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Improving Soil Fertility and Forage Productivity Through Bioorganic Fertilizer Substitution for Urea in *Pennisetum purpureum-Macroptilium atropurpureum* Association

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Abstract: Enhancing the productivity of forage crops while maintaining soil health remains a critical objective in sustainable agriculture. Excessive application of inorganic nitrogen (N) fertilizers, particularly urea, has contributed to soil degradation and environmental concerns, prompting the need for biologically sustainable alternatives. In this study, the effects of substituting urea with bioorganic fertilizer on soil quality and forage yield in an intercropping system of *Pennisetum purpureum* and *Macroptilium atropurpureum* were investigated. A randomized block design (RBD) was employed with six substitution treatments: no fertilizer (T), 0% substitution (S0), and 25% (S1), 50% (S2), 75% (S3), and 100% (S4) substitution of urea-N with bioorganic fertilizer. Each treatment was replicated four times, resulting in 24 experimental plots. Parameters evaluated included soil properties, populations of nitrogen-fixing bacteria (NFB) and phosphorus-solubilizing bacteria (PSB), and growth and biomass characteristics of the forage association. Substitution treatments significantly improved soil fertility indices. The highest soil organic carbon (SOC) (3.23%) was observed in S3, while total N content (Total N) in S2, S3, and S4 exceeded that of T and S0. Available phosphorus (P) was greatest in S3 and S4, and the highest cation exchange capacity (CEC) (24.08 me 100 g^{-1}) was recorded in S4. The S2 and S3 treatments yielded the highest leaf dry weights (1.55 and 1.49 kg plot^{-1}, respectively), stem dry weights (1.84 and 1.70 kg plot^{-1}), and total dry forage weight (3.38 and 3.19 kg plot^{-1}). Leaf-to-stem ratios and leaf areas in S2 and S3 were comparable to S0 and significantly greater than T. The lowest leaf area-to-total forage ratios (14.39 and 15.05 m² kg^{-1}) were also observed in these treatments. It was demonstrated that 50% and 75% substitution levels of urea-N with bioorganic fertilizer not only enhanced soil quality parameters but also significantly increased forage productivity compared to exclusive urea application. These findings underscore the potential of bioorganic fertilizer as a sustainable alternative to inorganic N sources, contributing to improved soil health, higher forage yields, and more resilient agroecosystems.

Keywords: Inorganic; Bioorganic; *Pennisetum purpureum*; *Macroptilium atropurpureum*; Fertilizer substitution

1. Introduction

Feed crops have been recognized as a critical component of the livestock production system, particularly within the upstream agricultural sector, due to their role in supporting national food security. For ruminant livestock, green fodder is the main source of feed. It not only functions as a satiety but also as a source of nutrition, namely protein, energy, vitamins, and minerals that have high nutritional value. The productivity of feed crops in producing green fodder is highly dependent on the pattern of plant association and the availability of nutrients. In ruminant rations, the portion of green fodder reaches 40-80% of the total dry matter of the ration or around 1.5-3% of the livestock's live weight, acting as a stimulant factor so that the rumen can function normally (Abdullah et al., 2005). Green fodder helps the digestive tract to function properly, creates a feeling of fullness (bulk), and encourages the release of digestive waste. Those functions cannot be replaced by concentrates (Pazla et al., 2023). According to Suarna et al. (2019), the availability of forage from grass and legumes as the main source of nutrition for ruminant livestock is currently inadequate.

Association (mixed) planting of grass and legumes is one way to increase the production and quality of forage. Mixed planting of grass with legumes has a higher potential to reduce soil degradation and weed invasion, increase soil fertility, have higher nutritional value, digestibility, degradability, and animal production potential than monoculture (Rusdy, 2021). One type of grass and legume that has the potential to be developed in association planting is hybrid elephant grass (*Pennisetum purpureum* 'Thailand') and siratro (*Macroptilium atropurpureum*). Hybrid elephant grass is more resistant to drought stress and is more nutritious than other elephant grass varieties (Turano et al., 2016). Siratro is a tropical legume that has auxin hormone at the base of the leaves and can exploit solar radiation more effectively in the process of root nodule formation, N fixation, and plant production. Siratro can also improve the growth, production, and quality of fodder in the surrounding area by the ability of siratro legume to be symbiotic with nitrogen-fixing bacteria (Hasan et al., 2019).

The efforts to increase crop productivity need to be synergized with soil fertility improvement through fertilization. N is generally considered the main nutrient that limits plant growth. Indonesia's global consumption of N fertilizer in the agricultural category continues to increase from 2.818 million tons yr^{-1} in 2015 and reached 3.541 million tons yr^{-1} in 2020 (FAO, 2021). Based on data from the Indonesian Fertilizer Producers Association (APPI), Indonesia's fertilizer consumption ranged from 10 million tons to 11 million tons between 2017 and 2021. Urea is the type of fertilizer most widely used domestically. From January to June 2022, the total consumption of urea fertilizer by agriculture, plantations, and industry in the country was 2.82 million tons (Rizaty, 2022). The continuous use of chemical fertilizers in high doses causes soil, water and air pollution as well as damage to the soil such as damage to soil structure and biodiversity, excessive nutrient leaching, water pollution (Anas, 2016), and soil degradation due to acidification and excessive accumulation of mineral nutrients in the soil (Zhao et al., 2018). This problem can be overcome by substituting inorganic fertilizers with organic ones. One of the organic fertilizers that has the potential to be utilized is bioorganic fertilizer. Bioorganic fertilizer is an organic fertilizer enriched by microbes, and the measurement parameters contain SOC and non-pathogenic multi-microbes that are synergistic with agricultural objectives (Suyani & Fatmawati, 2016). This type of organic fertilizer is made using raw materials (manure, crop straw, organic waste, etc.) and processed by functional microorganisms (He et al., 2024). This is important for the recovery of bio-based fertilizers from animal waste processing, with the aim of replacing synthetic N fertilizers as an important strategy to reconnect livestock and crop production, thereby closing the nutrient cycle and contributing to a more sustainable agricultural system (Sigurnjak et al., 2017).

Bioorganic fertilizer application has become more popular in some countries with intensive agricultural production and decreasing need for chemical fertilizers (Chen et al., 2022). Bioorganic amendment has been reported to reduce the level of chemical fertilizer use (Chen et al., 2022; Ye et al., 2020) and enhance plant growth with better soil quality (Wang et al., 2017; Ye et al., 2020). Substitution of inorganic fertilizers with bioorganic fertilizers, i.e., organic herbs, can increase the growth and production of tomato plants, with the best combination occurring at 40% inorganic (urea) and 60% organic herbs (Sari et al., 2019). Hu et al. (2022) concluded that compared to chemical fertilizers, 50% and 75% organic substitution levels significantly increased soil porosity, average weight diameter, SOC, catalase activity, and urease activity of soil microbes. Replacing 50% and 75% inorganic fertilizers with organic fertilizers significantly increased SOC, Total N, and soil enzyme activity (Yang et al., 2024).

