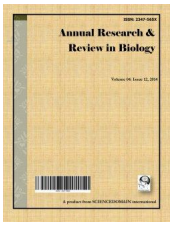

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Effect of Organic and Reduced Mineral Fertilisation on pH, Nutrient Content and Microbial Properties of Acid Soil

Daniel Kofi Boafo[1*], Boonsong Kraisornpornson[2], Somrak Panphon[1] and Bright Emmanuel Owusu[3]

[1]Division of Biology, Department of Science, Faculty of Science and Technology, Prince of Songkla University, Mueang Pattani, 94000, Thailand.
[2]Department of Agricultural Technology, Faculty of Science and Technology, Prince of Songkla University, Mueang Pattani, 94000, Thailand.
[3]Department of Information and Communication Technology/Mathematics, Faculty of Science and Technology, Presbyterian University College, Ghana.

Authors' contributions

This work was carried out in collaboration between all authors. Authors DKB and BK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SP and BEO managed the analyses of the study. Author DKB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was carried out to ameliorate acid sulphate soil (ASS), improve soil nutrient content and the soil quality by employing the use of organic and reduce mineral fertilisation.
Study Design: Treatments were arranged according to a completely randomised block design, in triplicates in a greenhouse.
Methodology: Three fertiliser regimes at three doses (mineral N, P, K (MIN-control (*CK*), *NPK*,

**Corresponding author: E-mail: boafo.psu@gmail.com;*

2NPK), empty fruit bunch compost (COM-*CK*, *EFB*, *2EFB*) and poultry manure (MAN-*CK*, *PM*, *2PM*)) were used. The effect of organic and reduce mineral fertilisation on soil pH, nutrient level, microbial count (colony forming unit (CFU)), microbial biomass (Cmic) and enzyme activity were investigated.

Results: The study revealed that the MAN (*PM* and *2PM*) led to a remarkable increase in bacterial and fungal CFU, Cmic, microbial activity, soil organic carbon (SOC) and nutrient content (N, P and K). MAN also stimulated enzyme activities (β-glucosidase (BG), acid phosphate (ACP) and protease (PRO)), but it did not affect the pH of the acid soil later. Soil pH decreased and *NPK* and *2NPK* were not significant. However, microbial count was decreased significantly at *2NPK* compared to the control even though the nutrient level was elevated to some extent. In COM, soil pH and nutrient level increased, but the fungal CFU, C_{mic} and BG were significantly low. Considering the high heavy metal content of the compost we ascribed these observations to a disturbance from the metal contamination due to high qCO_2 values at the end of the study, The effect of dose was most pronounced in MAN and least pronounced in MIN.

Conclusion: Our result suggests that, for amelioration and quality improvement of ASS of tropical coastal agroecosystem, manure fertilisation supplemented with lime or EFB compost (of good quality) could be recommended while maintaining favourable moisture conditions in the soil.

Keywords: Acid sulphate soil; empty fruit bunch; organic fertilisers; soil microbial properties.

1. INTRODUCTION

Soil acidity is a threat to coastal lands in South East Asia [1] with setbacks which are not only ecological but also economic and Peninsular Thailand is no exception. Organic matter (OM) decomposition by bacteria under anaerobic condition, reduces sulphate (SO_4^{2-}) from seawater and ferric (Fe^{3+}) from sediment to sulphide (S^{2-}) and ferrous (Fe^{2+}) ions, respectively. The primary end product is pyrite (FeS_2) [2], and it generate acidity upon oxidation (Acid sulphate soil (ASS)). At this point, Al and Fe toxicity is predominant, whereas plant nutrient content is low [1].

The reclamation and later fertilisation of acid soil are usually time-consuming and capital intensive as such soil in this areas are not entirely exploited agronomically. Improvement of acid soil is mostly made through the use of lime materials, but in cases where organic fertiliser is used, amelioration, nutrient status and soil quality improvement are considered. [3], suggested that manure can replace lime in the amelioration of acid soil.

