



Biofunctool® Approach Assessing Soil Quality under Conservation Agriculture and Conventional Tillage for Rain-fed Lowland Rice Systems in Cambodia

VENG SAR*

*Provincial Department of Agriculture, Forestry and Fisheries, Pailin province, Cambodia
Conservation Agriculture Service Center, Department of Agricultural Land Resources Management,
General Directorate of Agriculture, Cambodia
Faculty of Agriculture, Kasetsart University, Bangkok, Thailand
Email: sarveng@hotmail.com*

KANNIKA SAJJAPHAN

*Department of Soil Science and Center for Advanced Studies in Agriculture and Food,
Kasetsart University, Bangkok, Thailand*

WUTTHIDA RATTANAPICHAJ

*Department of Soil Science and Center for Advanced Studies in Agriculture and Food,
Kasetsart University, Bangkok, Thailand*

ALEXIS THOUMAZEAU

CIRAD, Systèmes de pérennes, University of Montpellier, Montpellier, France

VANG SENG

*Department of Agricultural Land Resources Management, General Directorate of Agriculture,
Phnom Penh, Cambodia*

FLORENT TIVET

CIRAD, AIDA, University of Montpellier, Montpellier, France

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Abstract Rice productivity is often limited by soil fertility depletion, water availability and access. Conservation Agriculture (CA) cropping systems have been designed and tested with the main objectives of restoring soil fertility, increasing productivity and profitability. This study assessed changes in soil health under rain-fed lowland rice under (i) conventional tillage (CT), (ii) CA (CA7: 7 years under CA) and (iii) green manure management for one year (CGM1) and for two years CGM2). Biofunctool®, a multi-functional soil assessment approach based on a set of seven soil indicators, was used to evaluate changes in three main soil functions (C transformation, nutrient cycling, and soil structure). In addition, soil chemical analyses were conducted in the 0-5, 5-10, 10-20 and 20-40 cm soil layers to assess changes in nutrient contents. Our results emphasized positive impacts of CA on C transformation, soil structure and nutrient contents. Soil organic carbon and total N were significantly higher ($p < 0.05$) under CA7 in the 0-5 cm layer with up to $+7.5 \text{ g C kg}^{-1}$ and $+0.74 \text{ g N kg}^{-1}$, respectively. Higher values of labile C and soil respiration ($p < 0.05$) were observed under CA in the 0-5 and 5-10 cm layers. More stable soil aggregates and improved VESS values ($p < 0.05$) were also observed under CA. CA and CGM had 2 to 3 times more available phosphorus than CT in the 0-5 cm layer, and higher values were observed under CA from a depth of 0-20 cm. Higher Ca, Mg and K contents were recorded under CA and CGM in the 0 to 40-cm soil layer. A SOC stabilization trend was observed in soils under CA (0-5 and 5-10 cm layers) while a SOC

mineralization trend was observed under CT and CGM. These results emphasize the positive impacts of CA on maintaining and/or enhancing soil health and in contributing to SOC accumulation. A diachronic analysis is now needed to assess the long-term on-farm impacts of CA on soil health and crop performances.

Keywords soil organic carbon dynamic, sustainable intensification, climate change adaptation

