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

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27 Index; in-field assessment; red Oxisol

28 **Abstract**

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

45 Introduction

46 In agriculture, the soil system has historically been seen as a medium for food production,
47 while it is also a source of further supporting, regulating and cultural services which are
48 crucial for human well-being (Adhikari and Hartemink, 2016). However, this system is widely
49 jeopardized and especially in Asia, where Montanarella et al. (2016) showed that soils are
50 under pressure for commodities production (i.e., maize, cassava) generating soil erosion, soil
51 organic carbon (SOC) depletion, and nutrient imbalance. These threats are confirmed in
52 Cambodia where in 2008, 43% of the territory was estimated to be degraded due to
53 intensification of agriculture after forest clearance, and that degradation affected almost 25
54 % of the population living on degraded areas (Bai et al., 2008). Under this context, there is a
55 need to promote sustainable agricultural management, to limit soil degradation and to
56 improve soil functioning.

57 As a response, it is recognized that Conservation Agriculture (CA) may strengthen a large set
58 of soil ecosystem services (Palm et al., 2014). CA is based on three technical principles which
59 are (i) minimum soil disturbance, (ii) retention of crop and cover crop residues and (iii) use of
60 diversified cropping patterns (FAO, 2014; Séguy et al., 2006). CA was promoted for providing
61 multiple beneficial effects on soil physical (Indoria et al., 2017; Patra et al., 2019), biological
62 (Lienhard et al., 2013; Mathew et al., 2012; Souza et al., 2018) and chemical properties (Ivy
63 et al., 2017; Parihar et al., 2018; Ranaivoson et al., 2017). Amongst soil chemical properties,
64 soil organic carbon (SOC) was widely studied under CA, due to its potential to adapt farming
65 systems to climate change and variability, and mitigate the effects of climate change through
66 carbon sequestration (Lal, 2013). Globally, these studies allowed to provide process-based
67 analysis of the soil physical, biological and/or chemical compartments, as affected by CA

68 practices. However, such analyses do not seem sufficient to propose consistent soil health
69 assessments.

70 Soil health was defined by Kibblewhite et al., (2008) as the capacity of a soil to produce a
71 good quantity and quality food and fibre together with the delivery of other ecosystem
72 services. However in most studies in the literature, all variables used to assess soil health are
73 treated without proper consideration of their role along the soil health causal chain. Indeed,
74 there is often no consideration of the cause/consequence relation between soil properties,
75 soil functions and soil ecosystem services (Ivy et al., 2017; Lienhard et al., 2013; Thierfelder
76 and Wall, 2012), while the realization of soil properties leads to the expression of soil
77 functions which allows a soil to provide ecosystem services (Kibblewhite et al., 2008; Su et
78 al., 2018). Also, many of the published studies do not, or weakly, consider the interactions
79 between the different soil compartments (e.g. Das et al., 2014) , which should be at the
80 heart of soil health assessments (Karlen et al., 1997; Kibblewhite et al., 2008; Rinot et al.,
81 2019). These classical approaches are also usually based on the accumulation of distinct soil
82 properties measurements (e.g. soil organic carbon, soil biodiversity...). The lack of consensus
83 on this causal chain, the different interaction between soil properties and the addition of
84 separated soil health indicators lead to different considerations of the components of soil
85 systems which may lead to misleading conclusions on soil functioning assessment. To fill this
86 gap, integrative approaches based on the results of soil physical-biological-chemical
87 interactions should be implemented (Kibblewhite et al., 2008; Vogel et al., 2018; Rinot et al.,
88 2019). Finally, assessments should be easily transferable to smallholder farmers,
89 development practitioners and land managers who are the first beneficiaries of soil health
90 (Idowu et al., 2008), but most studies rely on laboratory tools, protocols, and complicated

91 data-analysis interpretation which may limit the further dissemination of new practices
92 (Bünemann et al., 2018).

