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Research Article

Factors Governing Organic Amendments and NPK Fertilizers Effects on Sweet Maize in Old and Intensively Cultivated Experimental Farm

Faktor-Faktor yang Mengontrol Pengaruh Amelioran Organik dan Pupuk NPK pada Tanaman Jagung Manis di Kebun Percobaan Lama dan Dibudidayakan Secara Intensif

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Abstract: Applying organic amendment (OA) containing humic compounds (HC) and phytohormones is a promising solution to intensify sustainable food production under increasing global food needs, declining environmental carrying capacity and changing climate. However, most positive claims on OA efficacy often came from controlled, greenhouse experiments. The field trial was conducted on an intensively cultivated experimental farm station, Department of Soil Science and Land Resources, Faculty of Agriculture, IPB University. The OA testing was done on sweet maize (*Zea mays* L. saccharata) using a fractional factorial randomized block design by comparing five rates of the organic amendment (0, ½, 1, 1½, and 2 standards OA) with three rates of NPK fertilizer (0, 1, and ¾ standards NPK). The results revealed that a single OA application did not significantly boost the growth and biomass of sweet maize, especially when applied to an old and intensively cultivate and organically manured farm. OA had significant interaction with NPK at most of the yields and biomass parameters. Amending soils more than 12 L OA ha^{-1} could improve the sweet maize's growth and development while saving 25% NPK fertilizers. Linear mixed effect model and multivariate analysis uncovered higher heterogeneity in trial plots controlled maize growth, biomass, and agronomic effectivity, regardless of the given treatments. This study highlighted three important marks for future research: (1) soil plowing, harrowing and mixing must be intensively done across plots, (2) adequate HC contents must be increased from the OA current rate, and (3) the greater role of phytohormone in stimulating maize growth and production at the OA current rate.

Keywords: humic compounds, mixed effect models, organic amendment, PCA, pythohormones

Abstrak: Penerapan amelioran organik (OA) yang mengandung senyawa humat (HC) dan fitohormon adalah solusi yang menjanjikan dalam intensifikasi produksi pangan berkelanjutan di bawah meningkatnya kebutuhan pangan global, penurunan daya dukung lingkungan, dan perubahan iklim. Namun, sebagian besar klaim positif mengenai efikasi OA sering kali berasal dari eksperimen rumah kaca yang terkontrol. Uji coba lapangan dilakukan di kebun percobaan yang dibudidayakan secara intensif, Departemen Ilmu Tanah dan Sumberdaya Lahan, Fakultas Pertanian, IPB University. Pengujian OA dilakukan pada tanaman jagung manis (*Zea mays* L. saccharata) menggunakan Rancangan Acak Kelompok (RAK) fraksional faktorial dengan membandingkan lima takaran bahan organik (0, ½, 1, 1½, dan 2 standar OA) dengan tiga takaran pupuk NPK. (0, 1, dan ¾ standar NPK). Hasil penelitian menunjukkan bahwa aplikasi OA tunggal tidak nyata meningkatkan pertumbuhan dan biomassa jagung manis, terutama bila diaplikasikan pada lahan yang dibudidayakan sejak lama dan intensif serta dipupuk organik. OA memiliki interaksi yang signifikan dengan NPK pada sebagian besar hasil dan parameter biomassa. Pengolahan tanah lebih dari 12 L OA ha^{-1} meningkatkan pertumbuhan dan perkembangan jagung manis, sekaligus menghemat 25% penggunaan pupuk NPK. Model linier campuran terampat dan analisis multivariat mengungkap heterogenitas yang lebih tinggi di petak

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percobaan yang mengontrol pertumbuhan jagung, biomassa, dan efektivitas agronomi, terlepas dari perlakuan yang diberikan. Studi ini menyoroti tiga poin penting untuk penelitian di masa depan: (1) pembajakan, penggaruan dan pencampuran yang lebih intensif di seluruh plot, (2) kandungan HC yang memadai harus lebih ditingkatkan dari dosis OA saat ini, dan (3) peran fitohormon yang lebih besar dalam menstimulasi pertumbuhan dan produksi jagung pada dosis OA saat ini.

Kata kunci: senyawa humat, model linier campuran terampat, amelioran organik, PCA, fitohormon

INTRODUCTION

The constraints concerning soil quality often found in the humid tropics are naturally poor in nutrients (Funakawa *et al.* 2009; Watanabe *et al.* 2006) and the implementation of inappropriate and unsustainable land management (Nkonya *et al.* 2005). As consequences, agricultural lands suffer soil nutrients mining and the decline of crop production (Purwanto & Alam 2019; Gebremedhin *et al.*, 2022). Currently, the improvements of agricultural soils quality are heavily embraced, focusing on the increase of soil carbon stores and soil biodiversity as the central role of agricultural land in the global nutrient cycle, as well as sustainably fulfils the foods require by world's population (Koch *et al.* 2013; McBratney *et al.* 2014; Bouma 2020; Lehmann *et al.* 2020).

The agricultural sector faces multidimensional challenges, especially from increasing food demand in response to an increased human population. However, this great need collides with the shrinking of agricultural areas as a result of land conversion into built-up areas (Verburg *et al.* 1999; Rustiadi *et al.* 2020; Tri Harjanti and Hara 2020), the decreasing carrying capacity of the environment (Tarigan and Tukayo 2013), and the threat of climate change (Altieri and Nicholls 2017). As a result, sustainable intensification through soil quality improvement and the increase of food crops productivity is considered necessary.

