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Enzymes and C pools as indicators of C build up in short-term conservation agriculture in a savanna ecosystem in Cambodia

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ABSTRACT

Soil organic carbon (SOC) pools, particularly labile pools, and soil enzymes are good indicators of short-term impacts of soil management. We hypothesized that labile SOC pools drives C accumulation and enzyme activity can be an efficient indicator for C build up in short-term conservation agriculture. The aims of this study were to quantify the impacts of tillage and crop rotations with diverse crop residue inputs on changes in SOC labile pools and enzymatic activities in rice-, soybean- and cassava-based cropping systems designated as RcCS, SbCS and CsCS, respectively. The four treatments in each cropping system consisted of: conventional tillage (CT), no-till in which main crops (rice, soybean and cassava) were grown in a one-year frequency pattern (NT1) and, no-till in which the main crops were grown in bi-annual rotations with maize (NT2 and NT3). After 5 years experiment period, greater hot-water extractable organic C (HWEO-C) stocks of 61%, 55% and 53%, and permanganate oxidizable C (POX-C) stocks of 23%, 21% and 32% were attributed to NT than those in CT soils under RcCS, SbCS and CsCS, respectively, at 0–5 cm soil layer. The pyrophosphate extractable organic C (PEO-C) and chemically stabilized organic C (CSO-C) stocks were almost constant in each depth among treatments, except 0–5 cm in CsCS. The β-glucosidase activity was 18%, 28% and 49% greater in NT than those in CT soils at 0–5 cm under RcCS, SbCS, CsCS, respectively. Arylsulfatase activity was 36% and 39% greater in NT than in CT under SbCS and CsCS, respectively but no significant differences in RcCS. A strong and positive correlation ($P < 0.001$) between β-glucosidase and arylsulfatase activity with POX-C, HWE-C, SOC and total N indicated how these variables were inter-related. Comparison among three NT treatments, bi-annual crop rotations showed a better increasing trend of HWEO-C, POX-C and enzymatic activities than those with one-year frequency pattern. In conclusion, short-term NT crop rotations with permanent soil cover significantly increased the storage of HWEO-C and POX-C and enhanced β-glucosidase and arylsulfatase activities at the surface soil layer as a result of higher biomass-C input and the absence of soil disturbance.

1. Introduction

Agricultural management practices play an important role in the soil C sequestration process. The development of annual upland crops in Cambodia (i.e., maize, cassava, soybean and mungbean) soared from 217,106 ha in 2003 to 716,370 ha in 2012 (MAFF, 2013). Forest clearance to expand the agricultural land to satisfy the needs of expanding population exacerbated growing concern over land

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degradation. The challenge to evaluate land productivity, to improve soil health and to sequester soil C is necessary to define sustainable agricultural practices in this country.

Soil organic C (SOC) plays a crucial role in enhancing crop productivity as a result of its impacts on soil physical, chemical and biological properties. Conventional plow-based tillage (CT) hastens SOC oxidation due to greater exposure to microbial oxidation (Green et al., 2007). This process results from the breakdown of soil aggregates and marked changes in soil environment (i.e. temperature, moisture and oxygen) (Tivet et al., 2013a). Thus, it increases soil microbial biomass activity (Guo et al., 2013) and causes a drastic increase in C efflux from soil to the atmosphere (Lal and Logan, 1995).

SOC can be enhanced by crop rotations and no-till (NT) practices due to addition of biomass-C input into the soil via crop residues near the soil surface (Lal et al., 2003). Conservation agriculture (CA) holds tremendous potential for sustainable land management through the application of its three key principles: continuous minimal mechanical soil disturbance (no-tillage), permanent organic soil cover, and diversified crop rotations grown in sequence or associations (FAO, 2008). The impacts of CA has been reviewed as an improved agricultural practice to potentially sequester C into the soils in various regions.

To assess SOC dynamics under tillage and crop rotation systems, several indicators of SOC pools and enzymatic activities have been used. The SOC pool is highly diverse with contrasting turnover times, and stabilized or protected again microbial decomposition (Lützow et al., 2006). Active or labile pools exhibit the most rapid turnover times, being more sensitive to land use changes than total SOC (Campbell et al., 1997; Huang et al., 2008). For example, hot-water extractable organic C (HWEO-C) is a sensitive indicator of SOC quality and constitutes the readily-decomposable SOM (Ghani et al., 2003). Similarly, permanganate oxidizable carbon (POX-C) is an active SOC pool and is correlated to soil microbial activity including soil microbial biomass C (MBC), soluble carbohydrate C and total C (Weil et al., 2003).

Soil enzymes play a substantial role in organic matter mineralization through a wide range of metabolic processes (María et al., 2002) and their activities are sensors of SOM decomposition in the soil system by providing information about microbial status and soil physicochemical conditions (Sinsabaugh et al., 2008). NT high residue returns and crop rotations have been shown to enhance enzymatic activities. Soil enzymes such as Arylsulfatase and ß-glycosidase respond to soil management changes more quickly than other soil quality indicator changes and are detectable (Dick, 1994; Ndiaye et al., 2000). Balota et al. (2004) reported that under long-term experiment amylase, cellulase, acid phosphatase, alkaline phosphatase and arylsulfatase increased up to 68%, 90%, 46%, 61%, and 219% respectively in the surface layer of an Oxisol under NT cropping system in comparison to conventional tillage. Previous studies using enzymes such as dehidrogenase, cellulase and urease assays reported close relationship with soil physical and chemical properties (Vasconcellos et al., 2013), cellulase and ligninase with SOC dynamics (Cenini et al., 2015; Chen et al., 2017). Inagaki et al. (2016) reported that arylsulfatase and beta-glucosidase had a similar performance to indicate the SOC pool alterations (especially labile fractions) in a highly weathered Oxisol. Recently, Medeiros et al. (2017) reported that enzyme such as arylsulfatase, acid phosphatase and urease had a similar performance to identify the natural regenaration of the vegetation and the close relationship with C dynamic in a tropical region in northest Brazil.

At tropical temperatures, SOM is broken down ten times faster, allowing for more rapid biomass growth but resulting in a smaller soil carbon pool compared with temperate climate (Malhi et al., 1999). Short-term changes in total SOC due to soil management practices are often difficult to detect (Zotarelli et al., 2007). Recently, Hok et al. (2015) reported the importance of particulate organic C as an indicator of C storage in short-term CA. However, the short-term effects of CA on labile SOC pools like HWEO-C and POX-C and soil enzymatic activities remain debatable. We hypothesize that the combination of SOC pools and enzymatic activities can provide valuable information to assess the short-term SOC dynamics and the estimation over longer-term trends. Thus, this study aimed to quantify the impacts of short-term CA with diverse biomass-C inputs on changes in labile C (POX-C and HWEO-C) and soil enzymatic activities (β-glucosidase and arylsulfatase) in rice-, soybean- and cassava-based cropping systems.

