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ORIGINAL PAPER

Soil Carbon Fractions under Vetiver Grass in Australia and Ethiopia Relative to other Land Uses

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Abstract

The allocation of soil organic carbon (SOC) to its component fractions can indicate the vulnerability of organic carbon stocks to change. The impact of vetiver on the composition and distribution of SOC can provide a complete assessment of its potential to sequester carbon in soil.

Purpose: This study quantified the distribution and impact of SOC under vetiver and the allocation of SOC to particulate (POC), humus (HOC) and resistant (ROC) fractions differentiated based on particle size and chemical composition under vetiver grass compared with other plant types.

Methods: Carbon fractions were measured on soil samples collected from Australia and Ethiopia to a depth of 1.0 m under three plant communities (vetiver, coffee, and Australian native pastures). We used the MIR/PLSR spectra to estimate SOC fractions based on fractionated, and NMR measured values.

Results: The stocks of SOC fractions indicated significant differences in the proportion of labile POC to HOC across sites and vegetation types. The dominant carbon fraction was HOC (71%) for all vegetation types. The average carbon sequestration rate under vetiver 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.

Conclusion: Growing vetiver and undisturbed native pastures has on average a high accumulation rate of a more stable carbon (HOC) which is less vulnerable to change, and change was largely driven by the HOC fraction. We, therefore, recommend the use and promotion of perennial tropical grasses like vetiver and similar grasses and undisturbed native pastures as potential options to facilitate soil carbon sequestration.

Keywords Coffee · Humus · Pastures · Particulates · Resistant · NMR · MIR · POC · HOC · ROC · SOC

1 Introduction

Soil organic carbon conservation and sequestration is important for soil health, and therefore food security and environmental quality (Tebeje 2020). 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 and Guggenberger 2008) and nutrient availability (Chaplot 2021; and Chaplot and Smith 2023). 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

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and thus influence the stabilisation and net accumulation of SOC (Kaiser and Guggenberger 2008).

A strategy to enhance 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 and Lavania 2009; Tessema et al. 2021). 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; Tessema et al. 2022). 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; Tessema et al. 2021). 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 and Lavania 2009; Tessema et al. 2022). 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 (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) is 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. 2013a).

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, b). 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 1 m) 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 in Ethiopia 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.

2 Materials and Methods

2.1 Study Sites and Soil Samples

Soil samples were collected from the Gunnedah Research Centre (GRC), New South Wales, Australia (Fig. 1), and from Southwest Ethiopia (Fig. 2). 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 and to determine if similar patterns emerged in the SOC distribution and form in these two contrasting environments. 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 Southwest Ethiopia where vetiver was first introduced and widely used for conservation purposes. However, despite its wide use, vetiver, especially in Ethiopia, has not been studied for its contribution to soil carbon sequestration.

Gunnedah Research Centre is in north-western New South Wales (Fig. 1) 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 (Table 1). The centre has summer dominant annual rainfall of 638 mm, with an average temperatures of 24.6 and 12.2 °C, respectively. Soils at Gunnedah are moderately deep to deep Ferrosols [(Australian Soil Classification (ASC), (Isbell 2016) (United States Department of Agriculture (USDA) equivalent Oxisols, World Resource Base (WRB) equivalent Ferralsols)] on upper foot slopes with deep to very deep black soils (Vertosol - ASC, Vertisol-USDA and WRB) on lower slopes. The soil sample site was previously covered with C_3 crops (such as wheat and oats), but at the time of sampling (2014) the area was covered with mixed tropical and native pastures. The