Humic compounds/HC are recalcitrant and complex substances, mostly composed of the terminal stage of decomposed organic materials. HC constitutes around 60 – 80% of organic materials contained in soils (Vikram *et al.* 2022). Some studies detected that HC may harbor phytohormones or hormones-like substances, *i.e.*, auxin and giberellin (Muscolo *et al.* 1998; Arancon *et al.* 2006; Scaglia *et al.* 2016) and trigger the gene expression related to phytohormones secretion (Canellas *et al.* 2020; Souza *et al.* 2022), thus regulate plant metabolism and stimulate growth and development processes (Calvo *et al.* 2014; Berry and Argueso 2022). Studies on HC's applicability received great interest, particularly for their possibility of improving degraded lands resulting from natural and anthropogenic causes (Nan *et al.* 2016; Sharma and Singh 2019). Organic amendment/OA containing HC is widely known for its applicability in promoting plant growth and development (Khan *et al.* 2017; Bijanzadeh *et al.* 2019; Li *et al.* 2019; Boveiri Dehsheikh *et al.* 2020; Izhar Shafi *et al.* 2020), while subsequently improving soil qualities in term of physical, chemical, and biological characteristics (Li *et al.* 2019; Ampong *et al.* 2022; Ren *et al.* 2022; Zhao *et al.* 2022).

In spite of their positive findings, some scientists remain skeptical concerning the performance of commercially-derived HC on field deployment, particularly with regard to ineffective rates, nutritional inconsistency, and the lack of field trials (Quilty and Cattle 2011). Several researchers reported insignificant plants response on HC amendment (Çimrin and Yilmaz 2005; Shen *et al.* 2016; Mollah *et al.* 2020). Furthermore, there are reports suggesting adverse effects of inappropriate HC and its molecular species incorporation on plant physiology and metabolism, *e.g.*, generates cell death, reduces transpiration, and decreases root hydraulic conductivity and plant growth (Muscolo and Sidari 2009; Asli and Neumann 2010). Furthermore, research elucidating the incorporation of OA containing HC and phytohormones in soils at the field levels is considered scarce, especially in the humid tropic region. Much of our current understanding concerning the regulatory effect of OA containing HC and/or phytohormones on plants, especially on maize, primarily originated from the controlled laboratory, greenhouse, and hydroponic experiments (*e.g.,* those reviewed by Nardi *et al.* 2002, Olivares *et al.* 2017, and Akimbekov *et al.* 2021). Field trials were required to demonstrate these abovementioned (positive, nil, or negative) claims, especially at old, intensively cultivated, fertilized, and organically manured farms.

Therefore, this study aimed to test the effectivity of OA containing HC and phytohormones on maize at field level under following hypotheses: (1) moderate to high maize respond of OA and NPK combination and (2) plot position across slope gradients also primarily controls maize growth and yields, regardless of the OA and NPK treatments.

MATERIALS AND METHODS

Research Site and Soil Physicochemical Properties

The current trial was conducted at a relatively flat areat at a footslope, Cikabayan IPB University Experimental Farm, Dramaga, Bogor (Figure 1). The soil type in the research site is generally considered oxic-haplic latosols based on National Soil Classification System (Subardja *et al.* 2016) or oxic dystrudepts based on Soil Taxonomy (Soil Survey Staff 2014; Fuadi 2019). This juvenile soil was derived from andesitic volcanic tuff of Salak volcano. Then, it developed a cambic horizon while also had low base saturation (<50%) and clay exchange capacity (<24 meq $100g^{-1}$). This farm was firstly cleared and cultivated at early 1990s. Since then, the soils was cultivated intensively with various trials on maize (predominantly) involving organic and inorganic fertilizers and ameliorants.

Figure 1. The map of the research site

Before planting, the soil sample (±1 kg) representing the upper surface (0 – 30 cm) was compositely collected from all plots using a hand auger. The soil sample was then analyzed for their physical and chemical characteristics, including texture, actual and potential pH (1:5 H_2O and 1 N KCl, respectively), organic C (Walkley and Black), total N (Kjeldahl), available and potential P (Bray-1 and 25% HCl, respectively), CEC and exchangeable bases (1N NH_4OAc pH7), base saturation (KB), exchangeable Al and H (1 N KCl), as well as exchangeable Fe, Cu, Zn, and Mn (DTPA+TEA) at Soil Science and Land Resources Laboratory, Faculty of Agriculture, IPB University. The soil's physical and chemical properties are shown in Table 1. Based on the Soil Analysis Results Assessment Criteria (Eviati and Sulaeman 2009), the soil at the test site had low to very low exchangeable bases with an acidic pH. Meanwhile, soil organic C and total N contents were categorized as moderate. In contrast, Fe, Cu, Zn, and Mn content were classified as high. Moreover, soil Al saturation is considered very low. The conditions described above indicate that the soil in the study site had low fertility.

Table 1. Physical and chemical properties of soil at the study site

Soil Properties	**Value**	**Status**
Physical Properties		
Texture		
Sand (%)	9.94	Clay
Silt (%)	16.7	
Clay (%)	73.37	
Chemical Properties		
Organic C (%)	2.69	Moderate
Total N (%)	0.27	Moderate
C/N	9.96	Low
Potential P (mg kg^{-1})	283	Very High
Available P (mg kg^{-1})	8.43	Moderate
Exchangeable Bases ($cmol^+ kg^{-1}$) :		
K	0.69	High
Na	0.08	Very Low
Mg	0.98	Low
Ca	3.18	Low
Exchangeable Acid Cations ($cmol^+ kg^{-1}$)		
Al-dd	0.83	-
H-dd	0.57	-
CEC ($cmol^+ kg^{-1}$)	19.8	Moderate
Base Saturation (%)	24.89	Low
Al Saturation (%)	4.19	Very Low
DTPA-extracted Micronutrients (mg kg^{-1})		
Fe	46.1	High
Cu	3.83	High
Zn	8.53	High
Mn	116	High
pH KCl	4.18	-
pH H_2O	4.54	Acidic

Experimental Design of Organic Amendment

In this present study, an organic amendment (OA) containing humic compound/HC and phytohormones was used to ameliorate sweet maize (*Zea mays* L. Saccharata; Talenta variety) cultivation. The nutritional composition of the ameliorant is presented in Table 2. The Table showed that OA consisted of a 21.36% humic compound, supplied with 47.04 and 69.13 mg L^{-1} of auxin and gibberellin, respectively. The OA also had acidic pH and contained considerable Fe and Zn.

