

Biochemical proxies indicate differences in soil C cycling induced by long-term tillage and residue management in a tropical agroecosystem

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Abstract

Background & aim A potential benefit of conservation agriculture (CA) is soil organic carbon (SOC) accrual, yet recent studies indicate limited or no impact of CA on total SOC in tropical agroecosystems. We evaluated biochemical indicators of soil C cycling after 9 years (18 seasons) of contrasting tillage with and without maize residue retention in western Kenya.

Methods Potential activities of C-cycling enzymes (β -glucosidase, GLU; β -galactosidase, GAL; glucosaminidase, GLM; cellobiohydrolase, CEL), permanganate-oxidizable C (POXC), and soil organic matter (SOM) composition (by infrared spectroscopy) were measured.

Results POXC tended to be greater under reduced tillage and residue retention, but did not significantly differ among treatments ($\leq 2\%$ of SOC). Despite no significant differences in SOC concentrations or stocks, activities of all 4 C-cycling enzymes responded strongly to tillage, and to a lesser extent to residue management. Activities of GLU, GAL, and GLM were greatest under the combination of reduced tillage and residue retention relative to other treatments. Reduced tillage produced an enrichment in carboxyl C = O (+6%) and decreased polysaccharide C-O (-3.5%) relative to conventional tillage irrespective of residue management.

Conclusions Though enzyme activities and POXC are typically associated with SOC accrual, changes in soil C cycling at this site have not translated into significant

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differences in SOC after 9 years. Elevated enzyme activities may have offset potential SOC accumulation under CA. However, the ratio of C-cycling enzyme activities to SOC was higher under reduced tillage and residue retention relative to other treatments, indicating that stoichiometric scaling of SOC and enzyme activities does not explain absence of significant differences in SOC among tillage and residue managements. Potential factors that may explain the low SOC accrual rates in this tropical agroecosystem included the low, albeit realistic, levels of residue retention, nutrient limitations, and high temperatures favoring decomposition.

Keywords Conservation agriculture · Soil organic carbon · Enzyme activities · Glucosidase · Kenya · Tillage · Residue

Introduction

Conservation agriculture (CA) is aimed at improving soil and water conservation, soil health, and long-term crop productivity (Andersson and Giller 2012; Hobbs et al. 2008; Powlson et al. 2016). The combined use of reduced tillage, maintenance of soil cover, and crop rotation under CA has been reported to promote soil organic carbon (SOC) accrual (Brouder and Gomez-Macpherson 2014; Lal 2015; Palm et al. 2014). Such increases in soil C stocks under reduced tillage have been attributed to reduced soil mixing, aeration and slower turnover of SOC that is physically protected in soil aggregates (Chivenge et al. 2007; Janzen 2006; Six et al. 2000, 2002), thus leading to an increase in SOC levels, provided similar rates of organic matter (OM) inputs are maintained. However, recent meta-analyses have pointed out that long-term field studies comparing SOC stocks in CA and conventional tillage show variable results, ranging from increases, to no effect, to decreases in SOC (Gill et al. 2015; Palm et al. 2014; Powlson et al. 2016). Understanding the relative importance of different drivers (e.g. soil type, climate, tillage intensity, quantity and quality of OM inputs), and how they affect biochemical processes underpinning soil C decomposition, requires further study to explain this variation in response (Govaerts et al. 2009; Ogle et al. 2005). This is particularly the case in tropical agroecosystems for which there is a relative lack of studies on CA as well as drivers of C turnover (Batjes and Sombroek 1997; Ogle et al. 2005; Palm et al. 2014). In such systems, where there is a strong

need for cropping practices that adequately conserve soils and maintain productivity, the potential of CA to enhance SOC as compared to conventional tillage systems has been questioned (Cheesman et al. 2016; Giller et al. 2009; Govaerts et al. 2009; Palm et al. 2014; Powlson et al. 2016). No or limited effect of CA on SOC has been attributed to high OM decomposition rates (Ogle et al. 2005; Palm et al. 2014; Powlson et al. 2016; Scopel et al. 2013), as well as relatively low levels of residue input (Paul et al. 2013, 2015) that may limit CA induced SOC accrual as compared to temperate climates.

Enzyme activities represent potential rates of OM transformations (Burns et al. 2013; Tabatabai 2003). Since the stabilization of OM additions into SOC is mediated by microbial processing (Cotrufo et al. 2013; Kallenbach et al. 2016), the activity of enzymes that transform OM has been proposed to be a fundamental control on SOC accrual (Fontaine and Barot 2005; Lawrence et al. 2009; Schimel and Weintraub 2003; Sinsabaugh 2008). As a result, assays of soil enzyme activities can be used to help explain SOC response to managements such as tillage and OM inputs (Dick 1984; Stott et al. 2010). Potential activities of C-degrading enzymes have been shown to be more sensitive to management impacts than total SOC (e.g., Bergstrom et al. 1998; Marinari et al. 2006; Roldán et al. 2005; Sotomayor-Ramírez et al. 2009). Assaying a suite of enzymes involved in multiple points of decomposition pathways (e.g., cellulose to glucose) and diverse OM components (e.g., cellulose and chitin) can provide comprehensive understanding of C cycling (de la Paz Jimenez et al. 2002) which may help detect and explain management effects on total SOC. For example, cellobiohydrolase (CEL) facilitates the first step in degradation of cellulose, a prominent input to soils present in crop residues (Bandick and Dick 1999). Following depolymerization of cellulose, glucosidase (GLU) and galactosidase (GAL) hydrolyze the resulting oligosaccharides into low molecular weight sugars. Glucosaminidase (GLM) is involved in degradation of chitin, a compound present in fungal biomass, and is sensitive to management practices such as tillage that influence fungal populations (Ekenler and Tabatabai 2003; Mbutia et al. 2015). Despite the potential of C-cycling enzymes to elucidate CA effects on soil C cycling, only one study on CA to-date has examined such enzymes (GLU, GLM) in a temperate agroecosystem in which strong

increases in SOC occurred under reduced tillage and residue retention (Mbuthia et al. 2015).