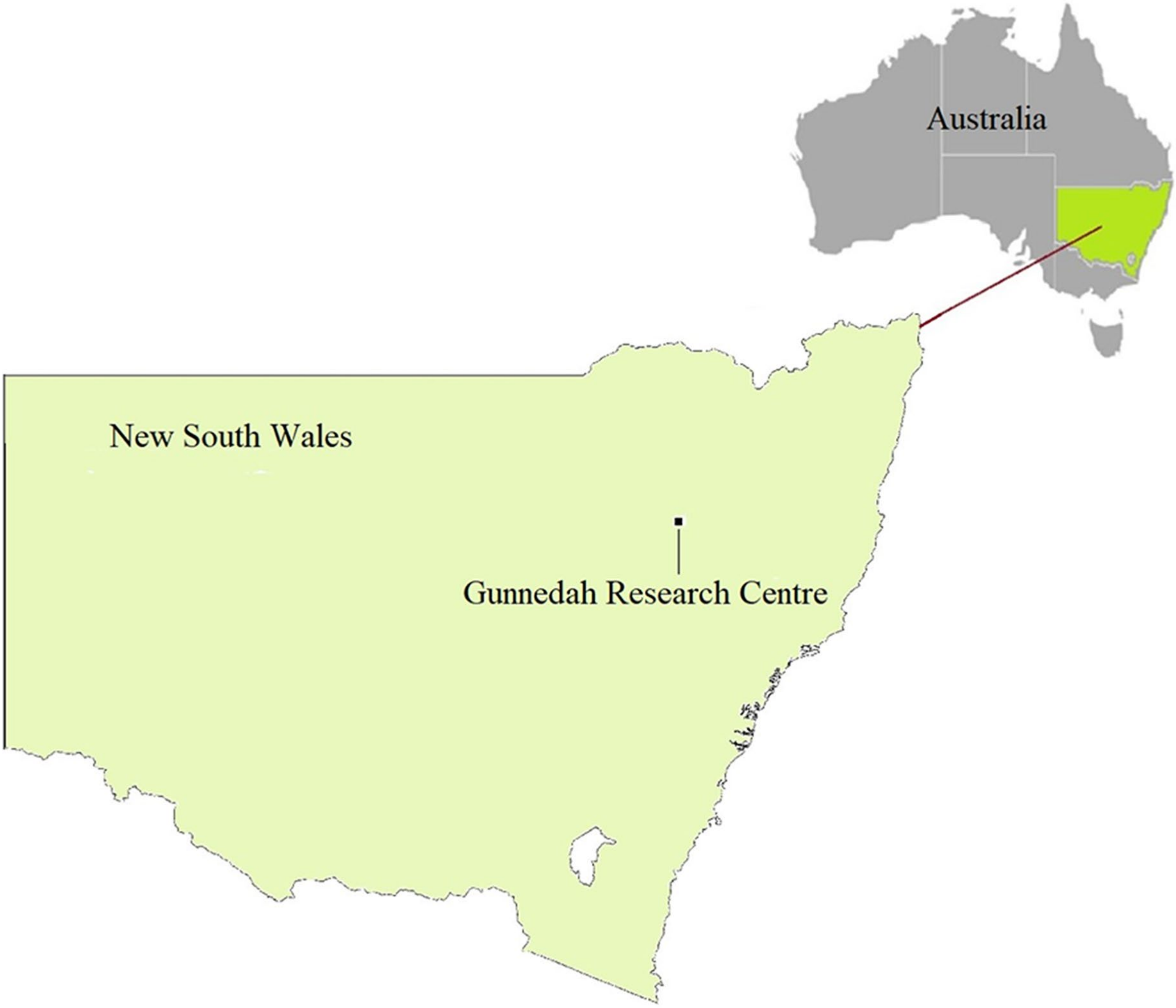

Fig. 1 Location map of Gunnedah research centre where soil samples taken from vetiver and native pastures, New South Wales, Australia