Table 2. Nutritional composition of OA used in this study

Parameter	**Unit**	**Value**
Organic C	%	14.01
Micronutrients:		
- Total Fe	mg kg^{-1}	275.00
- Available Fe	mg kg^{-1}	55.10
- Zn	mg kg^{-1}	8.50
pH	-	4.30
Escherichia coli	MPN ml^{-1}	< 3
Salmonella sp	MPN ml^{-1}	< 3
Heavy metals:		
As	mg kg^{-1}	< 0.026
Hg	mg kg^{-1}	<0.014
Pb	mg kg^{-1}	<0.080
Cd	mg kg^{-1}	<0.010
Cr	mg kg^{-1}	<0.001
Ni	mg kg^{-1}	<0.010
Other elements/compounds:		
Auxin	mg L^{-1}	47.04
Gibberellin	mg L^{-1}	69.13
Humic acid	%	13.30
Fulvic acid	%	8.06
Humic compound (calculation)	%	21.36

Notes: MPN = most probable number

A fractional factorial randomized block design (8 out of 15 combinations) had performed in this study. Four blocks/groups represented plot position across the slope. The entire experimental plots were 32, each occupying 25 m^2, resulting 480 m^2 in total. The experimental design, OA and NPK fertilizers rate, and application period are shown in Table 3. In accordance with the purpose, this study compared five rates of OA (0, ½, 1, 1½, and 2 standards of OA) with three rates of recommended NPK fertilization (0, 1 and ¾ standards of NPK), resulting in total 8 treatments combination.

The experimental field was plowed and prepared using a farm tractor. The OA was diluted in 5 L water prior to the application, then evenly spread over the soil surface and sprayed to plant leaves. Meanwhile, NPK fertilizers were placed into the soil surface, thoroughly mixed with a hand rake, and hoe to the required soil depth of approximately 20 cm. The OA was amended during and 28 days after planting/DAP, whereas NPK fertilizer was applied 0, 28, and 45 DAP. The sweet maize was planted with three seeds per hole at 5 cm depth using 70 x 30 cm spacing. At 28 DAP, the beds were raised around 20 cm high, followed by single underdeveloped individuals elimination in all plots, leaving only two plants per hole until the harvesting period.

Table 3. Experimental design

Treatments	**OA rates**		**NPK Fertilizers rates***	
	L ha^{-1}	**Abbrev.**	**Kg ha^{-1}**	**Abbrev.**
B0S0 (control)	0	0		0
B0S1	0	0	300, 200, 150	1 std
B1S1	6	½ std	300, 200, 150	1 std
B2S1	12	1 std	300, 200, 150	1 std
B3S1	18	1½ std	300, 200, 150	1 std
B4S1	24	2 std	300, 200, 150	1 std
B1S2	6	½ std	225, 150, 112.5	¾ std
B2S2	12	1 std	225, 150, 112.5	¾ std
Application Period	0 and 28 DAP		0, 28, and 45 DAP	

Notes: OA = organic amendment; asterisk (*) = NPK fertilization was used in the form of urea, SP36, and KCl, respectively; std = standard; DAP = days after planting

Ten individuals from ten planting holes were selected for plant sampling. The plant height/PH and leaf quantity/LQ were measured at the stages of vegetative (14, 35, and 42 DAPs) and generative (49 and 63 DAP). Furthermore, maize harvesting was conducted at 77 DAP, measuring biomass quantity/BQ, *i.e.,* maize stover wet weight/STV and wet weight of ear with (EWH) and without husks (ENH). The relative agronomic effectiveness (RAE) of OA was calculated using Engelstadt *et al.* (1974) approach with the following formula:

$$RAE = \frac{Production\ of\ maize\ from\ OA\ tested - control}{Production\ of\ maize\ from\ NPKstd\ fertilizer - control} \times 100\%$$

where the production includes all measured wet weight (therefore referred to as EAE_ENH, RAE_EWH, and RAE_STV).

Data Analyses

All statistical analyses were carried out in an R environment using RStudio and Minitab 20.3. This study performed local and general approaches to assess the regulatory effect of OA, NPK, and slope gradient (represented by block). Firstly, we run the general linear model/GLM and generalized linear mixed effect model/GLMM to conduct the first approach. Lastly, the latter general approach involves a multivariate ordination method.

The treatment effects (OA and NPK) and the position effect on the slope (block) as the environmental regulatory factor were evaluated sequentially using GLM and GLMM. In this study, we adopt the principle of completeness to accommodate the hypothesis. This allows us to expand the GLM's covariates to GLMM by incorporating block as random variables. However, it also agrees with the principles of parsimony, using the penalty of corrected-Akaike Information Criterion/AICc. The GLM models were used as a standard cut-off to build more complex GLMM models. Firstly, GLM models were optimized using four transformation types, *i.e.,* ordinary least squares, Poisson, quasiPoisson, and negative binomial, based on AICc (`AICcmodavg` package; Mazerolle *et al.* 2023) and adjusted coefficient of determination/adj-Rsq (`rsq` package; Zhang 2023). We selected the best GLM models based on the lowest AICc and highest Adj-Rsq. However, we carefully consider the goodness of fit based on adj-Rsq, since it will leads to apparent overfitting. Then, five GLMM models (fitted with maximum likelihood/ML estimator) were successively developed and ordered based on their AICc using `lme4` (Bates *et al.* 2022). The respective models consisted of three intercept models (OA, NPK, and block) and two full models with and without interaction. In the full LME models, OA and NPK were chosen as fixed factors. Meanwhile, the block was fitted as a random intercept. Furthermore, we also performed full GLMM models that fitted through Poisson and negative binomial distributions using the `MASS` package (Ripley *et al.* 2023). The GLM and GLMM models were compared, while the final model was selected based on the lowest AICc, considerable adj-Rsq, and suitability for our hypothesis. The entire GLMM final models were then refitted using a restricted maximum likelihood (ReML) estimator to provide unbiased estimates of the variance and covariance parameters.

