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Nutrient use efficiency in edamame (*Glycine max* L.) production with poultry manure and biofertilizer application

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ABSTRACT

Nutrient use efficiency (NUE), as one of the agriculture ecological efficiency measures, is an important concept to evaluate the production systems of the crop. NUE in organic farming is less studied. Large quantities of poultry manure are needed in organic farming, therefore biological fertilizer is considered to increase the efficiency of fertilizer use. The research was aimed at investigating the effects of poultry manure rates and biofertilizer on edamame yield and the NUE from two experiments at different times. The experiments were done in 2023 and 2024. Each experiment was arranged in a split plot block design with biofertilizer as the main plot (without and with) and poultry manure rates as subplot (0, 8, 16, and 24 ton·ha^{-1}), replicated 3 times. A combined analysis of variance from two experiments was done. The study investigated the edamame pod yield, agronomic efficiency based on manure rates and based on N, P, K supplied by manure (AE, AE_N, AE_P, AE_K), and partial factor productivity (PFP_N, PFP_P, PFP_K). The results revealed that filled pod number and weight of aboveground biomass increased with higher manure rates. The effect of interaction between biofertilizer, manure rates, and year was significant on pod weight per plant and edamame pod yield. The increases in pod weight and pod yield following manure rates showed different trends according to the presence or absence of biofertilizer and differences in experimental time. Higher increases in pod weight (141.5–293.3%) and pod yield (125.5–174%), following manure rates, occurred in the first experiment with biofertilizer. In Experiment 2, pod yield was not affected by manure rates either with or without biofertilizer. AE, AE_N, AE_P, and AE_K were significantly affected by the interaction of biofertilizer, manure rates, and year of the experiment; they decreased with increasing manure rates in the first experiment. Positive values of AE, AE_N, AE_P, and AE_K were found in the first experiment with biofertilizer. In contrast, in the 2nd experiment, positive values of AE, AE_N, AE_P, and AE_K were found without biofertilizer. PFP_N, PFP_P, and PFP_K decreased with higher poultry manure rates. Different soil characteristics and methods of biofertilizer application between two experiments may affect the NUE.

Keywords: agronomic efficiency, harvest index, leaf nutrient, organic farming, partial factor productivity.

INTRODUCTION

Edamame or vegetable soybean (*Glycine max* L. Merr.) is usually harvested at the R6-R7 stage when pods are still green but the seeds have fully developed. Good nutritional values of edamame that have been identified are isoflavones (Zeipina et al., 2017), dietary fiber (Johnson et al., 1999; Xu et al., 2012), polyunsaturated fatty acids, such as linoleic acid and linolenic acid (Kumar et al., 2006a). Rich in sugar contents is one of the things that makes edamame popular (Carneiro et al., 2021; Zeipina et al., 2017).

Edamame can be grown with poultry manure as an external source of nutrients. Poultry manure application can be comparable to that of urea in several crops' yields (Lin et al., 2016). The positive influences of poultry manure on crop production through improving soil properties, namely increasing soil organic carbon, pH, available P, K, and Zn. The improvement of soil properties was also found in biological characteristics (Kobierski et al., 2017) and physical characteristics such as reduced soil bulk density, temperature, and increased porosity and moisture content (Adeyemo et al., 2019; Agbede et al., 2017; Rasool et al., 2023). Poultry manure as the only external source of nutrients in organic farming has been studied for the production of *Sonchus arvensis* with 14 ton manure·ha^{-1} (Melati et al., 2021), okra with 18 ton manure·ha^{-1} (Fhonna et al., 2023), and bitter gourd with 30 ton manure·ha^{-1} (Jabary et al., 2023). To obtain the optimum yield of those crops, high dosages of poultry manure had been determined.

Continuous efforts are made to increase fertilizer use efficiency so the doses of fertilizer can be reduced. One way to increase fertilization efficiency is by providing biofertilizers. Biofertilizers are microbial inoculants, derived from plant roots and root zones, that can be applied to seeds, plant surfaces, or soil. The roles of biofertilizers in plant production are to improve soil fertility, increase nutrient availability, improve plant growth, and increase plant productivity. Another important role of biofertilizer is its ability to protect plants from pests and diseases and ameliorate biotic and abiotic stresses (Kumar et al., 2022; Nosheen et al., 2021). Biofertilizers can increase the availability of N, P, and K (Mitter et al., 2021; Ortega Pérez et al., 2023). The role of biofertilizers in plant growth promoting can contribute to the development of root hairs which in turn can improve water uptake (Magana et al., 2020; Singh et al., 2020; Weber et al., 2018).

Nutrient use efficiency (NUE) or fertilizer use efficiency is an important concept to evaluate the production systems of the crop (Gajanand et al., 2020). Several indices can be used to measure the nutrient use efficiency from the applied fertilizer to assess the response of a crop to a nutrient, they are recovery efficiency (RE), physiological efficiency (PE), internal utilization (IE), agronomic efficiency (AE), and partial factor productivity (PFP) (Dobermann, 2007). Various nutrient use efficiency (NUE) indices can also be grouped into fertilizer-based, plant-based, soil-based, isotope-based, ecology-based, and system-based. Each of the indexes has strengths and limitations to use in determining the nutrient use efficiency of the crops (Congreves et al., 2021). A similar calculation of nitrogen use efficiency (NUE) can be used to determine different nutrients, for example, phosphorus use efficiency (PUE) and other nutrients. The nutrient use efficiency is not only for calculating the efficiency due to fertilizer application (Mandic et al., 2015; Schütz et al., 2018) but can also as a result of crop cultivation arrangement (intercropping) (Suntari et al., 2023; Zhang et al., 2015). Improvement of NUE is also aimed at reducing the potential for excess fertilizer to pollute the environment.

