# Multi-functional assessment of soil health under Conservation Agriculture in Cambodia

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**Abstract**

As a response to the worldwide challenge raised by soil degradation, Conservation Agriculture (CA) was proposed to help restoring the three main soil functions, i.e. carbon transformation, nutrient cycling and structure maintenance. However, there is still a lack of integrative studies that assess the overall impact of CA on soil health. To fill the gap, Biofunctool®, a set of in-field indicators, was developed to monitor changes in soil biological functioning. In this study, Biofunctool® was used to assess the impact of a conventional tillage (CT) and three CA annual-based cropping systems on soil health on a Cambodian Oxisol. Eight indicators related to the three soil functions were monitored and integrated into a Soil Quality index (SQI), i.e. the Biofunctool® Index. Overall, we found that soil health was twice higher under the CA treatments than under CT treatment. Although it was similar in the three CA treatments, the contribution of each soil function to the soil health diverged. An analysis of soil carbon dynamics also showed that CA support short-term soil organic carbon stabilization compared to CT. This study demonstrates that Biofunctool® is a robust, relevant, time-and cost-effective in-field assessment tool that can be used in multiple ways including cropping system management, capacity building of local stakeholders, and policy dialogue.

## Introduction

In agriculture, the soil system has historically been seen as a medium for food production, while it is also a source of further supporting, regulating and cultural services which are crucial for human well-being (Adhikari and Hartemink, 2016). However, this system is widely jeopardized and especially in Asia, where Montanarella et al. (2016) showed that soils are under pressure for commodities production (i.e., maize, cassava) generating soil erosion, soil organic carbon (SOC) depletion, and nutrient imbalance. These threats are confirmed in Cambodia where in 2008, 43% of the territory was estimated to be degraded due to intensification of agriculture after forest clearance, and that degradation affected almost 25 % of the population living on degraded areas (Bai et al., 2008). Under this context, there is a need to promote sustainable agricultural management, to limit soil degradation and to improve soil functioning.

As a response, it is recognized that Conservation Agriculture (CA) may strengthen a large set of soil ecosystem services (Palm et al., 2014). CA is based on three technical principles which are (i) minimum soil disturbance, (ii) retention of crop and cover crop residues and (iii) use of diversified cropping patterns (FAO, 2014; Séguy et al., 2006). CA was promoted for providing multiple beneficial effects on soil physical (Indoria et al., 2017; Patra et al., 2019), biological (Lienhard et al., 2013; Mathew et al., 2012; Souza et al., 2018) and chemical properties (Ivy et al., 2017; Parihar et al., 2018; Ranaivoson et al., 2017). Amongst soil chemical properties, soil organic carbon (SOC) was widely studied under CA, due to its potential to adapt farming systems to climate change and variability, and mitigate the effects of climate change through carbon sequestration (Lal, 2013). Globally, these studies allowed to provide process-based analysis of the soil physical, biological and/or chemical compartments, as affected by CA practices. However, such analyses do not seem sufficient to propose consistent soil health assessments.

Soil health was defined by Kibblewhite et al., (2008) as the capacity of a soil to produce a good quantity and quality food and fibre together with the delivery of other ecosystem services. However in most studies in the literature, all variables used to assess soil health are treated without proper consideration of their role along the soil health causal chain. Indeed, there is often no consideration of the cause/consequence relation between soil properties, soil functions and soil ecosystem services (Ivy et al., 2017; Lienhard et al., 2013; Thierfelder and Wall, 2012), while the realization of soil properties leads to the expression of soil functions which allows a soil to provide ecosystem services (Kibblewhite et al., 2008; Su et al., 2018). Also, many of the published studies do not, or weakly, consider the interactions between the different soil compartments (e.g. Das et al., 2014) , which should be at the heart of soil health assessments (Karlen et al., 1997; Kibblewhite et al., 2008; Rinot et al., 2019). These classical approaches are also usually based on the accumulation of distinct soil properties measurements (e.g. soil organic carbon, soil biodiversity…). The lack of consensus on this causal chain, the different interaction between soil properties and the addition of separated soil health indicators lead to different considerations of the components of soil systems which may lead to misleading conclusions on soil functioning assessment. To fill this gap, integrative approaches based on the results of soil physical-biological-chemical interactions should be implemented (Kibblewhite et al., 2008; Vogel et al., 2018; Rinot et al., 2019). Finally, assessments should be easily transferable to smallholder farmers, development practitioners and land managers who are the first beneficiaries of soil health (Idowu et al., 2008), but most studies rely on laboratory tools, protocols, and complicated data-analysis interpretation which may limit the further dissemination of new practices (Bünemann et al., 2018).

Thoumazeau et al. (2019a) proposed Biofunctool®, an operational framework to address soil health assessment and its quantification in a holistic manner, taking into consideration soil component interactions. Biofunctool® does not address nor evaluate soil mechanisms, but proposes a methodology to assess the impact of agricultural practices on soil health by studying three main soil functions identified by Kibblewhite et al. (2008), namely (i) carbon transformation, (ii) nutrient cycling and (iii) structure maintenance. Biofunctool® consists in a core-set of 10 integrative, in-field, and low-tech indicators that may easily be transferred to land managers. In previous studies, Biofunctool® was applied and validated over a gradient of soil disturbance in Thailand, on rubber plantations (Thoumazeau et al., 2019b). In addition, part of the Biofunctool® set, two indicators (i.e. POXC and SituResp®) may provide further indications on soil carbon dynamics (mineralisation vs. stabilization, see Hurisso et al., 2016) which have a particular interest under the current environmental context (Lal, 2013).

The aim of our study is to make an integrative and quantified assessment of several CA-based cropping systems on soil health. To do so, the Biofunctool® set of indicators was used to assess the impact of contrasted agricultural practices on soil health in a long-term experiment in Cambodia uplands. Biofunctool® indicators were grouped according to the three main soil functions and finally integrated into a Biofunctool® Index. The impact of CA vs. CT cropping system on soil functions, indexes and short-term SOC dynamics were analysed. The potential applications of the Biofunctool® set are finally discussed and further methodological perspectives are proposed.