We, therefore, used standard GLM (multiple linear regression, similar to anova type III) to statistically differentiate the treatment levels under unbalanced experimental design. In this regard, Box-Cox transformed response values with optimum λ were fed to the models. Then, Tukey honesty differences (HSD) test was employed at 90% confidence intervals. Furthermore, similar method was performed to assess the differences of fixed factor levels given the difficulties of performing post-hoc tests on GLMM models. We included factors possessing higher P values but close to 0.1 to avoid unnecessary strict and illogical dichotomization (*e.g.,* Hurlbert and Lombardi 2009; Lew 2012). To evaluate the importance of the random factor towards the fixed factor and overall model, we also calculated fixed-Rsq and random-Rsq by decomposing the effects' explained variances following Nakagawa and Schielzeth (2012) through `rsq` package.

A principal component analysis/PCA was executed to accomplish the general approach using `factomineR` (Husson *et al.* 2020) and `factoExtra` packages (Kassambara and Mundt 2020). We selected five PCs to be visually presented in the scree plot. Considerable PCs were chosen based on "elbow points" of the variance explained by each successive PC, resulting in the first four PCs. The representation of each loaded variables of the main PCs were mapped and ordered in the form of contribution percentage plots. For visual convenience, we restricted the contribution to 8th highest variables. The grouping on observation plots used 90% multivariate confidence intervals based on treatments, OA, NPK, and groups.

RESULTS

Effect of the Treatments on Sweet Maize' Agronomic Performances

The effects of B+NPK treatments on sweet maize' agronomic performances varied widely according to the rate, observed parameters, and observation period, as shown in Table 4. In general, most combination treatments achieved statistical differences from control on PH at 35, 42, and 63 DAPs, LQ at 35 and 63 DAPs, and all of the BQs. Moreover, not all treatments exhibited remarkable differences with control, *i.e.,* B1S2, B2S2, and B4S1 treatments on PH at 35, 35, and 42 DAPs, respectively; B1S2 on LQ at 35 and 63 DAPs; B2S2 on LQ at 35 DAP; and B0S1 on ENH. Contrastingly, the treatments produced similar PH and LQ compared to the control at

14 and 49 DAPs, with the latter parameter also resulting in similar results at 42 DAP. We also found less notable effects of the treatments on the entire RAE.

According to Table 4, B3S1 treatment resulted in the highest PH at 35 and 42 DAPs, which was considerably different from the control (B0S0). Moreover, similar effects of the treatment were also observed in LQ at 35 and 63 DAPs. At 14 DAP, the B3S1 treatment achieved the highest LQ, yet no statistical differences compared to the control. B4S1 treatment yielded the highest PH at 14 DAP, heaviest ENH, and highest RAE_ENH; however, only the second parameter showed a remarkable difference from the control. At 49 and 63 DAPs, B1S1 treatment resulted in the highest PH, whereas only the latter was significantly different from the control. The highest result of LQ was also achieved by similar treatment at 42 DAP with an inconsiderable difference compared to the control. Maize fertilized with B2S2 treatment attained the heaviest EWH and highest RAE_EWH. Meanwhile, the heaviest STV and highest RAE_STV were produced by the B0S1 treatment.

Table 4. The effect of treatments on the sweet maize' agronomic performances

Agronomic Performances	**Treatments**							
	B0S0	**B0S1**	**B1S1**	**B2S1**	**B1S2**	**B2S2**	**B3S1**	**B4S1**
Plant Height (cm)								
14 DAP	24.5	25.6	25.0	25.1	24.7	25.3	25.9	**26.0**
35 DAP	72.7 b	87.3 a	89.7 a	86.1 a	81.8 ab	85.1 ab	**90.1 a**	89.6 a
42 DAP	88.0 b	107.6 a	111.2 a	104.1 a	102.0 a	103.6 a	**111.5 a**	111.3 ab
49 DAP	109.6	133.5	**142.5**	121.6	119.1	128.5	131.4	127.6
63 DAP	107.8 b	141.1 a	**145.0 a**	131.0 a	129.5 a	139.3 a	141.8 a	137.8 a
Leaf Quantity								
14 DAP	3.7	3.9	**4.0**	3.9	3.9	3.9	**4.0**	3.9
35 DAP	6.2 b	7.6 a	7.7 a	7.5 a	7.1 ab	7.4 ab	**7.8 a**	7.7 a
42 DAP	7.0	8.0	**8.3**	7.6	7.4	7.9	7.8	7.6
49 DAP	9.5	**10.7**	10.5	10.1	10.1	10.5	10.4	10.3
63 DAP	10.4 b	11.4 a	11.5 a	11.6 a	11.1 ab	11.4 a	**11.7 a**	11.3 a
Biomass Quantity (kg/plot)								
ENH	1.0 b	1.6 ab	2.1 a	2.0 a	1.7 ab	2.2 a	2.2 a	**2.3 a**
EWH	1.2 b	2.2 a	2.8 a	2.5 a	2.1 a	**3.0 a**	2.8 a	2.8 a
STV	9.1 b	**19.0 a**	18.6 a	16.4 a	14.9 a	15.9 a	17.0 a	17.1 a
Relative Agronomic Effectiveness (%)								
RAE_ENH		100.0	148.9	138.8	110.1	164.8	175.4	**186.1**
RAE_EWH		100.0	152.0	125.2	93.9	**175.8**	161.9	155.0
RAE_STV		**100.0**	93.4	69.7	56.8	67.2	79.0	80.0

Effect of OA and NPK Rates and Position on Slope on Sweet Maize' Agronomic Performances

The results presented in Table 5 exhibited no significant effect of OA amendment on the entire agronomic performance. However, the increase of OA rates tended to increase PH and LQ as shown by their incremental linear trends (Figures 2 and 3), particularly at 35 DAP and more. Furthermore, steep slopes satisfied second to fourth-degree polynomials curves were detected on BQ and RAE parameters, except for RAE_STV. Generally, the curves of both factors reached gentle slopes at 12 to 24 kg OA/ha (Figure 4).

