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Cowpea Crop Response to Mineral and Organic Fertilization in SE Spain

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Abstract: Mineral fertilization is considered to be useful for improving soil fertility and yields. However, its use is linked to global warming and soil and water pollution by its rapid mobilization. On the other hand, organic fertilization is recommended to maintain or improve soil organic carbon and total nitrogen stocks while contributing to climate change mitigation. The main goal of this study was to assess the effect of two different fertilizer types, mineral and organic, during three cowpea crop cycles on the soil's physicochemical properties, enzyme activities, crop yield, crop quality and nutritional composition when considering two cowpea cultivars (Feijão frade de fio preto (FP) and Feijão frade de fio claro (FC)). The use of mineral fertilizers was seen to contribute to improved soil fertility due to the increase in soil properties, such as recalcitrant carbon, total nitrogen, ammonium content, available K and available Mg. On the other hand, organic fertilizers only increased the nitrate content in the soil. There were no differences in terms of cowpea crop yield, quality and nutritional composition by fertilizer type. Thus, both fertilizer types contributed to the same crop yield and quality, and thus the use of organic fertilizers can result in a sustainable alternative for maintaining cowpea crop yield and quality.

Keywords: *Vigna unguiculata*; chemical fertilizers; organic fertilizers; carbon dynamics; nitrogen dynamics

1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) was first cultivated in Africa, and its cultivation has extended to tropical and semi-arid regions of the world [1]. Its seeds, leaves and green pods are valued for their nutritional properties, since its grains are rich in protein (about 25%), vitamins, fibers, minerals and other nutrients [2,3]. This legume is a drought-tolerant and warm weather crop which can be used not only in human food, but also for animal feed and the recovery of soil fertility through its use as green manure [4]. Cowpea as a legume crop needs a symbiotic association with effective nitrogen-fixing bacteria to attain maximal benefits from symbiotic N_2 fixation and thus save costly N fertilizers. Cowpea establishes symbiotic relationships with several species of the phyla Alpha- and Betaproteobacteria [5].

The use of mineral or organic fertilizers is directly related to soil fertility, soil carbon sequestration, greenhouse gas (GHG) emissions and crop yield. Mineral fertilizers play an important role in terms of the rapid increase of soil fertility and crop yield due to their high nutrient content and ease of availability [6]. However, this type of fertilization could not be the most effective or adequate, considering the maintenance of soil fertility over time, since an excessive application of mineral fertilizers can lead to low nutrient efficiency and deterioration of the soil and environment [7,8]. In terms of carbon sequestration, the use of mineral fertilizers has shown to have a rapid influence on the soil, since it can promote mineralization of a stable fraction of organic matter due mainly to their high N rates, or

it can increase carbon stocks by plant biomass production [9–11]. On the other hand, the application of N-rich mineral fertilizers used to sustain crop yield are responsible for 60% of global anthropogenic N_2O emissions. This greenhouse gas is primarily produced with the application of mineral fertilizers, providing a N source in a form directly available to the plant as NO_3^-, which can be reduced to gas by denitrifying microorganisms [12–14].

Organic fertilizers have higher organic matter and richer nutrient elements, although they are not readily available to the plant and they have to be microbially mineralized before becoming available to the plant [15]. In turn, the use of organic fertilizers has usually been associated with lower crop yields [16]. However, there are studies in which the application of manure acted as a better fertilizer than mineral fertilizers for increasing crop yields [17,18]. In terms of soil, organic fertilizers lead to the increase of carbon stocks, which enhances the benefits for plant growing and reduces soil erosion [19,20]. They can enhance the soil's physical properties such as aggregate stability, as well as the soil's biological and biochemical properties, and optimize the soil's microbial community structure [21–24]. In contrast to mineral fertilizers, the use of organic fertilizers results in positive long-term effects [10]. In addition, they can reduce the environmental impact in terms of GHG emissions, since organic farming uses N more efficiently by increasing the soil organic carbon (SOC) and N storage, and thus it is responsible for the lower GHG emissions [25,26].

According to the latter approaches, where cowpea was studied mostly in terms of symbiotic efficiency, stress response, genetic and morphological diversity or its nutritional characteristics, a cowpea crop was cultivated using mineral and organic fertilizers for three years. The objectives of this study were to (1) assess the effect of mineral and organic fertilizers on soil organic carbon content and pools, soil nutrients, soil aggregation, soil enzyme activities and the crop yield and quality parameters, and (2) infer if there is a relationship between soil fertility and crop yield and quality. We hypothesized that the use of mineral fertilizers would increase cowpea crop yield, since this is a source of nutrients directly available to the plant. However, the use of organic fertilizers would result in higher organic carbon and nutrient contents and thus higher microbial activity.

2. Materials and Methods

2.1. Study Site and Experimental Design

This study was carried out in the same field in Cartagena in southeastern Spain (37°41′ N 0°57′ E) for three years. The field experiment was designed as a complete randomized block with four replications, and the plot had a size of 100 m^2. Two local Portuguese cultivars (Feijão frade de fio preto (FP) and Feijão frade de fio claro (FC)) of cowpea (*Vigna unguiculata* (L.) Walp.) were grown during the spring and summer of 2014 (from 29 May 2014 to 13 August 2014), 2015 (from 3 June 2015 to 14 September 2015) and 2016 (from 1 June 2016 to 22 August 2016). This crop was drip irrigated, and two fertilizer types (mineral and organic) were used.

The soil was a Haplic Calcisol [27] with a clay loam texture, the main characteristics of which are shown in Table 1. The mean annual temperature of the study area was 18 °C, and the mean annual precipitation was 275 mm. Annual potential evapotranspiration surpassed 900 mm. Cowpea seeds were sown with a spacing of 100 cm between rows and 20 cm between plants (5 plants m^{-2}). No herbicide treatment was provided, and the crops were kept free of weeds through hand-hoeing when necessary.

