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

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Abstract

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

Key words: soil quality, physico-chemical properties, nematode indices, functional indicators, Biofunctool®, co-inertia analysis

1. Introduction

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

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

Biological analysis are more and more integrated in soil quality assessment programs [6], whereas they are still underrepresented in the literature [5]. This scientific enthusiasm to apply biological indicators makes it possible to enlarge and precise the global effect of land management or disturbance on soil systems [11]. Among biological approach, free-living soil nematodes are one of the most promising biotic assemblage to assess soil quality [12], [13]. Occupying key positions in the soil food web and being highly interactive with other soil organisms, nematodes are representative of numerous trophic groups, soil food web links and life strategies [14]. Based on nematode assemblage taxonomical analysis, nematodes indices have been widely used to describe soil food web response to soil disturbance or stress [15], [16]. Nematodes indices provide information on soil food web structure and maturity, decomposition pathway, as well as fertility levels and soil suppressivness to pathogens [17].

Using sets of independent soil physical, chemical or biological indicators might not be sufficient to reflect the complex nature of the soil system since it does not integrate emergent properties emerging from biotic-abiotic interaction [18], [19]. Several authors have underlined the need to develop functional approaches, also described as integrative approaches [20]. With functional approaches, indicators aim to target critical processes directly linked to one or more of the soil functions by measuring dynamic of state variable, related fluxes or transformations that are output of biotic-abiotic interactions [21]. The main distinction between functional approaches and other approaches is that functional approaches don't measure structure of soil biota or soil physico-chemical context, it does directly measure the result of their interaction with directly assessing soil functions, i.e., carbon transformation, nutrient cycling, structure maintenance [19], [20]. Biofunctool® is a recently developed set of indicators that were selected using expert opinion for their functional characteristics (i.e. measure the output of soil biotic-abiotic interactions) [19]. Biofunctool® set of indicators aims to provide a global overview of the effect of land management practices on three main functions of the soil (carbon transformation, nutrient cycling and structure maintenance).

In order to better understand the sensitivity and how those approaches may be complementary to assess soil quality, we decided to apply the three approaches (PC, biological, functional) together in the same site. We applied the three sets of indicators along a gradient of disturbance including cassava monoculture, rubber tree plantation of different ages and a forest in Chachoengsao province, Thailand. We hypothesize that i) the functional approach reflects physico-chemical and biological attributes of the soil, ii) the physico-chemical and biological approaches are complementary and when used independently, do not allow to provide a global vision of soil quality as they do not account for biotic-abiotic emergent properties.

2. Material and methods

2.1. Site description and experimental design

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

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

For experimental reasons, nematodes indicators were sampled only in cassava and rubber plots in 2014 whereas Biofunctool® and PC indicators were sampled in 2016. The two sampling years had similar climatic conditions and soil samples were collected at the end of the rainy season (November) to maximize soil biotic activities. Previous study done by the same team in this experimental site [23] demonstrated a weak change of biotic parameters (bacteria, fungi and macrofauna) within groups of plots having four years of difference. Based on this, and the fact that we focus on a contrasted gradient of disturbance with rubber plantations representing a rather stable environment, we assumed that time lapse of two years will have a negligible effect on our analyses. Moreover, we focus on relative changes in soil quality rather than absolute soil quality quantification. In all cases, the key rubber stand age affecting soil quality were integrated such as immature stage, intermediate mature stage and last mature stage [22], [23]. Implementing analysis of relative changes taking into account all stage thus seem robust to tackle this sampling year difference.

2.2. Measurements implemented for each set

2.2.1. Set of physico-chemical indicators (PC)

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

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

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

2.2.2. Set of nematodes indicators (Nem)

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

2.2.3. Biofunctool® set of indicators

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

For soil carbon transformation function, short-term turnover soil carbon pool was assessed directly in the field with Permanganate OXidizable Carbon method [32]. POXC is a processed fraction of labile soil organic carbon [33]. Soil organisms activity was evaluated with the Lamina baits test (Lamina, [34]) and the cast density measurement (Cast) at the soil surface. Lamina baits assess soil mesofauna activity through the decomposition of an organic substrate embedded in sixteen holes of a plastic stick and inserted into the topsoil during 15 days. Measuring the cast mass on a given soil surface allows to evaluate earthworm activities [19]. CO_2 release by microorganism was assessed with the SituResp® method [35]. SituResp® evaluates soil basal respiration in the field and is based on the color changes of a pH-sensitive gel over 24-h incubation.

For the nutrient cycling function, soil available mineral nitrogen (NminSoil) was determined from fresh soil extraction in a 1M KCl solution, quantifying soil available NO_3^- and NH_4^+ at one time. Nitrate dynamics in the soil was evaluated with the quantification of NO_3^- adsorbed on 6x2cm anion exchange membrane inserted at 8cm depth for 15 days (AEMNO3) [36], [37]. Nutrient cycling measurements in Biofunctool® set differs from PC set as it focuses on nutrient dynamics (i.e. available form of nitrogen on actual wet soil rather than on nutrient stocks on disturbed dried soil).

