Soil organic carbon and acid phosphatase enzyme activity response to phosphate rock and organic inputs in acidic soils of Central Highlands of Kenya

ABSTRACT

Aims: To evaluate the effects of phosphate rock and organic inputs on soil organic carbon and acid phosphatase activity.

Study design: The experiment was laid in Randomized Complete Block Design with seven treatments replicated thrice.

Place and duration of study: The study was conducted at Kigogo Primary school in Meru south sub-county, Tharaka Nithi County, Kenya. The experiment ran for two consecutive seasons the short rains of 2017 and long rains of 2018.

Methodology: There were seven treatments replicated thrice. the treatments included *Tithonia diversifolia*, Phosphate rock (PR), Goat manure, *Tithonia diversifolia* + Phosphate rock, Goat manure + Phosphate rock, Triple superphosphate + Calcium ammonium nitrate and a Control (no soil external inputs). The test crop was maize (Zea mays L.) H516 variety. Soil organic carbon followed modified Walkley and Black oxidation method while acid phosphatase enzyme activity was essayed following the method by Tabatabai and Bremner.

Results: Goat manure + phosphate rock, sole phosphate rock and use of goat manure significantly (P= .0001) increased soil organic carbon by 198, 100 and 71% compared to the control. *Tithonia diversifolia* reported a 3.4-fold increase in soil organic carbon compared to the control in short rains of 2017. Goat manure gave higher soil organic carbon by 135% compared to the control in the long rains of 2018. Goat manure + phosphate rock treatment significantly (P= .0002) increased the phosphatase activity by a difference of 1.12% compared with the control, with 2.14% decreases under TSP+CAN treatment compared to the control.

Conclusion: The results showed that integration of phosphate rock and manure could have a far-reaching influence on soil organic carbon and acid phosphatase activity thus could be recommended for improved soil productivity in *humic nitisols* in similar agro-ecological zones.

Keywords: enzyme activity, organic matter, mineral fertiliser, Tithonia diversifolia, goat manure

1. INTRODUCTION

Soil organic carbon is vital in improving biochemical and physical soil properties and sustaining fertility [1]. Fertilisation and combined use of mineral and organic amendments are universal management stratagem to increase soil quality and improve crop production [2]. Various field experiments have shown that the integrated application of organic fertiliser increases soil organic matter content and microorganism, which can control soil organic matter cycling and sequestration of carbon [3,4,5,6]. Soil extracellular enzymes are synthesised and discharged by roots and microorganisms and contribute to decomposition and soil organic matter formation [7]. However, low soil organic carbon, which is an index of soil organic matter has been observed in tropical soils, probably due to the high rate of soil oxidation [8]. Soil carbon dynamics is of concern as soil carbon pools, and quality significantly affects crop production in the tropical regions [9]. However, the interactive mechanism affecting SOC dynamics through stimulating enzyme activity by integrated utilisation of phosphate rock combined with organic inputs is particularly limited.

Organic fertiliser has shown a significant positive effect on organic carbon content additions in the topsoil [10]. Soil organic amendments have desirable results because they supply soil organic matter and other nutritive elements to the soil-plant system [11, 12]. Application of organic fertiliser, either as sole input or in combination with inorganic fertiliser increases soil organic matter resulting in high organic carbon concentration [13,14,15,16]. For instance, [17] showed that long-term application of inorganic fertiliser combined with manure input could increase soil organic carbon stock by 12 to 113% within 0 to 60 cm depth of the soil.

The green biomass of *Tithonia diversifolia* in comparison to other green biomass of trees and shrubs is relatively high in nutrients [18]. Therefore, *Tithonia diversifolia* is a good source of organic nutrients release and supply; hence, improves soil productivity [19]. For instance, [20] reported that *Tithonia diversifolia* could enhance soil physical and chemical properties; therefore, increasing nutrients in the soil. Contrary, inorganic fertiliser application is ineffective in improving soil quality mainly due to soil organic matter decline, imbalances in nutrient contents, soil physical degradation and increased cost and long-term acidic effect on soils [21]. Understanding the importance of animal manure and other organic inputs on soil organic carbon is of great agronomic and environmental importance. This warrants concerted efforts in improving the understanding of the effects of soil fertility ameliorating inputs on soil quality in the tropical regions, particularly Central Highlands of Kenya.

