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Organic Fertilization Improves Soil Multifunctionality in Sugarcane Agroecosystems

Sacha Delmotte [1,2,3,4,5,6,*], Caroline Brunel [3,4], Louise Castanier [1,2,7], Amélie Fevrier [7], Alain Brauman [5] and Antoine Versini [1,2]

1 CIRAD, UPR78, Recyclage et Risque, 97400 Saint-Denis, France; antoine.versini@cirad.fr (A.V.)
2 Recyclage et Risque, Univ Montpellier, CIRAD, 34398 Montpellier, France
3 CIRAD, UPR HortSys, 97455 Saint-Pierre, France; caroline.brunel@cirad.fr
4 HortSys, Univ Montpellier, CIRAD, 34398 Montpellier, France
5 IRD, Eco&Sols, Univ Montpellier, CIRAD, INRAE, IRD, Institut Agro, 34060 Montpellier, France; alain.brauman@ird.fr
6 CEFE, Univ Montpellier, CNRS, EPHE, IRD, 34090 Montpellier, France
7 eRcane, 97490 Saint-Denis, France; amelie.fevrier@ercane.re
* Correspondence: sacha.delmotte@umontpellier.fr

Abstract: Soil multifunctionality is closely tied to soil health, yet a comprehensive understanding of this link in agricultural soils is lacking. The aim of this study was to understand how long-term fertilization practices affect the provision of multiple services by comparing the multifunctionality of soils. The three objectives were to (i) determine whether the effect of fertilization is consistent across soil types, (ii) describe the effect of the different fertilizers on soil multifunctionality, and (iii) identify soil chemical properties that can be easily used proxies of soil multifunctionality. The descriptors belong to three functioning indexes associated with nutrient availability, carbon transformation, and soil structure maintenance. This study is the first to investigate the effect of a variety of organic fertilizers on the health of three soil types by combining physical, chemical, and biological indicators in sugarcane agroecosystems. An increase in soil multifunctionality was obtained, with no effect on yield. The effect of fertilizers was consistent across soil types. Filter mud and green waste compost significantly increased the multifunctionality and functioning indexes compared to mineral fertilizer. Modifications in soil properties did not fully explain the observed variations. Our results confirm the high potential of organic fertilization to improve multifunctionality and provide ecosystem services.

Keywords: multifunctionality; soil health; organic fertilization; sugarcane; Biofunctool®

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1. Introduction

The increasing quantity of organic waste generated by human activities can be valorized in agriculture to fuel a more circular economy [1]. Recycling organic waste as fertilizer reduces the need for the production and transport of mineral fertilizers, thereby reducing both the dependence on fossil fuels [2] and greenhouse gas emissions [3]. Sugarcane is one of the most widely grown crops in the world in terms of tonnage [4] and requires considerable quantities of nutrients usually supplied in the form of mineral fertilizers [5]. The use of organic fertilizers therefore represents an excellent opportunity in the face of the increasing scarcity of fossil fuels and the soaring costs of fertilizers, particularly in the outermost regions of the European Union that depend heavily on imports and fossil fuels [6]. Appropriate organic fertilization practices mitigate the risk of soil contamination [7,8] and antibiotic resistance [9,10], enhance crop nutrition and increase yields, and mitigate environmental pollution and improve soil health [11]. These practices are thus a key in the transition toward agroecosystem sustainability.

Fertilization is known to modify soil properties directly by adding nutrients and indirectly through effects related to biological activity [12]. By changing soil biotic and abiotic conditions, fertilization affects the interactions among the physical, chemical, and biological soil components, thereby regulating soil functioning [13,14]. In recent years, increasing scientific interest in organic fertilization has yielded insights into its impact on soil properties and the availability of nutrients, which relies on fertilizer quality [15,16]. In contrast to mineral fertilizers, the use of organic fertilizers generally benefits microbial community abundance, functions related to carbon and nitrogen cycling, and enzymatic activity involved in phosphorus availability [17], as well as enhances microbial biomass [18], organic matter content, and carbon stabilization [19]. In their review, Bhatt and colleagues [20] reported that the long-term application of organic fertilizers increased the availability of nitrogen, phosphorus, potassium, calcium, and magnesium in soil. Furthermore, organic fertilization was found to stimulate soil decompaction by enhancing porosity [18,21]. Thus, long-term organic fertilization is likely to enhance the sustainability of soil functioning through changes in physical, chemical, and biological soil properties [22]. Soil chemical properties could therefore serve as a proxy for soil multifunctionality, as previously investigated in biochar-amended soils [23].

Assessing the impact of fertilization practices on soil health, defined as the ability of soil to function and provide ecosystem services (sensu van Es and Karlen [24]), is a significant concern [25]. Soil multifunctionality is defined as the soil's capacity to simultaneously provide multiple key functions (e.g., organic matter transformation, nutrient cycling, and soil structuration) that support nutrient supply, primary production, climate regulation [26], erosion control, or water filtration [27]. Thus, soil multifunctionality ensures the provision of soil-based ecosystem services [28] and should be carefully considered in the development of sustainable agricultural systems. To enable a rapid yet comprehensive understanding of the soil functional state, sets of physical, chemical, and biological functional indicators were recently developed [29], some of which specifically focus on agricultural ecosystems [30].

An exhaustive assessment of the effect of distinct organic fertilizers on soil health that combines physical, chemical, and biological indicators is still lacking in sugarcane agroecosystems [31]. Beyond agricultural practices [32], sugarcane cultivation is known to have a negative impact on the physical and biological properties of the soil and on its functioning [33,34]. Sugarcane cultivation has a strong effect on the structure of the topsoil [35], primarily on the microstructure and porosity [36], due to the passage of agricultural machinery and tillage [37,38], and on the soil engineers that play a key role in maintaining soil structure, which is also affected by the changes in soil organic matter caused by these practices [39]. Managing straw removal can help mitigate these changes [40]. Understanding the effect of distinct organic fertilizers on sugarcane soil multifunctionality is indispensable to be able to direct fertilization toward improved ecosystem services provision and ensure the sustainability of this cropping system.

The purpose of the present study was to understand how long-term fertilization practices affect the capacity of the soil to provide multiple services, by comparing the multifunctionality of sugarcane soils in three experimental fields corresponding respectively to a Nitisol, a Cambisol, and an Andosol [41] located in Reunion Island (SW Indian Ocean). Multifunctionality was investigated using nine functional indicators of nutrient availability, carbon transformation, and soil structure maintenance (Figure S1). Our specific objectives were to (i) determine whether the effect of fertilization remained consistent across the three types of soil, (ii) describe the effect of the different fertilizers on soil multifunctionality, and (iii) identify variations in soil multifunctionality that could be approximated by soil chemical properties. We hypothesize that organic matter inputs enhance soil multifunctionality but with different effects related to their quality and that certain soil chemical properties could be used as proxies for soil multifunctionality.

