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## Relationships between physico-chemical, biological and functional approaches for soil quality assessment. A case study along a gradient of disturbance

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1 Relationships between physico-chemical, biological and functional approaches  
2 for soil quality assessment. A case study along a gradient of disturbance.

3 Pusanisa Heepngoen<sup>1,2,3</sup>, Alexis Thoumazeau<sup>2,3,4,5\*</sup>, Marie-Sophie Renevier<sup>2,6,7</sup>, Kannika Sajjaphan<sup>1,3\*</sup>,  
4 Frédéric Gay<sup>3,4,5</sup>, Alain Brauman<sup>2,6\*</sup>.

5 <sup>1</sup>Department of Soil Science and Center for Advanced Studies in Agriculture and Food, Kasetsart  
6 University, 10900 Bangkok, Thailand

7 <sup>2</sup>LMI LUSES, Land Development Department, 10900 Bangkok, Thailand;

8 <sup>3</sup>HRPP, Kasetsart University, 10900 Bangkok, Thailand;

9 <sup>4</sup>ABSys, Univ Montpellier, CIHEAM-IAMM, CIRAD, INRAE, Institut Agro, Montpellier, France

10 <sup>5</sup>CIRAD, UMR ABSys, F-34398 Montpellier, France

11 <sup>6</sup>Eco&Sols, Univ Montpellier, CIRAD, INRA, IRD, Montpellier SupAgro, F-34398 Montpellier, France;

12 <sup>7</sup>Faculté des Biogeosciences, Université de Lausanne, 1015 Lausanne, Switzerland;

13 \*Corresponding author:

14 Alexis Thoumazeau  
15 CIRAD – UMR ABSys  
16 TA B-34/02  
17 F-34398 Montpellier Cedex 5  
18 +33 (0)4 67 61 59 82  
19 alexis.thoumazeau@cirad.fr

20 Kannika Sajjaphan  
21 agrkks@ku.ac.th

22 Alain Brauman  
23 alain.brauman@ird.fr

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## 25 **Abstract**

26 The assessment of the impacts of land management on soil quality is crucial in the current  
27 environmental context. Among the many approaches available to assess soil quality, most of studies or  
28 monitoring programs consist in the measurement of stock measurements using an additive approach of  
29 physical, chemical and biological, parameters. More recently, functional methods have been developed  
30 to provide tools which better account the abiotic-biotic interactions. The objective of our study was to  
31 evaluate and compare the capacity of physico-chemical, biological and functional parameters to assess  
32 the effect of land management on soil quality over a gradient of disturbance based on rubber tree  
33 plantations in Chachoengsao province, Thailand. Three sets of indicators were applied based on i. soil  
34 physico-chemical analyses, ii. biological analyses, based on soil free-living nematode indices iii. functional  
35 analyses, with Biofunctool® indicators linked to three main soil functions (carbon transformation,  
36 nutrient cycling, soil structure maintenance). A soil quality index resulting from the aggregation of each  
37 set of indicators into a single score showed that functional assessment was the most sensitive to the  
38 gradient of disturbance. Co-inertia analysis between sets revealed a significant relationship between  
39 functional analysis and both physico-chemical and biological sets of indicators, whereas the two latter  
40 were not related. Our results validated the ability of the functional method to better reflect the  
41 complexity of the abiotic-biotic interactions of the soil system

42

43 Key words: soil quality, physico-chemical properties, nematode indices, functional indicators,

44 Biofunctool®, co-inertia analysis

## 45 1. Introduction

46 Human activities, especially land management for agricultural production, can cause severe disturbance  
47 to the soil. Those disturbances disrupt the ability of soil to function and consequently jeopardize its  
48 ability to provide ecosystem services such as food provision, biodiversity conservation, climate regulation  
49 [1]–[3]. In response to these threats, there is growing awareness of the need for sustainable agriculture  
50 to preserve and restore soil quality. Soil quality is recognized by the scientific community as “the capacity  
51 of a specific kind of soil to function [...]” [4]. However, the measurement of soil quality implies a huge  
52 number of tools and methodological approaches, reflecting the absence of agreement on soil quality  
53 assessments.

54 Physico-chemical (PC) indicators are still the most frequently used for soil quality assessment [5], for  
55 technical reasons and because they are paramount quantifiable indicators in an agronomical perspective  
56 such as soil nutrient quantification [6]. Approaches based on PC parameters often rely on the description  
57 of soil status with stock measurements, omitting soil dynamics and complexity [7]. Indeed, these  
58 approaches do not account for soil organisms which are the main driver of soil processes and are  
59 adaptive to short term changes [8]–[10].

60 Biological analysis are more and more integrated in soil quality assessment programs [6], whereas they  
61 are still underrepresented in the literature [5]. This scientific enthusiasm to apply biological indicators  
62 makes it possible to enlarge and precise the global effect of land management or disturbance on soil  
63 systems [11]. Among biological approach, free-living soil nematodes are one of the most promising biotic  
64 assemblage to assess soil quality [12], [13]. Occupying key positions in the soil food web and being highly  
65 interactive with other soil organisms, nematodes are representative of numerous trophic groups, soil  
66 food web links and life strategies [14]. Based on nematode assemblage taxonomical analysis, nematodes  
67 indices have been widely used to describe soil food web response to soil disturbance or stress [15], [16].  
68 Nematodes indices provide information on soil food web structure and maturity, decomposition

69 pathway, as well as fertility levels and soil suppressiveness to pathogens [17].

70 Using sets of independent soil physical, chemical or biological indicators might not be sufficient to reflect

71 the complex nature of the soil system since it does not integrate emergent properties emerging from

72 biotic-abiotic interaction [18], [19]. Several authors have underlined the need to develop functional

73 approaches, also described as integrative approaches [20]. With functional approaches, indicators aim

74 to target critical processes directly linked to one or more of the soil functions by measuring dynamic of

75 state variable, related fluxes or transformations that are output of biotic-abiotic interactions [21]. The

76 main distinction between functional approaches and other approaches is that functional approaches

77 don't measure structure of soil biota or soil physico-chemical context, it does directly measure the result

78 of their interaction with directly assessing soil functions, i.e., carbon transformation, nutrient cycling,

79 structure maintenance [19], [20]. Biofunctool® is a recently developed set of indicators that were

80 selected using expert opinion for their functional characteristics (i.e. measure the output of soil biotic-

81 abiotic interactions) [19]. Biofunctool® set of indicators aims to provide a global overview of the effect of

82 land management practices on three main functions of the soil (carbon transformation, nutrient cycling

83 and structure maintenance).

84 In order to better understand the sensitivity and how those approaches may be complementary to

85 assess soil quality, we decided to apply the three approaches (PC, biological, functional) together in the

86 same site. We applied the three sets of indicators along a gradient of disturbance including cassava

87 monoculture, rubber tree plantation of different ages and a forest in Chachoengsao province, Thailand.

88 We hypothesize that i) the functional approach reflects physico-chemical and biological attributes of the

89 soil, ii) the physico-chemical and biological approaches are complementary and when used

90 independently, do not allow to provide a global vision of soil quality as they do not account for biotic-

91 abiotic emergent properties.

## 92 2. Material and methods

### 93 2.1. Site description and experimental design

94 The study site was located around the Rubber Research Center of Chachoengsao, that belong to the  
95 Chachoengsao province in South-Eastern Thailand (13°41'N, 101°04'E). Soils are classified as Ultisols in  
96 the USDA soil classification and belong to the Kabin Buri series. Soil texture in the 0-10 cm layer is made  
97 of 21% clay, 21% silt and 58% sand. The study site is located under a tropical monsoon climate, with a  
98 mean annual temperature of 28°C and with mean annual precipitation of 1328 mm per year (Thai  
99 Meteorological Department). Information of the history and management of the study site are described  
100 in [22] and [23].