Every year, a surface application of 16,000 kg ha^{-1} of goat and sheep manure was carried out in all plots before sowing. This manure had the following characteristics: pH = 8.32 ± 0.07; electrical conductivity = 21.2 ± 1.01 mS cm^{-1}; total organic carbon content = 307 ± 11 g kg^{-1}; total nitrogen content = 13.4 ± 0.7 g kg^{-1}; P_2O_5 content = 1.8 ± 0.08%; and K_2O content = 4.6 ± 0.2%. Fertilizer application in the cowpea plots started between two or three weeks after sowing and continued until harvesting. In the cowpea crops, 30 kg ha^{-1} of N and 2.4 kg ha^{-1} of P_2O_5 were applied by mineral fertigation of ammonium nitrate (33.5% N) and monoammonium phosphate (61% P_2O_5, 12% N) and a commercial liquid organic fertilizer (Bombardier, Agroquímicos los Triviños, Spain; 10.7%

w/v N, 0.7% w/v P$_2$O$_5$) in the organic fertigation. Cowpea residues were removed from the field and not applied in the soil as green manure. The irrigation was established on the basis of the evapotranspiration rate, crop coefficient and climatic conditions of rainfall.

Table 1. Main soil characteristics. Values are mean $\pm$ standard deviation ($n = 4$).

Parameters [a]	
pH	8.40 ± 0.09
EC (dS m^{-1})	0.34 ± 0.07
SOC (%)	1.19 ± 0.18
Bulk density (Mg m^{-3})	1.01 ± 0.03
CEC (cmol kg^{-1})	7.80 ± 1.20
CaCO$_3$ (%)	30.2 ± 1.2
Clay (%)	34.5 ± 0.16
Silt (%)	21.3 ± 1.06
Sand (%)	44.2 ± 0.92
N$_t$ (%)	0.094 ± 0.007
NO$_3^-$ (mg kg^{-1})	156 ± 45
NH$_4^+$ (mg kg^{-1})	5.69 ± 1.12
Available P (mg kg^{-1})	30.0 ± 5.4
Exchangeable K (mg kg^{-1})	369 ± 63
Exchangeable Ca (mg kg^{-1})	2726 ± 126
Exchangeable Mg (mg kg^{-1})	606 ± 24
Exchangeable Na (mg kg^{-1})	301 ± 31

[a] EC: electrical conductivity; SOC: soil organic carbon; CEC: cation exchange capacity; and N$_t$: total nitrogen.

2.2. Soil and Plant Sampling

The soil was sampled after harvesting the cowpea crop for three successive years. All plots were sampled at 0–20 cm (A$_p$ horizon). Three random soil samples per plot were collected and homogenized to obtain a composite sample, which was air-dried for 7 days, sieved (<2 mm) and stored at room temperature until analyzed. Enzyme activities were also measured in the air-dried samples, since this property is medium-term stable in stored air-dried samples of Mediterranean semiarid soils [28].

The cowpea crop yield was determined by weighing all the pods in each plot harvested when the seeds were dried at the end of the crop cycle. The weight of 100 seeds and protein content in the seeds were recorded as crop quality parameters.

2.3. Soil Analyses

The following parameters were measured: bulk density by the cylinder method [29]; soil pH and electrical conductivity (EC) in deionized water (1:2.5 and 1:5 w/v, respectively); soil texture by the Bouyoucos method [30]; equivalent calcium carbonate using the volumetric method (Bernard calcimeter) [31]; SOC by the wet oxidation method using K$_2$Cr$_2$O$_7$ [32]; recalcitrant carbon (RC) and labile carbon (LC) by the method of double acid hydrolysis [33]; aggregate stability (AS) by the method proposed by [34], based on the application of simulated rainfall with a known intensity; total nitrogen (N$_t$) by the Kjeldahl method [35]; cation exchange capacity using BaCl$_2$ as an exchangeable salt [36]; NO$_3^-$, extracted with deionized water in a 1:10 soil–extractant ratio [37] and measured by ion chromatography (Metrohm 861); NH$_4^+$, extracted with 2 M KCl in a 1:10 soil–extractant ratio [37] and colorimetrically measured [38]; and available phosphorus (P), extracted according to the Burriel-Hernando method [39] using a Burriel-Hernando solution (0.2 g CaCO$_3$, 0.17 g MgCO$_3$, 5 mL glacial acetic acid and 0.2 mL H$_2$SO$_4$ in 2 L deionized water) in a 1:25 soil–extractant ratio. The available boron (B) was determined in the deionized water extract [40]. Exchangeable Ca, Mg, Na and K were determined in the BaCl$_2$ extract for CEC, and the P, B, Ca, Mg, Na and K concentrations were measured using ICP-MS (Agilent 7500CE). The β-glucosidase activity was based on the determination of p-nitrophenol released after incubation at 37 °C with β-D-glucopyranoside [41], while β-glucosaminidase

activity was based on the determination of p-nitrophenol released after incubation with p-nitrofenil-β-D-glucopyranoside at 37 °C [42]. The dehydrogenase activity was determined using p-iodonitrotetrazolium chloride as a substrate and measuring the absorbance of the iodonitrotetrazolium formazam (INTF) produced [43]. The arylesterase activity was based on the determination of p-nitrophenol released after incubation with p-nitrophenyl acetate at 37 °C [44]. The cellulase activity was assessed by the determination of gearbox sugars using amorphous cellulose as a substrate [45,46], and the urease activity was based on the determination of ammonium released after incubation of the soil with urea at 37 °C [47].