Because of its nutritional value and potential health benefits, the consumption of edamame has increased not only in Asia but also in the US (Yu et al., 2021). To meet the needs for edamame, the production needs to be increased, including through organic cultivation by using organic fertilizer. It is important to pay attention to the efficiency of inputs both for economic reason and concern for the environment. In organic farming practices, that apply large amounts of organic fertilizer, the effects of biofertilizer on nutrient efficiency have not been revealed yet. Therefore, the current study was aimed at investigating the effects of four levels of poultry manure and the application of biofertilizer on edamame soybean yield traits and the nutrient use efficiency from two experiments at different times. Nutrient use efficiencies were determined based on agronomic efficiency (AE) and partial factor productivity (PFP).

MATERIALS AND METHODS

Two experiments at different times have been conducted. The first experiment was carried out from January to April 2023, while the second experiment was in November 2023-March 2024 at IPB Experimental station in Cikarawang, Darmaga, Bogor, Indonesia (-6.5497175° S, 106.7287026° E). The region has a tropical climate with annual precipitation of 2000–3000 mm. The experimental site had Tropical Latosol soil (Alfisol). The chemical properties of the soil were analyzed before the application of manure (Table 1).

The experiment used edamame soybean seed from Biomax 1 variety, dolomite, laying hen manure, and commercial biological fertilizer (consortium of *Bacillus polymyxa, Pseudomonas flurescens, Rhizobium* sp). The chemical traits of poultry manure in presented in Table 2.

Table 1. Chemical properties of soil before manure application

Soil properties	2023	2024
pH H_2O	6.19	6.28
C-organic (%)	1.86	1.66
N-total (%)	0.25	0.22
P-available (ppm P_2O_5)	30.87	49.54
CEC (cmol/kg)	17.42	15.71
K-exc (cmol K/kg)	0.22	0.28
P-potential (mg P_2O_5/100g)	107.26	174.54
K-potential (mg K_2O/100g)	29.77	36.09

Table 2. Chemical properties of poultry manure applied in 2023 and 2024 and potential supply of nutrients for each dose of manure application

Manure properties	2023	Potential supply of nutrients from manure			2024	Potential supply of nutrient from manure		
		8 tons	16 tons	24 tons		8 tons	16 tons	24 tons
		(kg·ha^{-1})				(kg·ha^{-1})		
pH	7.31				8.09			
Moisture content	22.53 %				18.76 %			
C-organic	17.48 %				23.14 %			
N-total	1.43 %	88.6	177.3	265.9	2.14 %	139.1	278.2	417.2
P_2O_5 total	5.26 %	325.9	651.9	977.9	3.84 %	249.6	499.1	748.7
K_2O total	3.70 %	229.3	458.6	687.9	3.41 %	266.5	532.9	799.4

Experimental design

Experiments 1 and 2 applied a split-plot design with biological fertilizer (bio-fertilizer) as the main plot (with and without biofertilizer) and manure rates as sub-plot (0, 8, 16, dan 24 ton·ha^{-1}). Each treatment was replicated 3 times. The difference between Experiment 1 and 2 was the method of biofertilizer application.

Land preparation

Following the land preparation, dolomite lime and laying hen manure were spread over the soil surface and then mixed. Each plot size was 3×1.25 m (first experiment) and 3×1.5 m (2nd experiment). Each plot was separated by a distance of 50 cm.

Seed planting, application of bio-fertilizer, and harvest

Soybean seeds were planted with a 25×20 cm distance, 2 seeds per planting hole. In Experiment 1, seeds were soaked for 15 minutes in a biofertilizer solution with a concentration of 10 g·L^{-1} water, following the instructions of the product. In Experiment 2, seeds were soaked in a biofertilizer solution with a concentration of 10 g·L^{-1} water before planting. Additional application of biofertilizer was done twice. The first application was after planting, then at the V3 plant growth phase, with the concentration of 5 g·L^{-1} water as much as 100 mL per plant. Soybean pods were harvested immature between the R6 and R7 reproductive stages. At this stage, the pods are still green but the seeds have fully developed. At harvest, soybean plants were uprooted and then separated into pods and biomass.

Sample collection, measurements, and calculations

Ten plants were sampled to determine the pod number per plant, pod weight per plant, and fresh weight of plant biomass at harvest 70–72 days after planting (R6 stage). The Harvest index represents the ratio between the plant product and biomass (Eq. 1). To measure the nutrient efficiency, agronomic efficiency was calculated with Eq. 2–5, while the partial factor productivity was determined by Eq. 6–8 (Dobermann, 2007).

$$HI = \frac{WFPP\ (g)}{FWABH + PW\ (g)} \quad (1)$$

where: *HI* – harvest index, *WFPP* – weight of filled pod per plant, *FWABH* – fresh weight of aboveground biomass at harvest, *PW* – pod weight.

$$AE = \frac{Ym - Yo\ (ton)}{Manure\ applied\ (ton)} \quad (2)$$

where: *Ym* – yield with manure, *Yo* – yield without manure.

$$(AE_N) = \frac{Ym - Yo\ (kg)}{N\ supplied\ by\ manure\ (kg)} \quad (3)$$

where: AE_N – N – based agronomic efficiency, *Ym* – yield with manure, *Yo* – yield without manure.

$$(AE_P) = \frac{Ym - Yo\ (kg)}{P\ supplied\ by\ manure\ (kg)} \quad (4)$$

where: *AEp* – P – based agronomic efficiency, *Ym* – yield with manure, *Yo* – yield without manure.

$$(AE_K) = \frac{Ym - Yo\ (kg)}{K\ supplied\ by\ manure\ (kg)} \quad (5)$$

where: AE_K – *K* – based agronomic efficiency, *Ym* – yield with manure, *Yo* – yield without manure.

$$(PFP_N) = \frac{Pod\ yield\ (kg \cdot ha^{-1})}{N\ supplied\ from\ manure\ (kg \cdot ha^{-1})} \quad (6)$$

where: (PFP_N) – *N* – based partial factor productivity.

$$(PFP_P) = \frac{Pod\ yield\ (kg \cdot ha^{-1})}{P\ supplied\ from\ manure\ (kg \cdot ha^{-1})} \quad (7)$$

where: (*PFPp*) – *P* – based partial factor productivity.

$$(PFP_K) = \frac{Pod\ yield\ (kg \cdot ha^{-1})}{K\ supplied\ from\ manure\ (kg \cdot ha^{-1})} \quad (8)$$

where: (PFP_K) – *K* – based partial factor productivity.