2. Materials and methods

2.1. Site location and general description

The field experiments were initiated in 2009 and conducted in a Latosol equivalent to Oxisols in USDA-Soil Taxonomy or Ferralsols in FAO-Soil Classification (Crocker, 1962; Kubota, 2005) at Boskhnor Research Station in Kampong Cham Province, Cambodia (Latitude 12°12′30″N, longitude 105°19′7″E and 118 m elevation). The adjacent reference vegetation (RV) was located about 0.5 km from the experimental plots. Detailed descriptions of the site, experiments, below ground biomass-C estimation and soil sampling are reported in Hok et al. (2015).

The experiments distinctly comprised of (i) rice- (ii) soybean- and (iii) cassava-based cropping systems. The three-replicated experimental plots were laid out in randomized complete block design with four treatments (e.g., soil management systems) in each cropping system consisting of (i) conventional plow-based tillage systems (CT) where the main crops were planted in annual succession for rice and soybean (i.e., mungbean/rice–CT-R, sesame/soybean–CT-S) and mono-cropping for cassava (CT-C); (ii) no-till (NT) based systems where main crops were planted in a one year frequency pattern (NT1-R, NT1-S, NT1-C); and (iii) and (iv) NT based systems where main crops were planted in bi-annual rotations with maize, the two plots in these bi-annual rotations being NT2-R, NT3-R for rice, NT2-S, NT3-S for soybean and NT2-C, NT3-C for cassava. Additional information about the crop sequences in each treatment are reported in Hok et al. (2015). Details of the biomass-C input in the 5-year experiment period are presented in Table 1.

Basal P fertilizer application was done by surface banding with thermo phosphate (i.e., 16% P_2O_5, 31% CaO and 16% MgO), and fractioned top dressing on main crops for N and K, using urea (46% N) and potassium chloride (60% K_2O), respectively. The total fertilizer input (2009–2013) was 208 kg ha^{-1} P_2O_5, 253 kg ha^{-1} N, 180 kg ha^{-1} K_2O_5 for rice, 208 kg ha^{-1} P_2O_5, 115 kg ha^{-1} N, 300 kg ha^{-1} K_2O_5 for soybean, 208 kg ha^{-1} P_2O_5, 368 kg ha^{-1} N, 330 kg ha^{-1} K_2O_5 for cassava, and 208 kg ha^{-1} P_2O_5, 368 kg ha^{-1} N, 180 kg ha^{-1} K_2O_5 for maize.

Soil samples at seven depths (0–5, 5–10, 10–20, 20–40, 40–60, 60–80, and 80–100 cm) were collected during November 2011 and 2013. Bulk samples were oven-dried at 40 °C and passed by a 2 mm sieve. Due to a high clay content of the soil used in the experiment, it was assumed that the bulk density did not significantly change within this two-year period (2011–2013). Thus, soil bulk density (ρ_b) was measured only in 2011 and used to calculate PEO-C and CSO-C in 2011, and HWEO-C and POX-C stocks in both 2011 and 2013 by computing on an equivalent soil mass-depth basis described by Ellert and Bettany (1995).

2.2. Soil organic carbon pool extraction and analysis

Different SOC pools were isolated by (i) hot-water extractable organic C (HWEO-C), (ii) permanganate oxidizable C (POX-C), (iii) pyrosphospate extractable organic C (PEO-C) and (iv) the chemically stabilized organic C (CSO-C) extracted by H_2O_2 oxidation. The analyses of HWEO-C, POX-C and PEO-C were conducted in a sequence using the soil sample in the same tube.

Table 1
Land use, crop sequence, and carbon input in the five-year (2009–2013) experiment period.
(adapted of Hok et al., 2015, Table 1).

Land use[a]	Crop sequence[b]	Carbon input (Mg ha^{-1})	
		Cumulative	Annual
Rice-based cropping systems			
CT-Rc	Mb/R − Mb/R − Mb/R − Mb/R − Mb/R	14.07	2.81
NT1-Rc	Mt/R + St − Mt + Cr/R + St − St(2010)/R + St − St(2011)[¥]/R + St − Mt + St(2012)/R + St	34.39	6.88
NT2-Rc	Mt/R + St − Mt + Cr + St (2009)/M + St − Mt + Cr + St (2010)/R + St − St(2011)/M + St − St (2012)/R + St	33.10	6.62
NT3-Rc	Mt/M + St − Mt + Cr + St (2009)/R + St − St (2010)/M + St − St (2011)/R + St − St (2012)/M + St	36.13	7.23
Soybean-based cropping systems			
CT-Sb	Se/S − Se/S − Se/S − Se/S − Se/S	10.96	2.19
NT1-Sb	Mt/S + Brz − Brz(2009)/S + St − Mt/S + St + Sg − Mt/S + St − Sr + St (2012)/S + St + Sg	36.62	7.32
NT2-Sb	Mt+/S + St − Mt + Cr + St (2009)/M + St − Mt/S + St − Mt + Cr/M + St − Sr + St (2012)/S + St	35.47	7.09
NT3-Sb	Mt/M + Brz − Mt/S + St − Mt + Cr/M + St − St (2011)/S + St − Sg + Cr + St (2012)/M + St	37.77	7.59
Cassava-based cropping systems			
CT-Cs	C − C − C − C − C	8.06	1.61
NT1-Cs	C + St − C + St − C + St − C + St − C + St	19.54	3.91
NT2-Cs	C + St − Mt + St (2009)/M + St − St (2010)/C + St − Mt + Cr + St (2011)/M + St − St (2012)/C + St	22.22	4.44
NT3-Cs	Mt/M + St − C + St − Mt + Cr + St (2010)/M + St − C + St − Mt + Cr + St (2012)/M + St	26.19	5.24

[a] CT: conventional tillage; NT1: no-tillage in which main crops (rice, soybean and cassava) were grown in a one year frequency pattern; NT2 and NT3: no-tillage in which the main crops were grown in bi-annual rotations with maize.

[b] Mb: mung bean (*Vigna radiata*); R: rice (*Oryza sativa* L.); Mt: millet (*Pennisetum typhoides* Burm); St: *Stylosanthes guianensis*; Cr: *Crotalaria juncea*; M: maize (*Zea mays* L.); Se: sesame (*Sesamum indicum*); S: soybean (*Glycine max* (L.) Merr.); Brz: *Brachiaria ruziziensis* cv. ruzi; C: cassava (*Manihot esculenta*); Sg: sorghum (*Sorghum bicolor* L.).