Research on the substitution of inorganic fertilizers with bioorganic fertilizers represents a new approach that is increasingly important in the context of sustainable agriculture, the main novelty of which lies in the integration of biological and ecological approaches in soil fertility management to improve crop productivity and environmental sustainability. Despite the rapid progress in the development of bioorganic fertilizers, their full replacement of inorganic fertilizers still faces technical and socio-economic challenges. Integrative efforts are needed, involving further research, increased production capacity, farmer education, and policy support to accelerate the transition to a more sustainable agriculture.

The potential benefits and contributions of bioorganic fertilizers to agriculture in a broad sense and the environment have not been widely applied in the field due to the lack of scientific analysis studies and examples of their application, especially in feed crops in association planting. Therefore, research is needed to evaluate the effect of the urea substitution level with bioorganic fertilizers on soil quality and productivity of *Pennisetum purpureum* and *Macroptilium atropurpureum* association.

2. Methodology

2.1 Site Description

A field experiment on the effect of the urea substitution level with bioorganic fertilizers on *Pennisetum purpureum* and *Macroptilium artropurpureum* association was conducted in Pupuan village, Tegallalang, Gianyar, Bali, Indonesia, using two types of fertilizers, namely urea (46% N) and bioorganic (1.25% N). The results of soil analysis of the research site are presented in Table 1.

Table 1. Results of soil analysis before the research

Parameter	Unit	Soil Analysis Results	Criteria
pH:H_2O (1:2.5)		6.6	Neutral
Electrical conductivity (EC)	mmhos/cm	11.70	Very high
SOC	%	2.60	Medium
Total N	%	0.08	Very low
Available P	ppm	72.92	Very high
Available potassium (K)	ppm	213.98	Medium

Source: Soil Science Laboratory, Faculty of Agriculture, Udayana University, Denpasar, Bali (2023).

2.2 Experimental Design

A literature review was conducted to obtain research ideas and insights as well as an experimental framework. Preliminary research was conducted to obtain information on soil properties, the best N fertilizer doses, and plant associations to be used in the main research (Figure 1). The results of the preliminary research showed that the N fertilizer dose of 225 kg N ha^{-1} and the association pattern of one grass with two legumes were the best dose and association pattern, which were then applied to the main research. The main research was conducted by cultivating hybrid elephant grass (*Pennisetum purpureum*) and siratro legume (*Macroptilium atropurpureum*) with an association planting pattern of one grass with two legumes. The treatment of fertilizer substitution levels was carried out by substituting urea-N with bioorganic fertilizer according to the research design. After three months, the plants were cut, and the yield was measured. Simultaneously, soil fertility was evaluated to determine changes in soil properties and NFB and PSB populations caused by the substitution of urea with bioorganic fertilizer. All the data analysis was conducted using appropriate statistical methods to determine differences between treatments. The findings from this analysis were then synthesized to draw conclusions about the effectiveness of bioorganic fertilizers as an alternative fertilization and to propose recommendations for future research and practical applications in the sustainable cultivation of feed crops.

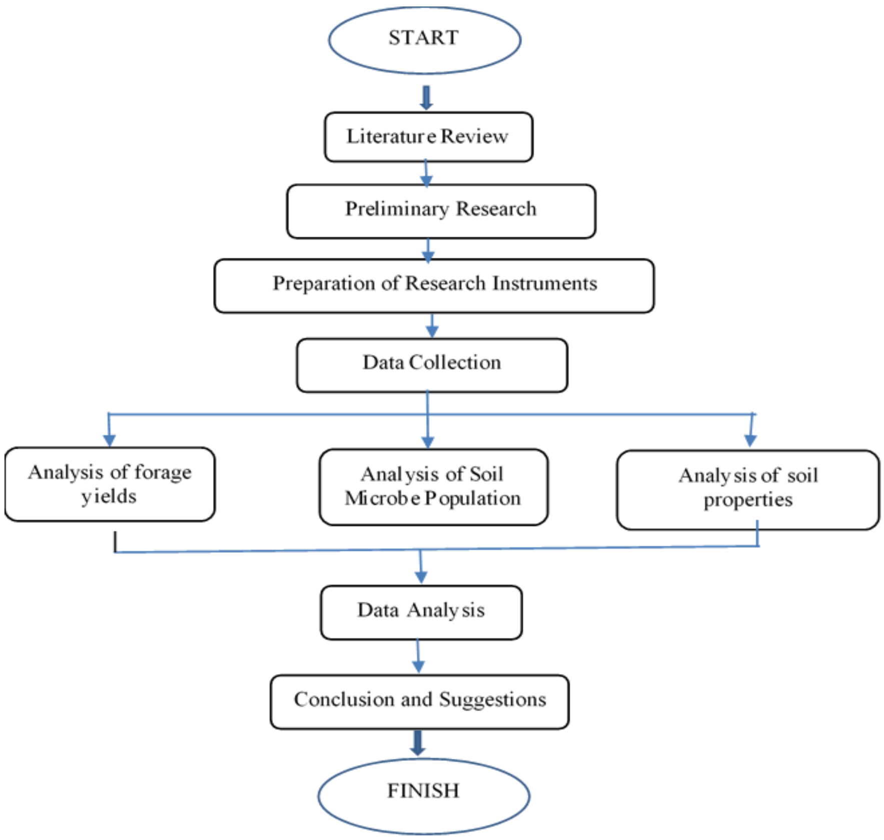

Figure 1. Research flow chart

Using an RBD with six treatments, the experiment was repeated in four blocks so that there were 24 experimental plots. Each plot was 7 m^2 (3.5 × 2 m), with a distance between plots of 0.5 m and a distance between blocks of 1 m (Figure 2). Six treatments of urea fertilizer substitution levels with bioorganic fertilizers consisted of no fertilizer (T), no substitution (S0), substitution of 25% (S1), 50% (S2), 75% (S3), and 100% (S4) against urea-N. Urea fertilizer application was based on preliminary research results, with the best results at a dose of 225 kg N ha^{-1}. Fertilization schemes for various treatments are detailed in Table 2.