NPK fertilization remarkably affected PH and LQ during observation, except in the second week after planting. Without RAE_STV, BQ and RAE also yielded similar results (Table 5). As can be observed in Figures 2 and 3, a 25% reduction of the NPK fertilization had insignificant differences in results on PH and LQ. ENH and EWH resembled these patterns, but the under-fertilized plot was also not significant to the unfertilized plot. On STV, each factor level was statistically different, with 1 std NPK recorded as the heaviest yield. Furthermore, OA and NPK interaction notably controlled PH at the two last observations, LQ at 42 and 49 DAPs, all BQ, and RAE on EWH and STV (Table 5).

Table 5. Effect of OA and NPK on RAE performance

Agronomic Performances	**Fixed Factors**			**Intercept**	**Random Factor**	**----------- Model Metrics -----------**			
	OA	**NPK**	**OA*NPK**		**Block**	**AICc**	**------------ Rsq ------------**		
	----------------- *P value* -----------------				*variance*		Adj	Fixed	Random
Plant Height									
14 DAP	0.65	0.38	0.67	**<0.01**	2.03	128.9	0.5	0.0	0.7
35 DAP	0.99	**<0.01**	0.37	**<0.01**	7.94	203.7	0.4	0.3	0.1
42 DAP	0.94	**<0.01**	0.33	**<0.01**	22.47	215.1	0.5	0.3	0.1
49 DAP	0.82	**0.09**	**0.06**	**<0.01**	67.86	249.6	0.2	0.6	0.2
63 DAP	0.98	**<0.01**	**0.04**	**<0.01**	84.07	229.2	0.6	0.3	0.3
Leaf Quantity									
14 DAP	0.61	0.65	0.66	**<0.01**	0.11	35.0	0.8	0.0	0.9
35 DAP	0.96	**<0.01**	0.29	**<0.01**	0.05	73.3	0.5	0.3	0.1
42 DAP	0.64	**<0.01**	**<0.01**	**<0.01**	0.21	76.2	0.6	0.3	0.1
49 DAP	0.71	**0.01**	**0.09**	**<0.01**	0.22	82.4	0.5	0.0	0.5
63 DAP	0.43	**<0.01**	0.64	**<0.01**	0.13	69.0	0.6	0.3	0.4
Biomass Quantity									
ENH	0.50	**0.15**	0.32	**0.01**	0.14	86.3	0.4	0.2	0.3
EWH	0.70	**<0.01**	**0.11**	**<0.01**	4.26	158.2	0.6	0.4	0.3
STV	0.47	**<0.01**	**0.08**	**<0.01**	3.42	143.2	0.8	0.6	0.3
Relative Agronomic Effectiveness									
RAE_ENH	0.23	0.34	0.59	**<0.01**	173.10	288.5	0.5	0.0	0.6
RAE_EWH	0.78	0.20	**0.11**	**<0.01**	173.10	235.3	0.2	0.0	0.2
RAE_STV	0.64	**0.02**	**0.14**	**<0.01**	90.05	227.0	0.3	0.2	0.1

In Table 5, the intercept was significant in all models. The variance of random factors in PH and LQ showed inclination trends as the observation time increased, culminating at 49 DAP. A similar trend was also observed for AICc. Adj-Rsq exhibited an irregular pattern against the observation period. In PH, the metric was highest at the last observation. Oppositely, its level was designated as the highest at the first LQ observation. BQ had moderate to high Adj-Rsqs, involving the highest and lowest Adj-Rsqs on STV and ENH, respectively. Furthermore, low Adj-Rsqs were detected on RAEs, especially at RAE_STV and RAE_EWH. On BQ and RAE, the trends of Adj-Rsq values were in contrast with AICc.

The fixed-Rsq gained considerably lower values than random-Rsq at the first observation in both PH and LQ. Similar patterns were observed at ENH, RAE_ENH, and RAE_EWH. However, the higher ratio of fixed- to random-Rsqs was exhibited by PH at the following observed DAPs, except at 63 DAP. In the last observation, the Rsq was comparable for both factors. For LQ, fixed-Rsqs were only higher than random-Rsqs during 35 and 42 DAPs. Moreover, the opposite pattern occurred in the following observations. The fixed-Rsqs were higher than random-Rsqs on EWH, STV, and RAE_STV (Table 5).