2.4. Plant Analyses

The plant samples were oven dried and ground (A11 Basic, IKA, Staufen, Germany) before incinerating at 500 °C. The ashes were dissolved in 0.6 N HNO_3 and analyzed for P, Ca, Mg, Na and K by ICP-MS (7500 CE, Agilent, Madrid, Spain). The nitrogen (N) content was determined by the Kjeldahl method [35]. NO_3^- was extracted with deionized water in a 1:50 plant–extractant ratio [37] and measured by ion chromatography (Metrohm 861, Metrohm Hispania, Madrid, Spain). The total organic carbon (TOC) was quantified by the total combustion.

2.5. Statistical Analyses

The data were checked to ensure normal distribution using the Kolmogorov–Smirnov test and log-transformed when necessary to ensure normal distribution. The data were submitted to two-way repeated ANOVA measures, with the year (2014, 2015 and 2016) as a within-subject factor and the cowpea cultivar (FP and FC) and fertilizer type (mineral and organic) as between-subject factors. The relationships among the properties were studied using Pearson's correlations. A principal components analysis (PCA) was performed with all data to study the structure of dependence and correlation established among the variables studied with both fertilizer types. Statistical analyses were performed with IBM SPSS version 22 software for Windows (IBM, New Yok, United States).

3. Results

3.1. Soil Physicochemical Properties

As a general pattern, the crop year significantly affected all soil properties, with general variations along the three crop cycles in most properties (Tables 2 and 3). The SOC, N_t, AS, RC, NO_3^- and P were higher in 2016 than in the first two years. The NH_4^+ showed significantly higher values during the first year, while LC, Ca, Mg, K and Na showed significantly higher values during the second year.

The cowpea cultivar only significantly influenced the RC and NH_4^+ (Table 2). Both soil properties were higher in the soils cultivated with FC than FP in 2015 and 2016. With regard to fertilizer type, the use of mineral fertilizers significantly contributed to higher values of N_t, RC, NH_4^+, Mg and K, mainly in 2015 and 2016 compared with the organic fertilizers. However, the NO_3^- content was higher using organic rather than mineral fertilizers. There was no significant effect of fertilizer type on the SOC, AS, LC, Ca, P and Na. The interaction of the year and fertilizer type was significant with respect to AS, RC and NO_3^-. AS and NO_3^- showed the highest values using organic fertilizers during 2016, while RC showed the highest values when using mineral fertilizers during 2016. The interaction of the year and cowpea cultivar was significant for the RC, which was higher in the soil cultivated with FC during 2016 than during the last two years. The interaction of the cowpea cultivar and fertilizer type did not affect any of the soil properties. Finally, the interaction of the three factors (year, cowpea cultivar and fertilizer type) was significant for NH_4^+, with the highest values in the FP cultivar using mineral fertilizers during 2016.

N_t was positively correlated with the RC ($r = 0.82$, $p < 0.01$). The Ca content was positively correlated with the Mg ($r = 0.96$, $p < 0.01$) and K contents ($r = 0.85$, $p < 0.01$). The Na content was negatively correlated with the available P ($r = -0.83$, $p < 0.01$).

Table 2. Soil organic carbon, total nitrogen, aggregates stability, recalcitrant carbon, labile carbon, ammonium and nitrate contents in the soil of cowpea crops using mineral and organic fertilizers over three years. Values are mean ± standard deviation (*n* = 4).

Cowpea Cultivar [a]	Fertilizer Type	SOC (g kg^{-1})	N$_t$ (g kg^{-1})	AS (%)	RC (%)	LC (%)	NH$_4^+$ (mg kg^{-1})	NO$_3^-$ (mg kg^{-1})
				2014				
FP	Mineral	13.2 ± 1.2	1.2 ± 0.1	24.7 ± 13.4	0.58 ± 0.06	0.74 ± 0.09	842 ± 134	6.7 ± 2.6
FC	Mineral	13.0 ± 0.4	1.1 ± 0.0	17.3 ± 4.7	0.56 ± 0.02	0.74 ± 0.05	576 ± 213	7.3 ± 1.0
FP	Organic	12.7 ± 1.0	1.1 ± 0.1	18.3 ± 6.5	0.59 ± 0.09	0.53 ± 0.22	473 ± 185	11.2 ± 4.1
FC	Organic	11.6 ± 0.7	1.0 ± 0.1	7.8 ± 4.4	0.53 ± 0.09	0.63 ± 0.07	499 ± 249	13.2 ± 3.6
				2015				
FP	Mineral	11.7 ± 0.4	1.1 ± 0.1	26.7 ± 1.7	0.69 ± 0.14	0.70 ± 0.49	58 ± 13	4.9 ± 0.6
FC	Mineral	11.2 ± 0.1	1.1 ± 0.1	22.6 ± 1.1	0.89 ± 0.02	0.70 ± 0.83	64 ± 16	6.9 ± 4.2
FP	Organic	11.6 ± 0.9	0.8 ± 0.0	14.7 ± 4.8	0.21 ± 0.08	0.95 ± 0.14	71 ± 48	8.7 ± 2.2
FC	Organic	12.6 ± 1.0	0.9 ± 0.1	14.7 ± 6.9	0.44 ± 0.25	0.82 ± 0.34	116 ± 68	5.8 ± 0.4
				2016				
FP	Mineral	14.6 ± 2.2	1.5 ± 0.1	50.7 ± 9.3	0.99 ± 0.13	0.47 ± 0.35	640 ± 110	10.4 ± 3.2
FC	Mineral	14.1 ± 1.6	1.4 ± 0.1	44.8 ± 6.8	1.12 ± 0.08	0.29 ± 0.08	644 ± 149	10.0 ± 1.1
FP	Organic	12.8 ± 2.2	1.2 ± 0.1	43.7 ± 9.9	0.92 ± 0.13	0.36 ± 0.09	315 ± 96	8.5 ± 1.5
FC	Organic	14.5 ± 1.1	1.3 ± 0.2	56.0 ± 3.0	1.04 ± 0.05	0.41 ± 0.10	424 ± 109	18.0 ± 2.8
				F-value [b]				
				Between Subjects				
Cowpea Cultivar (CC)		0.03 ns	0.04 ns	0.71 ns	13.67 **	0.05 ns	6.74 *	0.05 ns
Fertilizer Type (FT)		0.28 ns	24.28 **	2.87 ns	46.67 ***	0.11 ns	22.22 **	15.60 **
CC × FT		0.62 ns	0.26 ns	1.07 ns	0.02 ns	0.09 ns	2.45 ns	3.48 ns
				Within Subjects				
Year (Y)		21.13 ***	44.31 ***	128.76 ***	53.12 ***	4.85 *	9.85 **	44.03 ***
Y × CC		1.80 ns	1.05 ns	3.85 *	3.19 ns	0.11 ns	2.37 ns	1.26 ns
Y × FT		3.07 ns	1.78 ns	4.31 *	11.67 **	0.79 ns	1.42 ns	3.82 *
Y × CC × FT		2.58 ns	0.96 ns	2.99 ns	0.05 ns	0.22 ns	4.99 *	0.61 ns