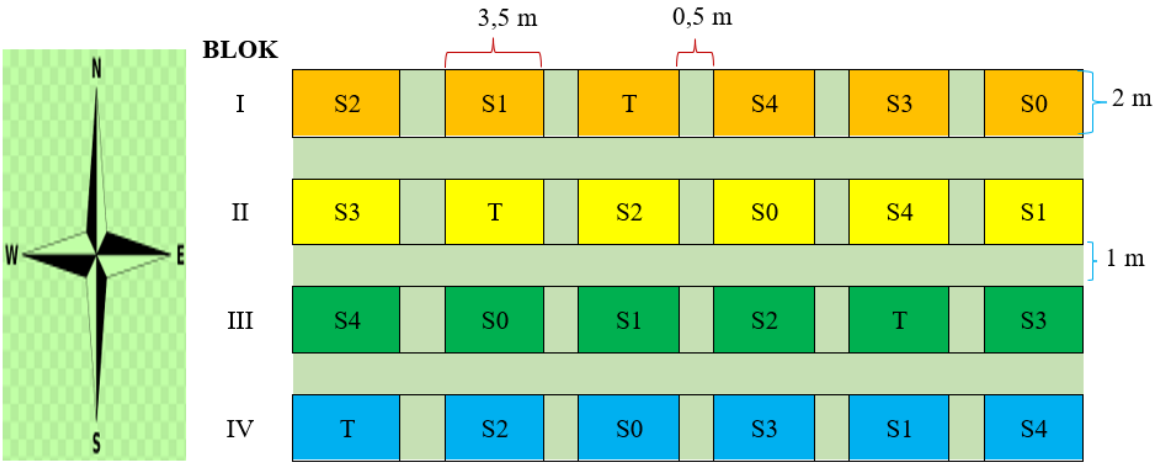

Figure 2. Field research plan

Table 2. Fertilization scheme for various treatments

Treatment Code	Treatment	Urea Fertilizer		Bioorganic Fertilizer		Total N $plot^{-1}$ (%)
		kg $plot^{-1}$	Total N (%)	kg $plot^{-1}$	Total N (%)	
T	No fertilizer	0.0000	0.0000	0.0000	0.0000	0.0000
S0	No substitution (urea: 225 kg N ha^{-1})	0.3424	0.1575	0.0000	0.0000	0.1575
S1	Substitution of 25% N	0.2568	0.1181	3.1500	0.0394	0.1575
S2	Substitution of 50% N	0.1712	0.0788	6.3000	0.0788	0.1575
S3	Substitution of 75% N	0.0856	0.0394	9.4500	0.1181	0.1575
S4	Substitution of 100% N	0.0000	0.0000	12.6000	0.1575	0.1575

Table 3. Results of bioorganic fertilizer analysis

Variable*	Unit	Analysis Results	Criteria	SNI 2019**
Chemistry and macronutrients[1)]				
pH:H_2O (1:2.5)		6.9	Neutral	4-9
EC	mmhos/cm	0.330	very high	-
SOC	%	43.90	very high	Minimum 15
Total N	%	1.25	very high	
Available P	ppm	632.44	very high	Minimum 2
Available K	ppm	849.60	very high	
Water content	%	25.18		10-25
CEC	me 100 g^{-1}	43.06		-
micronutrients[2)]				
- Total iron (Fe)	ppm	368.452		Maks. 15.000
- Available Fe	ppm	4.424		Maks. 500
- Zinc (Zn)	ppm	5.935		Maks. 5.000
E. coli[3)]	cfu/g or MPN/g	0		$< 1 \times 10^2$
Salmonella sp[3)]	cfu/g or MPN/g	0		$< 1 \times 10^2$
Functional microbes[4)]				$\geq 1 \times 10^5$
NFB	cfu/g	4.0×10^5		
PSB	cfu/g	2.3×10^4		

Note: [1)] Soil Science Laboratory, Faculty of Agriculture, Udayana University (2024); [2)] Analytical Laboratory, Udayana University (2024); [3)] Microbiology Laboratory, Udayana University (2024); [4)] Microbiology and APT Laboratory, Faculty of Animal Husbandry, Udayana University (2024); **Decree of the Minister of Agriculture of the Republic of Indonesia No. 261/KPTS/SR.310//M/4/2019.

2.3 Bioorganic Fertilizer

Bioorganic fertilizer was formulated from Balinese cow manure, which was mixed with urine and sawdust bedding. The formula was mixed, sieved, and fermented for two months with an effective fermenter. Before being applied, the bioorganic fertilizer was analyzed, as listed in Table 3.

2.4 Soil Sampling and Analyses

In terms of the collection of soil samples at harvesting, five soil subsamples for each plot were collected from a depth of 0–20 cm using a soil drill and mixed evenly to make a composite sample of about 1 kg. The samples were then transported and stored in the laboratory, air-dried, and sieved. The chemical properties of the soil samples were analyzed based on several parameters. pH (H_2O) and EC of the soil were determined using the H_2O 1:2.5 method, where the soil was mixed with distilled water in a 1:2.5 ratio. pH was measured using a pH meter, while EC was measured using a conductivity meter (Hendershot et al., 1993). SOC was analyzed using the Walkley and Black method, a widely used wet oxidation technique where potassium dichromate ($K_2Cr_2O_7$) in sulfuric acid (H_2SO_4) oxidizes organic matter, and the residual dichromate was titrated with ferrous sulfate to determine SOC (Walkley & Black, 1934). Total N was measured using the Kjeldahl method, which involves digesting soil samples with concentrated H_2SO_4 and a catalyst to convert organic N into ammonium, followed by distillation and titration to determine N concentration (Bremner, 1965). CEC was analyzed using the ^{1}N ammonium acetate (NH_4OAc) pH 7 method, where NH_4OAc was used to extract exchangeable cations, and the soil's capacity to retain and exchange cations was determined (Sumner & Miller, 1996). Similarly, base saturation (BS) was measured using the ^{1}N NH_4OAc pH 7 method, determining the percentage of exchangeable bases (Ca, Mg, K, and Na) relative to total CEC (Chapman, 1965). Total P was determined using the Bray-1 method, which extracts P with ammonium fluoride (NH_4F) and hydrochloric acid (HCl) to estimate plant available P (Bray & Kurtz, 1945). Likewise, Total K was analyzed using the Bray-1 method, which extracts K from the soil and quantifies it using atomic absorption spectrophotometry (AAS) or flame photometry (Thomas, 1982). These methods provide essential insights into soil fertility and nutrient availability for plant growth. The populations of NFB and PSB were determined using the pour method (Waluyo, 2019).

2.5 Collection of Plant Samples

Plants were harvested from 1 m^2 of randomly assigned tiles in each experimental plot. Samples were separated between leaves and stems, weighed, and dried at 70°C for 48 hours to obtain the leaf and stem dry weight. The total forage dry weight was calculated by adding the leaf dry weight to the stem dry weight. For leaf area calculation, five leaf samples each of *Pennisetum purpureum* and *Macroptilium artropurpureum* were randomly taken, weighed for the fresh weight of the leaf sample, and measured for the leaf area of the leaf sample. Leaf area per plot was calculated using the following formula:

$$LDP = \frac{LDS}{BDS} BDT$$

where, *LDP* is the leaf area plot^{-1}, *BDS* is the fresh weight of the leaf sample, *LDS* is the leaf area of the leaf sample, and *BDT* is the fresh weight of the total leaf.