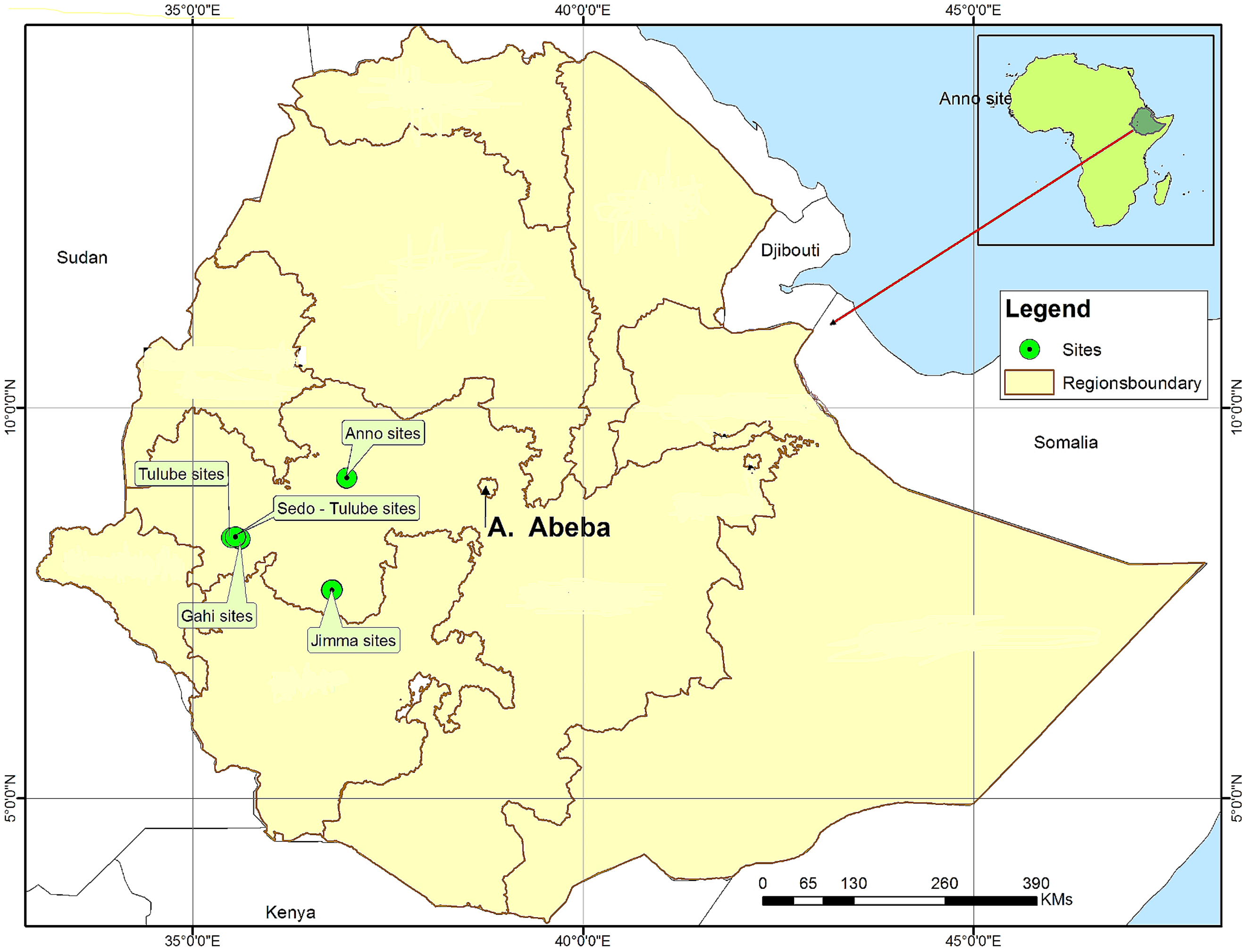

Fig. 2 Location map of the Anno, Jimma and Metu (Tulube and Ghi kebele's) sites in Ethiopia where the soil samples were taken from vetiver and coffee plantations

Table 1 Details of the study areas in Australia (Gunnedah) and Ethiopia (Jimma, Metu and Anno)

Site characteristics	Gunnedah	Anno	Jimma	Metu
Land hold/owned	Research centre	Private Agro-Industry	Public Research Centre	Farmers land
Location	31.03 °S and 150.27 °E	36° 35' 0.12"E 9° 3' 41.03"N	36° 46' 54.01"E 7° 40' 2.9"N	35° 19' 47.63"E 8° 11' 36.73"N
Altitude (m)	264	1881	1753	1669
Annual rainfall (mm)	638	1100	1561	1660–2200
Temperature (°c)	12.2–24.6	27 (max average)	9–28	12–27
Soil type	Ferrosols (upper foot) Vertosol (lower slope)	Nitosols	Eutric Nitosols	Leptosols, Nitosols
World Reference Base (WRB) Equivalent	Ferralsols - upper foot Vertisol - lower	Nitisol	Nitisol	Nitisol
Soil colour		Dark redish brown	Dark redish brown	Dark redish brown
pH	5–7	5–6	5–6	5–6
Cation exchange capacity (CEC)	--	Medium to high	Medium to high	Medium to high
Age of vetiver (years)	22	17	15	13
Purpose of vetiver plantation	Research	Conservation and income generation	Conservation and weed control	Conservation and income generation

three sample locations in Ethiopia (Anno, Jimma and Metu) had similar climates (mean annual rainfall and temperature) and Nitisols are dominant soil types to all three study areas and are equivalent to an Australian Ferrosol (Isbell 2016) (Table 1), 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 km^2 land area. The sites 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 (*Austro danthonia*), and windmill grass (*Chloris spp.*), both being established on sites previously under C_3 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. 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; Batjes 1998; Jobbágy and 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 (0–1 m) 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). A total of 499 (126 from Australia and 373 from Ethiopia) samples were collected. Each replicate sample for each soil depth were then bulked across plots to produce three composite samples and reduce the potential impact of spatial variability (Table 2).

2.2 Sample Preparation and Elemental Content Analyses

Sample preparation followed the procedures used by Baldock et al. (2013a and b). 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 ball mill) 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 1 M 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 pre-treated with 2% (by volume) phosphoric acid (H_3PO_4) 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 < 2 mm 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.

2.3 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 pre-processed as described by Baldock et al. (2013a). A principal component analysis (PCA) was applied to the acquired spectra to identify significant outliers and assess the extent of clustering of the soils by site or depth.

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.

Table 2 Number of total soil samples collected and composited for doil organic carbon (SOC) fractionation from Australia and Ethiopia

Country	Vegetation type		Number of all soils	Number of composite samples
Australia	Vetiver		63	21
	Native		63	21
		Sub total	126	42
Ethiopia	Vetiver		188	63
	Coffee		185	63
		Sub total	373	126
		Total samples	499	168

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 ($n = 12$).

Twelve soil samples were identified for full fractionation and then used to enhance the existing spectral database and the calibration. The 12 soils were fractionated according to Baldock et al. (2013a and b). Subsamples of < 2 mm 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 fraction (> 50 µm) 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 < 2 mm 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 MIR/PLSR predicted values of OC, POC, HOC and ROC contents used in subsequent analyses.

2.4 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 (Eqs. 1 and 2) using a non-linear least squares regression (NLS) procedure using an exponential decay model (Eq. 1) (where: a = values of the intercept, b = the slope (b > 0), x = soil depth, y = carbon).

$$y = a \times e^{-bx} \tag{1}$$

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 and Hauser 2013). Therefore, equivalent mass corrected for BD (mass per unit volume) 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 (%) (Eq. 2).

$$\begin{gathered} SOC\ Stocks\ (Mg\ C\ ha^{-1}) = \\ Soil\ layer\ (cm)\ X\ BD\ \left(g\ cm^{-3}\right)\ X\ C\ (\%) \end{gathered} \tag{2}$$

The relative carbon (OCs, POC, HOC and ROC) sequestration rates of vetiver grass was then calculated by dividing the carbon stock difference between vetiver and the coffee/native pasture (Eq. 3).

$$Relative\ Carbon\ Sequestration\ Rate\ = \frac{OC_{vetiver} - OC_{coffee/native}}{Age\ of\ Vetiver} \tag{3}$$

3 Results

3.1 Measured OC Content

Analysis of variance indicated that depth influenced soil carbon content across all sites ($P < 0.001$) (Fig. 3). The depth effect was indicated by a consistent decline in SOC content 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 changed with depth.