INTRODUCTION

Cambodia produced over eight million tons of paddy for a total cultivated area of ~ 3.3 million ha (MAFF, 2018). Rice exports jumped from 100,000 tons in 2010 to ~ 540,000 tons in 2016. Based on topographic position, rice agroecosystems range from upland rice, rainfed lowland or upper sandy terraces, flooded rice in the plains, receding rice, and deep-water rice. Soil fertility across the rice agroecosystems ranges from medium to low (Biswas *et al.*, 2017). Yields are often limited by low levels of soil nutrients, fluctuating water levels in the paddy field and related impacts on the form and availability of nutrients in the soil (Pheav *et al.*, 2005). Despite the increase in rice yields over the last two decades, rice yield and profitability of farming systems around the Tonle Sap lake are still low. In addition, increasing use of chemical fertilizers and pesticides are recorded both in flooded and irrigated conditions raising concerns about environmental and health issues, and food safety. The low level of diversification, the increasing use of pesticides (Flor *et al.*, 2018) and the combination of practices that deplete soil fertility (i.e., continuous ploughing, use of rotary tillers, low inputs of organic compounds, burning or removal of crop residue to feed cattle) call for the design and assessment of alternative rice-based cropping systems. There is an urgent need to promote diversified rice cropping systems to maintain and improve soil quality, increase farmers' incomes while simultaneously contributing to safer food production. Several studies have demonstrated the positive impacts of diversified conservation agriculture (CA) cropping systems which promote the accumulation of soil organic carbon (SOC) and improve soil fertility (Boddey *et al.*, 2010). Conservation agriculture (CA) is based on three technical principles with (i) minimum soil disturbance (i.e., no tillage), (ii) a permanent soil cover, and (iii) diversified cropping systems (FAO, 2014). Diversified CA cropping systems with high biomass-C inputs (Séguy *et al.*, 2006) insure a continuous supply of fresh organic compounds, thereby improving soil aggregation (Tivet *et al.*, 2013), increasing soil biodiversity (Lienhard *et al.*, 2013), and SOC content (de Moraes Sá *et al.*, 2015) while enhancing production and ecosystem services (Pittelkow *et al.*, 2015). Multifunctional soil assessments are needed to better understand the relationships between cropping system management and soil health. Thoumazeau *et al.* (2019 a, b) proposed an integrative, multifunctional approach, named Biofunctool®, that makes it possible to assess three main soil functions (i) carbon transformation, (ii) nutrient cycling, and (iii) soil structure, with a core set of in-field and low-tech indicators. Three indicators were used to assess the changes of the carbon transformation including the labile soil organic C fraction (permanganate oxidizable carbon: POXC) (Weil *et al.*, 2003), the basal soil respiration (SituResp®) (Thoumazeau *et al.*, 2017), and the soil biological activity using the bait lamina test (van Gestel *et al.*, 2003). Then, three indicators were used for the soil structure maintenance function by assessing soil aggregate water stability (AggSoil) at 0-5 and 5-10 cm depths (Herrick *et al.*, 2001), water infiltration (Beerkan) (Thoumazeau *et al.*, 2019b), and visual evaluation of soil structure (VESS) at 0-30 cm depth (Guimarães *et al.*, 2011). Finally, the nutrient cycling function was assessed by quantifying available N, P, Ca, Mg and K.

OBJECTIVE

We hypothesised that rice-based CA cropping systems have direct and positive effects on soil health, increase the main soil functions through C transformation, soil structure and nutrient contents. The

overall objective of the study was to conduct an integrative and quantified assessment of the relationships between contrasted rice cropping systems (i.e., conventional plough-based tillage (CT), CA and green manure management) and soil health on the flood plains of Lake Tonle Sap using the Biofunctool® approach.

METHODOLOGY

Study site:

In 2011, an on-farm experimental design was implemented in the hydromorphic plains in Kropour Kert village, Banan district, Battambang province (latitude 13°00'32.37" N, longitude 103°04'27.31"E, 18 m elevation, no slope). The soil in the 0-20 cm layer comprised 511 g kg⁻¹ clay, 339 g kg⁻¹ silt, 150 g kg⁻¹ sand and 5.19 pH (H₂O). The soil is classified as a Vertisol by the FAO and as clayey soil according to the USDA soil classification. Mean annual precipitation was 1,306 mm and the mean temperature was 27.5 °C.

Cropping systems:

Soil functions and soil physical-chemical characteristics were assessed for four main cropping systems (CA7, CGM1, CGM2 and CT). It should be noted that the on-farm assessment was based on an unequal number of fields under the same management, with two fields under CA management for 7 years (CA7), two fields under green manure management for one year (CGM1), three fields under green manure management for two years (CGM2), and seven fields under conventional plough-based management (CT). CA was based on no-tillage with a cover crop following wet season rice. Two main species were used, *Stylosanthes guianensis* (cv. Ubon Nina) and *Centrosema pascuorum* (cv. Cavalcade). Green manure management comprised ploughing the cover crop biomass into the soil a few weeks before rice was sown. Conventional tillage (CT) included ploughing (6-disc plough) and harrowing, and the residues of the previous crop were incorporated into the soil by ploughing or rotary tiller.