In both managed and unmanaged systems, pH of the soil is known to have a strong influence on soil microorganisms at a low pH [4]. Fungi dominate acid soil, and bacterial dominance in near neutral to slightly alkaline soils is well known [5]. Carbon-nitrogen (C/N) ratio is an indicator of soil organic carbon (SOC) quality. During microbial decomposition, C/N ratio does not only determines whether N is taken up or mineralised by microorganisms but also selectively determines which microbial groups (bacteria or fungi) may execute the decomposition of organic materials. Fertilisation affects microbial biomass, microbial respiration (activity) and enzyme activity [6-7] and their rapid stimulation upon fertilisation make them excellent candidates for assessing soil quality.

The study of the response of soil microbial and chemical properties upon fertilisation with organic and mineral in this region (southern-most part of Thailand) are lacking. As such, to enhance and improve the quality of the ASS soil both fertiliser regime and dose were considered in this study over a period of 4 months in a greenhouse. To test the hypotheses that (i) organic fertilisation will increase the pH level, microbial, nutrient properties relative to mineral fertiliser, while acidification is associated with mineral (urea) fertiliser will be lessened at reduced fertilisation rate, (ii) increasing the dose of different fertiliser treatments will differently stimulate the response of plant nutrient and also microbial properties.

2. MATERIALS AND METHODS

2.1 Experimental Design and Sampling

A Short-term incubation (29.2-32.0°C) study was conducted at Prince of Songkhla University Pattani campus, Thailand (from May 2016 to August 2016). The soil used in this study was Typic Sulphaquept [8] clay-textured soil (77.8 4% clay, 1.35% sand and 20.81% silt) with bulk density 1.06 g cm^{-3}. The soil was taken from Kho

po, Pattani, Thailand (6°46'N, 101°7'E; altitude 13 m a.s.l.). The experiment was made up of three fertilizer treatments with three doses i.e. mineral N, P and K fertilizer (MIN) - control (*CK*), *NPK*, *2NPK*, empty fruit bunch compost (COM) - *CK*, *EFB*, *2EFB* and poultry manure with litter (MAN) - *CK*, *PM*, *2PM* with each applied in triplicates. Pots (1.5 kg air-dry soil pot^{-1} with moisture at 40-45% water-holding capacity) in the greenhouse were arranged in a randomised complete block design. The source and amount of N, P and K with the mineral fertiliser were the source and amount known for oil palm growth in South East Asia region [9] (Table 1). While the rate of the compost and manure were conventional rate adopted by local farmers, expected to improve soil nutrient level. The amount of N, P and K input was fixed to be equivalent between COM and MAN pots although the measured values slightly differed (Table 1).

During sampling, 200 g fresh soil was collected from pots after the 1st and 4th month and divided into two subsamples. One subsample was air-dried and sieved through 2 mm sieve and stored for further analysis of chemical properties. The second subsample in moist condition was stored at 4°C for microbial analysis.

2.2 Laboratory Analyses

2.2.1 Chemical properties

Soil pH was measured in 1:5 ratio of soil: deionised water. Organic C and total N were measured by modified Walkley-Black method [10] and Kjeldahl method, respectively. Available phosphorus (AP) was measured colourimetrically by Bray-2 method [11]. Exchangeable potassium (EK) was determined using Atomic absorption spectrometer Aanalyst 100 (PerkinElmer, USA) after extracting soil with 1 M pH 7 NH_4OAc [12]. Total Ca, Mg, Zn, Fe and Cu in soil were determined after H_2SO_4-H_2O_2 digestion with Atomic absorption spectrometer.

2.2.2 Microbial count

The plate count method was used to estimate the population of bacteria and fungi. Nutrient agar (NA) and potato dextrose agar (PDA) supplemented with 300 mg L^{-1} streptomycin, were used for culturing bacteria and fungi [13], respectively. Two millimetres of micro-filtered (0.45 µm NYLON, VertiClean) streptomycin was added to 200 ml PDA and plated. Each dilution was plated in triplicates for each treatment. After the preparation of each media 1 ml of soil suspensions of serial dilution 10^{-3}-10^{-5} were thoroughly mixed with NA media in plates (pour plate method) and 0.1 ml of serial dilutions 10^{-2}-10^{-4} were evenly spread across the PDA media plates (spread plate method). Bacteria and fungi plates were incubated for 2 and 4 days at 35°C and 32°C, respectively. The microbial number was expressed as log CFU g^{-1} oven dry soil.