101 A randomized block design experiment was established consisting in five land management contexts,  
102 three replicates (blocks - smallholder's farmer plots) and three inner-plot replicates placed along a  
103 diagonal transect. Plots were approximately 2 km to each other (Supplementary reading 1). The five land  
104 management are (i) cassava (*M. esculenta*) (C), rubber tree plantations (*H. brasiliensis*) of different ages;  
105 (ii) 10 years old (Y), (iii) 13 to 17 years old (M) and (iv) >24 years old (hereafter O), and (v) a forest (F). The  
106 forest was uncultivated but partially degraded as it was part of the Chachoengsao Rubber Research  
107 Center domain. In total, we collected soils in 45 positions (5 land management x 3 true replicates x 3  
108 inner-replicates). Soil samples were collected either with an auger or with soil sampling cylinders. All  
109 measurements were performed on the 0–10cm soil layer, except for visual observation of soil structure  
110 (hereafter VESS) that applied to the first 25 cm depth.

111 For experimental reasons, nematodes indicators were sampled only in cassava and rubber plots in 2014  
112 whereas Biofunctool® and PC indicators were sampled in 2016. The two sampling years had similar  
113 climatic conditions and soil samples were collected at the end of the rainy season (November) to  
114 maximize soil biotic activities. Previous study done by the same team in this experimental site [23]  
115 demonstrated a weak change of biotic parameters (bacteria, fungi and macrofauna) within groups of

116 plots having four years of difference. Based on this, and the fact that we focus on a contrasted gradient  
117 of disturbance with rubber plantations representing a rather stable environment, we assumed that time  
118 lapse of two years will have a negligible effect on our analyses. Moreover, we focus on relative changes  
119 in soil quality rather than absolute soil quality quantification. In all cases, the key rubber stand age  
120 affecting soil quality were integrated such as immature stage, intermediate mature stage and last  
121 mature stage [22], [23]. Implementing analysis of relative changes taking into account all stage thus  
122 seem robust to tackle this sampling year difference.

## 123 **2.2. Measurements implemented for each set**

### 124 **2.2.1. Set of physico-chemical indicators (PC)**

125 The selection of the physico-chemical indicators was based on the critical review published by [5]. We  
126 selected the most frequently used indicators in the literature (frequency of soil quality indicators > 40%),  
127 based on figure 4 in the above-mentioned review (i.e. total C, total N, available P and K, pH, bulk density,  
128 soil moisture content). Only two small adaptations were made. The first is that soil texture was not  
129 included as a soil quality variable, as we did not consider soil texture to a sensitive indicator to land  
130 management, but rather an environmental filter describing inherent soil properties [22] and [24].  
131 Eventual influence of soil texture variation on the results was previously checked for Biofunctool® data  
132 set in [22] and proved negligible. The second modification was that Ca and Mg macronutrients were  
133 included in the set as they are crucial indicators of soil cation exchange capacity even if their frequency  
134 in the literature was 16.9% [5].

135 Fresh soil samples were weighed and dried at 105 °C for 24 h to measure soil moisture and bulk density.  
136 Laboratory analysis were performed by the Soil Laboratory of the Land Development Department in  
137 Bangkok. Soil samples were air-dried and then sieved at 2 mm. pH was determined in distilled water (1:1  
138 soil-water ratio). Available phosphorus was determined using the Bray II method [25]. K, Ca and Mg in  
139 soil solution were extracted by neutral 1 N ammonium acetate [26] and analysed by flame photometer

140 (Sherwood model 420) for K and Ca and by Atomic Absorption spectrophotometer (Shimadzu AA 6200)  
141 for Mg. For C<sub>tot</sub> and N<sub>tot</sub>, sub-samples of 2 mm sieved soils were finely ground (< 150 μm) and total C  
142 and N concentrations were analysed by dry combustion using an elemental CHN analyser (Thermo Flash  
143 2000) in the Eco&Sols laboratory in Montpellier, France.

#### 144 **2.2.2. Set of nematodes indicators (Nem)**

145 Nematodes were extracted from 250 g of soil (fresh weight) using an elutriation method [27] with one  
146 filter paper (Whatman, UK No.1, 125 mm diameter) and 50-micron pore size aperture sieve, before being  
147 fixed in 4% formaldehyde solution. The extraction-incubation phase lasted 48h under dark and control  
148 temperature room at 25 (±2) °C. Nematodes were counted and identified to the genus or family level in  
149 ELISOL laboratory in Montpellier, France. Nematodes were then classified into one of the five colonizer-  
150 persister functional groups based on [28], [29]. The abundance of nematodes was consistent enough to  
151 calculate indices with abundance average  $n = 433.8 \text{ ind} (\pm 341.73)$  for non-plant feeders and  $n = 160.04$   
152  $\text{ind} (\pm 100.39)$  for plant feeders. Six nematode indices were calculated : (i) Maturity Index (MI) [28], (ii)  
153 Plant-Parasitic Index (PPI) [30], (iii) Basal Index (BI), (iv) Enrichment Index (EI) and (v) Structure Index (SI)  
154 [15], (vi) Nematod Channel Ratio (NCR) [16]. In this study, the NCR was slightly modified in that  
155 facultative phytophagous nematodes (categories 1e and 1f from [31]) were counted as fungivorous  
156 nematodes. The above-mentioned indices reflect processes leading to ecosystem functioning, but do not  
157 indicate the magnitude of the processes [17]. We therefore added to the selected indices the nematode  
158 density representing the abundance of nematodes.



### 159 2.2.3. Biofunctool® set of indicators

160 In this study, the data set in [19] at “Chachoengsao” site was used for the Biofunctool® indicators. The  
161 sampling protocol and measurement methods for the ten indicators are described in [19] and briefly  
162 described below in this section.

163 For soil carbon transformation function, short-term turnover soil carbon pool was assessed directly in  
164 the field with Permanganate OXidizable Carbon method [32]. POXC is a processed fraction of labile soil  
165 organic carbon [33]. Soil organisms activity was evaluated with the Lamina baits test (Lamina, [34]) and  
166 the cast density measurement (Cast) at the soil surface. Lamina baits assess soil mesofauna activity  
167 through the decomposition of an organic substrate embedded in sixteen holes of a plastic stick and  
168 inserted into the topsoil during 15 days. Measuring the cast mass on a given soil surface allows to  
169 evaluate earthworm activities [19]. CO<sub>2</sub> release by microorganism was assessed with the SituResp®  
170 method [35]. SituResp® evaluates soil basal respiration in the field and is based on the color changes of a  
171 pH-sensitive gel over 24-h incubation.

172 For the nutrient cycling function, soil available mineral nitrogen (NminSoil) was determined from fresh  
173 soil extraction in a 1M KCl solution, quantifying soil available NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> at one time. Nitrate  
174 dynamics in the soil was evaluated with the quantification of NO<sub>3</sub><sup>-</sup> adsorbed on 6x2cm anion exchange  
175 membrane inserted at 8cm depth for 15 days (AEMNO3) [36], [37]. Nutrient cycling measurements in  
176 Biofunctool® set differs from PC set as it focuses on nutrient dynamics (i.e. available form of nitrogen on  
177 actual wet soil rather than on nutrient stocks on disturbed dried soil).

178 For soil structure maintenance function, the stability of surface (0-2cm) (AggSurf) and soil (2-10cm)  
179 (AggSoil) aggregates was evaluated after an immersion or slaking period in water [38]. Soil infiltration  
180 capacity was measured with the Beerkan test adapted from [39], pouring a fixed volume of 310mL of  
181 water in a 20cm diameter ring at the soil surface. Soil horizons structure was finally scored with the  
182 visual evaluation of soil structure (VESS) method [40].