2. Material and Methods

2.1. Study Sites and Experimental Design

This study was conducted at three sugarcane experimental sites in Reunion Island, established seven to five years prior to the measurements by the eRcane sugarcane research institute, with the aim of identifying an equivalence coefficient for different organic fertilizers (Figure 1B). We took advantage of these ongoing experiments to study the effect of organic fertilization on soil functioning. The first site was set up in 2014 near Saint Denis on the north of the island (20°54′10.4″ S 55°31′56.5″ E), under sprinkler irrigation. The median annual rainfall there is about 1500 mm per year [42], and the mean annual temperature is 23.7 °C [43]. The World Reference Base for Soil Resources classifies this soil as a Nitisol, which is a fine-textured material weathered from volcanic parent rock, dominated by kaolinite, halloysite, and iron oxides [41]. The second site was set up in 2015 on the west coast, near Piton Saint-Leu (21°12′48.8″ S 55°19′39.2″ E), also with sprinkler irrigation. The median annual rainfall there is 1250 mm per year, and the mean annual temperature is 19.9 °C. The soil is a Cambisol, characterized by the initial differentiation of horizons in the subsoil, changes in structure and color, alteration of the parent Andosol material, and high concentrations of Al and/or Fe [41]. The third site was set up in 2016 on the east coast, near Saint Benoit (21°05′38.8″ S 55°41′56.6″ E), with no sprinkler system. The median annual rainfall there is 4250 mm per year, and the mean annual temperature is 20.7 °C. The soil is classified as an Andosol composed of allophane, imogolite, and stable organo-mineral complexes [41]. Hereafter, the three sites are referred to as Nitisol, Cambisol, and Andosol, respectively. The chemical properties of the soils at the three sites are detailed in Table S1. At each site, fertilization practices were replicated in three 80 m^2 plots. Fertilization with pig manure or filter mud was compared to a mineral fertilization control at all three sites. Sewage sludge and green waste compost were specifically studied in the Nitisol and Cambisol, giving a total of 39 study plots in all (see Figure 1A). Each year, the quantities of fertilizer were determined to supply sugarcane with the equivalent of that supplied by the mineral control, based on the nitrogen content of the fertilizer and the mineral fertilizer equivalence coefficient estimated in temperate conditions. This means that the quantity of organic matter input varied from their quality for each site, but it did not affect yields (the yields and quantities of inputs are listed in Tables S1 and S2). The soil properties were analyzed each year, and the plots were limed when the pH was below 5.5.

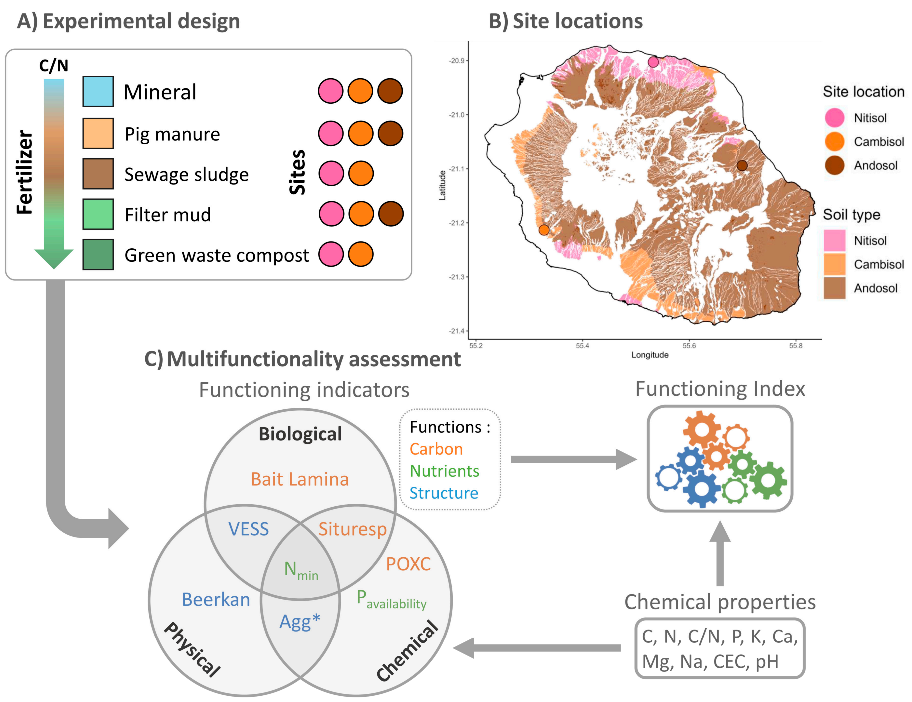

Figure 1. Overview of (**A**) the study design showing the fertilization practices used at each site, (**B**) the location of the sites and types of soil, and (**C**) the soil functions studied and analytical scheme. (*) Agg: aggregate stability.

2.2. Soil Chemical Analysis

Bulk soil was sampled in each plot (at a depth of 0–15 cm) at harvesting in November 2021 for soil chemical analysis in the Andosol and Cambisol sites and in February 2022 in the Nitisol site. Standard soil chemical analyses were performed in the CIRAD soil laboratory in Saint Denis. Due to the absence of carbonates, the soil organic carbon (g kg^{-1}) and total soil nitrogen (g kg^{-1}) were quantified using the Dumas combustion method (ISO 106994:1995 [44]) with an elemental analyzer (VarioMax Cube CNS, Elementar, Hanau, Germany). The C:N ratio was calculated from these measurements. The phosphorus content of the soil in Reunion Island, expressed in mg kg^{-1}, was always measured using the Olsen method modified by Dabin [45], despite its identified limitations as an indicator of available phosphorus [46]. The cation exchange capacity (CEC), in cmol kg^{-1}, as well as the concentration of exchangeable cations (K^+, Ca^{2+}, Mg^{2+}, and Na^+, in cmol kg^{-1}) were quantified using a solution of cobaltihexamine trichloride as the extraction reagent, according to standard ISO 23470:2018 [47]. Finally, the pH was quantified using the water and KCl method (ISO 10390:2021 [48]).

2.3. Indicators of Soil Nutrient Availability

The nutrient function was based on indicators of nitrogen and phosphorus availability provided by Serdaf, a soil-specific nutrient management expert system developed for sugarcane fertilization in Reunion Island [49].

Nitrogen availability (N_{min}) in each plot was assessed using Serdaf, which calculates soil nitrogen mineralization over a crop growth cycle, using the method proposed by Mary et al. [50]. This method estimates in situ soil mineralization from the in silico potential mineralization rate through normalized time considering the temperature and soil moisture. The in situ soil mineralization was obtained by multiplying the number of "normalized days" by the potential mineralization rate and by the soil nitrogen content. The parameters used to calculate the soil nitrogen content and the potential mineralization rate (C_m) have recently been updated for each type of soil [51].