2.6 Statistical Analyses

The experimental data were organized using Microsoft Excel. Data were analyzed using the SPSS 25 statistical software (IBM Crop., Chicago, IL, USA). One-way analysis of variance (ANOVA) was performed to determine significant differences between various treatments. The Duncan's Multiple Range Test (DMRT) at a 5% significance level ($P<0.05$) was applied for post hoc comparisons, identifying which treatments significantly differed from each other.

3. Results

3.1 Soil Properties

The soil properties at harvest are presented in Table 4, which shows that the soil pH ranges from slightly acidic to neutral (6.10-6.61) and tends to increase with increasing levels of fertilizer substitution. However, it is not statistically significantly different ($P>0.05$). EC ranges from 0.27 to 0.47 mmhos cm^{-1}, which is not statistically significantly different ($P>0.05$) in all treatments. SOC in all treatments of fertilizer substitution levels (S0-S4) is significantly higher than that of T. The highest SOC is in S3 (3.23%), significantly different (P<0.05) from all other treatments. Total N received by treatments T and S0 are 0.14% and 0.18%, respectively. All treatments of fertilizer substitution levels (S0-S4) show a significantly higher Total N in the soil (P<0.05) than T. Total N in S2, S3, and S4 is significantly different (P<0.05), higher than that of T and S0 (Figure 3).

Table 4. Soil properties at harvest

Variable	Treatment					
	T	S0	S1	S2	S3	S4
pH	6.30±0.21	6.10±0.06	6.12±0.07	6.52±0.29	6.61±0.12	6.61±0.27
EC (mmhos cm^{-1})	0.27±0.13	0.47±0.23	0.36±0.01	0.42±0.13	0.40±0.05	0.37±0.14
SOC (%)	2.11±0.06^{c}	2.47±0.01^{b}	2.46±0.01^{b}	2.48±0.02^{b}	3.23±0.09^{a}	2.68±0.30^{b}
Total N (%)	0.14±0.01^{d}	0.18±0.01^{b}	0.16±0.01^{c}	0.21±0.00^{a}	0.21±0.00^{a}	0.22±0.00^{a}
Available P (%)	31.02±11.24^{b}	28.48±6.31^{b}	38.65±3.34^{b}	49.95±11.33^{b}	73.85±6.55^{a}	87.40±3.30^{a}
Available K (%)	340.50±90.23	331.64±38.64	382.71±43.54	390.02±40.76	401.83±13.12	421.06±2.55
CEC (me $100g^{-1}$)	20.10±0.88^{bcd}	17.91±0.24^{cd}	17.68±0.17^{d}	20.21±0.28^{bc}	20.92±1.80^{b}	24.08±0.47^{a}
BS (%)	30.21±12.62	35.27±2.74	23.76±6.34	39.77±2.08	37.92±10.52	29.88±7.81

Note: Different superscript letters in each line indicate significant differences between treatments (P<0.05).

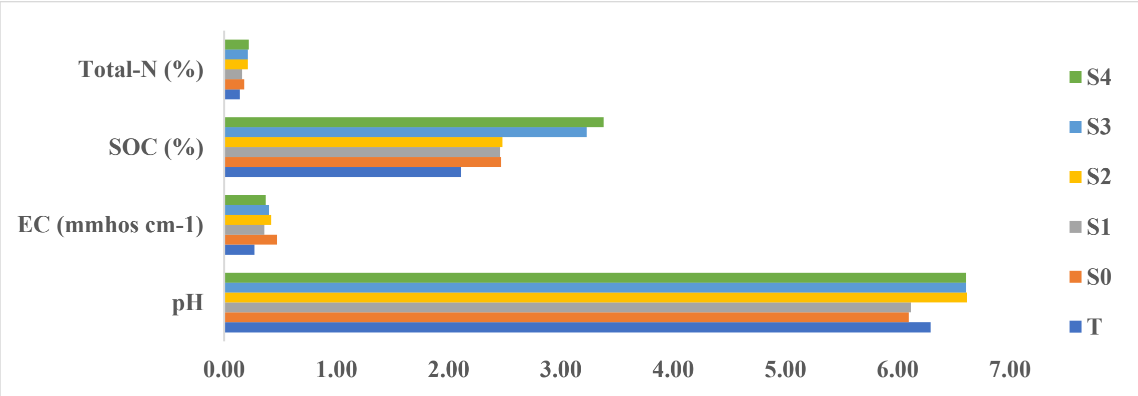

Figure 3. Soil properties (pH, EC, SOC, and Total N) at harvest

Available P content also shows significantly different results (P<0.05), with the highest found in S3 (73.85%) and S4 (87.40%), significantly different compared to other treatments. There is an increase in CEC with increasing levels of fertilizer substitution. The highest CEC is in S4 (24.08 me 100 g^{-1}), significantly different (P<0.05) compared to other treatments. BS ranges from 23.76% to 39.77% but is not statistically significantly different (P>0.05) (Figure 4).

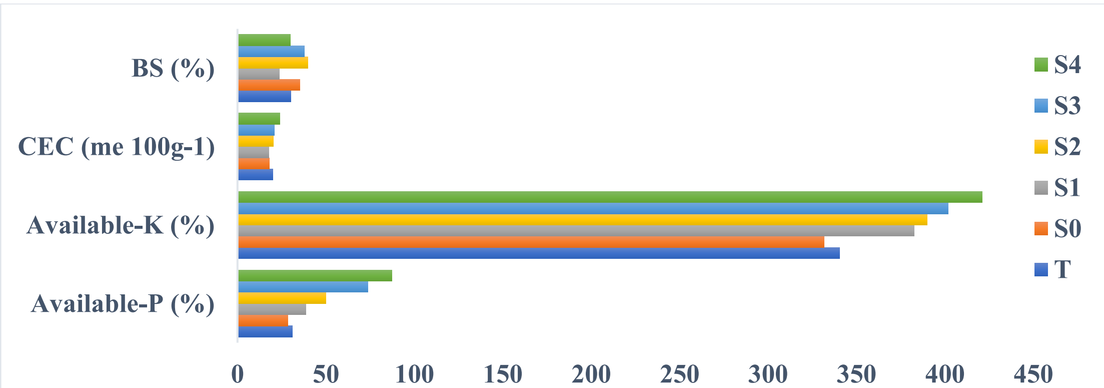

Figure 4. Soil properties (available P, available K, CEC, and BS) at harvest

3.2 Soil Microbial Population

Table 5 and Figure 5 present the population of soil microbes in the rhizosphere of *Pennisetum purpureum* and *Macroptilium atropurpurum* association. The highest NFB population is in S4 (19.2×10^{6}), significantly different from T, S0, and S1 (P<0.05), but not significantly different from S2 and S3 (P>0.05). The population of PSB ranges from 2.3×10^5 - 13.6×10^5, with the highest found in S0 (13.6×10^5), significantly different from all other treatments (P<0.05).