93 Thoumazeau et al. (2019a) proposed Biofunctool[®], an operational framework to address soil
94 health assessment and its quantification in a holistic manner, taking into consideration soil
95 component interactions. Biofunctool[®] does not address nor evaluate soil mechanisms, but
96 proposes a methodology to assess the impact of agricultural practices on soil health by
97 studying three main soil functions identified by Kibblewhite et al. (2008), namely (i) carbon
98 transformation, (ii) nutrient cycling and (iii) structure maintenance. Biofunctool[®] consists in
99 a core-set of 10 integrative, in-field, and low-tech indicators that may easily be transferred
100 to land managers. In previous studies, Biofunctool[®] was applied and validated over a
101 gradient of soil disturbance in Thailand, on rubber plantations (Thoumazeau et al., 2019b). In
102 addition, part of the Biofunctool[®] set, two indicators (i.e. POXC and SituResp[®]) may provide
103 further indications on soil carbon dynamics (mineralisation vs. stabilization, see Hurisso et
104 al., 2016) which have a particular interest under the current environmental context (Lal,
105 2013).

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

114 **2) Materials and methods**

115 **2.1) Site description and experimental design**

116 The experiment is located at the Bos Khnor experimental station that belongs to the General
117 Directorate of Agriculture (GDA), Bos Khnor Commune, Chamkar Leu District, Kampong
118 Cham province, in Cambodia (latitude 12°12'30" N, longitude 105°19'07"E, 118m elevation,
119 no slope). The history of the station was described in Hok et al. (2015). Soil is made of 68.2%
120 clay, 30.3% silt and 1.5% sand at 0-10 cm depth, and is classified as a Ferralsol or Red Oxisol
121 in the FAO and USDA soil classification, respectively. According to the Köppen classification,
122 the climate at the experimental site is Aw with rainfall concentrated during the wet season
123 (May to November). The mean annual precipitation (2009–2017) in the experimental site
124 was 1577 mm. The mean annual minimum and maximum temperatures were 23 °C and 32
125 °C respectively on the period 2009-2014 (NCEP, 2016). The experiment was implemented in
126 2009 by the Conservation Agriculture Service Center (CASC), Department of Agricultural
127 Land Resources Management (DALRM, GDA) under a partnership with the Centre de
128 Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD). A
129 randomized block design experiment was established consisting in four treatments, three
130 replicates with a plot dimension of 8 m × 37.5 m. The four treatments are (i) conventional
131 tillage (CT) system in which soybean (*Glycine mac (L) Merr.*) is cropped in annual succession
132 with sesame (*Sesamum indicum*), (ii) direct seeding mulch-based cropping system (CA) in
133 which soybean is cropped as a monoculture (CA 1), and (iii) bi-annual rotation of soybean
134 and maize (*Zea Mays*) under CA management (CA 2a and CA 2b). In 2017, due to inaccurate
135 density of soybean, rice-bean (*Vigna umbellata*) was used (Table 1). The experiment was
136 designed to compare the conventional tillage management represented by one cash crop
137 per year (soybean) under plow-based management (CT), with cropping systems involving
138 different cropping pattern and biomass-C inputs under no-tillage (CA 1, CA 2a and CA 2b).

139 **2.2) Cropping systems management**

140 2.2.1) Agroecology and rotations engineering

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

152 2.2.2) Agricultural practices

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

160 Since 2009, thermo phosphate (16% P₂O₅, 31% CaO and 16% MgO, 200 kg.ha⁻¹) has been
161 applied in March followed by potassium chloride (KCl, 0-0-60, 50 kg.ha⁻¹) mid-May. From

162 2009 to 2013, inorganic fertilization was based on the use of urea (46-0-0, 50 kg.ha⁻¹ at
163 sowing on soybean and maize, and 100 kg.ha⁻¹ for maize 25 days after sowing). N and P
164 fertilization was modified in 2014. Since that, a basal fertilizer is applied at sowing on
165 soybean (100 kg.ha⁻¹ of 18-46-0) and maize (150 kg.ha⁻¹ of 16-20-0) and a top-dressing with
166 urea is added on maize (100 kg.ha⁻¹). Fertiliser inputs are detailed in supplementary reading
167 1.