2.2.3 Microbial biomass

Microbial biomass carbon (C_{mic}) was determined using the chloroform fumigation-K_2SO_4 extraction method [14]. Fresh soil samples were extracted using 0.5 mol L^{-1} K_2SO_4 for 30 min on a rotary shaker after 24 h fumigation in the dark at 25°C. Microbial biomass C was then determined using dichromate oxidation method [15] and calculated using correction factors of 0.45 (*k*EC) [16].

2.2.4 Microbial activity

Basal and substrate-induced (amended with substrate (4 mg glucose g^{-1} soil)) respiration were measured by placing 10 g fresh soil in a beaker and incubating the sample in the dark at 25°C in an airtight jar along with 10 ml of 0.05 mol L^{-1} NaOH to absorb CO_2 released. The CO_2-C was determined by titration with 0.05 mol L^{-1} [13]. The results are expressed as µg CO_2-C g^{-1} h^{-1} oven dry soil and µg CO_2-C mg^{-1} C_{mic} h^{-1} metabolic quotient (qCO_2).

2.2.5 Enzyme activity

β-glucosidase [17], protease [18] and acid phosphatase [19] activities were measured in triplicate with substrate addition after incubation for controls. For β-glucosidase, moist soil was incubated with modified universal buffer (MUB) of pH 6.0 and 25 mM p-nitrophenyl-β-D-glucopyranoside (PNPG) as substrate. Similarly, acid phosphatase activity was assayed using MUB but at pH 6.5 with substrate p-nitrophenyl phosphate (PNPP). The quantity of p-nitrophenol released during an hour of incubation (37 °C) was measured at a wavelength of 410 nm with microplate reader EZ Read 200 (Biochrom, UK) and activities were expressed as µmol p-nitrophenol (p-NP) g^{-1} h^{-1} oven dry soil. Protease enzyme was measured by incubating fresh soil with 50 mM Tris-HCl buffer (pH 8.1) and 1% casein as substrate at 50 °C for 2 h. The tyrosine release was determined by Folin-Ciocalteu colourimetrically using microplate reader EZ

Read 200 (Biochrom, UK) at 700 nm and activity expressed as µmol tyrosine g^{-1} h^{-1} oven dry soil.

2.3 Statistical Analysis

Statistical analyses were executed using R version 3.3.1 [20] and data was log transformed where necessary. The effect of treatments, doses and sampling time (1st month and 4th month) on microbial and chemical properties was determined using a linear model with block as a random factor to cater for probable variability from the spatial distribution of pot on the greenhouse floor. Comparison of doses (single and double dose) with control was made using pairwise comparison of least square means (lsmean; lsmeans package) [21] with Tukey adjustment at $p < 0.05$. Principal component analysis (PCA) was performed using FactoMineR package [22] to assess the relationship (based on correlation matrix; $r > 0.50$) among soil properties. In the current study, PC1, PC2 and PC3 were considered for presentation of the loadings.

3. RESULTS AND DISCUSSION

Soil pH was higher in COM and MAN in the 1st month and lower in MIN and MAN at the end of the 4th month (Fig. 1A). Soil pH was altered in the short-term as pH at *PM*, and *2PM* decreased remarkably to initial soil value in the 4th month ($P = 0.04$). Increased soil pH following organic fertilisation (e.g. compost and manure) corroborates findings by [23], who reported an increase in soil pH from 4.5-7 after three months with 60 tha^{-1} EFB mulch application. The concomitant increase of soil pH brought about by organic fertiliser amendment in our study may be due to high Mg and Ca carbonates and bicarbonates that counter pH reduction [24]. Conversely, the significant decrease in pH in MAN at the end of the study may be due to organic and carbonic acids formed during decomposition of the organic fractions of the manure [25]. Even though pH showed decreasing trend among doses in MIN, the decrease was not significant relative to the control $P = 0.08$.