Table 5. Soil microbial population at harvest

Variable	Treatment						SEM
	T	S0	S1	S2	S3	S4	
	cfu g^{-1} tanah						
NFB	3.2×10^{6b}	2.6×10^{6b}	3.7×10^{6b}	10.0×10^{6ab}	10.5×10^{6ab}	19.2×10^{6a}	0.105
PSB	7.5×10^{5b}	13.6×10^{5a}	3.9×10^{5cd}	2.3×10^{5e}	3.1×10^{5de}	6.0×10^{5bc}	0.069

Note: Different superscript letters in each line indicate significant differences between treatments (P<0.05).

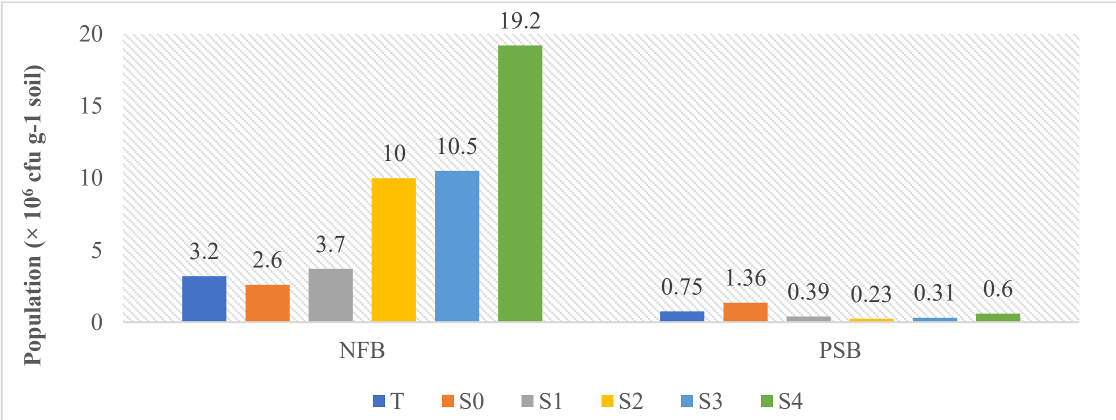

Figure 5. The population of soil microbes in the rhizosphere

3.3 Dry Weight Yield

Table 6 shows that all treatments of fertilizer substitution levels have significantly higher leaf dry weight, stem dry weight, and total forage dry weight (P<0.05) compared to T. The highest leaf dry weight is in S2 (1.55 kg $plot^{-1}$) and S3 (1.49 kg $plot^{-1}$), significantly different (P<0.05) compared to other treatments (subgraph (a) of Figure 6). S2 has the highest stem dry weight (subgraph (b) of Figure 6) and total forage dry weight (subgraph (c) of Figure 6), i.e., 1.84 and 3.38 kg $plot^{-1}$, respectively, significantly different (P<0.05) compared to T, S0, S1, and S4, but not significantly different (P>0.05) compared to S3.

Table 6. Dry weight yield of *Pennisetum purpureum* and *Macroptilium atropurpureum* association

Variable	Treatment					
	T	S0	S1	S2	S3	S4
	kg $plot^{-1}$					
Leaf dry weight	1.05 ± 0.04^c	1.22 ± 0.06^b	1.33 ± 0.10^b	1.55 ± 0.12^a	1.49 ± 0.06^a	1.22 ± 0.08^b
Stem dry weight	1.03 ± 0.07^c	1.34 ± 0.31^{bc}	1.39 ± 0.38^{bc}	1.84 ± 0.44^a	1.70 ± 0.18^{ab}	1.37 ± 0.29^{bc}
Total forage dry weight	2.08 ± 0.10^c	2.56 ± 0.36^b	2.72 ± 0.47^b	3.38 ± 0.50^a	3.19 ± 0.20^a	2.60 ± 0.37^b

Note: Different superscript letters in each line indicate significant differences between treatments (P<0.05).

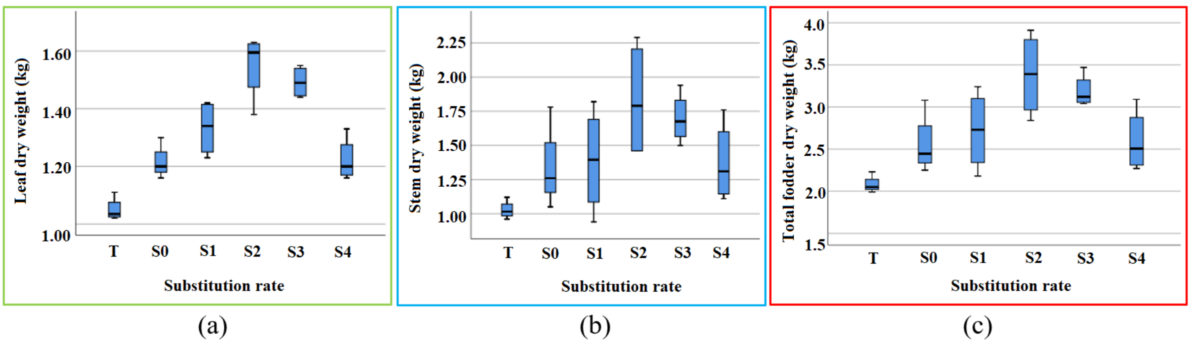

Figure 6. Effect of fertilizer substitution on (a) leaf dry weight, (b) stem dry weight, and (c) total forage dry weight

3.4 Growth Characteristics

Table 7 shows that the leaf/stem ratio value is the same in all treatments (subgraph (a) of Figure 7), with the highest leaf area produced in S2, which is significantly (P<0.05) higher than T, but not significantly different compared to S0, S1, S3 and S4 (subgraph (b) of Figure 7). The ratio of leaf area/total forage in S2 and S3 (14.39 and 15.05 $m^2 kg^{-1}$) is significantly lower compared to T, S1, and S4, but not significantly lower (P>0.05) compared to S0 (subgraph (c) of Figure 7).