### 183 2.3. Statistical analysis

184 Statistical analyses were computed using the version 3.6.3 of the R software [41]. Some outliers (within-  
185 plot replicate values) were observed in the PC and Nem sets and were removed. Within-plot replicate  
186 values were considered as outliers if they were out of the boxplot (lower than  $(Q1 - 1.5 * \text{Interquartile}$   
187  $\text{Range})$  or higher than  $(Q3 + 1.5 * \text{Interquartile Range})$ ) for, at least, two indicators in the same set. In total,  
188 16 values (only 2.7% of the data set) were removed from PC and Nem dataset. This data pre-processing  
189 was especially needed because of high data-set variability linked to the integration of inner-replicates in  
190 the analysis. For Biofunctool®, no outlier were removed as [22] already computed outlier analysis.  
191 First, univariate analysis was implemented and each indicator was studied separately using a linear-  
192 mixed effects model (package lme4, [42]). Treatment was defined as fixed factor and replicates (plots  
193 and inner-replicates) as random factors. After checking the normality of the model's residuals and  
194 homoscedasticity of variances' residuals, ANOVAs were run using the car package [43]. This was followed  
195 by posthoc mean comparisons, using Tukey with adjustment Bonferroni [44]. For three variables (BI,  
196 AggSurf, Lamina), preliminary conditions for the tests were not met, Kuskall-Wallis non-parametric tests  
197 were computed on plots averages in those specific cases.  
198 Then, principal component analyzes (PCA) [45] were performed on each set of indicators to analyze their  
199 response to land management systems. PCAs were made on within-plot replicate average values for PC  
200 and Biofunctool® and on replicates values for Nem. The significant effect of land management on the set  
201 of indicators was assessed through a between-class analyzes (BCA) with a Monte Carlos permutation test  
202 (999 permutations). The number of variable per set of indicators was different with seven variables for  
203 Nem, nine for PC and ten for Biofunctool®. This difference is too weak to explain eventual discriminatory  
204 potential between sets. Indeed, with sets of seven to ten variables, discriminatory differences will be  
205 more linked to variable sensitivity to land management and non-redundancy, than variable number in  
206 multivariate analysis.

207 To provide a comparison of the sensitivity of the three sets of indicators to the different land  
208 management, three soil quality indexes (SQIs) using separately PC, Nem and Biofunctool® dataset were  
209 calculated following [22]. The soil quality index (SQI) is a statistical method allowing the aggregation of  
210 several indicators into a single quality score based on weightings derived from multivariate analysis (PCA)  
211 [46]. SQI were calculated based on normalized within-plot replicate average values. Response curves for  
212 each indicator was determined based on literature and/or expert judgment (Supplementary reading 2).  
213 Analyses of variance (ANOVAs) on soil quality scores were performed to assess the effect of land  
214 management on SQIs. Preliminary assumptions (normality and homoscedasticity) were checked before  
215 implementing the ANOVAs. A post-hoc Tukey multiple test was performed when a significant effect of  
216 land management on SQIs was found [47].

217 Finally, co-inertia analysis (COIA) was used to characterize relationship between physico-chemical,  
218 biological and functional approaches. Based on a covariance optimization criterion, co-inertia analysis is  
219 a multivariate method that identify co-variant patterns between two sets of variables [48]. COIA aims at  
220 seeking axis that maximize the covariance between a group of variables of the two sets of variables. In  
221 this study, three co-inertia analysis were performed on (i) PC and Nem, (ii) Biofunctool® and PC, (iii)  
222 Biofunctool® and Nem PCAs. The strength of the relationships between the sets of indicators were  
223 evaluated with the RV coefficient and the statistical significance of the COIAs were tested with a Monte  
224 Carlo 999 permutation test on the sum of eigenvalues of the COIAs [48].

## 225 **3. Results**

### 226 **3.1. Analysis of the set of indicators**

#### 227 **3.1.1. Soil quality assessment using the set of PC indicators**

228 Univariate analysis in Table 1 raise two trends. First, an increase in Ctot, Ntot, P, Ca, Mg, pH was  
229 observed along the perturbation gradient, with significant differences detected between cassava crop

230 (C), old rubber plantation (O) and forest (F) ( $p < 0.05$ ). For K, BD and Moisture, no general trend were  
231 observed in accordance to the gradient.

232 The first axis of the PCA (Figure 1a) represents 48.6% of the total inertia and separates the forest from  
233 the other land management systems. Although the young (Y) and old (O) rubber plantations can be  
234 distinguished along the first axis, the intensive cash crop and rubber plantations ellipses overlap (Figure  
235 1a). Ctot, Ntot, Ca and Mg variables contribute the most to the first axis. The second axis represents less  
236 total inertia (25.2%) and is more related to inter-plot variability within cropland and perennial land  
237 management. P, K and Moisture indicators mostly explain this variability. The PCA ellipses in Figure 1a  
238 are in line with the SQI scores (Figure 1b). All the scores of the cassava and rubber plantation sites  
239 oscillated between 0.28 and 0.38 with no significant difference among them. With a score of 0.6, only  
240 the forest differed significantly from the other land management systems.

### 241 **3.1.2. Soil quality assessment using the set of Nem indicators**

242 All nematodes indicators showed a tendency to increase or decrease along the gradient of perturbation  
243 (Table 1). The structural index (SI) and enrichment index (EI) were higher in, respectively, young (Y) and  
244 old (O) rubber plantations than in cassava crops (C) ( $p < 0.05$ ) (Table 1). Nematode density and plant-  
245 parasitic were higher and lower respectively in old rubber plantations (O) than in the younger one (Y)  
246 ( $p < 0.05$ ).

247 The PCA analysis of nematode indices (Figure 2a) differs from the analysis of PC. Indeed, the Nem PCA  
248 better discriminates cassava crop (C) from mature (M) and old (O) rubber plantations along the first axis  
249 than the PCA for physico-chemical analysis (PC).

250 This first axis represents 53.1% of the total inertia and is related to the gradient of disturbance from  
251 annual cassava crop plantations to old rubber tree plantations. However, the first axis does not separate  
252 the rubber plantation (Y) ellipse from the C and M ellipses. Most of the variables contribute to the first  
253 axis, particularly the SI, MI and BI indices. The second axis represents 19.2% of the total inertia and, like

254 the PC indicators, is more related to inter-plot variability within each land management system. This  
255 variability is mostly explained by NCR\*. While the PCA shows a continuous gradient from cassava to old  
256 rubber plantation land management, the scores of the Nem SQI are slightly higher (0.49 to 0.74) but do  
257 not significantly differ between land management systems ( $p=0.063$ ) (Figure 2b).

### 258 **3.1.3. Soil quality assessment using the set of Biofunctool indicators**

259 Differences along the gradient of perturbation were mostly represented by an increase of soil labile  
260 carbon (POXC), soil respiration (SituResp<sup>®</sup>), soil aggregate stability (AggSoil), water infiltration (Beerkan),  
261 earthworm cast (Cast) and soil available nitrogen (NminSoil) with significant differences detected  
262 between land management (Table 1).

263 The first axis of the multivariate analysis (Figure 3a) represents most of the inertia (46.1%). Axis 1 is  
264 driven by the majority of variables, particularly soil respiration (SituResp<sup>®</sup>), labile carbon (POXC) and  
265 earthworm cast densities (Cast). This axis is directly connected with the gradient of disturbance from  
266 cassava (C) and young rubber plantations (Y) to the forest (F). The second axis represents only 14.4 % of  
267 the total variability and is related to inter-plot variability within each land management system. VESS and  
268 Lamina indicators in particular are linked to this spatial variability. The PCA analysis (Figure 3a)  
269 differentiated three main clusters, the first one grouping cassava (C) and the young rubber trees (Y), the  
270 second grouping mature and old rubber plantations (M and O) and the last one is represented by forest.  
271 This pattern can also be observed in the SQI results, with significant differences between the land  
272 management systems (Figure 3b). When plantations reach the age of 13 (O), a shift in soil functioning  
273 occurs with a 50% increase in soil quality. Finally, the forest reference (F) reached the highest score with  
274 a soil quality index of 0.79, nearly twice that of cassava (C) and young rubber plantations (Y).