Differences in soil enzyme activities are expected to be reflected by permanganate-oxidizable C (POXC), because POXC is thought to represent a microbially-processed, active C fraction – though not necessarily a labile one – and an early indicator of SOC accrual (Culman et al. 2012; Hurisso et al. 2016; Lucas and Weil 2012). POXC is strongly associated with changes in total SOC (Lucas and Weil 2012) and is sensitive to management practices that increase total SOC in the long-term, including tillage and OM additions (Culman et al. 2012; Hurisso et al. 2016; Quincke et al. 2007; Weil et al. 2003). In semi-arid regions, POXC was found to be more sensitive than total SOC to tillage management, and was increased under reduced tillage relative to conventional tillage (Melero et al. 2009). However, the response of POXC to tillage and residue components of CA has yet to be verified in tropical agroecosystems.

The activity of soil enzymes that drive soil C transformations could also impact the relative abundance of organic functional groups that constitute SOM (e.g., carboxyl C = O, ester C-O, aliphatic C-H). For example, decomposition of polysaccharides such as cellulose can result in decreased absorbance of polysaccharide C-O and greater relative absorbance of the oxidation product carboxyl C = O (Pérez et al. 2002). Shifts in SOM composition due to tillage and residue management can be characterized by diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy as relative changes in organic functional groups (Parikh et al. 2014).

The objective of this study was to evaluate the effect of tillage and residue management on biochemical indicators in a strongly weathered soil (Typic Rhodiudox) in western Kenya that could detect and explain differences in soil C cycling despite a lack of significant differences in total SOC after 9 years of CA practices (Margenot et al. 2017; Paul et al. 2013, 2015). This approach would potentially identify mechanisms explaining soil C response to CA in tropical agroecosystems. To this end, we quantified soil enzyme activities, POXC, and SOM functional group composition. We expected that biochemical indicators of C cycling would be more sensitive than total SOC to tillage and residue management and would provide us with a better understanding of potential changes in SOC accrual. Specifically, we hypothesized that relative to conventional tillage and residue removal, reduced tillage and residue retention

would increase POXC and elevate activities of C-cycling enzymes, which would be reflected in SOM functional group composition as enrichment of carboxyl C = O and a decrease in polysaccharide C-O.

Materials and methods

Site description

Our study was focused on a long-term CA trial in Nyabeda, western Kenya, which was established in March 2003 by the African Network for Soil Biology and Fertility (AfNet) and the International Center for Tropical Agriculture (CIAT). Extensive information on this field trial is provided by Paul et al. (2013, 2015). Briefly, the trial is located at 1320 m above sea level at 0° 7'46.96"N and 34°24'19.15"E, and experiences a mean annual temperature of 22.5 °C. Mean annual precipitation is 1727 mm (1997–2013), distributed bimodally over long rains (March–August) and short rains (September–January). The soil is a Typic Rhodiudox (WRB Ferralsol) with clay texture (663 g clay kg⁻¹, 151 g silt kg⁻¹, 167 g sand kg⁻¹) at 0–30 cm depth (Jelinski, unpublished).

The trial was established as a randomized block design with tillage and residue as main factors, each with two levels: conventional tillage (+T) or reduced tillage (-T), and residue retention (+R) or residue removal (-R). Each treatment occupied an individual plot (7 m × 4.5 m) across each of the four blocks. Maize (*Zea mays* L.) was cropped during the long rains followed by soybean (*Glycine max* L.) during the short rains. All plots were fertilized with 60 kg N ha⁻¹ as urea, 60 kg P ha⁻¹ as super triple phosphate (TSP), and 60 kg K ha⁻¹ as muriate of potash per growing season.

Under conventional tillage, the plots were prepared by hand hoeing to approximately 15 cm soil depth, mimicking practices used by smallholder farmers in this region (Kihara et al. 2011). Weeding was performed by hand hoe to 8 cm depth thrice per season. Under reduced tillage, the seedbed was prepared by hand hoeing to 3 cm, and hand pulling was used to weed thrice per season. Beginning in the 2009 long rainy period, herbicides (glyphosate and 2,4-dichlorophenoxyacetic acid) were applied to reduced tillage plots prior to planting, with subsequent weeding performed manually as described above. After harvest, maize residues were collected, dried, and

stored during the dry season (1 month). Within treatments with residue retention, chopped and dried maize residues were reapplied at a rate of 2 Mg ha⁻¹ immediately prior to soybean planting, and were incorporated by conventional tillage or remained on the soil surface as mulch under reduced tillage. Maize residue rates of 2 Mg ha⁻¹ achieve the minimum 30% soil surface coverage recommended by CA, and given maize yields in the region and competing demands on residue use (e.g., fodder), these rates are considered realistic maximums for western Kenya (Paul et al. 2013). Mean maize residue composition on an air-dried basis was 40.4% C, 0.36% N, 0.04% P, 0.98% K, with 0.51% polyphenols 4.0% lignin and 5.3% ash (Kihara et al. 2012).