Phosphorus deficiency ($P_{deficiency}$) was assessed in each plot using the doses of phosphorus fertilizer recommended by Serdaf. To establish a phosphorus recommendation for sugarcane, the expert system considers the soil phosphorus content, the soil pH, and the fixing capacity of each type of soil. The more soil phosphorus there is available for the crop, the lower the recommended dose. The phosphorus deficiency estimated by Serdaf was calculated based on the measured phosphorus content and pH. The method of calculating phosphorus deficiency for sugarcane fields in Reunion Island designed and implemented in the Serdaf tool is described in detail in Auzoux et al. [52].

2.4. Indicators of Soil Structure

Four indicators adapted from the Biofunctool® set [30] were used to describe soil structure. Measurements at the Andosol and Cambisol sites were taken after soil sampling in November 2021 and at the Nitisol site in February 2022. The soil samples were placed in individual polyethylene bags, transported in an icebox to minimize alterations of the material, and air dried. The aggregate stability at a depth of 0–2 cm (AggSurf) and at a depth of 2–10 cm (AggSoil), which provides information on the capacity of the soil to resist erosion [53], was assessed on six dried aggregates selected for sizes ranging from 6 to 8 mm. The resistance of aggregates to two successive 5 min periods of immersion in water followed by 5 s of agitation was observed, and a resistance score was attributed based on the method developed by Herrick et al. [54]. The highest score indicates the most resistant aggregates.

The water infiltration speed, which provides information on the infiltration rate, runoff, and erosion process [55], was assessed using the Beerkan method [56]. A consistent volume of water, equivalent to a 1 cm thickness (here 200 mL), was applied to the soil surface in a cylinder with a 15 cm diameter. The time that this volume of water took to infiltrate the soil was measured ten consecutive times. The water infiltration rate (mL min^{-1}) was calculated based on the infiltration curve established from these measurements. The highest value obtained indicates the best infiltration rate.

The Visual Evaluation of Soil Structure (hereafter VESS), which provides information on soil compaction and macrofaunal activity [57], was estimated at a depth of 0–20 cm using the method of Guimarães and colleagues [58]. The layers of soil were described based on the size and appearance of the aggregates and on porosity, using descriptive framework developed by Guimarães et al. [58]. The final score was obtained by averaging each layer, and the final values ranged from 1 (very friable soil without structure) to 5 (very compact soil).

2.5. Indicators of Soil Carbon Transformations

Three indicators adapted from the Biofunctool® set were used to describe soil carbon transformation [30]. The measurements and soil sampling were conducted simultaneously with those undertaken for soil structure analysis. The soil mesofauna and small macrofauna activity was assessed by examining substrate degradation using bait lamina sticks. These sticks are made of perforated plastic and initially filled with commercial organic substrate (70% cellulose, 27% bran flakes, and 3% active coal; Terra Protecta GmbH, Berlin). The sticks were planted vertically in groups of 7 with another strip as a control to check the degradation rate, spaced 30 cm apart. They were removed when approximately 80% of the substrate on the control stick was degraded (i.e., Nitisol: 14 days; Cambisol: 37 days; Andosol: 25 days). The mean decomposition rate of the remaining 7 sticks was calculated based on the method proposed by Gestel et al. [59] and von Törne [60]. High values indicate high fauna activity.

The short-term turnover of the soil carbon pool was investigated at a depth of 0-10 cm using the permanganate oxidizable carbon (POXC) test to measure the available energy of the system originating from biological activity [61]. Samples were collected and placed in individual polyethylene bags, transported to the laboratory in an icebox to minimize alterations of the material, and then air dried. The amount of potassium permanganate-oxidizable carbon was determined by exposing a 2.5 g sample of dry soil for 2 min to a solution of potassium permanganate at 0.02 mol L^{-1}, an oxidizing agent that reacts with readily oxidizable forms of active carbon. The potassium permanganate reduction reaction causes the absorbance to decrease proportionally to the increase in the amount of labile carbon, which is detected through spectrometry at 550 nm with an equation based on the calibration curve constructed from four successive dilutions of the stock solution, as presented by Culman et al. [61].

Basal soil respiration was assessed using the improved Situresp® method [62]. The principle is to assess the CO_2 emissions of a soil sample. This method assessed CO_2 emissions from a 98.17 cm^3 sample of fresh soil by measuring changes in color by spectrometry at 570 nm in a pH-sensitive gel in a 4.5 mL macro cuvette, induced by cresol red ($C_{21}H_{18}O_5S$) at 3.26×10^{-5} mol L^{-1}. The CO_2 emitted by the soil alters the pH of the gel, enabling estimation of the quantity of CO_2 emitted through spectrometry by calculating the difference between the initial absorbance and that after 24 h of incubation at 23 °C. To preserve the in-field soil structure and functioning as much as possible, the bulk soil density cylinder (0–5 cm deep and 5 cm in diameter) was incubated in a hermetically sealed 1 L glass pot. The absorbance is expressed per soil dry mass.

2.6. Computing the Soil Multifunctionality Index

Four functioning indexes (i.e., $FI_{structure}$, FI_{carbon}, $FI_{nutrient}$, and MFI) were aggregated from the 9 indicators (i.e., VESS, Beerkan, AggSurf, and AggSoil for $FI_{structure}$; bait lamina, POXC, and Situresp® for FI_{carbon}; and N_{min} and $P_{deficiency}$ for $FI_{nutrient}$) using a method adapted from Obriot and colleagues [63]. Briefly, indicator values were normalized per site based on desirability criteria: "more is better" for POXC, Situresp®, bait lamina, Beerkan, AggSurf, AggSoil, and N_{min}; "less is better" for $P_{deficiency}$; and "optimum" for VESS (optimal value = 1). AggSurf and AggSoil were reweighted as a unique indicator by averaging them before $FI_{structure}$ aggregation. FIs were calculated by averaging the indicator values [64], and the soil multifunctionality index (MFI) was calculated by averaging the

FI values. In each sub-plot, some indicators were measured three times (POXC, Situresp®, AggSurf, and AggSoil), some twice (bait lamina, VESS, and Beerkan), and some once on a composite sample of five sub-samples (soil analysis, N_{min}, and $P_{deficiency}$). Repeated measurements were averaged when required.

2.7. Statistical Approach

All statistical analyses were performed with R software (V4.2.2). First, for each site independently, the effects of fertilization practices on the FIs and MFI were evaluated using linear models with the lm function, ANOVA, and Tukey post hoc tests with emmeans and cld functions from lsmeans [65] and multcomp [66] R packages. Then, several subsets and data transformations (Table S3) were further used to investigate (i) the consistency of the fertilization effect on the indicators, FI, and MFI among soil types; (ii) the specific effect of fertilization on soil functioning; and (iii) the relationships between soil functioning and soil chemical properties. When required, the data were transformed with the bestNormalize function (bestNormalize R package) [67,68] to fit the model requirements, and the residual normality and homoscedasticity were tested with the shapiro.test and bptest functions (lmtest package), respectively [69].

To test whether the effects of fertilization practices on the indicators, FIs, and MFI were consistent across the three soil types (Table S4), linear models built with the lm function were used to assess the effect of fertilization practices, the type of soil, and their interaction. This analysis was limited to the subset (subset i) of fertilization practices used at all the sites (mineral, pig manure, and filter mud, n = 27, see Figure 1).