## 275 **3.2. Comparison between pairs of indicator sets**

### 276 **3.2.1. Co-variation between physico-chemical (PC) and nematode (Nem) indicators**

277 The co-inertia analysis (COIA) shows no significant co-structure between PC and Nem ( $RV= 0.37$ ,  $p > 0.3$ ).

278 The different land management are placed along the first axis in an order reflecting the gradient of  
279 disturbance. This first axis accounts for 78.62% of the total inertia (Figure 4a). Most Nem variables  
280 (except NCR\*) contribute to the first axis, while among PC variables, total carbon (Ctot) and total  
281 nitrogen (Ntot) contribute the most to the first axis (Figure 4b and 4c). The second axis accounts for  
282 16.32% of the total inertia. Among Nem variables, the decomposition pathway indicator (NCR\*) and the  
283 plant parasitic index (PPI) account for most to the inertia on the second axis, whereas among the PC, the  
284 second axis is driven by the soil bulk density (BD) and pH. The barycenters of PC and Nem in the mature  
285 rubber tree plantation (M) are overlapping, revealing a similar trend between the two sets of indicators  
286 for this land management system. However, some individuals in each land management system overlap  
287 the others.

288 **3.2.2. Co-variation between functional (Biofunctool®) and physico-chemical (PC)**  
289 **indicators**

290 The COIA revealed a significant co-structure between Biofunctool® and PC (RV coefficient=0.57,  $p < 0.02$ )  
291 (Figure 5a). The first axis of the co-inertia analysis accounts for 50.93% of the total inertia and is  
292 associated with the gradient of disturbance. The second axis accounts for 27.48% of the total inertia and  
293 is more related to differences between Biofunctool® and PC in the cassava system (C) and in mature  
294 rubber tree plantations (M). However, the barycenter of Biofunctool® and PC in young (Y) and old (O)  
295 rubber plantations completely overlap. The analysis differentiates cassava and young rubber tree  
296 plantations from the other plantations. However, individuals in mature and old plantations overlap.  
297 Figures 5b and 5c show that the main drivers of axis 1 are the infiltration rate (Beerkan) and labile carbon  
298 (POXC) for Biofunctool®. For PC, axis 1 is rather explained by total carbon (Ctot) and total nitrogen (Ntot).  
299 Concerning axis 2, the differences are mainly explained by the lamina bait decomposition as well as the  
300 soil available nitrogen (NminSoil) for Biofunctool® and by the pH for PC.

301 **3.2.3. Co-variation between functional (Biofunctool) and Nematode (Nem) indicators**

302 The COIA revealed the most significant co-structure between Biofunctool and Nem (RV=0.6,  $p < 0.01$ )  
303 (Figure 6a). The first axis of the co-inertia analysis accounts for 81.43% of the total inertia which is  
304 notably higher than that observed previously (part 3.2.2; Figure 5a). Axis 1 is generally linked to the  
305 gradient of disturbance and differentiates the three treatments. Concerning the variables, Figure 6b and  
306 6c show a more balanced distribution of the variables along the two axes for both Biofunctool® and  
307 Nem. Nevertheless, surface aggregate stability, water infiltration for Biofunctool® and the structural  
308 index (SI), basal index (BI) for Nem are more closely linked to the first axis than to the second whereas  
309 mineral nitrogen, cast density and exchangeable nitrate for Biofunctool® and the decomposition  
310 pathway (NCR\*) as well as nematode density for Nem are more related to the second axis.

311 **4. Discussion**

312 **4.1. How do the different soil quality assessment methods respond to the gradient of**  
313 **disturbance?**

314 **4.1.1. Case of physico-chemical indicators (PC)**

315 Among the soil quality datasets tested, the PC set showed the least potential ability to differentiate the  
316 land management systems. PC was able to highlight significant differences only between forest and the  
317 cash crops (cassava and rubber). Carbon content and soil nutrients (C<sub>tot</sub>, N<sub>tot</sub>, Ca and Mg) were the  
318 main variables that explained these differences. These results are in line with a panel of others studies  
319 that highlighted a decrease in soil chemicals between forest, mature rubber tree plantations [49]–[51]  
320 and intensive annual cropping systems [52]. Other chemicals (e.g. P and K) were rather linked to inter-  
321 plot variability, which was also observed by [53] for P content. This could be explained by variability of  
322 fertilization practices among smallholders [54].

323 The absence of a significant change in soil quality under different land use between intensive cash crop  
324 (cassava) and young rubber tree plantations, confirmed the results of previous studies conducted in the  
325 same agricultural and pedo-climatic context [23], [53]. We also confirmed the tendency of PC scores to  
326 increase with ageing rubber trees, as shown in recent studies [23], [53], [55]. However, the conditions  
327 and the land management systems we studied may not have met the sensitivity thresholds of the PC  
328 indicators. Indeed, tropical soils are well known for their low carbon and nutrient status [56]. This low  
329 stock status may make PC indicators more sensitive to uncontrolled variability than to management  
330 practices such as tillage, canopy closure, litter quantity/quality [57].

331 **4.1.2. Case of biological indicators (Nem)**

332 The Nem quality index was not significantly affected by land management ( $p=0.065$ ), despite the fact it  
333 tended to increase with the gradient. This cannot be explained by the slight differences in the system  
334 studied for each set (with and without forest), which had no impact on observed sensitivity  
335 (Supplementary reading 3 and 4). The structural index (SI), basal index (BI) and maturity index (MI) were  
336 the main drivers of the first axis related to the gradient of disturbance. This result suggests that, with the



337 gradient of disturbance (from cassava to rubber), the soil food web became more complex (decrease of  
338 BI) and more structured (increase of SI, and MI). The same trend was also observed by [58] in a similar  
339 rubber chronosequence. The high BI level combined with the low SI in the cassava plot revealed stress  
340 and limited resources mostly due to more intense cultivation practices (tillage, pesticides etc.) which  
341 may negatively affect the structure of the soil food web [15]. In contrast, more abundant resources  
342 (inputs of litter, carbon and nutrient contents) and improved soil environment in the mature rubber  
343 plantation may be responsible for the increased structural and maturity index (SI and MI) [59].

#### 344 **4.1.3. Case of functional indicators (Biofunctool®)**

345 Among all the sets of indicators tested, functional indicators was the most sensitive to the gradient of  
346 disturbance. The Biofunctool® quality index was sensitive both to the different land use under cassava  
347 and rubber and under rubber and forest. The increase in the Biofunctool® index along the  
348 chronosequence from the rubber to the forest was mainly driven by carbon transformation variables  
349 (SituResp®, POxC, Cast). This result is in line with results of previous studies that demonstrated an  
350 increase in the labile carbon pool along rubber chronosequences [51], [57], higher soil biota abundance  
351 such as microbial biomass [60] and more abundant soil fauna [23], [61]. Thus, concerning the soil  
352 structure maintenance function, the increase in the biomass of soil engineers with ageing rubber trees  
353 [23] together with the absence of tillage, may explain the positive evolution of soil structure parameters.  
354 These results point to an improvement in soil functioning in older rubber plantations, but their scores  
355 were nevertheless lower than those of the forest.

## 356 **4.2. How are the different soil quality index related?**

### 357 **4.2.1. Physico-chemical indicators vs Nematode indices (PC vs Nem)**

358 We observed a non-significant relationship between PC and Nem. Despite these trends, the results of  
359 non-significant relationships enable a better understanding of the specificities of each set of indicators  
360 alongside the soil compartment they focus on. Nem and PC may have different thresholds of sensitivity

361 to changes in land management. For example, the results of this study suggest that chemical parameters  
362 were more affected by long-term fertilizing practices, whereas nematode communities were more  
363 affected by change in organic matter content, a global feature of nematodes [62] related to both  
364 practices (tillage) and organic input (rubber tree litter). An approach based on physico-chemical  
365 parameters might not be sufficiently exhaustive to reflect soil functioning complexity. Hence, this  
366 validate that, both, physico-chemical and biological parameters should be measured to have a  
367 comprehensive insight of the effect of land management practices on the soil quality [5].