168 **2.3) Soil sampling and analysis**

169 2.3.1) Biofunctool® indicators

170 Biofunctool® consists in a core-set of ten functional indicators that directly assess three main
171 soil functions: carbon transformation, nutrient cycling and soil structure maintenance
172 (Thoumazeau et al., 2019a). However, due to on-field rodents destruction, two indicators of
173 the core-set, based on *in situ* incubation could not be analysed i.e., bait lamina and anion
174 exchange membranes. Also, even though earthworms were observed, cast could very hardly
175 be differentiated to soil aggregates due to the soil type and colour. In order to avoid
176 misleading measurements, cast indicator was not integrated in the set of measurements.
177 Notwithstanding these experimental inconveniences, Biofunctool® was applied as i) the bait
178 lamina is one of the less sensitive to land management and rather describe inter-plot spatial
179 heterogeneity (Thoumazeau et al., 2019b), ii) nutrient cycling function was adapted by
180 splitting the results of NH₄⁺ and NO₃⁻ from soil extraction, and iii) earthworm activity was
181 also indirectly integrated with other indicators such as water infiltration and aggregate
182 stability.

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

185 (SituResp[®], (Thoumazeau et al., 2017)), (2) for the nutrient cycling function, available
186 ammonium (NH₄⁺) and nitrates (NO₃⁻) from soil extraction with 1M KCl, and (3) for the soil
187 structure maintenance function: surface aggregates at 0-2 cm depth (AggSurf) and soil
188 aggregates at 2-10 cm depth (AggSoil) (Herrick et al., 2001), water infiltration (Beerkan)
189 (Lassabatère et al., 2006) and VESS at 0-30 cm depth (Guimarães et al., 2011).

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

194 2.3.2) Soil physico-chemical analysis

195 In addition to Biofunctool[®] indicators, further soil analysis were undertaken in the
196 laboratory. Soil bulk density was sampled at each sampling points and measured by the core
197 method (Blake and Hartge, 1986). Sub-samples of 2 mm sieved bulk soils were finely ground
198 (<150 µm) and analysed for total C and N concentrations by dry combustion method (Van
199 Moort and De Vries, 1970) using an elemental CHN analyser (Thermo Flash 2000). P
200 concentrations were measured with the P Olsen method (Olsen et al., 1954), and finally pH
201 was measured in water, using a 1:3 ratio (Table 2). All the analysis were implemented in
202 Eco&Sols laboratory, Montpellier, France.

203 **2.4) Statistical analysis**

204 Statistical analysis were computed using R software (R Development Core Team, 2008). Each
205 Biofunctool[®] indicator was first studied separately using a linear-mixed effects model
206 (package lme4, (Bates et al., 2015)). Treatment was defined as fixed factor and replicates
207 (plots and inner-replicates) as random factors. After checking the normality of the model's

208 residuals and homoscedasticity of variances' residuals, ANOVAs were run using the car
209 package (Fox and Weisberg, 2011). This was followed by post-hoc mean comparisons, using
210 Tukey with adjustment Bonferroni (Hothorn et al., 2008). As the VESS was sampled at 0-30
211 cm, a weighted score average was calculated and based on 0-10 cm depth only.

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

229 The crossed-analysis of POXC and SituResp® was implemented following the conceptual
230 framework proposed by Hurisso et al. (2016), based on the distribution of residuals of the
231 linear regression (lm) between POXC and SituResp®. Repartition of model's residuals

232 between treatments was assessed using one-way ANOVA followed by a post-hoc mean
233 comparison test, using HSD function (de Mendiburu, 2017). In addition, several t-test were
234 performed to assess if the mean value of residuals per treatment was statistically different
235 than 0. The “0 baseline” representing a state of equilibrium between mineralization
236 (negative values) and short-term SOC stabilization (positive values).

237 **3) Results**

238 ***3.1) Impact of Conservation Agriculture on soil functioning***

239 3.1.1) Impact of Conservation Agriculture on the three soil functions

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

243 For carbon transformation indicators, POXC (C labile) and SituResp[®] (basal soil respiration)
244 values were three times higher in CA 1 when compared with CT. The evaluation of C
245 dynamics (stabilization vs. mineralisation), using regression residuals between values of
246 POXC and SituResp[®], showed a gradual increase from CT to CA 1 (monoculture) to bi-annual
247 rotation (CA 2a and CA 2b). CT showed a significant C mineralization pattern (on average -
248 292.72) (Figure 1; supplementary readings 2). By contrast, among CA treatments, soil under
249 CA 2b exhibited a short-term SOC stabilization ($p < 0.05$) when no significant impact was
250 observed under CA 1 and CA 2a.