3.2 Mid-infrared (MIR) Spectra and PCA Analyses

The mid-infrared (MIR) spectra acquired for all samples are presented in Fig. 4. The MIR spectra showed significant signal intensity associated with organic materials e.g. CH_2 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 and Parthasarathy 2010) and carbonate signals are present at 2500 cm^{-1} (Bruckman and Wriessnig 2013) and are stable there. The other, much bigger signal of carbonates, which influenced neighbouring bands distinctly, appears at ca. 1400 cm^{-1}, and a further carbonate band appears at ca. 870 cm^{-1}. The spectra of the pure carbonate also show signals at 2900 and 2850 cm^{-1} (where the stretching signals of the aliphatic hydrocarbons are). Therefore, the aliphatic bands in the region of stretching vibrations are alternated by carbonates as well.

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 (Fig. 5a). 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 (Fig. 4b). All the carbonate soils (from Gunnedah) plotted towards the left on PC1 (Fig. 4c). The selection of samples for inclusion in the fractionation exercise of this study using the Kennard Stone algorithm covered the diversity in MIR spectra (Fig. 4d).

3.3 Partial Least Squares Regression (PLSR) Analysis

The relationship between measured and PLSR predicted square root transformed values of OC, POC, HOC and ROC are presented in Fig. 6 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

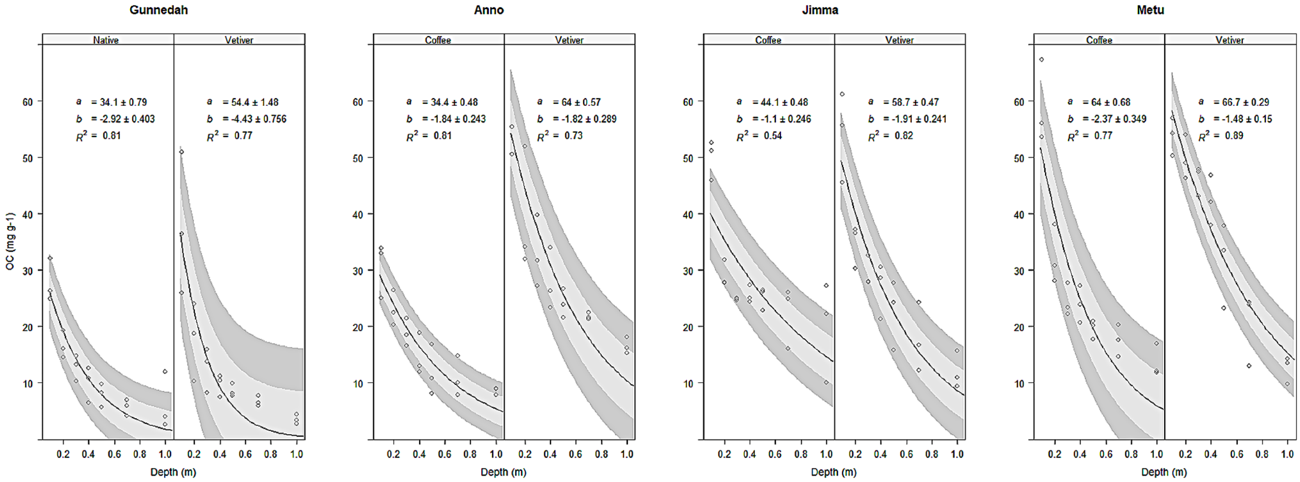

Fig. 3 Figure 3: Measured organic carbon (OC) content (mg per g) profile distribution under different plant types in Australia and Ethiopia. Plots show the raw data (o), a fitted exponential model (–) and confidence bands (1 and 2SE). Values of the intercept (a), slope (b) and Coefficient of determination (R^2).