Soil quality assessment:

According to the integrative view of the soil quality, the indicators used to assess changes in soil quality should be the result of soil biota-physical-chemical property interactions (Thoumazeau et al., 2019). The Biofunctool® approach was chosen to provide this integrative view and to describe the impacts of contrasted practices on soil quality. This approach is based on three main soil functions (i) soil carbon transformation, (ii) nutrient cycling and (iii) soil structure.

Soil sampling and soil physical-chemical analysis:

Soil samples were collected on January 25th, 2018 in four soil layers (0-5, 5-10, 10-20, and 20-40 cm) with three replicates per field. The Biofunctool® approach was applied to the 0-5 and 5-10 cm layers. In addition, the same two soil layers (0-5 and 5-10 cm) were sampled per subplot to assess water-stable aggregates. Total C and N concentrations were analysed using a dry combustion method with an elemental CHN analyser (Wright et al., 2008), available P (Bray II method) (Bray and Kurtz, 1945), and available K, Ca, Mg (AAS method) (Pyle et al., 1995). Available N was quantified on samples sieved at 2-mm using the Kjeldahl method (Craft et al., 1991).

Data analysis:

Statistical analysis was performed using R software (Dessau and Pippet, 2008). Each Biofunctool® indicator was first studied separately using a linear-mixed effects model (lme4 package, (Bates et al., 2015)). Treatment was defined as the fixed factor and replicates (plots and inner-replicates) as random factors. After checking the normality of the model residuals and the homoscedasticity of residual variance, ANOVAs were run using the car package (Fox and Weisberg, 2013). This was followed by post-hoc mean comparisons, using the Shapiro-Wilk test with Bonferroni adjustment (Hothorn et al.,

2008). After studying each indicator separately, the indicators were subjected to Principal Component Analysis (PCA) (FactoMineR package) (Lê et al., 2008).

RESULTS

Soil Chemical Analysis

Under CA7, higher SOC and N contents ($p < 0.05$) were recorded in the 0-5 cm soil layer, representing an increase of up to +7.5 g C kg⁻¹ and +0.74 g N kg⁻¹, respectively (Table 2). Higher N contents were observed in the 0-5 and 5-10 cm soil layers under CA7 than under CT and significant differences between treatments were observed in all soil layers. In addition, higher stratification of SOC and N contents were observed under CA7 and CGM2 compared with CGM1 and CT in the top soil layer (0-10 cm). SOC and N contents were more uniformly distributed under CT mainly due to the successive effect of ploughing which mixed the topsoil layers.

Table 1 Total soil organic carbon and nitrogen contents

Fields	Total OC (g kg ⁻¹)				Total N (g kg ⁻¹)			
	0-5	5-10	10-20	20-40	0-5	5-10	10-20	20-40
CA7	17.75 b	9.97 ns	7.19 ab	4.86 ns	1.76 b	1.09 b	0.81 ab	0.63 ab
CGM2	11.52 a	9.90 ns	7.35 b	5.05 ns	1.15 a	1.07 ab	0.86 b	0.67 b
CGM1	10.22 a	9.56 ns	5.57 a	4.11 ns	1.13 a	1.05 ab	0.78 ab	0.66 b
CT	10.75 a	9.22 ns	5.74 a	4.51 ns	1.02 a	0.91 a	0.66 a	0.53 ab

Note: CA7: 7 years CA (experiment, 2 fields, n = 6), CGM2: 2 years of cover crops as green manure (3 fields, n = 9), CGM1: 1 year of cover crops as green manure (2 fields, n = 6), CT: Conventional tillage as the control (7 fields, n = 21). Different letters indicate significant differences according to Tukey's test.