#### 368 **4.2.2. Soil functions vs physico-chemical indicators (Biofunctool® vs PC)**

369 The co-inertia analysis revealed a significant co-structure between soil functions and the physico-  
370 chemical index. Several functional and PC indicators co-evolve along the first axis representing the  
371 gradient of disturbance. POXC and AEMNO<sub>3</sub>, indicators of the carbon transformation and nutrient  
372 cycling function, were all positively related to the first axis and in line with total soil stocks (carbon and  
373 nitrogen). This result demonstrates the consistency of two approaches based either on total stocks or on  
374 available nutrients. The methods react to the same extent but would be differently affected by short-  
375 term changes in soil quality. Soil mineral nitrogen (N<sub>minSoil</sub>) and soil fauna activity (Lamina) for  
376 functional indicators were positively related to soil pH for PC, confirming previous studies [63], [64]  
377 showing that, in acid soils, pH increase leads to an increase in nitrogen mineralization.

#### 378 **4.2.3. Soil functions vs Nematode indices (Biofunctool® vs Nem)**

379 These two sets of indicators had the highest significant co-structure among all those tested. This result  
380 indicates that functional and biological indicators are sensitive to the same drivers and time patterns.  
381 They both reflect an increase in soil ecosystem stability along the rubber chronosequence through the  
382 correlation between nematodes indexes linked to ecosystem stability (SI and MI), soil structure  
383 (aggregate stability and water infiltration) and carbon transformation variables. The restoration of the  
384 soil system after the end of a soil disturbance caused by tillage practices may explain these relationships.

385 Indeed, the SI is based on the prevalence of omnivorous and predatory nematodes which are known to  
386 be sensitive to soil disturbance including tillage [65]. Similarly, aggregate stability and water infiltration  
387 are strongly influenced by physical disturbances of the soil [66], [67].

388 **4.3. Physico-chemical, biological, or functional: which indicator to use to tackle which**  
389 **question?**

390 Three sets of indicators were tested in this study. They are complementary and may answer different  
391 questions. The advantages and drawbacks of each method are detailed hereafter.

392 Physico-chemical analysis enables quantification of chemicals in a soil system, which are particularly  
393 needed to help land managers adjust fertilization to the crop nutrient needs. For example, quantification  
394 of soil nutrient deficiencies is a key to plant performances and should be investigated before providing  
395 agronomical advice [68]. Likewise, knowledge of soil carbon stocks is needed to better understand the  
396 global carbon balance and to link it with adaptation to - or mitigation of - climate change [69]. However,  
397 these methods provide a general picture of the soil system but do not include the biological  
398 compartment which is a key to understanding soil functioning. These methods are thus insufficient to  
399 assess soil quality following the definition proposed by [4].

400 Despite the cost and expertise required for analysis, free-living soil nematodes are known to be a  
401 promising biological indicators of soil quality. Thanks to the link established between taxonomy and  
402 functional groups, nematodes groups provide key elements on soil functions that are explored in this  
403 study. Nematodes bio-indicators are thus appropriate tools to measure the impact of a disturbance on  
404 soil functioning. However, identifying further links between the bio-indication and soil process dynamics  
405 or soil ecosystem services could be difficult. Despite interesting impact-based assessments, further  
406 description of soil functions, which are needed to qualify soil quality, remain difficult to tackle using bio-  
407 indication [70].

408 A useful compromise in the assessment of soil quality that has been poorly applied so far is to focus on  
409 functional indicators of soil quality that integrate interactions between soil abiotic and biotic

410 compartments. The Biofunctool® functional indicators study three key soil functions: carbon  
411 transformation, nutrient cycling and structure maintenance, to describe soil complexity. No specific  
412 expertise is required to apply the indicators that are easily implementable. However, this integrative  
413 functional approach does not allow the examination of the soil processes behind soil functions and is  
414 currently little used as a diagnostic tool, compared to PC approach. Biofunctool® also remains a  
415 comparative approach for use in a specific context, and would require additional databases to provide  
416 absolute soil quality scoring such as current physico-chemical [71], or biological indicators [72].

## 417 **5. Conclusion**

418 This paper compared three sets of indicators to assess the impact of a gradient of disturbance on soil  
419 quality and thus improves our understanding of the degree of sensitivity of each set along the gradient.  
420 Comparing the sets revealed how the indicators evolved together over the gradient studied. Linkages  
421 between the soil physico-chemical, biological and functional indicators confirmed that functional  
422 indicators (*e.g.* Biofunctool® indicators) reflects both soil physico-chemical properties and bio-indicators,  
423 and could represent a good compromise to monitor soil quality.

## 424 **Author contribution statement**

425 **Conceptualization:** Alexis Thoumazeau, Kannika Sajjaphan, Frédéric Gay, Alain Brauman. **Methodology:**  
426 Pusanisa Heepngoan, Marie-Sophie Renevier, Alexis Thoumazeau, Alain Brauman. **Field measurement:**  
427 Pusanisa Heepngoan, Marie-Sophie Renevier, Alexis Thoumazeau. **Data Curation:** Pusanisa Heepngoan,  
428 Marie-Sophie Renevier, Alexis Thoumazeau. **Writing - Original Draft:** Pusanisa Heepngoan, Marie-Sophie  
429 Renevier, Alexis Thoumazeau, Alain Brauman. **Writing - Review & Editing:** Marie-Sophie Renevier, Alexis  
430 Thoumazeau and Alain Brauman. **Supervision:** Kannika Sajjaphan, Frédéric Gay, Alain Brauman. **Project**  
431 **administration:** Kannika Sajjaphan, Frédéric Gay, Alain Brauman. **Funding acquisition:** Kannika  
432 Sajjaphan, Frédéric Gay, Alain Brauman.