The concentrations of N, P, and K in leaf tissues were determined from samples collected at the V3 growth stage. Leaves from two plants of each plot were oven-dried at 60 °C. Leaf N was determined with Kjeldahl, P with Spectrophotometer UV-VIS, and K with Atomic Adsorption Spectrophotometer (AAS). Analysis of N, P, and K in leaves, was done on treatment with biofertilizer.

Statistical analysis

The data were processed with analysis of variance using the statistical tool for agricultural research (STAR). A combined analysis of variance from two experiments was done with a Split Plot in Randomized Block Design. Three factors were used as sources of variance namely year (2023 and 2024), biofertilizer (without and with), and manure rates (0, 8, 16, 24 ton·ha^{-1}). The statistically significant differences between means were determined by the LSD test and the significance level was set at $p \leq 0.05$.

Data with concentrations of N, P, and K in leaves were analyzed by combined analysis of variance from two experiments in Randomized Block Design. Two factors were used as sources of variance namely year (2023 and 2024) and manure rates (0, 8, 16, 24 ton·ha^{-1}).

RESULTS

Plant biomass

Based on the result of the combined analysis of variance from the two experiments, attention was given to sources of variability that refer to the pooled error with a degree of freedom that is more than 6. The effects of manure rates were significant at $p < 0.01$ on the variables of plant biomass at 5 weeks after planting (5 WAP) and at harvest (9 WAP). Compared to control, the application of poultry manure increased pooled means of fresh and dry weight of aboveground biomass by 43.5–94.9% but there was no significant difference among manure rates. Poultry manure as much as 8, 16, and 24 ton ha^{-1} had increased the fresh weight of aboveground biomass at harvest by 24.4, 63.1, and 80.6% compared to control. The biomass weights were different among manure rates (Table 3).

Production components

For yield attributes, the significant effects of manure rates were found on filled pod number ($p<0.01$) and harvest index ($p<0.05$) (Table 4). The application of poultry manure with the rate of 16 and 24 ton·ha^{-1} increased the filled pod number by 64.2% compared to the control (without manure). The highest manure rate resulted in a decrease in harvest index by 6.8% compared to control (Table 4).

The significant effect of the interaction of year-biofertilizer-manure rates was found in pod weight per plant ($p<0.01$) and pod yield ($p<0.05$)

Table 3. The means of fresh weight and dry weight of aboveground biomass with different manure rates and biofertilizer treatments from the two experiments

Treatments	Fresh weight of aboveground biomass at 5 WAP (g)				Dry weight of aboveground biomass at 5 WAP (g)				Fresh weight of aboveground biomass at harvest 9 WAP (g)			
	2023	2024	pooled		2023	2024	pooled		2023	2024	pooled	
Biofertilizer												
+ biofertilizer	34.61	27.64	31.02		9.05	5.61	7.23		18.58	59.10	38.84	
- biofertilizer	38.23	26.99	32.37		9.46	5.67	7.45		18.42	50.00	34.21	
Manure rates (ton·ha^{-1})												
0	20.72	20.42	20.57	b	5.18	4.30	4.73	b	9.23	42.22	25.72	d
8	31.69	27.45	29.53	ab	8.40	6.11	7.21	a	13.62	50.37	31.99	c
16	51.21	28.98	39.32	a	13.23	5.91	9.23	a	24.21	59.67	41.94	b
24	46.10	33.18	39.37	a	11.19	6.34	8.61	a	26.94	65.94	46.44	a

Note: Numbers in the same column followed by different letters indicate significant differences based on LSD test at $p \leq 0.05$.

Table 4. The means of filled pod number and harvest index with different manure rates and biofertilizer treatments from the two experiments

Treatments	Filled pod number per plant				Harvest index			
	2023	2024	pooled		2023	2024	pooled	
Biofertilizer								
+ biofertilizer	18.7	24.0	21.4		0.66	0.52	0.59	
- biofertilizer	17.4	20.9	19.2		0.68	0.54	0.61	
Manure rates (ton·ha^{-1})								
0	11.4	18.2	14.8	b	0.68	0.55	0.61	a
8	14.7	20.8	17.8	b	0.71	0.54	0.62	a
16	22.1	26.4	24.3	a	0.66	0.54	0.60	ab
24	23.9	24.7	24.3	a	0.64	0.50	0.57	b

Note: Numbers in the same column followed by different letters indicate significant differences based on LSD test at $p \leq 0.05$.

(Fig. 1a and 1b). There are different trends of pod weight in response to the treatments. Without poultry manure but added with biofertilizer, pod weight per plant was 15.15 g (Experiment 2023) and 56.36 g (Experiment 2024). When poultry manure was added with biofertilizer, pod weight per plant from Experiment 1 (2023) increased between 141.5–293.3%, while from Experiment 2 (2024) pod weight increased between 16.4–25.4% as a result of manure application 8-24 ton·ha^{-1}, compared to without manure (Fig. 1a).

In contrast, without biofertilizer and poultry manure, pod weight per plant was 28.31 g (Experiment 2023) and 43.82 g (Experiment 2024). In the application of poultry manure without the addition of biofertilizer, the pod weight of edamame increased between 4.59–87.6% (Experiment 2023) and 15.5–52.83% (Experiment 2024) compared to the control without manure (Fig. 1a).