Soil Quality: C Transformation, Soil Structure and Nutrient Cycling

Carbon transformation:

Higher values of POXC and SituResp were observed in the 0-5 cm soil layer ($p < 0.05$) under CA7 than under CGM2, CGM1 and CT, representing higher labile-C inputs and soil biological activity of the mesofauna and microflora. No significant difference was observed for the bait lamina, however higher substrate degradation was observed under CA7.

Table 2 Soil carbon transformation

Fields	POXC		SituResp®		Lamina baits	
	(mg C. kg ⁻¹)		(Absorbance difference)		(Substrate degraded)	
	0-5	5-10	0-5	5-10	0-5	5-10
CA7	877.3 b	404.0 ns	0.71 b	0.25 b	0.53 ns	0.36 ns
CGM2	443.7 a	324.7 ns	0.33 ab	0.12 ab	0.38 ns	0.29 ns
CGM1	429.7 a	320.2 ns	0.29 a	0.13 ab	0.46 ns	0.40 ns
CT	428.0 a	290.2 ns	0.26 a	0.11 a	0.40 ns	0.33 ns

Note: CA7: 7 years CA (experiment, 2 fields, n = 6), CGM2: 2 years of cover crops as green manure (3 fields, n = 9), CGM1: 1 year of cover crops as green manure (2 fields, n = 6), CT: Conventional tillage as the control (7 fields, n = 21). Different letters indicate significant differences according to Tukey's test. POXC: permanganate oxidizable carbon (POXC); Situ Resp: Soil respiration

Soil structure stability:

A significant difference was observed in the 5-10 cm soil layer with higher values under CA7 emphasizing the higher soil aggregate stability (Table 4). A low water infiltration rate was observed in

all fields with no significant differences between fields. CA7 exhibited the better visual soil structure with a lower score.

Table 3 Soil structure stability

Fields	Aggregate (score)		Beerkan (ml.mn ⁻¹)	VESS (score)		
	0-5	5-10		0-5	5-10	10-20
CA7	5.20 ns	5.36 ns	4.22 ns	2.00 a	2.00 a	2.50 a
CGM2	5.59 ns	4.89 ns	3.78 ns	2.78 c	2.78 a	2.83 a
CGM1	5.69 ns	4.81 ns	4.17 ns	2.10 a	3.00 b	3.25 b
CT	5.41 ns	4.61 ns	3.34 ns	2.29 b	3.75 c	3.50 b

Note: CA7: 7 years CA (experiment, 2 fields, n = 6), CGM2: 2 years of cover crops as green manure (3 fields, n = 9), CGM1: 1 year of cover crops as green manure (2 fields, n = 6), CT: Conventional tillage as the control (7 fields, n = 21). Different letters indicate significant differences according to Tukey's test.

Nutrient contents:

Under CA7, higher concentrations of available nitrogen were recorded in the 0-5 cm soil layer ($p < 0.05$) and in the other soil layers except in the 20-40 cm layer (Table 5). Two to three times higher available phosphorus was measured in the 0-5 cm layer under CA7 and CGM1 than under CT and higher values were observed under CA7 in the three top soil layers. Higher Ca, Mg and K contents were recorded under CA7 and CGM2 in all soil layers (Table 5).

Table 4 Available phosphorus and nitrogen

Fields	P (mg.kg ⁻¹)				N (g.kg ⁻¹)			
	0-5	5-10	10-20	20-40	0-5	5-10	10-20	20-40
CA7	12.34 c	6.52 b	2.50 b	1.53 ns	1.28 b	0.74 ns	0.55 b	0.28 a
CGM2	5.75 a	4.03 a	1.85 ab	1.51 ns	0.78 a	0.70 ns	0.58 b	0.44 b
CGM1	9.51 b	5.66 b	1.73 ab	1.50 ns	0.64 a	0.59 ns	0.26 a	0.23 a
CT	4.51 a	3.60 a	1.45 a	1.25 ns	0.81 a	0.59 ns	0.36 a	0.32 a

Note: CA7: 7 years CA (experiment, 2 fields, n = 6), CGM2: 2 years of cover crops as green manure (3 fields, n = 9), CGM1: 1 year of cover crops as green manure (2 fields, n = 6), CT: Conventional tillage as the control (7 fields, n = 21). Different letters indicate significant differences according to Tukey's test.