SOC, TN, AP and EK contents were all affected by fertilisation and dose (Fig. 1C, 1D, 1E and 1F), and were mostly higher in the two organic fertiliser (COM and MAN) treatments compared to MIN, with dose effect being most pronounced in MAN. Higher addition of C, N, P and K (Table 1) with the organic fertilisers explains the higher nutrient content in MAN and COM. The COM and MAN treatments maintained SOC content at higher (i.e. +14.8%) levels compared to the MIN. Although the COM treatment received higher C input (~3 times greater) than MAN, there was no significant difference in their SOC content at the end of the 4th month. Considering the dissimilarities in the quality of substrate supplied by the organic fertilisers (Table 1), the humification and mineralisation dynamics of their organic constituents via microbial degradation [26] would be expected to be differently affected, hence cause a difference in SOC content.

Also, regardless of the high (agronomically pragmatic) rate of P in COM and MAN treatments (Table 1), only the MAN treatment increased AP content proportionally (Fig. 1E). [27], reported that manure P mineralisation is rapid in soils. The relatively low available P fraction in COM could be due to adsorption of P by oxides and hydroxides of Al and Fe along with clay mineral [28] in the ASS, thus rendering P less available. Sampling time had no significant effect on SOC ($P = 0.82$), AP ($P = 0.12$) and EK ($P = 0.50$) content. However, there was a significant decrease in TN to initial soil value, particularly in COM and MAN with a resultant increase in C/N ratio at the end of the 4th month (Fig. 1B and 1D; $P < 0.001$). The decrease in TN in the 4th month could be due to N loss via denitrification (most likely via microbial activity) resulting from anaerobic pockets near aerobic areas (area of high NO_3 concentration) in the soil, a condition suitable for denitrification [29].

Principal component (PC) analysis was employed (based on correlation matrix) to examine the relationship among soil properties, and all soil parameters measured in the study were included in the ordination process. The first principal component (PC1) axis accounted for 47.1% of the variation in the dataset (Table 3). The second and third principal components accounted for 22.3% and 12.5% of the variation, respectively, for a joined explanation of 81.9% of the total variation.

Bacterial CFU ($P < 0.001$) and fungal CFU ($P = 0.0002$) were affected by sampling time (Table 2). Relative to the MIN treatment, the CFU of bacteria increased on average by 12.4% in COM and 18.6% in MAN at the end of the study. Bacterial CFU increased as the dose applied increased in COM and MAN at the end of the study whereas *2NPK* showed a decrease in both bacterial (-3.4%) and fungal (-2.9%) CFU when

Table 1. Fertiliser application rate and selected chemical properties of soil and organic fertilisers

Treatment*	Fertilisation rate and C input‡				Field rate equivalent of mineral and organic fertilizers§			Chemical properties of soil and organic fertilisers#			
	N	P	K	C				Property (units)	Initial soil	Organic fertiliser	
	(mg pot^{-1} soil)									EFB	PM
					Mineral fertilizer (kg ha^{-1})			pH(1:5)	4.79 (0.06)	7.59 (0.05)	7.89 (0.16)
Mineral (MIN)					Urea	DAP	MOP	EC(dS m^{-1})	0.33 (0.05)	3.76 (0.10)	5.72 (1.02)
CK	0	0	0	0	0	0	0	C/N	26.1 (0.91)	37.5 (1.77)	12.9 (0.93)
NPK	100	20	150	0	130	65	195	SOC(g kg^{-1})	33.6 (1.20)	332.4 (11.8)	228.8 (9.54)
2NPK	200	40	300	0	260	130	390	TN(g kg^{-1})	1.29 (0.04)	8.86 (0.11)	17.8 (0.56)
								AP(g kg^{-1})	0.36 (0.02)	25.9 (1.06)	39.1 (4.34)
Compost (COM)					Organic fertilizer (t ha^{-1})			EK(g kg^{-1})	0.18 (0.03)	12.5 (0.45)	28.6 (0.98)
CK	0	0	0	0	0			Ca(mg kg^{-1})	0.01 (0.001)	56.7 (2.92)	17.0 (3.09)
EFB	203	596	287	7600	15			Mg(mg kg^{-1})	0.02 (0.001)	395.1 (5.03)	77.7 (4.99)
2EFB	407	1190	575	15300	30			Cu(mg kg^{-1})	0.28 (0.06)	5014.2 (297.7)	126.8 (22.1)
Manure (MAN)								Fe(mg kg^{-1})	123.6 (22.4)	15122.6 (656.3)	2599.5 (290.8)
CK	0	0	0	0	0			Zn(mg kg^{-1})	37.5 (2.17)	812.5 (40.6)	1366.1 (136.0)
PM	213	469	343	2700	8			CEC(cmol$_c$ kg^{-1})	29.5 (2.25)	ND	ND
2PM	427	939	685	5500	16			Al(cmol$_c$ kg^{-1})	2.03 (0.10)	ND	ND