[¥] St (*Stylosanthes guianensis)* left from the year in brackets./indicates relay cropping with varying planting dates; + indicates crops were planted side by side at the same planting date.

2.2.1. Hot-water extractable organic carbon (HWEO-C)

The HWEO-C was determined by the method adapted from Ghani et al. (2003). Briefly, 1.5 g of 2 mm-sieved bulk soil was weighed into 15 mL polypropylene centrifuge tubes. The sample was treated with 9 mL of distilled water for 16 h at 80 °C. Each tube was then shaken on a vortex shaker for 10 s to ensure that the HWEO-C released from the SOC was fully suspended in the solution. The tubes were centrifuged for 10 min at 4000 rpm. The SOC in the centrifuged extracts was oxidized by potassium dichromate in sulfuric acid and back titrated with ferrous sulfate.

2.2.2. Permanganate oxidizable carbon (POX-C)

The determination of POX-C is adapted from Tirol-Padre and Ladha (2004) and Culman et al. (2012). After the extraction of HWEO-C, the remaining supernatant in each tube was discarded and 10 mL of a stock solution of $KMnO_4$ (60 mM) was added to the sediments in the same tubes. The tubes were horizontally shaken on a table shaker at 200 rpm for 15 min at room temperature, and then centrifuged for 10 min at 4000 rpm. The extracted SOC content was determined with a spectrometer at 565 nm of absorbance. A standard curve was performed using pre-determined concentrations of $KMnO_4$ solutions and the samples SOC content was calculated through the relationship Absorbance x $KMnO_4$ concentration.

2.2.3. (Sodium) pyrophosphate extractable organic carbon (PEO-C)

The determination of PEO-C is adapted from Bascomb (1968) and McKeague et al. (1971), using only the samples collected in 2011. After removal of the $KMnO_4$ supernatant, the $KMnO_4$ residue in the sediments was washed out with deionized water for 3–4 times. Then, a third extraction was performed by adding 10 mL of 0.1 M sodium pyrophosphate ($Na_4P_2O_7$) solution into the same tubes. The tubes were horizontally shaken on a table shaker at 120 rpm for 6 h at room temperature, and then centrifuged for 10 min at 4000 rpm. The SOC in the centrifuged extracts was oxidized by potassium dichromate in sulfuric acid and back titrated with ferrous sulfate. The SOC dissolved in the pyrophosphate extract corresponded to the SOC associated with the active forms of Al and Fe.

2.2.4. Chemically stabilized organic carbon (CSO-C)

The determination of CSO-C was based on the method by Jagadamma et al. (2010) using only the samples collected in 2011. Briefly, 1 g of bulk soil was wetted with 10 mL of distilled water for 10 min. Then, 30 mL of H_2O_2 at 10% was added, and the solution was kept at 50 °C using a water bath. The oxidation was stopped when the frothing completely subsided. The sample was then washed and oven-dried at 40 °C until constant weight. The sample C determination was then performed by dry combustion using an elemental CN analyzer (TruSpec CN, LECO, St. Joseph, USA).

2.3. Assay of soil enzyme activities

The rationale to choose β-glucosidade and Arylsulfatase assay for this study was based on the experience in four highly weathered Oxisol under long-term tillage management experiment reported by Balota et al. (2004), by Inagaki et al. (2016, 2017) and in recent findings by Medeiros et al. (2017). In both situations, the β-glucosidade and Arylsulfatase had a close relationship with C dynamics and especially with labile fractions.

The soil enzyme activities were measured at 0–5, 5–10 and 10–20 cm depths using the same composite soil samples as used to analyze SOC pools.

2.3.1. β-glucosidase

Activity of *β*-glucosidase was assayed according to the method of Eivazi and Tabatabai (1988). Briefly, 1 g of dry soil (< 2 mm) was placed into a 50 mL flask, and then 4 mL of pH 6.0 of modified universal buffer (MUB) and 1 mL of 0.05 M *p*-nitrophenyl-*β*-D-glucoside (PNG) solution were added. The flask was swirled to fully mix the contents, stoppered, and incubated at 37 °C for 1 h. Then, 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.1 M pH 12 tris (hydroxymethyl) aminomethane (THAM) buffer were added to stop the reaction. The soil suspension was allowed to develop a yellow color and filtered. The color intensity was determined using a spectrophotometer at 400 nm *β*-glucosidase activity was reported on a dry soil basis with units of mg *p*-nitrophenol kg^{-1} soil h^{-1}.

2.3.2. Arylsulfatase

Arylsulfatase activity was assayed according to the method of Tabatabai and Bremner (1970). Briefly, 1 g of dry soil (< 2 mm) was placed into a 50 mL flask, and incubated with 4 mL of 0.5 M acetate

buffer (pH 5.8) and 1 mL of 0.05 M *p*-nitrophenol (PN) sulfate solution at 37 °C for 1 h. Then, 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M pH 12 NaOH were added to stop the reaction. The PN released was extracted and filtered, and the color intensity was determined using a spectrophotometer at 400 nm. Arylsulfatase activity was quantified as mass (mg) of *p*-nitrophenol being produced by enzymatic hydrolysis of potassium *p*-nitrophenyl sulfate during one hour incubation per unit mass (kg dry soil) (PNP equivalents).

2.4. Statistical analysis

The effects of cropping systems and soil use and management systems at each depth and in each cropping system on HWE-C and POX-C pools were assessed through a linear mixed effects model. The variable soil use and management systems were considered as the fixed effects, year as a repeated measure variable, and replication as the random variable. When significant difference was found on ANOVA in the fixed effect variables, LSD test ($p < 0.05$) was performed. The effects of crop based systems (rice – RcCS, soybean – SbCS, and cassava – CsCS) and soil management systems (CT-C, NT-1, NT-2 and NT-3) on C-PEOC and C-CSOC fractions and on β-Glucosidase and Arylsulfatase activities were accessed through a split-plot design and when significance was found on the ANOVA the LSD (95%) test was performed. The variables that affect activity of β-Glucosidase and Arylsulfatase were accessed through a linear multiple regression model using the backward procedure to select variables that contribute to increase the R^2. All statistical procedures were carried out using SAS v. 9.2.

3. Results

3.1. Soil organic carbon pools

For all three cropping system (rice – RcCS, soybean – SbCS, and cassava – CsCS), hot-water extractable organic C (HWEO-C), permanganate oxidizable C (POX-C) and pyrophosphate extractable organic C (PEO-C) stocks were affected by management systems and year (Table 2). Chemically stabilized organic C (CSO-C) stocks were not affected by management systems (Table 2).