[a] FP: Feijão frade de fio preto; and FC: Feijão frade de fio claro. [b] Significant at *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; and ns: not significant ($p > 0.05$). SOC: soil organic carbon content; N$_t$: total nitrogen; AS: aggregates stability; RC: recalcitrant carbon; and LC: labile carbon.

Table 3. Exchangeable calcium, magnesium, potassium and available phosphorus in the soil of cowpea crops using mineral and organic fertilizers over three years. Values are mean ± standard deviation (*n* = 4).

Cowpea Cultivar [a]	Fertilizer Type	Exc Ca (mg kg^{-1})	Exc Mg (mg kg^{-1})	Exc K (mg kg^{-1})	Exc Na (mg kg^{-1})	P (mg kg^{-1})
				2014		
FP	Mineral	3614 ± 291	91 ± 10	524 ± 138	680 ± 144	24.8 ± 7.5
FC	Mineral	3372 ± 30	84 ± 6	430 ± 43	554 ± 34	16.0 ± 3.5
FP	Organic	3414 ± 220	83 ± 2	422 ± 32	563 ± 143	30.6 ± 2.8
FC	Organic	3411 ± 112	85 ± 6	434 ± 117	565 ± 159	32.4 ± 8.1
				2015		
FP	Mineral	6471 ± 1158	1513 ± 290	927 ± 79	662 ± 64	25.0 ± 4.4
FC	Mineral	5555 ± 365	1222 ± 103	860 ± 31	506 ± 101	22.4 ± 5.9
FP	Organic	5308 ± 112	1185 ± 70	698 ± 145	635 ± 24	19.7 ± 1.5
FC	Organic	5396 ± 922	1140 ± 132	792 ± 168	569 ± 67	15.8 ± 3.2
				2016		
FP	Mineral	877 ± 186	190 ± 33	196 ± 30	177 ± 56	72.6 ± 9.4
FC	Mineral	925 ± 282	202 ± 76	205 ± 65	200 ± 111	66.0 ± 7.1
FP	Organic	979 ± 352	200 ± 84	167 ± 80	154 ± 80	89.9 ± 30.7
FC	Organic	673 ± 54	115 ± 12	127 ± 14	88 ± 12	94.5 ± 28.0

Table 3. *Cont.*

Cowpea Cultivar [a]	Fertilizer Type	Exc Ca (mg kg^{-1})	Exc Mg (mg kg^{-1})	Exc K (mg kg^{-1})	Exc Na (mg kg^{-1})	P (mg kg^{-1})
			F-value [b]			
			Between Subjects			
Cowpea Cultivar (CC)		2.16 ns	4.19 ns	0.68 ns	4.97 ns	0.32 ns
Fertilizer Type (FT)		3.26 ns	5.95 *	22.89 **	1.37 ns	4.21 ns
CC × FT		0.96 ns	0.61 ns	4.29 ns	0.55 ns	0.56 ns
			Within Subjects			
Year (Y)		292.13 ***	453.64 ***	107.37 ***	77.38 ***	80.21 ***
Y × CC		0.35 ns	2.01 ns	0.19 ns	0.60 ns	0.34 ns
Y × FT		1.42 ns	3.05 ns	0.81 ns	0.63 ns	3.82 ns
Y × CC × FT		1.45 ns	2.03 ns	0.76 ns	1.01 ns	0.21 ns

[a] FP: Feijão frade de fio preto; and FC: Feijão frade de fio claro. [b] Significant at *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; and ns: not significant ($p > 0.05$). Exc Ca: exchangeable calcium; Exc Mg: exchangeable magnesium; Exc K: exchangeable potassium; and Exc Na: exchangeable sodium.

3.2. Soil Enzyme Activities

Enzyme activities were significantly influenced by the crop year (Table 4). The β-glucosidase and cellulase activities showed the highest values during 2014. The dehydrogenase and arylesterase activities showed the highest values during 2015 and 2016, respectively. The activity of β-glucosaminidase was significantly higher during 2015 and 2016. The cowpea cultivar only significantly influenced β-glucosaminidase activity, with higher values in the FP than in the FC cultivar. The fertilizer type only significantly influenced the β-glucosidase activity, which was higher with the use of mineral fertilizer. The only interaction between factors that was significant was that of the year and fertilizer type for arylesterase activity. Arylesterase showed significantly higher activity in the cowpea cultivated with organic fertilizers during the first year. Dehydrogenase was positively correlated with Ca (r = 0.87, $p < 0.01$), Mg (r = 0.90, $p < 0.01$) and K (r = 0.88, $p < 0.01$). The other enzyme activities were not correlated with any other property.