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

255 It increased with depth for CT treatment (score increasing of 0.8) while it decreased for CA (-
256 0.7 score for CA 1 and CA 2a, -1.6 score for CA 2b).

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

260 3.1.2) Impact of Conservation Agriculture on global soil health

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

270 **3.2) Impact of rotation on soil functioning**

271 3.2.1) Impact of rotation on the three soil functions

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

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

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

282 3.2.2) Impact of rotation on global soil health

283 The Biofunctool® index values were not significantly different for the three CA, but the
284 contribution of each function to the index differed (Figure 3). The analysis of relative
285 contributions to the Biofunctool® index show that CA 1 and CA 2b have a rather similar
286 structure with (i) carbon transformation function contributing the most to the index (41-
287 39%), (ii) soil structure maintenance (35-33%), and (iii) nutrient cycling function contributing
288 the less (24-28%). Under CA 2a, functions were more evenly distributed, with relative
289 contribution of the three functions reaching around one third of the final index.

290 **4) Discussion**

291 This study allowed to assess soil health under conservation agriculture, following a multi-
292 functional approach, taking into account the interactions between the different soil

293 compartments based on a consistent set of soil indicators previously selected. The
294 integration of all indicators in a multivariate analysis enabled to understand how soil
295 functions are impacted by contrasted agricultural practices under a tropical climate on a
296 clayey Oxisol.

297 **4.1) Impact of Conservation Agriculture on soil health**

298 4.1.1) Impact of CA on carbon transformation function

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

312 4.1.2) Impact of CA on the nutrient cycling function

313 Soil total nitrogen is higher under CA systems than CT with significant difference when
314 comparing CT with CA 2a and CA 2b (Table 2). This trend is also observed for available NH₄⁺
315 and NO₃⁻. This result may be linked to the higher activities of decomposers and microbial

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

329 4.1.3) Impact of CA on the structure maintenance function

330 The absence of tillage combined with presence of soil surface residues, living root systems of
331 the cover crops during the dry season globally improved the structure maintenance function,
332 which confirms the conclusions made on the improvement of soil structure under CA
333 cropping systems (Hobbs et al., 2008; Indoria et al., 2017; Tivet et al., 2013). By contrast with
334 the study conducted by Castioni et al. (2018) on a Brazilian Oxisol (55% clay), VESS was less
335 sensitive to detect soil structure changes compared with aggregate stability. Thoumazeau et
336 al. (2019b) also found that VESS was not sensitive to land management based on a
337 perturbation gradient with very contrasted land uses. In our system, the superficial layer (0-5
338 cm) seemed to be the main part affected by CA practices. This soil layer may not have been

339 studied precisely enough with the VESS method to raise differences between systems. In
340 order to answer this issue, methodological improvements should be proposed to refine the
341 scoring methods and to readapt it to the studied system (Emmet-Booth et al., 2018). Then,
342 the better aggregate stability under CA is in agreement with several studies including those
343 by Nascente et al. (2015) and Ivy et al. (2017) under similar pedo-climatic conditions. Also, It
344 is recognized that CA-based cropping systems lead to stratification of soil aggregates and C
345 content (Mrabet, 2002; Patra et al., 2019; Tivet et al., 2013), with the highest accumulation
346 and aggregation on the surface layer. On the opposite, tillage disrupts top soil aggregates
347 which leads to a uniform aggregates and carbon distribution. The stratification ratio,
348 calculated as $\text{AggSurf}/\text{AggSoil}$ (Franzluebbers, 2002) ranged from 0.76 under CT to 1.42
349 under CA 2b. This stratification under CA can be directly attributed to the absence of soil
350 disturbance combined with higher biomass-C inputs from aboveground residues, root
351 systems that strengthen soil aggregation in surface layers and contribute to build up a more
352 stable aggregated soil matrix (de Oliveira Ferreira et al., 2018; Indoria et al., 2017; Six et al.,
353 2004). Despite a high variability among CA results, soil water infiltration tends to increase
354 under CA cropping systems. It is commonly recognized that water infiltration is improved
355 under no-tillage and residue cover (Powlson et al., 2011).