Table 7. Growth characteristics of *Pennisetum purpureum* and *Macroptilium atropurpureum* association

Variable	Treatment					
	T	S0	S1	S2	S3	S4
Leaf/stem ratio	1.03±0.03	0.94±0.17	1.01±0.22	0.88±0.19	0.89±0.11	0.91±0.13
Leaf area (m^2)	34.95±5.49^b	40.49±4.43^{ab}	45.37±5.14^a	48.58±6.41^a	47.89±4.09^a	45.33±4.12^a
Leaf area/total forage ratio ($m^2 kg^{-1}$)	16.75±1.91^{ab}	15.92±1.29^{bc}	16.84±1.20^{ab}	14.39±0.69^c	15.05±1.39^c	17.67±2.49^a

Note: Different superscript letters in each line indicate significant differences between treatments (P<0.05).

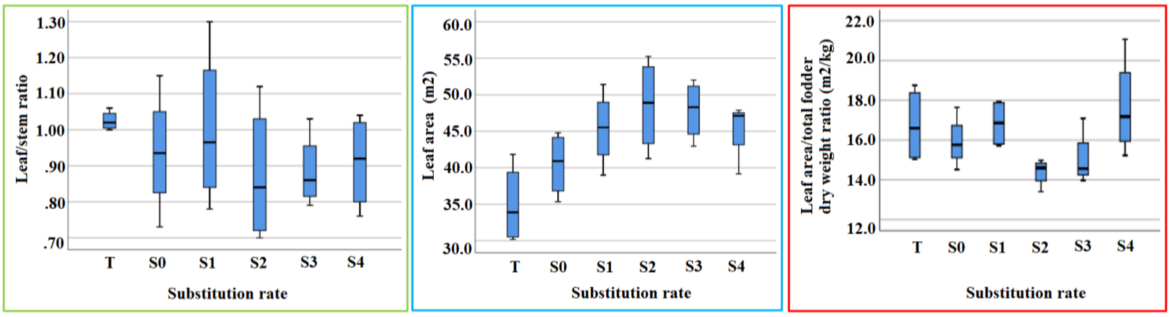

Figure 7. Effect of fertilizer substitution on (a) leaf/stem ratio, (b) leaf area, and (c) leaf area/total forage dry weight ratio

4. Discussion

4.1 Effect of Fertilizer Substitution on Soil Property Improvement

Soil properties are important indicators that reflect soil quality and health. This study revealed that substitution of urea fertilizer with bioorganic fertilizer improves soil quality by improving soil properties, especially at high substitution levels of 50-100% N. Increasing the substitution level of urea fertilizer with bioorganic fertilizer increases SOC, Total N (Table 4 and Figure 3), available P, and CEC (Table 4 and Figure 4). SOC at harvest is influenced by the treatment given and its utilization during the study by the plants and soil organisms involved. High SOC in S3 is attributed to the bioorganic fertilizer applied (Table 3). Therefore, the substitution of 75% N causes SOC to increase. This finding is in line with previous studies, which found that in rubber plantations, the treatment of replacing NPK fertilizer with 50% and 75% manure significantly increases SOC (Yang et al., 2024). SOC in S4 is lower than S3, allegedly caused by its utilization by NFB, whose population is higher in S4 (Table 5). SOC acts as an energy source for soil microorganisms, supporting the reproduction of microorganisms through the respiration process. The increase of SOC in the substitution of 50% and 75% N of inorganic fertilizer with bioorganic fertilizer in this study (17.54% and 53.08%, respectively) is higher compared to research results by Yang et al. (2024), where the increases are 7.15% and 3.46%, respectively. This may be influenced by the type of fertilizer and type of soil used in the study.

Higher Total N in S2-S4 (substitution level of 50-100% N) is attributed to the bioorganic fertilizer applied, containing Total N, which is classified as very high criteria, i.e., 1.25% (Table 3). Therefore, it could balance the N of the substituted urea fertilizer. Bioorganic fertilizers contain very high SOC, which can function as nutrients for plants and soil microbes. In addition, they contain NFB (Table 3). Therefore, when fertilizers are applied to the soil, NFB can survive, grow, and function to fix N from the free air to become available N in the soil. In addition, the provision of N fertilizer combined with bioorganic fertilizers reduces the rate of N loss, based on the results of previous studies which found that the rate of $_{15}$N fertilizer loss in the treatment of N fertilizer combined with organic fertilizers is significantly lower than N fertilizer without combination (Zheng et al., 2023). The available P content in S3 (73.85%) and S4 (87.40%) are highest, respectively higher 159.31% and 206.88% compared to S0, 138.07% and 181.75% compared to T (Table 4), this is influenced by the very high available P content in bioorganic fertilizers (Table 3). The CEC value also shows a significant increase compared to S0 (17.91 me $100g^{-1}$), especially at high substitution levels, i.e., S3 (20.92 me $100g^{-1}$) and S4 (24.08 me 100 g^{-1}). Higher CEC reflects higher soil fertility related to nutrient retention and exchange (Shifa et al., 2024). This increase in CEC can be attributed to organic matter added through bioorganic fertilizers undergoing decomposition and forming humus rich in functional groups such as carboxyl (-COOH) and phenolic (-OH), these groups provide a negative charge to soil particles, increasing the soil's ability to retain and exchange nutrient cations including Ca^{2+}, Mg^{2+}, and K^{+}. Application of various combinations of compost and biochar can increase soil CEC by 11.3% – 76.0% (Shifa et al., 2024). This finding adds to the evidence that bioorganic fertilizers can be a solution in reducing the use of inorganic fertilizers to increase feed crop productivity and support sustainable agriculture.

The findings in this study indicate that substitution of urea fertilizer with bioorganic fertilizers, especially at high levels (substitution of 50 and 75% N), is able to increase soil fertility through soil property improvement, especially by increasing Total N, SOC, pH, CEC, and NFB. These soil properties play a very important role in improving soil fertility and health. The increase in Total N is caused by bioorganic fertilizers, which contain N nutrients in very high criteria (Table 3), and also contain NFB that can remain alive and actively fix N when the fertilizer is applied. Application of bioorganic fertilizers increases soil pH and CEC because these fertilizers contain functional groups that can neutralize H^{+} ions in the soil, thereby increasing pH. The increase in CEC is influenced by the content of organic materials that have negatively charged groups to bind cations, which has implications for increasing soil CEC. The increase in the population of NFB comes from microbes in bioorganic fertilizers and microbes that are indeed in the soil, which can grow and develop due to the very high SOC in bioorganic fertilizers, acting as a source of nutrition for NFB. These results are supported by research results, which show that SOC has a positive correlation with the total population of soil microorganisms (Salsavira, 2024).

4.2 Effect of Fertilizer Substitution on Soil Microbial Population

Increasing the level of urea fertilizer substitution with bioorganic fertilizers increases the population of NFB (Table 5 and Figure 5), which is influenced by the very high SOC in bioorganic fertilizers (Table 3). SOC affects various soil properties such as structure, water retention capacity, nutrient availability, and microorganism activity. SOC is the carbon content contained in soil organic matter. Therefore, SOC reflects the presence of organic matter in the soil (Nopsagiarti et al., 2020). Microbial activity requires carbon as an energy source in converting organic N (Wijayanti & Prasetya, 2018), and there is a strong correlation between organic matter and bacterial community structure and diversity (Li et al., 2022).