The interaction of year-biofertilizer-manure rates affects the pod yield of edamame. Different trends of pod yield in response to the treatments were found. Without manure but applied with biofertilizer, pod yield was 4.27 ton·ha^{-1} (Experiment 2023) and 10.69 ton·ha^{-1} (Experiment 2024). With the addition of poultry manure 8–24 ton·ha^{-1}, edamame pod yield increased between 125.5–174% (Experiment 2023) but decreased by 1.03–8.04% in Experiment 2024 compared to without manure (Fig. 1b). However, pod yields were not different among manure rates in Experiment 2024. Without biofertilizer and poultry manure, pod yield was 7.65 ton·ha^{-1} (Experiment 2023) and 9.49 ton·ha^{-1} (Experiment 2024). The application of

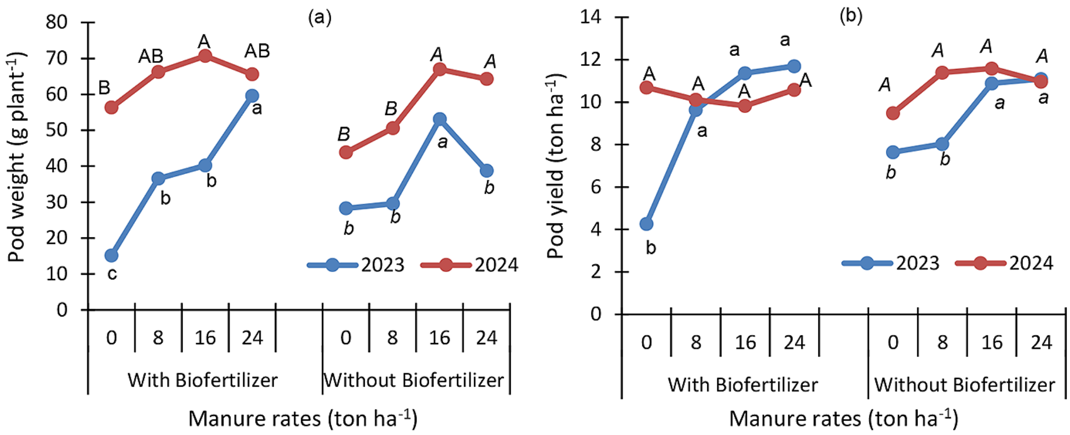

Figure 1. Interaction effects of manure rates, biofertilizer, and time of experiment on means of pod weight per plant (a) and pod yield (b). Different letters indicate significant differences based on LSD test at $p \leq 0.05$

poultry manure 8–24 ton·ha^{-1} increased pod yield by 4.97–44.97 % (Exp. 2023) and 15.6–22.13% (Exp. 2024) compared to control without manure (Fig. 1b). However, pod yields were not different among manure rates in Experiment 2024.

Agronomic efficiency

The effect of the interaction of year-biofertilizer-manure rates was significant on agronomic efficiency (AE) ($p<0.01$), AE based on N supplied by manure (AE_N) ($p<0.01$), AE based on P supplied by manure (AE_P) ($p<0.01$), and AE based on K supplied by manure (AE_K) ($p<0.05$). In Experiment 2023, with biofertilizer, the application of 8 ton of manure·ha^{-1} resulted in agronomic efficiency of as much as 0.623 ton of pod yield/ton manure (Fig. 2a). Increasing manure rates had increased pod yield (Fig. 1b) but the AE became lower. Given the 24 ton of manure·ha^{-1}, the AE decreased by 94% compared to AE from 8 ton·ha^{-1} (Fig. 2a). In Experiment 2024, with biofertilizer, the application of 8 ton·manure·ha^{-1} caused an agronomic efficiency of as much as -0.085 ton pod yield/ton manure (Fig. 2a) or there was a decrease in AE due to the slight reduction of yield (Fig. 1b). The reduction of AE became smaller with increasing manure rates. The AE was not different among 8, 16, and 24 ton of manure·ha^{-1}.

Without biofertilizer, the AE was 0.047 (Exp. 2023) and 0.238 (Exp. 2024) ton pod yield/ton manure. In 2023, the AE was not different among 8, 16, and 24 ton of manure·ha^{-1}, but in 2024, the AE with 24 ton of manure decreased by 41% compared to AE from 8 ton·ha^{-1} (Fig. 2a).Based on the amount of N, P, and K potentially supplied by poultry manure, the agronomic efficiency was determined for each nutrient. In Experiment 2023, positive values of AE_N (0.060 kg pod yield/kg N supplied), AE_P (0.017 kg·pod yield/kg·P supplied), and AE_K (0.023 kg·pod yield/kg·K supplied) were found from the application of 8 ton·manure·ha^{-1} with biofertilizer. Lower AE_N, AE_P, and AE_K were found with increasing manure rates (Fig. 2b, 2c, 2d). The values of AE_N, AE_P, and AE_K decreased by 9.2, 2.57, and 4.32% respectively.

Still in Experiment 2023, without biofertilizer, positive values of AE_N (0.004 kg·pod yield/kg·N supplied), AE_P (0.001 kg·pod yield/kg·P supplied), and AE_K (0.002 kg·pod yield/kg K supplied) were found from the application of 8 ton·manure·ha^{-1} (Fig. 2b, 2c, 2d), but they were smaller than those with biofertilizer. The AE_N, AE_P, and AE_K without biofertilizer were not different among 8, 16, and 24 ton of manure·ha^{-1}. Different trends were shown by Experiment 2024. Negative values of AE_N (-0.003 kg pod yield/kg·N supplied), AE_P (-0.002 kg·pod yield/kg·P supplied), and AE_K (-0.002 kg·pod yield/kg·K supplied) were found from the application of 8 ton·manure·ha^{-1} with biofertilizer. On the contrary, without biofertilizer, positive values were found for AE_N (0.010 kg·pod yield/kg·N supplied), AE_P (0.007 kg pod yield/kg·P supplied), and AE_K (0.007 kg·pod yield/kg·K supplied). These AE_N, AE_P, and AE_K were not different among manure rates.