Soil samples obtained by Paul et al. (2015) were used for additional measurements of POXC, enzyme activities, and DRIFTS. As described by Paul et al. (2015), soils were sampled 4 weeks before maize planting (prior to the long rain period) in year 9 (February 2012), corresponding to 18 cropping seasons since treatment implementation. For SOC, four soil subsamples per plot were sampled at 0–5, 5–

15 cm and 15–30 cm depths using 5 cm diameter cores to provide one composite sample per plot that was air-dried prior to analyses. Total SOC data from the same soil samples were published by Paul et al. (2015) and showed that there were no significant differences in total SOC concentrations nor stocks among tillage and residue treatments at the time of sampling, 9 years after trial establishment (Table 1). Values for SOC and bulk density at 0–15 cm depth were calculated based on values for 0–5 cm and 5–15 cm depths. For all other analyses, one composite soil sample per plot was sampled at 0–15 cm and 15–30 cm depth.

Permanganate-oxidizable C (POXC)

POXC was determined using the method of Weil et al. (2003) as modified by Culman et al. (2012). Briefly, duplicate air-dried and sieved (< 2 mm) 2.50 g soil samples were oxidized with 0.02 mol L⁻¹ KMnO₄ by 2 min shaking followed by 10 min incubation. Non-reduced Mn⁷⁺ was quantified by colorimetry (550 nm)

Table 1 Soil properties in year 9 (2012) of a conservation agriculture trial in western Kenya. Soil organic carbon (SOC) values are derived from measurements by Paul et al. (2015)

	SOC		SOC		C:N		pH	
	g kg ⁻¹		Mg ha ⁻¹				(0.01 M CaCl ₂)	
Depth 0–15 cm	mean	se	mean	se	mean	se	mean	se
-T - R	18.1	0.5 a	29.7	0.5 a	11.2	0.2 a	4.71	0.05 a
-T + R	20.2	1.2 a	32.6	2.0 a	11.3	0.0 a	4.77	0.05 a
+T - R	19.0	0.6 a	29.5	1.4 a	11.3	0.1 a	4.93	0.04 a
+T + R	19.2	0.2 a	29.2	0.9 a	11.3	0.1 a	4.92	0.04 a
Depth 15–30 cm								
-T - R	16.1	0.5 a	25.6	1.9 a	10.5	0.3 a	5.16	0.06 ab
-T + R	17.8	1.1 a	29.7	2.6 a	11.0	0.2 a	5.00	0.07 b
+T - R	18.1	0.5 a	30.0	1.0 a	11.0	0.2 a	5.09	0.04 ab
+T + R	18.0	0.4 a	29.6	1.6 a	11.2	0.2 a	5.27	0.03 a
Depth 0–30 cm								
-T - R	17.1	0.4 a	55.3	1.8 a	10.9	0.2 a	4.94	0.04 ab
-T + R	19.0	1.2 a	62.3	4.4 a	11.2	0.1 a	4.88	0.06 b
+T - R	18.5	0.5 a	59.5	2.1 a	11.2	0.1 a	5.01	0.03 ab
+T + R	18.6	0.1 a	58.8	2.3 a	11.2	0.1 a	5.10	0.03 a

Different letters indicate significant differences ($p < 0.05$)

se standard error, +T conventional tillage, -T reduced tillage, +R residue retention, -R residue removal

and POXC (mg kg^{-1} soil) was calculated as described by Culman et al. (2012).

Potential activities of C-cycling enzymes

To determine soil enzyme potential activities, a *para*-nitrophenyl linked substrate specific to an enzyme is incubated with soil in a buffered solution, and the release of *para*-nitrophenol (*p*NP) is determined colorimetrically to estimate the activity of soil enzyme. Potential activities were assessed for four C-cycling enzymes, β -glucosidase (Enzyme Commission 3.2.1.21; GLU), β -galactosidase (EC 3.2.1.23; GAL), glucosaminidase (EC 3.2.1; GLM), and cellobiohydrolase (EC 3.2.1.91; CEL) based on the method of Verchot and Borelli (2005), developed for weathered soils in western Kenya. Briefly, 1 g of air-dried soil (<2 mm) was incubated at 37 °C (1 h for GLU, GAL, GLM, and 2 h for CEL) in 50 mmol L⁻¹ acetate buffer (pH 5.0). While air-drying can affect soil enzyme activities in some regions (Sparling et al. 1986) others have reported to the contrary (Zornoza et al. 2006). Regardless of absolute effects, for a given soil type relative differences in enzyme activities among treatments are likely to persist after air-drying (Bandick and Dick 1999; Hinojosa et al. 2004). In regions with prolonged dry periods, such as western Kenya, air-drying is similar to conditions experienced by surface soils (Hinojosa et al. 2004). Soil enzymes such as GLU and GAL for which the majority of total activity is thought to be due to extracellular (matrix-bound) enzymes have been found to be least sensitive to air-drying (de Castro Lopes et al. 2015; Marando et al. 2012), including in weathered soils in the tropics (de Castro Lopes et al. 2015).

