a C₄ tropical perennial grass used extensively in Ethiopia for biological soil and water conservation and steep slope stabilization (Kebede & Yaekob, 2009; Abate & Simane, 2010; Terefe, 2011) and might also have potential as a biofuel (Lavania & Lavania, 2009; Mondyagu et al., 2012). Vetiver is a grass species with a large and deep root system and has been proposed as a candidate for long term carbon storage in agricultural production systems, through both carbon sequestration and reduced decomposition and loss of C through erosion (Lavania & Lavania, 2009). Singh et al. (2011) indicated the potential significance of carbon sequestration by vetiver and suggested that it could be a sustainable option to contribute to climate change mitigation. Despite the evidence relating to other tropical grass species, Lavania and Lavania (2009) and Singh et al. (2011) both highlighted the limited number of field estimates available for soil carbon sequestration potential using vetiver. Therefore, further research is needed to

quantify the effects of vetiver on SOC storage potential, particularly in deeper soil profiles (Tubiello et al., 2007).

This study examined SOC storage at three sites (Jimma, Metu and Anno) in Southwest, Ethiopia by quantifying SOC concentration and TOC quantity and distribution to 1.0 m depth in the soil profile under vetiver which is a C₄ grass and coffee (a major crop grown extensively in the study region). We aimed to determine: i) the concentration (SOC) and stock (TOC) of soil carbon under vetiver compared with adjacent coffee plantations, ii) the depth distribution of this carbon, and iii) the quantity of “new” carbon added by vetiver or source of the carbon using stable isotope analysis for the Jimma site (C₄ plant on a C₃ soil), where vetiver was preceded by a C₃ crop. The information generated from this study is intended to contribute to land management decisions for vetiver plantation systems in both Ethiopia and across tropical regions of the world.

Materials and methods

Site descriptions

Soil samples from vetiver and coffee plantations were taken from three study sites in southwest Ethiopia: Jimma, Metu, and Anno (Table 2 and Figure 6). The sample site at Jimma was located at the Jimma Agricultural Research Center (Melko). At Jimma the rainfall is bimodal with annual rainfall of 1561 mm with moisture deficit occurring from December to February each year (Kebede & Yaekob, 2009). The dark reddish brown soils of the area were formed in-situ from tertiary basalt and are classified as Eutric Nitisols (WRB) (FAO, 2014). These deep, clay soils have pH 5-6 and a medium to high cation exchange capacity (Kebede & Yaekob, 2009). The Metu study area included sites (farm lands owned by local farmers) at Gahi, Tulube and Sedo kebeles (the smallest administrative units in Ethiopia) near the town of Metu, IlluAbabora zone. The terrain is rolling with gentle slopes. Nitisols soil (WRB) (FAO, 2014) is dominant soil type in the area. Annual average rainfall is between 1660-2200 mm with the dry season occurring from November to February (Terefe, 2011). The Anno study area site was located at the Anno Agro-Industry PLC commercial seed (improved maize hybrid seed) producing and marketing farm, near the town of Bako. Anno has annual mean rainfall of 1100 mm, and the red soil is again classified as a Nitisols (WRB) (FAO, 2014). Since 1997, more than

300km (>500ha) of vetiver has been planted in strips ~at this farm as part of biological soil and water conservation measures (Table 2).

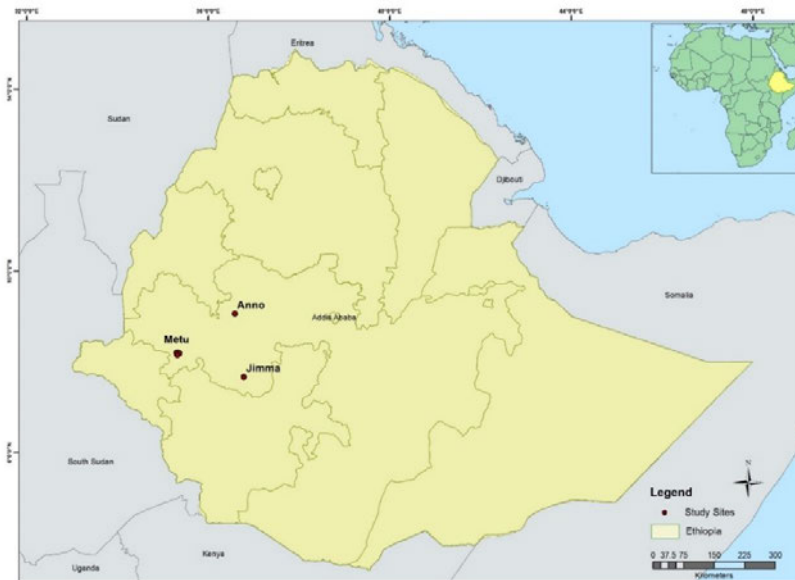


Figure 6: Location map of the study sites: Jimma zone (Jimma agricultural research centre), Metu district (Gehi, Tulube and Sedo kebeles in IlluAbabora zone) and Anno (Agro-Industry PLC) of the Oromia region in Southwest Ethiopia.

The Nitisols dominant at all three study areas are equivalent to an Australian Ferrosol (Isbell, 2016), to United States Department of Agriculture (USDA, 1999) Oxisol, and Nitisol (FAO, 2014). Nitisols are one of the most common soil types in Ethiopia, comprising 13.5% of the total 150,090 km² land area (Table 2).

Table 2: Locations, climate and soil types of the study areas in southwestern Ethiopia of the Oromia Region.

Site characteristics	Jimma	Metu	Anno
Site owned by	Public Research Center	Farmers	Private Agro-Industry
Location	36° 46' 54.01"E 7° 40' 2.9"N	35° 19' 47.63"E 8° 11' 36.73"N	36° 35' 0.12"E 9° 3' 41.03"N
Altitude (m)	1753	1669	1881
Rainfall (mm)	1561	1660-2200	1100
Temperature (°C)	9 – 28	12 - 27	27 (max average)
Soil type	Eutric Nitisols	Leptosols, Nitisols	Nitisols
WRB Equivalent	Nitisol	Nitisol	Nitisol
Age of vetiver (yrs.)	15 and 37	13 and 23	17
Purpose of vetiver plantation	Conservation and weed control	Conservation, income generation	Conservation, income generation

Experimental setting and soil sampling

We used three vetiver (C₄) and three coffee (C₃) plantation sites (Jimma, Metu and Anno) in southwestern Ethiopia. At these sites, vetiver had been planted on sites previously under C₃ crops as a biological soil and water conservation strategy. Soil sampling points within the areas were selected ensuring that each had an establishment history of more than 10 years (Table 2) which is recommended for soil carbon quantity and distribution inventory in studies of SOC and total nitrogen (TON) pools (Eswaran et al., 1993; Somebroek, 1993; Jobbágy & Jackson, 2000).

A nested plot experimental design was used for the soil sample collection using a paired site approach (Chan & McCoy, 2010; Sanderman et al., 2015) for vetiver and coffee plantations at each study site. The distance between sampling sites were: Jimma1 (coffee) to Jimma1 (vetiver) ≈ 450 m; Jimma1 (vetiver) to Jimma2 (vetiver) ≈ 815 m; Anno (coffee) to Anno (vetiver) ≈ 460 m; Metu1 (coffee) to Metu1 (vetiver) ≈ 3.60 km; Metu1 (vetiver) to Metu2 (vetiver) ≈ 7.46 km (Figure 7). Jimma2 (vetiver) and Metu2 (vetiver) sites were taken for vetiver age comparison on carbon storing potential. Three plots were sampled at each site and three soil cores were collected from each plot. The same

collection procedure was followed for all sites. Plots within vegetation types were separated by 20-30 m (based on the orientation of vetiver plantation on the farmlands), and three replicate core samples separated by 4.0 – 5.0 m were taken. Undisturbed soil cores (internal diameter = 50 mm) were taken to 1.0 m depth at each site and divided into seven depth increments (0-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.4, 0.4-0.5, 0.5-0.7 & 0.7-1.0 m). The age of vetiver plantations were 13 years at Jimma1, 15 years at Metu1, and 17 years at Anno. The age of coffee plantations were 14 years at Jimma1, 15 years at Metu1, and 17 years at Anno. Soil samples were also collected from the older age vetiver plantations at the Jimma2 (37 year old) and Metu2 (23 year old) sites to assess the impact of vetiver age and management on carbon storage potential. We grouped the vetiver age clusters into two for the purpose of analysis (~15 years as young and >20 years as old) at the Jimma site was preceded by C₃ vegetation (i.e. coffee which is a predominant crop in the area). The total samples for vetiver (younger) and coffee was 378 (3 sites x 3 plots x 3 cores x 7 depth x 2 plants), while the total samples of the older vetiver plantation was 105 (5 plots x 3 cores x 7 depth). In a small number of instances (5), data for some sample depths within cores were unavailable. This was the result of an insufficient soil sample for analysis, or the absence of collected samples due to bedrock at depth. Therefore, a total of 373 samples were collected for the younger age group. Hence, a combined total of 478 samples were collected from vetiver (young and old) and coffee plantations.



Figure 7: Soil samples were collected from vetiver and coffee plantations (1.0 m depth) in Jimma, Metu (Gahi, Tulube and Sedo kebeles) and Anno study locations in southwest Ethiopia of the Oromia Region. Soil sample points in the map represent vetiver (+) and coffee (◊) sample points at the three sites.

Sample preparation and analysis

Samples were oven dried at 40°C until a constant weight was obtained. Small subsamples (10g) were dried at 105°C to correct for oven dry moisture content. The 40°C dried samples were then crushed to pass through a <2mm sieve. Sieved soil samples (50g) were transported from Ethiopia to Australia for further analysis. Soil samples were further crushed using a ball mill to <200µm. Prior to analysis, soil samples were checked for the presence of inorganic carbonates (CaCO₃) using an acid pre-treatment (2% orthophosphoric acid H₃PO₄), for which all samples were negative. Soil sample analysis standards provided by UNE were then used to analyse SOC and TON concentration and δ¹³C (natural abundance of ¹³C) using a Sercon 20-22 continuous flow isotope ratio mass spectrometer (IRMS) connected to an ANCA-GSL sample preparation unit. For each sample, the ratio of ¹³C to ¹²C was determined against a known Vienna Pee Dee Belemnite (PDB) standard at the UNE Environmental Analysis Laboratory. Isotope ratios were expressed using the *delta* notation (δ) of units per mil or parts per thousand (‰) deviation from ¹³C:¹²C ratio using Equation 5 (Dalal et al., 2011a):

$$\delta^{13}\text{C} (\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \quad \text{Equation 5}$$

where R is the molar ratio of the heavy to light isotopes (¹³C/¹²C) of the sample or standard (Ehleringer et al., 2000; Dalal et al., 2011a).

Data processing and analysis

Bulk density (Equation 6) was corrected for moisture (105°C) and gravel (Equation 7) to calculate organic carbon stock for each sample at each depth. Total organic carbon stocks (Mg ha⁻¹) were calculated as a product of SOC%, bulk density and sampling depth (Batjes, 1998) (Equation 8) and the annual rate of TOC storage was calculated by dividing the TOC to the age of vetiver since establishment (Equation 9).

$$BD = \frac{m_{<2\text{mm}}}{v_{\text{total}}} \quad \text{Equation 6}$$

where *m* = mass and *v* = volume.

$$BD_{corrected} = \frac{m_{cann}}{(v_{total} - v_{gravel})} \quad \text{Equation 7}$$

where BD = bulk density, m = mass and v = volume.

$$TOC (Mg \text{ ha}^{-1}) = BD_{corrected} \times SOC\% \times Depth \quad \text{Equation 8}$$

$$TOC (Mg \text{ ha}^{-1} \text{ yr}^{-1}) = \frac{TOC (Mg \text{ ha}^{-1})}{Age} \quad \text{Equation 9}$$

For statistical analysis of TOC stock in the 0.5- 0.7m and 0.7-1.0 m depths, the sampling depth used in TOC calculations was adjusted from 0.2m and 0.3 m, respectively, to a representative 0.1 m in order to plot SOC distribution down the soil profile and mathematical model fitting.

For statistical analysis, data collected were grouped according to plant type (coffee-C₃ and vetiver-C₄), location (Jimma, Metu and Anno), plantation age (new [all ages between 13 and 17 years and categorized as 15 years] and old [20+ years]) and the seven depth increments. The data were also categorized into two groups, with group one being a comparison of similar aged vetiver and coffee plantations, and group two a comparison of old and new vetiver plantations to test if age had an effect on SOC storage potential of vetiver. We then conducted an analysis of TOC stocks across depth increments based on the effect of plant type, depth, age and soil depth:

1. Effect of plant types (vetiver and coffee) and depth
2. Effect of management duration (age) of vetiver establishment at Jimma and Metu sites

The differences found between sites were to be expected and between site differences were not considered further in the analysis. Statistical differences in SOC, TOC, $\delta^{13}\text{C}$ and their distribution in the soil profiles between vegetation type were tested using two-way analysis of variance (ANOVA) and nonlinear models using RStudio for Windows version (Version 00.99.879). Tukey's honestly significantly different test was performed when statistically significant differences ($P < 0.05$) were observed between factors. Preliminary data exploration of the relationship between SOC and its distribution down the soil profile

in vetiver and coffee revealed a nonlinear pattern. Therefore, a non-linear regression was applied using an exponential decay function (Equation 10),

$$y = a \times \exp(-bx) \quad \text{Equation 10}$$

where y = carbon, x = soil depth, a = y-intercept and b = decay constant (> 0).

Isotopic $\delta^{13}\text{C}$ signatures of -12.8 ‰ and -29 ‰ were determined for vetiver and coffee biomass, respectively. Where, the distinctive $\delta^{13}\text{C}$ values of vetiver and coffee enabled an estimation of the new C_4 carbon that had been added by vetiver with reference to the adjacent coffee plantation at Jimma site, while Anno and Metu sites were excluded for this calculation due to the absence of a clear history about the preceding crop which might be a C_4 plant (e.g. the adjacent sorghum). The $\delta^{13}\text{C}$ values determined from the Jimma site were therefore used to calculate the additional carbon contributed by the C_4 grass (vetiver) compared with the coffee plantation (Equation 11),

$$\text{C}_4 - \text{C} = \frac{\text{C}_x - \text{C}_3}{\text{C}_4 - \text{C}_3} \times 100 \quad \text{Equation 11}$$

Where, $\text{C}_4 - \text{C}$ is the C_4 plant (vetiver) derived carbon, C_x is measured $\delta^{13}\text{C}$, $\text{C}_3 - \delta^{13}\text{C}$ of the C_3 crop (coffee), C_4 is the $\delta^{13}\text{C}$ of vetiver. The assumption underpinning this calculation was that in the absence of vetiver the $\delta^{13}\text{C}$ value for the preceding C_3 crop was equivalent to C_3 plants (e.g. coffee).

Results

Effect of plant type and depth on SOC, TON, TOC and $\delta^{13}\text{C}$

SOC and TON concentrations

Analysis of variance showed a significant plant type and depth effect on SOC concentration ($P < 0.001$). The difference between plant types was indicated by a higher concentration of carbon under vetiver in all sites except Metu which was not significantly different compared with coffee (Figure 3a). This effect was observed across all depths although the largest differences occurred at the soil surface. Similar to SOC, TON concentration was also significantly affected by plant type and depth ($P < 0.001$). Vetiver also had a significantly higher soil TON concentration compared with coffee in all sites except at Metu, with the effect again occurring at all soil depths (Figure 8b).

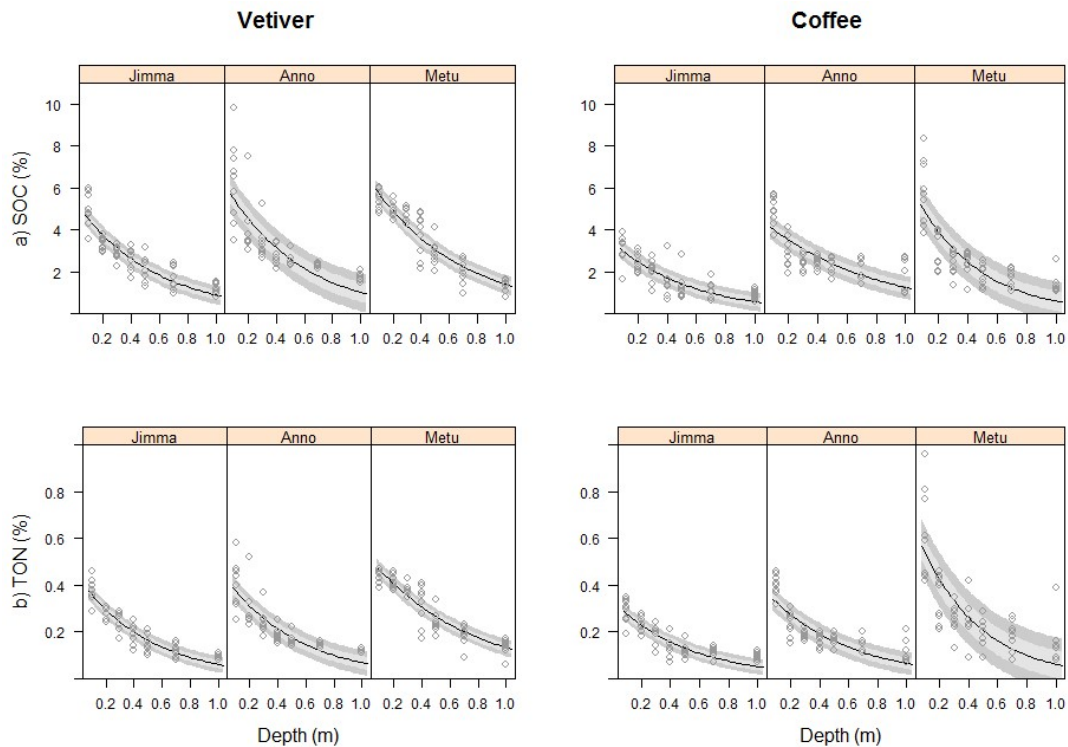


Figure 8: Variation of SOC (a) and TON (b) concentration in soil cores (1.0 m deep) collected from coffee plantations and from vetiver strips in three locations in Ethiopia. Plots show the raw data (O), a fitted exponential model (—), and confidence bands (1 and 2 SE).

TOC stock

Analysis of variance also indicated a significant plant type and depth effect on total carbon stock ($P < 0.001$). A significantly larger quantity of carbon was stored under vetiver compared with coffee, particularly in the deeper soils (Figure 9). Total organic carbon stock stored under vetiver at the Anno, Jimma and Metu sites was 255, 213, 318Mg C ha⁻¹, respectively (mean of 262 Mg C ha⁻¹ for vetiver), while for coffee the result was 185, 169 and 184 Mg C ha⁻¹ for the respective sites (mean 178Mg C ha⁻¹) to 1.0 m soil depth (Table 3). Hence, TOC of vetiver was significantly larger compared with coffee in all sites (i.e. +134, +70 and +44 for Metu, Anno and Jimma sites, respectively). The mean TOC stored across the full 1.0 m under vetiver was 47% (+84 Mg C ha⁻¹) higher compared with coffee. For both plant types across all sites a decrease in TOC was observed with increasing soil depth.

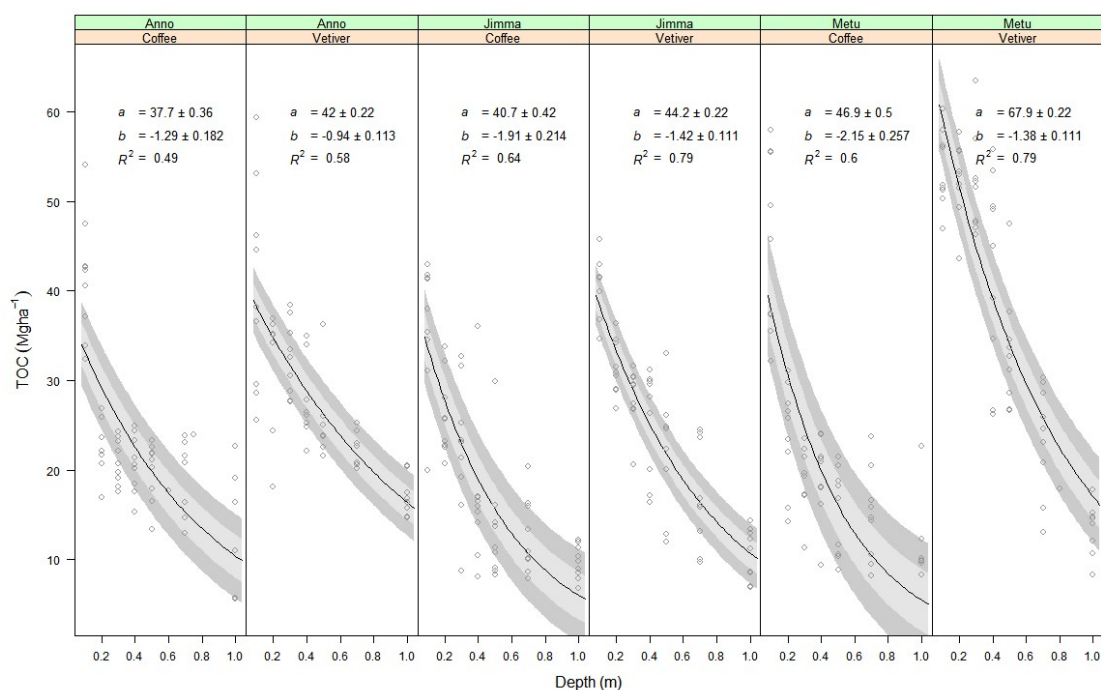


Figure 9: Variation of TOC stock (Mg ha⁻¹) in soil cores (1.0 m deep) collected from vetiver strips and an adjacent coffee plantation in three sites of Ethiopia. Plots show the raw data (O), a fitted exponential model (—) and confidence bands (1 and 2 SE). Values of the intercept (a), the slope (b) and Coefficient of determination (R²).

Table 3: The mean TOC (Mg ha^{-1}) and the Standard Error (SE) in 1.0 m soil profile for vetiver and coffee plantation sites at Anno, Jimma and Metu sites in southwest Ethiopia.

Plant type	Site	Mean TOC \pm SE (Mg ha^{-1})
Vetiver	Anno	255 \pm 6.68
	Jimma	213 \pm 10.94
	Metu	318 \pm 9.68
Coffee	Anno	185 \pm 12.99
	Jimma	169 \pm 12.87
	Metu	184 \pm 11.97

Carbon isotope ratios ($\delta^{13}C$)

Significant differences were observed in the $\delta^{13}C$ value between the soils under vetiver and coffee plantations and with depth increment ($P < 0.001$). Lower $\delta^{13}C$ values (more negative) were recorded at the soil surface and increased (less negative) with increasing soil depth (Figure 10). This increase in the $\delta^{13}C$ value was larger under the coffee plantation than under the vetiver (Figure 10a). Compared with coffee, $\delta^{13}C$ values of soils were significantly higher (less negative) for vetiver, in all soil layers and at all sites except a little higher (less negative) value of coffee at depth at the Metu site.