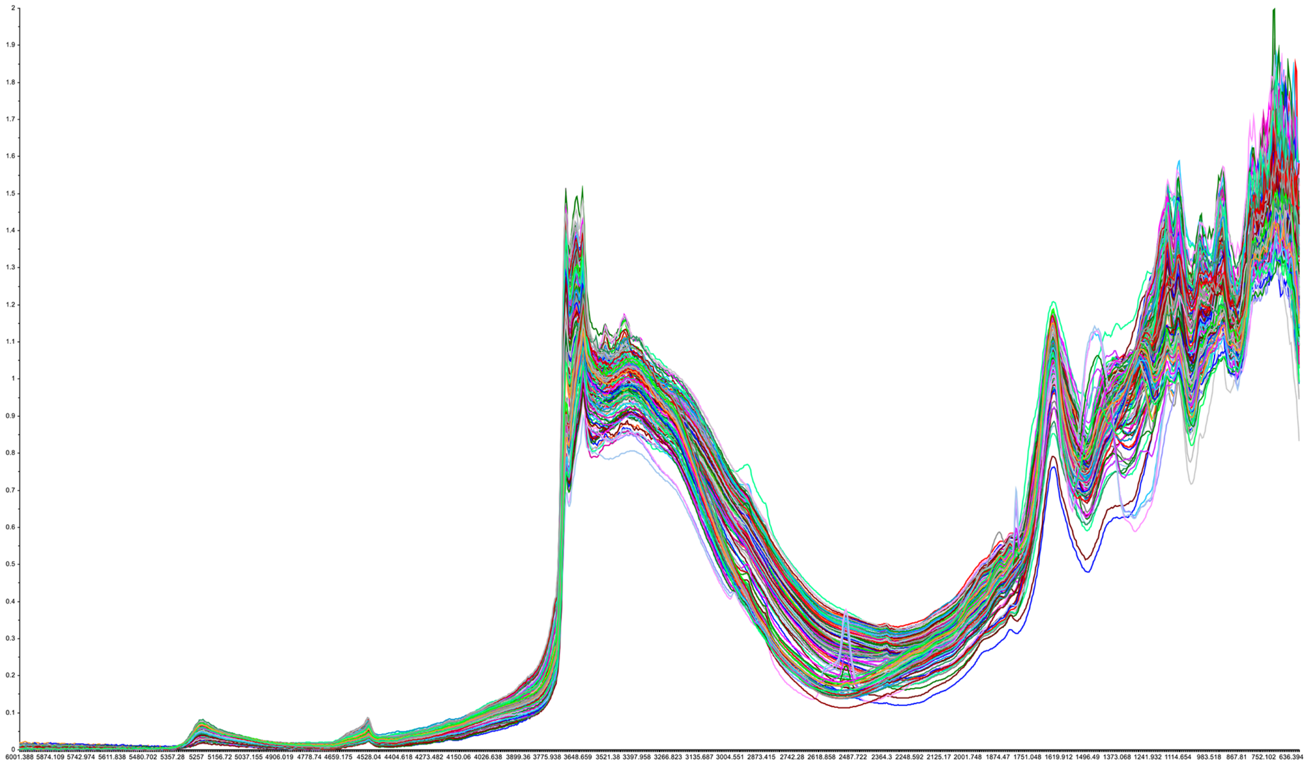

Fig. 4 Mid infrared (MIR) spectra acquired for all composite samples included in this study (Y-axis is Transmittance versus X-axis is wavelength (cm^{-1})

that all fractions were predicted reliably and with similar efficiency.

3.4 Carbon Stocks in Equivalent soil mass

3.4.1 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 (Fig. 7).

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

3.5.1 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 ($P < 0.001$) (Fig. 8) 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.

3.5.2 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 (Fig. 7). The difference was 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