356 4.1.4) Global impact of CA on the soil functions

357 The main difference observed in terms of soil functioning was observed between
358 conventional tillage (CT) and CA systems (CA 1, 2a and 2b). The Biofunctool® index showed a
359 better soil health under CA than under CT. Therefore, in addition to the positive impact on
360 numerous individual soil properties (Das et al., 2014; Hobbs et al., 2008; Kumar and Goh,

361 2000; Thierfelder and Wall, 2012), the multi-functional assessment of soil functions showed
362 an improvement of soil health under CA.

363 **4.2) Impact of rotation on soil health**

364 4.2.1) Impact of rotation on the three soil functions

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

380 No difference could be assessed between the three CA treatments for the nutrient cycling
381 function, despite nutrient cycling is usually significantly impacted by the nature of the
382 organic inputs and the long-term diversity of the cropping systems (Franchini et al., 2001;
383 Pavinato and Rosolem, 2008). At the time of the soil sampling in November 2017, residues

384 on the top soil of each cropping system were at different stages of decomposition and
385 mineralization. Under CA 2b, maize was harvested almost 4 weeks before the soil sampling.
386 Therefore, the decomposition process had started and, in addition, a new mix of cover crops
387 was sown (i.e., sorghum and sunnhemp) potentially leading to new rhizodeposites. By
388 contrast, under CA 1 and CA 2a, rice-bean was still under vegetative development and the
389 residues of former cover crops were already decomposed. Although this could not be
390 highlighted by our measurements, the residues' diversity (i.e., crops and mix of cover crops)
391 may have provided organic inputs with different characteristics that may have impacted
392 microbial communities build-up and turnover rate, thus differentiating kinetics of
393 decomposition processes and nutrients releases (Giacomini et al., 2003). Further
394 investigation on nutrient dynamics, as affected by rotations, should further be handled to
395 better understand the cycles. Also, the ion exchange membrane indicator could not be
396 measured in this study and prevented us to conduct a complete assessment of the nutrient
397 cycling function.

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

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

406 4.2.2) Global impact of rotation on soil health

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

420 ***4.3) Is Biofunctool® adapted to annual cropping system?***

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

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

437 **Conclusion**

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

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

457 **Conflict of interest**

458 None.

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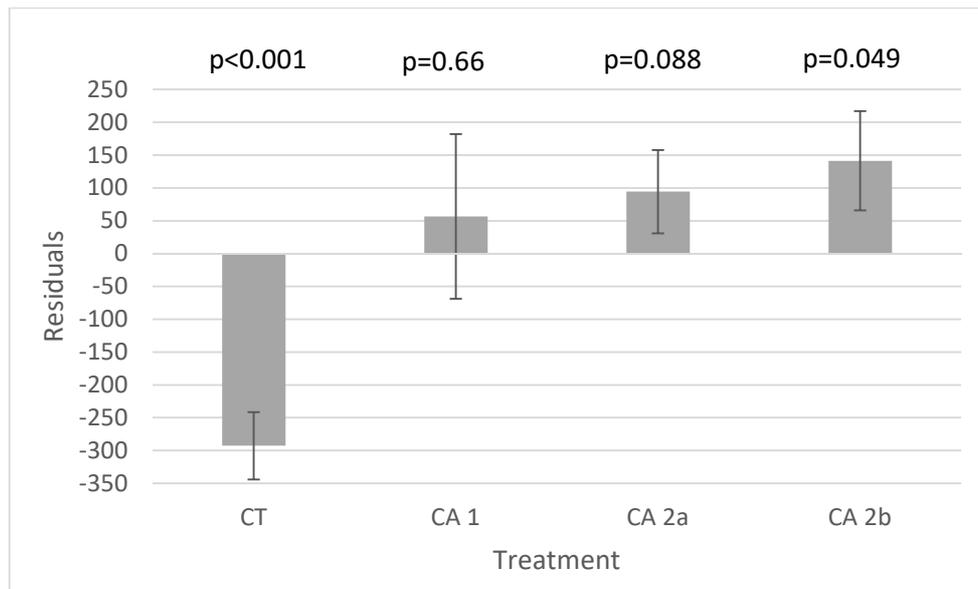


Figure 1. Mean values of regression residuals per treatment (n=9). (CT) Monoculture of soybean under conventional tillage, (CA 1) Monoculture of soybean under permanent cover crops, (CA 2a) Bi-annual rotation soybean-maize under conservation agriculture, and (CA 2b) Inversed bi-annual rotation maize-soybean 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 ($\alpha=0.05$).