* *CK: Control; EFB: Empty fruit bunch compost; PM: Poultry manure. Single dose (NPK, EFB, and PM) and double dose (2NPK, 2EFB and 2PM),* ‡ *The nutrient rate and C input with mineral fertiliser, empty fruit bunch and poultry manure,* § *Field rate of mineral and organic fertiliser.*

DAP: Diammonium phosphate; MOP: Muriate of potash (KCl)

Mean (± S.EM); ND = Not determined

compared to the control (Table 2). The increase in bacterial CFU may be due to easily degradable substrate C (microbial energy source) addition, since an increase in C resulting from high dose input may cause an increase in copiotrophic bacteria, capable of forming colonies on nutrient agar. Also, SOC was positively correlated with bacterial and fungal CFU on PC1.

Fungal CFU was similar to that of bacteria after the 1st month, but in the 4th month, changed to the order of MAN > MIN > COM (Table 2) with values of *EFB* and *2EFB* relatively lower than the unamended control. The observed decrease in fungal CFU at *EFB* and *2EFB* (4th month) doses is contrary to our anticipation, considering the C-rich EFB materials (high C/N) would be expected to favour fungi. An attribution of the low fungal values to heavy metal toxicity is possible, considering the high metal loading of the EFB material (77 and 230 µg Cu g^{-1} soil at *EFB* and *2EFB* doses, respectively) (Table 1). On the contrary, [30], showed that heavy metals decreased CFU for bacteria but not for fungi. The null effect of heavy metal toxicity on bacterial CFU in this study could be due to C from lysed fungal cells which increased bacterial CFU, hence offsetting the influence of heavy metal. Similarly, [31] reported a decrease in fungal CFU at $CuSO_4$ concentrations 0.25 to 0.5 mM. The reduced CFU of bacteria and fungi at *2NPK* (Table 2) could be due to ammonia (NH_3) toxicity caused by NH_4-forming mineral fertilisers when applied at increased doses [32].

Fertilisation only affected C_{mic} in the 4th month (MAN > MIN = COM) and dose in the 1st month (Table 2). When compared to the control, the increase in C_{mic} at *EFB* (P = 0.07) and *2EFB* (P = 0.09) fell short of significance in the 1st month, but there was a substantial decrease in C_{mic} at *2EFB* at the end of the study. High C and N along with the possible incorporation of microbial biomass with the manure may have led to the high C_{mic} in MAN. At the end of the study, C_{mic} in the MIN (*2NPK*) and COM (*2EFB*) treatments were similar to the control, although their SOC content remained unaltered. This is an indication that C_{mic} is a more sensitive indicator for the improvement of soil quality relative to SOC. Further, the low fungal CFU at *2EFB* which also reflected in C_{mic} agrees with the suggestion that decomposition pathway of EFB material is mainly of fungal origin [33]. The notable decrease in C_{mic} at the end of the 4th month could be due to heavy metal locked up in the compost structure during early stages of decomposition, which increased in their concentration during decay (metal contamination).

Similarly, the relatively lower BG in COM (Table 2) may be explained by the low fungal CFU since β-glucosidase activity is known to be mostly linked to fungi [34]. However, the drastic increase in BG (212% on average) at the end of the study (4th month) may stem from the fact that BG degrades labile cellulose and other carbohydrate polymers, reduce organic structures and molecular size, thus facilitating subsequent elevation of microbial enzyme activity including ACP and PRO activity. Though the high hydrolase enzyme activity could be ascribed to high organic carbon content, as seen by the significant and positive correlation between SOC and all three enzyme activities on PC1 (Table 3), the increase in BG and other enzymes in the 4th month did not cause significant (marginal, if any) shift or change in SOC. Similarly, [35] reported 2-4 folds increase in BG with only a minor change in SOC content following organic amendment. However, the increase in all treatments at the end of the study may be ascribed to both C/N ratio and pH. Acid phosphatase enzyme is predominant in acid soils [36] and C and N equilibrium (C/N ratio in this study: MIN ≈ COM ≈ MAN) may enhance soil ACP activity, as suggested by [37]. This is confirmed by the positive relationship between ACP and C/N and inverse relationship between ACP and pH on PC2 in our study (Table 3).