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

2.3. Statistical analysis

Statistical analyses were computed using the version 3.6.3 of the R software [41]. Some outliers (within-plot replicate values) were observed in the PC and Nem sets and were removed. Within-plot replicate values were considered as outliers if they were out of the boxplot (lower than (Q1 – 1.5*Interquartile Range) or higher than (Q3+1.5*Interquartile Range)) for, at least, two indicators in the same set. In total, 16 values (only 2.7% of the data set) were removed from PC and Nem dataset. This data pre-processing was especially needed because of high data-set variability linked to the integration of inner-replicates in the analysis. For Biofunctool®, no outlier were removed as [22] already computed outlier analysis.

First, univariate analysis was implemented and each indicator was studied separately using a linear-mixed effects model (package lme4, [42]). Treatment was defined as fixed factor and replicates (plots and inner-replicates) as random factors. After checking the normality of the model's residuals and homoscedasticity of variances' residuals, ANOVAs were run using the car package [43]. This was followed by posthoc mean comparisons, using Tukey with adjustment Bonferroni [44]. For three variables (BI, AggSurf, Lamina), preliminary conditions for the tests were not met, Kuskall-Wallis non-parametric tests were computed on plots averages in those specific cases.

Then, principal component analyzes (PCA) [45] were performed on each set of indicators to analyze their response to land management systems. PCAs were made on within-plot replicate average values for PC and Biofunctool® and on replicates values for Nem. The significant effect of land management on the set of indicators was assessed through a between-class analyzes (BCA) with a Monte Carlos permutation test (999 permutations). The number of variable per set of indicators was different with seven variables for Nem, nine for PC and ten for Biofunctool®. This difference is too weak to explain eventual discriminatory potential between sets. Indeed, with sets of seven to ten variables, discriminatory differences will be more linked to variable sensitivity to land management and non-redundancy, than variable number in multivariate analysis.

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

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

3. Results

3.1. Analysis of the set of indicators

3.1.1. Soil quality assessment using the set of PC indicators

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

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

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

3.1.2. Soil quality assessment using the set of Nem indicators

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

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

This first axis represents 53.1% of the total inertia and is related to the gradient of disturbance from annual cassava crop plantations to old rubber tree plantations. However, the first axis does not separate the rubber plantation (Y) ellipse from the C and M ellipses. Most of the variables contribute to the first axis, particularly the SI, MI and BI indices. The second axis represents 19.2% of the total inertia and, like the PC indicators, is more related to inter-plot variability within each land management system. This variability is mostly explained by NCR*. While the PCA shows a continuous gradient from cassava to old rubber plantation land management, the scores of the Nem SQI are slightly higher (0.49 to 0.74) but do not significantly differ between land management systems (p=0.063) (Figure 2b).

3.1.3. Soil quality assessment using the set of Biofunctool indicators

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

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

3.2. Comparison between pairs of indicator sets

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

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

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

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

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

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

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

4. Discussion

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

4.1.1. Case of physico-chemical indicators (PC)

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

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

4.1.2. Case of biological indicators (Nem)

The Nem quality index was not significantly affected by land management ($p=0.065$), despite the fact it tended to increase with the gradient. This cannot be explained by the slight differences in the system studied for each set (with and without forest), which had no impact on observed sensitivity (Supplementary reading 3 and 4). The structural index (SI), basal index (BI) and maturity index (MI) were the main drivers of the first axis related to the gradient of disturbance. This result suggests that, with the gradient of disturbance (from cassava to rubber), the soil food web became more complex (decrease of BI) and more structured (increase of SI, and MI). The same trend was also observed by [58] in a similar rubber chronosequence. The high BI level combined with the low SI in the cassava plot revealed stress and limitated resources mostly due to more intense cultivation practices (tillage, pesticides etc.) which may negatively affect the structure of the soil food web [15]. In contrast, more abundant resources (inputs of litter, carbon and nutrient contents) and improved soil environment in the mature rubber plantation may be responsible for the increased structural and maturity index (SI and MI) [59].

4.1.3. Case of functional indicators (Biofunctool®)

Among all the sets of indicators tested, functional indicators was the most sensitive to the gradient of disturbance. The Biofunctool® quality index was sensitive both to the different land use under cassava and rubber and under rubber and forest. The increase in the Biofunctool® index along the chronosequence from the rubber to the forest was mainly driven by carbon transformation variables (SituResp®, POxC, Cast). This result is in line with results of previous studies that demonstrated an increase in the labile carbon pool along rubber chonosequences [51], [57], higher soil biota abundance such as microbial biomass [60] and more abundant soil fauna [23], [61]. Thus, concerning the soil structure maintenance function, the increase in the biomass of soil engineers with ageing rubber trees [23]together with the absence of tillage, may explain the positive evolution of soil structure parameters.

These results point to an improvement in soil functioning in older rubber plantations, but their scores were nevertheless lower than those of the forest.

4.2. How are the different soil quality index related?

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

We observed a non-significant relationship between PC and Nem. Despite these trends, the results of non-significant relationships enable a better understanding of the specificities of each set of indicators alongside the soil compartment they focus on. Nem and PC may have different thresholds of sensitivity to changes in land management. For example, the results of this study suggest that chemical parameters were more affected by long-term fertilizing practices, whereas nematode communities were more affected by change in organic matter content, a global feature of nematodes [62] related to both practices (tillage) and organic input (rubber tree litter). An approach based on physico-chemical parameters might not be sufficiently exhaustive to reflect soil functioning complexity. Hence, this validate that, both, physico-chemical and biological parameters should be measured to have a comprehensive insight of the effect of land management practices on the soil quality [5].