To depict the effect of fertilization practices on the indicators, FIs, and MFI, mixed linear models were used with the lmer function in the lme4 R package [70]. To account for the non-independence of samples collected from the same sites, we included sites as a random effect in the models (Table S5). Group comparisons were performed with ANOVA (emmeans function) and Tukey post hoc tests (cld function) to detect significant differences. This analysis was limited to a subset (subset ii) of five different fertilization practices (i.e., mineral, pig manure, sewage sludge, filter mud, and green waste compost) used at two sites (i.e., Nitisol and Cambisol, n = 30, see Figure 1).

The full dataset (subset iii) was used to study the effect of soil chemical properties on the components of soil multifunctionality. First, the soil properties and indicator values were scaled for each soil type independently to exclude the site effect of the analysis. Next, an NMDS was built using the R function metaMDS in the vegan package [71] based on the Euclidean distance matrix (built with the vegdist function in the vegan R package) including all the indicators (i.e., VESS, Beerkan, AggSurf, AggSoil, bait-lamina, POXC, Situresp®, N_{min}, and $P_{deficiency}$) to observe any dissimilarity caused by fertilization. The variance in soil functioning explained by the soil chemical properties (i.e., CEC, Ca, pH, P, C/N, N, C, Mg, K, and Na) and their co-explanation were thus evaluated with the envfit function in the vegan package.

The most agronomically relevant soil chemical properties among those that best explain the dissimilar effects of fertilization practices were selected. From this step on, key soil chemical properties were used to partition the variance in the indicators, FIs, and MFI: pH, which provides information on the acid–base status of the soil (i.e., equilibrium between nutrient forms and affinities); the organic carbon content and C:N ratio, which rely on soil organic status (i.e., on the quantity and quality of soil organic matter [72]); and the calcium content, which refers to the soil mineral status (i.e., the quantity and diversity of mineral nutrients). The effects and the contribution of the chemical properties and their interactions in explaining soil functioning were tested using the Anova function in the car package [73] from linear models for each indicator and each functioning index. Pearson correlations and R-squared values were computed to investigate the relationship between the components of soil multifunctionality and selected chemical properties using the corr.test function in the psych R package [74], and the variables were transformed using the bestNormalize function if normality was not met.

Finally, the relationships between the MFI and the properties of the fertilizers were investigated using linear models between the MFI and input quantities (expressed as a percentage of the maximum quantity) or the C:N ratio (as a proxy of input quality), scaled per site with z scores. The relationships between fertilization and yields were tested using linear models following the presented method as described above. The fertilization practices did not influence the yields at the three sites studied here (Table S1), which is why we choose to only focus on the effect of the different fertilizers on soil multifunctionality.

3. Results

3.1. Consistency of the Effect of Fertilization Practices across Soil Types

The consistency of the effect of fertilization on soil functioning across soil types was investigated with linear models on a data subset that accounts for the three fertilizers used with all three soil types (Table S4). Most variation in the MFI was explained by the type of soil ($p_{\text{value}} < 0.001$ ***, $r^2 = 0.42$) and to a lesser extent by fertilization ($p_{\text{value}} < 0.002$ **, $r^2 = 0.27$). No effect of the interaction was detected ($p_{\text{value}} = 0.753^{\text{ns}}$, $r^2 = 0.03$), which is evidence for the consistency of the effect of the fertilizers on multifunctionality across sites and soil types.

The FI_{carbon} and FI_{nutrient} functions were also mainly explained by the type of soil ($p_{\text{value}} < 0.001$ ***, $r^2 > 0.45$). Interestingly, both $FI_{\text{structure}}$ and POXC were mainly driven by the soil type and were not significantly affected by long-term fertilization practices ($p_{\text{value}} > 0.05^{\text{ns}}$, $r^2 < 0.12$). Individually, all the indicators of each function were explained by the effect of the soil type and some of them (i.e., Situresp®, bait lamina, N_{min}, and $P_{\text{deficiency}}$) by the fertilization effect. Regarding $P_{\text{deficiency}}$, fertilization practices explained more variation than the type of soil ($r^2 = 0.52$ and $r^2 = 0.27$, respectively), and an interaction effect between fertilization and soil type was observed ($p_{\text{value}} > 0.018$ *, $r^2 = 0.01$). This interaction did not affect either the consistency of the FI_{nutrient} and MFI response or lead to significant differences between fertilization practices, despite a more pronounced phosphorus deficiency for pig manure on Cambisol (Figure S2). It is also worth noting that yields were not affected by the fertilization practices.

3.2. Effect of Fertilization Practices on the Different Components of Soil Functioning

To describe the effect of fertilization practices on the different components of soil multifunctionality (Figure 2, Table S5), ANOVA and Tukey post hoc tests were performed on mixed models.

The MFI was significantly lower in soil fertilized with mineral fertilizer, pig manure, and sewage sludge than in soil fertilized with filter mud and green waste compost. Green waste compost increased the soil MFI the most, i.e., by ca. 60% compared to mineral fertilization. Carbon transformation was also enhanced by treatments comprising filter mud and green waste compost, with a maximum difference of +68%. Similar patterns were observed for bait lamina and POXC (60% increase). No significant relationship was found between Situresp® and fertilization practices. Similar to carbon transformation, nutrient availability was also improved by treatments with filter mud and green waste compost, with a maximum difference of +195%. Interestingly, filter mud and green waste compost both enhanced N_{min} by up to +113%, while only filter mud significantly affected $P_{\text{deficiency}}$ by −83%. The structure function, such as the VESS, was significantly improved by green waste compost, i.e., by +33%. Beerkan, AggSurf, and AggSoil were not affected by long-term fertilization practices.

Overall, the MFI was improved in soil fertilized with organic inputs characterized by a higher C:N ratio, and this was especially true for the carbon and nutrient function indicators. Accordingly, the relations between the MFI and the quantity and quality of organic matter inputs were further investigated. The quantity of inputs of organic matter was closely related to the MFI ($r^2 = 0.57$) while the quality, here approximated by the C:N ratio of the fertilizers, was less so ($r^2 = 0.42$). These two relations are plotted in Figure S3.