## 2) Materials and methods

### *2.1) Site description and experimental design*

The experiment is located at the Bos Khnor experimental station that belongs to the General Directorate of Agriculture (GDA), Bos Khnor Commune, Chamkar Leu District, Kampong Cham province, in Cambodia (latitude 12°12'30" N, longitude 105°19'07"E, 118m elevation, no slope). The history of the station was described in Hok et al. (2015). Soil is made of 68.2% clay, 30.3% silt and 1.5% sand at 0-10 cm depth, and is classified as a Ferralsol or Red Oxisol in the FAO and USDA soil classification, respectively. According to the Köppen classification, the climate at the experimental site is Aw with rainfall concentrated during the wet season (May to November). The mean annual precipitation (2009–2017) in the experimental site was 1577 mm. The mean annual minimum and maximum temperatures were 23 °C and 32 °C respectively on the period 2009-2014 (NCEP, 2016). The experiment was implemented in 2009 by the Conservation Agriculture Service Center (CASC), Department of Agricultural Land Resources Management (DALRM, GDA) under a partnership with the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD). A randomized block design experiment was established consisting in four treatments, three replicates with a plot dimension of 8 m × 37.5 m. The four treatments are (i) conventional tillage (CT) system in which soybean (*Glycine mac (L) Merr.*) is cropped in annual succession with sesame (*Sesamum indicum*), (ii) direct seeding mulch-based cropping system (CA) in which soybean is cropped as a monoculture (CA 1), and (iii) bi-annual rotation of soybean and maize (*Zea Mays*) under CA management (CA 2a and CA 2b). In 2017, due to inaccurate density of soybean, rice-bean (*Vigna umbellata*) was used (Table 1). The experiment was designed to compare the conventional tillage management represented by one cash crop per year (soybean) under plow-based management (CT), with cropping systems involving different cropping pattern and biomass-C inputs under no-tillage (CA 1, CA 2a and CA 2b).

### *2.2) Cropping systems management*

2.2.1) Agroecology and rotations engineering

Soybean in conventional tillage (CT) represents the standard cropping system of smallholder farmers. Under CA 1, *Stylosanthes guianensis* (cv. CIAT 184) was used as cover crop and seeds are broadcasted 30 days before soybean (rice-bean in 2017) maturation. *S. guianensis* started growing at the end of the wet season, passed the dry season and was terminated at the end of June using a roller crimper with cutting discs. Soybean (rice-bean in 2017) was then sown mid-July. Under the bi-annual rotation (CA 2a and 2b), *S. guianensis* was associated with maize at sowing, and *S. guianensis* and *Sorghum bicolor* seeds were broadcasted 30 days before soybean maturation. All crops and cover crops used were grown under rainfed conditions. The cropping sequence is presented in Table 1. As a result of those cropping patterns, CA treatments receive annually from 2.5 to 3 times more biomass-C inputs (above-ground biomass resulting from main crops and cover crops) than CT (Table 1).

2.2.2) Agricultural practices

Tillage for CT treatment consists in disc ploughing to a 15–20 cm depth prior soybean cultivation. Soybean, rice-bean in 2017, and maize were sown with a no-till planter (Fitarelli or Vence Tudo). Under CA management, crop residues of the main crops and cover crops are left on the soil surface after the harvest. In case of poor development of the cover crops during the dry season, sorghum, pearl millet (*Pennisetum typhoides*) and/or sunn-hemp (*Crotalaria juncea*) are intercropped for 60 to 75 days with *S. guianensis* to increase the biomass-C inputs early in the wet season.

Since 2009, thermo phosphate (16% $P_2O_5$, 31% CaO and 16% MgO, 200 kg.ha$^{-1}$) has been applied in March followed by potassium chloride (KCl, 0-0-60, 50 kg.ha$^{-1}$) mid-May. From

2009 to 2013, inorganic fertilization was based on the use of urea (46-0-0, 50 $kg.ha^{-1}$ at sowing on soybean and maize, and 100 $kg.ha^{-1}$ for maize 25 days after sowing). N and P fertilization was modified in 2014. Since that, a basal fertilizer is applied at sowing on soybean (100 $kg.ha^{-1}$ of 18-46-0) and maize (150 $kg.ha^{-1}$ of 16-20-0) and a top-dressing with urea is added on maize (100 $kg.ha^{-1}$). Fertiliser inputs are detailed in supplementary reading 1.

### ***2.3) Soil sampling and analysis***

2.3.1) Biofunctool® indicators

Biofunctool® consists in a core-set of ten functional indicators that directly assess three main soil functions: carbon transformation, nutrient cycling and soil structure maintenance (Thoumazeau et al., 2019a). However, due to on-field rodents destruction, two indicators of the core-set, based on *in situ* incubation could not be analysed i.e., bait lamina and anion exchange membranes. Also, even though earthworms were observed, cast could very hardly be differentiated to soil aggregates due to the soil type and colour. In order to avoid misleading measurements, cast indicator was not integrated in the set of measurements. Notwithstanding these experimental inconveniences, Biofunctool® was applied as i) the bait lamina is one of the less sensitive to land management and rather describe inter-plot spatial heterogeneity (Thoumazeau et al., 2019b), ii) nutrient cycling function was adapted by splitting the results of $NH_4^+$ and $NO_3^-$ from soil extraction, and iii) earthworm activity was also indirectly integrated with other indicators such as water infiltration and aggregate stability.

All in all, we analysed 8 indicators with (1) for the carbon transformation function: Permanganate oxidizable carbon (POXC, (Weil et al., 2003)) and basal soil respiration

(SituResp®, (Thoumazeau et al., 2017)), (2) for the nutrient cycling function, available ammonium ($NH_4^+$) and nitrates ($NO_3^-$) from soil extraction with 1M KCl, and (3) for the soil structure maintenance function: surface aggregates at 0-2 cm depth (AggSurf) and soil aggregates at 2-10 cm depth (AggSoil) (Herrick et al., 2001), water infiltration (Beerkan) (Lassabatère et al., 2006) and VESS at 0-30 cm depth (Guimarães et al., 2011).

Soil samples were collected in November 2017 and the sampling was done at 0-10 cm depth (except for the VESS). Three sampling points (inner-replicates) were collected per plot representing 36 soil samples for the Biofunctool® analysis (except for the VESS indicator with only one replicate per plot collected).