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

The co-inertia analysis revealed a significant co-structure between soil functions and the physico-chemical index. Several functional and PC indicators co-evolve along the first axis representing the gradient of disturbance. POXC and AEMNO3, indicators of the carbon transformation and nutrient cycling function, were all positively related to the first axis and in line with total soil stocks (carbon and nitrogen). This result demonstrates the consistency of two approaches based either on total stocks or on available nutrients. The methods react to the same extent but would be differently affected by short-term changes in soil quality. Soil mineral nitrogen (NminSoil) and soil fauna activity (Lamina) for functional indicators were positively related to soil pH for PC, confirming previous studies [63], [64] showing that, in acid soils, pH increase leads to an increase in nitrogen mineralization.

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

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

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

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

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

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

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

A useful compromise in the assessment of soil quality that has been poorly applied so far is to focus on functional indicators of soil quality that integrate interactions between soil abiotic and biotic compartments. The Biofunctool® functional indicators study three key soil functions: carbon transformation, nutrient cycling and structure maintenance, to describe soil complexity. No specific expertise is required to apply the indicators that are easily implementable. However, this integrative functional approach does not allow the examination of the soil processes behind soil functions and is currently little used as a diagnostic tool, compared to PC approach. Biofunctool® also remains a comparative approach for use in a specific context, and would require additional databases to provide absolute soil quality scoring such as current physico-chemical [71], or biological indicators [72].

5. Conclusion

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

Author contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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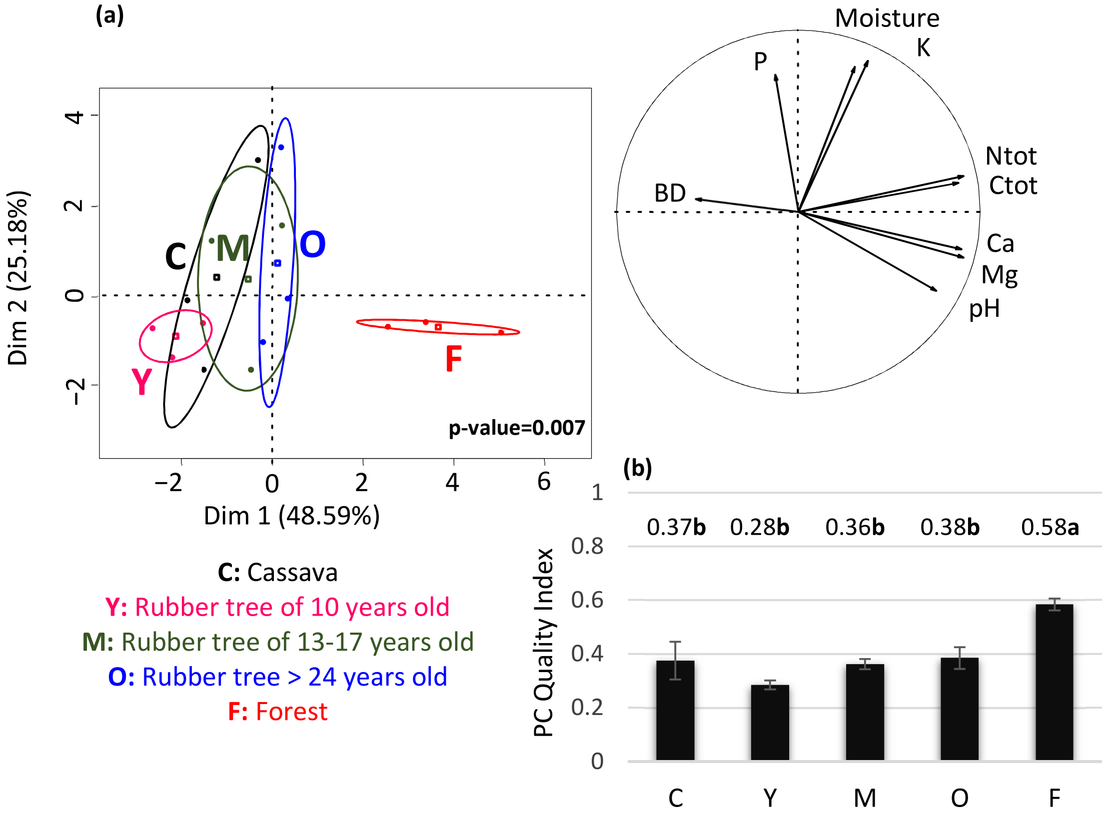


Figure 1: Land management impacts on physico-chemical indicators. Part a) is the individual and correlation circle graphs obtained from the PCA. With BD=bulk density, Ntot=total N, Ctot=total C. Part b) is the Quality Index obtained from SQI methodology with mean (n=3) ±SD. Different letters refer to significant differences, after the Tukey post-hoc test with $p < 0.05$.