Partial factor productivity

Manure rates significantly affect the partial factor productivity of N (PFP_N) ($p<0.001$) and of K (PFP_K) ($p<0.01$), while P (PFP_P) was influenced

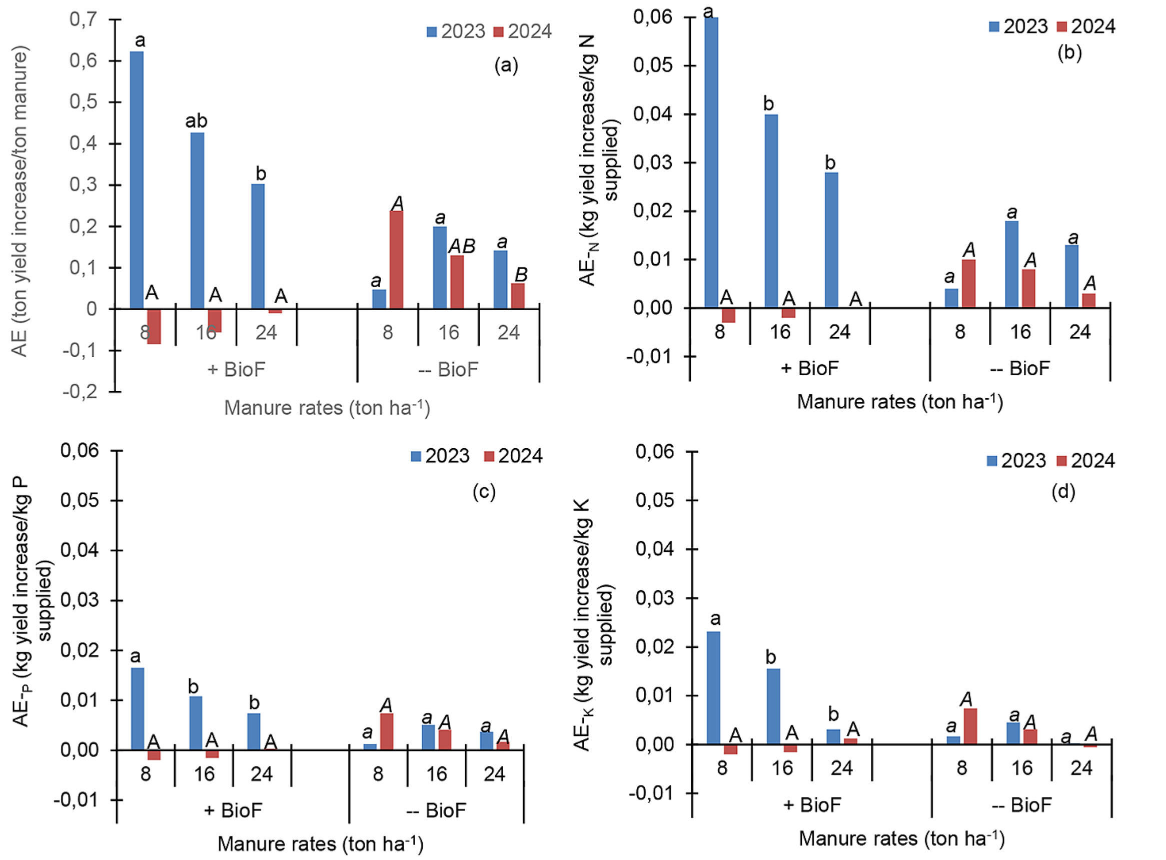

Figure 2. Interaction effects of manure rates, biofertilizer, and time of experiment on Agronomic Efficiency (AE) based on manure dose (a), AE based on N- (b), AE based on P- (c), and AE based on K- (d) supplied from manure. Different letters indicate significantly different based on LSD test at $p \leq 0.05$

by an interaction between year and manure rates (Table 5). PFP_N, PFP_P, and PFP_K decreased with increasing manure rates. Given 24 ton of manure·ha^{-1}, PFP_N, PFP_P, and PFP_K decreased by 61.2, 57, 66.6, and 61.9%, respectively, compared to 8 ton of manure ha^{-1}. PFP_P from Experiment 2024 was higher than in 2023.

Leaf nutrient content

Combined analysis of variance from two experiments for data of concentration of N, P, and K in leaves resulted in significant effects of manure rate on leaf K ($p<0.05$) (Table 5). Nitrogen and phosphorus contents in soybean leaves were not different among manure rates, while the potassium content

Table 5. Effects of manure rates on partial factor productivity (PFP) based on N, P, K applied from manure

Treatments	PFP-N				PFP-P					PFP-K			
	2023	2024	Pooled		2023		2024		Pooled	2023	2024	Pooled	
Biofertilizer													
+ biofertilizer	72.23	44.49	58.36		19.64		24.79		22.22	27.92	23.22	25.57	
Biofertilizer	64.59	49.96	57.28		17.56		27.84		22.70	24.96	26.08	25.52	
Manure rates (ton·ha^{-1})													
8	99.6	77.3	88.5	a	27.1	a	43.1	a	35.1	38.5	40.4	39.4	a
16	62.7	38.5	50.6	b	17.1	b	21.5	b	19.3	24.3	20.1	22.2	b
24	42.9	25.8	34.3	c	11.7	c	14.4	c	13.0	16.6	13.5	15.0	c

Note: Numbers in the same column followed by different letters indicate significant differences based on LSD test at $p \leq 0.05$.

was significantly affected by manure rates (Table 6). The application of 16 ton of manure·ha^{-1} increases K by 42% compared to control.