2.3.2) Soil physico-chemical analysis

In addition to Biofunctool® indicators, further soil analysis were undertaken in the laboratory. Soil bulk density was sampled at each sampling points and measured by the core method (Blake and Hartge, 1986). Sub-samples of 2 mm sieved bulk soils were finely ground (<150 mm) and analysed for total C and N concentrations by dry combustion method (Van Moort and De Vries, 1970) using an elemental CHN analyser (Thermo Flash 2000). P concentrations were measured with the P Olsen method (Olsen et al., 1954), and finally pH was measured in water, using a 1:3 ratio (Table 2). All the analysis were implemented in Eco&Sols laboratory, Montpellier, France.

### *2.4) Statistical analysis*

Statistical analysis were computed using R software (R Development Core Team, 2008). Each Biofunctool® indicator was first studied separately using a linear-mixed effects model (package lme4, (Bates et al., 2015)). Treatment was defined as fixed factor and replicates (plots and inner-replicates) as random factors. After checking the normality of the model's residuals and homoscedasticity of variances' residuals, ANOVAs were run using the car package (Fox and Weisberg, 2011). This was followed by post-hoc mean comparisons, using Tukey with adjustment Bonferroni (Hothorn et al., 2008). As the VESS was sampled at 0-30 cm, a weighted score average was calculated and based on 0-10 cm depth only.

After studying each indicator separately, they were computed within a Principal Component Analysis (PCA) (FactomineR package, (Lê et al., 2008)) based on the mean of their three replicates (except for $NO_3^-$, see Figure 2). The last step of analysis consisted in calculating the Biofunctool® index, for which the methodology was defined by Obriot et al. (2016) and Thoumazeau et al. (2019a). This indicators' aggregation followed by scorings have already been handled by many authors (Armenise et al., 2013; Askari and Holden, 2015; Ngo-Mbogba et al., 2015; Yu et al., 2018). In our case two adaptations to the original scoring methodology of Obriot et al. (2016) and Thoumazeau et al. (2019a) were computed. First, concerning the scoring function of the VESS indicator, it was rather considered as an optimum at a score of 2.5 than following "the less the better" response curve. According to expert opinion, it was agreed that a highly friable soil corresponded to a disaggregated soil and thus could not be associated to an optimum. Then, in the study, a statistical PCA variable weighting was implemented to provide the same weight to each soil function. This allowed to study the absolute contribution of each function to the total index, rather than focusing only on relative evolution along the treatment studied. After calculation of the index, a variance analysis of the contribution of each soil function to the final score was run using one-way ANOVA.

The crossed-analysis of POXC and SituResp® was implemented following the conceptual framework proposed by Hurisso et al. (2016), based on the distribution of residuals of the linear regression (lm) between POXC and SituResp®. Repartition of model's residuals between treatments was assessed using one-way ANOVA followed by a post-hoc mean comparison test, using HSD function (de Mendiburu, 2017). In addition, several t-test were performed to assess if the mean value of residuals per treatment was statistically different than 0. The "0 baseline" representing a state of equilibrium between mineralization (negative values) and short-term SOC stabilization (positive values).

## 3) Results

### *3.1) Impact of Conservation Agriculture on soil functioning*

#### 3.1.1) Impact of Conservation Agriculture on the three soil functions

All the indicators were sensitive to the agricultural practices studied, except the visual structure evaluation (VESS) and nitrate concentration ($NO_3^-$) (Table 3). The other six sensitive indicators showed significant higher values under CA compared to CT.

For carbon transformation indicators, POXC (C labile) and SituResp® (basal soil respiration) values were three times higher in CA 1 when compared with CT. The evaluation of C dynamics (stabilization vs. mineralisation), using regression residuals between values of POXC and SituResp®, showed a gradual increase from CT to CA 1 (monoculture) to bi-annual rotation (CA 2a and CA 2b). CT showed a significant C mineralization pattern (on average -292.72) (Figure 1; supplementary readings 2). By contrast, among CA treatments, soil under CA 2b exhibited a short-term SOC stabilization ($p<0.05$) when no significant impact was observed under CA 1 and CA 2a.

For the structure maintenance function, results showed that soil of CT treatment tended to be less structured (score of VESS = 1.3), significantly less stable (AggSurf and AggSoil) and with a significant lower infiltration rate of water (2 times) compared to CA treatments. Also, for each treatment, an opposite pattern of aggregate stability with soil depth was observed.

It increased with depth for CT treatment (score increasing of 0.8) while it decreased for CA (-0.7 score for CA 1 and CA 2a, -1.6 score for CA 2b).

For the nutrient cycling function, only $NH_4^+$ concentrations significantly differed between treatments. In CA treatments, $NH_4^+$ was almost two times higher than in the CT treatment (on average 15 mg.kg$^{-1}$ vs 8.3 mg.kg$^{-1}$).

3.1.2) Impact of Conservation Agriculture on global soil health

In order to gather all the functional parameters, a principal component analysis (PCA) was undergone (Figure 2). The first and second PCA axes represented 45.7% and 19.5% of the total variability respectively. The PCA differentiated CA and CT and the difference was mainly related to three indicators linked to the soil functions studied with POXC (C transformation), $NH_4^+$ (nutrient cycling), and AggSurf (soil structure). The Biofunctool® index values for CA treatments had average score of 0.64 ($p<0.05$), which is about twice the score of CT treatment (Figure 3). Carbon transformation function was the most contributing function explaining the differences in the index. Its contribution was more than two times higher in the CA treatments.

### ***3.2) Impact of rotation on soil functioning***

3.2.1) Impact of rotation on the three soil functions

The indicators of carbon transformation function, POXC (C labile) and SituResp® did not differ between the three CA treatments (Table 3). Only CA 2b showed a significant C stabilisation dynamic (p=0.049) (Figure 1). For the two other CA treatments, although the regression mean residuals were positive, they were not significant (p>0.05) (Figure 1).

For the nutrient cycling function, $NO_3^-$ concentrations appeared higher in CA 2a and CA 2b (Table 3). However, due to high data variability, these differences were not significant. No difference was noted in the $NH_4^+$ concentrations between the three CA treatments.

For the structure maintenance, no difference was found between the three CA treatments for VESS and aggregate stability, except for water infiltration which was almost two times higher under CA 1 than under CA 2b (p<0.05) (Table 3).