## 433 **Declaration of competing interest**

434 The authors declare that they have no known competing financial interests or personal relationships that  
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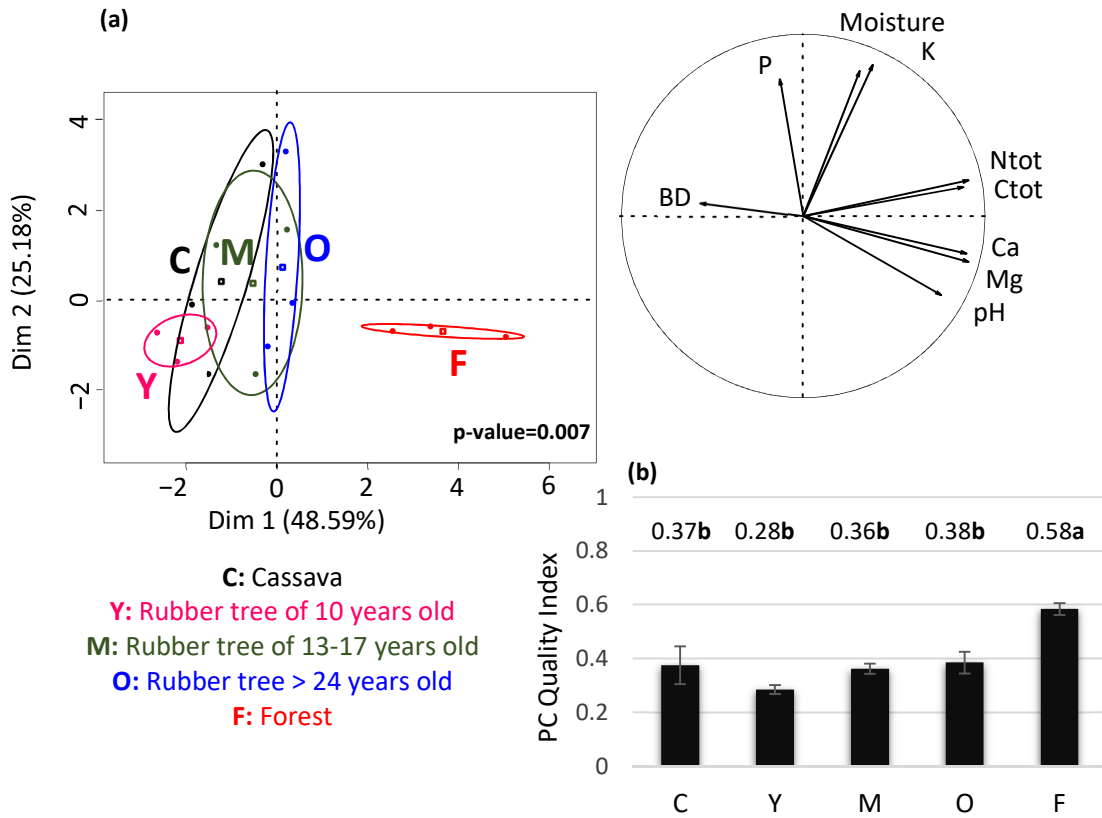
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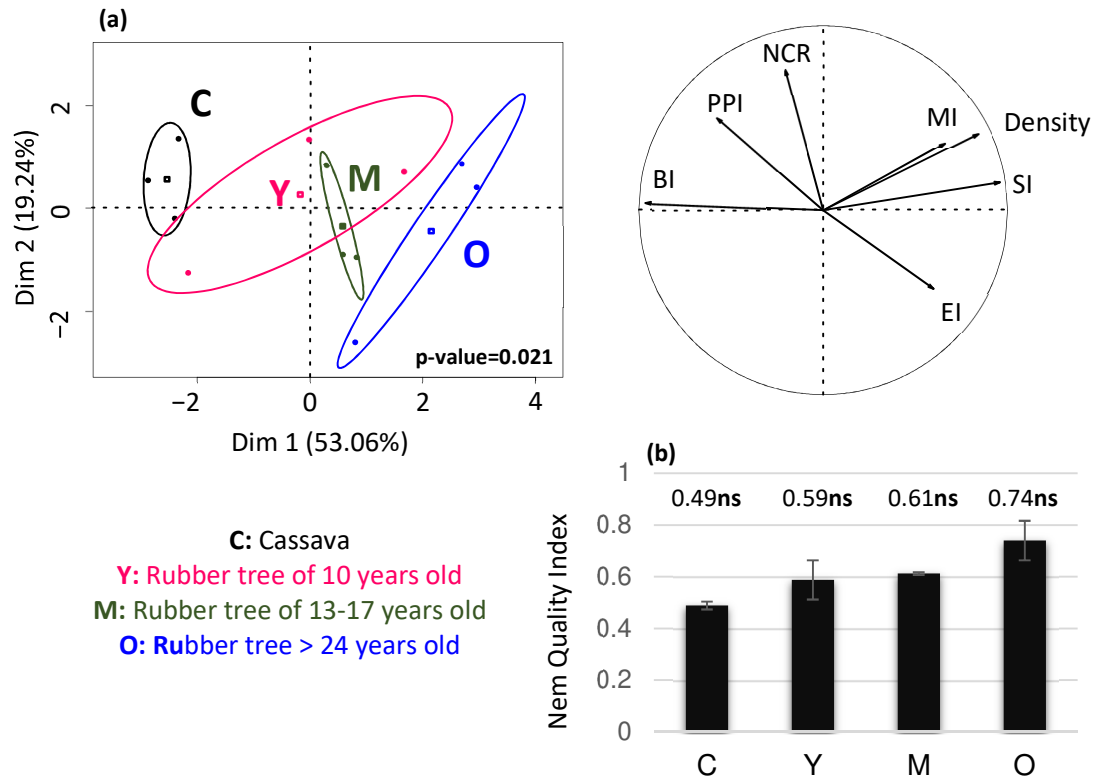
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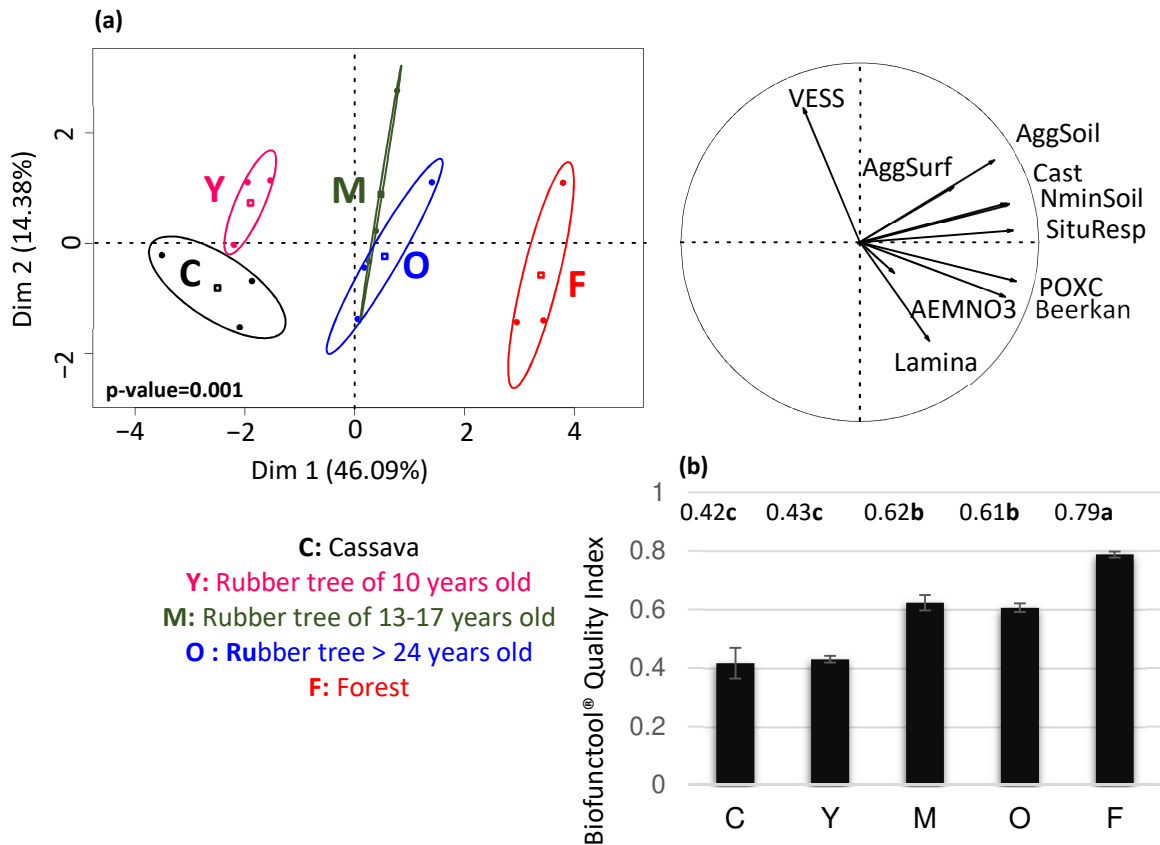
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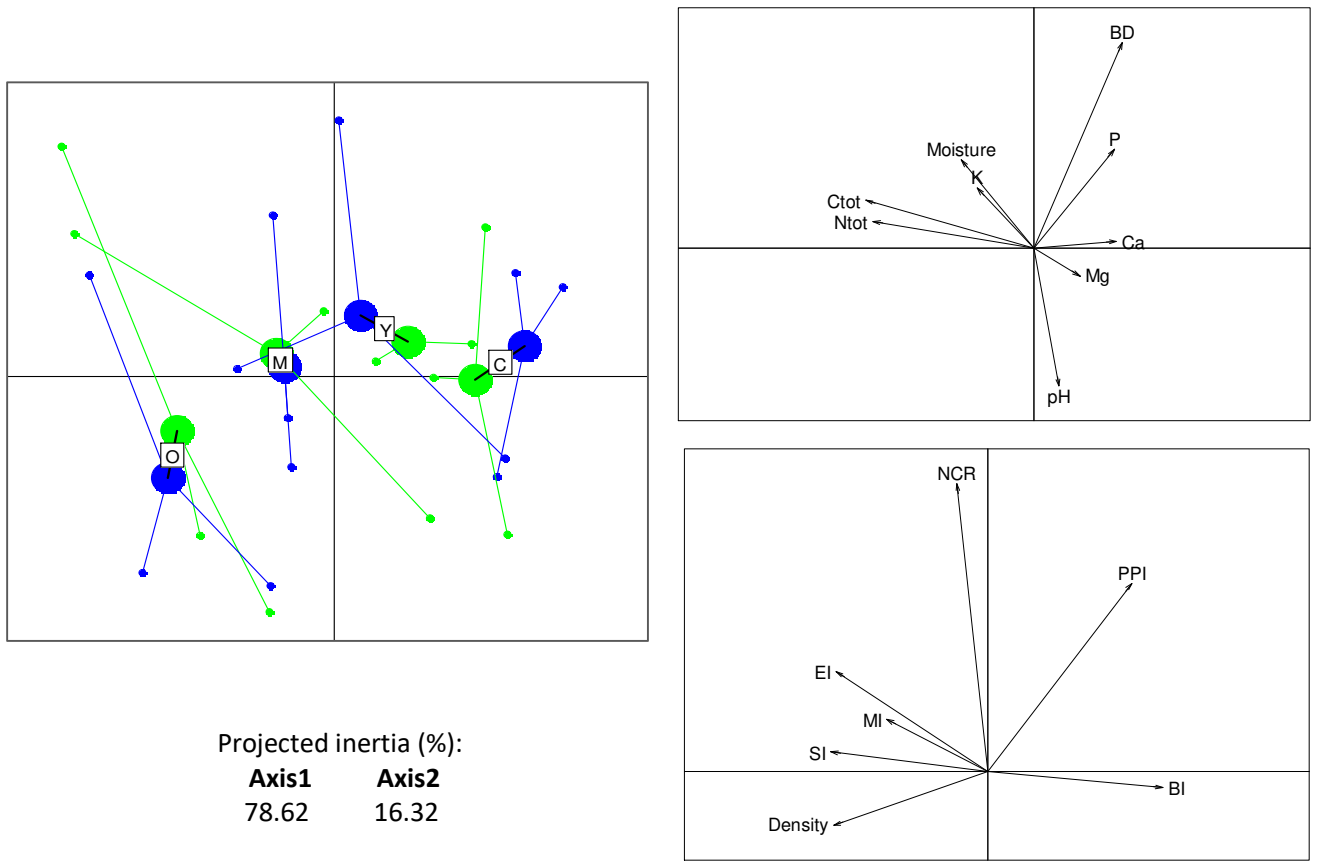
**Figure 1:** Land management impacts on physico-chemical indicators. Part a) is the individual and correlation circle graphs obtained from the PCA. With BD=bulk density, Ntot=total N, Ctot=total C. Part b) is the Quality Index obtained from SQI methodology with mean (n=3)  $\pm$ SD. Different letters refer to significant differences, after the Tukey post-hoc test with  $p < 0.05$ .