3.1.1. Rice-based production systems

After five cropping seasons, significant effects of management systems treatments on hot-water extractable organic C (HWEO-C) stocks were detected in both 2011 and 2013 ($P < 0.05$) (Table 3). In 2011, HWEO-C stock under NT2-Rc and NT3-Rc were significantly greater than under CT-Rc. In 2013, NT soils contained on average 61% higher HWEO-C stocks than CT-Rc at the topsoil. The RV soil had significantly higher HWEO-C stocks than the plow tillage treatments at 0–5 and 5–10 cm depths in both 2011 and 2013 (Table 3). Soil under RV contained 69, 63, 44 and 40% higher HWEO-C stocks than CT-Rc, NT1-Rc, NT2-Rc and NT3-Rc, respectively, at the surface layer in 2011, and also had 48 and 39% greater HWEO-C than CT-Rc and NT-Rc soil at 5–10 cm depth. Similar to 2011, at 0–5 cm depth, soil under RV had 96 and 21% higher HWEO-C stocks than those under CT-Rc and NT-Rc soils, respectively. Considering the 100 cm as a single stratum, NT-Rc soils showed an increasing trend compared CT-Rc soil. On average, soils under NT-Rc had 10 and 20% more HWEO-C stocks in 2011 and 2013, respectively.

Significant differences among management systems treatments on permanganate oxidizable C (POX-C) stocks were observed at 0–5 and 5–10, 40–60 and 60–80 and 80–100 cm depths in both 2011 and 2013 (Table 4). NT-Rc systems resulted in a higher trend of increasing POX-C stocks in the subsoil layers compared to CT-Rc systems in 2013. Considering the 100 cm as a single stratum, NT-Rc soils reserved 7 and 14% more POX-C stocks than CT-Rc soil in 2011 and 2013, respectively.

Differences in management systems did not significantly affect the changes in stocks of pyrophosphate extractable organic C (PEO-C) and chemically stabilized organic C (CSO-C) in all depths after 3 years of management practices (Table 5). However, the PEO-C stocks in the RV was greater than in the tillage systems for 0–5, 5–10 and 10–20 cm soil layer. Different from PEO-C, no significant differences between RV and cultivated soils were detected for CSO-C stocks. Considering the 100 cm as a single stratum, RV soil had 4.0 and 1.17 Mg ha^{-1} more PEOC and CSO-C stocks, respectively than cultivated soils. Overall, the mean portions of the SOC pools for RV and all treatments and depths ranked in the order CSO-C > POX-C > PEO-C > HWEO-C.

3.1.2. Soybean-based production systems

Significant effects of soil use and management systems on HWEO-C stocks were detected at 0–5 and 5–10 cm depth in both 2011 and 2013 (Table 3). Considering the 100 cm as a single stratum, there were no significant differences in HWEO-C stocks between RV and cultivated soils. However, bi-annual crop rotation treatments tended to increase more HWEO-C than conventionally tilled soils after 5 years.

At 0–5 cm layer, on average for 2011, NT-Sb soils stored 14% more POX-C than CT-Sb. After two additional years, NT-Sb soils had 21% greater POX-C stocks than CT-Sb soil. Soils under RV had greater POX-C stocks than under CT-Sb and NT-Sb by 54 and 35% in 2011, and 54 and 27% in 2013, respectively. Considering the 100 cm as a single stratum, the POX-C stocks were almost constant among RV and management systems treatments. However, from 2011 to 2013, POX-C stocks increased by 5, 2 and 5% in NT1-Sb, NT2-Sb and NT3-Sb soils, respectively, whereas NT3-Sb had 12% POX-C stock significantly higher than CT-Sb.

Soils under RV significantly had 39% greater PEO-C stocks than cultivated soils only at the surface soil layer (Table 4). In contrast, RV and cultivated soils had no significant differences in CSO-C stocks at all depths. Considering the 100 cm as a single stratum, PEO-C and CSO-C stocks did not differ between RV and cultivated soils.

3.1.3. Cassava-based production systems

Significant effects of management systems treatments on HWEO-C stocks were detected at 0–5 and 5–10 cm depth in both 2011 and 2013 (Table 3). Soils under RV had greater HWEO-C than CT-Cs and NT-Cs by 69 and 48% in 2011, and 88 and 24% in 2013, respectively. Considering the 100 cm as a single stratum, RV and cultivated soils did not significantly differ in both sampling times. The increase in HWEO-C was observed in NT-Cs systems and the stocks ranked in the order RV > NT-Cs > CT-Cs.

The differences in management systems resulted in significant effects on POX-C stocks at 0–5 and 5–10 cm depths (Table 4). Furthermore, NT2-Cs and NT3-Cs showed a trend of greater accumulation of POX-C in the 40–100 depths interval in the two sampling times. Considering the 100 cm as a single stratum, no significant differences in POX-C stocks were detected among soil use and management. However, the soils under RV and bi-annual crop rotations treatments (NT2-Cs and NT3-Cs) showed an increasing trend compared to CT-Cs soil.

PEO-C stocks were influenced by management systems at 0–5 and 5–10 cm depths but the significant differences in CSO-C stocks were not detected in all soil depths (Table 5). Average PEO-C stocks under NT2-Cs and NT3-Cs were 12 and 7% greater than those under CT-Cs and NT1-Cs, respectively, at 0–5 cm soil layer. An increasing trend under NT-Cs systems was observed at 5–40 cm depth interval. RV soils showed greater PEO-C stocks than cropping systems. The different trend of PEO-C under RV and cultivated soils was not apparent in the deeper soil layers. Similar to rice- and soybean-cropping systems, CSO-C stocks at cassava-cropping systems were almost constant among treatments in all depths. Considering the 100 cm as a single stratum, PEO-C and CSO-C stocks were almost constant between RV and cultivated soils after 3 years. It is clear that short-term NT practices with different crop rotations did not alter the changes in PEO-C and CSO-C in 100 cm soil depth.

Table 2
Effect of management systems (M) and year (Y) on SOC pools and enzyme activity in 0- to 100 cm depth under three cropping systems in a high weathered Oxisol in Cambodia.