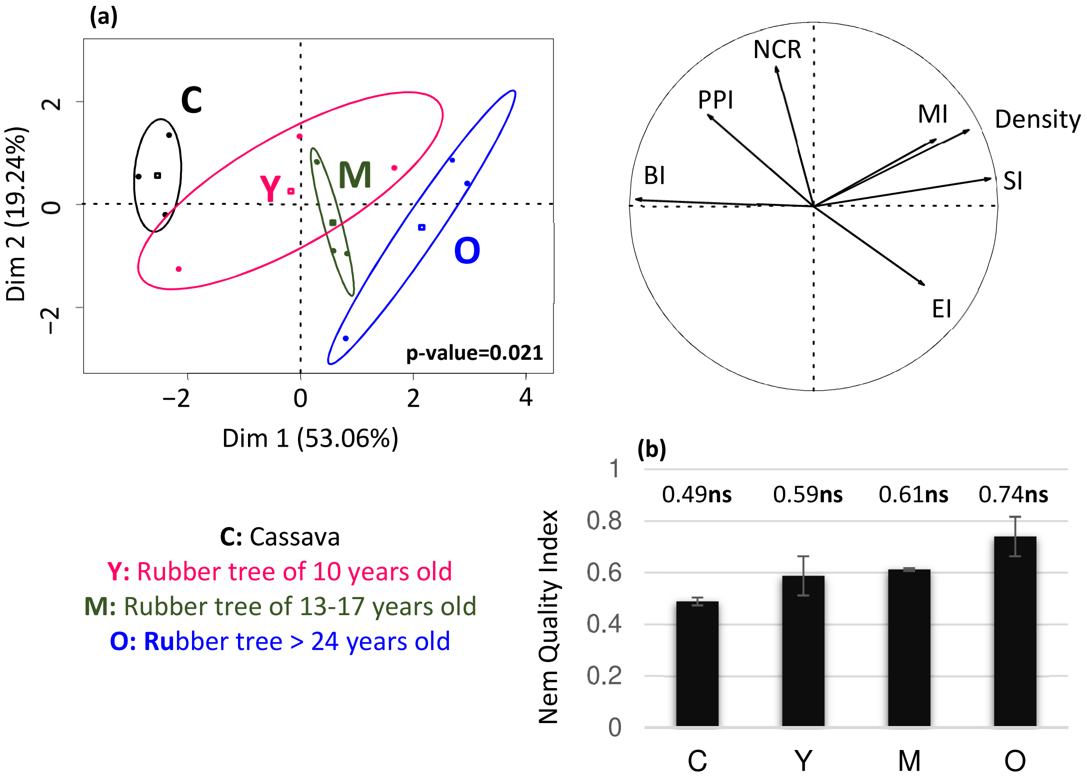


Figure 2: Land management impacts on soil nematodes indices. Part a) is the individual and correlation circle graphs obtained from the PCA. With BI=basal index, Density=nematode abundance, EI=enrichment index, MI=maturity index, NCR=nematode channel ratio, PPI=plant-parasitic index. Part b) is the Quality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