3.3. Cowpea Yield, Crop Quality and Nutritional Characteristics

As a general trend, the year had a significant effect on the cowpea yield and quality and on the nutritional characteristics (Tables 5 and 6). The cowpea crop showed the lowest yield in 2015, which was related to a serious virosis episode in this crop during this crop cycle. The cowpea yield and weight of 100 seeds were highest during 2016, while the Ca, Mg, K and P contents showed higher values during 2015. The cowpea cultivar significantly affected the cowpea yield and weight of 100 seeds. The cowpea yield was significantly higher in the FC cultivar, while the weight of 100 seeds was higher in the FP cultivar. The fertilizer type did not significantly affect the cowpea yield, crop quality or nutritional characteristics, indicating that both organic and conventional fertilizers contributed to the same crop yield and quality every year. The interaction of the year and fertilizer was significant for Ca, Mg, K and P, which showed higher values using organic fertilizers during 2015. The other interactions among factors were not significant. The cowpea yield was negatively correlated with Ca (r = −0.83, $p < 0.01$), Mg (r = −0.81, $p < 0.01$), K (r = −0.88, $p < 0.01$) and P (r = −0.86, $p < 0.01$).

Table 4. Dehydrogenase, β-glucosaminidase, β-glucosidase, arylesterase and cellulase activities in the soil of cowpea crops using mineral and organic fertilizers for three successive years. Values are mean ± standard deviation (n = 4).

Previous Cowpea Cultivar [a]	Fertilizer Type	Dhs (µmol INTF g^{-1} h^{-1})	Glm (µmol PNP g^{-1} h^{-1})	Glu (µmol PNP g^{-1} h^{-1})	Aryl (µmol PNP g^{-1} h^{-1})	Cel (nmol Gearbox Sugars g^{-1} h^{-1})
				2014		
FP	Mineral	0.16 ± 0.05	0.12 ± 0.04	0.59 ± 0.07	128 ± 32	171 ± 50
FC	Mineral	0.18 ± 0.04	0.11 ± 0.05	0.54 ± 0.13	97 ± 30	190 ± 47
FP	Organic	0.16 ± 0.09	0.15 ± 0.04	0.48 ± 0.05	176 ± 45	165 ± 96
FC	Organic	0.13 ± 0.01	0.11 ± 0.02	0.45 ± 0.10	173 ± 60	172 ± 51
				2015		
FP	Mineral	0.79 ± 0.13	0.29 ± 0.02	0.52 ± 0.10	70 ± 16	23 ± 17
FC	Mineral	0.71 ± 0.04	0.17 ± 0.06	0.36 ± 0.08	84 ± 20	30 ± 24
FP	Organic	0.73 ± 0.20	0.24 ± 0.11	0.28 ± 0.04	84 ± 30	22 ± 11
FC	Organic	0.89 ± 0.25	0.21 ± 0.01	0.41 ± 0.03	56 ± 20	22 ± 12
				2016		
FP	Mineral	0.04 ± 0.01	0.24 ± 0.08	0.36 ± 0.06	131 ± 44	1 ± 2
FC	Mineral	0.02 ± 0.01	0.21 ± 0.12	0.38 ± 0.12	154 ± 14	5 ± 1
FP	Organic	0.02 ± 0.00	0.27 ± 0.06	0.24 ± 0.09	105 ± 51	6 ± 2
FC	Organic	0.02 ± 0.00	0.12 ± 0.05	0.36 ± 0.11	125 ± 33	7 ± 3
				F-value [b]		
				Between Subjects		
Cowpea Cultivar (CC)		0.137 ns	9.41 *	0.10 ns	0.00 ns	0.22 ns
Fertilizer Type (FT)		0.04 ns	0.03 ns	6.81 *	0.47 ns	0.10 ns
CC × FT		1.34 ns	0.24 ns	4.22 ns	0.04 ns	0.07 ns
				Within Subjects		
Year (Y)		146.77 ***	10.28 **	17.09 ***	14.55 ***	72.74 ***
Y × CC		0.20 ns	0.91 ns	1.58 ns	1.09 ns	0.07 ns
Y × FT		0.47 ns	0.35 ns	0.10 ns	5.74 *	0.13 ns
Y × CC × FT		1.22 ns	2.24 ns	2.41 ns	0.82 ns	0.01 ns

[a] FP: Feijão frade de fio preto; and FC: Feijão frade de fio claro. [b] Significant at *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; and ns: not significant ($p > 0.05$). Dhs: Dehydrogenase; Glm: β-glucosaminidase; Glu: β-glucosidase; Aryl: Arylesterase; and Cel: cellulase.

Table 5. Crop yield and quality of cowpea crops using mineral and organic fertilizers for three successive years. Values are mean ± standard deviation (n = 4).