3.2.2) Impact of rotation on global soil health

The Biofunctool® index values were not significantly different for the three CA, but the contribution of each function to the index differed (Figure 3). The analysis of relative contributions to the Biofunctool® index show that CA 1 and CA 2b have a rather similar structure with (i) carbon transformation function contributing the most to the index (41-39%), (ii) soil structure maintenance (35-33%), and (iii) nutrient cycling function contributing the less (24-28%). Under CA 2a, functions were more evenly distributed, with relative contribution of the three functions reaching around one third of the final index.

## **4) Discussion**

This study allowed to assess soil health under conservation agriculture, following a multi-functional approach, taking into account the interactions between the different soil compartments based on a consistent set of soil indicators previously selected. The integration of all indicators in a multivariate analysis enabled to understand how soil functions are impacted by contrasted agricultural practices under a tropical climate on a clayey Oxisol.

### *4.1) Impact of Conservation Agriculture on soil health*

#### 4.1.1) Impact of CA on carbon transformation function

The increase of POXC confirms the measurements of Hok et al. (2018) on the same experimental trial, who found more labile carbon under CA systems at 0-10 cm depth. On a similar experiment in Brazil, Sá et al. (2014) also observed on average two times more POXC under the no-till system up to 20 cm when compared with plough-based management. For soil respiration measurements, SituResp® values tended, or were significantly higher under CA, thus showing an higher soil microbial activity linked to the improvement of microorganisms habitat and the increase in soil labile carbon (Balota et al., 2004; Wang et al., 2003). When integrating POXC and SituResp®, CT and CA treatments follow a dynamic in line with the results of Hurisso et al. (2016) i.e. CT tends to mineralize fresh organic carbon, when CA treatments tend to stabilize C inputs. Also, the results follow the trends of the current literature highlighting the potential for carbon accumulation under CA management systems (Lal, 2015; Powlson et al., 2016), mainly due to increased carbon inputs on superficial horizons (Fujisaki et al., 2018; Virto et al., 2012).

#### 4.1.2) Impact of CA on the nutrient cycling function

Soil total nitrogen is higher under CA systems than CT with significant difference when comparing CT with CA 2a and CA 2b (Table 2). This trend is also observed for available $NH_4^+$ and $NO_3^-$. This result may be linked to the higher activities of decomposers and microbial communities under CA when compared with CT (Baudoin et al., 2009). Hok et al. (2018), on the same experiment, emphasized that the β-glucosidase and aryl-sulfatase activities are significantly higher under CA 2a and CA 2b when compared with CT at 0-5 cm depth. Indeed no till practices stimulate the microbial biomass, enzyme activities and denitrification processes especially in the upper soil surface (Baudoin et al., 2009; Bergstrom et al., 1998; Rabary et al., 2008). The increase in $NH_4^+$ and in some extend $NO_3^-$, may be related to several factors including (i) the increase N inputs through symbiotic $N_2$-fixing pathways, (ii) diversity and amount of organic inputs that may enhance soil decomposers and microbial communities activities (iii) no tillage practices that affect microbiota activities linked to the N cycle (nitrifiers and denitrifiers) in the top soil (Krauss et al., 2017). In a study conducted in middle-west region of Madagascar, Zemek et al. (2018) observed under low inputs rice-based cropping systems incorporating one-year long *Stylosanthes guianensis* fallow, a total N uptake of 225 kg.ha$^{-1}$, with 55% originating from the soil and 45% from the atmosphere.

4.1.3) Impact of CA on the structure maintenance function

The absence of tillage combined with presence of soil surface residues, living root systems of the cover crops during the dry season globally improved the structure maintenance function, which confirms the conclusions made on the improvement of soil structure under CA cropping systems (Hobbs et al., 2008; Indoria et al., 2017; Tivet et al., 2013). By contrast with the study conducted by Castioni et al. (2018) on a Brazilian Oxisol (55% clay), VESS was less sensitive to detect soil structure changes compared with aggregate stability. Thoumazeau et al. (2019b) also found that VESS was not sensitive to land management based on a perturbation gradient with very contrasted land uses. In our system, the superficial layer (0-5 cm) seemed to be the main part affected by CA practices. This soil layer may not have been studied precisely enough with the VESS method to raise differences between systems. In order to answer this issue, methodological improvements should be proposed to refine the scoring methods and to readapt it to the studied system (Emmet-Booth et al., 2018). Then, the better aggregate stability under CA is in agreement with several studies including those by Nascente et al. (2015) and Ivy et al. (2017) under similar pedo-climatic conditions. Also, It is recognized that CA-based cropping systems lead to stratification of soil aggregates and C content (Mrabet, 2002; Patra et al., 2019; Tivet et al., 2013), with the highest accumulation and aggregation on the surface layer. On the opposite, tillage disrupts top soil aggregates which leads to a uniform aggregates and carbon distribution. The stratification ratio, calculated as AggSurf/AggSoil (Franzluebbers, 2002) ranged from 0.76 under CT to 1.42 under CA 2b. This stratification under CA can be directly attributed to the absence of soil disturbance combined with higher biomass-C inputs from aboveground residues, root systems that strengthen soil aggregation in surface layers and contribute to build up a more stable aggregated soil matrix (de Oliveira Ferreira et al., 2018; Indoria et al., 2017; Six et al., 2004). Despite a high variability among CA results, soil water infiltration tends to increase under CA cropping systems. It is commonly recognized that water infiltration is improved under no-tillage and residue cover (Powlson et al., 2011).

4.1.4) Global impact of CA on the soil functions

The main difference observed in terms of soil functioning was observed between conventional tillage (CT) and CA systems (CA 1, 2a and 2b). The Biofunctool® index showed a better soil health under CA than under CT. Therefore, in addition to the positive impact on numerous individual soil properties (Das et al., 2014; Hobbs et al., 2008; Kumar and Goh, 2000; Thierfelder and Wall, 2012), the multi-functional assessment of soil functions showed an improvement of soil health under CA.

