Evaluation of Green Manure Cropsand Their Effects on Mineralizable Nitrogen and Changes of Nutrient Contents in the Soil

ABSTRACT

To evaluate the green manure crops those can promote soil properties and the ability of nitrogen (N) mineralization in paddy soil, a pot experiment was conducted at the Department of Agronomy, Yezin Agricultural University, Naypyidaw, Myanmar from April to August 2022. The experimental design was a randomized complete block design with three replications. Ten green manure cropswere sown as treatments: soybean (*Glycine max (L.) Merr*), green gram (*Vigna radiata L. Wildzek.*), black gram (*Vigna mungo L. Hepper.*), cowpea (Bocate) (*Vigna catjang*), cowpea white (*Vigna unguiculata (L.) Walp.*), lab lab bean (*Lablab purpureus (L.) Sweet.*), mayflower bean (*Phaseolus vulgaris L.*), rice bean (*Vigna umbellata*), sunn hemp (*Crotalaria juncea L.),* and dhaincha (*Sesbania bispinosa (Jacq.)*). At the flowering stage, various green manures were incorporated into the soil, and their growth characters were measured along with the mineralizable N, total N, phosphorus (P_2O_5), and potassium (K_2O) contents of the soil. The results indicated that rice bean, sunn hemp, and dhaincha had higher shoot and root length, shoot and root fresh weight, and dry weight compared to other varieties. In addition, they contain a higher percentage of total N content and total P_2O_5, and consequently, its C:N ratio was lower. After incorporating green manures into the soil, the mineralizable N increased gradually from 0 weeks to 10 weeks and then, the nutrient values decreased. Among green manures, sunn hemp, dhaincha, and rice bean were found to have higher mineralizable N (%) and applicable mineralized N than others. In conclusion, the incorporation of green manures into the soil improves the N, P_2O_5, and K_2O contents of the soil, along with the mineralizable N content. Among green manure crops, dhaincha, rice bean, and sunn hemp were superior to other varieties due to higher biomass yield, mineralizable N (%), and chemical compositions, especially total N content.

Keywords; Green manures, Soil nutrient contents, Mineralizable nitrogen, Biomass

1. INTRODUCTION

The fertility of the soil in Myanmar has deteriorated and the production of crops has declined. This may be due to several factors such as the increased use of inorganic fertilizers, the long-termmono-cropping pattern, weather conditions, and the failure to reuse plant residues. The yearly application of higher or excessive doses of inorganic or chemical fertilizers can lead to acidification, damage to the soil's structure, water depletion, and changes in the population and activity of microbes in the soil [1];[2]. Most farmers harvest the whole plant without leaving any residue, which can be used for the nextseason's crop and soil nutrition. As a result, the level of organic matter in the soil decreases, leading to poor soil properties. Several researchers have pointed out that incorporating green manures (GMs) or crop residue can improve the physical condition of the soil. This is achieved by building up organic matter, decreasing bulk density, and increasing total pore space, water-stable aggregates, and hydraulic conductivity of the soil [3]; [4]. GM is usually a specific species of legume plant, such as a tree, a bush, a vine, a crawling plant, or algae that farmers plant to maintain or improve soil fertility, control weeds, or for many other reasons. GM canbe beneficial for crops by improving the contentof nitrogen (N), phosphorus (P), and potassium (K). It also benefits the soil structure, texture, water holding capacity, nutrient losses, fertilizer use by plants,andmicrobial population andactivity. The use of GMs increases the nutrient retention capacity and improves soil structure and microbial activity [5]. In mono-rice paddy fields, fast-growing leguminous GMs that can fix atmospheric N may offer opportunities to increase and sustain productivity and income in the rice-based cropping system, and their incorporation into soils is beneficial due to their adaptability to different rice-based cropping patterns [6]. Among GMs, dhaincha and sunn hemp are well known in South Asia for their capability of nodule formation and N fixation, and they produce relatively higher organic matter [7].

The objective of the study was to evaluatethe mineralization efficiency of tested GM crops based on their biomass yield and chemical composition by incorporating their biomass into the soil. Specifically, the study will explore how GMs impact the mineralization of N in the soil and the soil's physicochemical properties. However, the contribution of GMs to N in cropping systems is dependent on various factors such as the species, biological N fixation, and the growth of GMs based on the climate, soil, and management of residues. The amount of GM biomass can also vary depending on the plant type,growth stageand leafy conditions. Therefore, this study has two objectives. Firstly, to selectthe superior GMs for further cropping systems based on their high nutrient content and biomass yield. Secondly, to determine how GMs affect the mineralized N pattern and soil changes based on their biomass and chemical composition in the plant parts of GMs.