Table 5 Available potassium, calcium and magnesium

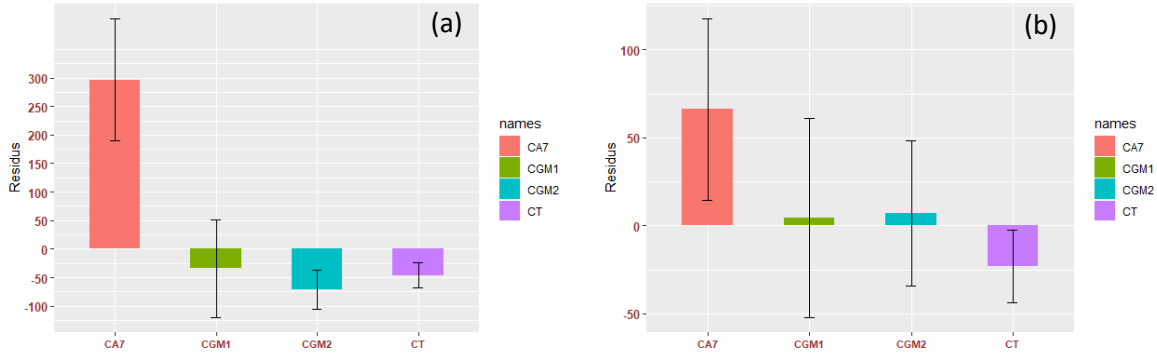
Fields	K (mg.kg ⁻¹)				Ca (mg.kg ⁻¹)				Mg (mg.kg ⁻¹)			
	0-5	5-10	10-20	20-40	0-5	5-10	10-20	20-40	0-5	5-10	10-20	20-40
CA7	27.27 c	11.70 ns	9.97 b	10.77 ns	926.6 c	840.7 b	812.8 b	747.5 c	118.8 c	106.2 b	103.3 b	98.7 ab
CGM2	22.93 bc	9.47 ns	10.37 b	10.93 ns	804.0 bc	794.6 b	809.0 b	714.4 bc	105.6 bc	106.9 b	132.9 c	124.3 b
CGM1	17.33 bc	8.60 ns	6.46 a	8.94 ns	648.1 ab	632.3 a	524.9 a	548.9 a	79.8 ab	78.5 a	75.4 a	77.1 a
CT	15.63 a	10.24 ns	8.60 ab	10.41 ns	687.1 a	692.5 a	609.0 a	630.8 ab	79.9 a	84.6 a	84.3 a	93.1 a

Note: CA7: 7 years CA (experiment, 2 fields, n = 6), CGM2: 2 years of cover crops as green manure (3 fields, n = 9), CGM1: 1 year of cover crops as green manure (2 fields, n = 6), CT: Conventional tillage as the control (7 fields, n = 21). Different letters indicate significant differences according to Tukey's test.

SOC Stabilization vs. SOC Mineralization

Due to the unbalanced design, the trends and hypothesis presented hereafter need to be confirmed in a medium to long-term study. We nevertheless present our results here as the relationship between POXC and SituResp is a promising decision-making tool to assess SOC dynamics. A SOC stabilization

trend was only observed under CA7 (0-5 and 5-10 cm soil layers) (Fig. 1 a and b). For CT and CGM, a trend of SOC mineralization was observed with negative values of the residues of the linear relationship between POXC and SituResp. The SOC mineralization trend under CT could result from successive ploughing and lower inputs of biomass compared with CA. In addition, disruption of soil aggregates due to ploughing exposed SOC to microbial communities and to mineralization processes (Chen et al., 2009).