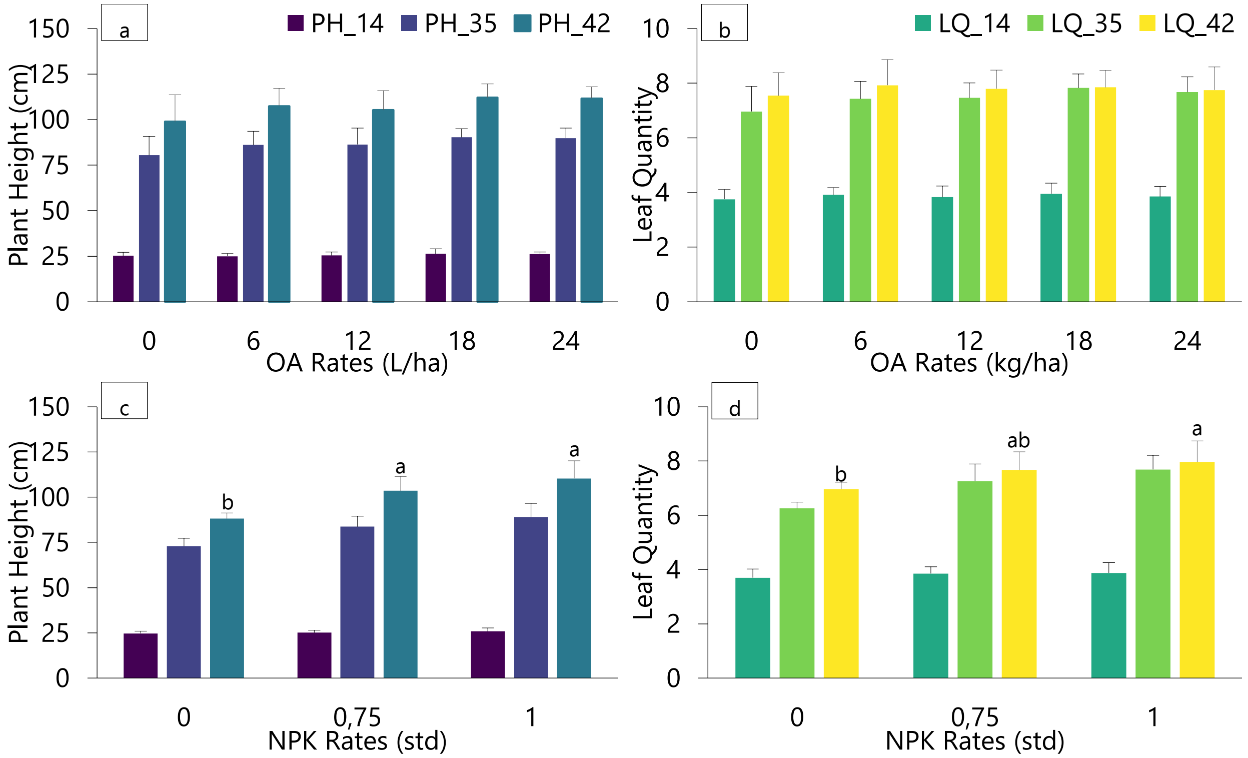

Figure 2. The effect of OA (upper graphs) and NPK (lower graphs) on sweet maize' plant height (a, b) and leaf quantity (c, d) at the vegetative stage.

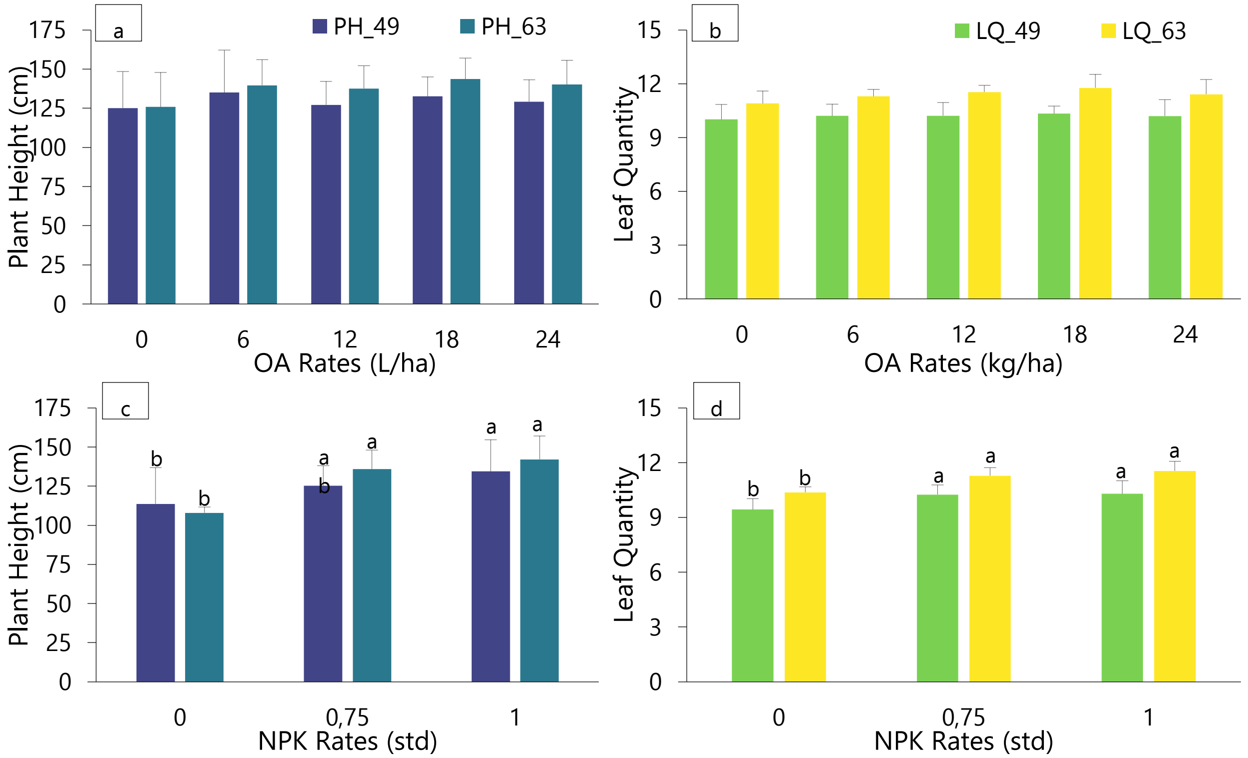

Figure 3. The effect of OA (upper graphs) and NPK (lower graphs) on sweet maize' plant height (a, b) and leaf quantity (c, d) at the generative stage

Figure 4. The effect of OA (left) and NPK (right) rates on sweet maize' biomass quantity (a, b) and relative agronomic effectiveness (c, d)

Multivariate Analysis of the Effect of Treatments, OA and NPK Rates on Sweet Maize' Agronomic Performances

The multivariate analysis containing principal component explained variances, covariate contributions, and grouping was presented in Figure 5. Based on Figure 5a, there were four PCs that primarily constitute the dataset' variance, totaling about 90.9%. The fist PC, explaining the most data variance (66.3%; Figure 5b), was loaded significantly by three PHs, two LQs, and all BQs. The entire significant PHs and LQs on PC1 were from observation following five weeks. The early observations on PH and LQ gained notable contribution on the remaining PCs, that possessed minor contribution on the dataset variance (Figures 5c, 5d, and 5e).