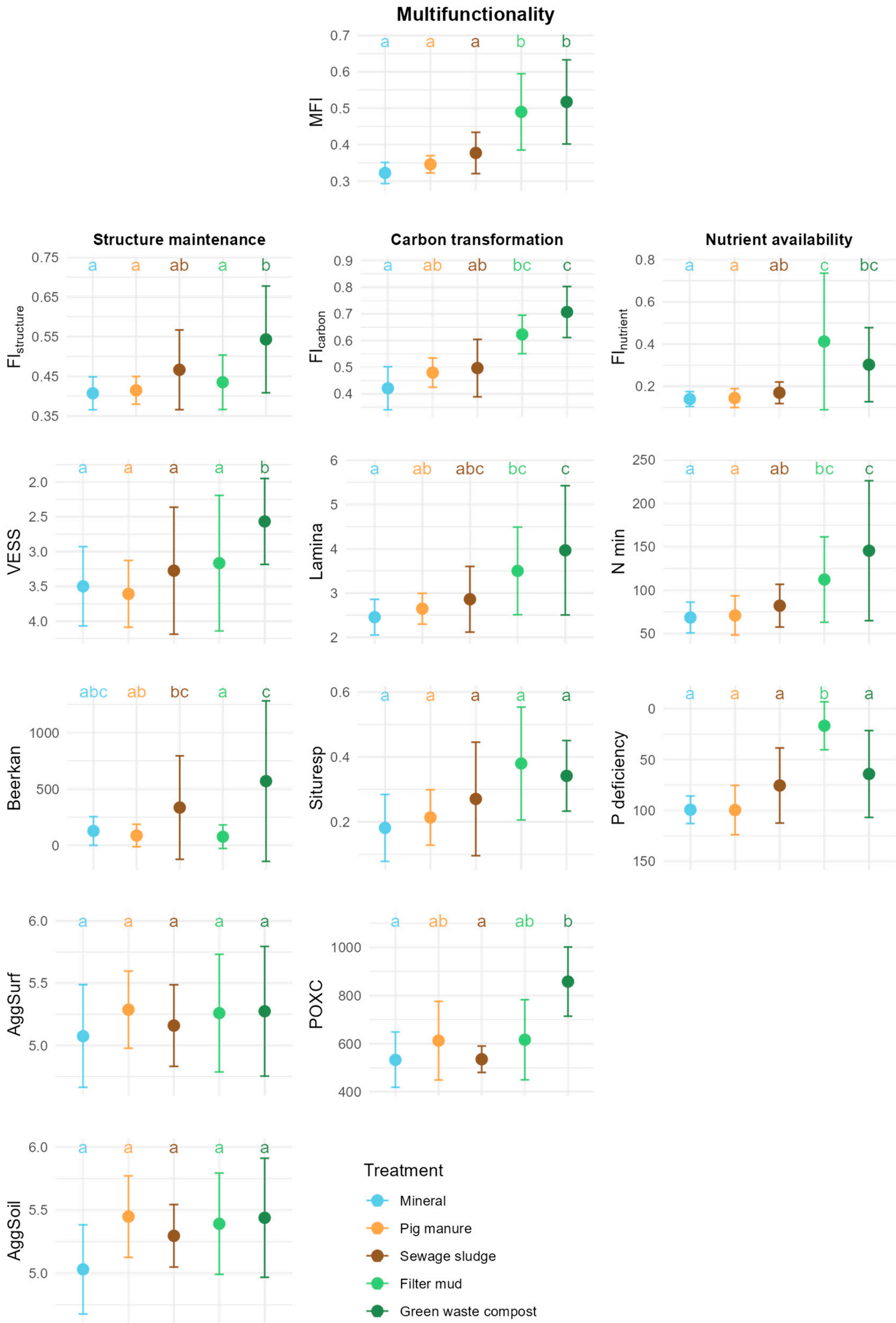

Figure 2. Differences induced by fertilization practices in functional indicators, soil functions, and index of multifunctionality (MFI and FIs). Mean values (dots) and standard errors (error bars) are shown, and letters indicate significant differences at $p_{\text{value}} < 0.05$ tested by ANOVA and the Tukey post hoc test (n = 6) on subset ii.

3.3. Linking Variation in Soil Multifunctionality Induced by Fertilization with Soil Chemical Properties

Relations between variations in soil functioning and soil properties were investigated (Figure 3, Table S6) by fitting environmental vectors (i.e., CEC, Ca, pH, P, C/N, N, C, Mg, K, and Na) onto an ordination of soil functioning. Except for K and Na, all soil chemical parameters were significantly related to soil functioning patterns (Table S6). The calcium concentration (r^2 = 0.55), soil solution pH (r^2 = 0.54), C/N ratio (r^2 = 0.46), and organic carbon concentration (r^2 = 0.43), which were important in explaining global functioning, were specifically selected for further study of the relations between soil functioning and chemical properties.

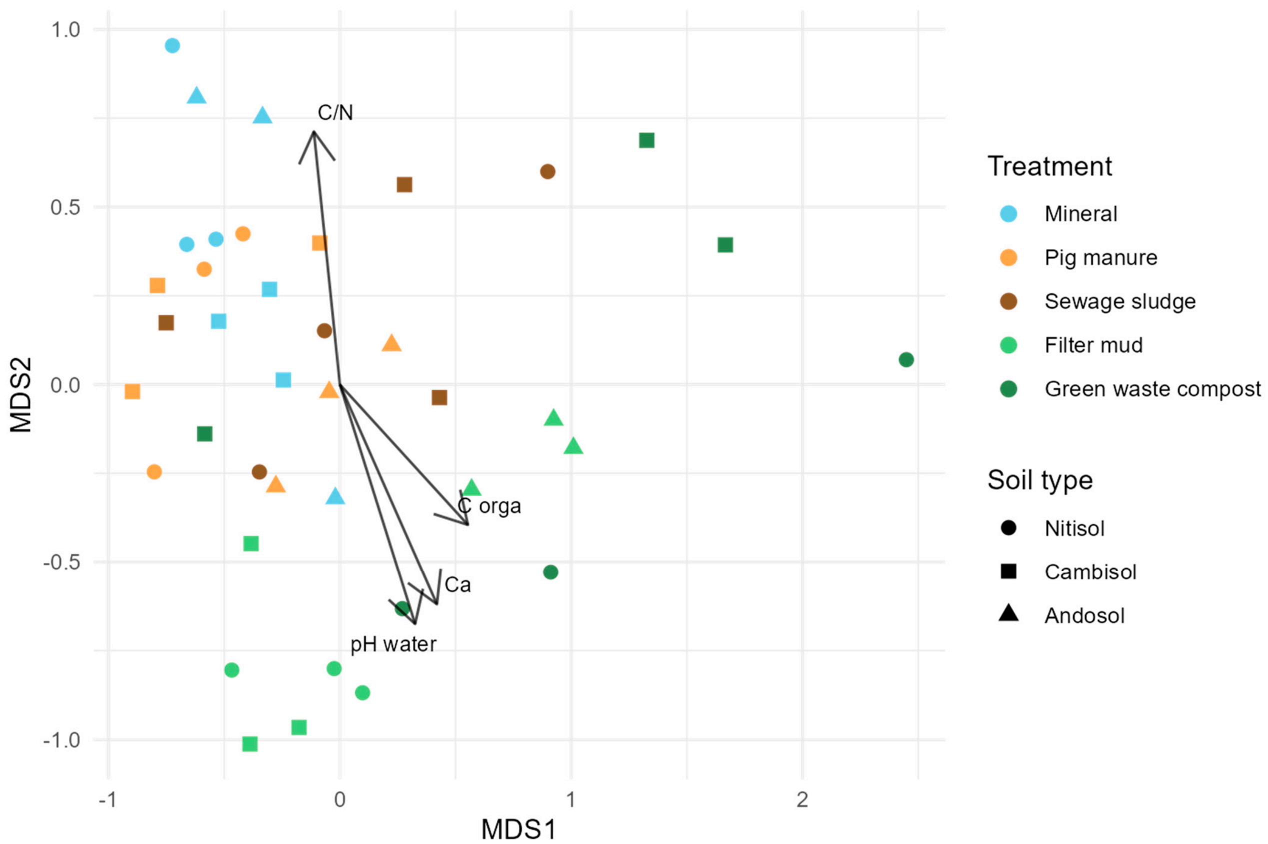

Figure 3. Non-metric multi-dimensional scaling of soil functioning based on Euclidean dissimilarity distances corrected for soil type variance (n = 39) using subset iii. The relation with chemical properties is indicated by vectors on the ordination.