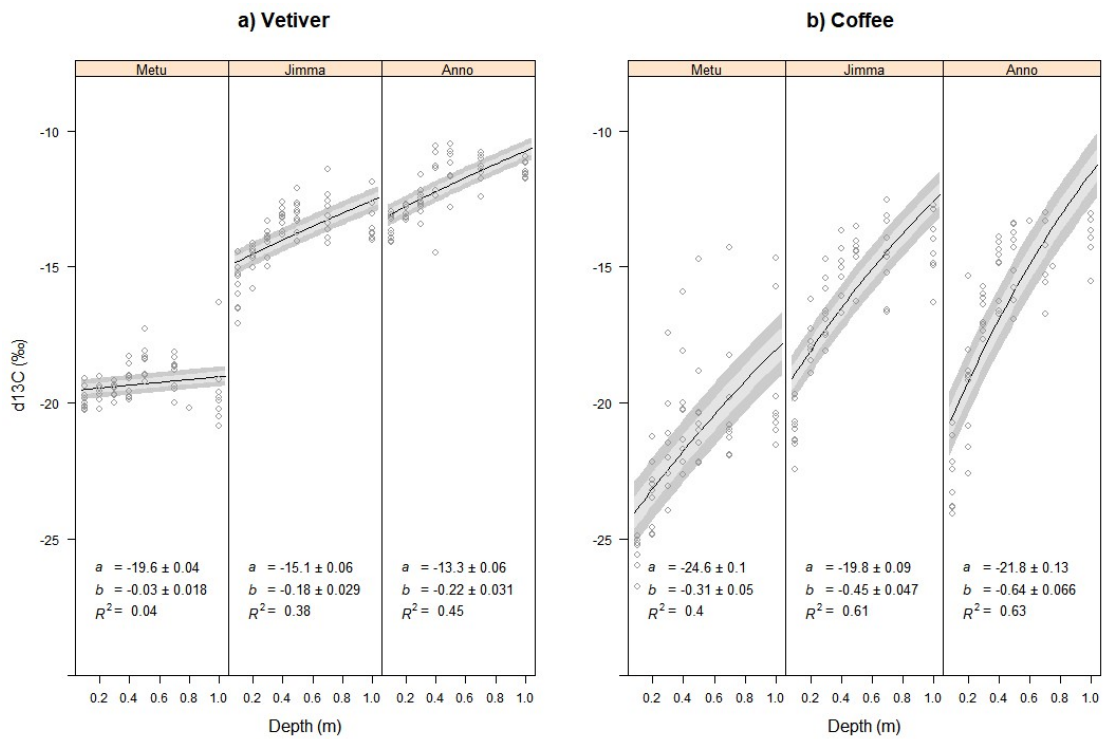


Figure 10: Variation of the carbon isotope ratios ($\delta^{13}C$) in soil cores (1.0 m deep) under vetiver (C_4) plantation and an adjacent coffee plantation at Metu, Jimma and Anno, Ethiopia. Plots show the raw data (O), a fitted exponential model (—) and 95% confidence bands (1 and 2 SE).

Effect of Age of Vetiver on SOC, TON, TOC, $\delta^{13}\text{C}$ and New Carbon

SOC and TON concentrations

Analysis of variance indicated a significant age (since establishment) and depth effect on SOC concentration ($P < 0.001$) in vetiver plantations. A higher concentration of carbon was recorded in younger vetiver plantations (15 years) compared with the older plantations (20+ years) for both sites. This was more pronounced at Metu where there was a difference in depth, particularly in the upper 0.3 m of the soil profile (Figure 11a). The SOC declined with increasing depth for younger and older plantations of vetiver. Similarly, age and depth had significant effects on TON concentration ($P < 0.001$). Patterns of TON concentrations were like SOC concentrations where higher TON concentrations were observed in the younger plantations (Figure 11b), where the effect largely occurred in the upper soil depth. The TON concentration also consistently declined with depth for both recent and older plantations.

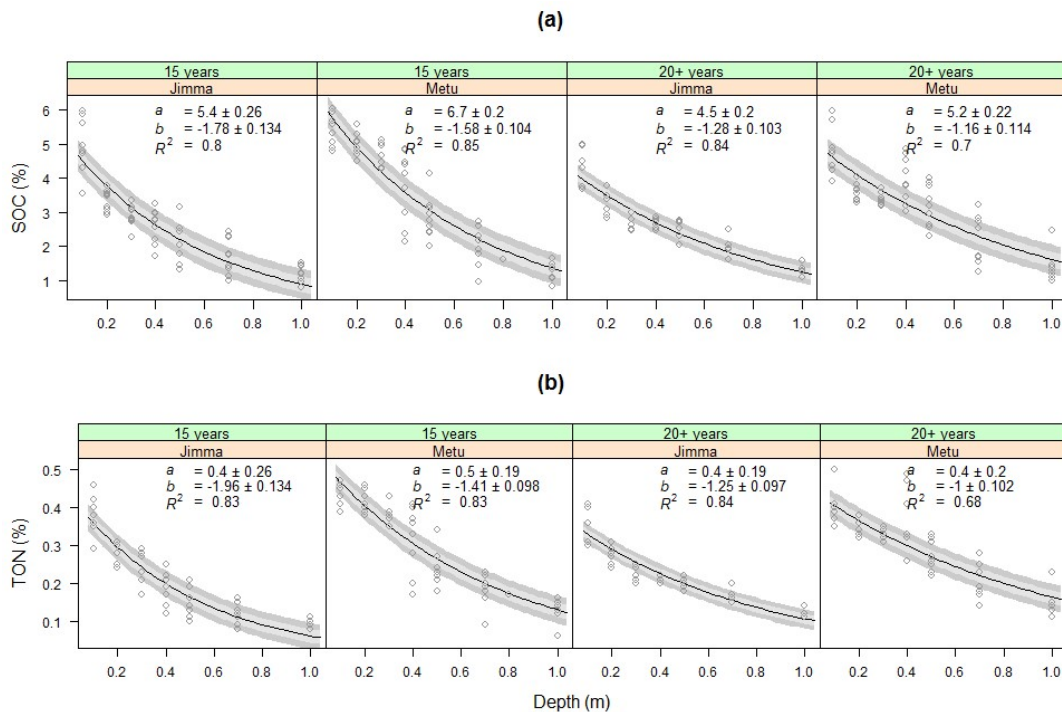


Figure 11: Variation of SOC (a) and TON (b) concentrations in soil cores (1.0 m soil profile) collected from vetiver strips of two age categories in two locations in Ethiopia. Plots show the raw data (\circ), a fitted exponential model ($-$) and confidence bands (1 and 2 SE). Values of the intercept (a), the slope (b) and Coefficient of determination (R^2).

TOC stock

Analysis of variance indicated that age and depth had significant effects on total organic carbon stocks. Differences were indicated by a higher TOC stock in the younger vetiver plantations compared with the older vetiver plantations only at Metu sites (Figure 12). The average additional total carbon stored on annual basis by the younger vetiver was 213 and 318 Mg C ha⁻¹ at a rate of 16.4 and 21.2 Mg ha⁻¹ yr⁻¹ at Jimma and Metu sites, respectively, whereas under the older plantations the additional total carbon stocks was 250 and 289 Mg C ha⁻¹ over the plantation period which is a lower accumulation rate of 6.8 and 12.6 Mg ha⁻¹ yr⁻¹ compared with the younger plantation, respectively (Table 4).

Table 4: TOC (Mg ha⁻¹) and carbon accumulation rate (Mg ha⁻¹ yr⁻¹) in 1.0 m soil profile for different age of vetiver plantation sites at Jimma and Metu sites in southwest Ethiopia.

Site	Age of vetiver (years)	TOC (Mg ha ⁻¹)	Rate (Mg ha ⁻¹ yr ⁻¹)
Jimma	13	213 ± 10.94	16.4
	37	250 ± 9.09	6.8
Metu	15	318 ± 9.68	21.2
	23	289 ± 10.1	12.6

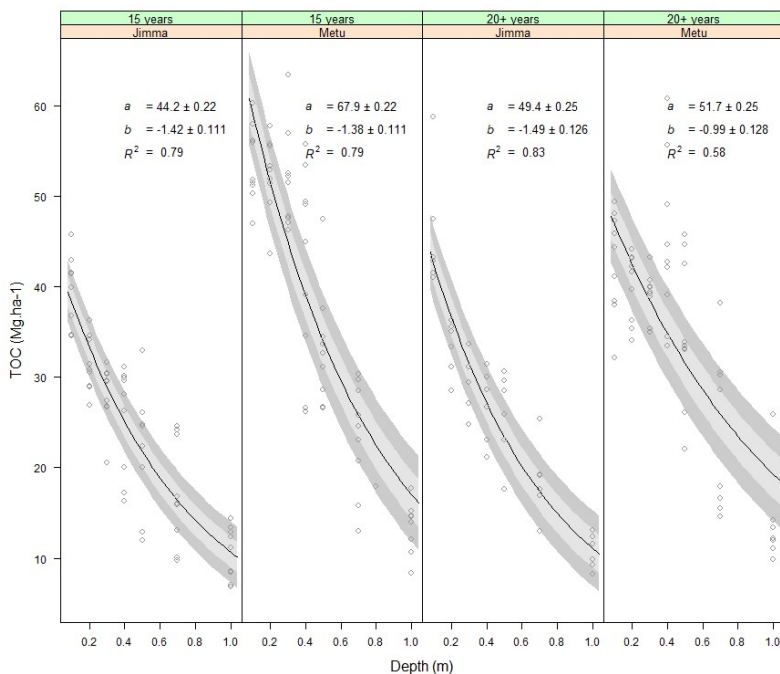


Figure 12: Variation of TOC stock in soil cores (1.0 m deep) collected from vetiver strips of contrasting ages in three locations in Ethiopia. Plots show the raw data (O), a fitted exponential model (—) and confidence bands (1 and 2 SE). Values of the intercept (a), the slope (b) and Coefficient of determination (R²).

Stable Carbon Isotopes ($\delta^{13}C$)

Significant differences were observed in the $\delta^{13}C$ values for which both age and depth were significant factors (Figure 13). The difference was indicated by higher $\delta^{13}C$ values (less negative) under the old vetiver at the soil surface and at Jimma site compared with the young vetiver plantations. The change in the $\delta^{13}C$ values was reflected between and within age categories. Both recent and older plantations showed an increase in the $\delta^{13}C$ values (less negative) with increasing soil depth, except the older plantation at one of the sites (Metu) which showed a decrease with increasing soil depth.

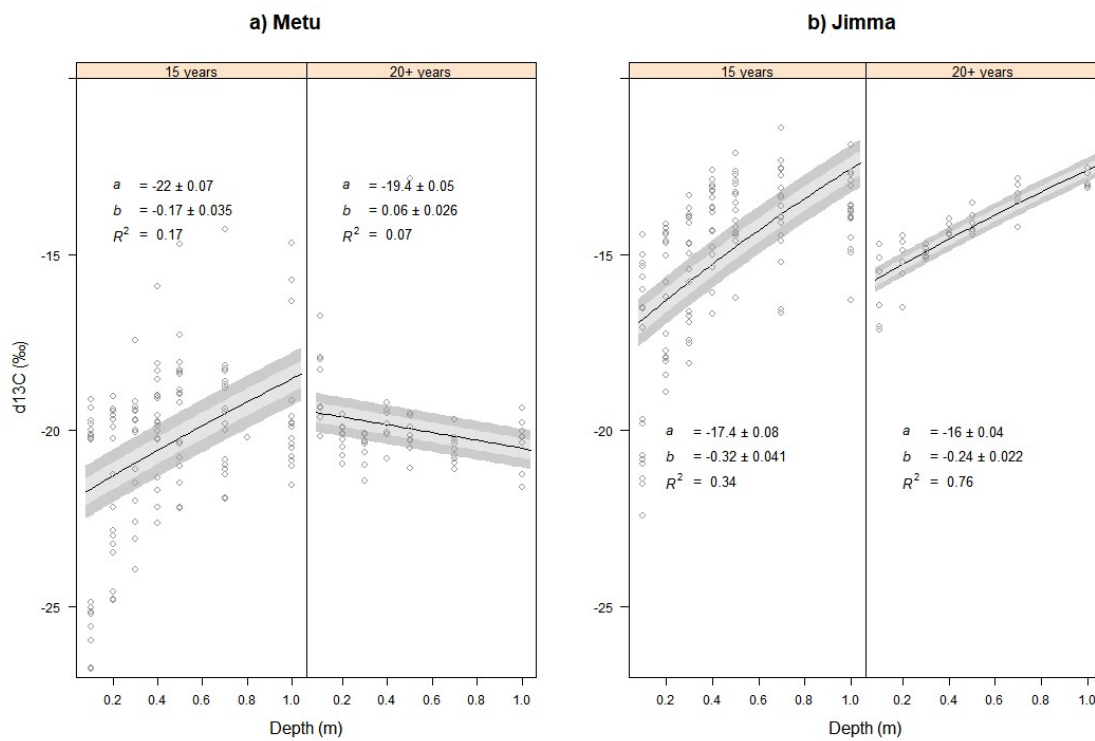


Figure 13: Variation of $\delta^{13}C$ in soil cores (1 m deep) collected from vetiver strips of contrasting ages in three locations in Ethiopia. Plots show the raw data (O), a fitted exponential model (—) and confidence bands (1 and 2 SE). Values of the intercept (a), the slope (b) and Coefficient of determination (R^2).

New carbon input by vetiver at different age of establishment at Jimma

Using the coffee plantation $\delta^{13}\text{C}$ value as a baseline, significant differences were observed in the new carbon added by vetiver between the young and old vetiver plantations at Jimma (Figure 14). Comparing the two age groups, 93% and 90% of the original C_3 carbon has been replaced by the new C_4 carbon from the young and old vetiver plantations, respectively. By considering the age of establishment (young = 13 year and older = 37 year) at Jimma, the $\delta^{13}\text{C}$ values helped to calculate the new carbon replaced which was ($6.2\% \text{ year}^{-1}$) and ($2.4\% \text{ year}^{-1}$) by the younger and older vetiver plantation, respectively of the original carbon on annual basis (2.5 x) higher. The new carbon added by the younger vetiver has shown a higher $\delta^{13}\text{C}$ value than by the older vetiver plantation at all soil depths except at deeper soil profile.

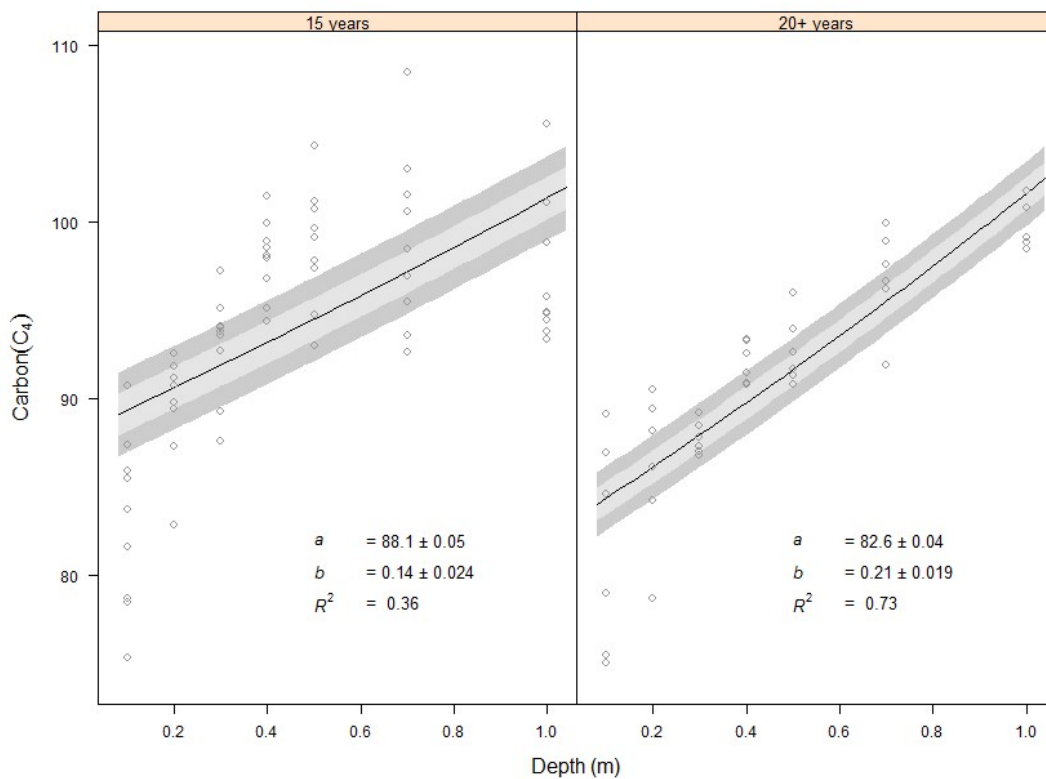


Figure 14: Variation of new C_4 carbon in soil cores (1.0 m deep) under vetiver of two age groups of plantations at Jimma site, Ethiopia. Plots show the raw data (o), a fitted exponential model (—) and 95% confidence bands. Values of the intercept (a), the slope (b) and Coefficient of determination (R^2).

Discussion

Effect of plant type and depth on carbon concentration, stock and $\delta^{13}\text{C}$

Carbon concentration and stock

Vetiver accumulated higher concentrations of SOC in almost all sites studied, which resulted in a higher total stock (+84 Mg ha⁻¹) compared with the coffee plantation through the whole sampling depth (1.0 m). Although SOC concentration (Figure 8) was not significant at Metu, TOC stock was significantly different between plant types at this site presumably due to the difference in the bulk density and environmental factors such as temperature. Hence, the need to express results in equivalent soil mass is crucial for the TOC stock. In this regard numerous tropical grasses have been reported to accumulate large quantities of SOC due to their high above-ground biomass (and root mass) production (Hansen et al., 2004b; Chan & McCoy, 2010; Poeplau & Don, 2013; Robertson et al., 2013; Robertson et al., 2015). *Miscanthus* for example had a higher total soil carbon storage of (131.3 Mg C ha⁻¹) over 13 years compared with arable cropland (105.8 Mg C ha⁻¹) (Dondini et al., 2009). The larger carbon concentration and stock in the soils we studied was most pronounced near to the soil surface, which could be as a result of a more organic matter (litter and roots) contributed at the soil surface because of the exponential decline in root concentration (Waisel & Eshel, 2002), and the decrease in organic matter decomposition with increasing depth.

Stable Carbon Isotopes ($\delta^{13}\text{C}$)

The results showed that plant type and depth as significant factors determining the $\delta^{13}\text{C}$ value. The sites planted with vetiver had a higher $\delta^{13}\text{C}$ through the whole soil profile compared with the coffee plantations which is an indication of significant quantity of C₄ carbon addition through the whole profile, possibly through the high root biomass (Ehleringer et al., 2000). The higher $\delta^{13}\text{C}$ value (less negative) demonstrated that pre-existing C₃ carbon is being replaced by C₄ carbon. The addition of new carbon might have been countered by decomposition of C in the profile. So, the addition of TOC and indeed new C₄ is only a balance between these competing processes. The lower $\delta^{13}\text{C}$ values in the surface soil layers which showed a progressive increase with increasing soil depth at Jimma site is a commonly observed effect resulting from increasing litter decomposition and humus formation with

depth (Ehleringer et al., 2000; Chen et al., 2005; Ma et al., 2009). The progressive increase in $\delta^{13}\text{C}$ value with increasing depth agrees with previously reported data stating a decrease of $\delta^{13}\text{C}$ with increasing soil depth (Ehleringer et al., 2000; Badeck et al., 2005; Schwendenmann & Pendall, 2006).

Effect of Age of vetiver on SOC, TON, TOC, $\delta^{13}\text{C}$ and New Carbon

Carbon concentration and stock

Studies suggest that management duration or the age at which the soil is covered with vegetation has a significant impact on changes in the amount of soil carbon stored over time (Batjes, 2000; Yan et al., 2007; Abberton et al., 2010b). Neill et al. (1997) also stated that long term carbon accumulation in pasture lands is determined by the length of time the land remains under pasture, regardless of specific mechanisms such as soil physical and chemical process. However, Conant et al. (2001) noted that duration only explained a small amount of soil carbon variability in response to management changes, with climate and disturbance accounting for other sources of variability. The current study indicated that the younger vetiver plantation had larger SOC and TON concentration which was also the case for TOC stock. The difference in TOC stock between the young and old vetiver plantations were not comparable due to some management regime which is in agreement with (Conant et al., 2001). Hence, in this study the sites responded differently and the factors which have affected the soil carbon dynamics might have been related to environmental factors such as high rainfall (Metu is wetter) and management or farming practices in the respective areas.

$\delta^{13}\text{C}$ and New carbon annual addition rates by vetiver at Jimma

Our results from Jimma indicated that the age of vetiver plantation and soil depth had an effect on the $\delta^{13}\text{C}$ value, which is a signal of the addition of C_4 derived new carbon. Both the young and old plantations had a higher $\delta^{13}\text{C}$ (less negative) values at Jimma, increasing further with increasing soil depth which is an implication of vetiver modifying carbon at depth (Ehleringer et al., 2000). The $\delta^{13}\text{C}$ signature indicated that much more $\delta^{13}\text{C}$ depleted carbon has been added to the soil under vetiver grass. The lower $\delta^{13}\text{C}$ values at the surface soil layers which increased with depth for both age categories is a commonly observed trend resulted from increasing organic carbon decomposition except at Metu, which is a progressive enrichment in $\delta^{13}\text{C}$ values. The younger vetiver plantation has added a significant

quantity of soil carbon through the whole profile compared with the older vetiver plantation. This indicates that at the earlier growth stage of vetiver, SOC gain was large and faster may be due to the higher photosynthesis process associated with its fast growth and the fast root system establishment. In addition, perhaps in the early years the associated farm management practices such as adding inputs could have contributed to the faster SOC gain. However, addition of C₄ carbon diminished as plants age. The higher $\delta^{13}\text{C}$ values in younger vetiver compared to older plantation could be due to the change in $\delta^{13}\text{C}$ signature with age and microbial decomposition. Hence, the shift was an effect of the combination of both accumulation processes associated with C addition and decomposition.

The effect of age of establishment of vetiver reflected on the nature of carbon and the new carbon added in the soil. The younger vetiver plantation performed better in terms of adding new carbon compared with the older plantation which was reflected on the TOC stock and the rate at which the new carbon added on an annual basis. A mean C₄-C sequestration rate of $(0.94 \pm 0.024 \text{ Mg C ha}^{-1} \text{ yr}^{-1})$ was found by the younger vetiver, which conforms with rate at which carbon sequestered by a similar age *Miscanthus* $(0.78 \pm 0.19 \text{ Mg C ha}^{-1} \text{ year}^{-1})$ (Poeplau et al., 2013). However, the older vetiver plantation showed a much lower mean C₄-C sequestration rate $(0.16 \pm 0.019 \text{ Mg C ha}^{-1} \text{ yr}^{-1})$ which was (~6x) less suggesting that after a certain age the rate at which the new carbon being added by vetiver started declining over the years (Neill et al., 1997; Ehleringer et al., 2000). This could be related to the physical and chemical processes such as decomposition and other plant growth factors at different planting 'ages' and specific factors related to climate (temperature and rainfall) and management practices. The newer plantations are more vigorous thus increasing biomass productivity both above- and below-ground and the way vetiver strips managed regarding cutting, moving and grazing could have contributed to the difference in soil carbon between the two age groups.