Assays were performed with a final substrate concentration of 10 mmol L⁻¹ per g soil for GLU, GAL, and GLM, and 5 mmol L⁻¹ per g soil for CEL. Triplicate negative controls (no soil) were included. Reactions were terminated with 4 mL of 0.1 mol L⁻¹ tris(hydroxymethyl)aminomethane (THAM, also referred to as Tris) buffer (pH 12.0), and 1 mL of 0.5 mol L⁻¹ CaCl₂. THAM buffer was used instead of sodium hydroxide (NaOH) to avoid potential abiotic cleavage of *p*NP-linked substrates (Turner and Haygarth 2005). Assays were centrifuged (2113 × RCF, 3 min) to remove sediment and *p*NP in

the supernatant was quantified colorimetrically using absorbance at 410 nm. Mean absorbance of the negative controls was subtracted from absorbance of soil assays. Potential activities of C-cycling enzymes were expressed on a soil basis ($\mu\text{mol g}^{-1} \text{h}^{-1}$) and normalized to SOC ($\mu\text{mol pNP g C}^{-1} \text{h}^{-1}$).

Diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy

Relative changes in SOM composition were evaluated by DRIFT spectroscopy using an approach previously applied for soils in western Kenya under differing tillage management (Verchot et al. 2011). Spectra were collected on neat soil samples (no KBr dilution) loaded into an aluminum well. Absorbance spectra were corrected against a solid aluminum blank in ambient air as the background using a Nicolet 6700 spectrometer (Thermo Scientific, Waltham, MA) with a deuterated triglycine sulfate (DTGS) detector and a diffuse reflectance accessory (Pike AutoDIFF, Pike Technologies, Madison, WI). Spectra were calculated as the mean of 400 scans across 4000–650 cm⁻¹ at 4 cm⁻¹ resolution. Triplicate spectra were collected on separate soil samples to account for potential heterogeneity in well packing (Terhoeven-Urselmans et al. 2010) and averaged to obtain a final spectrum for each soil.

To assess SOM composition, DRIFT spectra were evaluated for six absorbance bands used in previous studies to characterize SOM in weathered soils, including western Kenya (Lehmann et al. 2007; Verchot et al. 2011): phenol and/or alcohol O-H and amine N-H at 3433 cm⁻¹, aliphatic C-H at 2930 cm⁻¹, carboxylic C=O at 1780 cm⁻¹, aromatic C=C with possible ketone and/or quinone C=O and amide C=O contributions at 1625 cm⁻¹, aliphatic C-H at 1373 cm⁻¹, and polysaccharide C-O at 1018 cm⁻¹. Though absorbance at these bands includes mineral contributions, constant mineralogy across soil samples allows attribution of relative differences in absorbance to organic functional groups (Demyan et al. 2012; Margenot et al. 2016; Verchot et al. 2011). To quantify relative differences in band absorbance, for each spectrum the absorbance intensity of each of the six bands was normalized as the percent absorbance intensity of the sum of absorbance intensities of the six bands (Haberhauer et al. 1998; Verchot et al. 2011).

Statistical analyses

Two-way analysis of variance (ANOVA) was performed to evaluate tillage and residue treatment effects on soil variables, with two levels each for tillage (+/−) and residue retention (+/−) using Proc GLM in SAS v9.4 (Cary Institute, NC). First, an exploratory model was used to test for tillage × residue interactions ($y = \text{tillage residue block tillage} \times \text{residue}$). For variables that did not show a significant ($p < 0.05$) interaction of tillage and residue treatments, a readjusted model excluding this interaction was used. Soil measures were analyzed independently for two soil depths (0–15 cm and 15–30 cm). Linear correlation analysis (Pearson's correlation co-efficient, R) was used to evaluate relationships of C-cycling enzyme activities with POXC and total SOC using Proc CORR.

Results

Treatment effects on POXC

Tillage and residue management did not influence POXC concentration, nor the proportion of total SOC represented by POXC (Table 2). POXC was generally higher at 0–15 cm depth (mean 386 mg kg^{−1}) than at 15–30 cm depth (mean 306 mg kg^{−1}), and represented ≤2.1% of total SOC.

Treatment effects on C-cycling enzyme activities

Potential activities of all 4 C-cycling enzymes were impacted by tillage and residue management (Fig. 1). Enzyme activities responded more strongly to tillage than residue management, and were greater under reduced tillage compared to conventional tillage. The combination of reduced tillage and residue retention (−T + R) yielded similar or greater potential activities of C-cycling enzymes as other treatment combinations, and the combination of conventional tillage and residue removal (+T-R) did not necessarily result in the lowest enzyme activities.