The relations between functioning indicators and selected soil parameters were then investigated using Pearson correlations (Figure 4A) and variance partitioning with linear models (Figure 4B). The MFI was positively related to the organic carbon content (r^2 = 0.48), pH (r^2 = 0.35), and calcium content (r^2 = 0.43) and negatively related to the C:N ratio (r^2 = 0.35), but the respective effect of these soil properties accounted for less than 10% of the observed variation. The increase in nutrient availability was mainly related to the organic carbon content (r^2 = 0.47). Nitrogen mineralization, which mainly increased with organic carbon (r^2 = 0.79), was the functional indicator that was best explained by the soil chemical properties. The regulation of $P_{deficiency}$ was mainly associated with variation in the C:N ratio (r^2 = 0.40) and calcium content (r^2 = 0.45). The transformation of carbon was positively related to organic carbon (r^2 = 0.43). About two-thirds of the variation in the bait laminas was explained by the chemical properties, of which the most important part was explained by the organic carbon content (r^2 = 0.34). Situresp® and POXC were weakly related to soil chemical properties but mainly to pH (r^2 < 0.2). None of the four selected chemical properties was a significant explanatory variable for soil structure. VESS was significantly related to organic carbon content (r^2 = 0.25). To sum up, the organic carbon content, C:N ratio, and, to a lesser extent, pH and Ca, were important variables in explaining several functioning indicators, FIs, and the MFI. For most descriptors, about half of the total variation in the MFI induced by fertilization practices was not explained by the selected soil chemical properties.

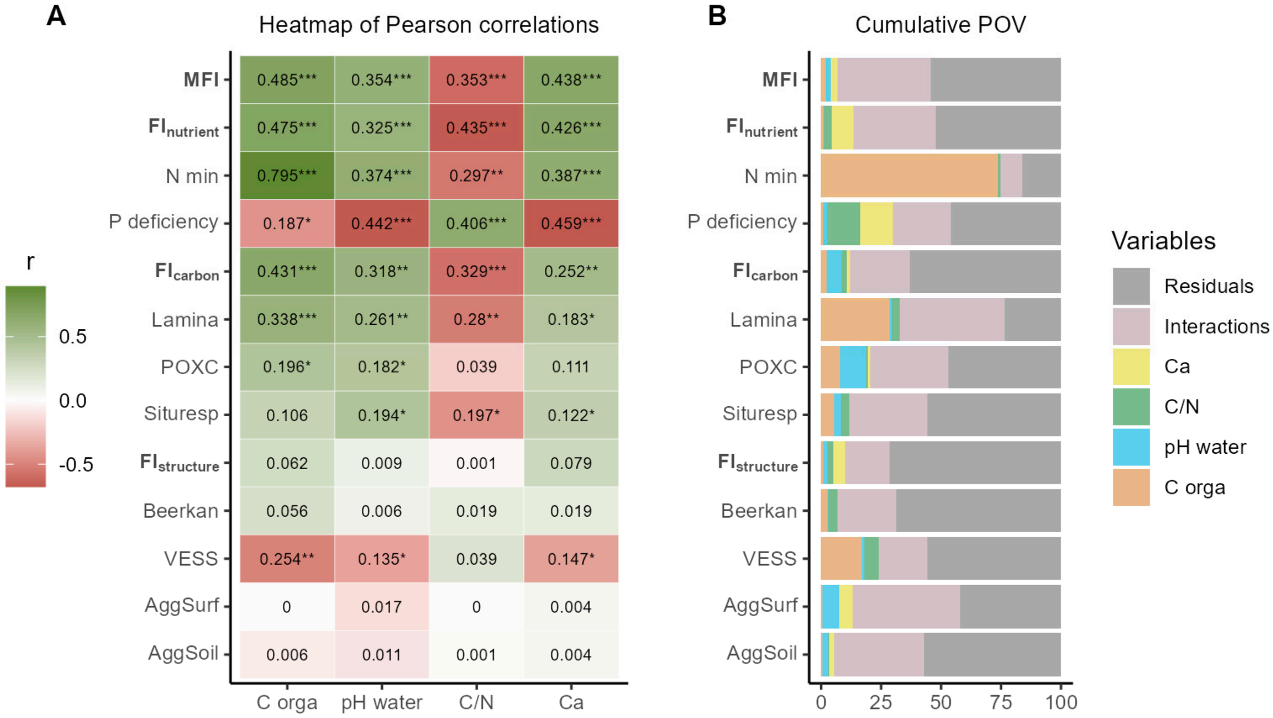

Figure 4. (**A**) Heatmap of Pearson correlations between multifunctionality components and soil chemical properties with significance labels per coefficient (*, **, and ***; $p < 0.05$, 0.005, and 0.0005) and adjusted R-squared values; (**B**) variance partitioning of indicators and their interactions by chemical properties (n = 39) scaled per soil type on subset iii.

4. Discussion

4.1. Responses of Soil Functioning Indicators to Organic Fertilization

Research is lacking on the long-term effect of organic fertilization on soil functioning in cultivated sugarcane systems [31], and this study is the first to assess the impact of a variety of organic fertilizers on soil multifunctionality in an Andosol, a Cambisol, and a Nitisol. Consequently, to discuss the functioning of agroecosystems fertilized with organic matter, we are obliged to review the literature on other soil types and other cropping systems. The effects of organic fertilizers used for the cultivation of sugarcane were generally consistent across the three different soil types studied. In the case of other types of soil, fertilization may have different effects by inequitably altering soil biological communities, their associated metabolism [75], and soil physicochemical properties [76].