### ***4.2) Impact of rotation on soil health***

4.2.1) Impact of rotation on the three soil functions

Part of the carbon transformation function, POXC, although defined as a "soil fraction that is sensitive to management" (Bongiorno et al., 2019; Culman et al., 2012), was not sensitive to the crop rotation under CA in our case. This confirms the results of Hok et al. (2018) who found similar POXC stocks in the three CA treatments in 2013 (2.5 $Mg.ha^{-1}$ for CA 1, 2.5 $Mg.ha^{-1}$ for CA 2a and 2.6 $Mg.ha^{-1}$ for CA 2b). Similarly, basal soil respiration did not allow to distinguish CA treatments. Thus, even though these indicators (POXC and SituResp®) are recognized to be sensitive to land management, the rotation effect was not highlighted. This result emphasized that high biomass-C inputs under CA management, root systems deposition and rhizodeposites of crops and cover crops are predominant over the rotational pattern. Despite no difference among individual indicators, when crossing POXC and SituResp®, the CA treatments seemed to reach an equilibrium between short-term SOC mineralization and stabilization. Within the CA treatments, CA 2b was the only that showed a significant carbon stabilization. These information on carbon dynamics followed the observation of Hok et al. (2015), who noted a higher increase in CA 2b SOC stock in 0-5 cm depth in comparison with the other treatments.

No difference could be assessed between the three CA treatments for the nutrient cycling function, despite nutrient cycling is usually significantly impacted by the nature of the organic inputs and the long-term diversity of the cropping systems (Franchini et al., 2001; Pavinato and Rosolem, 2008). At the time of the soil sampling in November 2017, residues on the top soil of each cropping system were at different stages of decomposition and mineralization. Under CA 2b, maize was harvested almost 4 weeks before the soil sampling. Therefore, the decomposition process had started and, in addition, a new mix of cover crops was sown (i.e., sorghum and sunnhemp) potentially leading to new rhizodeposites. By contrast, under CA 1 and CA 2a, rice-bean was still under vegetative development and the residues of former cover crops were already decomposed. Although this could not be highlighted by our measurements, the residues' diversity (i.e., crops and mix of cover crops) may have provided organic inputs with different characteristics that may have impacted microbial communities build-up and turnover rate, thus differentiating kinetics of decomposition processes and nutrients releases (Giacomini et al., 2003). Further investigation on nutrient dynamics, as affected by rotations, should further be handled to better understand the cycles. Also, the ion exchange membrane indicator could not be measured in this study and prevented us to conduct a complete assessment of the nutrient cycling function.

The Beerkan indicator differentiated CA 1 and CA 2b, and the rice-bean cultivated in both plots in 2017 (CA 1 and CA 2a) may have a positive impact on water infiltration. The other indicators of structure maintenance and nutrient cycling were hardly sensitive to the rotation, therefore showing the importance of no-tillage and biomass–C inputs rather than the rotation itself.

Eventually we noted an improvement of inter-connected functions like C transformation, soil structure maintenance, and nutrient cycling. This strengthens adaptive capacities under CA systems.

4.2.2) Global impact of rotation on soil health

Even if the Biofunctool® index did not differ significantly under CA, contrasted patterns were observed. Rotation seemed to induce differences in the balance between soil functions. The contributions of each function to the index were more balanced under CA 2a, with a higher contribution of nutrient cycling than the others. However, the absence of significant difference on this soil function in the index could be explained by an insufficient complexity of the bi-annual rotation when compared with CA 1 where globally similar cover crops were used. To answer this hypothesis, further studies with more complex cropping patterns should be implemented. Also, CA 2a and CA 2b showed different patterns despite being a bi-annual rotation since 2009. Similar differences among a bi-annual rotation under CA systems were observed on an alkaline soil in the Battambang province, Cambodia (Tivet, pers. com.). The differences observed between CA 2a and CA 2b could be linked to the number of years of the cropping pattern that has a significant impact on soil functioning despite being a bi-annual rotation since 2009, and/or to a legacy effect from the past use of the plots.

### *4.3) Is Biofunctool® adapted to annual cropping system?*

Until now, the only published data on Biofunctool® came from a study in Thailand, implemented over a contrasted gradient of disturbance in perennial cropping systems (rubber plantations) and sandy soils (65 to 77% of sand) (Thoumazeau et al., 2019a; 2019b). In our study, Biofunctool® was used on annual-based cropping systems in a very different soil context (highly clayey red Oxisol). Globally, the Biofunctool® index was sensitive to contrasted agricultural practices (perennial/annual, till/no-till) and the conclusions were consistent with other studies from the literature. This confirms the tools' robustness and the relevance.

However, the Biofunctool® index did not raise difference between the monoculture and rotations under CA systems. The impact of rotation on soil health is thus rather low emphasizing that the biomass-C inputs (above-ground and below-ground) from the cover crops are predominant over the rotational pattern. The indicators that could not be implemented (Ion exchange membrane, Lamina and Cast) may have provided important information on carbon transformation or nutrient cycling function among the index. In the future, and in such contexts, those Biofunctool® indicators should be modified, or better protected to avoid on-field destruction.

## Conclusion

The study was a first application of Biofunctool® under contrasted annual cropping systems and showed to be well-adapted to an annual cropping system under tropical climate conditions. The multi-functional study of soil health globally raised large differences between tillage-based management and CA management on soil functioning. All tested indicators improved under CA compared with CT (except VESS that was less sensitive to soil management), with a soil quality index twice higher under CA. These conclusions globally confirm the trends in literature on the improvement of soil health under CA. Also, although biomass-C inputs and tillage were more important than the rotation pattern in the improvement of soil health, the presence of rotation in the management pattern seemed to induce a better balance between soil functions.

Further, Biofunctool® supports awareness raising of development practitioners and policy-makers on the negative impacts of current cultivation practices and demonstrates the positive impacts of alternatives cultivation methods such as CA on soil health. After the methodological development and the first applications in Thailand, Cambodia was another pilot country for Biofunctool® implementation. The Biofunctool® index was a good communication tool, easy to explain to a range of stakeholders (i.e., smallholder farmers, development practitioners, and policy-makers), and thus appears to be an accurate pathway to guide decision-making to improve cropping systems management, performances and efficiency.

## Conflict of interest

None.