Figure 5f showed that the agronomic performances significantly responded to the given treatments when compared to the control. However, highly overlapped circles were exhibited on all treatments without control, except for some treatments (i.e., B1S2 vs. B1S1 and B3S1) that presented slight and observable differences. Furthermore, highly overlapped circles were shown by OA rates without control (Figure 5g). There were considerable agronomic differences in the addition of the two highest OA rates compared to the control, as shown by their slightly overlapped circles. The two highest NPK rates yielded remarkably different agronomic performances when compared to the control. Nevertheless, both the rates' circles and point distributions were highly overlapped. Similar patterns were observed at maize planted at blocks 2 and 4, whereas both blocks were located adjacent to block 3 (Figure 5i). Meanwhile, block 1 showed distant separation from other blocks.

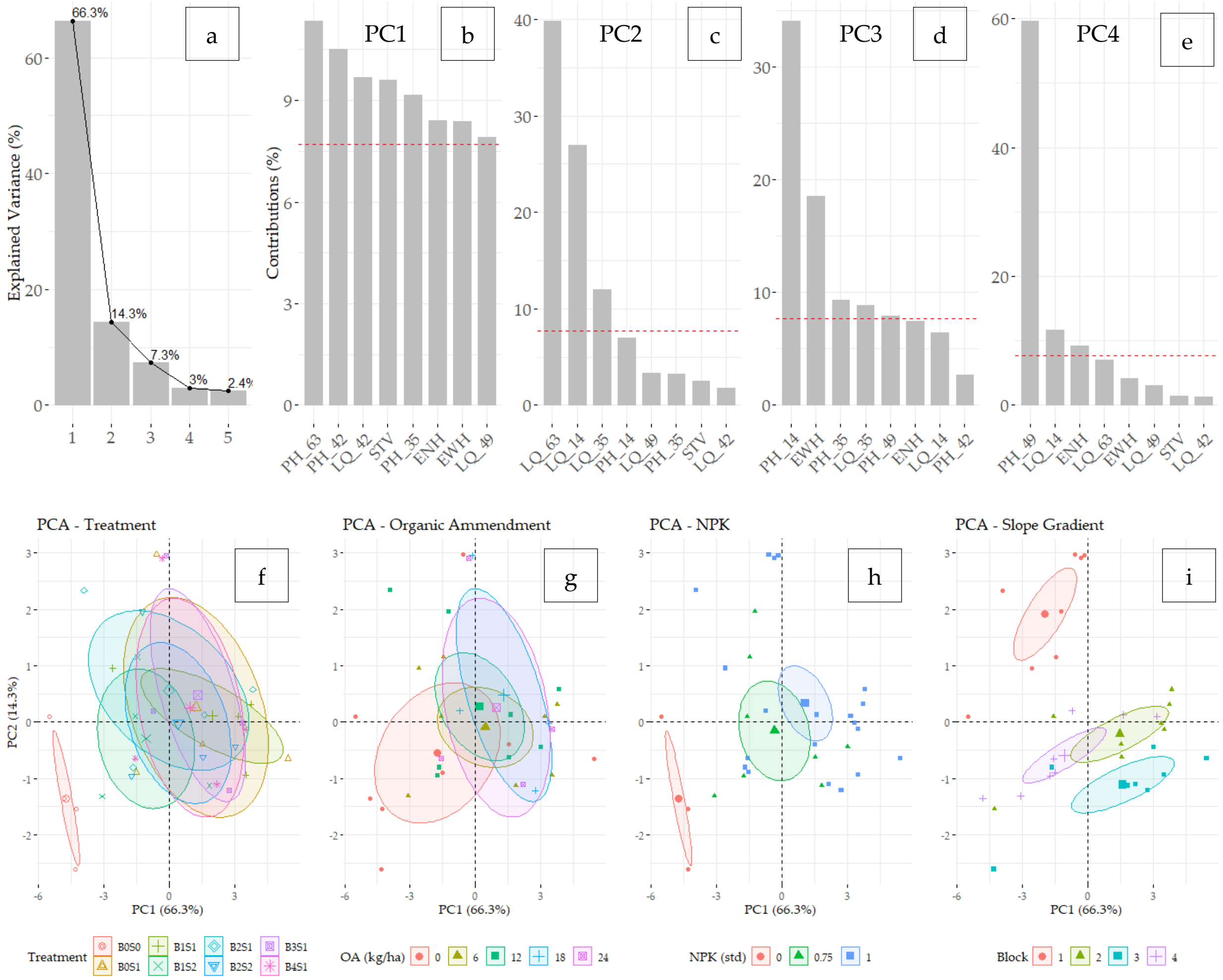

Figure 5. Multivariate analysis of the result data presenting scree plot of the explained variance percentage of the fifth highest PCs (a), contribution plot of the eighth highest variables over the fourth highest PCs (b, c, d, e), and observation plot with four groupings based on treatments (f), OA (g), NPK (h), and block/slope (i)

DISCUSSION

This study provided field evidence of combined OA (containing HC and phytohormones) amendment and NPK fertilization effects on sweet maize' agronomic performances on old and intensively cultivated experimental farm and high plot heterogeneity. Amending more than 12 L OA ha^{-1} could improve the sweet maize' growth and development. Furthermore, current NPK fertilization in our trial site could be 25% reduced under OA incorporation.

Our results suggested that relying on only OA application did not significantly boost the growth and biomass of sweet maize. Nevertheless, amending OA is considered beneficial, as suggested by its significant interaction with NPK at most biomass and yield parameters (Table

5). Moreover, adding higher OA rates tended to increase plant growth (as presented by PH and LQ) at the vegetative and generative stages (Figures 2 and 3) and most biomass and yield parameters (Figure 4). These results partially support previous positive outcomes of OA containing HC and phytohormones at the field level. Therefore, this study's results also agreed with the skeptical perspective, in terms of inadequate HC content at the current recommended rate.