Conclusion

Our results show that the contribution of vetiver was high due to an increased SOC and particularly TOC concentration and the total carbon stock through the whole sampled depth (1.0 m). Vetiver accumulated higher concentrations of SOC and higher total stock compared with coffee plantations. The higher $\delta^{13}\text{C}$ values under vetiver also suggest that this C_4 species with a distinctively higher $\delta^{13}\text{C}$ signature added a significant quantity of new carbon through the whole soil profile. The continuous addition of new carbon into the soil has therefore, particularly at the earlier age of vetiver plantation, resulted in the larger total carbon and suggested that C_3 carbon replacement by C_4 (vetiver) carbon particularly in the surface soil layers. Hence, there is a need to continue advancing knowledge on the potential of tropical perennial pasture species to influence land management decisions in tropical and sub-tropical regions of the world.

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(To appear at the end of each thesis chapter submitted as an article/paper)

We, the Research PhD candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated in the *Statement of Originality*.

	Author's Name (please print clearly)	% of contribution
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STATEMENT OF ORIGINALITY

We, the Research PhD candidate and the candidate's Principal Supervisor, certify that the following text, figures and diagrams are the candidate's original work.

Type of work	Page number/s
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CHAPTER 5: Functional Links between Soil Organic Carbon, Biomass and Decomposition of Vetiver (*Chrysopogon zizanioides*) Grass in three Different Soils

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Abstract

Below-ground plant biomass is a primary factor that can contribute to soil carbon sequestration, both at the surface and at depth. Perennial grasses, particularly those that have a deep root system, are likely to contribute significantly to soil carbon. However, we have a limited knowledge of how their root and shoot decomposition differs and their contribution to soil carbon sequestration. Vetiver (*Chrysopogon zizanioides*) is a C₄ perennial tropical grass that can produce a large root system which might contribute significantly to soil carbon. In this study, we examined the above- and below-ground biomass production and the relative decomposition of vetiver grass. Vetiver plant material was grown under glasshouse conditions and subsequently incubated for 206 days and ANCA-GSL a combined elemental analyser was used for the gas analysis. The results from this study confirmed the large biomass production potential of vetiver grass over a short period of time even in soils with low fertility (161 and 107 Mg ha⁻¹ fresh and 67.7 and 52.5 Mg ha⁻¹ dry shoot and root biomass, respectively). Shoot to root biomass production ratio was 1.43 and 1.25 for the fresh and the dry biomass production, respectively. Vetiver root materials decomposed more rapidly compared with the shoot material regardless of where they were sampled (depth) from, which could be attributed to the lower C:N ratio of the roots than the shoots. This finding therefore, suggests that for vetiver, the large root biomass produced does indeed contribute more to the soil carbon accumulation and the faster decomposition of root litter is crucial in releasing the carbon in the root litter and would also speed up its contribution to stable soil organic matter. Hence, planting vetiver and similar tropical perennial grasses on degraded and less fertile soils could be a good strategy for carbon sequestration and to rehabilitate degraded soils.

Key Words: Root, Shoot, Soil type, Australia

Introduction

Soils globally are important in sequestering atmospheric carbon and can thus significantly affect greenhouse gas flux (Batjes, 1996). Retention of organic matter (OM) in soil is however, largely controlled by environmental variables, the nature of the OM, and its spatial distribution and interactions with other soil constituents. Therefore, maximizing the carbon input, and minimizing the rate of organic matter decomposition after deposition in soil, are two important factors that can help to increase the amount of carbon sequestered from the atmosphere (Reichle et al., 1999).

Plant production and decomposition determine carbon inputs to the soil profile, and as such, plant shoot and root allocation (above- and below-ground, respectively), as well as allocation of roots between shallow and deep soil layers, can result in a very different soil carbon distribution with depth in the profile (Jobbágy & Jackson, 2000). Tropical perennial grasses grow continually and are adapted to a wide range of soil and climate conditions (Reichle et al., 1999; McKenzie & Mason, 2010). For many years, the International Center for Tropical Agriculture (CIAT) has been working on selecting tropical grasses with deep and massive root systems that can exploit nutrients and water from deeper soil profiles (Fisher et al., 1994; Fisher et al., 2007). McKenzie and Mason (2010), indicated that deep soil profiles with fertile subsoil allow deep root penetration into the subsoil where the environment is cooler and less likely to promote organic carbon decomposition than in topsoils. Belowground biomass is therefore believed to be a primary vehicle for soil carbon storage (Kuzyakov, 2002; Nguyen, 2003; Kell, 2011). Hence, perennial grasses, due to their deep root systems, might contribute significantly to soil carbon (Fisher et al., 1994; Fisher et al., 2007), via biomass inputs and slow mineralization processes due to slow OM turnover at depth (Monti & Zatta, 2009). Studies also report that a large root biomass can support substantial soil microorganism populations and their metabolic processes, and thus contribute significantly to soil organic matter decomposition and carbon turnover (Kuzyakov, 2002). A precise relationship between root biomass and soil organic carbon (SOC) is not, however, easy to establish because soil OM decomposition depends on several interacting factors including climate, litter quality, water and nutrient availability, soil type/texture and biotic activity (Scherer-Lorenzen et al., 2007; Bills et al., 2010; Zatta et al., 2013).

Litter quality factors important to decomposition and mineralization include the chemical composition of the organic matter (e.g. C:N ratio), whereby litter with higher concentrations of nutrients and lower concentrations of lignin will decompose more rapidly (Walela et al., 2014). Soil texture, and in particular clay content, can assist in the physical protection of SOC within soil aggregates and therefore suppress decomposition and promote SOC storage (Bronick & Lal, 2005). Bacteria and fungi are primary decomposers in soils, and soil structure and texture can be a dominant control over

decomposition as they affect accessibility of microbes to the soil substrate and OM. These factors are reflected in different decomposition rates between different types of soil (Van Veen & Kuikman, 1990). However, in decomposition studies, much attention has been given to biotic and abiotic (temperature and moisture) factors rather than soil structure and texture which are clearly linked (Van Veen & Kuikman, 1990; Van Groenigen et al., 2014). Clay content is associated with factors such as plant growth and moisture and a larger retention of carbon. For example, clayey soils have on average slower decomposition rates and higher retention of OM than sandy soils and a negative correlation between clay content and decomposition of crop residues is often found (Van Veen & Kuikman, 1990). McKenzie and Mason (2010), similarly stated that clay soil types in general result in a slower rate of decomposition compared to sandy soils.

There are a number of below-ground factors that moderate OM decomposition. For example soil moisture content is essential for decomposition, although excessive moisture can lead to anaerobic conditions and reduced the rates of OM breakdown because of a lack of oxygen for soil organisms compared to soils exposed to the atmosphere (McKenzie & Mason, 2010). de Wit et al. (2014), showed that reduced tillage can also promote SOC sequestration by limiting soil disturbance, which reduces decomposition by aeration. However, Chendev et al. (2014), indicated that accelerated OM decomposition due to coarse textured soils in warm temperatures and low water holding capacity can limit plant growth which can result in low SOC. Scheffer and Aerts (2000), stated that roots and rhizomes can play a major role in cycling of carbon and nutrients. But, Amougou et al. (2012) indicated that abscised leaves (in their case, of *Miscanthus*) can contribute more to the soil carbon accumulation than rhizomes or roots. Beuch et al. (2000), similarly mentioned that *Miscanthus* roots, compared to shoots, have less readily decomposable soluble compounds. Hence, moisture, reduced tillage, soil texture, temperature and below-ground biomass are the factors playing key roles in the SOC sequestration and decomposition. Studies on the decomposition of belowground plant parts are therefore important to fully understand carbon cycling.

In this study, we examined vetiver's (*Chrysopogon zizanioides*) above- and below-ground biomass production where plants were grown under glasshouse conditions in sandy soil, in addition to the relative decomposition rates of the grass shoot and root biomass when incubated with three Australian soil textures (sand, silt and clay) with different initial properties (e.g. texture, pH and SOC). The aim of this study was to: Quantify the above- and below-ground biomass of vetiver grown in sandy soil under glasshouse conditions; Measure the relative difference in the rate of decomposition of the above- and below-ground biomass of vetiver grass and determine the effect of contrasting soil types on root and shoot decomposition.

Materials and Methods

Experimental setting and design

To determine the above- and below-ground biomass production and relative decomposition of vetiver (*Chrysopogon zizanioides*) grass, an experiment was undertaken from late 2014 through to 2016 at the University of New England, Australia. Specimens of vetiver were collected in March 2014 from the NSW Office of Environment and Heritage, Gunnedah Research Centre, NSW where the vetiver had been established for more than 20 years. Specimens were vegetatively propagated and then maintained in a UNE glasshouse until required. Prior to the experiment, a three-month pilot experiment was conducted in a glasshouse where vetiver was planted in 12 small pots (0.3 m height x 0.12 m diameter) with a sand textured soil. Within three months, vetiver roots had reached the bottom of the pots, therefore, we estimated that a six-month growth period would be suitable for vetiver root extension in 1.0 m length pots for the subsequent experiment.

Treatment description

A sandy loam soil collected from Kirby/ Newholme Farm, UNE was used for biomass production so that the root biomass could be recovered easily. The soil was collected from the UNE Newholme Farm and had a sandy loam texture and extremely low fertility (see Table 5 for soil characterization). We added 4 g of multi-grow fertilizer (10.1% N, 3.5% P, 5.5% K, 16.3% S and 7.8% Ca) via surface fertilizer application to pots at the same point as root cuttings (~ 6 cm depth) to provide starter nutrients to the 10 replicate pots (radius = 6 cm, height = 1.0 m, area = 113.04 cm²). The resultant biomass from five randomly selected pots was used to measure shoot and root biomass production, and fresh biomass (refrigerated) from two randomly selected pots supplied plant material for the decomposition experiment (Table 6). For each pot, a single vetiver plant was split and cut to a 0.06 m root and 0.12 m shoot length, then planted and watered to upto 60% Field Capacity (FC)—follow-up watering to the same moisture content was conducted every second day of the experiment under ideal ambient temperature and humidity glasshouse condition.

Table 5: Physical and chemical characteristics of the soils used for the biomass and decomposition experiments

Soil description	Based on texture	Sand %	Silt %	Clay %	SOC%	$\delta^{13}\text{C}$ ‰	%TN	$\delta^{15}\text{N}$	C:N	pH
Sand	Sandy loam	76.30	10.50	13.20	1.06	-18.39	0.09	0.37	0.09	4.4
Silt	Silty clay loam	45.00	25.00	30.00	2.25	-19.80	0.20	0.37	0.44	5.2
Clay	Silty clay	19.00	26.00	55.00	2.03	-19.23	0.14	0.37	0.29	6.4

Table 6: Biomass assessment design used for biomass and decomposition experiment

Experiment	No. of pots	Reps	Biomass	Materials used	Activity	Analysis
Biomass production	5	5	Fresh	Shoot and Root	Harvesting	Measure and weighing
		5 ¹	Dry	Shoot and Root		
Decomposition ²	2	4	Fresh	Shoot	Incubation	GC-MS
		4 x 7 depth	Fresh	Roots		
		4	None	Soils		

1. The 5 dried reps were the 5 fresh reps dried after fresh mass was recorded

2. Fresh plant supply only for further decomposition study

Sample collection, preparation & analysis

Biomass Assessment

The extent of root growth and its extension through the entire soil volume in selected pots was determined by CT-scanning during month six of the experiment to re-confirm a sufficient root mass had established in the pot and plants were suitable for harvesting—any further extension may have been restricted by the pot. At the end of the experimental period (213 days), five of the 10 pots were randomly selected and vetiver harvested for above- and below- ground biomass assessment while the other five were used for the decomposition experiment. Soils from each pot were divided into the following seven depth increments to vertically differentiate the root biomass: 0 - 0.1 m, 0.1 - 0.2 m, 0.2 - 0.3 m, 0.3 - 0.4 m, 0.4 - 0.5 m, 0.5 - 0.7 m, and > 0.7 m and the whole root mass extracted from each by washing with distilled water. Fresh shoots and roots were weighed to provide fresh biomass, then dried at 70 °C to provide dry biomass. A total of 5 shoot biomass samples and a total of 35 root biomass samples (5 x 7 depth increments) were used for biomass production analysis. Shoot to root ratio ($\text{g dry matter}^{-1} \text{ m}^{-2}$ of each depth increment), shoot/root length (m) and number of stems were counted and measured.

Decomposition Study

The decomposition study was conducted in 2016 for 206 days to assess vetiver shoot and root biomass decomposition when applied to three different soil types: sandy loam (Kirby sand_Chromosol), Silt (Dalkieth_Chromosol) and clay (Clarke's farm_Dermosol) (particle size distribution detailed on Table 5). The three different soil types had different initial properties such as clay content, initial SOC (%) and pH. The field capacity was calculated as 0.7027, 0.3576 and 0.3377 g water g^{-1} soil for the clay, sand and silt soil textures, respectively. We used sealed polypropylene jars (250 ml) with lids fitted with septa to facilitate gas sampling during incubation of soil and plants material. The fresh biomass was supplied from two pots randomly selected from the 10 replicate pots established during the biomass assessment, and resultant shoot and root (divided into the seven depth

increments detailed above) biomass were refrigerated until the incubation installation. Fresh shoot and root (7 depth increments) biomass samples (0.05 gm) were chopped to between 5–10 mm and then added to the soil surface (25 g) in containers (250 ml) (see Table 9 for ratios), with four replicates of each soil to biomass mixture. Four replicate controls of each soil type with no biomass material added were also included, along with four blank containers (N = 112) (Table 8). Containers were placed in a constant temperature (25 °C) cabinet in the dark. Six gas samples were taken from each vessel during the incubation period (at Day 7, 16, 42, 83, 134 and 206). At each sampling time, 12ml vacutainers were evacuated and a gas sample extracted from the headspace within each container. Following this, jars were opened to the ambient air and watered to achieve 60% field capacity. Septa were then replaced and jars re-sealed and returned to the constant temperature environment until the next sampling time.

Table 7: Properties of vetiver biomass

Vetiver	SOC (%)	$\delta^{13}\text{C}$	TN %	C:N
Shoot	44.10	-11.59	1.49	66
Root	34.22	-13.87	0.61	21

CO₂ evolved per day was evacuated for each measurement period following Equation 14, and this value was calculated as an average CO₂ evolved per day over each sampling period. An ANCA-GSL combined elemental analyser and gas purification module that produces clean gas samples for a 20-20 isotope ratio mass spectrometer was used for analysis of the gases. The experiment measured CO₂ evolved from soil only and soil plus fresh (shoot and root) biomass through time for four replicate samples: soil only (sand, silt, clay); shoot x soil (sand, silt, clay) and root x soil (sand, silt, clay), with values from blank containers subtracted from treatment values (Table 8).

Table 8: Decomposition experiment design including blanks and the three soil types with no biomass added for reference, and soils (sand, silt and clay) with shoot and root (seven depth increments) biomass added.

	Sand	Silt	Clay	Soil type	Rep	Depth	Total Sample
Blank	-	-	-	-	4	-	4
Soil	Sand	Silt	Clay	3	4	-	12
Shoot	Shoot + Sand	Shoot + Silt	Shoot + Clay	3	4	-	12
Root	(Root* + Sand)	Root* + Silt	Root* + Clay	3	4	7	84
	root (0-10 cm) + sand	root (0-10 cm) + silt	root (0-10 cm) + clay				
	root (10-20 cm) + sand	root (10-20 cm) + silt	root (10-20 cm) + clay				
	root (20-30 cm) + sand	root (20-30 cm) + silt	root (20-30 cm) + clay				
	root (30-40 cm) + sand	root (30-40 cm) + silt	root (30-40 cm) + clay				
	root (40-50 cm) + sand	root (40-50 cm) + silt	root (40-50 cm) + clay				
	root (50-70 cm) + sand	root (50-70 cm) + silt	root (50-70 cm) + clay				
	root (>70 cm) + sand	root (>70 cm) + silt	root (>70 cm) + clay				
Total no. of samples							112
* Root at a specific soil depth							

Table 9: Decomposition experiment materials used and ratios

Materials used	Size	Ratio
Container	250 ml	
Soils (sand, silt, clay)	25 g	1:10 (soil: container)
Shoot biomass (fresh)	0.05 g	1:500 (shoot: soil)
Root biomass (fresh)	0.05 g	1:500 (root: soil)

Data analysis

For shoot and root biomass production we calculated the mean for the five replicate shoot samples and five replicate root samples for each of the seven depth increments. Root and shoot biomass are reported as mass per unit volume of soil in the soil pots [pot surface area x pot height (1.0 m)] in kg m⁻³ accumulated during the 7 month growth period. Where, the diameter of the pot was 0.12m.

For the decomposition experiment, CO₂ evolved was calculated by deducting the blank from the measured samples. Adjustments were made during gas chromatography (GC) measurement for container volume, soil mass and number of days of incubation (Equation 12). Carbon dioxide evolved per gram of soil was calculated following Equation 13 and results presented in mg C g⁻¹ soil day⁻¹, where the CO₂ evolved was converted to C (mg) using Equation 14.

$$CO_2(\text{released}) = \frac{[CO_2(\text{measured}) - CO_2(\text{blank container})]}{\text{Duration(Days)}} \quad \text{Equation 12}$$

$$CO_2 = \frac{CO_2(\text{released})}{\text{gm Soil}} \quad \text{Equation 13}$$

$$C(\text{mg}) = 3.67CO_2 \quad \text{Equation 14}$$

The statistical analyses were performed using R version 3.3.2. One-way analysis of variance (ANOVA) was performed to test if there was an effect of time on decomposition and to determine differences between different soil types. Tukey's honestly significantly different (HSD) test was then performed to determine statistically significant differences (P < 0.05). Non-linear regression was used analyse the variation in response variables over time. An exponential decay function (Equation 15) was used,

$$y = a \times \exp(-bx) \quad \text{Equation 15}$$

where y = carbon, x = soil depth, a = y-intercept and b = decay constant (> 0).

Results

Vetiver biomass production assessment

During the seven-month growing period, vetiver produced a mean total biomass (root plus shoot) of 2.68 kg m⁻³ fresh and 1.2kg m⁻³ dry biomass (Table 10). The above- and below-ground biomass was (1.61 ± 0.218 kg m⁻³) and (1.07 ± 0.128 kg m⁻³) fresh and (0.67 ± 0.101 kg m⁻³) and (0.53 ± 0.054 kg m⁻³) dry biomass, respectively. This translated to a shoot to root biomass ratio of (1.43) for the fresh biomass and (1.25) for the dry biomass (Table 10). On a per hectare basis, if planted at densities equal to the pot area, the mean total biomass for shoots would equate to 161 Mg ha⁻¹, and the root biomass 107 Mg ha⁻¹ fresh biomass and 67.7 Mg ha⁻¹ dry shoot and 52.5 Mg ha⁻¹ dry root biomass. Biomass decreased exponentially with depth in the soil profile (Table 11). Mean shoot length was 1.54 m and the roots penetrated to 0.86 m in the 1.0 m pot depth, and the average number of tillers produced was 14 per each planted tiller for the growing period (213 days).

Table 10: Mean and ratio of vetiver dry and fresh biomass (shoot to root) production (kg m⁻³) plantation in 1.0 m pot in a sand soil for the experimental period (7 month).

Plant allocation	Fresh biomass (kg m ⁻³)	Dry biomass (kg m ⁻³)
Above-ground biomass	1.61± 0.218	0.67± 0.101
Below-ground biomass	1.07± 0.128	0.53± 0.054
Total biomass	2.68± 0.344	1.2± 0.151
Shoot-to-Root ratio	1.43	1.25

Table 11: Mean fresh and dry root biomass production for the seven root depth increments of a 1.0 m pot

Root depth (cm)	Fresh (g)	Dry (g)
0-10	56.2± 29.9	28.9± 13.5
10-20	17.7± 7.1	9.4± 1.7
20-30	14.6± 6.6	7.1± 2.8
30-40	12.5± 6.0	5.3± 2.7
40-50	8.4± 4	3.6± 1.8
50-70	9.3± 7.8	3.7± 3.2
>70	2.8± 5.4	1.6± 3.1

Vetiver biomass decomposition

Relative rate of decomposition between root and shoot

Analysis of variance showed a significant difference in the rate of decomposition of vetiver root and shoot biomass in all soil types (Figure 15). The difference between shoot and root decomposition rate was indicated by a higher rate of decomposition for vetiver roots compared with the shoot biomass in the clay soil type ($P < 0.001$). The difference between shoot and root decomposition was not consistent through the decomposition time period for the clay soil. Both root and shoot biomass decomposition differed between soil types where decomposition was higher in the clay soil type compared with the sand and silt soils. For the soils (sand, silt and clay) only treatment without vetiver biomass addition, carbon evolved was significantly different depending on time ($P < 0.001$), and this was most pronounced during the first seven days. Carbon evolved from the clay soil was also higher for the first seven days due to the difference in the initial organic matter content between the soils.

Table 12: Rate of vetiver biomass (above and below ground biomass) decomposition ($\text{mg C g}^{-1} \text{ soil}$) in three soil textures (sand, silt and clay). Values of the intercept (a), the slope (b) and Coefficient of determination (R^2)

	a			b			R ²		
	Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay
Soil	210 ±45	256±43	400±109	-0.04±0.015	-0.02±0.006	-0.04±0.02	0.53	0.53	0.49
Shoot	186±43	342±44	484±159	-0.03±0.013	-0.02±0.005	-0.06±0.031	0.47	0.68	0.51
Root	177±23	224±17	830±104	-0.05±0.01	-0.02±0.003	-0.1±0.014	0.35	0.42	0.64

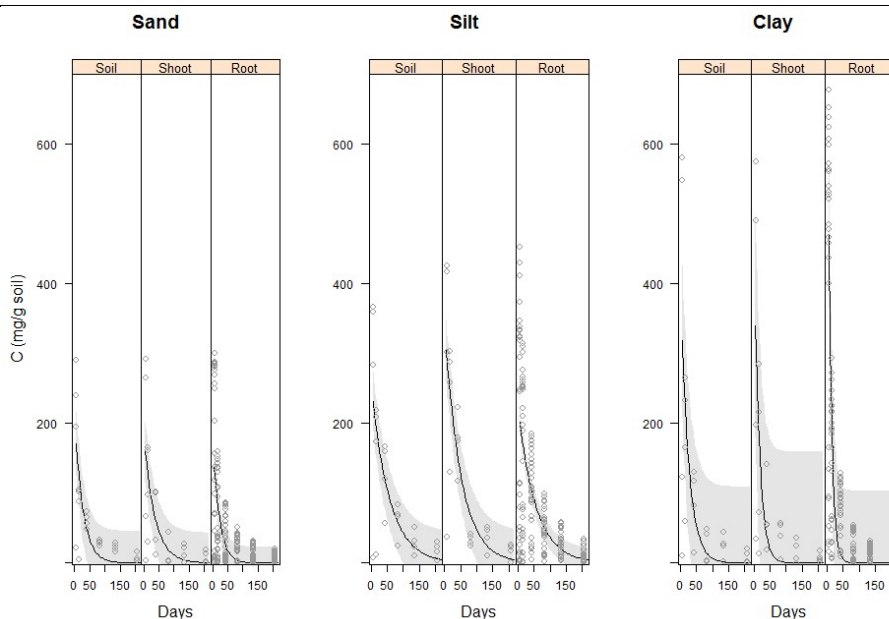


Figure 15: Carbon ($\text{mg C g soil}^{-1} \text{ day}^{-1}$) evolved from vetiver shoot and root decomposition in three soil textures (sand, silt, clay) during the 206 day incubation period. Vetiver biomass was used from the glasshouse experiment and incubated at 25°C constant temperature at UNE. Plots show the raw data ($^\circ$), a fitted exponential model ($-$) and 95% confidence bands.