Cropping	Depth	SOC pools and enzyme activity [b]									
Systems[a]		HWEO-C			POX-C			PEO-C	CSO-C	βeta-G	Aryl-S
		M	Y	M vs Y	M	Y	M vs Y	M	M	M	M
	(cm)										
RcCS	0–5	**	*	ns	**	**	**	**	ns	**	**
	5–10	*	ns	ns	**	**	*	**	ns	**	**
	10–20	ns	ns	ns	ns	**	ns	**	ns	ns	ns
	20–40	ns	ns	ns	ns	ns	ns	ns	ns	–	–
	40–60	ns	ns	ns	ns	ns	ns	ns	ns	–	–
	60–80	ns	ns	ns	ns	ns	ns	ns	ns	–	–
	80–100	ns	ns	ns	ns	ns	ns	ns	ns	–	–
	0–100	ns	ns	ns	ns	*	ns	*	ns	–	–
SbCS	0–5	**	*	ns	**	ns	ns	**	ns	**	**
	5–10	*	*	ns	ns	ns	ns	ns	ns	**	**
	10–20	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	20–40	ns	ns	ns	ns	ns	ns	ns	ns	–	–
	40–60	ns	ns	ns	ns	ns	ns	ns	ns	–	–
	60–80	ns	ns	ns	ns	ns	ns	ns	ns	–	–
	80–100	ns	ns	ns	ns	ns	ns	ns	ns	–	–
	0–100	ns	ns	ns	ns	ns	ns	ns	ns	–	–
CsCS	0–5	**	*	ns	**	**	**	**	ns	**	**
	5–10	*	ns	ns	*	ns	ns	*	ns	**	**
	10–20	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	20–40	ns	ns	ns	ns	ns	ns	ns	ns	–	–
	40–60	ns	ns	ns	ns	ns	ns	ns	ns	–	–
	60–80	ns	ns	ns	ns	ns	ns	ns	ns	–	–
	80–100	ns	ns	ns	ns	ns	ns	ns	ns	–	–
	0–100	ns	ns	ns	ns	ns	ns	ns	ns	–	–

*and ** represents P < 0,05 and P < 0,01, respectively.
ns = Non significant.
[a] RcCS – Rice cropping system; SbCS – Soybean cropping system; CsCS – Cassava cropping system.
[b] HWEO-C = hot-water extractable organic C; POX-C = permanganate oxidizable C; PEO-C = pyrophosphate extractable organic C; CSO-C = chemically stabilized organic C; βeta-G = β-glucosidase activity; Aryl-S = arylsulfatase activity.

3.2. Soil enzymatic activities (β-glucosidase and arylsulfatase)

β-glucosidase activity was significantly influenced by management systems for the three cropping systems (i.e., rice – RcCS, soybean – SbCS and cassava – CsCS) in the 0–5 and 5–10 cm depths (Table 6). β-glucosidase and arylsulfatase activities were significantly increased by the NT and crop rotation practices compared to conventionally tilled system at 0–5 cm depth under SbCS and CsCS experiments. At this depth, the increase in β-glucosidase activity ranged on average from 18, 28 and 48% under NT when compared with CT for RcCS, SbCS and CsCS, respectively. No significant differences were observed between management systems in the 10–20 cm soil layer. Under RcCS, arylsulfatase activity was not found to be significantly different among management systems at all depths after 3 years (Table 6). In contrast, at 0–5 cm depth, arylsufatase activity was significant higher under bi-annual crop rotations treatments (NT2 and NT3) than CT under both, SbCS and CsCS experiments. This percentage ranged from 174 and 102% for CT-Sb and from 241 and 138% for CT-Cs. Differences in β-glucosidase and arylsulfatase activities between RV and NT soils ranged from 119 and 61% for RcCS, from 114 and 68% for SbCS, and from 130 and 71% for CsCS. Both enzymatic activities showed strong correlation ($P < 0.001$) with SOM pools (e.g., HWEO-C, POX-C, PEO-C, CSO-C, SOC and total nitrogen – TN) (Table 7). Multiple linear regression analysis indicated that TN, POX-C and PEO-C for the β-glucosidase ($\beta\text{eta-G} = -37.3 + 18.5(\text{TN}) + 12.7(\text{POX-C}) + 3.8(\text{PEO-C})$; $R^2 = 0.91$; $P < 0.001$) and, HWEO-C, POX-C, PEO-C and SOC for arylsulfatase ($\text{Aryl-S} = -8.9 + 13.6(\text{HWEO-C}) + 2.7(\text{POX-C}) + 1.5(\text{PEO-C}) + 0.3(\text{SOC})$; $R^2 = 0.83$; $P < 0.001$), were the most important variables for prediction of enzymatic activities.

4. Discussion

4.1. Changes in hot-water extractable organic C, permanganate oxidizable C, pyrophosphate extractable organic C and chemically stabilized organic C

In the present study, HWEO-C and POX-C were able to differentiate the impact of short-term NT cropping systems. We observed a significant increase in HWEO-C and POX-C stocks after five years of the three NT crop rotations with cover crops in the surface soil layer in the three cropping systems. The possible contributing factor could be the continuous supply of biomass-C input in the NT systems. It might influence an increase in these two labile SOC pools due to higher root input, which could stimulate the microbial activity (Lienhard et al., 2013). The increase in HWEO-C could contribute to the changes in SOC due to its positive correlation with SOC (Sparling et al., 1998). On the other hand, CT practices decreased HWEO-C stocks in the topsoil by 14%, 7% and 1% in rice-, soybean- and cassava-based cropping systems, respectively, in two years (2011–2013) (Table 2). The possible explanation could be the conventional plow-based tillage with less biomass-C input compared to NT systems. It might have led to a decrease in the supply of carbohydrates for microorganisms and soil enzyme activity resulting in a reduction in microbial biomass-C (MBC). Salinas-Garcia et al. (2000) observed that the greater concentration of MBC under NT practices than under CT after 6 years in a dry tropical region of Mexico resulted from a higher accumulation of crop residues at the soil surface.

Rhizodeposition of root mass and exudates greatly influences C turnover in soils, which can affect the net accumulation HWEO-C in soil rhizosphere (Ghani et al., 2003). An increasing trend of HWEO-C accumulation in the subsoil layers under NT systems was observed compared to conventionally tilled soils. This was probably due to the

Table 3
Hot water-extractable organic C (HWEO-C) stock in 1990 (RV), 2011 and 2013 at 0–100 cm depth under three cropping systems in a high weathered Oxisol in Cambodia.