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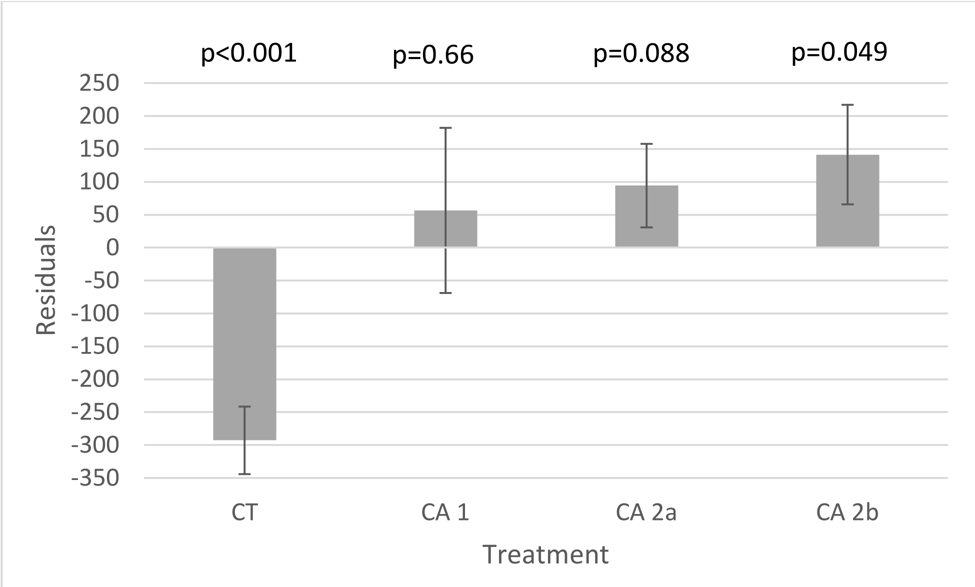


**Figure 1.** Mean values of regression residuals per treatment (n=9). (CT) Monoculture of soybean under conventional tillage, (CA 1) Monoculture of soybean under permanent cover crops, (CA 2a) Bi-annual rotation soybean-maize under conservation agriculture, and (CA 2b) Inversed bi-annual rotation maize-soybean under conservation agriculture. Regression was made between values of POXC and SituResp® (see supplementary readings 2). Residuals mean values below zero represent a trend of mineralizable soil organic C, the above-zero values reflect a trend of short-term SOC stabilization. Vertical line represents the standard error per treatment. The p-values correspond to t-test analysis of divergence from 0 ($\alpha$=0.05).

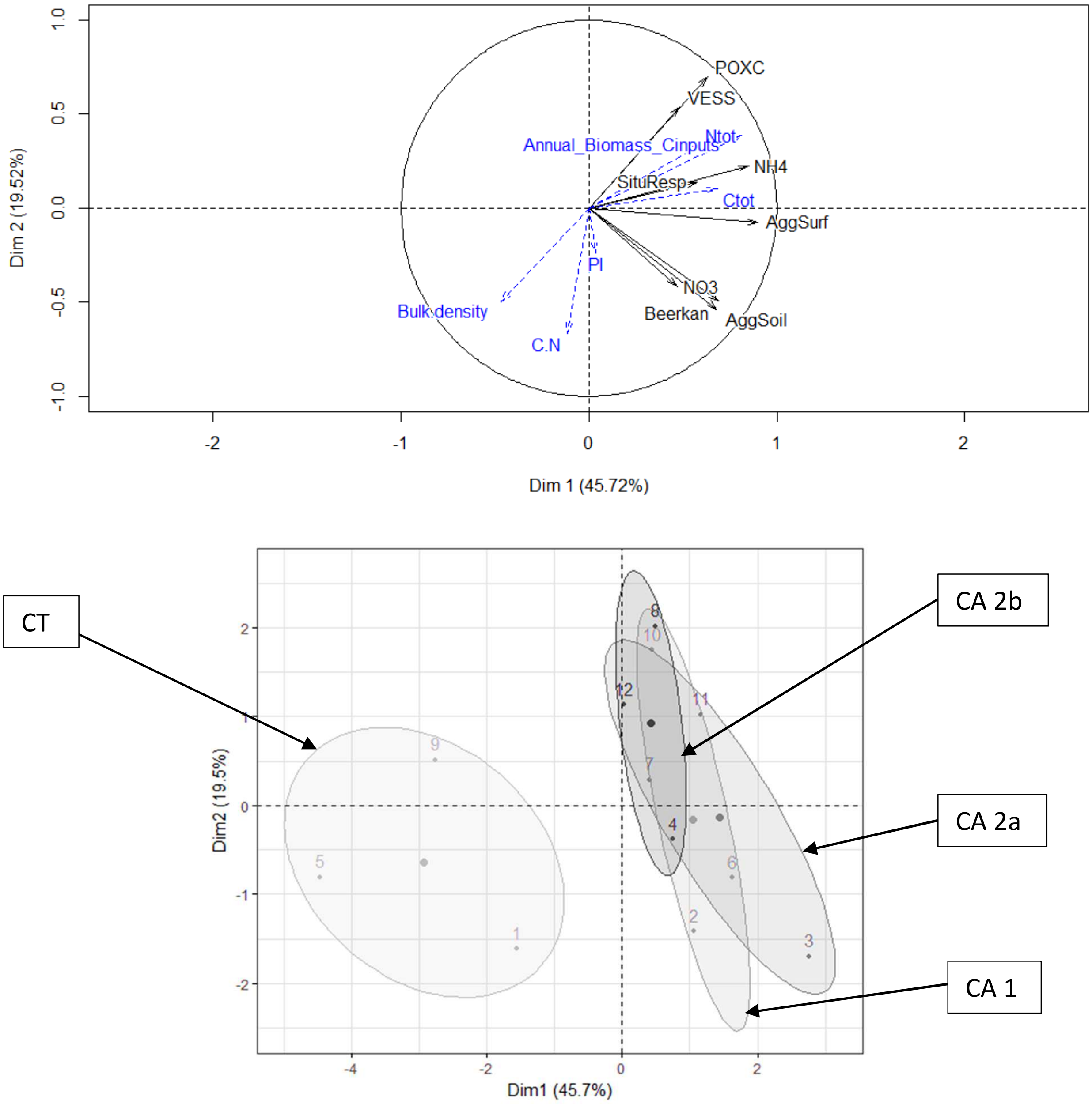


**Figure 2. Principal component analysis for the impacts of contrasted cropping systems on soil health**.

Top– Correlation circle. Values in blue are supplementary values, they are not used in the calculation of the PCA.

Bottom – Graph of individuals. (CT) Monoculture of soybean under conventional tillage, (CA 1) Monoculture of soybean under permanent cover crops, (CA 2a) Bi-annual rotation soybean-maize under conservation agriculture, and (CA 2b) Inversed bi-annual rotation maize-soybean under conservation agriculture.