Cowpea Cultivar [a]	Fertilizer Type	Crop Yield (kg ha^{-1})	Protein Content in Seeds (%)	Weight of 100 Seeds (g)
		2014		
FP	Mineral	2333 ± 133	20.06 ± 1.25	21.27 ± 1.66
FC	Mineral	2490 ± 167	19.66 ± 3.79	18.73 ± 0.59
FP	Organic	2135 ± 306	19.64 ± 1.78	18.07 ± 0.70
FC	Organic	2371 ± 161	18.54 ± 2.17	18.27 ± 0.81
		2015		
FP	Mineral	100 ± 52	19.48 ± 1.93	19.63 ± 1.40
FC	Mineral	216 ± 144	20.15 ± 0.57	20.53 ± 2.29
FP	Organic	121 ± 70	23.86 ± 3.57	24.11 ± 5.95
FC	Organic	366 ± 152	21.55 ± 0.98	22.80 ± 3.03
		2016		
FP	Mineral	3033 ± 757	21.05 ± 0.74	27.79 ± 0.89
FC	Mineral	4166 ± 1171	19.72 ± 1.80	21.08 ± 1.06
FP	Organic	2533 ± 321	20.75 ± 1.71	25.11 ± 0.50
FC	Organic	3366 ± 1167	18.94 ± 0.64	21.87 ± 1.38
		F-value [b]		
		Between Subjects		
Cowpea Cultivar (CC)		6.48 *	2.01 ns	9.72 *
Fertilizer Type (FT)		1.82 ns	0.50 ns	0.08 ns
CC × FT		0.01 ns	0.87 ns	0.96 ns
		Within Subjects		
Year (Y)		95.76 *	2.71 ns	13.14 ***
Y × CC		2.03 ns	0.17 ns	3.51 ns
Y × FT		1.35 ns	3.47 ns	4.27 ns
Y × CC × FT		0.13 ns	0.39 ns	1.31 ns

[a] FP: Feijão frade de fio preto; and FC: Feijão frade de fio claro. [b] Significant at *** $p < 0.001$; * $p < 0.05$; and ns: not significant ($p > 0.05$).

Table 6. Nutrient content of cowpea seeds using mineral and organic fertilizers for three successive years. Values are mean ± standard deviation (*n* = 4).

Cowpea Cultivar [a]	Fertilizer Type	N (g kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	K (mg kg^{-1})	P (mg kg^{-1})
				2014		
FP	Mineral	32.10 ± 2.01	754 ± 76	1985 ± 56	2977 ± 368	4651 ± 267
FC	Mineral	31.45 ± 6.06	882 ± 67	1953 ± 48	13,468 ± 579	4751 ± 173
FP	Organic	31.43 ± 2.85	716 ± 116	2029 ± 205	13,300 ± 486	4735 ± 658
FC	Organic	29.66 ± 3.47	853 ± 38	2015 ± 56	13,801 ± 175	4886 ± 199
				2015		
FP	Mineral	31.17 ± 3.09	2205 ± 397	3889 ± 457	28,302 ± 2575	11,208 ± 1264
FC	Mineral	32.24 ± 0.91	2111 ± 86	3905 ± 346	28,321 ± 2832	10,225 ± 1174
FP	Organic	38.17 ± 5.71	3068 ± 642	4575 ± 744	32,436 ± 5906	10,911 ± 1783
FC	Organic	34.48 ± 1.57	2834 ± 109	4822 ± 311	32,963 ± 3009	11,735 ± 2724
				2016		
FP	Mineral	33.69 ± 1.19	412 ± 65	1276 ± 134	3711 ± 230	1855 ± 115
FC	Mineral	31.55 ± 2.88	604 ± 356	1826 ± 1110	4962 ± 2441	2481 ± 1220
FP	Organic	33.21 ± 2.73	233 ± 88	599 ± 217	2135 ± 596	1067 ± 298
FC	Organic	30.31 ± 1.03	349 ± 103	877 ± 317	3133 ± 772	1566 ± 386
				F-value [b]		
			Between Subjects			
Cowpea Cultivar (CC)		2.01 ns	0.20 ns	0.81 ns	0.46 ns	0.23 ns
Fertilizer Type (FT)		0.50 ns	3.91 ns	0.00 ns	1.18 ns	0.01 ns
CC × FT		0.87 ns	0.14 ns	0.00 ns	0.00 ns	0.48 ns
			Within Subjects			
Year (Y)		2.71 ns	271.24 ***	235.52 ***	503.09 ***	221.67 ***
Y × CC		0.17 ns	0.14 ns	1.08 ns	0.13 ns	0.26 ns
Y × FT		3.47 ns	14.96 ***	14.44 ***	6.47 **	1.35 ns
Y × CC × FT		0.39 ns	0.07 ns	0.35 ns	0.02 ns	0.71 ns

[a] FP: Feijão frade de fio preto; and FC: Feijão frade de fio claro. [b] Significant at *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; and ns: not significant ($p > 0.05$).