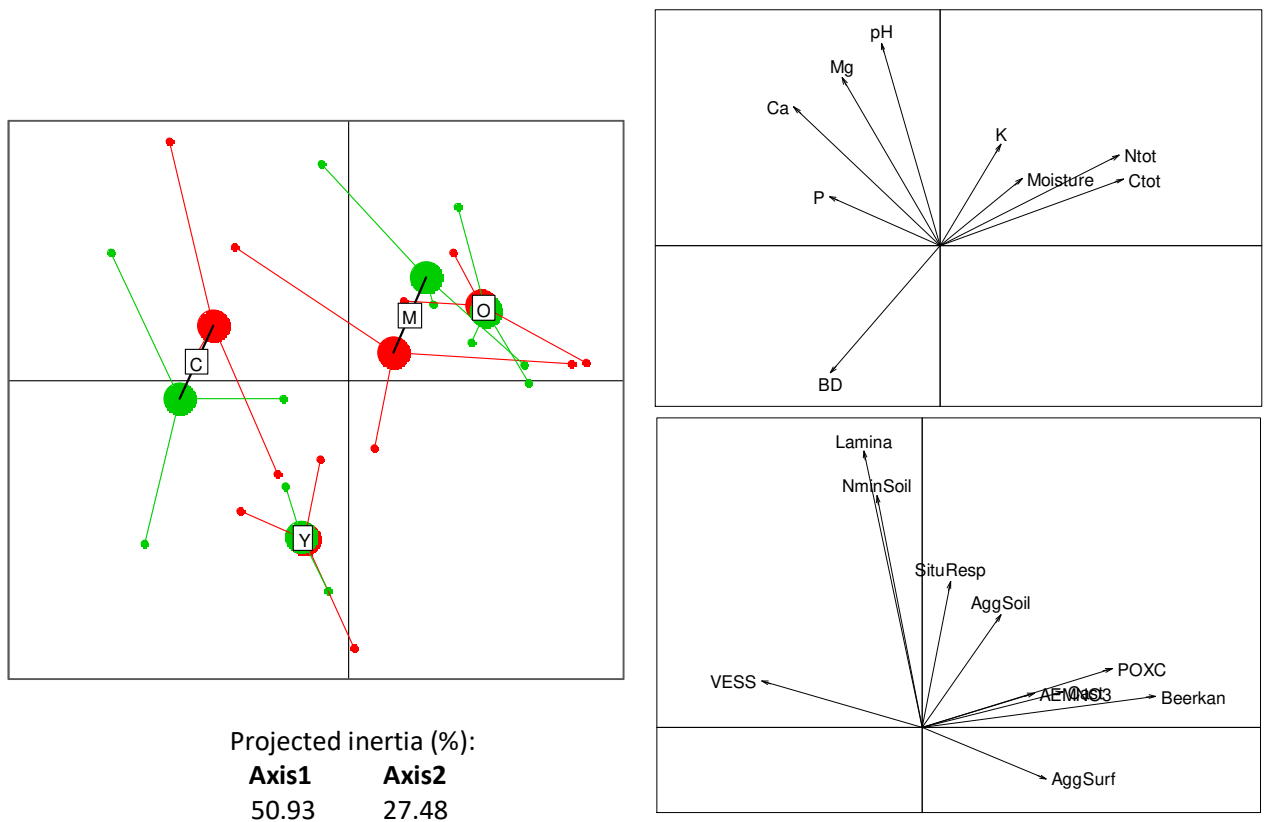
**Figure 2:** Land management impacts on soil nematodes indices. Part a) is the individual and correlation circle graphs obtained from the PCA. With BI=basal index, Density=nematode abundance, EI=enrichment index, MI=maturity index, NCR=nematode channel ratio, PPI=plant-parasitic index. Part b) is the Quality Index obtained from SQI methodology with mean (n=3) ±SD. Different letters refer to significant differences, after the Tukey post-hoc test with  $p < 0.05$ .



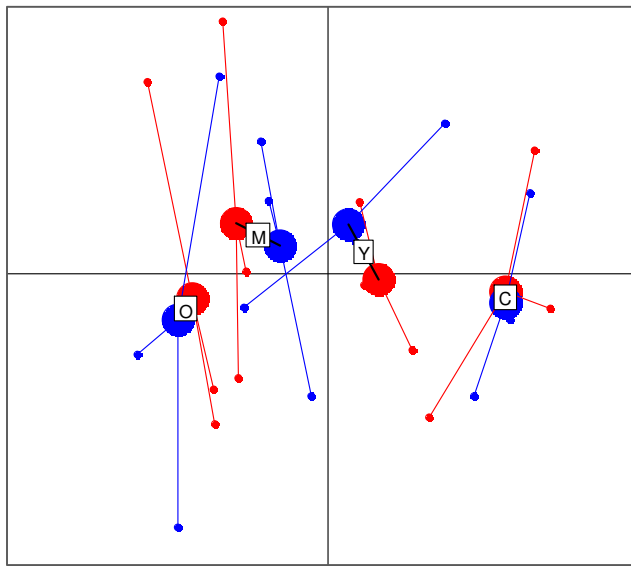
**Figure 3:** Land management impacts on the Biofunctool functional indicators, referring to Thoumzau et al. (2019). Part a) is the individual and correlation circle graphs obtained from the PCA. With AggSoil=aggregate stability (2-10cm), AggSurf=aggregate stability (0-2cm), AEMNO3=NO<sub>3</sub><sup>-</sup> fixed on anion exchange membrane, Beerkan=infiltration rate, Cast=cast density, Lamina=Bait Lamina, NminSoil=soil available nitrogen (NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>), POXC=Permanganate OXydizable Carbon, SituResp=basal soil respiration, VESS=visual evaluation of soil structure. Part b) is the Quality Index obtained from SQI methodology with mean (n=3) ±SD. Different letters refer to significant differences, after the Tukey post-hoc test with p < 0.05.