Effect of soil type on carbon evolved

For the sand, silt and clay soils without vetiver addition, carbon evolved followed a double exponential decay curve where it began with rapid phase and then slow phase ($P < 0.001$), and this was most pronounced during the first seven days. Carbon evolved from the clay soil was the highest during for the first seven days compared with the silt and the sand. The analysis indicated that the carbon evolved from the clay soil type was significantly higher compared with silt and sand soil types ($P = 0.001$). For all three soil types, C evolution began to plateau after Day 43 (Figure 16).

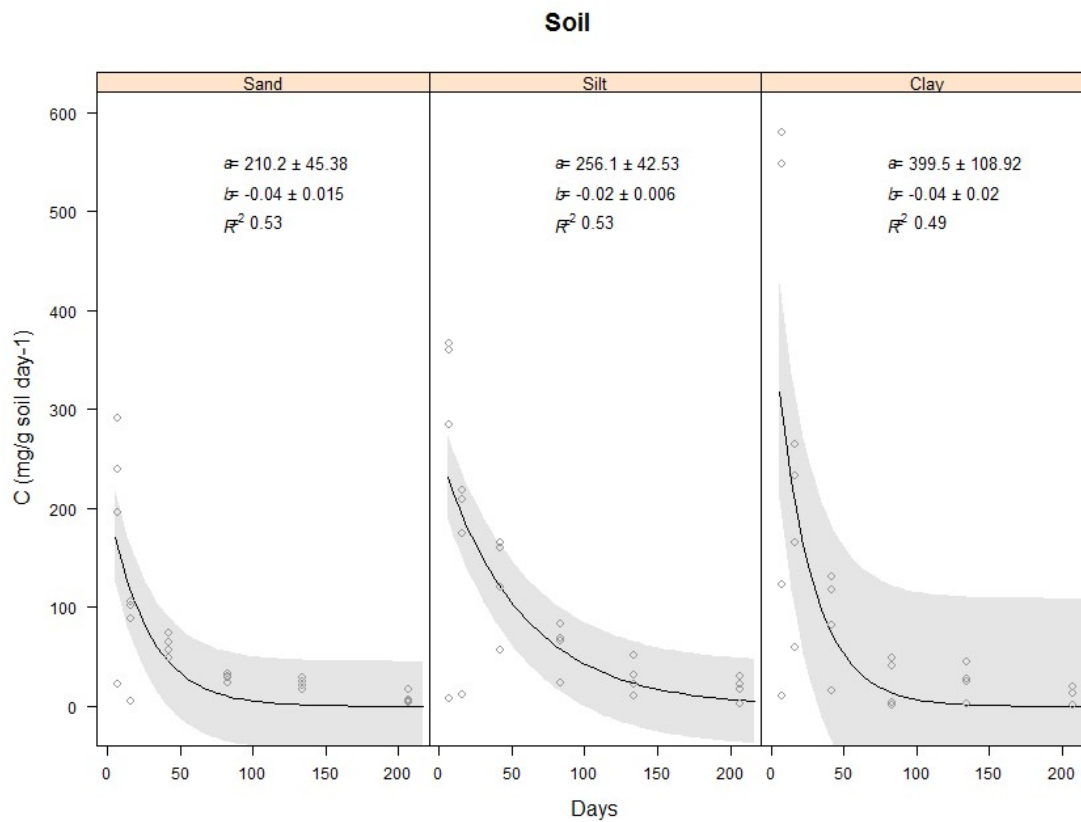


Figure 16: Carbon ($\text{mg C g soil}^{-1} \text{ day}^{-1}$) evolved from the soils (sand, silt, clay) without biomass addition during the 206 day decomposition period. Where soils only incubated at 25°C constant temperature at UNE. Plots show the raw data (o), a fitted exponential model (—) and 95% confidence bands. Values of the intercept (a), the slope (b) and Coefficient of determination (R^2).

Effect of soil type on carbon evolved from vetiver shoots

Analysis of variance indicated that the shoot decomposition rate was affected by soil type. CO₂ evolved from the clay soil was the highest at 323.9 mg C day⁻¹ for the first 7 days, compared to 294.9 mg C day⁻¹ for the silt and 156.5 mg C day⁻¹ for the sand. The analysis indicated that the difference between the shoot decomposition in the clay and silt soils were significantly different (P= 0.001), and that both were significantly higher than in the sand (P= 0.001). The total carbon evolved from shoot decomposition in the clay soil was the most rapid, particularly during the first seven days, and as with the soil only, slowed only after Day 43 (Figure 17).

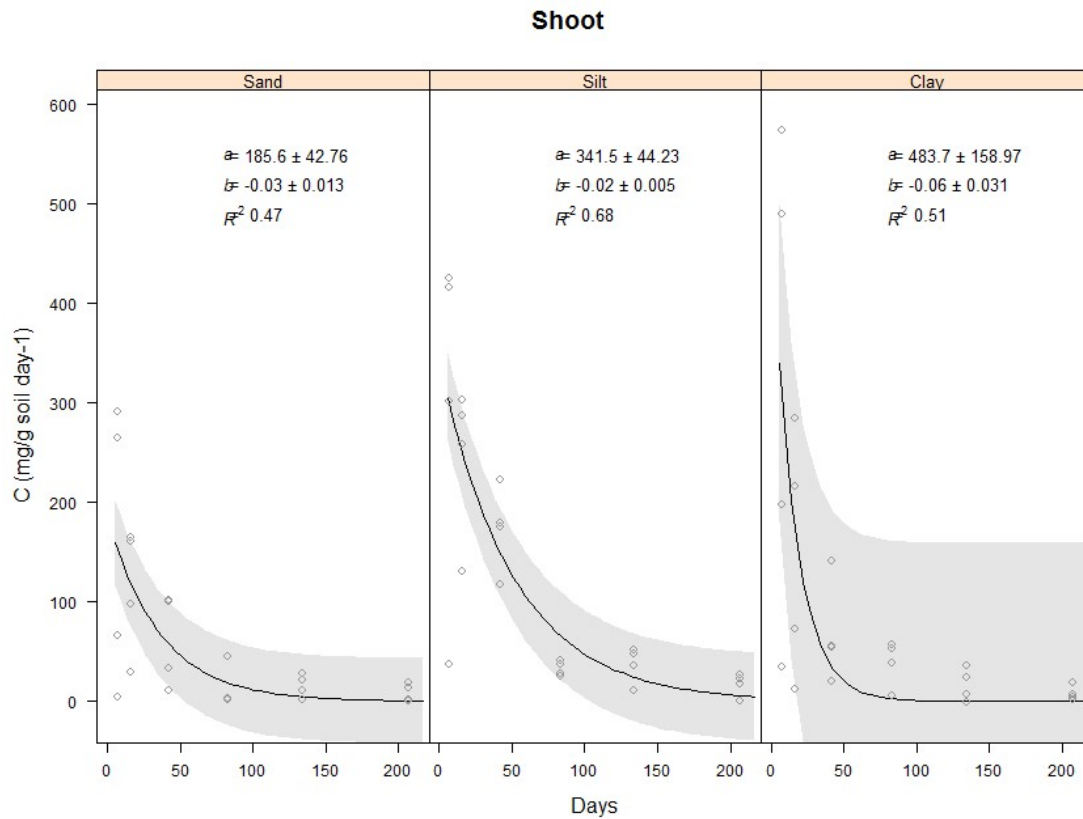


Figure 17: Carbon (mg C g soil⁻¹ day⁻¹) evolved from the decomposition of vetiver shoot biomass in three soil texture classes (sand, silt, clay) during the 206 days decomposition period. Where vetiver shoot used from the glasshouse experiment and incubated at 25°C constant temperature at UNE. Plots show the raw data (o), a fitted exponential model (-) and 95% confidence bands. Values of the intercept (a), the slope (b) and Coefficient of determination (R²).

Effect of soil type on total carbon evolved from vetiver roots

Analysis of variance indicated that the amount of carbon evolved from vetiver root decomposition, for all depths combined, was affected by the soil type and time ($P < 0.001$). However, the differences between soil types were only in the early stages of the experiment. For soil type, carbon evolved from vetiver root decomposition in the clay soil (average for first 7 days $413.18 \text{ mg C day}^{-1}$) was higher compared to the silt (average for first 7 days $205.37 \text{ mg C day}^{-1}$) and sandy (average for first 7 days $142.34 \text{ mg C day}^{-1}$) soils ($P < 0.001$) (Figure 18).

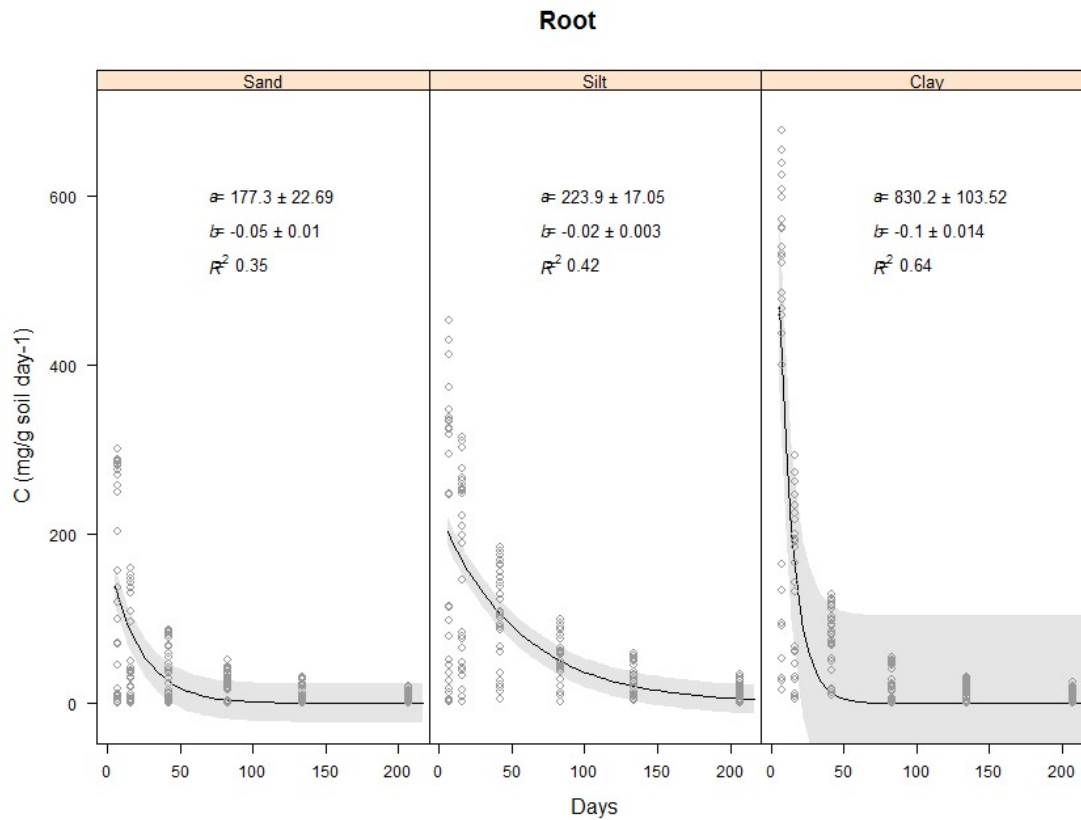


Figure 18: Carbon ($\text{mg C g soil}^{-1} \text{ day}^{-1}$) evolved from blank soils and from the decomposition of the whole vetiver root biomass in three soil texture classes (sand, silt, clay) during the 206 incubation period. Where vetiver root biomass used from the glasshouse experiment and incubated at 25°C constant temperature at UNE. Plots show the raw data (o), a fitted exponential model (—) and 95% confidence bands. Values of the intercept (a), the slope (b) and Coefficient of determination (R^2).

Vetiver root decomposition was also analysed for individual biomass produced at different soil depth taken through the 1.0 m pots which were divided into 7 depth increments (0-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.4, 0.4-0.5, 0.5-0.7 & 0.7-1.0 m). Therefore, vetiver root decomposed at the same rate regardless of where they are sampled (depth) and was consistent (Figure 19). Hence, there was no significant difference between decomposition in the clay and silt soils. The proportion of the variance in the carbon evolved predicted by time was above $R^2 = 0.6$ for the clay and lower for the silt and sand (Table 13).

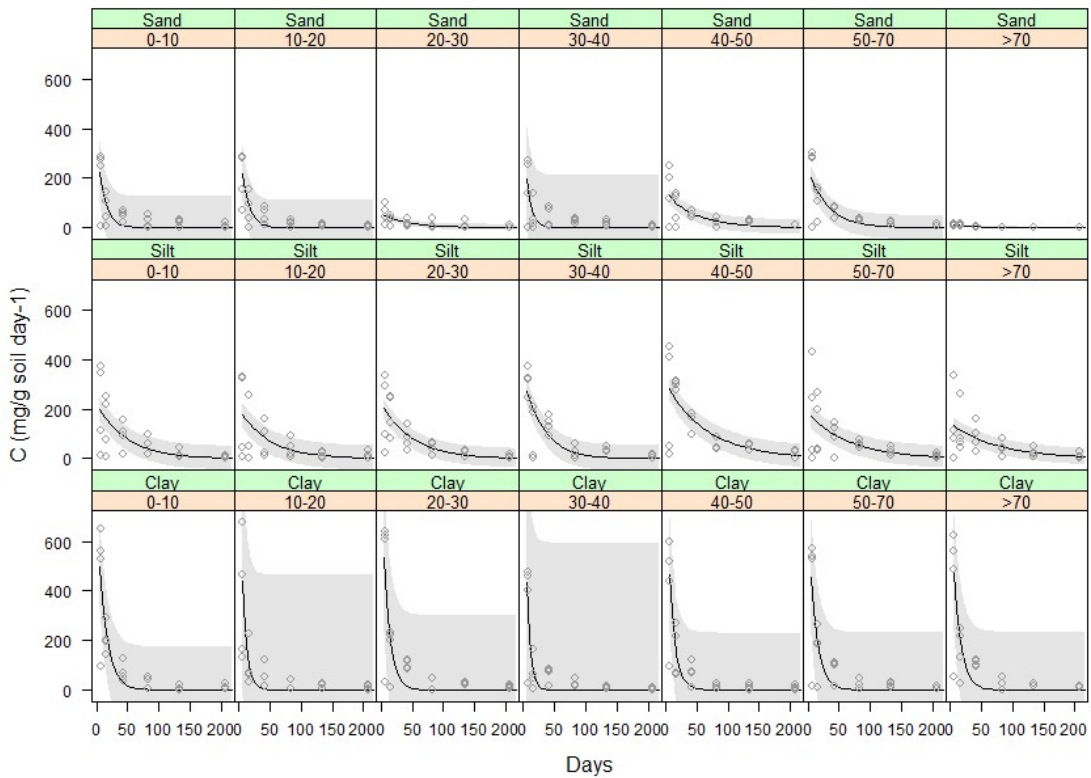


Figure 19: Carbon ($\text{mg C g soil}^{-1} \text{ day}^{-1}$) evolved from the decomposition of vetiver root biomass of seven depth increments in three soils during the 206 days. Where vetiver root biomass used from the glasshouse experiment and incubated at 25°C constant temperature at UNE. Plots show the raw data (o), a fitted exponential model (—) and 95% confidence bands.

Table 13: Rate of carbon evolution from vetiver below ground biomass decomposition in three soil textures (sand, silt and silty clay). Values of the intercept (a), the slope (b) and Coefficient of determination (R^2).

Root Depth (cm)	a			b			R^2		
	Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay
0-10	364 ± 125	225 ± 48.5	756 ± 173	-0.090 ± 0.04	-0.021 ± 0.01	-0.074 ± 0.02	0.55	0.44	0.74
10-20	355 ± 110	198 ± 50.5	967 ± 466	-0.090 ± 0.03	-0.020 ± 0.01	-0.141 ± 0.06	0.62	0.35	0.6
20-30	58 ± 12.7	226 ± 38.8	919 ± 301	-0.020 ± 0.01	-0.020 ± 0.01	-0.097 ± 0.04	0.42	0.54	0.64
30-40	399 ± 212	324 ± 53.2	1130 ± 595	-0.130 ± 0.06	-0.031 ± 0.01	-0.172 ± 0.07	0.42	0.65	0.64
40-50	142.0 ± 3	305 ± 46.7	791 ± 230	-0.021 ± 0.01	-0.015 ± 0.01	-0.095 ± 0.03	0.41	0.54	0.7
50-70	242 ± 49	184 ± 44.3	734 ± 232	-0.032 ± 0.01	-0.015 ± 0.01	-0.085 ± 0.03	0.55	0.31	0.62
70-100	115 ± 2.6	144 ± 33.0	764 ± 234	-0.033 ± 0.01	-0.013 ± 0.01	-0.086 ± 0.03	0.65	0.32	0.63
0-100	177 ± 23	224 ± 17.0	830 ± 104	-0.500 ± 0.01	-0.02 ± 0.003	-0.1 ± 0.014	0.35	0.42	0.64

Discussion

Biomass production assessment

Many studies have shown that vetiver has the potential to produce a large amount of biomass both above- and below-ground, and have therefore suggested it has the potential to store additional carbon (Lavania, 2003; Tomar & Minhas, 2004; Gaspard et al., 2007; Singh & Dagar, 2009; Singh et al., 2013). Hence, we recorded significantly more vetiver above- and below-ground biomass production across a seven month growing period (67.7 Mg ha⁻¹ and 52.5 Mg ha⁻¹, respectively) compared with all previous reported data. Tomar and Minhas (2004), who examined different cultivars of vetiver in India for two years growing period, found 72.6 to 78.7 Mg ha⁻¹ shoot and 1.12 to 1.71 Mg ha⁻¹ root biomass, and a total mean dry biomass for vetiver 30.3 Mg ha⁻¹ yr⁻¹. In contrast, a study by Neal et al. (2009) indicated the dry biomass production potential of vetiver as only half (17 Mg ha⁻¹ yr⁻¹), of the value reported by Tomar & Minhas, (2004). Vetiver (*Chrysopogon zizanioides*) has been reported to produce 28.62 Mg ha⁻¹ yr⁻¹ dry shoot and 1.56 Mg ha⁻¹ yr⁻¹ dry root biomass in Thailand and India, respectively (Kaveeta et al., 2002; Singh et al., 2013). For *Andropogon guayanus*, a similar tropical perennial grass a 43 Mg ha⁻¹ yr⁻¹ of above ground biomass in the South American Savannah (Fisher et al., 1994) was reported. For another similar grass, *Miscanthus*, Amougou et al. (2012), indicated 18.5 to 21 Mg ha⁻¹ yr⁻¹ dry biomass production potential in Northern France which on average is equivalent to vetiver dry biomass (17 Mg ha⁻¹ yr⁻¹).

In this study, we also found an average of 14 tillers per plant which is significantly higher than previously reported results. For example Xu (2005), reported only 4-6 tillers per vetiver plant in the northern subtropics of China and Kaveeta et al. (2002), reported 7-8 tillers/plant for four ecotypes of vetiver grown in China which is on average half of the result under this study.

The variation in biomass production reported in the literature is probably a consequence of the specific growing conditions (i.e. optimum moisture, nutrient supply and temperature) (Singh & Dagar, 2009), and variations in the genetic potential of the germplasm used (Lavania, 2003). Also, the planting density used to calculate biomass production does not take into account any spacing. If biomass production in our trial was applied as a standard vetiver planting rate of 88.5 plants ha⁻¹ (Chairoj & Roongtanakiat, 2004), the potential production would be 120 Mg ha⁻¹, which is still significantly higher than the values reported by other workers above.

Our plants were grown in an ideal glasshouse environment, with a complete fertilizer and regular watering. These conditions did not occur in some other studies (Percy & Truong, 2003; Wagner et al., 2003; Singh & Dagar, 2009) which may explain why our shoot and root biomass levels are much higher than those reported for vetiver grown in field conditions. Our result indicates that when grown in carbon and nutrient depleted soils, but with good agronomic practices during establishment (i.e. nutrient addition, regular watering), vetiver has the potential to produce a large amount of biomass.

Vetiver biomass decomposition

Vetiver roots decomposed more rapidly in the clay soil type compared with vetiver shoots. This is due in part to the lower C:N ratio of the roots compared with the higher C:N ratio of the shoot biomass (21 and 66, respectively). A comparative study between buried and mulched vetiver shoots by Chairoj and Roongtanakiat (2004), demonstrated that the rate of decomposition was higher in buried shoots compared to mulched vetiver shoots, and this can be due to more contact between the plant biomass and soil. Incorporation of plant residues can affect soil moisture, temperature, organic carbon concentration and microbial activity, all of which can further influence decomposition of plant material (Liu et al., 2011). In our study, the biomass was placed on the soil surface where the root and shoot material had less soil contact and the comparison was between the above and below ground biomass and a rapid decomposability of the vetiver root was observed compared with the shoot biomass. So, it might be expected that incorporation of root or shoot material would increase decomposition rate. The rapid decomposition of vetiver root can potentially explain the exponential decrease in soil carbon with increasing soil depth and a rapid decomposition rate of vetiver in the soil may result in high carbon turnover. Hence, the higher decomposition of vetiver root litter is crucial because it releases the carbon in the root litter and would also speed up its contribution to stable soil organic matter. On the other hand, the slowness of vetiver shoot decomposition could hold the C in decomposing litter longer but would also slow down its contribution to stable soil organic matter this is in agreement with (Guo et al., 2006).