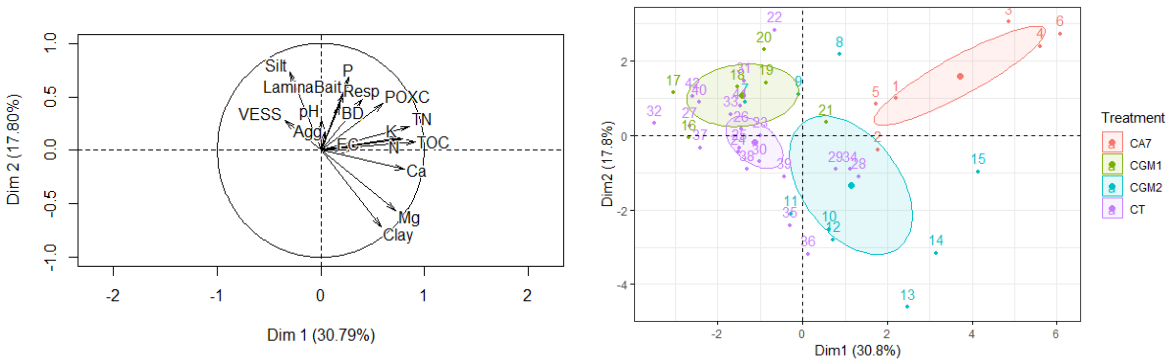


Note: CA7: 7 years CA (experiment), CGM2: 2 years of cover crops as green manure incorporating into the soil by tillage, CGM1: 1 year of cover crops as green manure incorporated into the soil by tillage, CT: Conventional tillage as the control. Regression was made between values of POXC and SituResp® (see Appendix 6). Residual mean values below zero represent a trend of mineralizable soil organic C, above zero values reflect a trend of short-term SOC stabilization. The vertical lines represent the standard error per treatment.

Fig. 1 Mean values of regression residuals per treatment in the 0-5 cm (a) and 5-10 cm (b) soil layers

Principal Component Analysis

A principal component analysis (PCA) was conducted. Three main clusters were identified (1) CT and CGM1, (2) CGM2 and (3) CA7 (Fig. 2). The first axis separates CA7 from CGM1 and CT, while the second axis separates CA7 from CGM2. It should be noted that the main indicators of the Biofunctool® such as POXC, SituResp and lamina baits are correlated with long-term CA management (CA7). Other Biofunctool indicators of soil structure stability (i.e., VESS and soil aggregates) contributed less to both axes. As observed previously, CT and CGM1 are characterised by lower TOC, TN, Ca, Mg contents and SituResp compared with CA7 and CGM2.



Note: CA7: 7 years CA (experiment), CGM2: 2 years of cover crops as green manure, CGM1: 1 year of cover crops as green manure, CT: Conventional tillage

Fig. 2 Principal component analysis of the impacts of cropping systems on soil functions and soil chemical properties in the 0-10 cm soil layer

DISCUSSION

This study assessed changes in soil quality under conventional plough-based and conservation agriculture management, using a multi-functional approach integrating a set of seven soil quality indicators related to three main soil functions (C transformation, nutrient cycling, and soil structure) (Thoumazeau et al., 2019). Soil physical-chemical analyses were also performed. Including all the indicators in a multivariate analysis made it possible to understand how soil functions in a clayey Vertisol in the flood plains of Tonle Sap lake are affected by contrasted agricultural practices under a tropical climate.

Impacts of Contrasted Cropping Systems on Soil Chemical Properties

The main soil indicators were positively affected by long-term CA practices (CA7) with an increase in soil organic C and N contents, an increase in labile-C (POXC), an improvement of soil aggregation that can protect SOC from microbial oxidation compared with under CT. However, a SOC accumulation trend under CA cropping systems was only detected in the surface soil layer confirming the results of the study conducted by Hok et al. (2015). Soil organic C and N contents were stratified with depth under CA compared with CT as a consequence of crop residues being left on the surface of the soil, thereby regulating fluctuations in soil temperature and moisture, and limiting the SOC decomposition rate (Franzluebbers, 2008).