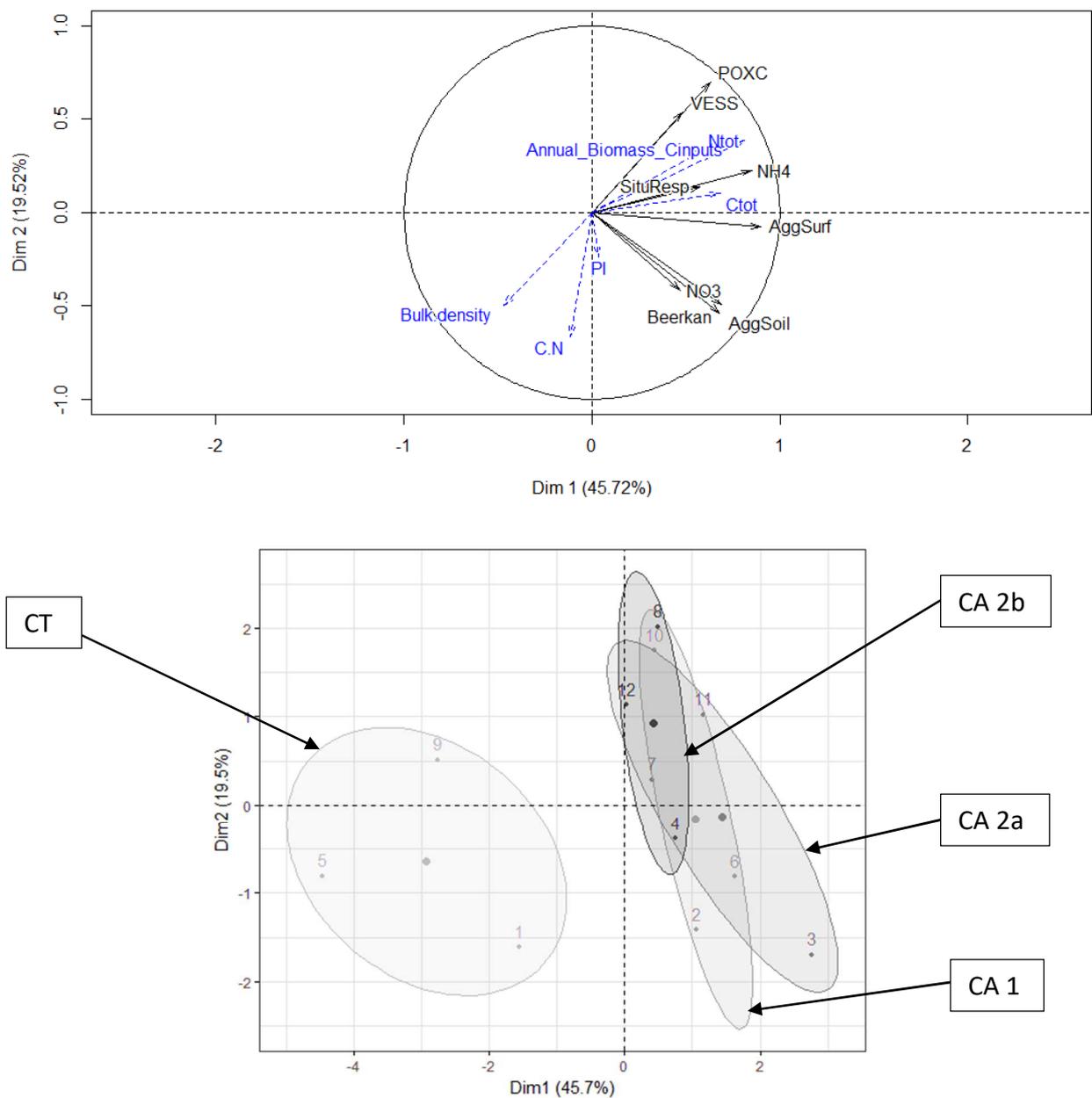
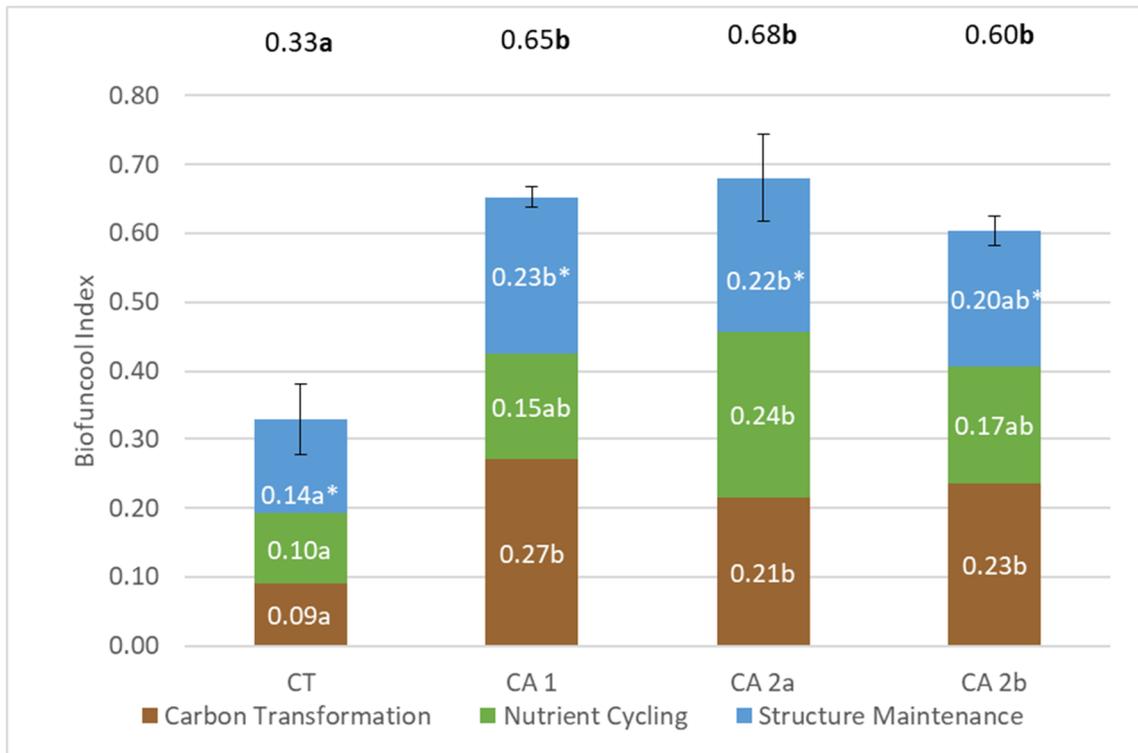