The population of PSB is relatively lower than that of NFB, which is influenced by the status of soil P nutrients. Before the study, the status of soil P nutrients showed very high criteria, while that of N nutrients was classified as very low criteria (Table 1), indicating the dominant presence of NFB. The population of PSB in all urea fertilizer substitution with bioorganic fertilizer treatments (S1-S4) is lower than T and S0, and it is significantly highest in S0 (Table 5), influenced by higher available P (Table 4). Increasing available P in the soil can affect the population of PSB through a negative feedback mechanism. The increasing concentration of available P causes a decrease in the need for plants and microorganisms to dissolve P from insoluble sources, thereby reducing the activity and growth of PSB. This is supported by the results of previous studies, which show that inoculation of PSB increases the density of PSB that reaches its peak on the 14^{th} day, but decreases until the 42^{nd} day, even though the availability of diphosphorus pentoxide (P_2O_5) increases (Widjaja, 2022). In addition, it can be caused by the application of urea, which is a source of N that is quickly available when fertilizer is applied to the soil. Therefore, it can be utilized more quickly by certain groups of PSB as a source of nutrients. This is also supported by the results of soil analysis at harvest, which showed that Total N in S0 is significantly ($P<0.05$) lower than that in S2, S3, and S4 (Table 4), indicating that N is utilized not only by plants but also by PSB. On the other hand, bioorganic fertilizers contain organic materials that require decomposition by soil microorganisms to release nutrients available to plants. This decomposition process takes time and can cause competition between microorganisms for nutrient sources, which may inhibit the specific growth of PSB.

4.3 Effect of Fertilizer Substitution on Forage Dry Weight Yield

The efforts to increase plant productivity with fertilization must maintain soil quality and health. The produced leaf dry weight, stem dry weight and total fodder dry weight which are significantly higher in all fertilizer substitution treatments (S0, S1, S2, S3 and S4) compared to T, indicating that the application of fertilizer with N content of 225 kg ha^{-1} (15.75 kg N $plot^{-1}$) can significantly increase crop yields. This occurs because the Total N of the soil at the research site, based on the results of soil analysis, is classified as very low (0.08%), while other nutrients are in the medium to very high criteria (Table 1). Therefore, the application of N fertilizer can significantly increase the yield of cultivated plants. N directly affects the rate of photosynthesis and growth, and ultimately affects the yield and utilization of light energy. The N element is the nutrient that is needed the most compared to other nutrients (Chairunnisak et al., 2018). In plants, N functions as the main component of protein, hormones, chlorophyll, vitamins, and essential enzymes, and makes up 40-50% of the dry weight of protoplasm (Nurhayati, 2021).

Treatment of S2 has the highest leaf dry weight, followed by S3 (Table 6 and subgraph (a) of Figure 6), influenced by the significantly higher leaf area compared to T, and tended to be higher than S0 (Table 7). Plants with a large leaf surface can result in the factors needed by plants for photosynthesis being easily fulfilled so that the photosynthesis process can run more optimally (Sakya & Rahayu, 2010). The increase in the rate of photosynthesis enhances the production of dry matter in plants so that the assimilates translocated to plant parts can also increase (Sakya et al., 2015). The higher the photosynthesis results, the greater the accumulation of food reserves translocated to produce plant dry weight (Gardner et al., 1985). The size of the leaf area determines the amount of light interception that affects photosynthesis and photosynthate accumulation (Dai et al., 2001).

The significantly higher leaf dry weight in S2 and S3 indicates that substitution of 50% and 75% urea-N with bioorganic fertilizer increases the leaf dry weight yield higher than the use of urea without 25% or 100% N substitution. This is because at the substitution levels of 50% and 75%, there is a balance of nutrients according to the needs of plants and soil microbes, so that it can support optimal plant productivity. Bioorganic fertilizers are beneficial for plants and soil. For plants, bioorganic is useful for stimulating root development, increasing the amount of chlorophyll and photosynthetic activity, accelerating growth, increasing plant productivity, and improving plant quality and yield (Setiadi, 2012). It is further explained that for soil, bioorganic is useful for adding macro- and micro-nutrients, loosening the soil, increasing water-binding capacity, improving CEC, saving fertilizer use, increasing nutrient absorption, activating soil microbes, preventing aluminum (Al) and Fe poisoning, and dissolving bound phosphate. The findings in this study are supported by previous research, which shows that replacing 50% and 75% of inorganic fertilizers with organic fertilizers can significantly increase SOC, Total N, and soil enzyme activity (Yang et al., 2024). Application of bioorganic fertilizers to reduce chemical fertilizer applications can significantly improve soil fertility and tomato yield and quality (Ye et al., 2020).

S2 has the highest stem dry weight, indicating that the treatment of a 50% N substitution level of urea with bioorganic fertilizer can increase the stem dry weight better than urea without substitution. The high stem dry weight in S2 is attributed to the significantly different ($P<0.05$) higher leaf dry weight (Table 6) and the leaf area, which tends to be the widest (Table 7). Heavier and wider leaves allow for higher photosynthesis capacity, so that the carbohydrates produced can increase. The results of the photosynthesis process are used by plants for growth and increase plant carbohydrates as a component of dry weight. Higher leaf area and leaf dry weight have the potential to produce higher photosynthate, so more can be stored in the stem as a food reserve, resulting in higher dry weight of stems. The total forage dry weight is the sum of the leaf dry weight and the stem dry weight. Therefore, the results are also highest in S2 and S3 (Table 6 and subgraph (c) of Figure 6). The accumulation and distribution of dry matter are the basis for the formation of crop yields and the key to various agronomic actions to regulate yields (Shi et al., 2021).

4.4 Effect of Fertilizer Substitution on Growth Characteristics

The leaf/stem ratio is not significantly different (Table 7 and subgraph (a) of Figure 7), indicating that the fertilizer substitution treatment does not affect the quality of the forage yield. The substitution level of 25-100% urea-N with bioorganic fertilizer produced forage with the same quality as the application of urea without substitution in terms of the leaf/stem ratio. The ratio of leaf dry weight to stem dry weight is influenced by the value of leaf dry weight and stem dry weight. That is, the higher the leaf dry weight at the same or lower stem dry weight, the higher the ratio value can be. The higher the leaf/stem ratio value means the higher the portion of leaves compared to the portion of stems. This value indicates the quality of forage. It is said to have good quality if it has a high ratio of leaf dry weight to stem dry weight.