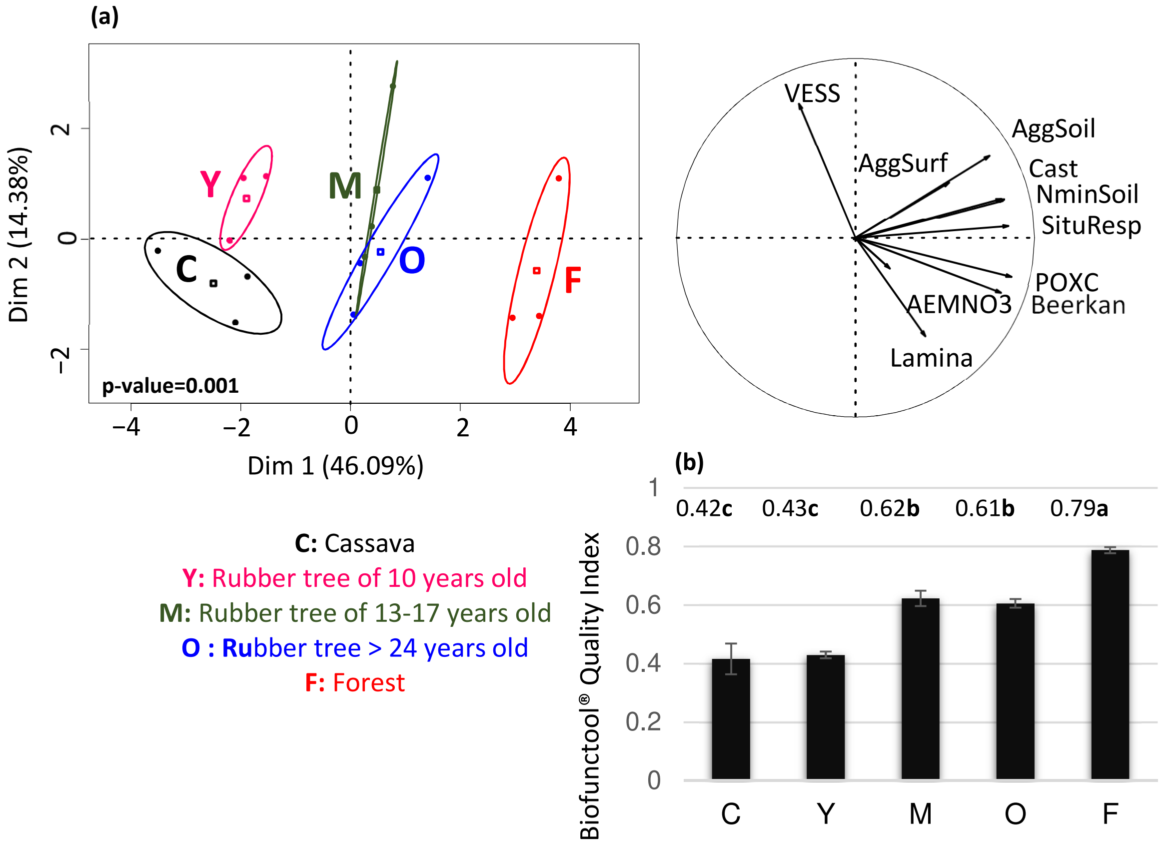


Figure 3: Land management impacts on the Biofunctool functional indicators, referring to Thoumazeau et al. (2019). Part a) is the individual and correlation circle graphs obtained from the PCA. With AggSoil=aggregate stability (2-10cm), AggSurf=aggregate stability (0-2cm), AEMNO3=NO_3^- fixed on anion exchange membrane, Beerkan=infiltration rate, Cast=cast density, Lamina=Bait Lamina, NminSoil=soil available nitrogen (NO_3^-, NH_4^+), 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 $p < 0.05$.

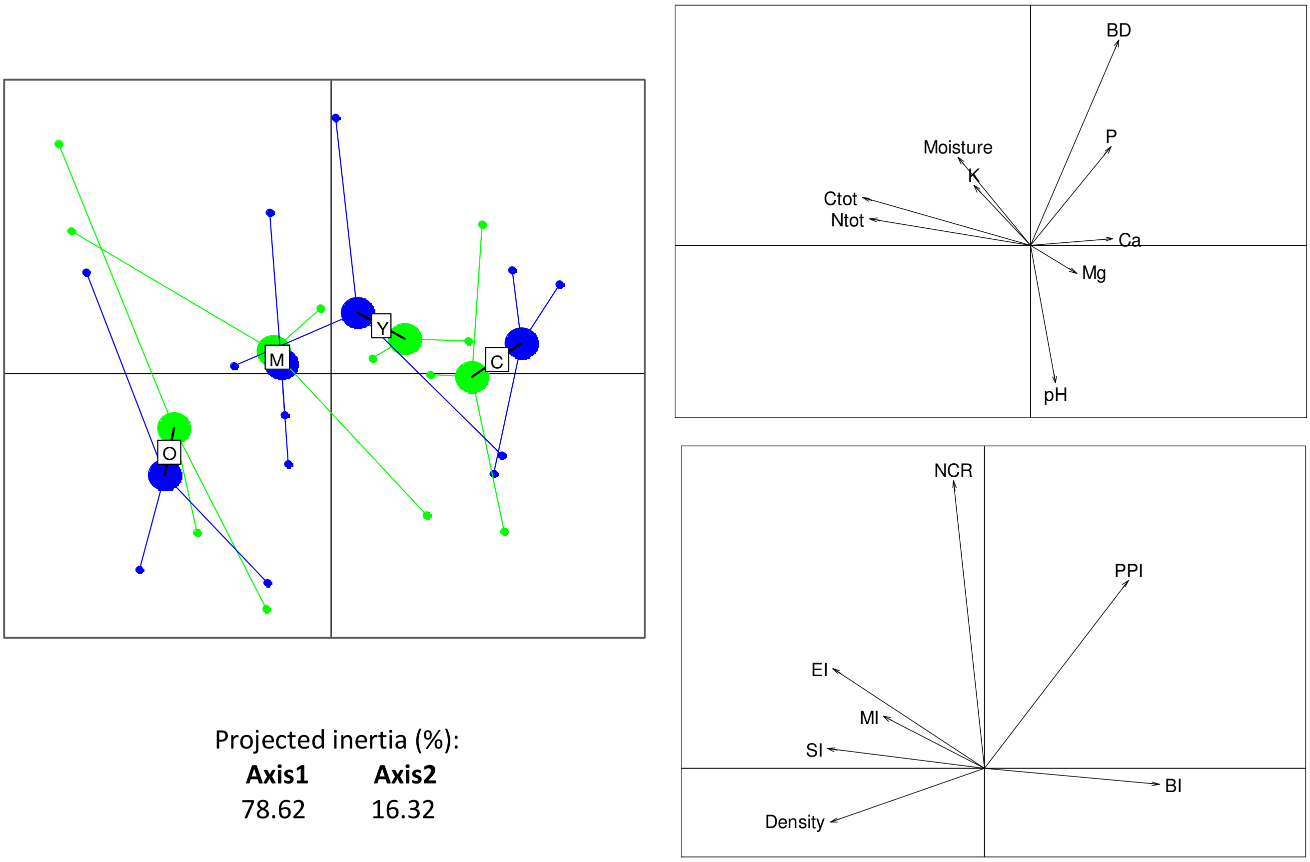


Figure 4: Co-inertia analysis between physico-chemical (PC) parameters and nematodes indices (Nem). Green and blue circles represent the PCA sample map according to, respectively, PC and Nem indicators (a). The biggest circle are barycenters. O, M, Y and C are the land uses names.