Figure 2. Principal component analysis for the impacts of contrasted cropping systems on soil health.

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.

Note: 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.



Contributions to the Biofunctool Index

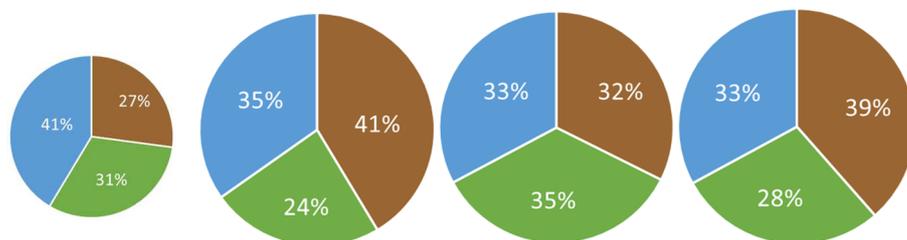


Figure 3. Biofunctool Index per treatment. (CT) Monoculture of soybean under conventional tillage, (CA 1) Monoculture of soybean under permanent cover crops, (CA 2a) Bi-annual rotation soybean-maize under conservation agriculture, and (CA 2b) Inversed bi-annual rotation maize-soybean under conservation 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: *Crotalaria 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.

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}	
	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 $\log(\text{Total Carbon})$ 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 NO₃⁻ and NH₄⁺. Letters indicate significant differences according to Tuckey test.

Treatment	Carbon transformation				Structure maintenance								Nutrient cycling			
	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 NO₃ without normality of residuals (p-value of shapiro test < 0.05)