A C:N ratio of 30 is commonly regarded as a threshold for predicting whether net N mineralization (< 30) or net N immobilization (> 30) occurs following crop residue addition, although this empirical parameter can vary from one soil to another (Li et al., 2013). Plant materials of different C:N ratios affect bacterial and fungal growth differently, leading to further variations in the C:N ratio of newly produced microbial biomass (Vinten et al., 2002; Rousk & Bååth, 2007). Irrespective of soil aeration, Li et al. (2013), reported that soil N₂O production was generally lower using plant materials with high C:N ratios compared to those with low C:N ratios. The C:N ratio of vetiver is generally reported as 19 - 79 (Lakshmanaperumalsamy et al., 2006), and in this study it was 66 for shoot and 21 for roots. This suggests that N mineralization was likely for the root material while immobilisation of N was more likely to occur for shoot material, with related effects on the rate of decomposition and the storage of carbon in the soil. The rapid decomposition of root was only in the earlier part of the experiment and it was independent of the depth sampled.

Carbon evolved from both shoot and root biomass in the three different soil types indicated that soil type can affect the rate at which above- and below-ground vetiver biomass decomposes. CO₂ evolution was greater in clay soils and decomposition was more rapid than in the sand and silt soils for both shoot and root biomass. This effect was more pronounced in the first 50 days, before it started levelling out for all three soil types for the next 150+ days, similar to other findings (Wattanaprat et al., 2006; Lavania & Lavania, 2009; Wang et al., 2017). Although a study by Bronick and Lal (2005) showed that clay content can suppress decomposition and promote SOC storage by increasing the physical protection of SOC within soil aggregates, in our study the biomass was placed on the soil surface, minimising the potential protective effects of the soil aggregates in the clay. Instead, the faster decomposition in the clay soil compared with the silt and sand was more likely due to different initial nutrient and moisture levels, pH, structure, biological activity and a combination of these (Scherer-Lorenzen et al., 2007; Bills et al., 2010; Zatta et al., 2013).

Roots decomposed faster than shoots but this was not consistent through the whole experiment period which could be an initial flush of labile C (with lower C:N) followed by a slowing and convergence of decomposition of shoot and root behavior. The implications of the results from study are therefore, roots decompose faster compared to shoots however, they decompose at the same rate regardless of where they are sampled (depth) from. The higher root decomposition rate compared with shoot decomposition rate might be attributable to the C:N ratio.

Conclusion and Implications

The results from this study confirmed the large biomass (both above- and below-ground) production potential of vetiver grass over a short period of time even in soils with low fertility. The application of vetiver shoots and roots biomass on the surface of three soils with contrasting textures has also decomposed differently over time and the decomposition was more rapid in the clay soil compared with the sand and silt soils. However, the rate of decomposition of vetiver roots was more rapid than the shoots in all soil types. Besides, the high biomass production potential, the more rapid decomposition rate of vetiver root materials regardless of where they were sampled (root depth) from could be attributable to the lower C:N ratio of the vetiver roots compared with the vetiver shoots. Hence, the larger carbon storage through the depth and deeper soils could be a contribution from the vetiver roots than the shoots. This finding therefore, suggests that for vetiver, the large root biomass produced does indeed contribute more to the soil carbon accumulation than the shoots not only to the soil organic matter. This is due to the faster decomposition of vetiver root litter which is crucial in releasing the carbon in the root litter and would also speed up its contribution to stable soil organic matter. Hence, planting vetiver and similar tropical perennial grasses on degraded and less fertile soils could be a good strategy for carbon sequestration and to rehabilitate degraded soils. We therefore, suggest that farmers need to be encouraged to plant vetiver and similar tropical perennial grasses on degraded soils and marginal lands to facilitate rehabilitation and carbon sequestration. Further research also needs to be conducted to investigate the mechanisms and impacts of potential tropical grasses like vetiver.

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CHAPTER 6: Predicted Contents of Soil Carbon Fractions under Vetiver Grass in Australia and Ethiopia

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Abstract

The allocation of soil organic carbon to its component fractions can provide an indication of the vulnerability of organic carbon stocks to change. This study quantified the distribution of soil carbon in particulate, humus and resistant fractions differentiated on the basis of particle size and chemical composition under vetiver grass compared with other vegetation types. Vetiver is a perennial grass growing widely in tropical and sub-tropical regions with a large above and below-ground biomass production potential. Hence, our study aimed to quantify the impact of vetiver on the vertical soil profile distribution of SOC stock and its allocation to POC, HOC and ROC fractions. Soil organic carbon fractions were measured on soil samples collected from Gunnedah, Australia and from Southwest, Ethiopia to a depth of up to 1.0 m under three different plant communities (vetiver, native pasture and coffee). We used the MIR/PLSR spectra to predict soil organic carbon fractions based on fractionated and NMR measured values. The stocks of soil carbon fractions indicated significant differences between the labile POC to the HOC across site and vegetation types. The dominant carbon fraction was HOC (71%) for all vegetation types which indicates the less vulnerability of the carbon in the HOC component fraction given its less labile nature. The average carbon sequestration rate under vetiver ranged for OC was -2.64 to +7.69 Mg C ha⁻¹ yr⁻¹, while for the POC, HOC and ROC was 0.04 to +1.17, -3.36 to +4.64 and -0.35 to +1.51 Mg C ha⁻¹ yr⁻¹, respectively. Our results therefore indicated that growing vetiver has on average a high accumulation rate of the more stable carbon (HOC) which is less vulnerable to change and to use this in the carbon accounting program can be feasible. We therefore, suggest that countries in tropical regions should promote the use of perennial tropical grasses such as vetiver especially on degraded lands as a potential option to facilitate carbon sequestration and environmental rehabilitation.

Key words: Anno, coffee, humus, Jimma, native pastures, particulate, resistant, Metu, NMR, MIR, SOC

Introduction

Soil organic carbon (SOC) conservation and sequestration is important for soil health, and therefore food security and environmental quality. Sequestration rates of carbon in soil are determined by carbon inputs and losses and the resulting net equilibrium (Lal et al., 2007; Lal, 2015). Inputs of carbon to the soil can occur at the soil surface (e.g. shoot residues) and within the soil profile (e.g. roots and root exudates). Rate and quantity of carbon accumulation will depend on mechanisms that can stabilise carbon against decomposition (Kaiser & Guggenberger, 2003). Soil mineral composition and particle size distribution provide control over the amount and reactivity of mineral surfaces available to adsorb SOC within a soil horizon and thus influence the stabilisation and net accumulation of SOC (Kaiser & Guggenberger, 2003).

A strategy for enhancing the amount of organic carbon stored in soils is to identify and implement management practices that lead to an accumulation of the more stable forms of SOC at depth in the soil profile where rates of decomposition are lower (Nepstad et al., 1992; Batjes, 1998). One such strategy involves perennial tropical grasses, which are known to produce large above- and below-ground biomass (e.g. up to 100–120 Mg ha⁻¹) (Lavania & Lavania, 2009). Due to their large biomass, it is believed that these grasses can translocate large quantities of carbon to their root system and consequently increase SOC stocks (Zimmermann et al., 2012). Tropical perennial grasses therefore represent a potential option facilitating soil carbon sequestration, particularly with cropland conversion to pasture, which is widely recognised as a mechanism for accumulating SOC (Clifton-Brown et al., 2007; Conant, 2012; Zimmermann et al., 2012). For example, Dondini et al. (2009), compared *Miscanthus* grass and arable crop land, demonstrating a higher SOC in different aggregates throughout the soil profile under *Miscanthus*, which they attributed to the input of new carbon and low disturbance in the *Miscanthus* grass.

Vetiver, is a grass species that is widely distributed in tropical & sub-tropical regions of the world. It is a multipurpose grass and is extensively used for soil conservation (Gaspard et al., 2007; Singh et al., 2011). Due to its fast growing nature and large biomass production, it has been recommended as a candidate for facilitating carbon sequestration in soil while also being an effective solution for environmental degradation (Lavania & Lavania, 2009). However, research quantifying the impact of vetiver on carbon sequestration in soil and the allocation of carbon to SOC fractions remains limited (Gaspard et al., 2007).

SOC is comprised of numerous fractions with variable physical and chemical properties that can influence rates of turnover and accumulation in the soil (Bol et al., 2009; Poeplau et al., 2013). Due to a number of physical and chemical mechanisms and processes occurring in the soil system, organic

carbon can be transformed from biologically accessible forms of organic matter into more stable forms that are resistant to degradation processes and remain in the soil environment for long periods (Hobley et al., 2016; Sanderman et al., 2016). Management practices can alter both the magnitude of decomposable carbon inputs to soil and subsequent rates of decomposition and therefore influence the type and quantity of SOC present. Allocating SOC to component fractions defined by variations in chemical and physical properties can provide an indication of its resilience, potential susceptibility to decomposition and vulnerability to change (Baldock et al., 2013a; Gollany et al., 2013; Guimarães et al., 2013; Page et al., 2014). A number of fractionation methods are commonly used to differentiate SOC that is protected from biological decomposition by physical or chemical mechanisms associated with soil organo-mineral complexes (Gollany et al., 2013). One approach to allocate SOC to biologically significant fractions has used variations in particle size and chemical composition to distinguish three components: 1) particulate organic carbon (POC) defined as the organic carbon associated with 0.050 – 2 mm soil particles and dominated by individual pieces of fresh and decomposing plant residues, 2) humus organic carbon (HOC) defined as the organic carbon associated with <0.050 mm soil particles and dominated by mineral associated organic carbon and 3) resistant organic carbon (ROC) defined as the organic carbon associated with soil particles <2 mm but having a polyaromatic chemical structure consistent in form with charcoal (Skjemstad et al 2004, Baldock et al 2013).

Mid infrared (MIR) spectroscopy used in conjunction with partial least squares regression (PLS) and a calibration dataset of analytical values can provide an accurate, rapid, cost effective and simple method (compared to traditional laboratory methods) to derive estimates of the content and composition of SOC (Janik et al., 2007; Baldock et al., 2013a; Baldock et al., 2013b). Procedures developed by Baldock et al. (2013b), provide a means of quantifying the allocation of SOC to its component POC, HOC and ROC fractions. Hobley et al. (2016), indicated that depth was a key factor affecting the content of all three fractions in soil, with proportions of SOC allocated to POC decreasing while the HOC increased with increasing depth. This study also suggested that POC was a significant contributor to SOC content, reporting that SOC was less strongly associated to the HOC and ROC fractions, with climate and soil physical and chemical properties more important as explanatory variables describing the contributions of the fractions to SOC. Furthermore, Hobley et al. (2016), indicated that human influences (land-use change and management) were not important in defining the proportion of the fractions or in controlling SOC stability.

Our study aimed to quantify the impact of vetiver on the vertical soil profile distribution (to 1m) of SOC stock and its allocation to POC, HOC and ROC fractions compared to that under native pastures at Gunnedah, Australia and coffee plantations in Southwest Ethiopia. We aimed to compare and contrast the effects of vetiver on soils by comparison with locally relevant land-use types in these two

contrasting environments – an experimental site in Australia and sites in Africa where vetiver has routinely been used as a practical soil conservation practice. Quantifying the impact of vetiver on the composition (allocation to fractions) and vertical distribution of SOC in addition to total SOC stocks provides a more complete assessment of its potential to sequester carbon in soil.

Materials and Methods

Study sites and Soil samples

Soil samples were collected from the Gunnedah Research Center (GRC), New South Wales, Australia (Figure 20), and from South West Ethiopia (Figure 21). These locations were selected to examine and compare the effects of Vetiver in a controlled experimental environment (Australia) and in an African environment (Ethiopia) where Vetiver has been widely used in the agricultural landscape. The specific study locations within the respective countries were selected due to a longer history of establishment of vetiver grass. This is particularly true in the South West Ethiopia where vetiver for the first time introduced and widely used for conservation purposes. However, despite its wide use vetiver specially in Ethiopia has not been studied for its contribution to soil carbon sequestration.

Gunnedah Research Centre is located in northwestern New South Wales (see location map) in SE Australia located at 31.03 °S and 150.27 °E in a landscape dominated by ridges of Carboniferous-Permian sandstones and conglomerates, Permo-Triassic and Tertiary basalts. Annual rainfall at the GRC is 638 mm (summer dominant) and the average maximum and minimum temperatures are 24.6 and 12.2 °C. Soils at Gunnedah are moderately deep to deep Ferrosols (Australian Soil Classification, (Isbell, 2016) (USDA equivalent Oxisols, WRB equivalent Ferralsols) on upper foot slopes with deep to very deep black soils (Vertisol-ASC, Vertisol-USDA and WRB) on lower slopes. The vegetation of the area is dominated by open woodland and grassland vegetation. The land use in and around the area is predominantly grazing on steeper slopes and cropping on deeper soils with pastures used in a rotation system. Gunnedah site where the samples collected from was previously covered with C₃ crops (such as wheat and oats), but currently the area is covered with mixed tropical and native pastures. The three sample locations in Ethiopia (Anno, Jimma and Metu) had similar climates (mean annual rainfall: 1100, 1561 and 1660-2200 mm, respectively and temperature: 27, 9 – 28 and 12 – 27 °C, respectively) and Nitisols are dominant soil types to all three study areas and are equivalent to an Australian Ferrosol (Isbell, 2016), to United States Department of Agriculture (USDA, 1999) Oxisol, and Nitisol of the World Reference Base (FAO, 2014). Nitisols are one of the most common soil types in Ethiopia, comprising 13.5% of the total 150,089.5 km² land area. These sites (Anno, Jimma and Metu) are differed in their management and land ownership (private large scale farming systems, a research centre and smaller farmlands, respectively).

The samples from Gunnedah were from a vetiver (*Chrysopogon zizanioides*) plantation established in 1992 and from a surrounding mixed native pasture established in 1993 consisting of Queensland blue grass (*Dicanthium sericeum*), slender bamboo (*Austrostipa verticillata*), wallaby grass (*Austrodanthonia*), and windmill grass (*Chloris spp.*), where both planted on sites previously under C₃ crops. The samples in Ethiopia were collected from Vetiver (*Chrysopogon zizanioides*) strips and under coffee plantations at three locations (Anno, Jimma and Metu). Soil samples were collected in June 2014 from Gunnedah and in February/March, 2015 from Ethiopia.

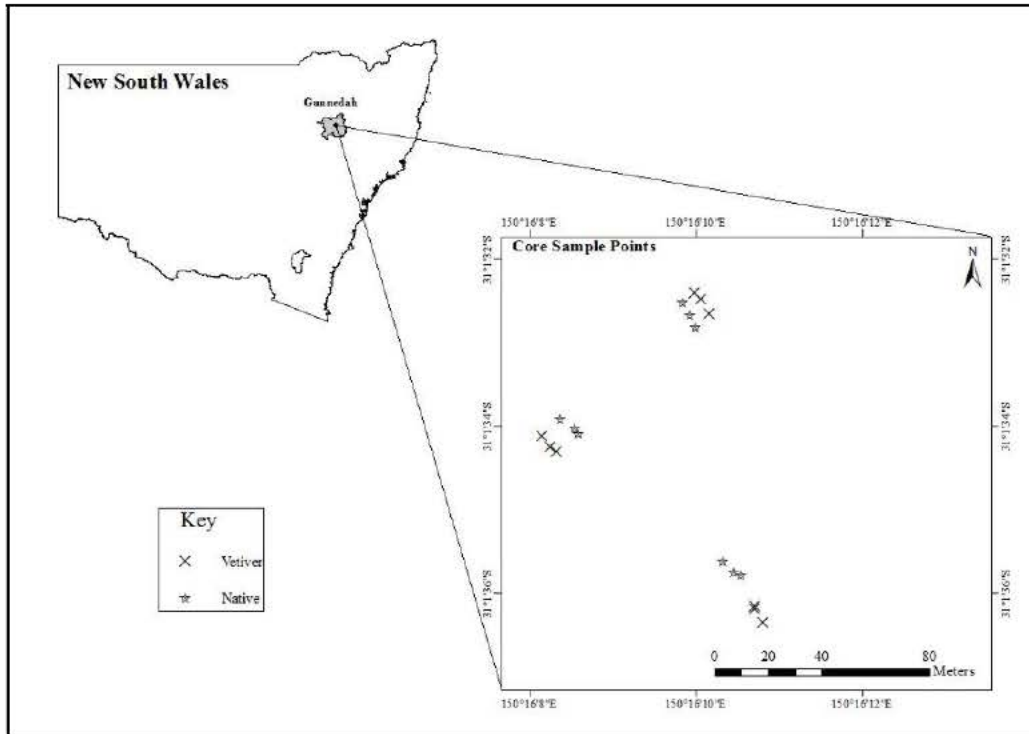


Figure 20: Locations where 0-1m soil cores under Vetiver (X) and native pastures (*) at the Gunnedah Research Center, New South Wales, Australia.

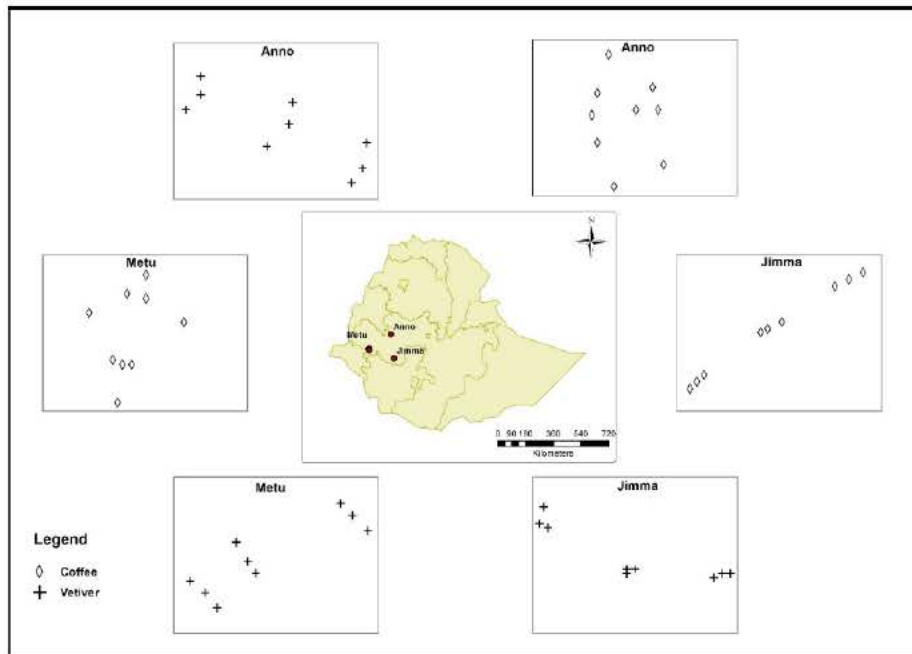


Figure 21: Locations where 0-1m soil cores were collected from under Vetiver (+) and coffee plantations (◇) (1.0 m depth) at the Jimma, Metu and Anno study sites in the Oromia Region of SW Ethiopia.

Sample locations were selected to ensure an establishment history of more than 10 years for all vegetation types which is recommended for assessing management impacts on the quantity and distribution of SOC (Eswaran et al., 1993; Somebroek, 1993; Batjes, 1998; Jobbágy & Jackson, 2000). The sample locations in Ethiopia represented locations where vetiver was introduced as a soil and water conservation method. Within each site three plots were established for each vegetation type and three replicate samples were taken from each plot. Soil cores (0-1m) were collected in 50 mm ID steel cores and then divided into seven depth increments (0-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.4, 0.4-0.5, 0.5-0.7 & 0.7-1.0 m). The total number of samples collected from Gunnedah, Australia, was 126 (1 site x 2 grasses x 3 plots x 3 reps x 7 depth increment = 126) and 373 for Ethiopia (3 sites x 2 plants x 3 plots x 3 reps x 7 depth with 5 samples being missed due to bedrock at the maximum depth), to give an overall total of 499 samples for all vegetation types and sites combined. Each of the three replicate samples for each depth were then bulked across plots to produce three composite samples. The compositing was performed to reduce the potential impact of spatial variability. A total of 168 composite samples were then used to quantify the stocks, composition and vertical distribution of SOC (Table 14).

Table 14: Number of soil samples collected and composited for the fractionation work

Country	Vegetation	No. of all soils	Composite Samples
Australia	Vetiver	63	21
	Native	63	21
	<i>Sub total</i>	126	42
Ethiopia	Vetiver	188	63
	Coffee	185	63
	<i>Sub total</i>	373	126
Total No. of samples		499	168

Sample preparation and elemental content analyses

Sample preparation followed the procedures used by Baldock et al., (2013a); Baldock et al., (2013b). Samples were oven dried at 40 °C and crushed to <2 mm. A subsample of the <2 mm dried soil was finely ground (using a ballmill) to <200 µm. Total carbon (TC) content of each dried and finely ground soil was determined using LECO TruSpec Series Carbon and Nitrogen analyser. Each soil sample was tested for the presence of carbonates (using 1M HCl) and 11 soils were found to contain carbonates. An additional subsample (0.8 g) of each of the 11 dried and finely ground carbonate containing soils was pretreated with 2% (by volume) phosphoric acid (H₃PO₄) to remove carbonate, dried at 40°C and its carbon content redetermined (LECO TruSpec). This approach allowed the acquisition of total carbon (TC), organic carbon (OC) and inorganic carbon (IC) contents for the dried soil samples. The moisture content of each <2mm dried sample was determined by drying subsamples at 105 °C and the oven dry equivalent soil mass and content of OC was calculated following correction of air dry soil mass to oven dry mass with all contents reported in mg g⁻¹ oven dry soil.

MIR spectroscopy and soil organic carbon fractionation

The following sequence of activities was used to acquire, analyse and predict OC, POC, HOC and ROC contents of the ground composite soil samples using MIR spectroscopy.

MIR spectra were acquired for all fine ground (< 200 µm particle size) samples using MIR spectroscopy instrumentation and processes following Baldock et al. (2013a). All MIR spectra were preprocessed as described by Baldock et al (2013a). A principal components analysis (PCA) was applied to the acquired spectra to look for significant outliers and assess whether or not any clustering of the soils by site or depth existed.

The OC content data was combined with the respective MIR spectra of all samples and a partial least squares regression (PLSR) analysis was completed to quantify the ability to derive a predictive MIR/PLSR algorithm for OC content and to provide the basis for selecting a subset of samples for the subsequent soil OC fractionation analyses. The Kennard-Stone algorithm was applied to the scores plot of the PLSR analysis and a subset of representative samples that accounted for the variance in both the MIR spectra and the OC contents were selected (n=12).

The 12 soils identified were fractionated according to Baldock et al., (2013a); Baldock et al., (2013b). Subsamples of < 2mm soil (two 10 g replicates) were added to 500-mL containers. A 50 ml volume of a sodium hexa-metaphosphate (NaHMP) solution (5 g L⁻¹ solution) was added to each 10 g of sample and shaken overnight to disperse the soil. All samples were then passed through a 50 µm sieve using an automated wet sieving system (Fritsch analysette 3 –Body steel/RF Mesh S-steel/RF) and the soil was separated into fine (< 50 µm) and coarse (> 50 µm) fractions. The coarse (> 50 µm) fraction containing sand particles and particulate organic material and the fine fraction (< 50 µm) were dried at 40°C and weighed. The > 50 µm soil samples were ground to < 200 µm using a ball mill. The OC and TN contents of the dried and ground coarse fraction and the dried fine fraction were determined by analysis on a LECO (TruSpec Series). Mass recovery and carbon recoveries in the two fractions were calculated. Additional 10 g subsamples of the < 2mm soils were also fractionated to provide the material required for solid-state NMR analysis to allow the contents of POC, HOC and ROC to be determined as defined by Baldock et al (2013a). Prior to NMR analysis, the particulate carbon in the coarse fractions was separated from the sand on the basis of the difference in density and the fine fractions were pre-treated with Hydrofluoric acid (HF) according to Skjemstad et al. (1994) to concentrate organic carbon and remove paramagnetic materials.