2. MATERIALS AND METHODS

2.1 The experimental site, design, and treatments

A pot experiment was conducted at the Department of Agronomy, Yezin Agricultural University (YAU), Naypyidaw, Myanmar from April to August 2022 to evaluate the impact of ten GM crops on biomass yield, nutrient contents, and N mineralization. The GM cropsinclude green gram, black gram, soybean, cowpea (Bocate), white cowpea, lab lab bean, mayflower bean, rice bean, sunn hemp, and dhaincha. Seeds were sourced from the Department of Agricultural Research(DAR), Myanmar. In the Randomized Complete Block (RCB) design, ten GMs were planted as treatments with three replications.

2.2 Preparation of pot

The soil that had been used for cultivating rice annually was taken and passed through a 2 cm sieve to remove rocks and inert materials. Around 17 kg of soil was then added to each plastic pot measuring 1 foot in width and 1 ft in height (0.28 m²). The soil in each pot watered for 2 days to moisten before sowing the seeds.

2.3 Green manure cultivation

Ten GMs were planted in each pot, with a rate of 10 seeds per pot. After 14 days, the plants were thinned out, leaving only five plants in each pot. The soil moisture level in all pots was maintained by watering as needed in the morning and evening.

2.4 Measurement of shoot length, root length, biomass, and physico-chemical properties of GMs

During the flowering stage of each variety, the plants were cut, and their shoot and root lengths were measured. After that, the fresh weight of each plant part (shoot and root as separately) was measured immediately and then oven-dried at a temperature of 70℃ for 48 hours.Then, the total fresh weight and dry weight were calculated as the summation of the weights of the shoot and roots.

To measure the chemical composition of GMs, 2 grams of each part were collected and used to measure the moisture content, N, P_2O_5, and K_2O. The oven-dried samples of shoot and root parts were ground into a fine powder using a sample mill (Cyclotec 1093, Foss, Hilleroed, Denmark). The N was measured using the Kjeldahl distillation method, and C was measured using a CHN coder (MT-5, Yanaco) with three replications. For the measurement of P_2O_5 and K_2O, the ground shoot and root were digested separately with three replications, using the Molybivanado phosphoric acid and Wet digestion with HNO_3 methods, respectively [8].

2.5 Measurement of mineralizable nitrogen from soil incorporated by green manures

During the flowering stage, GMs were cut and chopped into small pieces, about 2-4 cm in size. The samples obtained from the GMs were used to determine the nutrient content and biomass yield. The remaining parts of the GMs were mixed thoroughly into the soil, at a rate of 2 kg per pot (based on fresh weight). The soil was watered equally in the morning and evening to maintain moisture at the field capacity for all pots. The pots were placed under similar conditions as paddy fields.
Soil samples were collected from each pot at 0, 5, 10, and 15 weeks after the incorporation of GMs. Mineralized N, including ammonium (NH_4–N) and nitrate (NO_3–N), were measured by indophenol and Cataldo methods, respectively, after each incubation period [9]; [10]. N mineralization (%) from each GM will be calculated as the equation [9];

N mineralization (%) = Mineralized N (NH_4-N+NO_3-N) [treatment – control] ÷ Applied N ×100

2.6 Measurement of physicochemical properties of experimental soil

Soil samples were collected from crop fields on the YAU campus. A composite surface soil sample (0-20 cm) was taken from 13 points along an S-shaped route in a crop field. The collected soil samples were bulked, dried at room temperature, cleared of rocks and coarse organic materials, and ground to pass through a 2-cm sieve. They were then thoroughly mixed for pot preparation. A small amount of soil sample was sub-sampled by sieving through a 2-mm sieve for physical and chemical analysis. The physicochemical properties of the soil were measured before and after the incorporation of GMs in the pot experiment. The soil was analyzed for physical properties such as Bulk density and Porosity by the Core sampling method [11], Electrical conductivity (ECe) (dSm-1) by ECe method [12], and Sand (%), silt (%), Clay (%) and Texture class by international pipette method [13]. For chemical analysis, soil samples were analyzed for Soil pH by glass electrode as described by [14]. Organic carbon by Wet digestion method [15]. Available nitrogen by extraction and distillation method stated by [16].Available phosphorus by Olsen bicarbonate method [17]. Available potassium by Ammonium acetate extraction method [18]. and Cation exchange capacity (CEC) by the leaching method [19].

3. Statistical analysis

The data were subjected to an analysis of variance (ANOVA). The mean values of treatments were compared using Tukey's honestly significant difference (LSD) test at a 5% probability level using Statistix software (ver. 8.0; Analytical Software, Tallahassee, FL, USA).