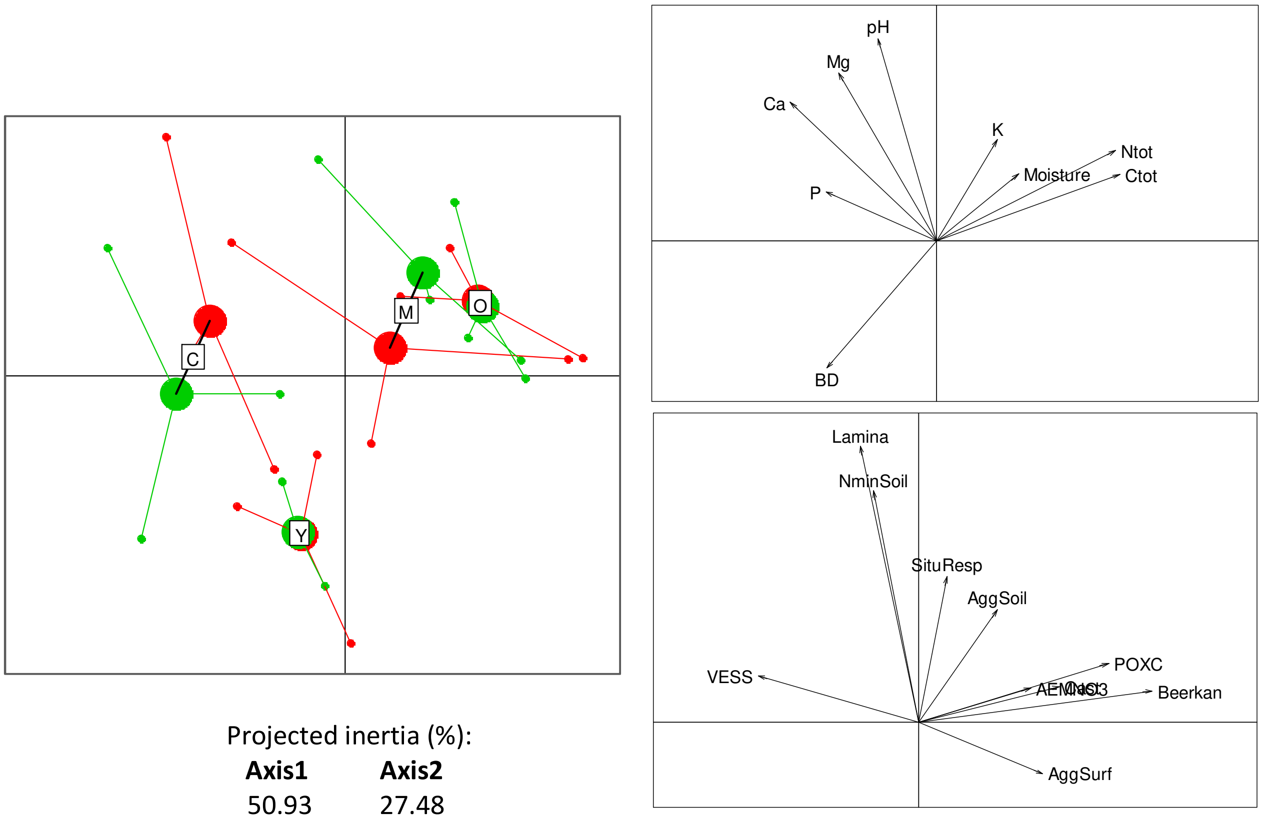


Figure 5: Co-inertia analysis between physico-chemical parameters (PC) and Biofunctool® indicators and. Red and green circles represent the PCA sample map according to, respectively, Biofunctool® and PC indicators (a). The biggerstcircle are barycenters. O, M, Y and C are the land uses names.