Similar trends in treatment responses occurred at both depths for GLU, GAL and GLM, and though activities were higher at 0–15 cm depth, relative differences among treatments were more pronounced at 15–30 cm depth. At the 0–15 cm depth reduced tillage

Table 2 Soil permanganate-oxidizable carbon (POXC) in a 9-year conservation agriculture trial in western Kenya under conventional tillage (+T) and reduced tillage (−T), and residue retention (+R) and residue removal (−R)

	POXC			
	(mg kg ^{−1})		(% of SOC)	
	mean	se	mean	se
Depth 0–15 cm				
− T − R	371	27	2.06	0.19
− T + R	387	13	1.85	0.09
+ T − R	370	17	2.04	0.13
+ T + R	361	7	1.88	0.03
<i>p</i> -value				
Tillage	0.862	ns	0.977	ns
Residue	0.554	ns	0.182	ns
Tillage × Residue	0.464	ns	0.870	ns
Depth 15–30 cm				
− T − R	284	13	1.77	0.10
− T + R	348	20	1.96	0.11
+ T − R	306	34	1.69	0.17
+ T + R	286	22	1.59	0.12
<i>p</i> -value				
Tillage	0.427	ns	0.129	ns
Residue	0.400	ns	0.770	ns
Tillage × Residue	0.083	ns	0.660	ns

se standard error, SOC soil organic carbon, ns not significant at $p < 0.05$

increased potential activities of GLU by 21% ($p = 0.003$), GAL by 18% ($p = 0.002$), GLM by 24% ($p = 0.034$), and CEL by 47% ($p = 0.007$) relative to conventional tillage. Residue retention impacted enzyme activities depending on tillage management. In tandem with reduced tillage, residue retention stimulated enzyme activities of GAL by 21% and GLM by 26% compared to residue removal. Under conventional tillage, residue retention elevated CEL activity by 97% compared to residue removal.

The impact of tillage and residue management on C-cycling enzyme activities extended to 15–30 cm depth. Reduced tillage increased GLU by 27% ($p = 0.051$) and GLM by 52% ($p = 0.017$) relative to conventional tillage. Opposite to the trend at 0–15 cm depth, at 15–30 cm depth residue removal stimulated CEL by 43% compared to residue retention ($p = 0.021$) regardless of tillage ($T \times R p = 0.20$). When combined with reduced

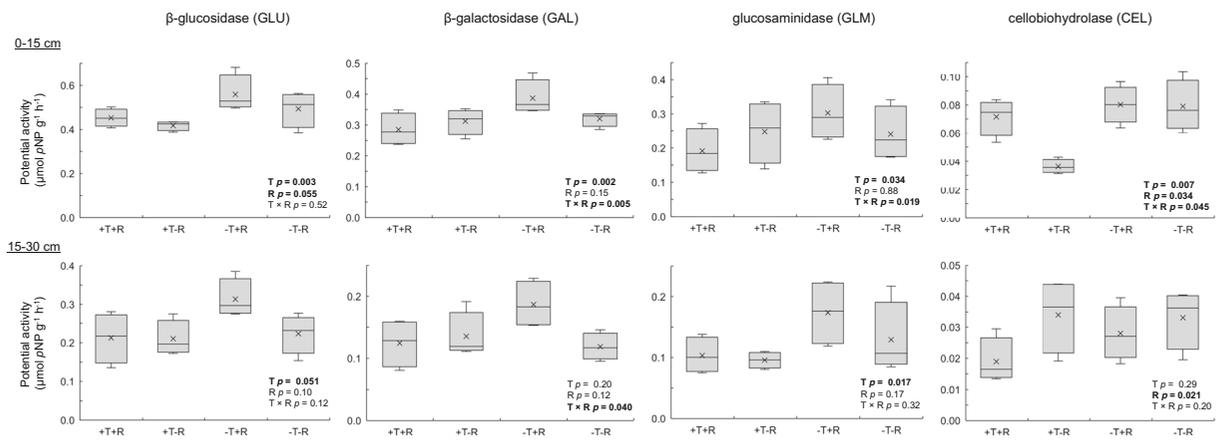


Fig. 1 Activities of C-cycling enzymes in a 9-year conservation agriculture trial in western Kenya under conventional tillage (+T) and reduced tillage (–T), and residue retention (+R) and residue removal (–R)

tillage, addition of residues also increased GLM activity by 57% ($T \times R p = 0.040$).

While POXC was positively correlated with total SOC ($R = 0.59$, $p = 0.0003$), potential activities of C-cycling enzymes were more strongly associated with POXC ($R = 0.75$ – 0.60) than with total SOC ($R = 0.60$ – 0.36 ; Table 3). When normalized to SOC ($\mu\text{mol pNP g C}^{-1} \text{ h}^{-1}$), potential activities of C-cycling enzymes expressed similar response to tillage and residue managements (Supplementary Fig. 1).

DRIFT spectroscopic characterization of SOM composition

Tillage, but not residue management alone, produced small but significant changes in the functional group composition of SOM (Table 4). At 0–15 cm depth, reduced tillage decreased polysaccharide C–O

(1018 cm^{-1}) by 3.5% and increased the relative absorbance of carboxyl C = O (1780 cm^{-1}) by 6%, as well as aliphatic C–H (2930 cm^{-1}) by 4%. Relative to conventional tillage, the proportion of phenol and/or alcohol O–H and amide N–H (3433 cm^{-1}) decreased by 1.6% under reduced tillage combined with residue retention at 0–15 cm depth, and by 2.3% under reduced tillage at 15–30 cm depth.