Predictive PLSR algorithms for OC, POC, HOC and ROC contents were derived and applied to the MIR spectra acquired for all soils included in this study. The PLSR algorithms were constructed by adding the measured soil carbon fractions data and MIR spectra collected for the 12 fractionated soils to that associated with a subset of soils from the CSIRO soil fractions database (SFD). The subset of SFD soils was selected by applying the PCA model developed for the SFD soils to all soils included in this study. The principal component (PC) scores obtained were then projected onto the PCA applied to the SFD soils. All SFD soils having similar combinations of PC1 and PC2 scores to those obtained for soils included in this study were selected from the SFD (n= 129). A square root transformation was applied to the analytical data (OC, POC, HOC and ROC contents). PLSR algorithms were derived for each form of organic carbon and validated using full cross-validation. The PLSR algorithms were then applied to all MIR spectra acquired for the soils included in this project to produce estimates of the square root transformed OC, POC, HOC and ROC contents. The

predicted values were then back transformed to produce the the MIR/PLSR predicted values of OC, POC, HOC and ROC contents used in subsequent analyses.

Statistical analysis

All chemometric analyses (spectral transformations, PCA and PLSR) were completed with the Unscrambler X (CAMO, Norway) software. All statistical analyses were completed using the R statistical software (version 3.3.2) with the RStudio interface (Version 1.0.136). Statistical analysis was undertaken to detect differences in OC, POC, HOC and ROC between vegetation types and soil depth increments. Statistical differences were tested in a two-way analysis of variance (ANOVA) using Tukey's HSD as a post-hoc analysis, to determine statistically significant differences ($P < 0.05$) between different vegetation types and depths. Vegetation type and depth were tested as the key explanatory factors defining whether significant main or interaction terms were found, for OC measured, OC, POC, HOC and ROC predicted in (mg C g soil^{-1}) and TOC stock (Equation 17 and 18) using a non-linear least squares regression (NLS) procedure using an exponential decay model (Equation 16) (where: a = values of the intercept, b = the slope ($b > 0$), x = soil depth, y = carbon).

$$y = a \times e^{-bx} \quad \text{Equation 16}$$

The bulk density (BD) was calculated as the oven dry mass of soil < 2 mm divided by the volume of soil collected. Total carbon stock for the measured and predicted OC was then expressed on an equivalent mass basis after Sanderman et al., (2009) to correct for differences in BD between plots/sites. Equivalent soil mass was used to balance unequal bulk density and avoid comparison of compacted soil with a less compacted soil, the depth of assessment was adjusted to assess a consistent mass of soil (Wendt & Hauser, 2013). Therefore, equivalent mass corrected for BD (mass per unit volum) across sites, vegetation types and soil depth increment. This gives the carbon stocks of a defined soil mass or in a specific depth layer for different vegetation types and sites. The TOC stock (Mg C ha^{-1}) was then calculated by multiplying each soil depth by the BD and the carbon content (%) (Equation 2).

$$\text{SOC Stocks (Mg C ha}^{-1}\text{)} = \text{Soil layer (cm)} \times \text{BD (g cm}^{-3}\text{)} \times \text{C (\%)} \quad \text{Equation 17}$$

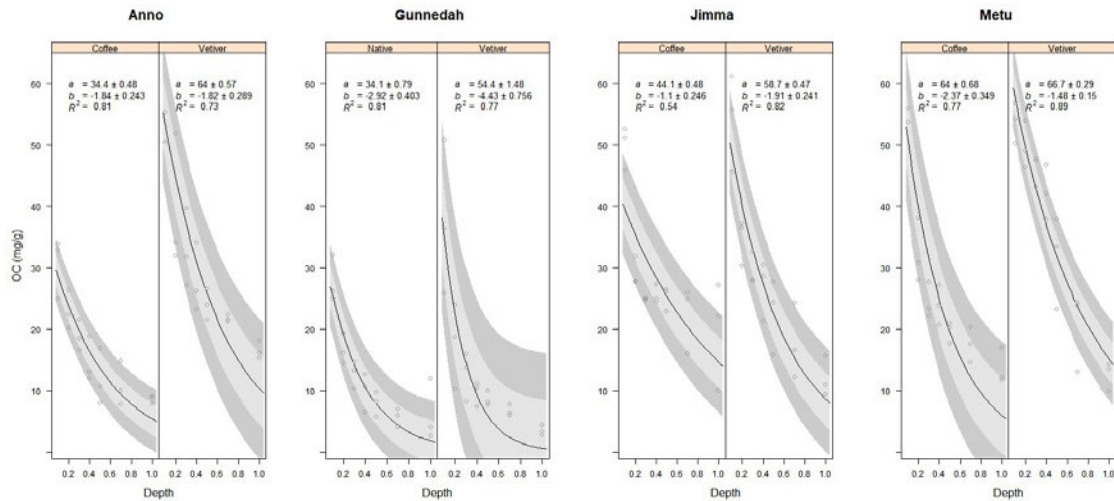
Carbon sequestration rate of vetiver grass was then calculated by deviding the carbon stock difference between vetiver and the coffee/native pasture (Equation 18).

$$\text{Carbon Sequestration Rate} = \frac{\text{OC}_{\text{vetiver}} - \text{OC}_{\text{coffee/native}}}{\text{Age of Vetiver}} \quad \text{Equation 18}$$

Results

Measured OC content

Analysis of variance indicated that depth influenced soil carbon content across all sites ($P < 0.001$) (Figure 22). The depth effect was indicated by a consistent decline in SOC concentration with increasing soil depth. However, vegetation type had an effect only at Anno ($P < 0.001$) in Ethiopia, indicated by a consistently higher SOC content under vetiver compared with the coffee. However, the OC content for vetiver declined more rapidly compared with the other vegetation types i.e concentration of the surface was higher under vetiver. The higher carbon content under vetiver grass was not consistent across the depth and the direction of carbon content change with depth.



ANOVA Effect	P-value	ANOVA Effect	P-value	ANOVA Effect	P-value	ANOVA Effect	P-value
Depth	<0.001	Depth	<0.001	Depth	<0.001	Depth	<0.001
Vegetation	<0.001	Vegetation	-	Vegetation	-	Vegetation	-
D X V	-	D X V	-	D X V	-	D X V	-

Figure 22: Measured contents of OC in mg g^{-1} soil and the profile distribution in soil cores (1.0 m depth) under vetiver and native pastures in Gunnedah Research Center, Australia and from under vetiver and coffee vegetations in southwest Ethiopia. Plots show the raw data (o), a fitted exponential model (—) and confidence bands (1 and 2 SE). Values of the intercept (a), the slope (b) and Coefficient of determination (R^2).

MIR spectra and PCA analyses

The mid-infrared spectra acquired for all samples are presented in Figure 23. The MIR spectra showed significant signal intensity associated with organic materials (e.g. CH₂ stretching at 2923 and 2850 cm⁻¹, carboxyl C at 1708 cm⁻¹, amide C at 1660 and 1556 cm⁻¹(Janik et al., 2007) and minerals e.g. kaolinite at 3400 and 3650 cm⁻¹ (Saikia & Parthasarathy, 2010) and carbonate at 2500 cm⁻¹ (Bruckman & Wriessnig, 2013).

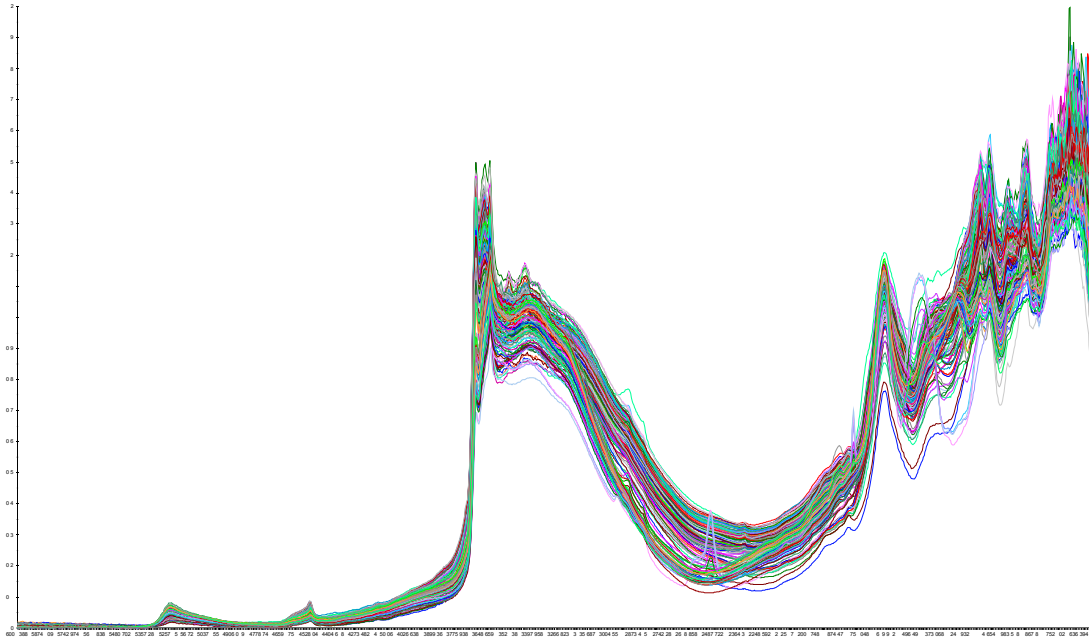


Figure 23: MIR spectra (6000-600 cm⁻¹) acquired for all composite samples included in this project.

The principal components analysis of the MIR spectra showed that the soils from each site differed given their relative position along the PC1 and PC2 axes which indicated a total of 97% of the spectral variance (Figure 24a). A strong predictor along PC1 indicated the presence of kaolinite with those samples sitting to the right (Kaolinite rich Ethiopian soils) having higher PC1 scores than the Gunnedah soils (Figure 5b). All the carbonate soils (from Gunnedah) plotted towards the left on PC1 (Figure 24c). The selection of samples for inclusion in the fractionation exercise of this study using the Kennard Stone algorithm covered the diversity in MIR spectra (Figure 24d).

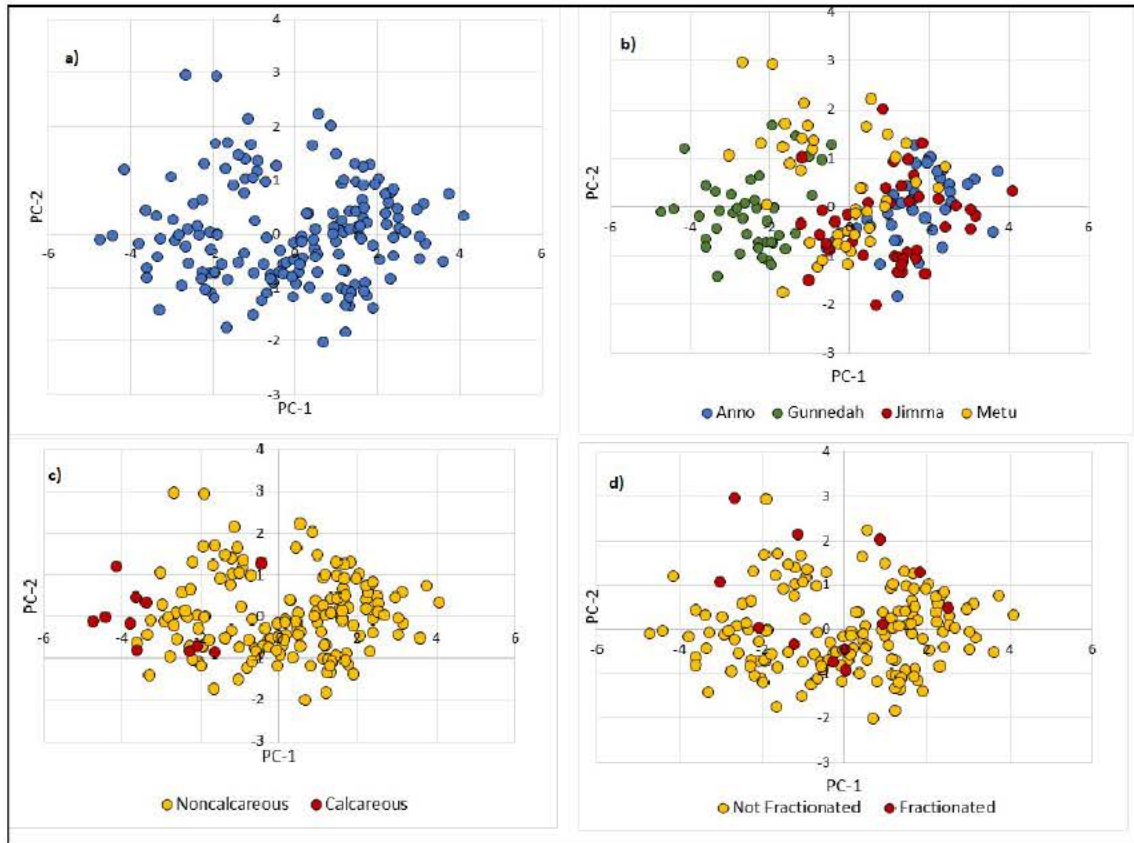


Figure 24: The principal components analysis of the MIR spectra. No grouping applied (a), grouping by site (b), grouping by carbonate/non carbonate (c) and grouping by included or excluded in the samples that were fractionated (d).

PLSR Analysis

The relationship between measured and PLSR predicted square root transformed values of OC, POC, HOC and ROC are presented in Figure 25 and were categorized by the slope, offset, the R^2 (proportion of variance indicated) and the root mean square error (RMSE). The PLSR model for the sqrtOC, sqrtPOC, sqrtHOC and sqrtROC models generated R^2 values of 0.94, 0.78, 0.83 and 0.80, and RMSE values of 0.45, 0.65, 0.48 and 0.42, respectively. The results indicated that all fractions were predicted reliably and with similar efficiency.

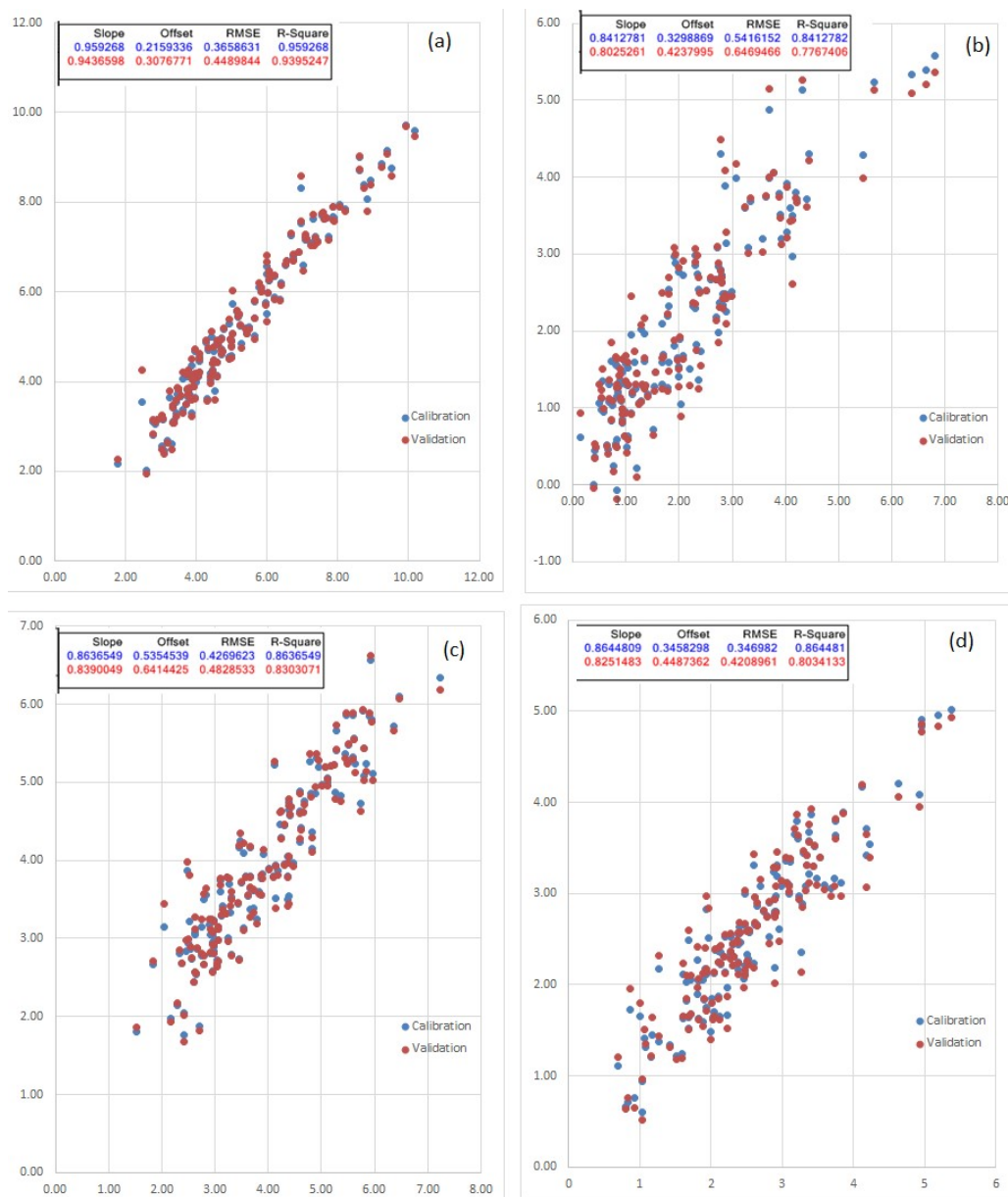


Figure 25: PLSR derived prediction algorithms for the sqrtOC (a), sqrtPOC (b), sqrtHOC (c) and sqrtROC (d).

Carbon stocks in equivalent soil mass

Measured carbon stock

Analysis of variance indicated that only vegetation type was a significant factor influencing OC stocks at Anno ($P < 0.001$), Gunnedah ($P < 0.001$) and Metu ($P < 0.001$) sites but not at Jimma. The difference in the vegetation type was indicated by the higher SOC stock under vetiver compared with coffee and native pastures at the sites where the significant effect was found. At Jimma ($P = 0.015$) a significant interaction between the effects of depth and vegetation types was shown for the measured OC influencing the OC stock, while no significant interaction was shown between the depth and vegetation type effects in all sites (Figure 26).

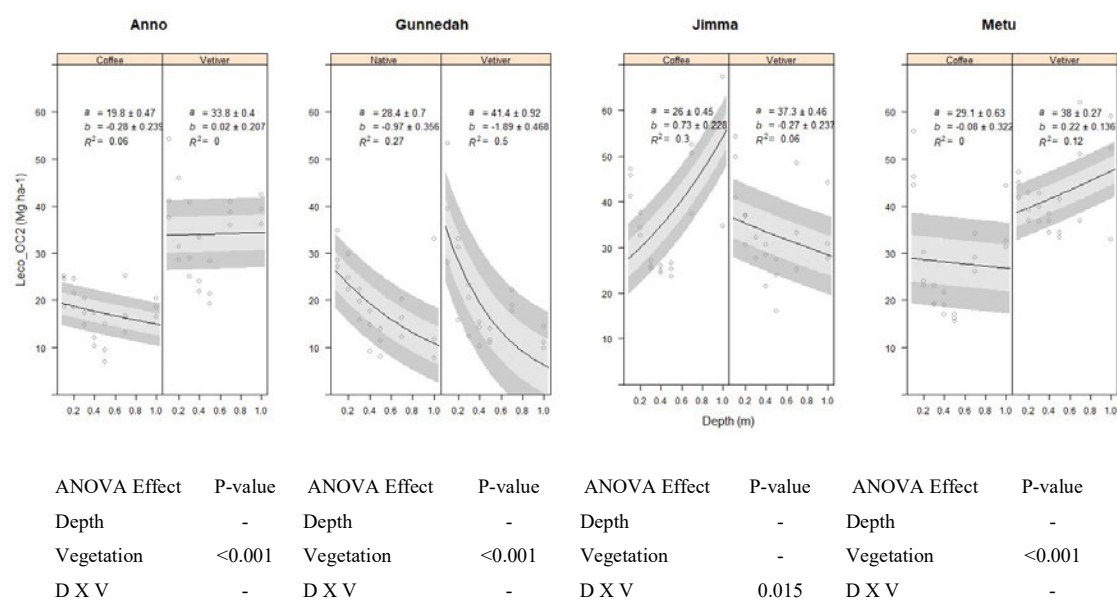
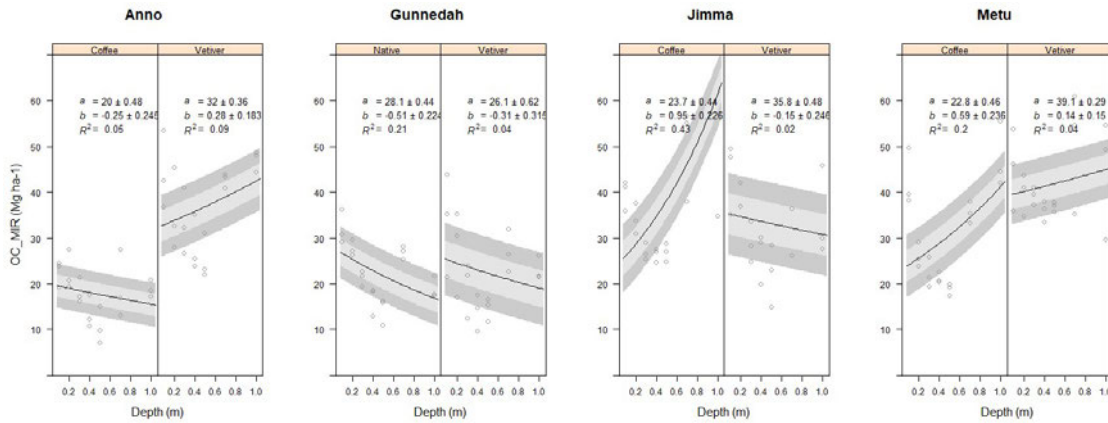


Figure 26: The predicted stocks of soil organic carbon (OC) in Mg ha^{-1} and the profile distribution in soil cores (1.0 m depth) under vetiver, native pastures and coffee plantation in Australia and Ethiopia. Plots show the raw data (o), a fitted exponential model (—) and confidence bands (1 and 2 SE). Values of the intercept (a), the slope (b) and Coefficient of determination (R^2).