All fertilizer substitution level treatments can increase leaf area (Table 7 and subgraph (b) of Figure 7), which, at a substitution level of 25-100% urea-N with bioorganic fertilizer, tends to be wider than urea fertilizer without substitution. This is because bioorganic fertilizer acts as a source of nutrients, improving soil structure and increasing microbial activity, nutrient availability, and fertilization efficiency. Many previous studies have been conducted to determine the effect of organic fertilizer on leaf area, which shows that this fertilizer can replace some chemical fertilizers to encourage leaf growth (Li et al., 2015). The results of previous studies show that the leaf area of wheat given organic fertilizer combined with compound fertilizer at the flowering stage is significantly higher than that of compound fertilizer alone and without fertilizer treatment (Ma et al., 2012). Leaves are important organs for photosynthesis to produce organic matter for plant growth and are also important indicators to determine whether plants can produce high yields (Ji et al., 2021; Zhang et al., 2022).

Treatments of S2 and S3 (50% and 75% N) substitution levels of urea with bioorganic fertilizers produced lower leaf area/total forage ratio (Table 7 and subgraph (c) of Figure 7), indicating that these treatments can produce higher total forage dry weight at the same leaf area compared to other treatments. The magnitude of the leaf area/total forage ratio is influenced by the leaf area and total forage dry weight produced in a certain area. The lower the leaf area/total forage ratio, the more effective the photosynthesis process. Therefore, the same leaf area can produce higher total forage. The provision of organic fertilizers can effectively increase leaf growth and the photosynthetic capacity of plants (Guo et al., 2022; Li et al., 2022; Zheng et al., 2023), because organic substitution can ensure a balanced supply of inorganic and organic nutrients throughout the growing season, promoting plant growth and nutrient absorption and increasing yield components (Geng et al., 2019; Shen et al., 2020).

It was found in this study that the combination of inorganic N fertilizer (urea) with bioorganic fertilizer can significantly increase plant yields compared to the control (without fertilizer) and the application of single inorganic or single bioorganic fertilizers. The highest results are in treatments S2 (substitution of 50% N) and S3 (substitution of 75% N), with the same forage quality (leaf/stem ratio). Bioorganic fertilizers increase plant yields because of their ability to increase soil fertility physically, chemically, and biologically, as indicated by improvements in soil properties, especially Total N, SOC, soil pH, CEC, and NFB, which synergistically improve the soil environment to support increased plant growth and yields.

The combination of inorganic fertilizers with bioorganic fertilizers can overcome their respective weaknesses and bring out their respective advantages. Inorganic fertilizers quickly replenish mineral elements, while bioorganic fertilizers improve soil fertility by improving the physical, chemical, and biological properties of the soil, thereby ensuring adequate fertility supply, increasing crop yields and quality, and protecting soil and environmental health to realize sustainable agricultural development. Bioorganic fertilizers are organic fertilizers enriched by microbes, the measurement parameters of which contain SOC and non-pathogenic multi-microbes that are synergistic with agricultural objectives (Suyani & Fatmawati, 2016). Through three consecutive years of field experiments, it was found that substitution of organic fertilizers to 12%, 18% and 24% N and P content of conventional fertilizers (133 kg P_2O_5 ha^{-1} and 300 kg N ha^{-1}) increased wheat yield productivity and soil quality compared to conventional fertilization, and also increased the diversity of bacterial communities and tissue complexity, without causing the risk of heavy metal pollution in the soil (He et al., 2024). Different organic fertilizers combined with N fertilizers can reduce the loss of N and increase the absorption and utilization of N fertilizers (Zheng et al., 2023). Bioorganic fertilizer is a type of organic fertilizer made using raw materials (manure, crop straw, organic waste, etc.) and processed by functional microorganisms (He et al., 2024). The application of bioorganic fertilizers has become more popular in several countries with intensive agricultural production, decreasing the need for chemical fertilizers (Chen et al., 2022). Bioorganic amendments have been reported to control Fusarium wilt or weeds (Li et al., 2023; Wu et al., 2015), reduce the use rate of chemical fertilizers (Chen et al., 2022; Ye et al., 2020), and enhance plant growth with improved soil quality (Wang et al., 2017; Ye et al., 2020).

5. Conclusions

In the association cultivation of *Pennisetum purpureum* and *Macroptilium atropurpureum*, substitution of urea fertilizer with bioorganic fertilizer significantly improves soil quality by improving soil properties, especially at high substitution levels of 50-100% N. The findings indicate that increasing the substitution level of urea fertilizer with bioorganic fertilizer in S3 and S4 increases SOC by up to 3.23% and 2.68%, respectively, compared to 2.11% in control treatments (T), reflecting 53.08% and 27.01% improvement, and compared to 2.47% in urea fertilizer without substitution treatments (S0), reflecting 30.77% and 8.50% improvement. Total N in S3 (0.21%) and S4 (0.22%) is also significantly higher compared to T (0.14%) and S0 (0.18%); likewise, available P respectively increases to 73.85% and 87.40% from 31.02% in T and 28.48% in S0. Additionally, the highest population of NFB is in S4 (19.2×10^6), compared to 3.2×10^6 in T and 2.6×10^6 in S0. Furthermore, treatments of S2 (50% N substitution) and S3 (75% N substitution) significantly produced the highest yield of leaf dry weight (1.55 kg $plot^{-1}$ and 1.49 kg $plot^{-1}$), stem dry weight (1.84 kg $plot^{-1}$ and 1.70 kg $plot^{-1}$), total forage dry weight (3.38 kg $plot^{-1}$ and 3.19 kg $plot^{-1}$) and leaf area (48.58 m^2 and 47.89 m^2), the lowest leaf area/total forage ratio (14.39 m^2 kg^{-1} and 15.05 m^2 kg^{-1}), respectively, and the same leaf/stem ratio. Thus, bioorganic fertilizer can be used as an alternative solution in reducing dependence on inorganic fertilizers to improve soil fertility and health, increase the productivity of forage crops, and support sustainable agriculture.

Author Contributions

Conceptualization, N.G.K.R. and I.W.S.; methodology, N.G.K.R. and I.W.S.; software, N.G.K.R..; validation, N.G.K.R., I.W.S., N.N.S. and I K.M.B.; formal analysis, N.G.K.R.; investigation, N.G.K.R.; resources, N.G.K.R.; data curation, N.G.K.R..; writing—original draft preparation, N.G.K.R..; writing—review and editing, N.G.K.R.; visualization, N.G.K.R.; supervision, I.W.S., N.N.S. and I.K.M.B.; project administration, N.G.K.R.; funding acquisition, N.G.K.R. All authors have read and agreed to the published version of the manuscript.

Data Availability

The data utilized to substantiate the conclusions of this research can be obtained from the corresponding author upon request.

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Conflicts of Interest

The authors declare no conflict of interest.

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