In accordance with the results of Hok et al. (2015) and Pheap, Lefevre et al. (2019), under CA, SOC and TN contents were higher in the topsoil layer. Zhang and He (2004) reported a gradual increase in SOC contents in the 0-15 cm layer in the first 30 years of rice cropping in South-East Asia. The highest accumulation of SOC and TN were associated with the higher biomass input and less disturbance (CA7) compared with other practices (CGM1, CGM2 and CT).

Impact of Contrasted Cropping Systems on Three Soil Functions

Carbon transformation:

Part of the carbon transformation function, POXC, defined as a “soil fraction that is sensitive to cropping system management” (Pheap *et al.*, 2019) was indeed affected by the different practices with higher carbon contents measured in the 0-5 cm layer under CA7. Hok et al. (2018) reported an increase in POXC in the 0-10 cm layer under CA systems. In a similar experiment in Brazil, Sá et al. (2015) observed twice higher POXC under the no-till system down to a depth of 20 cm compared with under plough-based management. The increase in the top soil layer could be attributed to the difference in distribution of crop and cover crop residues between CA7 and other practices (Chatterjee and Lal, 2009) and due to a smaller amount of crop residues returned to the soil under CT than under CA7.

Concerning soil respiration, our results are in accordance with those of Pheap, Lefèvre et al. (2019), who reported that SituResp® values tended to be, or were significantly higher under CA, due to increased soil microbial activity linked to improved microorganism habitats and an increase in soil labile carbon. Used in combination, POXC and SituResp® play complementary roles by providing a framework for evaluating the relative dynamics of soil organic C stabilisation and mineralisation processes in agroecosystems (Hurisso et al., 2016). As observed by Pheap, Lefevre et al. (2019), CT tends to mineralise fresh organic carbon, while CA7 tends to stabilise C inputs.

Soil structure:

The absence of tillage combined with higher biomass inputs resulted in significant changes in soil physical properties, specifically improved soil aggregation (Lal and research, 1997). Soil physical properties improved rapidly under CA compared with under tillage-based systems. More stable aggregates in the top soil layers and better soil porosity are generally observed under CA (Busari et al.,

2015). As observed in our study, VESS was less sensitive to changes in the soil structure compared with water aggregate stability. This result is also in agreement with the results of other studies including those by Castioni et al. (2018) on a Brazilian Oxisol (55% clay) and by Thoumazeau et al. (2019b) for different land uses in Thailand.

Nutrient cycling and contents:

Ca, Mg and K contents were higher in the 0-10 cm soil layer under CA7 and CGM2 than under other practices. It is widely reported in the literature that CA with long-term use of cover crops recycle significant amounts of nutrients through the diversity of roots systems and aboveground biomass deposition. Continuous decomposition of plant residues enriches the main nutrient contents of the soil. Busari et al. (2015) reported that exchangeable Ca, Mg, and K were significantly higher in the surface soil under CA than under CT. Lower P, K, Ca and Mg contents were recorded in CT soils possibly due to the inversion of the top soil due to ploughing, which shifts less fertile subsoil to the surface with, in addition, possible leaching (Ali et al., 2006).

CONCLUSION

A multifunctional soil assessment was conducted using the Biofunctool® approach to compare the impacts of contrasted rice cropping systems on soil health. Long-term CA cropping systems (CA7) and early green manure management for two consecutive years (CGM2) had positive impacts on the main soil functions compared with short-term green manure management (CGM1) and CT. These results showed that POXC, SituResp®, and VESS are sensitive in-field indicators of the impacts of annual rice cropping systems on soil health. By contrast, soil aggregate stability, beerkan, and lamina baits are less sensitive to changes. The relationship between POXC and SituResp® revealed a SOC accumulation trend under long-term CA management and is thus a promising in-field and low-cost indicator to assess the dynamics of soil organic C (stabilisation vs. mineralisation). The comparison conducted here, with an unbalanced number of fields among the different cropping systems, only highlights potential trends in changes in soil fertility. Additional studies are thus needed along with a diachronic analysis to assess the long-term on-farm impacts of CA on soil health, crop performances and on the provision of ecosystem services.

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