Given the crucial role that soil enzymes play in the biochemical soil system functioning, it has been suggested as an important soil quality indicator [22]. Environmental conditions influence decomposer enzyme activities and hence, the decomposition dynamics of applied organic fertiliser. Besides, soil microbial activities are affected by sub-optimal prevailing conditions, nutrient limitation or energy deficiency and organic matter inaccessibility due to its sparse density or association with reactive soil mineral surfaces [23]. For instance, before P is taken up by the plant roots, its hydrolysis from organic P to inorganic P must take place. In the hydrolysis process, and P cycling as a whole, the phosphatase enzyme plays a vital role [24]. The application of phosphate rock improved the phosphatase enzyme of three cereal plant species reflecting the deliberate P discharge that improved microbial activities, thereby increasing the availability of P [25].

Long-term use of organic amendments influences soil properties [26], which significantly affect SOM turnover. However, the inadequate experimental data is rather contentious and no common agreement on the effect of integrated approach utilising phosphate rock and organic amendments. Therefore, understanding the importance of integrated application of organic input and phosphate rock on soil organic carbon is of great agronomic and environmental importance. More insight is required on soil response to integrated manure and phosphate rock. Thus, the objective of this study was to evaluate the effect of phosphate rock and organic inputs on soil organic carbon and phosphatase enzyme activity. We hypothesised that application of organic amendments and phosphate rock would increase soil organic carbon and phosphatase activity in the acidic soils of the Central Highlands of Kenya.

2. MATERIAL AND METHODS

2.1 Site description

The study was conducted at Kigogo Primary School (00◦23’S, 37◦38’E) in Meru South Sub-county, Tharaka-Nithi County, Kenya. The sub-county falls within agro-ecological zones upper midland zone two (UM2) and upper midland zone three (UM3) and lies at an altitude of 1500 m above sea level on the eastern slope of Mount Kenya (Figure 1)