Our results presented above were somewhat contradictory with previous findings on old farms. Due to nutritional and physical constraints (Lemenih *et al.* 2005; Sa and Lal 2009; Nunes *et al.* 2023; Pinheiro and Nunes 2019), plants cultivated at old farms likely respond to organic amelioration (*e.g.*, Moebius-Clune *et al.* 2011). Oppositely, higher soil fertility on younger farms reportedly causes insignificant differences among OA types (Güerena *et al.* 2016). Currently, no reports of these differences in OA (containing HC) rates, whereas was on our old trial, generated similar results (Table 5; Figures 2, 3, and 4). OA amendment effect in our study, therefore, was possibly masked by high residual nutrients and plot heterogeneity. Long-term intensive fertilization and organic amelioration consequently maintained the soil fertility as indicated by moderate C and N, as well as high P residue and micronutrient contents (Table 1; Kihanda *et al.* 2007). Even though our plots were located on a relatively flat slope, they also possessed high heterogeneity with respect to slope gradient, revealed by higher to comparable variance explained by random against fixed factors (Table 5) and clear separation on the block compared to OA-based multivariate groupings (Figures 5g, 5h, and 5i). To attain more homogeneous land characteristics and minimize this masking effect in the future, block 1 must be excluded from the trial experiments or be intensively plowed, harrowed, and mixed with soils from the three other plots.

Mollah *et al.* (2020) suspected that higher rainfall also contributed to the dissipation of the HC effect in soils. Furthermore, another strong explanation for the low OA effect could be attributed to the moderate content of C and N in soils (Table 1) generated from long-term extensive manure addition. These indicate a higher presence of organic materials, thus accumulating higher recalcitrant organic materials which is the building block of HC (Luan *et al.* 2019). Taking soil bulk density of 1 g cm^{-3}, 58% of organic materials/OM is constituted by C, 30% OM is comprised of HC, and 40% effective planting beds of 25 m^{2} plot area, at the worst case, generating around 28 kg HC contained by soils per plot at upper 20 cm. In porewater, the concentration of HA were ranging from tens to a few hundred mg L^{-1} (Chen and Aviad 1990). This naturally occurred soils and porewater contained HC, however, highly exceeds OA maximum rate (2 std; Table 3) which contains only 0.013 L HC plot^{-1}. Our results suggested that at our current rates, OA's stimulatory effect on maize growth and production might be contributed predominantly by auxin, gibberellin, or other phytohormones-like substances (Scaglia *et al.* 2016), rather than HC. The sprayed OA that concentrated on maize organs and soil adjacent to the root system also could trigger gene expression related to hormonal secretion, which can stimulate plant growth and development (Souza *et al.* 2022).

This study results provide feedback for the company to concentrate HC in their OA product, given by the advantageous effect of HC amendment reported by previous researchers (Boveiri Dehsheikh *et al.* 2020; Izhar Shafi *et al.*, 2020; Ampong *et al.* 2022; Ren *et al.* 2022). HC amendment improves soil physical properties by increasing soil aggregation and porosity through molecular and physical bridges. Moreover, HC provides additional adsorption complexes that can retain nutrients, thus, increasing their availability and preventing leaching (Li *et al.* 2019; Xu *et al.* 2021). This study also found that OA amendment interacted with NPK fertilization (Table 5; Figure 3.3), corroborating with other findings (Seadh *et al.* 2012; Shen *et al.*

2016). Humic acid/HA, which is a part of HC, can limit the urease activity, lower urea hydrolysis rate (Dong *et al.* 2009), and form a stable complex with the urea amide group (Guo *et al.* 2022); generating higher N fertilization efectivity and suppress N_2O emission. Similar effectivity results have also been reported on P fertilization (Seyedbagheri 2010; Bejarano Herrera *et al.* 2016). An improvement of soil bio-physicochemical properties mediated by HC provided indirect support for further increase in crop growth and production (Khan *et al.* 2016; Bijanzadeh *et al.* 2019; Li *et al.* 2019), particularly in infertile and degraded soils (Khaled and Fawy 2011; Mora *et al.* 2014; Wulandari *et al.* 2019; Deng et al. 2021; Wandansari *et al.* 2023). In the long term view, the potential advantage of HC amelioration may promote the stabilization of organic matter in soils and restrict greenhouse gas emissions from agricultural fields. Nevertheless, excessive HC amendment must also be avoided, considering their adverse effects on plant physiology and metabolism (Muscolo and Sidari 2009; Asli and Neumann 2010).

A strong effect was exhibited by NPK fertilization on most of the maize's agronomic performances (Table 5), suggesting significant plant response on 75% and 100% fertilization compared to unfertilized plots (Figures 2, 3, and 4). Long-term intensive fertilization resulted in large residual P in soil (Table 1), as P might be complexed by sesquioxides under low pH conditions (Fink 2016). Based on this research, the addition of concentrated HC combined with 75% NPK fertilization could maintain maize productivity. Previous studies revealed the role of HS in releasing residual and complexed nutrients, *i.e.,* P (Hua *et al.* 2008; Liu *et al.* 2023) under acidic or high sesquioxides soils and Fe (Zanin *et al.* 2019) and Zn (Morais *et al.* 2021) under alkaline or calcite-ameliorated soils.

CONCLUSIONS

In our field trial, a single OA application did not significantly boost the growth and biomass of sweet maize, especially when applied to an old and intensively cultivate and organically manured farm. OA had significant interaction with NPK at most of the yields and biomass parameters. Amending soils more than 12 L OA ha^{-1} could improve the sweet maize's growth and development while saving 25% NPK fertilizers. Higher heterogeneity in trial plots was governed by plots' position across slope gradients controlled maize growth, biomass, and agronomic effectivity. This study suggested inadequate HC content of OA at the current rate, while also indicating the greater role of phytohormone in promoting maize growth and production.

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