Predicted Carbon Stocks (OC, POC, HOC and ROC)

Predicted OC stock

Analysis of variance indicated that vegetation type had a significant effect on the predicted OC stock at Anno ($P < 0.001$) and Metu ($P < 0.001$) (Figure 27) sites. The vegetation effect was indicated by a higher OC stock under vetiver compared with coffee in both sites, where this factor for the predicted OC varied spatially between sites. In addition, no significant interaction between depth and vegetation type were obtained for the predicted OC stock at all sites which indicates a no difference in depth profile characteristics for vetiver and coffee. The predicted OC stock declined with increasing soil depth for vetiver at Gunnedah and Jimma sites. Similarly, for the corresponding plants at Anno (coffee) and Gunnedah (native pasture) predicted OC showed a decrease with increasing soil depth. While, at Jimma and Metu sites both vetiver and coffee showed an increase with increasing soil depth. For vetiver however, OC declined with depth at Jimma and increased at Metu. There were also minor differences observed between the measured and predicted OC stocks.

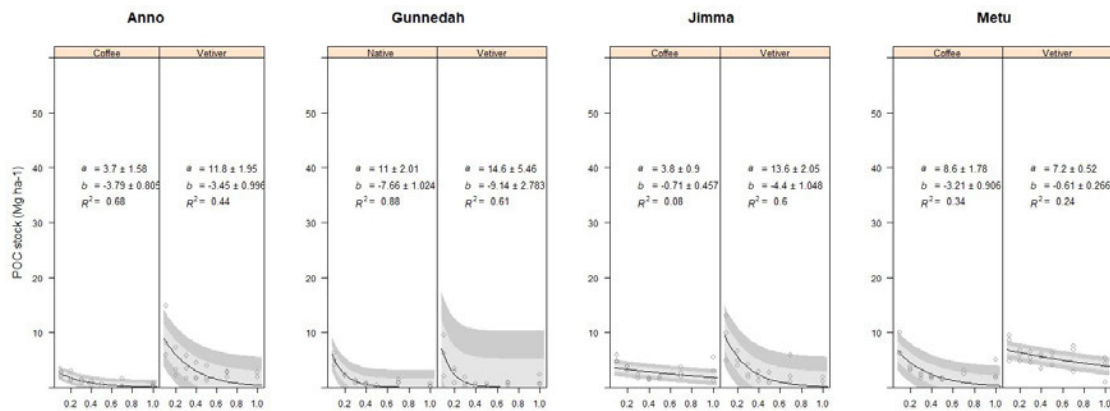


ANOVA Effect	P-value	ANOVA Effect	P-value	ANOVA Effect	P-value	ANOVA Effect	P-value
Depth	-	Depth	-	Depth	-	Depth	-
Vegetation	<0.001	Vegetation	-	Vegetation	-	Vegetation	<0.001
D X V	-	D X V	-	D X V	-	D X V	-

Figure 27: The predicted stocks of soil organic carbon (OC) in $Mg\ ha^{-1}$ and the profile distribution in soil cores (1.0 m depth) under vetiver, native pastures and coffee plantation in Australia and Ethiopia. Plots show the raw data (o), a fitted exponential model (—) and confidence bands (1 and 2 SE). Values of the intercept (a), the slope (b) and Coefficient of determination (R^2).

Particulate organic carbon (POC) stock

Analysis of variance indicated a significant effect of depth on the POC stock at Anno ($P > 0.001$), Gunnedah ($P < 0.001$), Jimma ($P > 0.001$) and Metu ($P = 0.005$) sites (Figure 28). The difference was indicated by a higher POC stock at the surface declining with increasing soil depth in all sites. However, vegetation was a significant factor only at Anno ($P < 0.001$) and Metu ($P < 0.001$) sites. These effects were indicated by a significantly higher POC stock under vetiver at Anno site at the surface but at Metu site it was higher through the whole sampled depth with exponential decline. Depth and vegetation showed a significant interaction effect on the POC stock only at Jimma ($P = 0.002$) site, indicating differences in depth profile characteristics between vegetation types. Depth was a significant factor for the POC in all sites while vegetation was a factor only at Anno and Metu sites.

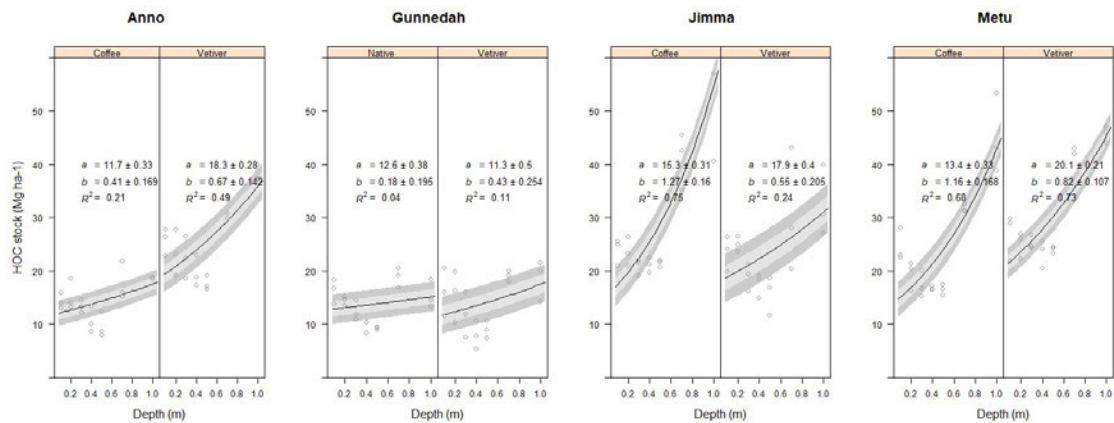


ANOVA Effect	P-value	ANOVA Effect	P-value	ANOVA Effect	P-value	ANOVA Effect	P-value
Depth	>0.001	Depth	<0.001	Depth	>0.001	Depth	0.005
Vegetation	<0.001	Vegetation	-	Vegetation	-	Vegetation	<0.001
D X V	-	D X V	-	D X V	0.02	D X V	-

Figure 28: The predicted stocks of POC ($Mg\ ha^{-1}$) and the profile distribution in soil cores (1.0 m depth) under vetiver, coffee and native pastures in Gunnedah, Australia and southwest Ethiopia. Plots show the raw data (o), a fitted exponential model (—) and confidence bands (1 and 2 SE). Values of the intercept (a), the slope (b) and Coefficient of determination (R^2).

Humus organic carbon (HOC) stock

Analysis of variance indicated a significant depth and vegetation type effect on the HOC stocks at Anno ($P < 0.001$, both effects), Jimma ($P < 0.001$; $P = 0.011$, respectively) and Metu ($P < 0.001$; $P = 0.005$, respectively) sites, but neither depth nor vegetation types had significant effect on HOC at the Gunnedah site. The depth difference was indicated by an exponential increase of the HOC stock with increasing soil depth in all Ethiopian sites. The vegetation difference was indicated by a higher HOC stock under vetiver compared with coffee throughout the depth profile in Ethiopian sites particularly at the Anno site. Depth and vegetation type interactions were found at Anno ($P = 0.038$) and Jimma ($P = 0.0028$) sites (Figure 29), indicating different depth profile characteristics between vegetation types.



ANOVA Effect	P-value	ANOVA Effect	P-value	ANOVA Effect	P-value	ANOVA Effect	P-value
Depth	<0.001	Depth	-	Depth	<0.001	Depth	<0.001
Vegetation	<0.001	Vegetation	-	Vegetation	0.011	Vegetation	0.005
D X V	0.038	D X V	-	D X V	0.0028	D X V	-

Figure 29: The predicted stocks of HOC in Mg ha^{-1} and the profile distribution in soil cores (1.0 m depth) under vetiver, coffee and native pastures in Gunnedah, Australia and southwest Ethiopia. Plots show the raw data (o), a fitted exponential model (—) and confidence bands (1 and 2 SE). Values of the intercept (a), the slope (b) and Coefficient of determination (R^2).

Resistant Organic Carbon (ROC) stock

Both depth and vegetation type had significant effects on the ROC stock at both Anno ($P= 0.0427$ and $P< 0.001$, respectively) and Metu ($P= 0.0114$ and $P< 0.001$, respectively). The difference was indicated by a higher ROC under vetiver compared with coffee through the depth profile, especially at the surface at the Metu site. But, there was no significant effect of either depth or vegetation type on the ROC stock at Gunnedah and Jimma sites. In addition, significant depth by vegetation interaction effect was found in all sites but not for Gunnedah (Figure 30), indicates that the different depth profile characteristics between vegetation types. Both vegetation type and depth were significant factors affecting the ROC stock at Anno and Metu sites but not at Gunnedah and Jimma sites.

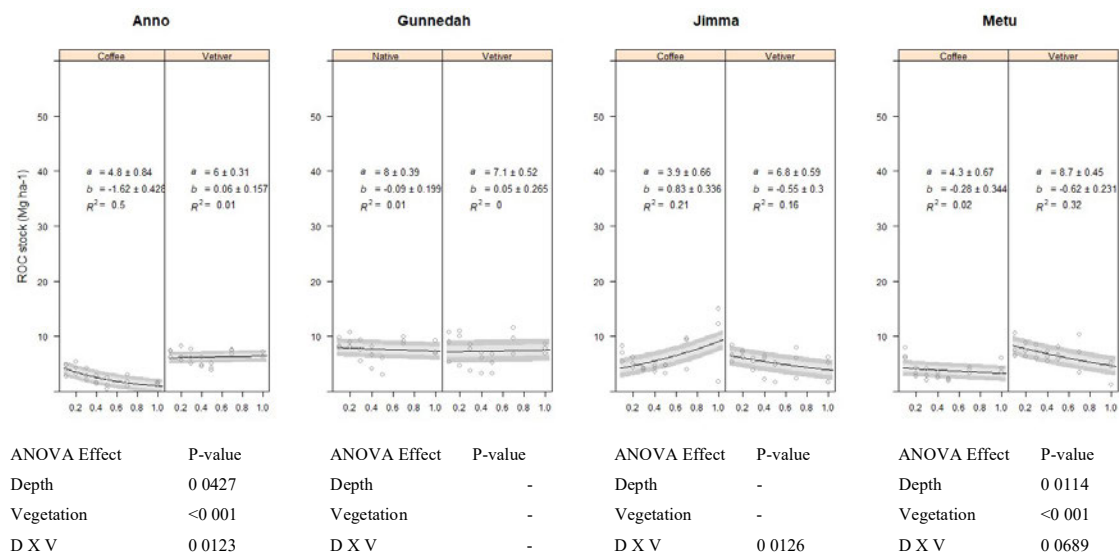


Figure 30: The predicted stocks of ROC ($Mg\ ha^{-1}$) and the profile distribution in soil cores (1.0 m depth) under vetiver, coffee and native pastures in Gunnedah, Australia and southwest Ethiopia. Plots show the raw data (o), a fitted exponential model (—) and confidence bands (1 and 2 SE). Values of the intercept (a), the slope (b) and Coefficient of determination (R^2).

Carbon stock of the measured (OC) and predicted (OC, POC, HOC and ROC) to a total of 1.0 m soil depth for the vegetation types studied are presented in the Table 15 below. A large part of the OC was stored in the HOC fraction under all the vegetation types and in all sites. However, there was a difference in the HOC stock between vegetation types expressed in higher HOC stock under vetiver at Anno ($+78\ Mg\ ha^{-1}$) and Metu ($+40\ Mg\ ha^{-1}$) compared with coffee, while at Jimma the HOC stock stored was higher under coffee ($+43\ Mg\ ha^{-1}$) compared with vetiver. However, there was no difference in HOC stock between vetiver (only $+2\ Mg\ ha^{-1}$) and native pastures at Gunnedah. Over all, there were differences in the carbon fractions between sites, where the impact of vetiver was much higher in Ethiopian locations compared with Gunnedah. The ratio of POC to HOC indicates the vulnerability of carbon to change and in this study the result showed a very low value for all the vegetation types and in all sites. This indicates lower vulnerability of the carbon stock to change or

turnover due to the larger proportion stored in the more resistant form of carbon fraction (HOC) (Table 15).

Table 15: Measured OC and predicted (OC, POC, HOC and ROC) carbon stocks (Mg ha⁻¹) in 1.0 m soil depth

Site	Vegetation	Measured (Mg ha ⁻¹)		Predicted (Mg ha ⁻¹)			Vulnerability POC:HOC
		Leco_OC	OC_MIR	POC	HOC	ROC	
Anno	Coffee	122 ± 24	125 ± 25	7 ± 3	100 ± 12	18 ± 6	0.08
	Vetiver	238 ± 27	256 ± 22	27 ± 5	178 ± 11	43 ± 4	0.15
Gunnedah	Native	134 ± 34	158 ± 11	11 ± 0	96 ± 3	54 ± 7	0.11
	Vetiver	143 ± 32	159 ± 30	12 ± 5	98 ± 14	51 ± 11	0.12
Jimma	Coffee	262 ± 31	268 ± 39	20 ± 2	207 ± 21	42 ± 12	0.09
	Vetiver	231 ± 37	234 ± 46	25 ± 9	164 ± 28	37 ± 12	0.15
Metu	Coffee	196 ± 16	213 ± 11	22 ± 3	171 ± 6	27 ± 3	0.13
	Vetiver	295 ± 35	292 ± 25	39 ± 7	211 ± 9	47 ± 4	0.18

Through the whole soil depth studied large proportion (60-80%) of the total OC was stored in the form of HOC fraction under all vegetation types and in all sites while the rest i.e. 12-34% and 5-13% of the total OC stored in the ROC and POC fractions, respectively (Table 16). The HOC proportion however was higher under coffee compared with the vetiver but not at Gunnedah where the HOC under vetiver and native pasture were similar.

Table 16: Proportion of OC stored in the form of particulate, humus and resistant organic carbon under different vegetation types in Australia and Ethiopia

Site	Vegetation	OC proportion in each fraction (%)		
		POC	HOC	ROC
Anno	Coffee	5.6	80.0	14.4
	Vetiver	10.5	69.5	16.8
Gunnedah	Native	7.0	60.8	34.2
	Vetiver	7.5	61.6	32.1
Jimma	Coffee	7.5	77.2	15.7
	Vetiver	10.7	70.1	15.8
Metu	Coffee	10.3	80.3	12.7
	Vetiver	13.4	72.3	16.1
Average		9	71	20

Using Equation 18 (Table 17) there was a difference in carbon sequestration rate between vetiver grass and the other adjacent crops (coffee and native pastures). All carbon fractions and sites under vetiver showed a gain of carbon but not at the Jimma site relative to coffee and native pasture which could be an indication of slower turnover of C as a result of vetiver plantation. The sequestration by vetiver grass for the measured and predicted OC across all locations fall between -2.64 to +7.69 Mg C

ha⁻¹ yr⁻¹, while for the particulate, humus and resistant organic carbon was 0.04 to +1.17, -3.36 to +4.64 and -0.35 to +1.51 Mg C ha⁻¹ yr⁻¹, respectively.

Table 17: Carbon Sequestration (Mg C ha⁻¹ yr⁻¹) by Vetiver where the age of Vetiver Anno=17, Gunnedah 22, Jimma=13 and Metu=15 years at the time of sampling

Sites	Measured OC	Predicted OC	POC	HOC	ROC
Anno	6.83	7.69	1.17	4.64	1.51
Gunnedah	0.40	0.07	0.04	0.07	-0.12
Jimma	-2.32	-2.64	0.39	-3.36	-0.35
Metu	6.57	5.23	1.1	2.63	1.34

Discussion

The Impact of Vegetation on TOC stocks

Previous studies have widely recognised the use of perennial tropical grasses as an alternative land management practice and a strategy to enhance accumulation of large quantities of soil carbon due to their large biomass (Clifton-Brown et al., 2007; Dondini et al., 2009; Lavania & Lavania, 2009; Conant, 2012; Zimmermann et al., 2012). In the current study, when soil carbon values were expressed as TOC stock on an equivalent soil mass basis, vetiver had accumulated a higher organic carbon stock, shown on both measured (Anno, Gunnedah and Metu) and predicted (Anno and Metu) TOC stocks despite the spatial variability for the predicted OC stocks (Table 14). The statistically different organic carbon stock between vetiver and the corresponding coffee and native pastures between sites (Ethiopia and Australia) suggests that these different plant types, established at different time (planting years prior to sampling) and with the different soil conditions (carbonate and Kaolinite) were not equally effective at storing additional soil organic carbon over this time. The higher OC under vetiver probably resulted from the new carbon input due to its deep root system, root respiration and organic matter inputs (Dondini et al., 2009). Increases of OC stocks were found all through the soil profile but significance of the increase under vetiver diminished with depth. This effect was strongest at the soil surface implying that surface litter inputs dominate the sites.

Vetiver was effective at increasing soil carbon concentration and stocks relative to coffee in most sites under study. However, vetiver and coffee at Jimma and native pasture at Gunnedah were established at the same time and on similar soil condition but showed no difference. The lack of differences in the soil carbon stock between these plant types in those specific sites could also have been influenced by soil and environmental factors and farming practices (e.g. tillage, biomass removal, altered hydrology from irrigation, nutrient inputs from fertiliser).

Our results therefore, confirm that using vetiver a tropical perennial grass as a land management option does indeed result in accumulation of additional carbon relative to the previous land use but that, vetiver performs in a similar way to the other plant types studied at Gunnedah and Jimma sites.

The change of stocks of different soil carbon fractions

Differences in the allocation of SOC to its component fractions can be used to define the potential vulnerability of SOC stocks to temporal change. Hence, the MIR prediction we used to derive estimates of the contents and composition of soil OC provided reliable predictions of the contents of soil OC and various soil carbon fractions (Janik et al., 2007; Zimmermann et al., 2007). Our result showed a higher amount of OC allocated to HOC fraction under vetiver in most study locations which is in agreement with the study undertaken by SCaRP in Australia (Baldock et al., 2013a; Baldock et al., 2013b) in terms of the dominant fractions but not specific to vetiver. This study therefore, has shown differences between sites in the carbon fractions, where the impact of vetiver was much higher in Ethiopian locations compared with Gunnedah. In this study we have found 71, 20 and 9 % proportion accounted of the total carbon present in the humus, particulate and resistant organic carbon, respectively while other studies have reported 56, 26 and 19% OC in the respective fractions in Australia (Baldock, 2013a). Hence, our study showed a significantly higher proportion of HOC fraction which makes it an important finding in terms of using vetiver as a potential climate change mitigation option because of the more stable and less vulnerable nature of the HOC fraction. Hence, HOC was the dominant carbon fraction for vetiver and all vegetation types on average (71%) which is the most stable fraction and less vulnerable to change/turnover than POC. Our result is therefore, much higher for the HOC fraction (> 15%) than the result obtained by other workers conducted in Australia on agricultural lands which indicated an average allocation of 56% of the total carbon in the HOC fraction (Baldock et al. 2013a & b). In this study, the significant variations in the stocks of carbon fractions could suggest rapid change of the labile (POM) to the more stable (HOM) fraction in all study locations and vegetation types. The variation in the amount and stocks of carbon in biological forms could occur due to the soil and environmental factors and as well as farming practices.

The TOC stock was influenced by depth and vegetation where the higher quantity or proportion of TOC stock was mainly contributed by the HOC fraction. The ratio of POC to HOC which was low could indicate that there is either limited inputs of POC or that POC is decomposed quickly. Even though there was an effect of vegetation type influencing the quantity or proportion of HOC, the interaction of depth by vegetation could have an impact on the accumulation of this fraction which implies that there might be different depth profile characteristics between vegetation types such as pH (Wilson et al., 2010). Other studies have indicated that different management practices can cause

differences in the amount of carbon sequestration potentials (Hutchinson et al., 2007; Sanderman et al., 2009), which equally could apply to the different soil carbon fractions and changes in the stocks of the different soil carbon fractions. The TOC stock under vetiver to a deeper soil sampling profile we used (1.0 m) was high from the previous studies due than the sample depth used by the previous studies (0.3 m) despite the similar fractionation processes we used which suggests the importance of considering sampling of deeper soil profiles in soil carbon inventory (Baldock et al., 2013a; Baldock et al., 2013b).

Baldock et al. (2013b) proposed that the ratio of POC to HOC stocks could provide an indication of vulnerability with increasing ratios being indicative of a greater vulnerability given the more labile nature of the POC fraction. Our result (ratio of POC to HOC stocks) therefore, indicates a limited vulnerability to change of the total soil carbon under vetiver due to higher proportion of the HOC fraction which is less labile in nature and how stocks of fractions fit well in with SCaRP measured results with the Australian soils (Baldock et al., 2013a; Baldock et al., 2013b). This result can tell us that the carbon can stay in the soil for longer time.

Vertical distribution of carbon stocks

Depth was a key factor affecting the contents of carbon fractions, particularly for the POC and the HOC stocks with increasing soil depth. The proportion of SOC allocated to the POC fraction decreased while the HOC fraction increased with increasing depth under all vegetation types. Our results are therefore, in agreement with Hobley et al. (2016), who indicated depth as a key factor affecting the content of all three fractions in soil, with proportions of SOC allocated to POC decreased while the HOC increased with increasing depth. Hence, in this study SOC was less strongly associated with POC and ROC fractions, while Hobley et al. (2016), reported a weaker association of HOC and ROC with SOC as climate, soil physical and chemical properties could be more important as explanatory variables than depth. The presence of HOC fraction contributes more to the accumulation of TOC stock which is a mechanism by which SOC builds through the whole soil profile. Furthermore, Hobley et al. (2016), indicated that human influences (land-use change and management) were not important in defining the proportion of the fractions or in controlling SOC stability. Dondini et al. (2009), also compared *Miscanthus* grass and arable crop land, demonstrating a higher SOC in different aggregates throughout the soil profile under *Miscanthus*, which they attributed to the input of new carbon and low disturbance in the *Miscanthus* grass. Our result has therefore, showed an exponential change in carbon stocks with increasing soil depth. Therefore, vertical distribution is important because the carbon in the different fractions is differently susceptible and this could lead to different stabilities and vulnerability of soil carbon in soil carbon accounting schemes.

Implications of the Predicted Carbon Fractions under Vetiver

In our study the dominant fraction in almost all sites (Australia and Ethiopia) was the humus organic carbon (HOC) for all vegetation types (vetiver, native pasture and coffee plantations) which is more stable form of carbon and less vulnerable to rapid change than the POC. Vetiver had a higher amount of humus organic carbon fraction accumulated compared with the POC and ROC carbon fractions in almost all sites (Australia and Ethiopia) considered in this study. This result implies that vetiver can accumulate the more stable form of carbon fraction which is less susceptible to rapid change/turnover. Therefore, growing vetiver could be a feasible strategy which has an implication for the high rate of stable carbon accumulation. Our result showed that measured and predicted OC across all locations fall between -2.64 to $+7.69$ Mg C ha⁻¹ yr⁻¹, while for the particulate, humus and resistant organic carbon was 0.04 to $+1.17$, -3.36 to $+4.64$ and -0.35 to $+1.51$ Mg C ha⁻¹ yr⁻¹, respectively (Table 17). The result demonstrated that under vetiver the carbon loss was less which implies that vetiver production resulted in slower turnover. This result can therefore, be an indicative to a large potential in carbon sequestration where, the dominant carbon added by vetiver doesn't change rapidly.