Correlation among variables

The correlation matrix revealed that pod yield positively correlated with weight of aboveground biomass at 5 weeks after planting (V3 growth phase) ($p<0.001$). This correlation shows that the plant performance at V3 growth phase can determine the edamame pod production. This correlation matrix also shows that the agronomic efficiency AE, AE-N, and AE-P, positively correlated to pod yield which means that the higher the agronomic efficiency, the higher the yield of edamame (Fig. 3).

DISCUSSION

The impact of poultry manure application

The current study showed that the use of poultry manure as a source of nutrients in organic farming can support the production of edamame soybeans. The increase in manure rates increases the potential supply of nutrients to the soybean plants (Table 2) and results in the increase of N and P (not significant) and K in leaf tissues (significantly increases by 42%) (Table 6). Nitrogen, phosphorus, and potassium are the three major nutrients required by plants. Nitrogen is the main essential nutrient for plants, a component of the protein. The important role of nitrogen in plant

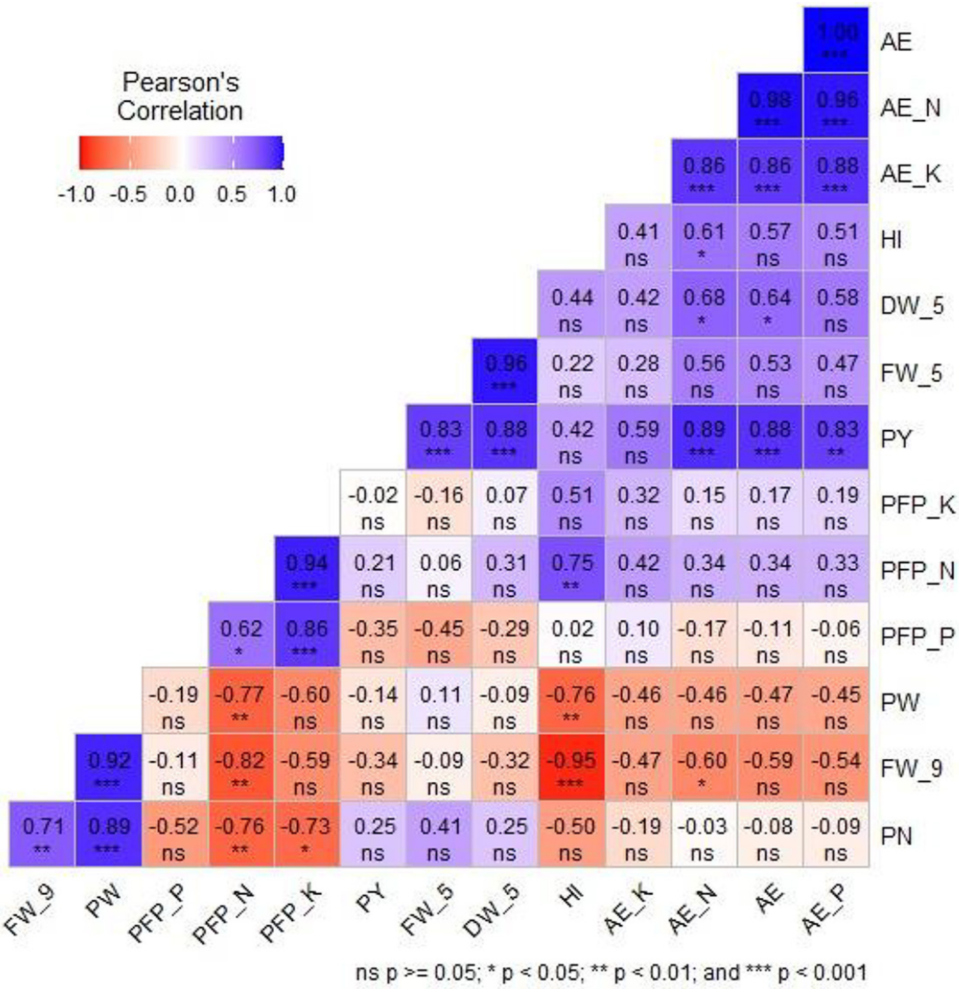

Figure 3. Correlation matrix depicting relationships between edamame pod yield and different variables. FW_5: fresh weight of aboveground biomass at 5 weeks after planting; DW_5: dry weight of aboveground biomass at 5 weeks after planting, FW_9: fresh weight of aboveground biomass at harvest (9 WAP); PN: filled pod number; PW: pod weight per plant; PY: pod yield; HI: harvest index; AE: agronomic efficiency based on manure; AE_N: AE based on N supplied from manure; AE_P: AE based on P supplied from manure; AE_K: AE based on K supplied from manure; PFP_N: nitrogen use efficiency; PFP_P: phosphorus use efficiency; PFP_K: potassium use efficiency

Table 6. Means of concentration of N, P, and K in soybean leaves with different manure rates

Manure rates (ton·ha^{-1})	Leaf N (%)			Leaf P (%)			Leaf K (%)			
	2023	2024	Pooled	2023	2024	Pooled	2023	2024	Pooled	
0	4.30	4.04	4.17	0.32	0.38	0.35	0.90	1.73	1.31	b
8	4.35	3.85	4.10	0.33	0.37	0.35	1.37	1.72	1.54	ab
16	4.43	4.15	4.29	0.32	0.38	0.35	1.74	1.98	1.86	a
24	4.65	4.29	4.47	0.32	0.40	0.36	1.73	1.94	1.84	a

Notes: numbers in the same column followed by different letters indicate significant differences based on LSD test at $p \leq 0.05$.

physiology is because N is the major component of chlorophyll, so it determines the photosynthetic rate of the plant which in turn the plant productivity (Fathi, 2022). In the process of plant growth, nitrogen controls cell division and cell elongation (Luo et al., 2020; Roggatz et al., 1999). Phosphorus is a structural component of nucleic acid, as a component of ADP, and functions in carbohydrate transfer (Hawkesford et al., 2012). Unlike nitrogen and phosphorus, potassium is a free cation and not a component of molecular structure, but K also plays a key role in plant growth and photosynthetic (Sardans & Peñuelas, 2021), especially as osmoregulation which is important for stomata movement and cell extension (Hawkesford et al., 2012). In the current experiment, the supply of N, P, and K may have improved the photosynthetic of the plants, therefore increasing the aboveground biomass at 5 weeks after planting (Table 3). The aboveground biomass at Vn stage positively correlated to the pod yield and indicates that the increase in plant biomass has increased pod yield.