Cropping	Depth	Soil use and managment[b]								
Systems[a]		RV 1990	CT 2011	NT1	NT2	NT3	CT 2013	NT1	NT2	NT3
	(cm)	HWEO-C stock, Mg ha^{-1}								
RcCS	0–5	0.49 A	0.29 Db	0.30 CDb	0.34 BCb	0.35 Bb	0.25 Da	0.37 CDa	0.40 BCa	0.44 Ba
	5–10	0.43 A	0.29 C	0.30 BC	0.32 B	0.31 B	0.25 C	0.33 BC	0.34 B	0.37 B
	10–20	0.50	0.54	0.49	0.46	0.50	0.44	0.54	0.54	0.61
	20–40	0.85	0.81	0.85	0.74	0.79	0.76	0.81	0.80	0.85
	40–60	0.73	0.57	0.74	0.77	0.69	0.68	0.76	0.77	0.77
	60–80	0.53	0.45	0.57	0.60	0.59	0.53	0.63	0.56	0.57
	80–100	0.55	0.54	0.55	0.66	0.63	0.47	0.62	0.55	0.58
	0 − 100	4.08	3.49	3.79	3.89	3.86	3.38	4.06	3.96	4.19
SbCS	0 − 5	0.49 A	0.30 Cb	0.31 Bb	0.34 Bb	0.36 Bb	0.28 Ca	0.42 Ba	0.42 Ba	0.46 Ba
	5–10	0.43 A	0.29 Cb	0.27 BCb	0.26 BCb	0.32 ABb	0.27 Ca	0.34 BCa	0.38 BCa	0.39 ABa
	10–20	0.50	0.49	0.47	0.49	0.47	0.46	0.49	0.49	0.53
	20–40	0.85	0.87	0.81	0.83	0.87	0.83	0.89	0.78	0.82
	40–60	0.73	0.58	0.71	0.71	0.74	0.78	0.65	0.71	0.74
	60–80	0.53	0.49	0.51	0.65	0.71	0.70	0.63	0.68	0.63
	80–100	0.55	0.53	0.42	0.54	0.54	0.64	0.54	0.62	0.70
	0 − 100	4.08	3.56	3.50	3.82	4.01	3.96	3.96	4.07	4.26
CsCS	0–5	0.49 A	0.29 Cb	0.30 Bb	0.34 Bb	0.35 Bb	0.26 Ca	0.37 Ba	0.40 Ba	0.42 Ba
	5–10	0.43 A	0.29 B	0.29 B	0.29 B	0.30 B	0.26 B	0.29 B	0.34 B	0.36 B
	10–20	0.50	0.47	0.48	0.47	0.44	0.48	0.55	0.56	0.53
	20–40	0.85	0.80	0.90	0.76	0.81	0.74	0.82	0.89	0.82
	40–60	0.73	0.54	0.70	0.76	0.62	0.66	0.71	0.73	0.75
	60–80	0.53	0.49	0.66	0.63	0.62	0.53	0.64	0.61	0.63
	80–100	0.55	0.51	0.51	0.58	0.63	0.48	0.61	0.54	0.59
	0–100	4.08	3.39	3.83	3.84	3.77	3.41	3.99	4.07	4.09

Uppercase letters in the same line and at the same year refers to the difference between soil use and managment (RV, CT, NT1, NT2 and NT3) at $P < 0.05$ by LSD. Lowercase letters within the same line and in the same soil use and managment indicate difference among 2011 and 2013 at $P < 0.05$ by LSD.

[a] RcCS – Rice cropping system; SbCS – Soybean cropping system; CsCS – Cassava cropping system.

[b] RV – reference vegetation; CT – conventional tillage; NT – no-till, NT1, NT2 and NT3 refers no-till associate with cropping systems described at Table 1.

Table 4
Permanganate oxidizable C (POX-C) stock in 1990 (RV), 2011 and 2013 at 0–100 cm depth under three cropping systems in a high weathered Oxisol in Cambodia.

Cropping	Depth	Soil use and managment[b]								
Systems[a]		RV 1990	CT 2011	NT1	NT2	NT3	CT 2013	NT1	NT2	NT3
	(cm)	POX-C stock, Mg ha^{-1}								
RcCS	0–5	1.65 A	1.03 Ca	1.18 Ba	1.16 Bb	1.19 Bb	1.14 Ca	1.34 Ba	1.37 Ba	1.41 Ba
	5–10	1.30 A	0.99 Ca	1.11 Bb	1.07 CBb	1.13 Bb	1.06 Ba	1.22 Aa	1.22 Aa	1.27 Aa
	10–20	2.12	1.98 b	2.09 b	2.01 b	2.09 b	2.05 a	2.35 a	2.38 a	2.33 a
	20–40	3.52	3.40	3.41	3.47	3.41	3.32	3.58	3.84	3.97
	40–60	3.15	2.95	3.12	3.37	3.04	2.99	3.27	3.42	3.49
	60–80	2.97	2.65	2.78	3.00	2.78	2.74	2.99	3.12	3.02
	80–100	2.99	2.60	2.70	3.12	2.69	2.72	2.89	3.11	3.02
	0–100	17.71	15.60 b	16.38 b	17.20 b	16.33 b	16.02 a	17.63 a	18.46 a	18.50 a
SbCS	0–5	1.65 A	1.07 C	1.18 B	1.22 B	1.27 B	1.07 C	1.29 B	1.26 B	1.34 B
	5–10	1.30	1.09	1.09	1.08	1.10	1.12	1.17	1.15	1.22
	10–20	2.12	2.09	2.12	2.13	2.15	1.94	2.12	2.17	2.27
	20–40	3.52	3.59	3.55	3.59	3.87	3.62	3.70	3.75	4.04
	40–60	3.15	3.29	3.15	3.37	3.52	3.31	3.48	3.41	3.54
	60–80	2.97	2.97	2.95	3.24	3.06	3.00	2.96	3.19	3.33
	80–100	2.99	2.93	2.91	3.23	3.04	2.91	3.10	3.23	3.25
	0–100	17.71	17.03	16.94	17.86	17.99	16.96	17.81	18.16	18.98
CsCS	0–5	1.65 A	0.97 Ca	1.00 Ca	1.16 Bb	1.13 Bb	0.93 Ca	1.05 Ca	1.29 Ba	1.33 Ba
	5–10	1.30 A	0.99 C	1.01 C	1.14 B	1.12 B	1.02 C	1.03 C	1.19 B	1.16 B
	10–20	2.12	2.03	1.89	2.18	2.10	1.89	1.95	2.20	2.22
	20–40	3.52	3.52	3.36	3.73	3.62	3.49	3.44	3.74	3.80
	40–60	3.15	2.95	3.18	3.49	3.34	3.10	3.09	3.16	3.32
	60–80	2.97	2.77	2.97	3.22	3.09	2.88	3.02	3.10	3.09
	80–100	2.99	2.78	2.98	3.28	3.09	2.80	2.91	2.98	2.94
	0–100	17.71	16.01	16.39	18.20	17.49	16.12	16.48	17.67	17.86

Uppercase letters in the same line and at the same year refers to the difference between soil use and managment (RV, CT, NT1, NT2 and NT3) at $P < 0.05$ by LSD. Lowercase letters within the same line and in the same soil use and managment indicate difference among 2011 and 2013 at $P < 0.05$ by LSD.

[a] RcCS – Rice cropping system; SbCS – Soybean cropping system; CsCS – Cassava cropping system.

[b] RV – reference vegetation; CT – conventional tillage; NT – no-till, NT1, NT2 and NT3 refers no-till associate with cropping systems described at Table 1.

Table 5
Pyrophosphate extractable organic carbon (PEO-C) and chemically stabilized organic carbon (CSO-C) stock in 1990 (RV) and 2011 at 0–100 cm depth under three cropping systems in a high weathered Oxisol in Cambodia.