The functioning indicators of soil structure were weakly affected by organic fertilization, despite repeated applications over a period of 5 to 7 years. No effect of organic fertilization on soil structure was observed in a 15-year-long study [77], while Naveed et al. [78] detected changes in the soil water holding capacity, porosity, and aeration after 106 years of organic fertilization. This underlines the fact that the modification of soil structure is a long-term biologically mediated process [79]. The extent of the observed shifts in the functioning indicators of soil structure depended on the type of fertilizer. The application of green waste compost led to soil decompaction and improved water infiltration, probably because of the high carbon content (Table S1). Naveed and colleagues [78] found a strong link between an increase in the organic carbon content and porosity in soils fertilized with manure compared to soils treated with mineral fertilizer, while Brar and colleagues [80] found a strong link between the soil carbon content and the rate of water infiltration. Indeed, fertilizers with a higher carbon content are known to positively affect mesofauna activity and the abundance of soil engineers, leading to improved soil structure [81,82]. Concerning soil aggregates, the addition of organic matter has already been shown to enhance aggregate stability, with a strong effect that starts in the first weeks following inputs of organic matter and then diminishes as it stabilizes over months and years [83]. However, probably due to the high clay content [84,85] or allophane content [84,86] of the soils studied here, the aggregates were highly stable irrespective of the type of soil or fertilization practices used. Indexing soil aggregate stability is thus not relevant for soils in Reunion Island.

The functioning indicators of carbon transformation were influenced by organic fertilizers, with slight improvements observed with pig manure and sewage sludge and substantial improvements with filter mud and green waste compost compared to with mineral fertilization. Decomposition assessed using bait lamina was highest with filter mud and green waste compost, which provide the highest organic matter inputs. This result is in line with those obtained by Zhou et al. [87], who showed a positive relation between decomposition and the amount of organic matter inputs using different quantities of green waste digestate in a long-term experiment. The highest inputs increased microorganism feeding activity and the organic carbon content. The latter decreased with mineral fertilizer. In the present study, there was no clear increase in the labile carbon content in soils with pig manure, sewage sludge, filter mud, or mineral fertilization, and only green waste compost significantly improved the labile carbon content. These results are in line with the findings of Hwang et al. [88]. In their study, inputs of compost increased the labile carbon content in soil more than manure and mineral fertilizer. The authors suggest that this increase has a beneficial effect on microorganism activities as a readily available carbon source and enhances the physical structure of the soil. Although non-significant, we found a higher basal respiration in soil amended with filter mud and green waste compost. More pronounced responses have been observed in other experiments. For instance, Chang et al. [89] measured high basal respiration and microbial activity with the application of green waste and compost in a 12-year experiment on loamy soil.

The functioning indicators of nutrient availability were affected by organic fertilization. Increases in the nitrogen content and decreases in soil phosphorus deficiency were found in soil amended with filter mud and green waste compost. When amended in large quantities, organic matter increases nutrient availability [90]. For instance, in two very long-term experiments (>100 years of organic fertilization), organic matter inputs resulted in correlated increases in both the soil organic carbon and nitrogen [19,91]. These findings can be explained by the release of nutrients from soil organic matter thanks to the enzyme activities of the microbial communities involved in nitrogen mineralization processes [90,92]. We observed a similar trend for soil phosphorus deficiency that decreased with the application of filter mud and, to a lesser extent, with applications of sewage sludge and green waste compost. The organic matter in the filter mud contains on average more phosphorus than other fertilizers (Table S2). Moreover, the balance between available and unavailable phosphorus is also influenced by its solubilization, which, in turn, is driven by the pH of the soil solution and by the concentration of calcium in the soil [93–96]. These two parameters may help explain the higher concentration of available phosphorus in the soil, as shown in other sugarcane field experiments [97,98].

Some indicators appear to be better suited and easier to use to evaluate the long-term effect of fertilization on the multifunctionality of the types of soil studied here. Visual evaluation of soil structure has emerged as the most sensitive, user-friendly, and integrative indicator [99] of soil structure. The assessment of mesofauna decomposition using bait lamina was the best indicator of carbon transformation and, unlike other methods, requires minimal equipment when set up. Nitrogen availability was preferred for the assessment of nutrient availability because its sensitivity to fertilization was more consistent across soil types.

4.2. Organic Fertilizers Affect Distinct Soil Functions

The fertilizer we studied affected long-term soil structure, carbon transformation, and nutrient availability in different ways. Compared to mineral fertilizer, pig manure had no positive effect on soil structure or on the ability of the soil to supply nutrients, suggesting that the main effect of pig manure took place directly after spreading. In the present study, pig manure only slightly influenced carbon transformation. In the systematic review of Yost et al. [100], the authors showed that pig manure clearly enhanced carbon transformation despite the different durations and pedoclimatic contexts. For instance, pig manure was reported to enhance soil health and fertility by increasing the soil organic matter content and carbon stabilization through the transformation of organic matter mediated by microbial activity that strongly depends on nitrogen availability [19]. Similarly, sewage sludge only slightly improved all the soil functions studied here compared to mineral fertilization. Simoes-Mota and colleagues [101] previously showed that nitrogen and phosphorus availability, soil porosity, organic matter content, and earthworm activity were improved by application of sewage sludge and increased associated functions. Sewage sludges indeed have positive effects on the physical, chemical, and biological properties of soils [102], and their potential ability to reduce soil phosphorus deficiency was highlighted in this study.

Filter mud did not affect soil structure but significantly enhanced carbon transformation and nutrient availability. Chattha et al. [103] showed that composted sugarcane filter mud increased the soil organic matter content and its chemical properties, indicating improved carbon transformation [104]. An increase in soil nitrogen and a decrease in phosphorus deficiency is an expected effect of filter mud, which is known to contain large quantities of phosphorus and easily mineralizable organic nitrogen. When applied directly to the field, filter mud has indeed been found to enhance soil fertility by changing the composition of bacterial communities [105], increasing the quantities of nitrogen and phosphorus, and improving the cation exchange capacity [106,107].

In addition to enhancing nutrient availability and carbon transformation similar to filter mud, green waste compost was the only fertilizer studied that clearly improved soil structure. This simultaneous effect on soil biological activities and physical properties has already been reported after seven years of application of green waste compost using multicriteria analyses in wheat–maize rotations [63]. An improvement in soil fertility and in the organic status of a sugarcane cultivation system was reported in an Australian clayey soil altered only four years after a single subsoil (20–25 cm) application of green waste compost due to an increase in organic carbon and nitrogen content [108]. Such changes might be due to the physical and chemical nature of organic matter, which is stabilized during the composting of green wastes, and is composed of bigger particles than filter mud and resulted in a more stable soil structure.

Although the type of fertilizer played an important role in driving soil functioning, the quantity and the quality of organic matter applied, assessed by the C:N ratio, were also positively correlated with multifunctionality (Figure S3). Our experimental design did not allow us to distinguish whether the quantity or quality of the organic matter was the most important driver, as the inputs of fertilizers were calculated based on their quality. Testing several quantities for each fertilizer would have been informative and should be evaluated in future experiments. However, this approach was already partially applied by Zhou et al. [87], who found a positive relation between the activity of decomposers and the quantity of fertilizer using green waste digestate. This suggests that providing more organic matter, at least that with a high C:N ratio, is likely to favor biological activities, thereby enabling higher soil multifunctionality.