*<u>Note:</u> $NO_3^-$ showed extreme values probably due to the unequal repartition of residues between the different treatments. Therefore, we chose to keep them in the analysis, but as the variability was high (CV=95% and 186% for CA 2a and CA 2b respectively), the median was used to run the PCA.*

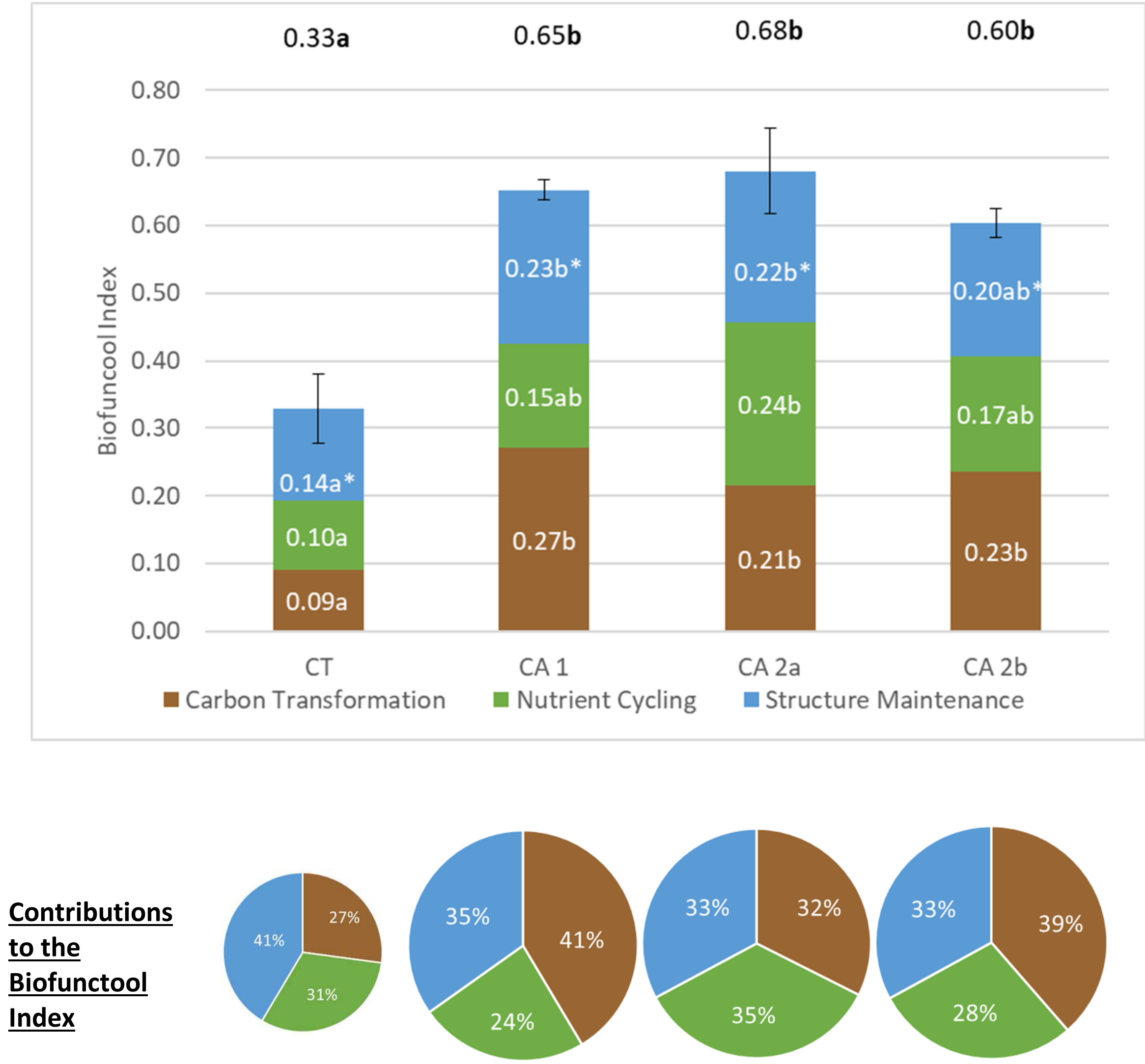


**Figure 3.** Biofunctool Index per treatment. (CT) Monoculture of soybean under conventional tillage, (CA 1) Monoculture of soybean under permanent cover crops, (CA 2a) Bi-annual rotation soybean-maize under conservation agriculture, and (CA 2b) Inversed bi-annual rotation maize-soybean under conservation agriculture. Standard error of the index is represented for each treatment. Below are shown the relative contribution of each of the three soil functions studied. Different letters mention significant differences at $p < 0.05$ for Carbon Transformation and Nutrient cycling. **p<0.1 for Structure maintenance.*

**Table 1.** Cropping system per treatment, cumulative and annual biomass-C inputs from 2009 to 2017. Adapted from Hok *et al.*, (2018)

| | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | **Biomass-C inputs ($Mg.ha^{-1}$)** Cumulative | Annual |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **CT** | Se/Sb | Se/Sb | Se/Sb | Se/Sb | Se/Sb | Se/Sb | Se/Sb | Se/Sb | Rb | 29.66 | 3.29 |
| **CA 1** | Mt /Sb+Brz | Brz(2009) /Sb+St | Mt /Sb+St+Sg | Mt /Sb+St | Sg+St(2012) /Sb+St+Sg | St+Sg(2013) /Sb+St+Sg | St+Sg(2014) /Sb+St+Sg | St+Sg(2015) /Sb+St+Sg | St+Sg(2016) /Rb* | 73.45 | 8.16 |
| **CA 2a** | Mt+Cr+St/ Sb+St | Mt+Cr+St(2009) /Mz+St | Mt /Sb+St | Mt+Cr /Mz+St | Sg+St(2012) /Sb+St | St(2013) /Mz+St | St(2014) /Sb+St+Sg | St+Sg(2015) /Mz+St | St(2016) /Rb* | 87.04 | 9.67 |
| **CA 2b** | Mt /Mz+Brz | Mt/Sb+St | Mt+Cr /Mz+St | St(2011) /Sb+St | Sg+Cr+St(2012) /Mz+St | St(2013) /Sb+St+Sg | St+Sg(2014) /Mz+St | St(2015) /Sb+St+Sg | St+Sg(2016) /Mz+Sg+Cr | 90.04 | 10.01 |

(CT) Monoculture of soybean under conventional tillage, (CA 1) Monoculture of soybean under permanent cover crops, (CA 2a) Bi-annual rotation soybean-maize under conservation agriculture, and (CA 2b) Inversed bi-annual rotation maize-soybean under conservation agriculture.