Cropping	Depth	Soil use and managment[b]									
Systems[a]		RV 1990	CT 2011	NT1	NT2	NT3	RV 1990	CT 2011	NT1	NT2	NT3
	(cm)	PEO-C stock, Mg ha^{-1}					CSO-C stock, Mg ha^{-1}				
RcCS	0–5	1.89 a	1.10 b	1.06 b	1.17 b	1.14 b	2.55	2.98	2.63	2.66	2.64
	5–10	1.48 a	1.03 b	0.97 b	1.13 b	1.07 b	2.48	2.63	2.38	2.36	2.51
	10–20	2.68 a	1.91 b	1.80 b	1.92 b	1.74 b	5.09	4.73	4.61	5.02	4.64
	20–40	4.04	3.16	3.25	3.10	3.03	9.64	9.88	9.21	8.96	9.44
	40–60	2.83	2.21	2.17	2.14	2.06	8.43	8.76	8.16	8.60	8.28
	60–80	1.63	1.64	1.62	1.33	1.22	7.75	7.35	7.37	7.55	7.65
	80–100	1.41	1.26	1.30	1.21	1.07	7.56	6.83	7.13	7.28	7.08
	0–100	15.96 a	12.31 b	12.17 b	12.00 b	11.33 b	43.50	43.17	41.49	42.43	42.24
SbCS	0–5	1.89 a	1.33 b	1.35 b	1.39 b	1.38 b	2.55	2.61	2.64	2.60	2.57
	5–10	1.48	1.35	1.35	1.37	1.37	2.48	2.44	2.58	2.39	2.50
	10–20	2.68	2.50	2.61	2.63	2.57	5.09	4.78	4.59	4.58	4.85
	20–40	4.04	3.77	3.76	3.95	3.98	9.64	9.34	9.97	9.27	9.66
	40–60	2.83	2.54	2.49	2.65	2.80	8.43	8.66	8.81	9.21	9.11
	60–80	1.63	1.34	1.42	1.45	1.44	7.75	7.42	7.41	8.02	8.09
	80–100	1.41	1.21	1.07	1.25	1.29	7.56	7.30	7.29	8.24	7.75
	0–100	15.96	14.04	14.04	14.69	14.84	43.50	42.55	43.29	44.31	44.53
CsCS	0–5	1.89 a	1.17 b	1.22 b	1.31 b	1.31 b	2.55	2.60	2.52	2.62	2.52
	5–10	1.48 a	1.18 c	1.21 bc	1.30 bc	1.33 ab	2.48	2.45	2.50	2.49	2.53
	10–20	2.68	2.24	2.29	2.39	2.48	5.09	5.04	4.87	4.64	5.05
	20–40	4.04	3.83	4.04	4.03	4.13	9.64	9.33	9.36	9.39	9.54
	40–60	2.83	2.75	2.81	2.59	2.67	8.43	8.84	8.89	9.06	8.79
	60–80	1.63	1.82	1.61	1.60	1.80	7.75	7.69	7.81	7.66	7.58
	80–100	1.41	1.67	1.53	1.39	1.47	7.56	7.53	7.71	7.15	8.05
	0–100	15.96	14.66	14.72	14.61	15.19	43.50	43.48	43.66	43.02	44.07

Uppercase letters in the same line refers to the difference between soil use and managment (RV, CT, NT1, NT2 and NT3) at $P < 0.05$ by LSD.
[a] RcCS – Rice cropping system; SbCS – Soybean cropping system; CsCS – Cassava cropping system.
[b] RV – reference vegetation; CT – conventional tillage; NT – no-till, NT1, NT2 and NT3 refers no-till associate with cropping systems described at Table 1.

incorporation of deep-rooted cover crops such as Congo grass, millet, sorghum, and sunhemp into crop rotations under NT practices in the three cropping systems. Continuous input of root biomass and exudates from these cover crops could contribute to the increase in HWEO-C under NT systems. Séguy et al. (2006) reported that SOC in the subsoil could be sequestered by higher SOC rhizodeposition of the deep rooting systems such as Congo grass and sorghum and *Crotalaria* sp. Similarly, NT systems also significantly increased POX-C. Soils under NT systems averagely had 20%, 21% and 32% greater POX-C stocks than those obtained in conventionally tilled soils at the 0–5 cm soil layer under rice-, soybean- and cassava-based cropping systems, respectively, after 5 years. This was probably the fact that accumulation of POX-C results

Table 7
Bivariate Pearson correlation coefficients between β-glucosidase and arylsulfatase activities with soil organic matter pools, n = 39.

	HWEO-C	POX-C	PEO-C	CSO-C	SOC	TN
β-glucosidase	0.93***	0.96***	0.79***	0.52***	0.88***	0.96***
Arylsulfatase	0.95***	0.92***	0.76***	0.71***	0.93***	0.89***

HWEO-C: hot-water extractable organic C; POX-C: permanganate oxidizable C; PEO-C: pyrophosphate extractable organic C; CSO-C: chemically stabilized organic C; SOC: soil organic carbon; TN: total nitrogen.
*** $P < 0.001$.

Table 6
β-glucosidase (βeta-G) and arylsulfatase (Aryl-S) activities in 1990 (RV) and 2011 at 0–20 cm depth under three cropping systems in a high weathered Oxisol in Cambodia.

Cropping	Depth	Soil use and managment[b]									
Systems[a]		RV 1990	CT 2011	NT1	NT2	NT3	RV 1990	CT 2011	NT1	NT2	NT3
	(cm)	βeta-G (mg *p*-nitrofenol kg^{-1}soil h^{-1})					Aryl-S (mg *p*-nitrofenol kg^{-1}soil h^{-1})				
RcCS	0–5	80.1 a	31.0 c	35.7 cb	36.6 b	37.2 b	26.6 a	15.8 b	16.1 b	16.7 b	16.8 b
	5–10	50.3 a	27.9 b	29.4 b	29.1 b	29.2 b	19.7 a	12.3 b	12.0 b	12.4 b	13.4 b
	10–20	25.1	19.0	19.8	18.8	20.0	9.9	7.7	7.5	7.7	8.1
SbCS	0–5	80.1 a	29.2 c	35.6 cb	38.3 b	38.2 b	26.6 a	13.2 c	15.3 cb	19.4 b	19.1 b
	5–10	50.3 a	28.7 b	28.9 b	28.1 b	29.7 b	19.7 a	12.7 b	13.3 b	13.6 b	13.7 b
	10–20	25.1	21.2	21.7	20.6	21.2	9.9	8.1	8.1	7.8	8.3
CsCS	0–5	80.1 a	23.5 c	30.9 cb	36.2 b	37.6 b	26.6 a	11.2 d	13.5 cd	16.5 bc	16.7 b
	5–10	50.3 a	24.4 c	26.4 cb	31.1 cb	32.9 b	19.7 a	12.2 b	13.0 b	13.5 b	14.0 b
	10–20	25.1	20.8	19.5	21.8	22.4	9.9	7.3	7.3	7.8	8.7