4.3. Soil Chemical Changes Poorly Explain Multifunctionality

First, it is important to note that the existence of numerous methods for calculating multifunctionality indexes leads to slightly different results. Some methods have already been tested in sugarcane systems in Brazil [34], but these differences did not substantially alter either the results themselves or their interpretations. In our study, soil functioning was largely structured by the study site (Table S4) and, to a lesser extent, by the fertilization practices. This highlighted the structuring effect of the climate on soil multifunctionality, thereby also highlighting the limited effects of organic fertilizers, bounded by inherent soil properties. The changes to key properties (i.e., Δ C, C/N, pH, and Ca) induced by fertilization explained the multifunctionality and its components.

Of the four selected soil properties, the carbon content was the best covariate of soil multifunctionality, as it explained around half of the total variation. An increased soil carbon content and a lower C:N ratio also led to long-term increases in the mineral nitrogen content and reduced phosphorus deficiency, likely due to the transformation of organic matter through biological activity [109]. This is in line with the results of the present study that point to the close correlations between the carbon content and the C:N ratio with bait lamina, the quantity of labile carbon, or basal respiration. Several studies highlighted the importance of the quality of soil organic matter and its stoichiometry in driving basal respiration processes [110–112]. Luo et al. [113] also showed that carbon transformation processes were mainly driven by the quantity of inputs and soil organic carbon content. As in the present study, organic fertilization was found to improve the organic status of soils [20], to enhance the supply of soil nitrogen [19] as well as biological activity [114], and to improve soil structure [115] and water circulation [80]. The important contribution of the soil carbon content to multifunctionality has already been demonstrated in other studies [116]. The authors documented the strong link between the soil carbon content, soil multifunctionality, and functional diversity. In so doing, they underlined the importance of microbial communities in ensuring sustainable soil functioning in agroecosystems.

The pH, which is determined by the acid–base status of the soil, was, similar to the carbon content, closely linked to soil multifunctionality and its nutrient and carbon components. Variations in the Ca content, which reflect the mineral status of the soil, explained basically the same functioning properties as the pH. Close relations between soil pH and nitrogen [117] or pH and phosphorus availability [95] have been found in other studies, recalling that pH can affect nutrient availability by influencing the equilibrium between their available and unavailable forms. Our results are in line with this point, especially through phosphorous availability, although the pH is not sufficient to explain phosphorous availability. Moreover, numerous studies [104,118–120] found that pH modulates microbial activity, regulates fauna communities, alters organo-mineral associations, and affects aggregate stability. Taken together, this points to a close relationship between pH and carbon transformation.

Variations in soil chemical properties induced by fertilization explained about half of the variability in soil multifunctionality. Previous research has identified biotic factors that must be taken into consideration to enhance existing explanations of multifunctionality [121–123]. Indeed, the effects of agricultural practices on soil multifunctionality are mainly indirect, driven by changes in soil chemical properties that result in shifts in the soil community structure and composition [12,121,124,125]. Taking biological activity into consideration appears to be indispensable to describe and understand the drivers of soil multifunctionality [81,124,126]. The higher sensitivity of indicators associated with biological activities (i.e., bait lamina, POXC, N_{min}, $P_{deficiency}$, and VESS) identified in this study supports the hypothesis that biological communities contribute significantly to soil multifunctionality. Further studies that jointly investigate biotic and abiotic factors are now needed to advance our understanding of the underlying soil multifunctionality mechanisms.

5. Conclusions

This study demonstrated a consistent effect of organic fertilization on soil multifunctionality across three different types of soil used to cultivate sugarcane in Reunion Island. The use of different types of fertilizer affected soil multifunctionality, highlighting the beneficial effects of fertilizers containing organic matter with a high C:N ratio, while other types had similar effects to those of mineral fertilizer. Functions related to the transformation of applied organic matter, nutrient availability, and its transfer to the soil exhibited higher sensitivity, whereas soil structure displayed stronger inertia. A longer period of applying fertilization practices could help detect the effect of organic fertilizers on soil structure. Moreover, a good proxy of soil multifunctionality was not identified among any of the four soil chemical properties studied. Thus, the variations in chemical properties alone did not sufficiently explain the effect of fertilization practices on soil functioning. The experimental design did not allow us to distinguish whether the beneficial effect was due to the quantity or the quality of the organic matter. However, as in this study, fertilization practices are designed as a function of the nutrient content of the organic matter, and our findings are important for farmers. They clearly demonstrate that for equivalent nitrogen inputs and hence similar yields, the use of organic fertilizers with higher C:N ratios enhanced soil multifunctionality, principally through nutrient availability and carbon transformation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14112475/s1, Table S1: Mean values and standard deviations of sugarcane yield in fresh matter (FM) and soil chemical properties in dry matter (DM) in the year the indicator measurements were calculated independently for each type of soil according to each fertilization practice (n = 3). The variables are yield, organic carbon (C), total nitrogen (N), C:N ratio (C/N), total phosphorus (P), exchangeable potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), cation exchange capacity (CEC), and acidity (pH); Table S2: Characterization of fertilizers and inputs of nutrients in each fertilization treatment and each soil type. The characteristics of the organic fertilizers are grouped in the mean value of inputs per site. The characteristics are the quantity provided (OM input), C:N ratio (C/N) representing the quality of organic matter, mineral fertilizer equivalence coefficient for nitrogen (C_{min} N), total nitrogen (N total), available nitrogen (N available), mineral fertilizer equivalence coefficient for phosphorus (C_{min} P), total phosphorus (P total), available phosphorus (P available), total and available potassium (K), calcium (Ca), magnesium (Mg), and organic matter acidity (pH). Values are expressed relative to dry matter (DM); Table S3: Summary of the different datasets used and associated figures, model building, quality of fit, and transformation of the variables. If it was necessary, the response variable x was transformed before building the models to respect validity conditions; Table S4: Effect of fertilization practices over sites and on soil functions (n = 3): significance of and variance partition for linear models on subset i; Table S5: Effect of fertilization practices on soil functions: significance for and variance partition for mixed effect models on subset ii (5 fertilization practices and 2 sites; n = 6), with *p* value labels per coefficient (ns, *, **, and ***: $p > 0.05$, ≤ 0.05, ≤ 0.005, and ≤ 0.0005); Table S6: Relation of chemical properties in shaping soil functioning: coefficient of determination and p value (ns, *, and **: $p > 0.05$, ≤ 0.05, and ≤ 0.005) for each soil parameter (n = 39); Figure S1: Schematic description of the functioning indicators used to determine structure maintenance, nutrient availability, and carbon transformation; Figure S2: Differences induced by fertilization practices in $P_{deficiency}$. Measurements (dots), mean values (rhomboid), and standard deviations (error bars) are shown, and letters indicate significant differences at a *p* value < 0.05 tested by ANOVA and the Tukey post hoc test (n = 3) on subset ii between mineral (blue), pig manure (orange), and filter mud (green) for each soil type; Figure S3: Linear regressions between multifunctionality and (A) maximal quantity and (B) C/N ratio of organic matter z score site scaled (n = 39) on subset iii.

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