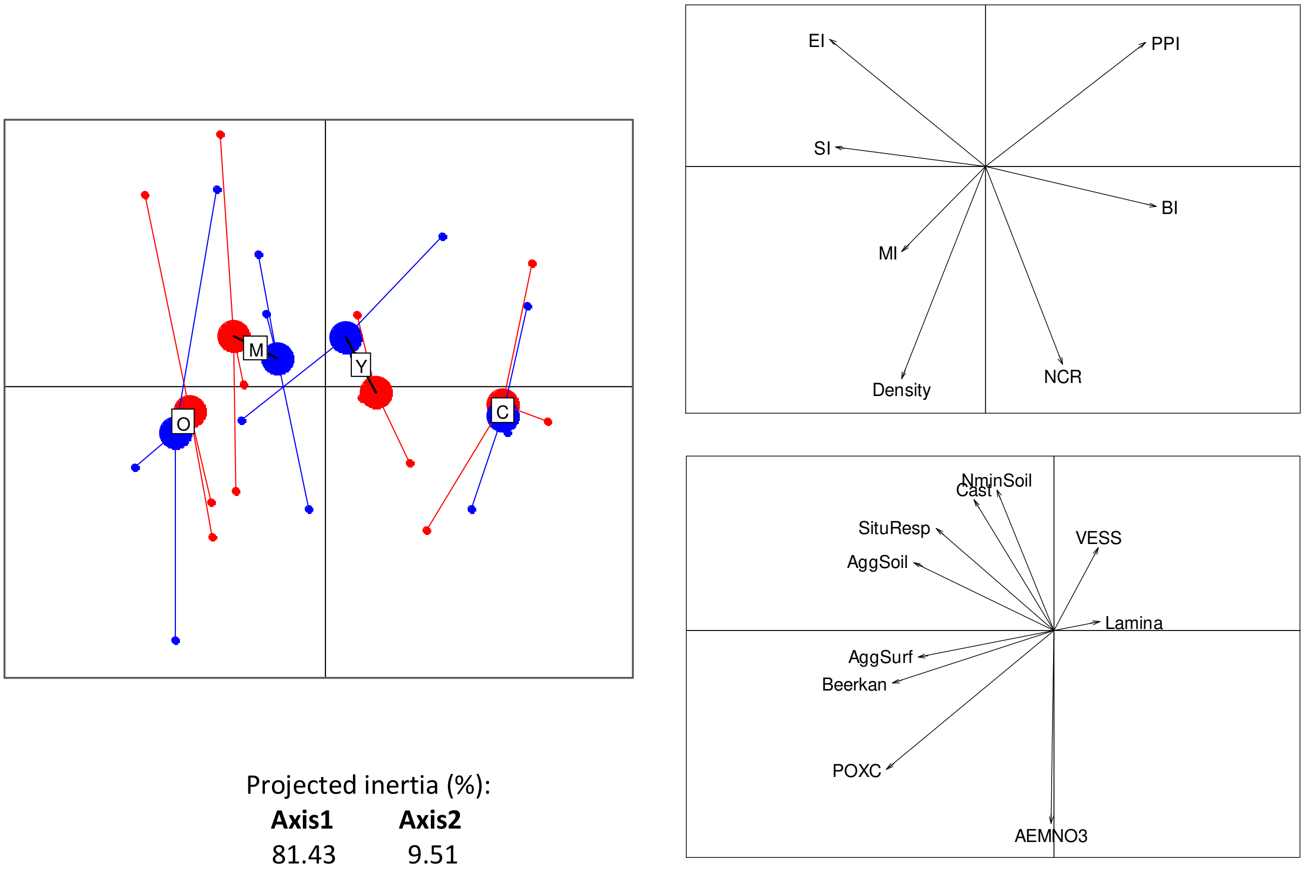


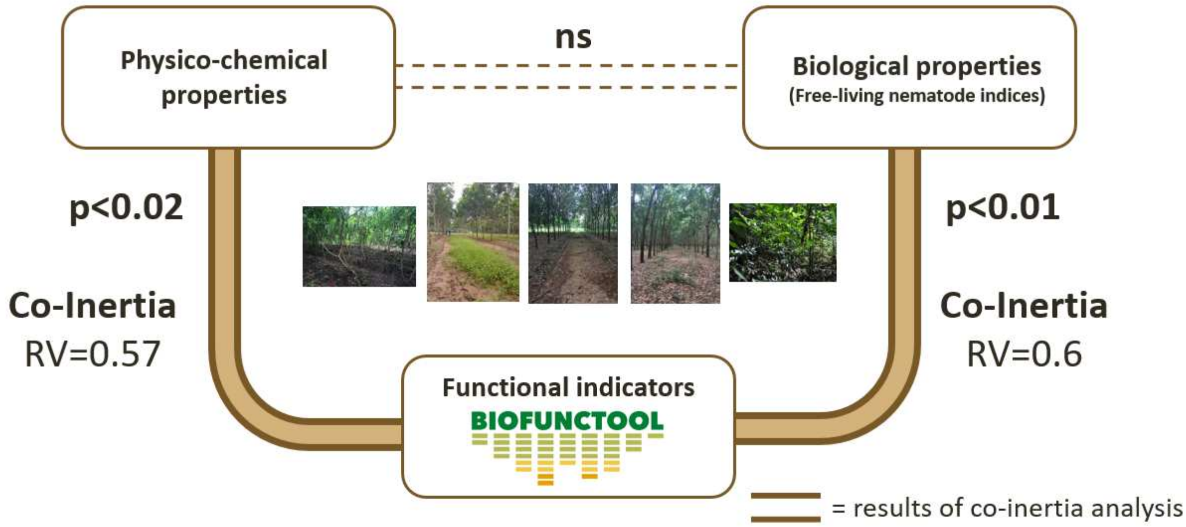
Figure 6: Co-inertia analysis between nematodes indices (Nem) and Biofunctool indicators. Red and blue circles represent the PCA sample map according to, respectively, Biofunctool® and Nem indicators (a). The bigger circle are barycenters.