Poultry manure contains N, P, and K and the potential supply of nutrients (Table 2) from each manure dose may have improved the soil properties. The organic carbon from poultry manure, which is in good values (18–22 %) (Table 2) can increase the soil organic carbon. The soil organic carbon accumulation can accelerate the formation of soil aggregation (Zhao et al., 2023). Improvements in soil nutrient availability as a result of organic fertilizer application, are caused by improvements in the biological, physical, and chemical properties of the soil (De Sousa Lima et al., 2021). Poultry manure affects soil properties by increasing soil pH, soil organic carbon, and available P, exchangeable Ca, Mg, and K. It has been reported earlier that increasing poultry manure doses increased the concentration of N, P, and K in leaf tissues of soybean (Soremi et al., 2017), and increased grain yield of soybean (Ahmadi & Arien, 2022). The application of poultry manure can be considered environmentally friendly because the organic fertilizers release nutrients slowly (Asadu et al., 2024).

Edamame productivity

The interaction effect of year-biofertilizer-manure rates affects filled pod weight per plant and pod yield (Fig. 1). Different times of experiment showed different responses of pod weight and pod yield to manure rate treatments with or without biofertilizer. In Experiment 2023, the increase of pod weight per plant and pod yield were higher when combined with biofertilizer than without biofertilizer (Fig. 1). On the contrary in 2024, the higher increase of pod weight and pod yield with the absence of biofertilizer. Figure 1 shows that all values of pod weight are higher in 2024 than in 2023. The results may relate to the soil properties of these two experiments (Table 1). There are some improvements in soil characteristics in 2024, namely slightly higher soil pH, higher total P and available P, and higher total K and exchangeable K. Better soil characteristics before the initial experiment in 2024 may have contributed to the better response of the plants to the application of poultry manure which resulted in higher biomass then pod weight per plant in 2024 than 2023. It was reported earlier that the influence of residue from previous manure increased wheat productivity (Dhaliwal et al., 2023), while residual compost increased radish yield especially in the area with high soil fertility (Lanna et al., 2018).

In Experiment 2023, pod weight per plant and pod yield increased significantly with the addition of poultry manure combined with biofertilizer. The effect of biofertilizer, which is a consortium of *Bacillus polymyxa* (*Paenibacillus polymyxa*), *Pseudomonas flurescens, Rhizobium* sp, may have contributed to promoting plant growth due to the increase of soil nutrient availability and phytohormone. *Paenibacillus polymyxa*, is a gram-positive bacterium, that has a broad host range so it is a potential PGPR that can promote plant growth (Jeong et al., 2019; Timmusk et al., 2005). *Pseudomonas fluorescens* is a gram-negative bacteria and one of the genera that are capable of solubilizing insoluble soil P (Anand et al., 2016) into available form (Mitter et al., 2021) and it can promote plant growth (Sahu et al., 2018). Rhizobium is a symbiotic N2-fixing organism that can convert N_2 from the atmosphere into NH_3 (Yang et al., 2022), promote nodulation, nitrogen fixation, nutrient uptake, plant growth, and seed yield of soybean (Htwe et al., 2019). The positive effect of the combination of poultry manure and biofertilizer has been reported to increase the yield of *Stevia rebaudiana* (Lozano-Contreras et al., 2021).

The effect of biofertilizers on edamame production may be more prominent in the experiment in 2023 than in 2024. The current study showed that the soil properties before the initial experiment in 2023 had slightly lower soil pH, lower total P, available P, total K, and exchangeable K than in 2024. This soil status may cause plants to get better benefits from the presence of biofertilizers. Several factors determine the efficacy of biofertilizers that can be grouped into edaphic and environmental, inoculant-related, and plant-related factors (Malusà et al., 2016). In edaphic/environmental factors, usually under low nutrient availability, plant growth and yield respond better to biofertilization (Da Costa et al., 2014) indicating that the plant gets the maximum benefit from the added microbes (Mitter et al., 2021). Previous studies reported that high soil N inhibits the nodulation of rhizobia (Thilakarathna & Raizada, 2017), while high soil P limits the success of AMF colonization (Jansa et al., 2009).

The different trends of plant's response to the manure rates in 2023 and 2024 can relate to several possibilities. First, there is a residual effect of poultry manure from experiment 2023. Secondly, different methods of microbe inoculation may determine the efficacy of biofertilizers. From inoculant-related factors, there are several critical points for the success of inoculation. The types of microbe, the concentration and formulation of biofertilizer, and the method of delivery of the biofertilizer (Mitter et al., 2021). The application method of biofertilizer (on seed or into the soil) and the time and frequency of application determine the success of microbe inoculation (Parnell et al., 2016). In the current study in experiment 2023, biofertilizer was inoculated on the seed, while in experiment 2024, biofertilizer was delivered on the seed and into the soil (twice). The application of biofertilizer as seed coating may have resulted in better root colonizing and facilitated better nutrient supplies to the plants (Mujeeb et al., 2022), but the combination of application methods can also improve plant growth (Abdiani et al., 2019; Mujeeb et al., 2022). However, it is not yet known whether the application of biofertilizers 3 times in experiment 2024 has an impact on the indigenous microorganisms. The impact of biofertilizers on soil microbial diversity is generally positive (Samantaray et al., 2024) but the possible antagonistic interaction is also being concern (Mitter et al., 2021).