High microbial count and activity triggered by substrate addition (both C and N) and high proteinaceous substrate level could be responsible for the high PRO activity in COM and MAN (Table 1). [38], observed a strong correlation between N from organic matter and protease activity. This is further supported by the significantly positive relationship between TN and PRO on PC1 (Table 3). Moreover, the organic fertilisers may have added enzymes to the soil via organic residue input.

Both BR (Table S1) and qCO_2 (Table 2) were higher in COM (at *2EFB*). According to [39], fungi inhibition increases CO_2 release suggesting high microbial activity when fungi decreases and bacteria increases in acid soil. The above statement is in line with a report from [40] that microbes in contaminated soils are under stress and as such use C less efficiently, causing more CO_2 to evolve per unit substrate (high qCO_2 values in COM). The high qCO_2 in the compost

treatments could also be due to reduced N content at the end of the study. On PC2, there was a significant yet positive relationship between C/N and qCO_2 and has this been suggested to due microbial N-mining. Microbes burn readily available C to gain energy for further acquisition of N from more recalcitrant OM [41]. Furthermore, BR is linked to autochthonous microbial population (K-strategists), while SIR depends on zymogenous microbial community (r-strategists), which exhibit a tremendous increase in numbers in response to substrate addition. The increased BR in MIN and decreased SIR in MAN treatment (Table S1) at the end of the incubation period could mean an effective shift in microbes from r-strategists to K-strategists in the acid soil [42].