Discussion

POXC response to tillage and residue management

Our hypothesis that the combination of reduced tillage and residue retention would produce greatest mean POXC, especially at 0–15 cm depth, was not confirmed. Similar to total SOC, POXC tended to be higher under the treatment of reduced tillage with residue retention (CA), but did not differ significantly from other tillage and residue managements. According to previous studies, POXC can be an indicator of SOC accrual (Hurisso et al. 2016; Lucas and Weil 2012; Weil et al. 2003), and this was confirmed at this site, with no significant change in either C pool after 9 years of CA, suggesting little C accrual under any of the treatments.

POXC has been interpreted as a ‘processed’ C fraction based on its strong correlations with fine (53–250 μm) particulate organic C (POC) and heavy ($>1.7 \text{ g cm}^{-3}$) POC fractions across diverse cropping, climate and soil conditions in the USA (Culman et al. 2012), and also as an ‘active’ C fraction because it is strongly correlated with soil respiration and microbial

Table 3 Associations between potential activities of C-cycling enzymes and C fractions in soils (0–15 and 15–30 cm depths) in year 9 of a conservation agriculture trial in western Kenya

	POXC		total SOC	
	R	p	R	p
GLU	0.75	<0.0001	0.49	0.0042
GAL	0.75	<0.0001	0.60	0.0003
GLM	0.62	0.0002	0.45	0.010
CEL	0.60	0.0003	0.36	0.041

GLU β -glucosidase, GAL β -galactosidase, GLM glucosaminidase, CEL cellobiohydrolase, POXC permanganate-oxidizable carbon, SOC soil organic carbon

Table 4 Relative absorbance of organic functional groups assessed by diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy of soils in a 9-year conservation agriculturetrial in western Kenya. Spectra were collected on neat (no KBr dilution) samples. Significant differences ($p < 0.05$) determined by Tukey's test are indicated by *

	3433 cm ⁻¹		2930 cm ⁻¹		1780 cm ⁻¹		1625 cm ⁻¹		1373 cm ⁻¹		1018 cm ⁻¹	
	phenol, alcohol O-H, amide N-H		aliphatic C-H, stretch		carboxyl C = O		aromatic C = C, amide C = O		aliphatic C-H, bend		polysaccharide C-O	
	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se
Depth 0–15 cm												
– T – R	17.0	0.1	12.9	0.2	11.5	0.3	19.5	0.3	17.4	0.1	21.8	0.1
– T + R	16.7	0.1	13.0	0.3	11.6	0.4	19.3	0.3	17.3	0.1	22.1	0.4
+ T – R	16.9	0.1	12.5	0.1	10.9	0.2	19.5	0.1	17.3	0.1	22.8	0.3
+ T + R	17.1	0.1	12.4	0.2	10.8	0.3	19.7	0.1	17.3	0.1	22.7	0.3
<i>p</i> -value												
Tillage	0.150		0.035	*	0.034	*	0.256		0.787		0.022	*
Residue	0.783		1.000		0.898		0.859		0.677		0.638	
Tillage × Residue	0.046	*	0.641		0.725		0.327		0.944		0.521	
Depth 15–30 cm												
– T – R	17.1	0.2	13.0	0.5	11.4	0.7	19.0	0.4	17.0	0.2	22.4	0.8
– T + R	16.9	0.1	12.5	0.2	11.0	0.2	19.3	0.2	17.3	0.2	22.9	0.2
+ T – R	17.4	0.2	13.0	0.3	11.2	0.3	19.3	0.1	17.0	0.2	22.2	0.4
+ T + R	17.5	0.1	12.8	0.4	10.9	0.6	19.3	0.2	17.0	0.0	22.6	0.6
<i>p</i> -value												
Tillage	0.013	*	0.739		0.714		0.736		0.318		0.629	
Residue	0.755		0.316		0.505		0.576		0.473		0.375	
Tillage × Residue	0.280		0.696		0.866		0.607		0.29		0.91	

se standard error, *T* tillage, *R* residue

biomass (Weil et al. 2003) and manure decomposition (Tatzber et al. 2015). This apparent contradiction reflects the fact that decomposition is inherently associated with C loss through microbial respiration, but at the same time favors stabilization of C in soil as decomposition products (Cotrufo et al. 2013).

Though POXC did not significantly differ among treatments at our study site, reduced- or no-tillage and/or residue retention have been found to increase POXC in temperate and tropical agroecosystems (Awale et al. 2013; Carbonell-Bojollo et al. 2015; Culman et al. 2012; López-Garrido et al. 2014; Plaza-Bonilla et al. 2014), even without increases in total SOC (e.g., Melero et al. 2009). The positive correlation of POXC and C-cycling enzyme activities may reflect decomposition of OM by enzymes (Li et al. 2016). POXC has been found to positively associate with C and N mineralization, microbial biomass (Culman et al. 2012; Wade et al. 2016), and maize and vegetable yields across a diversity of

soils and climates (Culman et al. 2013; Huriisso et al. 2016).

Changes in SOM composition

Shifts in organic functional groups in SOM detected by DRIFT spectroscopy indicate tillage and residue effects on soil C cycling. Changes in relative absorbance of organic functional groups were statistically significant, but minor (<7%). However, even small changes in SOM composition can reflect alteration of soil C cycling (e.g., Erhagen et al. 2013; Haberhauer et al. 2000; Shi et al. 2006). Despite the semi-quantitative nature of DRIFT spectroscopy, the changes in relative absorbance of selected organic functional groups identify relative differences in SOM composition that are consistent with soil enzyme activities. Lower relative polysaccharide C-O and greater relative carboxyl C = O under reduced tillage are consistent with elevated activity of

polysaccharide-degrading enzymes (CEL, GLU) because oxidation of polysaccharides can be detected as increased absorbance of carboxyl C = O relative to polysaccharide C-O (Ernakovich et al. 2015; Pérez et al. 2002).