3.4. Interrelationships between Soil and Crop Properties

The PCA performed with the studied soil physicochemical properties, enzyme activities, cowpea crop yield, crop quality and nutritional characteristics using both fertilizer types showed that 83.3% of the total variation could be explained by the first five PCs. PC1 (which explained 33.2% of the variation) and PC2 (which explained 19.1% of the variation) did not separate the mineral and organic fertilizers (Figure 1A). PC1 was related to soil-exchangeable K, Mg and Ca, soil NH_4^+, the nutrient content in the seeds and soil enzyme activities (Table 7). PC2 was related to soil B, soil AS, exchangeable Na, bioavailable P and enzyme activities. PC3, which explained 15.5% of the variation, slightly separated the mineral (positive scores) and organic fertilizers (negative scores) (Figure 1B). PC3 was related with higher values of EC, N_t and SOC and lower values of pH (Table 7). PC4 (which explained 9.5% of the variation) and PC5 (which explained 5.9% of the variation) did not separate the mineral and organic fertilizers (Figure 1B,C) and were related to the protein content in the seeds, the weight of the seeds (PC4) and the soil LC (PC5). Therefore, PCA confirmed the lack of differences between both types of fertilizers in terms of crop properties and most soil properties during the three crop cycles.
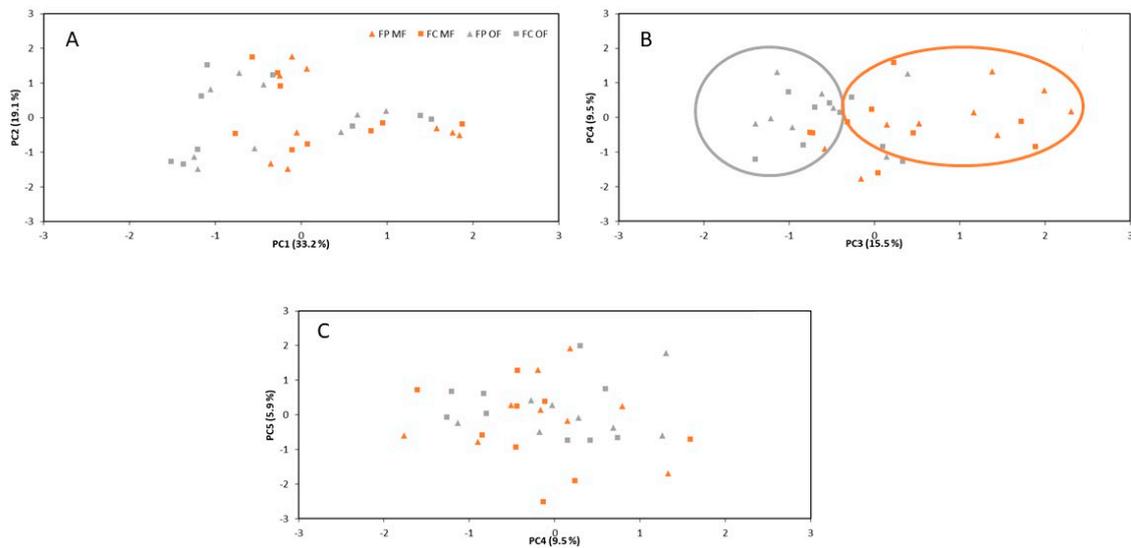
Figure 1. PCA factor scores of variations in the soil's physicochemical properties, enzyme activities, cowpea crop yield, crop quality and nutritional characteristics, considering different cowpea cultivar and fertilizer types. The figure type represents the cowpea cultivar (triangle: FP cowpea cultivar; and square: FC cowpea cultivar), and the color represents the fertilizer type (orange: mineral fertilizer; and gray: organic fertilizer). FP: Feijão frade de fio preto; FC: Feijão frade de fio claro; MF: mineral fertilizer; and OF: organic fertilizer. (**A**) **; (**B**) **; (**C**) **. ** $p < 0.01$.

Table 7. Matrix of the PCA of the soil's physicochemical properties, enzyme activities, crop yield, crop quality and nutritional characteristics of the cowpea crop using different fertilizer types for three years.

Variance Explained	PC1 (33.2%)	PC2 (19.1%)	PC3 (15.5%)	PC4 (9.5%)	PC5 (5.9%)
Exc K (mg kg^{-1})	0.884	0.286	−0.269	−0.068	0.051
Exc Mg (mg kg^{-1})	0.876	−0.208	−0.365	0.065	0.094
Dhs Activity (μmol INTF g^{-1} h^{-1})	0.852	0.033	−0.376	0.167	0.175
P in Seeds (mg kg^{-1})	0.849	0.173	−0.385	0.037	0.205
Mg in Seeds (mg kg^{-1})	0.827	0.143	−0.325	0.107	0.229
K in Seeds (mg kg^{-1})	0.822	0.229	−0.420	0.070	0.183
Ca in Seeds (mg kg^{-1})	0.807	0.064	−0.391	0.161	0.262
Exc Ca (mg kg^{-1})	0.797	0.372	−0.378	−0.029	0.162
Crop Yield (kg ha^{-1})	−0.773	−0.081	0.427	−0.044	−0.269
Ure Activity (μmol NH$_4^+$ g^{-1} h^{-1})	0.749	−0.386	0.049	0.106	−0.165
NH$_4^+$ (mg kg^{-1})	−0.672	−0.187	−0.052	−0.119	0.054
Aryl Activity (μmol PNP g^{-1} h^{-1})	−0.603	0.141	0.135	−0.327	0.015
B (mg kg^{-1})	0.211	0.928	−0.121	−0.05	0.131
Cel Activity (nmol gearbox sugars g^{-1} h^{-1})	−0.280	0.855	−0.013	−0.120	0.058
AS (%)	−0.361	−0.723	0.354	−0.016	−0.220
Exc Na (mg kg^{-1})	0.557	0.723	−0.115	−0.016	0.209
P (mg kg^{-1})	−0.605	−0.658	0.167	0.047	−0.211
β-Glm Activity (μmol PNP g^{-1} h^{-1})	0.332	−0.587	−0.042	0.071	0.276
RC (%)	−0.261	−0.584	0.400	−0.232	−0.473
β-Glu Activity (μmol PNP g^{-1} h^{-1})	0.087	0.532	0.238	−0.516	0.217
EC (μS m^{-1})	−0.449	0.077	0.822	−0.063	−0.069
pH	0.236	0.347	−0.798	−0.057	0.127
NO$_3^-$	−0.496	0.354	0.731	0.00	−0.182
N$_t$ (g kg^{-1})	−0.338	−0.433	0.688	−0.218	−0.193
SOC (g kg^{-1})	−0.338	−0.344	0.653	−0.046	0.305
Protein in Seeds (%)	−0.368	−0.029	−0.056	0.942	0.091
N in Seeds (g kg^{-1})	0.187	−0.029	−0.056	0.942	0.091
Weight of 100 Seeds (g)	−0.026	−0.556	0.235	0.581	0.177
LC (%)	0.261	0.242	−0.132	0.154	0.784

Exc K: exchangeable potassium; Exc Mg: exchangeable magnesium; Dhs: Dehydrogenase activity; Exc Ca: exchangeable calcium; Ure: urease activity; Aryl: Arylesterase activity; Cel: cellulose activity; AS: aggregates stability; Exc Na: exchangeable sodium; Glm: β-glucosaminidase activity; RC: recalcitrant carbon; Glu: β-glucosidase activity; EC: electrical conductivity; N$_t$: total nitrogen; SOC: soil organic carbon; and LC: labile carbon.