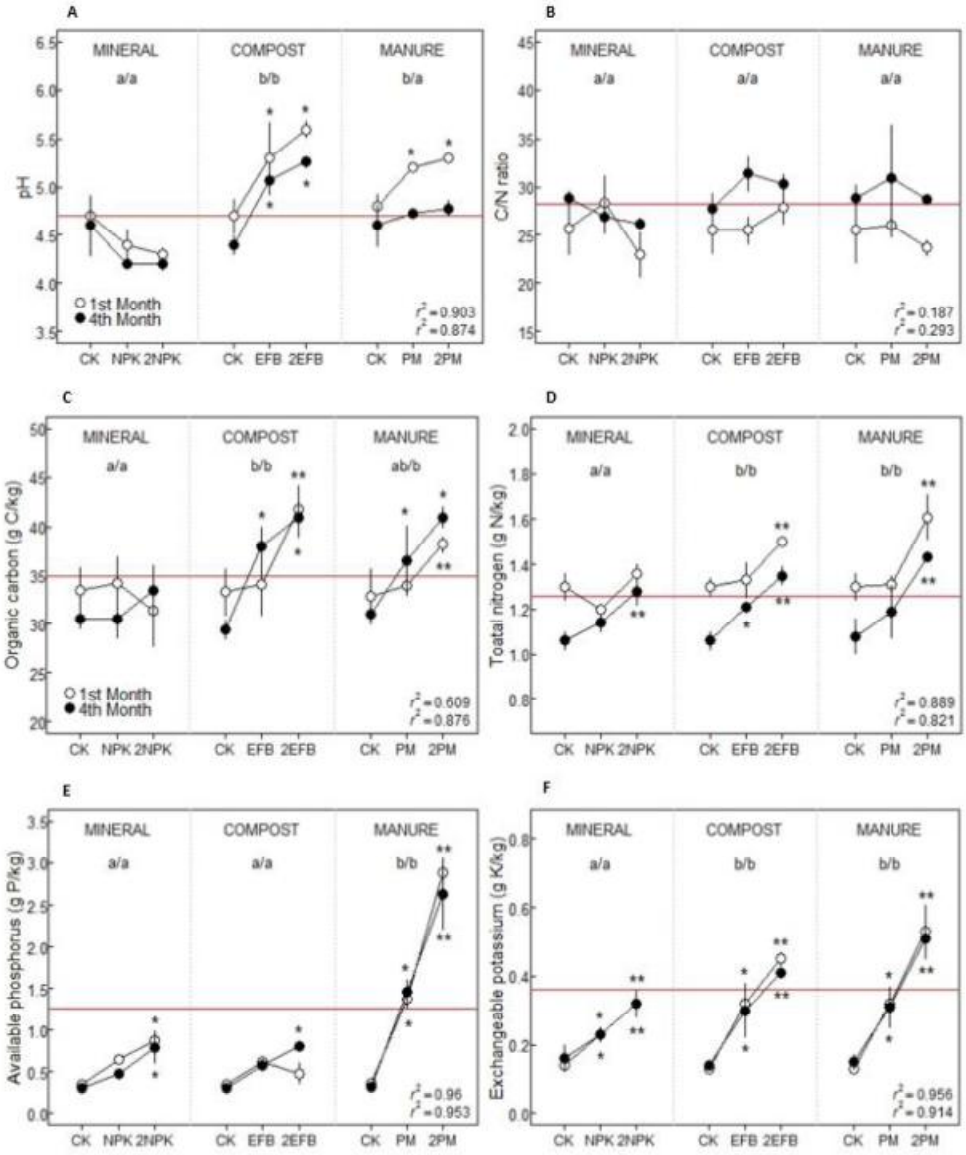

Fig. 1. (A) pH, (B) C/N ratio, (C) SOC, (D) TN, (E) AP and (F) EK in 1st month (opened points) and 4th months (closed points) affected by treatments (MIN, COM and MAN) at varying doses. Horizontal line denotes overall mean. Points are means (± 95% CI) and treatment effect with different letters (left = 1st month/right = 4th month) show difference at $P < 0.05$. Dose effect of single and double compared to the control is designated by "*" and double dose from both single and control are ""**

Table 2. Mean of bacterial and fungal CFU, C_{mic}, metabolic quotient (qCO_2), BG, ACP and PRO activity affected by fertiliser regime and dose from 1 month and 4 months after fertilisation

Treatment	Month = 1st							Month = 4th						
	Bacteria	Fungi	C_{mic}	qCO_2	BG	ACP	PRO	Bacteria	Fungi	C_{mic}	qCO_2	BG	ACP	PRO
	(log CFU g^{-1} soil)		(µg C g^{-1})		(µmol product g^{-1} h^{-1})‡			(log CFU g^{-1} soil)		(µg C g^{-1})		(µmol product g^{-1} h^{-1})		
Fertilisation														
MIN	6.27a	4.65a	484.4a	2.68a	0.03a	1.10a	0.27a	6.13*a*	4.88*b*	273.8*a*	7.59*b*	0.25*b*	3.29*a*	0.67*a*
COM	7.04b	4.90b	374.6a	5.38b	0.04a	1.47b	1.12b	6.89*b*	4.61*a*	136.9*a*	19.5*c*	0.11*a*	3.55*b*	1.37*b*
MAN	7.33c	5.95c	579.2a	3.63b	0.12b	2.23c	1.94c	7.27*c*	5.56*c*	533.0*b*	2.75*a*	0.31*c*	3.89*c*	1.33*b*
P-value	***	***	n.s.	**	***	***	***	***	***	***	***	***	***	**
Dose														
Control (CK)	6.28a	4.80a	183.6a	3.96a	0.02a	0.95a	0.28a	6.21*a*	4.87*a*	257.5*a*	5.21*a*	0.08*a*	1.58*a*	0.59*a*
Single	6.79b	5.26c	467.4b	3.49a	0.05b	1.49b	1.00b	6.74*b*	4.96*b*	322.7*a*	8.31*ab*	0.25*b*	3.63*b*	1.10*b*
Double	6.97c	5.07b	491.2b	4.30a	0.08b	1.71b	1.23b	6.79*b*	5.07*b*	306.4*a*	11.6*b*	0.27*b*	3.51*b*	1.15*b*
P-value	***	***	***	n.s.	***	***	***	***	***	n.s.	0.020	***	***	***
Fertilisation x Dose														
CK	6.28A	4.80A	183.6A	3.96A	0.02A	0.95A	0.28A	6.21A	4.87A	257.5A	5.21A	0.08B	1.58B	0.59B
MIN														
NPK	6.20A	4.96A	435.9*A	2.50A	0.04A	1.18A	0.24A	6.27A	5.03*A	303.2A	7.72A	0.25*B	3.32*B	0.56B
2NPK	6.34B	4.60*A	532.9*B	2.87A	0.03A	1.01A	0.30A	5.99**A	4.73**A	244.5A	7.46A	0.24*B	3.25*B	0.78B
COM														
EFB	7.00*B	5.18*B	374.5A	4.92A	0.04A	1.46A	1.18*A	6.78*A	4.63*A	176.0A	14.5A	0.10B	3.75*B	1.29*A
2EFB	7.08*A	4.62**A	374.5B	5.82A	0.05A	1.48A	1.06*A	7.00**A	4.59*A	97.8*A	24.4*B	0.11B	3.35*B	1.45*A
MAN														
PM	7.17*A	5.93*B	592.0*A	3.06A	0.08*A	1.84*A	1.59*A	7.16*A	5.22*A	489.0*A	2.68A	0.39*B	3.82*B	1.46*A
2PM	7.50**A	5.97*A	566.3*A	4.21A	0.15**A	2.63*A	2.30*A	7.38**A	5.89**A	577.0*A	2.81A	0.47*B	3.94*B	1.21*A
P-value	***	***	n.s.	n.s.	***	**	***	***	***	**	**	***	**	**
r^2 -Adjusted	0.953	0.978	0.664	0.161	0.843	0.727	0.916	0.989	0.990	0.618	0.677	0.917	0.959	0.601