Relative enrichment in aliphatic C-H absorbance under reduced tillage, independent of residue management, has been reported previously (Ding et al. 2002; Veum et al. 2014). For example, in western Kenya, minor but significant enrichment in aliphatic C-H (+0.14%) was detected after 7 years of no-tillage relative to conventional tillage in coarse, but not fine-textured, soils (Verchot et al. 2011). The association of aliphatic C-H with POXC and total SOC could reflect contribution of microbial biomass and metabolic products enriched in aliphatic C-H (e.g., lipid bilayer) to SOC (Cotrufo et al. 2013; Grandy and Neff 2008; Mambelli et al. 2011; Throckmorton et al. 2015). As aliphatic C-H and POXC were positively correlated in this and other studies (Veum et al. 2014; Margenot et al. 2015), elevated aliphatic C-H but not POXC under reduced tillage at our site could mean that changes in SOM composition were insufficient to influence this active C fraction.

C-cycling enzyme response to CA practices

A stronger impact of tillage than residue management on enzyme activities may be explained by the low residue rates used in this study, because other studies have found changes in SOC under CA to be most strongly driven by OM input rates rather than by tillage (Jat et al. 2012; Mangalassery et al. 2015). In addition to providing a C source, surface residue retention moderates soil moisture and temperature conditions, which can increase microbial activity (Govaerts et al. 2007; Jat et al. 2012). Enzyme activities tended to show similar trends by treatments across enzyme types and depths, with elevated activity under reduced tillage and residue retention (CA). Though enzyme activities were lower, a stronger relative response of enzyme activities (GLU, GAL, GLM) to tillage and residue treatments at 15–30 cm depth suggests greater sensitivity of soil C cycling at subsurface depths to CA practices. CEL activities exhibited a unique depth-specific pattern, with low activity under reduced tillage and residue retention at 0–15 cm depth, yet higher activity at 15–30 cm depth. It is not clear why CEL activities exhibited this treatment- and depth-response.

Though bacteria and fungi produce enzymes in the cellulose degradation pathway (GLU, GAL, CEL), fungi have been proposed to be responsible for the majority of GLU (Rhee et al. 1987) and CEL (Baldrian and Šnajdr 2011; Kjølner and Struwe 2002) activities by secretion of these enzymes, in particular under no-tillage (van Capelle et al. 2012; Zuber and Villamil 2016). Fungi can further influence GLM indirectly because this enzyme degrades chitin derived from fungal biomass (Parham and Deng 2000). Positive associations of fungal abundance or biomass and GLM activity (Miller et al. 1998; Rodriguez-Kabana et al. 1983) may be enhanced under minimal soil disturbance (Jansa et al. 2003; van Capelle et al. 2012). Activity of GLM could potentially serve as an early indicator of SOC accrual, because chitin is more resistant to degradation in soils than cellulose and may contribute to greater SOC under reduced tillage compared to conventional tillage (Guggenberger et al. 1999; Nakas and Klein 1979; Schreiner et al. 2014; Six et al. 2006). At 0–15 cm depth, the activity of GAL was most sensitive to treatments and was more strongly correlated with total SOC than GLU ($R = 0.60$ versus 0.49 , respectively). Activity of GLU is often used as an indicator of soil C cycling because it is considered to be in equilibrium with SOC (i.e., GLU:SOC) in stable systems and tends to be strongly correlated with SOC (Stott et al. 2010). Equal or greater sensitivity of activities of other C-cycling enzymes to management practices that influence SOC underscores the importance of assaying activities of multiple enzymes.

In order to improve understanding of management impacts on soil enzyme activities and C cycling, future work should consider relationships between soil enzyme activities and genes encoding these enzymes (Trivedi et al. 2016). This approach can identify enzyme origins (e.g., fungal versus microbial; Lagos et al. 2016; Ragot et al. 2017) and how microbial community response to management may be implicated in changes in enzyme activity and soil C (Cui et al. 2015).

Soil C cycling response to CA

Differences in rates of C transformations represented by enzyme activities can be interpreted in two ways with respect to minor changes in SOC. *Sensu stricto*, elevated enzyme activities indicate higher potential rates of C turnover. This could antagonize SOC accrual if enzymatic transformation rates, a reflection of microbial C

demand, surpass C input rates. This may well be the case in this trial given the low, albeit realistic for western Kenya smallholders (Giller et al. 2009; Guto et al. 2012), rates (2 Mg ha^{-1}) of relatively low quality maize residues (high C:N). Trends in enzyme activities may reflect higher decomposition rates under reduced tillage and with residue retention. At the same time, elevated enzyme activities signify a greater potential for SOC accrual because microbial processing products are more favorably stabilized as SOC (Cotrufo et al. 2013; Grandy and Neff 2008; Mambelli et al. 2011; Throckmorton et al. 2015).