Nutrient use efficiency

In the present study, nutrient use efficiency is presented as agronomic efficiency based on manure rates (AE) and based on the amount of N (AE_N), P (AE_P), and K (AE_K) potentially supplied by the added poultry manure (Fig. 3). Different times of experiment show different trends of AE, AE_N, AE_P, and AE_K.

The nutrient use efficiency, measured as agronomic efficiency (AE, AE_N, AE_P, and AE_K), was found higher in Experiment 2023 where biofertilizer was combined with the application of poultry manure, compared without biofertilizer. Without biofertilizer, the with 16 and 24 ton of manure were not significantly different over 8 ton (Fig. 3). This shows that there is a positive effect of a combination of poultry manure and biofertilizer on the productivity of edamame soybeans. Generally, organic fertilizers have a positive effect on rhizosphere microorganisms (Malusà et al., 2016). The consortium of *Bacillus polymyxa* (*Paenibacillus polymyxa*)*, Pseudomonas flurescens, Rhizobium* sp, may have contributed to promoting plant growth due to the increase of soil nutrient availability and phytohormone.

In Experiment 2023, combined with the application of biofertilizer, the AE decreased significantly at 24 ton of manure ha^{-1}, while the AE_N, AE_P, and AE_K decreased significantly at 16 ton of manure ha^{-1} over 8 ton ha^{-1}. The effect of biofertilizer, which is a consortium of *Bacillus polymyxa* (*Paenibacillus polymyxa*)*, Pseudomonas flurescens, Rhizobium* sp, may have contributed to the increase of soil nutrient availability. Therefore, a further increase in manure rates does not increase production as much as the difference between low rate of manure and no fertilizer. Several factors can cause low nutrient use efficiency, they are leaching loss, gaseous losses, immobilization of fertilizer nutrients, the interaction between different fertilizers, physical and chemical characteristics of soil, soil temperature that influence uptake of N, P, and K, soil moisture, and the fertilizer characteristic itself (Mishra et al., 2023). In line with the current study, it has been previously reported that higher fertilizer rates resulted in lower nutrient (N) use efficiency in common beans (Argaw et al., 2015), and wheat (Sharma et al., 2022). In the current study, the nutrient use efficiency values decreased with higher manure rates because they measured the increase in edamame production between fertilizer and control. However, it can be seen that edamame yield increases more with biofertilizers (Fig. 1) which indicates biofertilizers increase the efficiency of manure application. The positive role of biofertilizer has also been reported previously that the application of plant growth-promoting bacteria (PGPB) can reduce the poultry manure dose from 18 to 12 ton ha^{-1} for okra fruit production (Magana, 2020).

Several approaches can be considered to improve nutrient use efficiency in organic farming including to improve plant morphological dan physiological characteristics and cultivation techniques. The agronomical approach is essential to improve the nutrient use efficiency in plants to make them economically more feasible as well as to prevent environmental damage due to excess fertilizer. Improvement in cultivation technique including the use of different types of fertilizer (e.g. bio-fertilizers, granule organic fertilizer), nutrient management improvement, modern technology (e.g. precision farming by determining the fertilizer recommendation based on soil testing), and agronomic practices (e.g. cropping pattern) (He et al., 2009; Javed et al., 2022; Mishra et al., 2023).

Different trends of AE, AE_N, AE_P, and AE_K were shown in Experiment 2024. Only AE significantly decreased with higher manure rates. In contrast to Experiment 2023, the combination of poultry manure and biofertilizer did not have a positive effect on AE, AE_N, AE_P, and AE_K of Experiment 2024. The efficacy of biofertilizers is determined by, among others, the plant physiological traits and soil characteristics (Malusà et al., 2016). Plants can modify the compound released from the roots and select the specific bacterial communities (Marschner et al., 2004). Root exudates are important to initiate the effect of the rhizosphere in seedling and lateral root emergence. Therefore, to increase the efficacy of biofertilizer, biofertilizer should be delivered on seeds and seedlings (Malusà et al., 2016). In Experiment 2023, biofertilizer was applied to seeds, while in 2024, biofertilizer was delivered to the seed and into the soil (twice). The difference in delivering biofertilizer may have impacted the different efficacy of biofertilizer when combined with poultry manure in 2024 although repeated applications of biofertilizer during the growing season of the plant have been recommended (Malusà et al., 2016).

Partial factor productivity PFP_N, PFP_P, and PFP_K decreased with higher manure rates (Table 7). The PFP can be used to provide an idea of how production can be increased or maintained with minimal input of nutrients (Congreves et al., 2021). The present study showed that to increase edamame productivity, the nutrient supply from poultry manure cannot be minimized yet.

CONCLUSIONS

The study revealed that poultry manure, as a source of nutrients, can support organic edamame soybean production in the two experiments. A combination of poultry manure and biofertilizer determines the different responses of soybean plants from different times of experiment. Higher rates of manure increased pod yield with biofertilizer than without biofertilizer in the first experiment but not in the subsequent experiment. Agronomic efficiency was higher with biofertilizer than without biofertilizer in the first experiment but vice versa in the 2nd experiment. In general, AE decreased with higher manure rates. Partial factor productivity decreased with increasing rates of poultry manure. It is important to increase nutrient use efficiency in organic farming including by looking for more suitable types of microbes or by combining various nutrient sources. The study shows that the application of poultry manure in organic farming needs to be combined with biofertilizer, especially on land that has not accumulated high levels of organic matter. However, in the next planting season, the application method of biofertilizer, in combination with poultry manure, needs to be carefully reviewed. The current study showed that 16 ton·ha^{-1} of poultry manure is sufficient for edamame production with the addition of biofertilizer.

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