Uppercase letters in the same line refers to the difference between soil use and managment (RV, CT, NT1, NT2 and NT3) at $P < 0.05$ by LSD.
[a] RcCS – Rice cropping system; SbCS – Soybean cropping system; CsCS – Cassava cropping system.
[b] RV – reference vegetation; CT – conventional tillage; NT – no-till, NT1, NT2 and NT3 refers no-till associate with cropping systems described at Table 1.

from the rate of C inputs from plant biomass, a major source of SOC, returned to the soil and the absence of soil disturbance under NT management that reduced SOC mineralization. Even three years longer but in the same soil type and climatic condition, these results are consistent with the study by Tivet et al. (2013b) who reported a significantly increased HWEO-C and POX-C at 0–5 cm soil depth after eight-year of intensive NT systems (e.g. diversity of cover/relay crops and high annual biomass input). Similar effects were also observed in the study by Stine and Weil (2002) in a tropical region of south central Honduras who found POX-C was highly correlated to SOC and soils under NT contained greater POX-C than CT. The consistent effect of NT crop rotations with cover crops on HWEO-C and POX-C, after 5 years of management suggests that these two labile SOC pools may be useful in assessing soil changes particularly the soil surface layer due to short-term NT crop rotations.

In the present study, PEO-C was almost constant in each depth among treatments in rice- and soybean-based cropping systems. However, it showed an increase under bi-annual crop rotations treatments at 0–5 cm depth in cassava-based cropping systems. The PEO-C stocks averagely comprised of 16% of SOC stocks in 2011 (data not shown) and were comparable to POX-C stocks in all cropping systems. It demonstrates a potential of this clayed Cambodian Oxisol to function as a sink for SOC that could be related to the formation of complexes with the active forms of Fe and Al.

Likewise, CSO-C was almost constant among treatments at each soil layer and these results are in accord to those reported by Tivet et al. (2013b) in a subtropical and tropical Oxisol (southern and central Brazil, respectively), and by Eusterhues et al. (2005) in the temperate Cambisol and Podzol (northern Bavaria, Germany). This finding could be explained that the amount of young plant residue-derived SOC added to the soil from crop residues did not affect CSO-C with 3 years, suggesting that CSO-C in the C baseline could be related to chemical and morphological structure of SOM and chemical and physical nature of the soil minerals. The clay contents of soils used in the present study were almost constant in each depth in the three cropping systems (data not shown). In general, this study shows a slight decrease with increasing depths in the three cropping systems. The slightly higher CSO-C in the surface layers in this study was probably due to the fresh aliphatic plant materials resistant to H_2O_2 oxidation (Eusterhues et al., 2005) because the oxidation process was done with the bulk soil without prior removal of the labile SOC pool.

4.2. Changes in β-glucosidase and arylsulfatase activities

The enzyme activities in soil systems are sensitive indicators to provide information on the impact of land use management and cropping systems (Rabary et al., 2008). In this study, it is consistent that tillage and crop rotation practices affected β-glucosidase activity in the surface soil layer when NT had 18, 28 and 49% higher β-glucosidase activity than CT in rice-, soybean- and cassava-based cropping systems, respectively. This could be explained by the fact that biomass-C input supplies from crop residues were the readily available substrate such as carbohydrate that could increase this enzyme activity. A similar finding was also observed by Green et al. (2007) who found that the β-glucosidase activity in the soil under a NT corn-common bean rotation was 82% significantly greater than under disk plow management in the 0–5 cm depth after five-year NT practices in a red Latosol in the tropical Savannah. In the present study, we also observed a decrease in β-glucosidase activity with increasing depths. NT systems mostly maintained an increasing trend over conventionally tilled soil, except under NT3-C, which was already significantly greater than that obtained in the CT-C soil. Although the residues were not mechanically incorporated with the soil, the restitution of crop residues on the soil surface led to a slow incorporation of organic materials into the soil. Together with root biomass and exudates, the significant increase in β-glucosidase activity in the subsoil layers might be apparent with longer time. Thus, NT crop rotations with permanent soil cover provide a good potential to enhance β-glucosidase activity in the subsoil layers as shown by results of this and previous studies.

Likewise, NT practices maintained greater arylsulfatase activity in the surface layer in soybean- and cassava-based cropping systems. Although they did not differ in rice-based cropping systems, NT practices still showed an increased trend of 5% compared to conventionally tilled soil. This greater arylsulfatase activity is likely due to the increase of microbial biomass from the higher crop residues under NT systems due to its relations to an increase HWEO-C and POX-C. High organic matter inputs via crop residues tend to increase soil microbial biomass due to continuous provision of energy sources for microorganisms (Vaughan and Ord, 1985). In contrast to the result of this study, Green et al. (2007) reported that there was no significant changes in arylsulfatase activity under five-year NT systems compared to disk plow systems in tropical Savannah. This was probably due to low biomass-C input since the study was conducted in a corn-common bean rotation without incorporation of other forage crops as soil cover. However, the study in temperate soils by Gajda et al. (2013), in eastern Poland, indicated that arylsulfatase activity under eight-year NT systems was two- to threefold greater than that obtained under traditional tillage at 0–15 cm soil layer as a result of higher organic C input via plant residues. The significant effect of NT crop rotations with cover crops on β-glucosidase and arylsulfatase activities in this study suggests that the two enzymes are good indicators to assess the effect of short-term NT crop rotations on the biological activity of soil, particularly the soil surface layer.

5. Conclusions

This study shows that short-term NT crop rotations with permanent soil cover are likely to play a substantial role in increase storage of HWEO-C and POX-C and improve β-glucosidase and arylsulfatase activities, especially at the 0–5 cm soil layer. When comparing among NT systems, bi-annual crop rotations might be recommended as an appropriate crop rotation scheme in Cambodian Latosols. The results emphasize the positive impact of the absence of soil disturbance under NT and the importance of the plant and their residues cropped in association in NT crop rotations to significantly accumulate more labile SOC pools and change the biological functioning of the soil, with higher soil enzyme activities in the surface layers. The increase in the two labile SOC pools (HWEO-C and POX-C) were able to differentiate the impact of short-term NT cropping systems and had a positive correlation with increase of SOC stock. Thus, these two labile SOC pools and soil enzymes could serve as sensitive indicator of management effects on SOC dynamics of short-term changes in agricultural management practices.

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