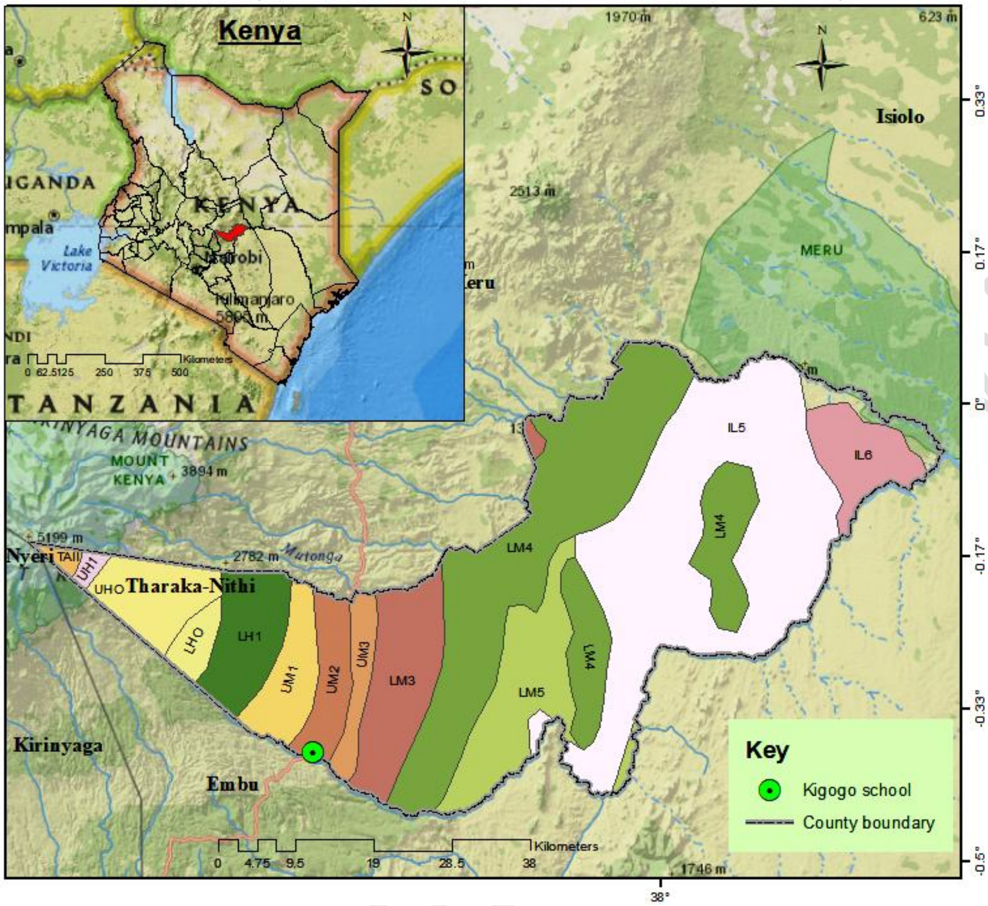

Figure 1: Map of the study area

Annual rainfall ranges from 1200 mm to 1400 mm and a yearly mean temperature of 20°C. There are two cropping seasons annually with long rains (LR) season from March through June and short rains (SR) season from October to December. The soils are primarily Humic nitisols, typically deep weathered with moderate to high inherent fertility and clay soil texture [27, 28]. The site falls within a predominantly maize growing zone with small landholdings ranging from 0.1 to 2 ha and an average of 1.2 ha per household [29]. Smallholder mixed farming activities characterise agriculture comprising of food crops, cash crops, trees and livestock [29]. The initial soil chemical properties of the study site are as shown in Table 1.

Table 1: Initial soil properties of the experimental site (0-15cm) in Meru South

Parameter	**Value**
Soil pH	5.01
Exch. Acidity (me %)	0.30
Total Nitrogen (%)	0.2
Total Org. Carbon (%)	2.21
Phosphorus (Mehlich) (ppm)	10
Sodium (me %)	0.16
Calcium (me %)	1.40

Magnesium (me %)	2.41
Manganese (me %)	1.03
Copper (ppm)	1.23
Iron (ppm)	14.6
Zinc (ppm)	11.2

Cumulative rainfall received during the experimental period was 608.5 mm during the short rains of 2017 and 1250.5 mm in the long rains of 2018 (Figure 2). A continuous decline in the rainfall amount was observed throughout the cropping seasons. Based on the predictable rainfall dates 15th March and 15th October [28], the SR17season and LR18 season onset dates were within the reported range. Overall, SR17 was a dry season with earliest cessation dates making the season to be shorter with 46 days length. LR18 season was relatively a wet season with early-onset dates and latest cessation dates making the season the longest with the highest cumulative rainfall (1250.5 mm) with a well-distributed rainfall (Figure 2).

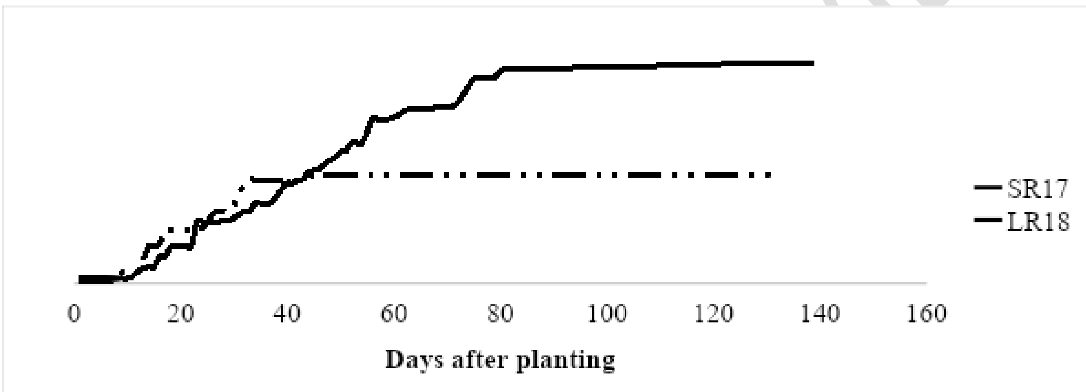

Figure 2: Cumulative rainfall distribution as observed in Meru South during SR17 and LR18 season