Within columns, different lowercase letters (non-italicized = 1st and italicized = 4th month) indicate significant difference at P < 0.05. Within rows, different uppercase letters indicate significant difference between 1st and 4th month at p < 0.05. Significant dose effect of single and double doses compared to the control (P < 0.05) are designated by "" and effect of double dose from both single dose and control (P < 0.05) are designated by "**".*

*Coefficient of determination (r^2) and ** P < 0.01, *** P < 0.001 and n.s. = not significant.*

‡ BG and ACP = µmol p-NP g^{-1} h^{-1}; PRO = µmol tyr g^{-1} h^{-1}; qCO_2 = µg CO_2-C mg^{-1} C_{mic} h^{-1}

Table 3. Correlation between variables and principal components, the Eigenvalues and percent rate of explained variability of each component

Parameter	PC1	PC2	PC3
Chemical			
pH	0.578*	n.s.†	-0.723*
SOC	0.700*	0.509*	n.s.
TN	0.776*	n.s.	n.s.
C/N	n.s.	0.641*	n.s.
AP	0.972*	n.s.	n.s.
EK	0.904*	n.s.	n.s.
Microbial			
Bacterial CFU	0.922*	n.s.	n.s.
Fungal CFU	0.792*	-0.537*	n.s.
MBC	0.566*	-0.675*	n.s.
BR	0.556*	n.s.	n.s.
SIR	0.894*	n.s.	n.s.
qCO_2	n.s.	0.840*	n.s.
Enzyme			
BG	0.570*	n.s.	0.770*
PRO	0.845*	n.s.	n.s.
ACP	0.557*	0.514*	0.596*
Eigen value	7.926	3.969	2.035
Variance (%)	47.1	22.31	12.53
Cumulative variance (%)	47.1	69.41	81.94

*† n.s. = not significant– loading (r) < 0.50. *Significance*

4. CONCLUSION

Contrary to our hypothesis, poultry manure (MAN) did not alter pH, however, in corroboration with our hypothesis, the urea-containing mineral NPK fertiliser (MIN) did not significantly decrease the pH. SOC**,** TN, AP and EK content were notably higher in MAN and COM compared to MIN. The MAN treatment, in particular, improved and maintained SOC at a higher level, which consequently led to a rapid boost in the microbial count (bacterial and fungal CFU), activity (SIR and BR), enzyme (BG, ACP and PRO) and soil fertility, thus improving soil quality. The dose effect was most pronounced with poultry manure (*CK* < *PM* < *2PM*) and least pronounced in mineral fertilisation. The plate count methods have limitations. Future work on microbial communities using culture-independent approaches (*q*PCR, sequencing, and so forth) is needed for our acid soil under field conditions to determine whether the results obtained here can be generalised across different ASS.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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