A second, compatible interpretation is that potential enzyme activities scale stoichiometrically with SOC content (Sinsabaugh 2008; Sinsabaugh et al. 2014; Sinsabaugh and Follstad Shah 2012). In this view, enzyme activities are a response to substrate availability, and therefore increase with an increase in C substrate. Even so, the reciprocal microbial-SOC interplay that is facilitated through enzyme activities means these activities can serve as sensitive indicators of changes in SOC (Green et al. 2007; Stott et al. 2010; Tian et al. 2010). Significant differences in GLU, GAL and GLM activities among treatments were positively associated with non-significant trends in POXC and SOC, in support of previous studies in temperate systems that potential activities of soil enzymes such as GLU can be early indicators of SOC accrual (de la Paz Jimenez et al. 2002; Stott et al. 2010).

However, similar trends in enzyme activities normalized to SOC ($\mu\text{mol } p\text{NP g C}^{-1} \text{ h}^{-1}$) among tillage and residue treatments as on a soil mass basis ($\mu\text{mol } p\text{NP g soil}^{-1} \text{ h}^{-1}$) suggests that enzyme activities did not simply scale with SOC. It has been proposed that a GLU:SOC ratio greater than $122 \mu\text{mol } p\text{NP g C}^{-1} \text{ h}^{-1}$ indicates SOC accrual, $122\text{--}72 \mu\text{mol } p\text{NP g C}^{-1} \text{ h}^{-1}$ indicates SOC equilibrium, and less than $72 \mu\text{mol } p\text{NP g C}^{-1} \text{ h}^{-1}$ indicates SOC loss (Stott et al. 2010). Based on this index, empirically developed for temperate systems, GLU:SOC at our tropical site would suggest SOC loss under all treatments. It is difficult to interpret the values of GLU:SOC based on the index proposed by Stott et al. (2010) given strong differences in soil and climate type, and because the lack of baseline activity of GLU (and other C-cycling enzymes) when the trial was established. In order to better ground understanding of soil enzyme activities as indicators of SOC dynamics, future research should examine changes in C-cycling enzyme activities and SOC over time in order to better

understand how changes in enzyme activities correspond to changes in SOC.

Observed differences in enzyme activities and SOM composition, but not total SOC, could be interpreted as changes in soil C transformations that have not translated to significant changes in total SOC. A possible and obvious explanation is that C inputs (residue rates) are too low. This mass balance approach is based on the concept of SOC as the difference between C inputs and outputs, and so SOC accrual requires that inputs exceed outputs (Janzen 2006; Kong et al. 2005). However, the effects of residue rates on SOC are not straightforward, in particular when residue quality and fertilization are considered. For example, minimum residue rates for maintaining SOC tend to be lessened by N fertilization (Lemke et al. 2010), and fertilizer N inputs at our site are considered sufficient for maize production (Kihara and Njoroge 2013).

An additional possibility is limitation of SOC accrual by non-C nutrient elements, conceptualized as the “inorganic nutrient cost” of SOM accrual (Richardson et al. 2014). In this view, limitation of soil microbes by N, P, and S leads to mineralization of SOM to access these nutrients, entailing C loss. Supplying these nutrients averts C mineralization, and can yield rapid increases in stable (fine-fraction) SOC and efficiency of C stabilization (Kirkby et al. 2013, 2016; Kirkby et al. 2014; Kirkegaard et al. 2014; Manzoni et al. 2012). This effect is especially aggravated by surface stratification of nutrients under reduced tillage, as observed for P at our site (Margenot et al. 2017), and in weathered soils with inherently low fertility (Kirkby et al. 2014). Background fertilization at our trial was based on regional recommendations (KARI 1994; Kihara and Njoroge 2013) of N, P, and K, but even then yielded low available P levels (Margenot et al. 2017) and did not include S and Ca despite their likely limitation (Kihara and Njoroge 2013). Soil nutrient constraints may thus contribute to the observed disparity between greater SOC accrual under CA in temperate versus tropical agroecosystems that is typically attributed to differences in climate-driven rates of OM decomposition (e.g., Powlson et al. 2016).

Conclusion

At a 9-year trial in western Kenya, tillage and residue treatments that yielded non-significant differences in

total SOC showed no significant differences in POXC. However, a significant response of soil enzyme activities as biochemical indicators of soil C cycling was detected. Greatest potential activities of enzymes occurred under the combination of reduced tillage and residue retention prescribed by CA, including the sequence of decomposition of cellulose to glucose. Consistent with elevated activities of C-cycling enzymes, SOM enrichment in carboxyl C = O and aliphatic C-H was detected for this treatment. Strong positive correlations of C-cycling enzyme activities with POXC and SOC suggest that potential enzyme activities can serve as indicators sensitive to minor differences in soil C in weathered soils in tropical climates. These results support the hypothesis that reduced tillage, and to a lesser extent residue retention (at relatively low, albeit regionally realistic, rates), induce differences in soil C cycling relative to conventional tillage and residue removal. Elevated enzyme activities may have offset soil C inputs, which would entail greater nutrient ‘functionality’ of SOC. Non-significant trends in SOC after 9 years of treatment indicate at best a very slow rate of SOC accrual, which may be explained by low residue quantity and/or nutrient limitation in combination with tropical climatic conditions of high humidity and temperature. Future research should quantify the extent to which these potential constraints (co-)limit SOC accrual in tropical agroecosystems on weathered soils so as to develop management solutions (e.g., residue retention rates, N and P availability) that best support long-term soil fertility and productivity in these systems.

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