Table 2: Rainfall characteristics of Meru south during SR17 and LR18 seasons

Parameter	SR17	LR18
Onset Date	12th Oct 2017	3rd Mar 2018
Cessation Date	25th Nov 2017	4th Aug 2018
Length of the season	46	155
Total rainfall(mm)	608.5	1250.5
5 to 10 days	1	2
11 to 15 days	1	2
More than 15 days	0	1
Total dry spell	2	5

2.2 Experimental layout and management

The field experiment was laid on September 2017, just before the onset of the short rains 2017 (SR17) season in a randomised complete block design (RCBD). There were seven treatments replicated thrice giving a total of 21 plots (Table 3).

Table 3: Treatment description in the experimental site at Meru South

		P rate(Kg ha^{-1})		
Treatment	**P source**	**Organic**	**Inorganic**	**Total**
Goat Manure	Goat manure	60	-	60
TSP+CAN	TSP	-	60	60
Control	-	-	-	-
Phosphate Rock	PR	-	60	60
T.diversifolia + PR	*T. diversifolia* and PR	20	40	60
Goat manure + PR	Goat manure and PR	20	40	60
T. diversifolia	*T. diversifolia*	60	0	60

PR= phosphate rock, TSP= Triple superphosphate, CAN=Calcium ammonium nitrate, T.diversifolia= Tithonia diversifolia

The plot dimensions were 6 m by 4.5 m with a 1 m wide alley separating plots within a block, and a 2 m wide alley between blocks. The test crop was maize (Zea mays L.), H516 variety. The experiment ran for two consecutive cropping seasons; short rains 2017 (SR17) and long rains season 2018 (LR18). The planting spacing was 0.75 m by 0.50 m, inter and intra rows, respectively. Three maize seed were planted per hill and later thinned to two after emergence, to achieve the recommended plant population of 53,333 plants ha^{-1} [29]. A sample of the goat manure and tithonia diversifolia were analysed for P and N content amounts before application.

Table 4: Chemical composition (%) of organic materials applied in the treatments

Organic Input	C	N	C:N ratio	P	Ca	Lignin	Polyphenol
Tithonia diversifolia	42	3.1	13.4	0.3	2.1	14	3.16
Manure	36	1.8	20.0	0.4	0.9	21	0.83

Goat manure and T. diversifolia were applied and incorporated into the plots two weeks before the onset of the season. The inorganic fertiliser used were phosphate rock (PR) and Triple superphosphate (TSP). Phosphate rock was applied one week before planting while TSP + CAN was applied during planting. The standard agronomic practices such as weeding, soil erosion control were followed throughout the seasons. The experimental plots were maintained weed-free throughout the seasons.

2.3 Soil sampling

Composite soil samples were collected from each plot at the start of the experiment and at the end of each season using a zigzag method at a depth of 0-15 cm using Eijkelkamp Gouge. The samples were then transported from the field sites in cool boxes and stored at 4 °C in a refrigerator for soil organic carbon and phosphatase enzyme activity analysis.

2.4 Laboratory analyses

Soil organic carbon was determined following modified Walkley and Black oxidation method [30]. A 0.5 g of soil sample was treated with ten ml of 1 N K2Cr2O7 in a 500 ml conical flask and slightly swirled to disperse the soil. A 20 ml concentrated sulphuric acid was then added, placed on the asbestos and left for 30 minutes. 200 ml deionised water was then added, followed by three drops of “ferroin" indicator and the titration done with FeSO4. For quality control purposes, and as part of the protocol to standardise K2Cr2O7 solution, two blanks (without soil) were included for each batch run.

Acid phosphatase enzyme activity was essayed following the method by [31]. The procedure involved the spectrophotometric determination at the wavelength of 400 nm of the phenyl phosphatase solution (pNPP) released by 1 g of soil after 30 minutes of water bath incubation at a temperature of 37°C. 1 g soil samples were placed in a 50ml Erlenmeyer flasks and treated with 0.25 ml toluene. Then, 1 ml pNPP was added with 4 ml of tris buffer (pH 6.5). The content was mixed and incubated for one hour at 37°C. afterwards, the contents were mixed and filtered through Whitman no.2 filter paper. The filtrate was treated with 1 ml CaCl2 (0.5m) and 4ml NaOH (0.5M) to stop the reaction. For the controls, samples were treated with 1 ml pNPP after addition of stop solutions. All measurements were done in triplicate. The data obtained were expressed as mg-pNPP h-1 g-1 soil.