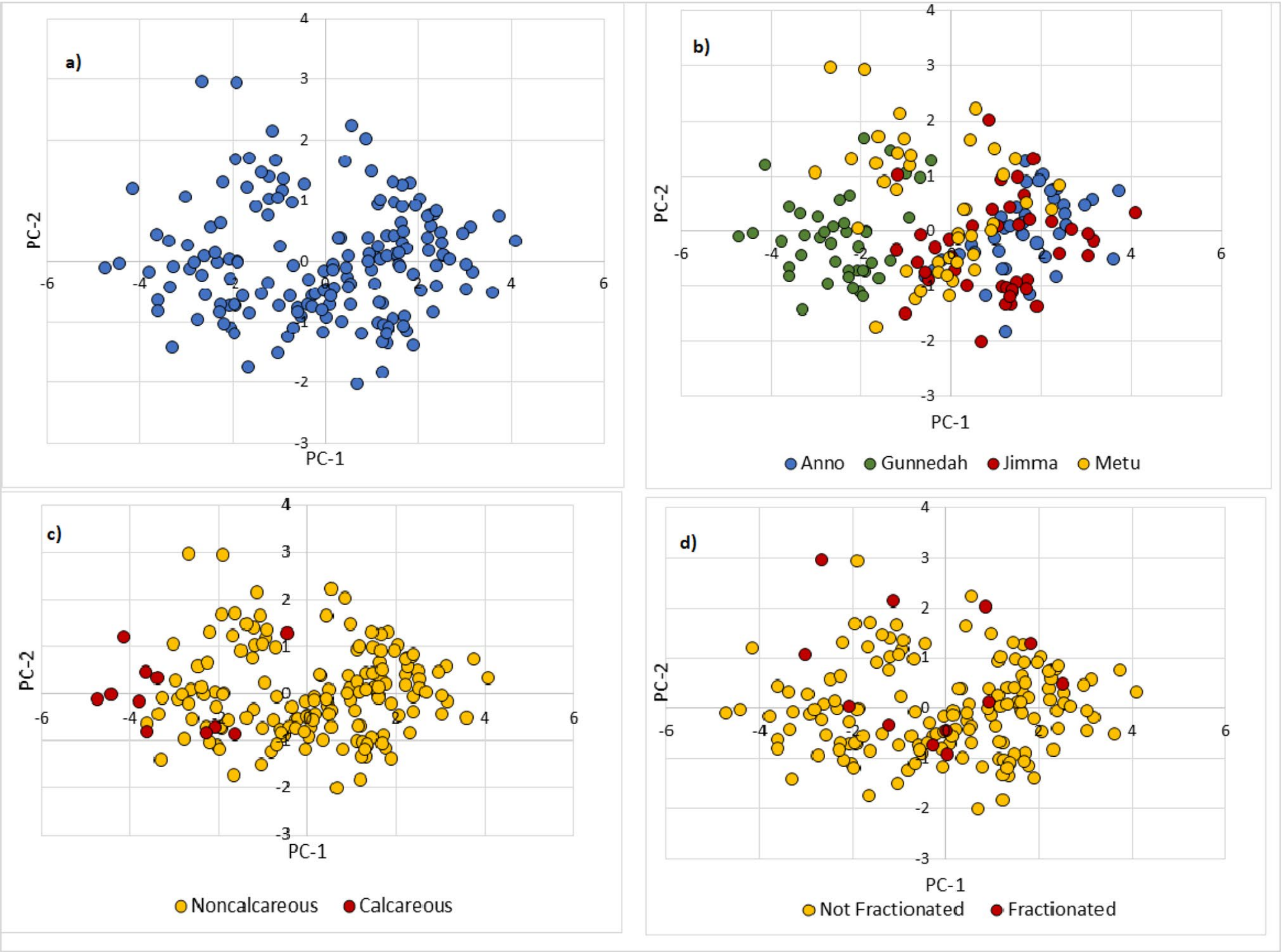

Fig. 5 The principal components analysis of the Mid infrared (MIR) spectra. No grouping applied (**a**), grouping by site (**b**), grouping by carbonate/non-carbonate (**c**) and grouping by included or excluded in the samples that were fractionated (**d**)

Metu ($P < 0.001$) sites. These effects represented 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.

3.5.3 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 (Fig. 8) indicating different depth profile characteristics between vegetation types.

3.5.4 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 (Fig. 7), indicating that the different depth

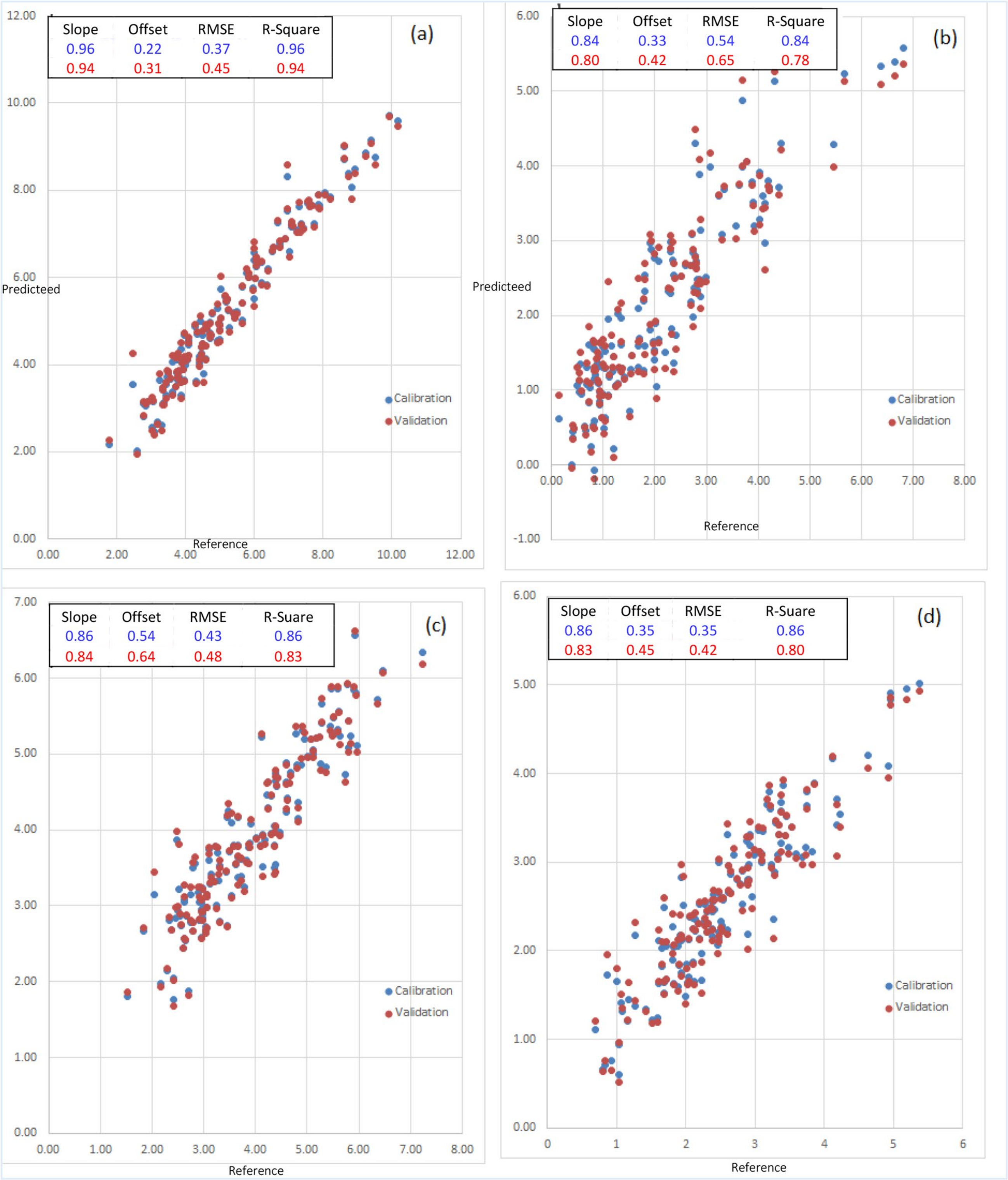

Fig. 6 Partial least squares regression (PLSR) derived prediction algorithms for the sqrtOC – organic carbon (**a**), sqrtPOC – particulate organic carbon (**b**), sqrtHOC – humic organic carbon (**c**) and sqrtROC – resistant organic carbon (**d**)