4. Discussion

The inclusion of a cowpea crop for three years increased the soil's properties, accompanied by an enhance in the crop yield and quality over time. Cultivation of the cowpea acts as a source of different types of C through rhizodeposition, which stimulates the growth of rhizosphere microorganisms [48] and facilitates nutrient availability for the plant [49]. In addition to this, we observed the effects of the specific cowpea cultivar on the soil properties, soil enzyme activities and crop yield and quality. This is not surprising, as previous reports have observed that the release of root exudates is controlled by the plant genotype [50,51]; thus, the plant genotype could have different effects on the soil properties and soil enzyme activities. On the other hand, this effect would be even larger if cowpea residues were used as green manure, increasing the nutrients' availability through mineralization of its residues [52–54]. In this regard, in this study, we observed a negative relationship between the exchangeable cations and crop yield. In fact, the lowest cowpea crop yield was observed during 2015, which coincided with the highest nutrient availability in the soil (Tables 3 and 5). This could be explained by the fact that soil sampling was carried out after two weeks of harvesting, and thus the higher the cowpea production and the higher the quantity of nutrients absorbed from the soil by the plants to sustain that production, reducing their concentrations in the soil at the end of the crop cycle. These results indicate that there was no nutrient limitation under either of the fertilized systems, conventional or organic. Since soil nutrients are the main factors limiting the crop yield, a high crop yield mainly depends on nutrient availability through fertilizer application [55], also being highly efficient with the use of organic fertilizers.

As far as the fertilizer type is concerned, both had a similar effect on the microbial activity, with similar soil enzyme activities. In turn, this study showed through PCA that the application of mineral and organic fertilizers had the same effect on the soil's properties (Figure 1). However, mineral fertilizers contributed to higher RC and N_t contents, which was not associated with a higher crop yield. This may be explained by the fact that the application of mineral fertilizers provides a source of N directly available for crop growth, and this N input acts as a driver for the C cycle in soils since it increases biomass production. Thus, it maintains and builds up the SOC stocks [56–58]. On the other hand, organic fertilizers contributed to an increase in NO_3^- content in the soil. This result may be explained by the fact that the application of fertilizers in the cowpea crop concluded three weeks before soil sampling, and the use of organic fertilizers produced a greater residual effect than mineral fertilizers by continuous mineralization and nitrification in the soil [59]. In fact, some authors have reported that the residual effect from mineral N fertilizers after a long-term fertilization experiment was low, or even that no effect was observed [60–62].

The lack of the expected results with improved soil physicochemical and biological properties with the use of organic fertilizers could be explained by the fact that these fertilizers release nutrients slowly, and only a fraction of the N and other nutrients become available to plants in the first year after application [63,64]. However, the nutrient availability was enough to provide high crop yields, with similar values to those with mineral fertilizers. In terms of crop yield, the literature reviewed showed that mineral fertilization of the soil is useful for improving yields through the increase of SOC and N stocks [65,66]. However, this type of fertilization could not be the most effective in light of a high environmental cost [67]. In addition to this, a higher soil N concentration following mineral fertilization may induce microbe-mediated N transformation processes, leading to high N_2O emissions [12] or the lack of synchrony between the N release and crop demand, which can stimulate N_2O emissions [68]. As a consequence, the use of organic fertilizers has been strongly recommended as a replacement for some or all mineral fertilizers, since this kind of fertilization not only includes mitigation of global warming [69], but their use also results in the reduction of NO_3^- and can improve crop yields. Despite this, the results of our study indicated, on one hand, that the use of organic fertilizers led to a higher NO_3^- content in the soil due to a greater residual effect, and on the other hand, there were no significant differences in the crop yield, crop quality or nutritional characteristics

due to the fertilizer type (Figure 1). In this context, the use of organic fertilizers may be beneficial to crop yields in the long term [70]. However, there are a number of scientific papers which conclude that the use of organic fertilizers alone as substitutes for mineral fertilizers is not enough to maintain the productivity of high-yielding crops [71,72], while an effective solution could be the partial substitution of mineral fertilizers with organic fertilizers [73]. Irrigation and fertilization are two important factors for obtaining higher productivity [74]. Nevertheless, some other factors such as the cultivar, climate and soil type are also responsible for changing the yield. In this study, we confirmed that the crop yield was indeed influenced by the cowpea cultivar.

5. Conclusions

In conclusion, our results show that the cowpea cultivar influences the soil's physicochemical properties and crop yield and quality. Mineral fertilizers increased the RC, nitrogen forms (N_t and NH_4^+) and nutrient availability (Mg and K), but the concentration of soil nutrients with organic fertilizers was also suitable for providing high crop yield and quality. As a consequence, there were no significant differences in the cowpea yield, crop quality or nutritional characteristics by fertilizer type. Thus, although mineral fertilizers increased the soil organic matter and some nutrients in the short term, the nutrient availability and microbial activity were high with the use of both types of fertilizers, leading to the same crop yield.

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