2.5 Data analysis

Diagnostic checks on the potentially influential and outlying random and residual effects were executed on the data using the studentized residual approach in SAS version 9.4 [32]. This allowed quick graphical checks of fitted residuals in assessing distributional assumptions such as normality and variance homogeneity. The data was then subjected to Analysis of variance (ANOVA) and the mean separation was done using Duncan’s Multiple Range Test at $P \leq .05$.

3. RESULTS AND DISCUSSION

3.1 Soil organic carbon

Soil organic carbon (SOC) content was significantly (P= .0001) influenced by the treatments in all the seasons (Table 5). In the SR17, Tithonia diversifolia significantly increased SOC by 3.4-fold compared to the control. Application of goat manure + phosphate rock, sole phosphate rock and sole goat manure also showed significant increases in SOC by 198, 100 and 71% respectively, compared with the control during SR17 (Table 5). During LR18 season, sole goat manure application gave higher SOC by 135% compared with the control. Goat manure + phosphate rock increased SOC by 114% compared to the control. TSP + CAN and Tithonia diversifolia increased SOC by 63.4% and 50% respectively compared to the control LR18 (Table 5).

Table 5: Effects of various treatments on soil organic carbon for SR17 and LR18 in Meru South

Treatment	**Soil Organic Carbon (mg C g^{-1} soil)**	
	SR17	LR18
TSP + CAN (60 kg P ha^{-1})	1.80[e]	2.19[c]
Goat manure (20 kg P ha^{-1}) + PR (40 kg P ha^{-1})	3.75[b]	2.88[ab]
PR (60 kg P ha^{-1})	2.52[c]	2.97[a]
Tithonia diversifolia (60 kg P ha^{-1})	1.86[e]	2.58[b]
Tithonia diversifolia (20 kg P ha^{-1}) + PR (40 kg P ha^{-1})	4.26[a]	2.01[c]
Goat manure (60 kg P ha^{-1})	2.16[d]	3.15[a]
Control (no input)	1.26[f]	1.34[d]
P-value	.0001	.0001

PR=Phosphate rock, TSP=Triple superphosphate, CAN=Calcium ammonium nitrate Means with the same letters in each column are not statistically different at $P \leq .05$

3.2 Acid Phosphatase Activity

There was no significant treatment effect observed in acid phosphatase enzyme activity in SR17. In the subsequent season (LR18) treatments significantly (*P*=.0002) influenced the acid phosphatase enzyme activity (Figure 3). The highest acid phosphatase enzyme activity was observed under goat manure + phosphate rock treatment with a significant (*P*=.0002) difference of 1.12% compared to the control. Sole goat manure increased phosphatase enzyme activity by 0.8% above the control. Conversely, TSP + CAN decreased acid phosphatase enzyme activity by 2.14% compared to the control.