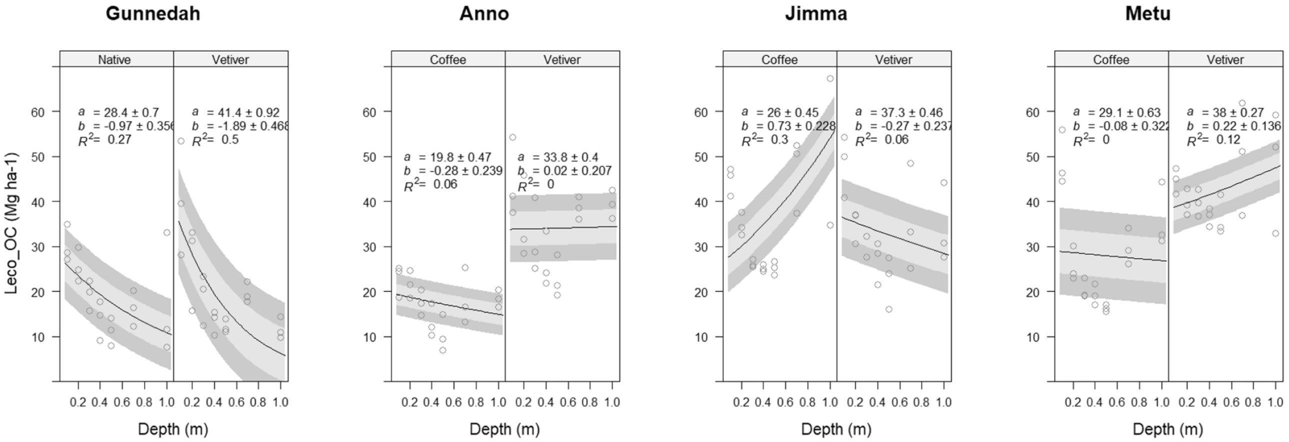

Fig. 7 The predicted stocks of soil organic carbon (OC) and soil profile distribution under the different plants in Australia and Ethiopia. Plots show the raw data (o), a fitted exponential model (–) and confidence bands (1 and 2 SE). Values of the intercept (a), the slope (b) and Coefficient of determination (R^2)

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.

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 3 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. Overall, 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 3).

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 4). 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.

Using Eq. 3 there was a difference in carbon sequestration rate between vetiver grass and the other adjacent plant types (Table 5). All carbon fractions at all sites, except Jimma, showed a gain of carbon under vetiver relative to the other plants. 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.

4 Discussion

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 production potentials (Dondini et al. 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. The statistically different OC 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