**Figure 4:** Co-inertia analysis between physico-chemical (PC) parameters and nematodes indices (Nem). Green and blue circles represent the PCA sample map according to, respectively, PC and Nem indicators (a). The biggest circle are barycenters. O, M, Y and C are the land uses names.

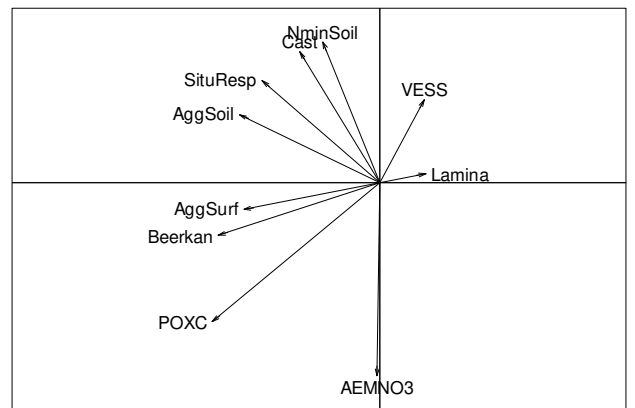
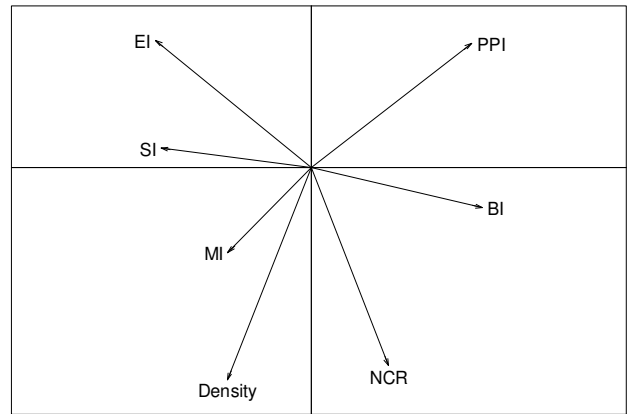


**Figure 5:** Co-inertia analysis between physico-chemical parameters (PC) and Biofunctool® indicators and. Red and green circles represent the PCA sample map according to, respectively, Biofunctool® and PC indicators (a). The bigger circle are barycenters. O, M, Y and C are the land uses names.



Projected inertia (%):

Axis1	Axis2
81.43	9.51



**Figure 6:** Co-inertia analysis between nematodes indices (Nem) and Biofunctool indicators. Red and blue circles represent the PCA sample map according to, respectively, Biofunctool® and Nem indicators (a). The bigger circle are barycenters.



	Physico-chemical (PC)									Nematods (Nem)						Functional (Biofunctool®)										
	BD (g.cm <sup>-3</sup> )	Ctot (%)	Ntot (%)	P (mg.kg <sup>-1</sup> )	K (mg.kg <sup>-1</sup> )	Ca (mg.kg <sup>-1</sup> )	Mg (mg.kg <sup>-1</sup> )	pH <sub>wat.</sub>	Moist. (%)	Density (nb ind.)	EI	SI	BI	MI	PPI	NCR	AEMNO3 (μg.cm <sup>-1</sup> .d <sup>-1</sup> )	AggSoil (Score)	AggSurf (Score)	BeerK. (Score)	Cast (g.m <sup>-1</sup> )	Lamina (%deg.d <sup>-1</sup> )	Nmin (mg.kg <sup>-1</sup> )	POXC (mgC.kg <sup>-1</sup> )	SituResp (Score)	VESS (Score)
C	1.46ab (±0.07)	0.71a (±0.13)	0.06a (±0.01)	33.50b (±32.04)	47.39ab (±27.32)	267.78a (±116.07)	52.83a (±18.74)	4.86a (±0.39)	17.05ns (±2.80)	483.00ab* (±59.27)	41.15a* (±10.26)	35.10a* (±8.48)	44.43ns* (±7.75)	2.18ns* (±0.09)	2.74ab* (±0.31)	0.47ns* (±0.03)	1.26ns (±0.85)	1.61a (±0.70)	2.89ns (±1.85)	3.90a (±1.42)	43.80a (±32.06)	2.37ns (±1.97)	2.55a (±1.70)	570.67a (±156.50)	0.89ab (±0.14)	2.60ab (±0.39)
Y	1.52a (±0.10)	0.79ab (±0.09)	0.07ab (±0.01)	10.06ab (±3.08)	28.11a (±6.48)	136.50a (±92.73)	35.83a (±17.25)	4.59a (±0.30)	15.37ns (±1.98)	319.50a (±199.87)	49.60ab (±10.53)	65.12b (±26.66)	24.50ns (±15.69)	2.70ns (±0.54)	2.68b (±0.27)	0.45ns (±0.09)	1.22ns (±0.52)	2.67ab (±1.44)	6.00ns (±0)	3.44ab (±1.33)	77.38ab (±77.70)	0.47ns (±0.29)	1.83a (±1.08)	577.97a (±167.85)	0.83a (±0.16)	2.59ab (±0.39)
M	1.47ab (±0.06)	0.99bc (±0.25)	0.08bc (±0.02)	11.38ab (±9.71)	46.88ab (±22.31)	187.72a (±107.30)	42.44a (±15.66)	4.82a (±0.40)	17.88ns (±2.29)	467.63ab (±168.40)	58.63ab (±6.31)	70.57b (±11.63)	19.28ns (±5.90)	2.53ns (±0.31)	2.50ab (±0.18)	0.46ns (±0.12)	1.35ns (±0.82)	4.00b (±1.46)	5.17ns (±1.66)	4.50bc (±1.00)	282.36cd (±186.49)	1.61ns (±0.37)	4.63a (±1.90)	766.54b (±130.98)	1.00ab (±0.13)	2.73b (±0.79)
O	1.38b (±0.07)	1.04c (±0.18)	0.09c (±0.01)	14.67ab (±13.95)	55.33b (±20.55)	153.00a (±63.22)	40.39a (±13.73)	4.79a (±0.41)	18.05ns (±2.29)	907.53b (±530.36)	61.09b (±17.51)	83.42b (±11.63)	11.38ns (±5.16)	2.81ns (±0.51)	2.37a (±0.27)	0.42ns (±0.11)	1.68ns (±0.78)	3.56b (±1.65)	6.00ns (±0)	5.30c (±1.58)	137.53bc (±136.87)	1.68ns (±0.35)	3.26a (±2.18)	791.95b (±134.81)	1.01b (±0.11)	2.17a (±0.46)
F	1.40a (±0.10)	1.34d (±0.14)	0.12d (±0.01)	8.89a (±4.61)	55.28b (±23.15)	724.31b (±184.34)	146.33b (±35.81)	5.50b (±0.53)	17.25ns (±2.82)	NA	NA	NA	NA	NA	NA	NA	1.33ns (±0.77)	3.94b (±1.24)	6.00ns (±0)	9.57d (±5.05)	318.43d (±200.46)	2.71ns (±1.26)	7.17b (±3.64)	1082.04c (±3.64)	1.18c (±0.05)	2.27a (±0.37)

Table 1: Univariate analysis for each land management. C=cassava (*M. esculenta*), Y=rubber tree plantations (*H. brasiliensis*) of 10 years, M= rubber tree plantations of 13 to 17 years, O=rubber tree plantations of 24 years and F=forest. Letters indicate significant differences according to Tuckey test. Variability within treatment is expressed with standard deviation. n=9 per land management, only n=3 (composite soil sample per plot) for nematodes under cassava for experimental reasons (\*). Linear model on plot's average (without nested design) were computed for this calculations.