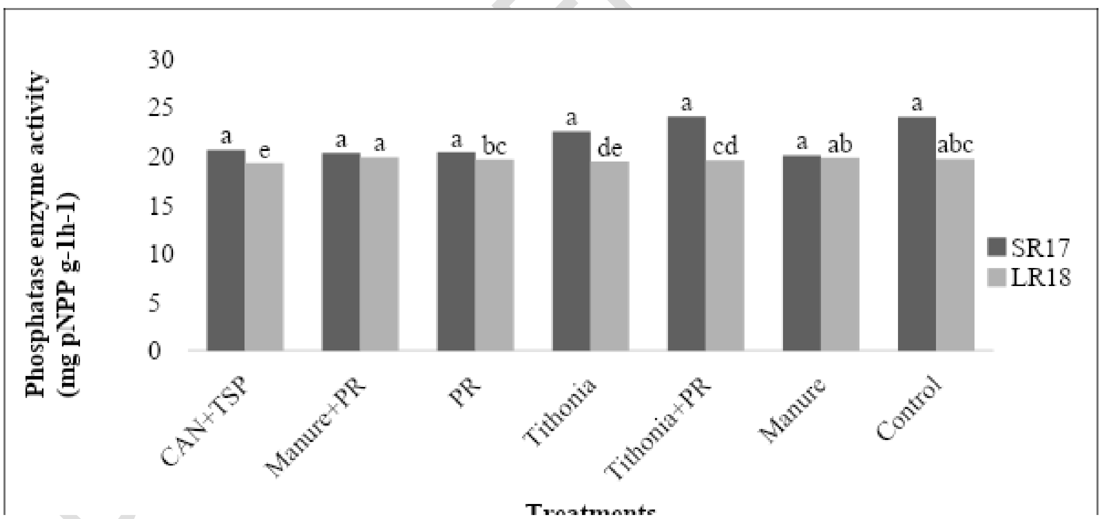

Figure 3: Effects of various treatments on acid phosphatase enzyme activity (mg pNPP g^{-1} soil hr^{-1}) in Meru South

3.3 Soil organic carbon

Soil organic carbon is a strong determinant of the soil biological, chemical and physical properties; thus the quality of the soil [15]. We observed high SOC under treatment with goat manure + phosphate rock. This could be attributed to the enhanced decomposition of organic carbon due to the priming effect motivated by microbial use of exudate C from the roots [33, 34]. Additionally, this could also be credited to the steady supply of mineralised carbon, which improved soil microbial activity [35, 36]. After two-decade fertilisation with NPK plus manure, [17] reported increases in soil organic carbon content. Similarly, [37] reported improved soil organic carbon by 37.8% with the use of poultry manure at 10 t ha^{-1} for two years. Consistent to the findings of this study are previous studies by [38, 39] who reported that sole use of organic materials or in combination with inorganic fertiliser over a long time significantly enhanced soil organic carbon compared to the initial condition. Similar results were reported by [40] who observed significant increases in the organic carbon content with application of manure for seventeen years.

Comparative to the initial soil condition, goat manure application significantly increased soil organic carbon. This can be ascribed to the availability of more humified and recalcitrant carbon forms in the animal manure compared to other organic fertilisers. Based on the C/N ratios of the manure (20) and *Tithonia diversifolia* (13.5) (Table 4), it implies that manure has more recalcitrance carbon compared to *Tithonia diversifolia* hence it maintained high SOC to the end of the experiment. Additionally, this can also be credited to the high P content of the manure (Table 4).

Phosphorus promotes the plant root growth hence the increased root biomass returning the more organic residue to the soil leading to increased SOC. The SOC increases under manure has also been linked to microbial growth and activity [41, 42, and 43]. Manure can improve soil organic matter by supplying a great source of nutrients and carbon for the soil microorganisms [44]. After conducting a long term experiment for 40 years in Hungarian soils, [45] reported 8.2% higher total organic carbon with farmyard manure comparative to NPK fertiliser. These assertions are in line with [46] who also observed 2.1-3.3% increase of organic carbon content under pig manure amended soils comparative to unfertilized treatments.

We observed an upsurge in SOC under *tithonia diversifolia*. This may have resulted from the high organic carbon content of *tithonia diversifolia* (Table 4). Additionally, this could be attributed to the buildup of soil organic matter, available N, P and K nutrients and the provision of micronutrients [47]. The low C/N ratio of *tithonia diversifolia* (Table 4) implies a rapid decomposition rate. Comparatively, the soils had low N, Ca and SOC (Table 1). In another study, *tithonia diversifolia* improved soil organic matter concentration, the P availability and total nitrogen [48]. Also, the lignin content of *tithonia diversifolia* from great decomposition has been stated as its strong point as an organic matter source [49].

Increases in SOC under phosphate rock treatment may be ascribed to increases in nutrient contents as well as the carbon input decomposition rate [50]. Also, this could be attributed to soil moisture content that influenced the SOC accumulation through the amount of plants carbon release to soil [51]. The findings of this study are in agreement with [52], who reported that soil phosphorus showed an increase in SOC by 150% compared to unfertilized controls. Long-term use of chemical phosphorus, nitrogen and potassium fertiliser increased SOC by 11-66% [53, 54].