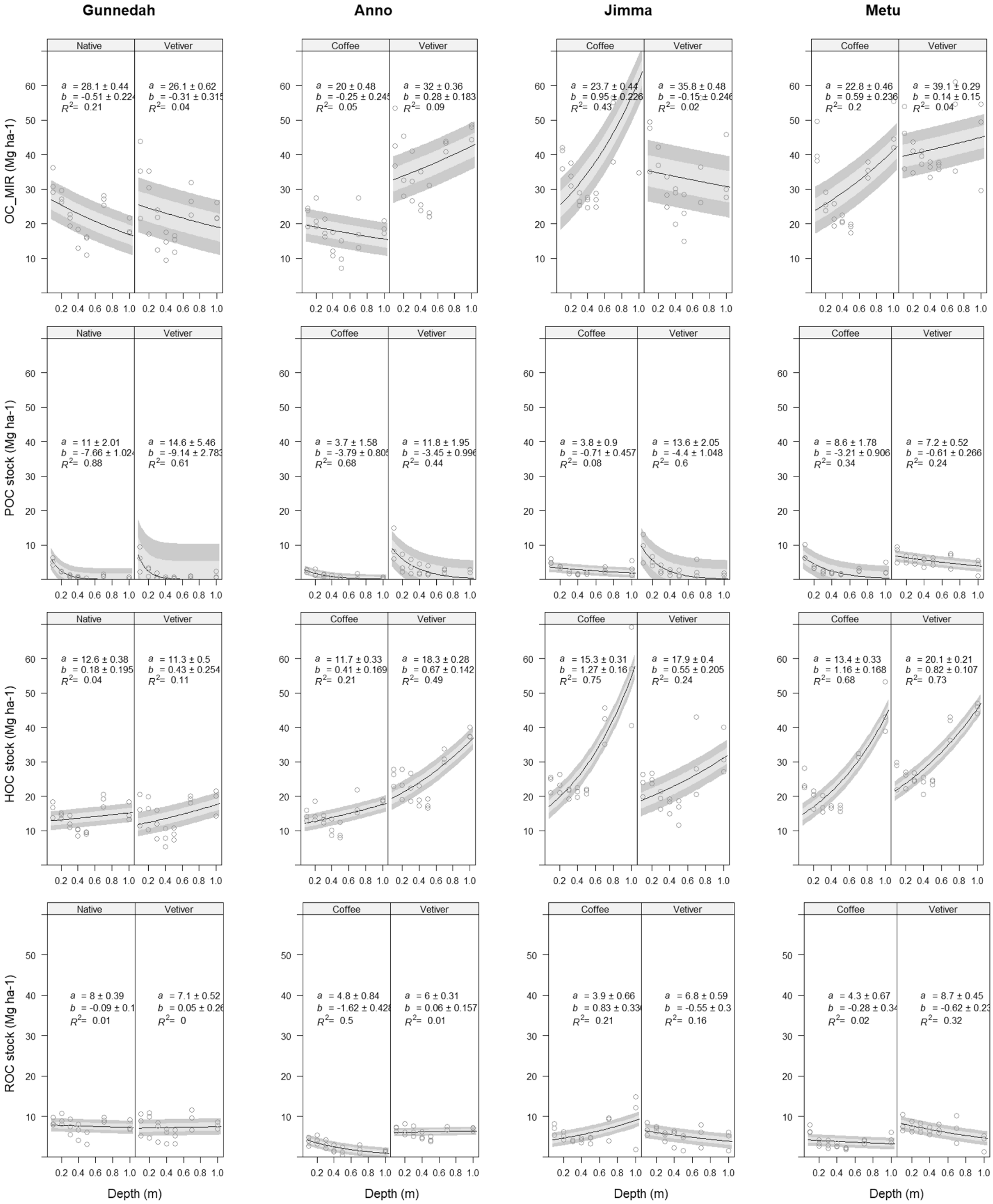

Fig. 8 The predicted stocks of soil organic carbons: organic carbon - OC (top), particulate organic carbon - POC (second from top), humic organic carbon - HOC (second from bottom) and resistant organic carbon - ROC (bottom) and profile distribution under the different plant types in Australia and Ethiopia. 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)

Table 3 Measured organic carbon (OC) and Mid infrared (MIR) spectroscopy predicted organic carbon (OC), particulate organic carbon (POC), humic organic carbon (HOC) and resistant organic carbon (ROC) stocks (mg ha^{-1}) in 1.0 m soil depth

Site	Vegetation	Measured (Mg ha^{-1})	MIR Predicted (Mg ha^{-1})				Vulnerability POC: HOC
		Leco_OC	OC	POC	HOC	ROC	
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
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
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

Table 4 Proportion of organic carbon (OC) stored in the form of particulate organic carbon (POC), humic organic carbon (HOC) and resistant organic carbon (ROC) organic carbon under different vegetation types in Australia and Ethiopia

Site	Vegetation	OC proportion in each fraction (%)		
		POC	HOC	ROC
Gunnedah	Native grass	7.0	60.8	34.2
	Vetiver	7.5	61.6	32.1
Anno	Coffee	5.6	80.0	14.4
	Vetiver	10.5	69.5	16.8
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

Table 5 Soil organic carbon (OC) carbon fractions: particulate organic carbon (POC), humic organic carbon (HOC) and resistant organic carbon (ROC) sequestration potential of vetiver grass relative to coffee and Australian native grasses

Sites	Carbon Sequestration (Mg ha^{-1} yr^{-1})				
	Measured OC	Predicted OC	POC	HOC	ROC
Gunnedah	0.40	0.07	0.04	0.07	-0.12
Anno	6.83	7.69	1.17	4.64	1.51
Jimma	-2.32	-2.64	0.39	-3.36	-0.35
Metu	6.57	5.23	1.1	2.63	1.34

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 more effective at increasing soil carbon concentration and stocks in Ethiopia relative to coffee in all sites except at Jimma. However, vetiver and coffee at Jimma and the native pasture at Gunnadah showed no difference which could be due to the similar establishment time and soil conditions. 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). Our results, therefore, confirm that using vetiver 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.

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. 2006). 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 et al. 2013a). 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. Humus organic carbon 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 occurr due to the soils and environmental factors 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 are 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 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 a longer time.

For the vertical distribution of the 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, shown 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.

Overall, our study showed that the dominant fraction in all sites (Australia and Ethiopia) was the humus organic carbon (HOC) for all vegetation types (vetiver, native pasture and coffee plantations) which is a 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) under 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. 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.

5 Conclusion

In conclusion, vetiver has proven to be a valuable asset in accumulating stable soil organic carbon fractions, notably humus organic carbon (HOC), which exhibits long-term stability. Across sites in Australia and Ethiopia, vetiver showcased a superior capacity for HOC accumulation compared to particulate organic carbon (POC) and resistant organic carbon (ROC) fractions. This suggests its potential for enduring carbon sequestration. Our study also reveals that vetiver plantation leads to slower carbon turnover, with measured and predicted organic carbon levels falling within defined ranges across locations. The predominance of HOC in vetiver underlines its suitability for sustained carbon storage, particularly in deeper soil profiles. This emphasizes vetiver's potential role as a sustainable solution for soil health improvement and engagement in carbon accounting initiatives such as the emerging voluntary carbon markets, particularly in developing regions like Africa. We therefore recommend the plantation of perennial grasses like vetiver and native pastures as a viable strategy for soil carbon sequestration in accordance with prompting further exploration and application of advanced carbon measurement techniques in diverse agro ecologies and land use systems.

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