Cr: *Crotolaria juncea;* Mt: millet (*Pennisetum typhoides Burm*); St: *Stylosanthes guianensis*; Mz: maize *(Zea mays L.*); Se: sesame (*Sesamum indicum*); Sb: soybean (*Glycine mac (L) Merr.*); Brz: *Brachiaria ruziziensis cv. Ruzi*; Rb: rice-bean (*Vigna umbellata*); Sg: sorghum (*Sorghum bicolor L.*) left from the year in brackets. "/" indicates relay cropping with varying planting dates; "+" indicates crops planted in association (same or staggered sowing dates). * In 2017, due to inaccurate density of soybean, rice-bean (*Vigna umbellata*) was used.

**Table 2.** Soil characteristics per treatment. (CT) Monoculture of soybean under conventional tillage, (CA 1) Monoculture of soybean under permanent cover crops, (CA 2a) Bi-annual rotation soybean-maize under conservation agriculture, and (CA 2b) Inversed bi-annual rotation maize-soybean under conservation agriculture. Letters indicate significant differences according to Tuckey test. S.E stands for standard error, n=9 per treatment.

| | Total Carbon* ($g.kg^{-1}$) | | Total Nitrogen ($g.kg^{-1}$) | | C/N | | Inorganic Phosphorus ($g.kg^{-1}$) | | Bulk Density ($g.cm^{-3}$) | | $pH_{water}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | mean | S.E | mean | S.E | mean | S.E | mean | S.E | mean | S.E | mean | S.E |
| CT | 21.3 **a** | 0.7 | 1.68 **a** | $5.4.10^{-2}$ | 12.7 **bc** | 0.2 | $7.5.10^{-2}$ **ab** | $4.3.10^{-3}$ | 1.27 **ns** | $2.9.10^{-2}$ | 6.02 **ns** | 0.07 |
| CA 1 | 22.6 **a** | 0.6 | 1.74 **a** | $4.8.10^{-2}$ | 13.0 **c** | 0.1 | $8.2.10^{-2}$ **b** | $4.3.10^{-3}$ | 1.22 **ns** | $2.0.10^{-2}$ | 6.01 **ns** | 0.10 |
| CA 2a | 23.7 **b** | 0.6 | 1.92 **b** | $5.0.10^{-2}$ | 12.4 **ab** | 0.1 | $7.2.10^{-2}$ **ab** | $4.7.10^{-3}$ | 1.18 **ns** | $2.3.10^{-2}$ | 6.02 **ns** | 0.12 |
| CA 2b | 23.6 **b** | 0.4 | 1.94 **b** | $3.7.10^{-2}$ | 12.2 **a** | 0.1 | $6.2.10^{-2}$ **a** | $2.6.10^{-3}$ | 1.19 **ns** | $3.2.10^{-2}$ | 5.89 **ns** | 0.06 |
| ANOVA | $p<0.01$ | | $p<0.001$ | | $p<0.001$ | | $p<0.01$ | | $p=0.1$ | | $p=0.6$ | |

** The statistical analysis was made using log(Total Carbon) in order to gather all the hypothesis needed to run the ANOVA.*

**Table 3.** Biofunctool® indicators per treatment. (CT) Monoculture of soybean under conventional tillage, (CA 1) Monoculture of soybean under permanent cover crops, (CA 2a) Bi-annual rotation soybean-maize under conservation agriculture, and (CA 2b) Inversed bi-annual rotation maize-soybean under conservation agriculture. The analysis was made in 0-10 cm depth, except for AggSurf (0-2 cm) and AggSoil (2-10 cm). n=9 for each treatment except for VESS where n=3 per treatment (no inner-replicate). Due to the loss of one sample, n=8 instead for CT of $NO_3^-$ and $NH_4^+$. Letters indicate significant differences according to Tuckey test.

| | Carbon transformation | | | | Structure maintenance | | | | | | | | Nutrient cycling | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | POXC (mgC.kgsoil$^{-1}$) | | SituResp® (Absorbance difference) | | VESS (Score) | | Beerkan (ml.min$^{-1}$) | | AggSurf (Score) | | AggSoil (Score) | | $NO_3$ (mg.kg$^{-1}$) | | $NH_4$ (mg.kg$^{-1}$) | |
| | mean | S.E | mean | S.E | mean | S.E | mean | S.E | mean | S.E | mean | S.E | mean | S.E | mean | S.E |
| CT | 272.1 **a** | 51.3 | 0.12 **a** | 3.2.10$^{-2}$ | 2.1 **ns** | 0.3 | 124.7 **a** | 19.3 | 2.5 **a** | 0.4 | 3.3 **a** | 0.4 | 0.5 **ns** | 5.7.10$^{-2}$ | 8.3 **a** | 0.6 |
| CA 1 | 723.5 **b** | 112.2 | 0.43 **b** | 9.3.10$^{-2}$ | 2.3 **ns** | 0.2 | 263.7 **b** | 25.6 | 5.4 **b** | 0.3 | 4.7 **b** | 0.5 | 0.5 **ns** | 5.5.10$^{-2}$ | 13.0 **b** | 1.1 |
| CA 2a | 704.9 **b** | 63.7 | 0.26 **ab** | 5.5.10$^{-2}$ | 2.5 **ns** | 0.1 | 214.7 **ab** | 40.7 | 5.5 **b** | 0.2 | 4.8 **b** | 0.2 | 2.6 **ns** | 0.8 | 15.8 **b** | 0.9 |
| CA 2b | 762.1 **b** | 78.5 | 0.29 **ab** | 6.3.10$^{-2}$ | 2.4 **ns** | 0.1 | 140.9 **a** | 33.5 | 5.5 **b** | 0.2 | 3.9 **b** | 0.4 | 2.1 **ns** | 1.3 | 14.9 **b** | 0.8 |
| ANOVA | p<0.001 | | p=0.01 | | p=0.4 | | p<0.001 | | p<0.001 | | p<0.001 | | p=0.1* | | p<0.001 | |

**Based on the assumption that ANOVA is rather resistant to non-normality, an ANOVA was run for $NO_3$ without normality of residuals (p-value of shapiro test < 0.05)*