The observed increases in SOC under TSP + CAN could be credited to the built-up of plant residues and the root carbon input [55]. In other studies, NPK has influenced the SOC storage even in little amounts [56, 57]. Inversely, results published by [58] reported that the use of balanced inorganic fertiliser could only sustain soil organic matter with no major changes. With the use of data from 30 long-term study sites, [59] reported that inorganic fertiliser at the rate of 200 or 400 kg ha-1 N yr-1 did not increase soil organic carbon in the entire soil profile with respect to unfertilized control. In another study, NPK treatment reported decreases in SOC by 5-6% under Chinese Aridisols [60]. Also, more than 60 years of use of different soil input reported decreases in SOC under the sole application of NPK [61].

3.4 Phosphatase enzyme activity

Enzymes are essential to soil component catalyzing the transformation interrelated to decomposition and nutrient turnover. Their activities in the soil can be considered as a measure of soil condition [62]. During the two experimental seasons, the short rains of 2017 were poorly distributed, and the organics were a better alternative for the soil biochemical properties enhancement. The long rain season of 2018 was generally a wet season (Figure 2) implying the significant responses of acid phosphatase enzymes. In our study, acid phosphatase activity increased under goat manure + phosphate rock and sole use of goat manure compared to the control (Figure 3). This may have resulted from increased soil organic carbon (Table 5). Higher enzymatic activities under great organic matter regimes have been cited [63]. Likewise, [64] ascribed this to the combined effect of stabilisation of phosphatase enzyme to humic substances and increased microbial biomass with an increased concentration in TOC.

In that context, the release of phosphatase from roots reflects great microbial activities [65] since plant roots secrete organic compounds that stimulate microbial activity in the rhizosphere hence the enzymatic activity [66,67]. These results are in accord with previous studies [64, 68] who observed enhanced enzymatic activities with increased microbial biomass and a positive correlation between, enzymes and soil organic carbon due to activated enzymatic activities by manure and other organic amendments.

Manuring has been observed to enhance enzymatic activities in the soil [45]. This may have major implication in soil fertility and C cycling in extremely weathered soil environs. Also, [69] in their study on the effect of manure and biochar concluded that the use of manure in the short term improved enzymatic activities compared to biochar. Results of [70] reported high phosphatase activity under organic management compared to conventional management. The significant increases in phosphatase activity during the long rain season can also be linked to increased moisture content (Figure 2 and Table 2). In the same context, a Hungarian study assessing soil enzyme activity in response to organic matter management reported increased phosphatase activities in spring coincidental with great soil moisture [62].

The decreases in phosphatase activity under CAN + TSP may be due to low soil organic carbon input (Table 5) and enhanced fertiliser-induced decomposition of SOC [71]. The addition of inorganic fertiliser alone was not sufficient to activate the microorganisms and to increase crop root biomass. After conducting a three consecutive season, on-farm experiment under *humic nitisols* cultivated with maize, [64] reported no effect on phosphatase activity with the sole application of mineral fertiliser and decreased in some treatments. Decreases in enzyme activities with application of mineral fertiliser are cited [72]. Similarly, decreases in bacterial diversity have also been reported under long-term use of inorganic fertiliser [73, 74]. The assertions of this study are in line with the findings of [75] who reported decreases in the bacterial diversity under a long term inorganic fertiliser application, which also implied reduced enzymatic activities.

4. CONCLUSION

The treatments with goat manure resulted in higher soil organic carbon and acid phosphatase enzyme activity compared with sole mineral fertiliser, indicating the supremacy of organics in soil biochemical properties improvement due to their beneficial role relative to use of P in the water-soluble fertiliser. It was observed that the use of goat manure or combinations with phosphate rock enhanced SOC and phosphatase enzymes while sole use of *tithonia diversifolia* increased the soil organic carbon. Subsequently, use of manure has a wide range of advantages compared to mineral fertiliser for enhancing soil biochemical properties. Thus, the use of goat manure and combinations with phosphate rock could be a long-term strategy to enhance soil organic carbon and enzymatic activity.

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