Conclusion

In conclusion, vetiver a perennial tropical grass which is widely growing in tropical and subtropical regions of the world has shown a buildup of high soil organic carbon stock to a 1.0 m soil depth. Due to its large biomass and deep root system, vetiver had shown potential soil carbon stocks to a 1.0 m soil depth predominantly the more stable carbon fraction (i.e. HOC). SOC stock is predominantly allocated to the humus organic carbon (HOC) fraction which is the more stable and less vulnerable to change or turnover. While, the most labile (POC) and the resistant (ROC) carbon fractions had a small contribution to the total SOC stocks compared to HOC. All carbon fractions were located more at surface and declined with depth increment. Hence, the dominant carbon fraction (HOC) under vetiver is the most stable and changes quite slowly implying that the most dynamic process can buildup may be in the first 10 years. The result from this study can therefore, be an indicative to a large potential for carbon storage, where, the dominant carbon fraction (HOC) added by vetiver doesn't change in rapidly and could have high residence time deep in soil profiles. We therefore, suggest that growing vetiver has a proven potential for accumulation of the more stable carbon (HOC) to the deeper soil depth profile. Hence, vetiver could be a potentially sustainable option to improve soil health and could be a feasible scheme which could lead farmers especially those in the developing nations to participate in the carbon accounting program. We therefore, suggest that countries specially in tropical regions to encourage and promote plantation of perennial tropical grasses such as vetiver especially on degraded lands as potential option to facilitate carbon sequestration and environmental rehabilitation. The wide assumptions about vetiver as a potential candidate for facilitating soil carbon

sequestration therefore, is a possibility for viable recommendation to encourage a more rigorous application of the cutting-edge carbon measurement techniques on similar potential grasses at a wider scale and in different agro ecologies.

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CHAPTER 7: General Conclusions and Future Research

Introduction

Globally, there is a widespread land degradation associated with deterioration of natural resources contributing to increasing poverty. A key indicator of land degradation is soil carbon, which has declined as a result of human induced disturbance such as vegetation clearing, soil disturbance, cultivation and erosion with negative effects on production and productivity (Shiferaw et al., 2013). Loss of soil carbon has local and regional consequences for food security and livelihoods due to compromised soil and landscape resilience and productivity, as well as global consequences for climate change. Conversely, maintaining and increasing soil carbon has a combined effect of sustaining soil health, agro-ecosystem function, productivity and potentially contributing to climate change mitigation efforts (Sommer & Bossio, 2014). Addressing land degradation by increasing soil carbon sequestration is therefore a win-win strategy to achieve poverty eradication and climate change mitigation.

Research has shown that adopting natural resource management innovations can potentially increase farm productivity and soil health (Freibauer et al., 2004; Lal, 2004a; Rabbi et al., 2013a). Land management to improve soil organic matter content, condition and productivity is therefore, a key strategy to safeguard agricultural production, food supply and environmental quality (Conant et al., 2001; Glover et al., 2008). Soil carbon sequestration through the use of plant species with high photosynthetic efficiency, deep roots and high biomass production is one of the important options for more stable subsoil carbon storage. Plant biomass is a primary source that can contribute to soil carbon sequestration, both at the surface and at depth, and an important component of this is the below-ground plant biomass. Perennial grasses, particularly those that have a deep root system are likely to contribute significantly to soil carbon storage (Hansen et al., 2004a; Clifton-Brown et al., 2007; Poepflau & Don, 2013). Cropland conversion to pasture has a demonstrated capacity to store additional soil carbon and perennial tropical pastures appear to have particular value in this regard (Fisher et al., 1994; Batjes, 1998; Conant et al., 2001; Conant, 2012; Sanderman et al., 2013a; Sanderman et al., 2013b). A range of tropical pasture species have been investigated for their soil carbon storage potential (Sanderman et al., 2014), but vetiver grass is currently attracting particular attention (Singh et al., 2011), given its extensive use globally and its large biomass production. Vetiver grass therefore, has considerable, as yet unquantified, potential for long term carbon storage (Lavania & Lavania, 2009; Brown et al., 2011; Singh et al., 2011; Gao et al., 2016).

The aim of this PhD research was therefore; to review the current knowledge gaps and current research needs with regard to the carbon storage potential of tropical pastures and particularly to

estimate the soil carbon sequestration potential of vetiver (*Chrysopogon zizanioides*). Data were therefore generated to elucidate the viability of using vetiver for soil carbon sequestration given its wide distribution and extensive use in tropical countries and, in particular, Ethiopia where the current agricultural policy environment has identified land management innovations as key entry point to achieve co-benefits of resilient agriculture, poverty alleviation, and climate change mitigation.

Synthesis of the main findings and Implications

A series of research questions were examined under this PhD research work: a) The soil carbon content and depth distribution down the soil profile under vetiver compared with native and tropical pastures and cropland soil (Chapter 3); b) the impact of vetiver grass on carbon sequestration and its SOC input and the quantity of SOC attributable to vetiver (C₄ carbon) compared with soil dominated by pre-existing (C₃) Carbon (Chapter 4); c) The above- and below-ground biomass production and the relative rate of decomposition of vetiver shoots and roots in different soil types (Chapter 5) and d) The amount of allocation of soil carbon to particulate, humus and resistant fractions differentiated on the basis of particle size and chemical composition (Chapter 6).

In this final chapter, the main research findings are summarized and the implications are discussed within the respective chapters in the following sections.

Soil Carbon Storage Potential of Tropical Pastures: A Review (Chapter 2)

This section reviewed and synthesized the current literature relating to the important principles, subject areas and studies related to tropical grasses, and particularly vetiver for carbon storage and assessed the work undertaken in the area.

A number of tropical grasses are adapted to a wide range of conditions and are potential candidates to contribute to climate change mitigation efforts through soil carbon sequestration. This is due to their rapid establishment, high biomass production, continual/year round and fast growth rates, and deep-rooted systems. Using tropical grasses also has a low cost of implementation, rehabilitates degraded land and most importantly, improves soil health/productivity through increasing soil organic carbon (SOC) which in addition help countries especially those in the developing world, such as Ethiopia, to contribute to climate change mitigation efforts through increasing SOC. In addition, opportunities such as biomass energy use and land rehabilitation efforts could encourage the wide use of tropical pastures as a climate change mitigation option.

Tropical grasses are therefore potential candidates to contribute to climate change mitigation efforts through additional SOC storage. The existing literature on tropical grasses potential for soil carbon

sequestration provide positive evidence and encourage further investigations on: cropland conversion to tropical perennial pasture as an important strategic option to improve soil health and soil carbon storage; the extent of SOC contribution from tropical grass species and carbon inventory in deeper soil profiles; the rate of soil carbon turnover and cycling of the new carbon added and the extent to which it is retained in the soil system; the best management practices, site specific policies and technological options which can reverse and have positive effect on soil carbon storage; vetiver though seems to be an all-round winner and to have considerable as yet not quantified potential for carbon sequestration. The work presented in this thesis aimed to address some of the knowledge gaps highlighted in this literature review to quantify the effects of planting vetiver grass on SOC storage potential by sampling soil profiles (to 1.0 m) with the main objective of examining the quantity, nature, fractions and distribution of SOC compared with other plant types.

Soil Carbon Storage and Distribution under Vetiver (*Chrysopogon zizanioides*) using Stable Isotope Analysis (Chapter 3)

This chapter examined the amount and depth distribution of carbon stored under vetiver grass compared with native, other tropical pastures and cropland soil (as a reference) in Australia. This work indicated that Vetiver has the potential to increase SOC concentration and total carbon stock (Mg ha^{-1}) compared with that of cropping management. A larger TOC stock was found under vetiver compared with tropical pasture and cropping soils. However, when the age of these pastures was accounted for on an annual carbon storage basis, the SOC storage potential of vetiver, Australian native pastures and other tropical pastures was equivalent over time. For all plant types, a decrease in organic carbon concentration was observed with increasing soil depth but a larger TOC stock of carbon was found under vetiver through the whole soil profile.

Soils under vetiver at Gunnedah, Australia had a higher (less negative) $\delta^{13}\text{C}$ value compared with native, tropical pastures and cropping soils. Both litter and root mass probably have contributed to the additional TOC stock (43.5%) under vetiver and this grass has a considerable potential for carbon sequestration, particularly on carbon depleted soils. However, its potential would not appear to exceed that of native pasture nor to other tropical pastures on an annual basis. The higher $\delta^{13}\text{C}$ value under vetiver through the soil profile indicated that new (C4) carbon was being contributed down the soil profile by vetiver with a net addition rate of ~2% per annum. However, this result suggests that while carbon was indeed being added to the soil, associated decomposition of both old and presumably new carbon resulted in only a modest increase in the total carbon stock. This study has also shown the importance of considering deeper soil sampling to investigate effects of these grasses especially where these are deep rooted and high biomass producing plant species.

In Australia, there is a need to further consider tropical perennial pasture species to include in cropland to pasture conversion practices to both improve the soil health and the status of soil carbon and the quality of animal feedstock. We believe that vetiver, due to its wide adaptability, rapid and efficient establishment and high bioass has particular considerable potential for carbon sequestration, particularly on carbon depleted soils. Internationally, there is also a need for further work to explore the potential of tropical perennial grass species including vetiver to influence land management decisions using perennial grasses and hence contribute to climate change mitigation action and restore degraded lands in tropical and sub-tropical regions of the world.

Soil Carbon Storage Potential and Depth Distribution under Vetiver Grass in SW Ethiopia and its Implications (Chapter 4)

This chapter quantified the amount and depth distribution of soil carbon stored under vetiver compared with coffee plantations in southwest Ethiopia. This chapter further provides the total soil carbon, $\delta^{13}\text{C}$ values and the new carbon added by vetiver grass. This was done to explore effects of vetiver grass in an African (specifically in Ethiopian) environment where vetiver has been extensively used for soil and water conservation purposes.

Vetiver grass, planted on soil bunds in the southwest Ethiopian highlands showed a larger SOC concentration and TOC stock (262Mg C ha^{-1}) compared with coffee (178 Mg C ha^{-1}) through the whole sampled profile (1.0 m). The SOC concentration and total carbon stock declined with increasing soil depth for both plant types. Vetiver grass showed higher (less negative) $\delta^{13}\text{C}$ values at the soil surface increasing with increasing soil depth through the whole soil profile which indicated the replacement of C_3 carbon by C_4 carbon, through the whole soil profile but especially in the surface soil layers (Ehleringer et al., 2000). The higher $\delta^{13}\text{C}$ values under vetiver suggests that this C_4 species with a distinctively higher $\delta^{13}\text{C}$ signature added a significant quantity of soil carbon through the whole profile. This study also showed a continuous addition of new carbon in the soil under vetiver but there is significant carbon turnover in the soil system resulting, in only a modest increase in carbon storage overall. In this environment (Ethiopia), vetiver is better than coffee at storing carbon even though coffee not regularly cultivated. Hence, we suggest that interplanting coffee with vetiver might be feasible.

For vetiver, there was a progressive increase in the $\delta^{13}\text{C}$ value with increasing soil depth which conflicts with the value found in Gunnedah, Australia (Chapter 3), where vetiver showed a decrease in $\delta^{13}\text{C}$ with increasing soil depth. The results found in these sites however, agrees with previously reported data stating a common trend observed in a decrease of $\delta^{13}\text{C}$ with increasing soil depth (Ehleringer et al., 2000; Badeck et al., 2005; Schwendenmann & Pendall, 2006). The difference in

$\delta^{13}\text{C}$ values between the Gunnedah and Ethiopian sites could have existed because of the difference in environmental conditions such as soil type (Ferrosols and Nitisol, respectively), the rainfall and management related issues present between the sites under this study.

In countries like Ethiopia soils are carbon depleted as a result of severe land degradation. However, if appropriate land management practices and carbon input measures are undertaken these soils are believed to have great potential for storing additional carbon (WB, 2008; Abebe et al., 2012; Shiferaw et al., 2013; Shiferaw et al., 2015; Solomon et al., 2015). Hence, knowledge of soil carbon storage potential and change as a function of land management practices can offer the opportunity to better manage land for enhanced productivity and could contribute to the goals of enhancing carbon storage in soils (SIDA, 2010a; Brown et al., 2011; Solomon et al., 2015; Rimhanen et al., 2016). Therefore, soil carbon sequestration potentials of the use of vetiver grass has shown to have the capacity to store additional carbon through the whole profile in this environmental condition. Hence, this result is crucial to successfully achieve co-benefits of resilient agriculture and climate change mitigation via soil carbon sequestrations at regional and country-wide scales. The information generated from this study intended to contribute to the need of continuing of advancing knowledge on the potential of tropical perennial grass species to influence land management decisions in Ethiopia and across tropical regions of the world.

Functional Links between SOC, Biomass and Decomposition of Vetiver grass in Soils (Chapter 5)

This chapter investigated the above- and below-ground biomass production and relative rate of decomposition of the root and shoot biomass. Hence, it provides the above- and below-ground biomass production potential of vetiver grass under a glasshouse condition and further provides a relative decomposition of root and shoot biomass. This study helps understand which part (the above- or below-ground biomass) of the plant contributes to carbon accumulated in the soil.

Below-ground plant biomass is a primary factor that can contribute to soil carbon sequestration, both at the surface and at depth. Tropical perennial grasses, particularly those that have a deep root system such as vetiver, are proposed to contribute significantly to soil carbon. The results of this study, confirmed the large biomass (both above- and below-ground) production potential of vetiver grass over a short period of time, even in low fertile soil. Biomass values were quantified as 161 and 107 Mg ha^{-1} of fresh and 67.7 and 52.5 Mg ha^{-1} of dry shoot and root biomass, respectively. Root mass production declined exponentially with depth in the soil. Shoot to root biomass production ratio was 1.43 and 1.25 for the fresh and the dry biomass production, respectively.

Vetiver root materials were found to have a rapid decomposition rate regardless of where the root material were sampled (root depth). The high vetiver root biomass production and the distinctively high (less negative) $\delta^{13}\text{C}$ signature (Chapter 3 and 4, respectively) could explain the significant quantities of soil carbon added through the whole soil profile. This study suggests that roots are significant contributors to the SOC concentration and TOC stock through the depth than the shoots. Hence, the exponential decline in the SOC concentration and TOC stock is linked with the exponential decline in root biomass production and distribution with increasing soil depth.

Vetiver root material decomposed more rapidly (i.e humified more rapidly) and hence contribute to SOC cycling more than the shoot material. The more carbon release from the root-soil interaction could therefore be the reason for the high carbon turnover under vetiver grass though there is an evidence in the continuous new carbon addition (Chapter 3 and 4). This finding therefore, indicated that for vetiver, the large root biomass produced does indeed contribute more to the soil carbon accumulation not only to the soil organic matter which was confirmed after the decomposition of vetiver shoot and root biomass applied on soil surface. This is due to the faster decomposition rate of vetiver root litter which is crucial in releasing the carbon in the root litter and would also speed up its contribution to stable soil organic matter. In addition, the more rapid vetiver root decomposition can clarify the reason why there is an exponential decrease in the quantity of roots with increasing soil depth. Hence, although its often assumed that SOC is contributed from the surface down the soil profile this result suggests that roots are more significant.

This study has therefore contributed to the need of evidence we have on the how root and shoot decomposition differs and their relative contribution to soil carbon sequestration in this case under vetiver. Hence, planting and the use of vetiver and similar tropical perennial grasses on degraded and less fertile soils could be an important strategy to consider as an alternative land management practice to halt land degradation and enhance soil carbon sequestration. We therefore suggest that farmers need to be encouraged to plant vetiver and similar tropical perennial grasses on degraded soils and marginal lands to facilitate land rehabilitation and enhance soil carbon sequestration. Further rigorous research also needs to be conducted to investigate the mechanisms and impacts of other potential tropical perennial grass species similar to vetiver to influence land management decisions in tropical and sub-tropical regions of the world.

Predicted Contents of Soil Carbon Fractions under Vetiver Grass in Australia and Ethiopia (Chapter 6)

This chapter quantified the impact of growing vetiver on the vertical soil profile distribution (to 1m) of SOC stock and its allocation to POC, HOC and ROC fractions compared to native pastures at Gunnedah, Australia and coffee plantations in Southwest Ethiopia. In addition, it compared and contrasted the effects of vetiver on soils by comparison with locally relevant alternative land-use types in these two contrasting environments – an experimental site in Australia and sites in Ethiopia where vetiver has routinely been used as a practical soil conservation technique. This would also be used to quantify the impact of vetiver on the composition (allocation to fractions) and vertical distribution of SOC in addition to total TOC stocks which provided a more complete assessment of its potential to sequester carbon in soil.

In the current study, results were estimated from MIR yet for TOC results were very similar to those reported in previous chapters when soil carbon values were expressed as TOC stock on an equivalent soil mass basis, vetiver was again more effective at increasing soil carbon concentration and stocks relative to coffee in most sites despite the spatial variability for the predicted OC stocks. Increases of OC stocks were found through the whole soil profile but significance of the increase under vetiver diminished with depth. This effect was strongest at the soil surface implying that vetiver root litter inputs are more likely contributed to the OC stocks in the sites (Dondini et al., 2009).

The allocation of SOC to its component fractions can offer an indication of potential vulnerability of organic carbon stocks to temporal change. Studies have shown that different management practices can cause differences in the amount of carbon sequestration potentials (Hutchinson et al., 2007; Sanderman et al., 2009), which equally could apply to the different soil carbon fractions and changes in the stocks of the different soil carbon fractions. We therefore, used MIR prediction to derive estimates of the contents and various compositions of SOC fractions (following (Janik et al., 2007; Zimmermann et al., 2007)). Vetiver in this study, showed a buildup of OC stock predominantly allocated to the humus organic carbon (HOC) fraction which is the more stable carbon fraction compared with in the most labile POC fraction. In this study we found 71, 20 and 9 % proportion accounted of the OC present in the HOC, POC and ROC, respectively while other studies have reported 56, 26 and 19% OC in the respective fractions in Australia (Baldock et al., 2013a; Baldock et al., 2013b). Hence, the more HOC suggests a more rapid accumulation and humification of vetiver material than under most systems. The accumulation information is therefore, suggesting that the large root decomposition of vetiver root material leads to the more HOC increase (rather than POC) and therefore, a loss of carbon through respiration resulting in the modest TOC increase. In this study, SOC was also less strongly associated with POC and ROC fractions, whereas Hobley et al. (2016),

reported a weaker association of HOC and ROC with TOC as climate, soil physical and chemical properties could be more important as explanatory variables. This study, has also demonstrated significant variations between sites in the proportions of carbon fractions, where the impact of vetiver was much higher in Ethiopian locations compared with Gunnedah, Australia which imply that plantation of vetiver grass needs to be encouraged in the land management decisions of the country.

Baldock et al. (2013b) proposed that the ratio of POC to HOC stocks could provide an indication of vulnerability with increasing ratios being indicative of a greater vulnerability given the more labile nature of the POC fraction. The ratio of POC to HOC stock in this study, showed a more limited vulnerability to change of OC due to the high proportion of HOC fraction under vetiver than the usual proportion given its less labile nature which could help the carbon stay in the soil for longer time and changes quit slowly. On the other hand, the significant variations in the stocks of carbon fractions could suggest rapid change of the labile POM to the more stable HOM fraction in all study locations and vegetation types but POC:HOC was lower for vetiver which resulted in a more rapid root decomposition. The variation in the amount and stocks of carbon in biological forms could occur due to the soil and environmental factors and farming practices in the area.

According to Hobley et al. (2016), depth is a key factor affecting the content of all three carbon fractions. Our study indicated that contents of carbon fractions, particularly POC and HOC stocks were influenced by soil depth. However, the proportion of OC allocated to the POC fraction decreased with increasing depth while the HOC fraction increased with increasing depth under all vegetation types. Similarly Dondini et al. (2009), demonstrated a higher SOC in different aggregates throughout the soil profile under *Miscanthus* a similar grass to that of vetiver. The vertical distribution is therefore, important because the carbon in the different fractions is differently susceptible and this could lead to different stabilities and vulnerability of soil carbon in soil carbon accounting schemes. In this study we used a deeper soil sampling depth (1.0 m) than the standard used for soil carbon inventory because of the deep root system of vetiver grass which indicated the importance of considering sampling of deeper soil profiles in soil carbon inventory especially for plant types having a deep root production potential (Baldock et al., 2013a; Baldock et al., 2013b).

The wide assumptions about tropical perennial grass species such as vetiver as a potential candidate for facilitating soil carbon sequestration is viable. Our results demonstrated that using vetiver as a land management option does indeed effect in accumulation of the more stable carbon fraction (HOC) to even the deeper soil depth profiles. Hence, growing vetiver could be a feasible strategy which has an implication for the high rate of stable carbon accumulation and could potentially be a potential climate change mitigation option. This can make vetiver a potentially sustainable option to improve soil health and could be a feasible scheme which could lead farmers especially those in the developing

nations to participate in the carbon accounting program. We therefore, suggest that countries especially in tropical regions to encourage and promote plantation of vetiver grass especially on degraded and marginal lands as potential option to facilitate carbon sequestration and environmental rehabilitation.

Recommendations for further research work

There are many reports highlighting the potential of tropical grasses in increasing SOC. However, in this work it has been demonstrated that vetiver grass has an important role in storing large TOC stock, has the potential to add new carbon despite high rates of turnover; produce high biomass and have high root to shoot decomposition which might be a reason for high turnover rates and larger organic carbon accumulation in the more resistant (hemic organic carbon fraction) carbon pool throughout the 1.0 m soil profile and has considerable potential for both restoration of soil health and for storing additional soil carbon to offset greenhouse gas emissions.

Nevertheless, further research work is needed to:

- Elucidate the impact of different factors (e.g. temperature, soil type, elevation) on SOC concentrations in soils at a landscape scale
- Further investigate the importance of cropland conversion to tropical perennial pastures as strategic option to improve soil health and soil carbon storage
- Determine biomass production of vetiver considering plant spacing and other effects (e.g. climate, competition...etc) under a field condition to test if the glasshouse experiment can be replicated under field conditions.
- Investigate the potential of vetiver as a biofuel plant due to its massive biomass production potential
- Determine the mechanism of carbon addition and turnover rates of different carbon fractions and develop models using fractionation and total carbon generated
- Assess the variations in SOC concentrations under pastures due to pasture management influencing carbon storage amounts
- Assess best management practices, site specific policies and technological options which can reverse and have positive effect on soil carbon storage are essential
- Continue advancing knowledge on the potential of tropical perennial grass/pasture species for carbon sequestration to influence land management decisions in tropical and sub-tropical regions of the world

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