	Physico-chemical (PC)								
	BD (g.cm^{-3})	Ctot (%)	Ntot (%)	P (mg.kg^{-1})	K (mg.kg^{-1})	Ca (mg.kg^{-1})	Mg (mg.kg^{-1})	pH$_{wat.}$	Moist. (%)
C	1.46**ab** (±0.07)	0.71**a** (±0.13)	0.06**a** (±0.01)	33.50**b** (±32.04)	47.39**ab** (±27.32)	267.78**a** (±116.07)	52.83**a** (±18.74)	4.86**a** (±0.39)	17.05**ns** (±2.80)
Y	1.52**a** (±0.10)	0.79**ab** (±0.09)	0.07**ab** (±0.01)	10.06**ab** (±3.08)	28.11**a** (±6.48)	136.50**a** (±92.73)	35.83**a** (±17.25)	4.59**a** (±0.30)	15.37**ns** (±1.98)
M	1.47**ab** (±0.06)	0.99**bc** (±0.25)	0.08**bc** (±0.02)	11.38**ab** (±9.71)	46.88**ab** (±22.31)	187.72**a** (±107.30)	42.44**a** (±15.66)	4.82**a** (±0.40)	17.88**ns** (±2.29)
O	1.38**b** (±0.07)	1.04**c** (±0.18)	0.09**c** (±0.01)	14.67**ab** (±13.95)	55.33**b** (±20.55)	153.00**a** (±63.22)	40.39**a** (±13.73)	4.79**a** (±0.41)	18.05**ns** (±2.29)
F	1.40**a** (±0.10)	1.34**d** (±0.14)	0.12**d** (±0.01)	8.89**a** (±4.61)	55.28**b** (±23.15)	724.31**b** (±184.34)	146.33**b** (±35.81)	5.50**b** (±0.53)	17.25**ns** (±2.82)

	Nematods (Nem)						
	Density (nb ind.)	EI	SI	BI	MI	PPI	NCR
C	483.00**ab*** (±59.27)	41.15**a*** (±10.26)	35.10**a*** (±8.48)	44.43**ns*** (±7.75)	2.18**ns*** (±0.09)	2.74**ab*** (±0.31)	0.47**ns*** (±0.03)
Y	319.50**a** (±199.87)	49.60**ab** (±10.53)	65.12**b** (±26.66)	24.50**ns** (±15.69)	2.70**ns** (±0.54)	2.68**b** (±0.27)	0.45**ns** (±0.09)
M	467.63**ab** (±168.40)	58.63**ab** (±6.31)	70.57**b** (±11.63)	19.28**ns** (±5.90)	2.53**ns** (±0.31)	2.50**ab** (±0.18)	0.46**ns** (±0.12)
O	907.53**b** (±530.36)	61.09**b** (±17.51)	83.42**b** (±11.63)	11.38**ns** (±5.16)	2.81**ns** (±0.51)	2.37**a** (±0.27)	0.42**ns** (±0.11)
F	NA	NA	NA	NA	NA	NA	NA

	Functional (Biofunctool®)									
	AEMNO3 (µg.cm^{-2}.d^{-1})	AggSoil (Score)	AggSurf (Score)	Beerk. (Score)	Cast (g.m^{-2})	Lamina (%deg.d^{-1})	Nmin (mg.kg^{-1})	POXC (mgC.kg^{-1})	SituResp (Score)	VESS (Score)
C	1.26**ns** (±0.85)	1.61**a** (±0.70)	2.89**ns** (±1.85)	3.90**a** (±1.42)	43.80**a** (±32.06)	2.37**ns** (±1.97)	2.55**a** (±1.70)	570.67**a** (±156.50)	0.89**ab** (±0.14)	2.60**ab** (±0.39)
Y	1.22**ns** (±0.52)	2.67**ab** (±1.44)	6.00**ns** (±0)	3.44**ab** (±1.33)	77.38**ab** (±77.70)	0.47**ns** (±0.29)	1.83**a** (±1.08)	577.97**a** (±167.85)	0.83**a** (±0.16)	2.59**ab** (±0.39)
M	1.35**ns** (±0.82)	4.00**b** (±1.46)	5.17**ns** (±1.66)	4.50**bc** (±1.00)	282.36**cd** (±186.49)	1.61**ns** (±0.37)	4.63**a** (±1.90)	766.54**b** (±130.98)	1.00**ab** (±0.13)	2.73**b** (±0.79)
O	1.68**ns** (±0.78)	3.56**b** (±1.65)	6.00**ns** (±0)	5.30**c** (±1.58)	137.53**bc** (±136.87)	1.68**ns** (±0.35)	3.26**a** (±2.18)	791.95**b** (±134.81)	1.01**b** (±0.11)	2.17**a** (±0.46)
F	1.33**ns** (±0.77)	3.94**b** (±1.24)	6.00**ns** (±0)	9.57**d** (±5.05)	318.43**d** (±200.46)	2.71**ns** (±1.26)	7.17**b** (±3.64)	1082.04**c** (±3.64)	1.18**c** (±0.05)	2.27**a** (±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.



Physico-chemical properties
ns
Biological properties
(Free-living nematode indices)
p<0.02
Co-Inertia
RV=0.57
p<0.01
Co-Inertia
RV=0.6
Functional indicators
BIOFUNCTOOL
= results of co-inertia analysis