4. Results and discussion

4.1 Physicochemical properties of the soil before and after the experiment

Before the experiment, the soil was analyzed, and the results are shown in Table 1. The pH (1:2.5) was found to be 5.77, indicating moderate acidity. The electrical conductivity was very low at 0.071 mS/cm, and the soil had very low levels of organic carbon (0.764%) and total nitrogen (0.093%). The exchangeable cations present in the soil were low levels of Ca^{++} (9.62 meq/100g), medium levels of Mg^{++} (3.427 meq/100g), high levels of Na^{+} (1.251 meq/100g), and low levels of K^{+} (0.132 meq/100g). The soil had a medium cation exchange capacity of 14.43 meq/100g, low available P (2.803 ppm), low available K_2O (6.254 ppm), bulk density of 1.195, and sandy loam texture with sand (63.28%), silt (20.89%), and clay (15.83%).
After incorporating GMs, it was observed that the soil's pH, organic C, total N, available P, and available K levels had slightly increased even in one season.

Table 1. Physicochemical properties of experimental soil before and after the experiment

No.	Treatment	pH (1:2.5)		Organic carbon (%)		Total N (%)		Available P (ppm)		Available K_2O (ppm)	
		Before	After	Before	After	Before	After	Before	After	Before	After
1	Green gram	5.78	6.78	0.76	0.86	0.09	0.28	2.8	6.04	6.25	46.13
2	Black gram	5.78	6.19	0.76	0.88	0.09	0.71	2.8	7.42	6.25	52.49
3	Soybean	5.78	7.34	0.76	0.78	0.09	0.89	2.8	9.34	6.25	47.24
4	Cowpea bocate	5.78	7.03	0.76	0.78	0.09	0.60	2.8	6.22	6.25	41.80
5	White cowpea	5.78	6.75	0.76	0.63	0.09	0.62	2.8	5.78	6.25	32.38
6	Mayflower bean	5.78	6.19	0.76	1.37	0.09	0.63	2.8	7.90	6.25	41.78
7	Rice bean	5.78	6.10	0.76	0.94	0.09	0.49	2.8	6.50	6.25	46.33
8	Dhaincha	5.78	7.16	0.76	0.80	0.09	0.35	2.8	5.54	6.25	30.54
9	Sunn hemp	5.78	6.18	0.76	0.76	0.09	0.37	2.8	9.52	6.25	48.58
10	Lab lab bean	5.78	7.31	0.76	0.79	0.09	0.71	2.8	5.12	6.25	31.29

2.7 Measurement of shoot length and root length of green manure

The shoot length of plants measured at the 50% flowering stage was significantly different among tested GMcrops ($P<0.05$). Sunn hemp had the longest shoot length (117.35 cm), followed by dhaincha (91.53 cm), and rice bean (77.87 cm) whereascowpea (Bocate) had the lowest shoot length18.23 cm, among themvarieties listed in(Table 2). This difference in shoot length could be attributed to variations in the genetic makeup and growth patterns of these GM crops, as well as differences in climate, season, and site [20].

The root length of GM crops was measured at the flowering stage, before chopping the plant and incorporating it into the soil. The results found that the root length of all testedGM cropswas not significantly different at $P<0.05$. Numerically, dhaincha had the longest root length (21.32 cm), followed by sunn hemp (16.37 cm), lab lab bean (14.53 cm), rice bean (14.25 cm), and cowpea (Bocate) had the shortest root length (10.95 cm) (Table 2). Longer root length can absorb more atmospheric nitrogen, which can improve the physical and chemical composition of the soil. GMs can fix atmospheric nitrogen through N-fixing bacteria associated with their roots [21].
In terms of shoot length, root length, and biomass yield, sunn hemp, dhaincha, and rice bean showed higher results than other varieties, indicating that they could be suitable for cultivation before crop cultivation.

Table 2. Shoot length (cm), root length (cm), and number of nodules of green manure crops at the flowering stage

No.	Treatments	Shoot length (cm)	Root length (cm)
1	Green gram	24.07 e	12.21b
2	Black gram	18.69 e	11.65b
3	Soybean	40.21 de	12.28 b
4	Cowpea Bocate	18.23 e	10.95 b
5	White cowpea	36.30 de	17.90ab
6	May Flower Bean	65.57 bcd	12.83 b
7	Rice Bean	77.87 bc	14.25 ab
8	Dhaincha	91.53 ab	21.32 a
9	Sunn hemp	117.33 a	16.37 ab
10	Lab Lab Bean	47.60 cde	14.53 ab
	$LSD_{0.05}$	30.49	7.60
	Pr> F	**	*
	CV%	33.07	30.69

** = significantly difference at 1% level and *= significantly difference at 5% level.

4.2 Shoot and root's fresh and dry weight

The shoot and root fresh weight (g $plant^{-1}$)of the tested GM varieties did not show significant differences between each other at a ($P<0.05$). Dhaincha showed the highest shoot fresh weight (50.93 g), followed by rice bean (41.83 g), sunn hemp (35.37 g), and white cowpea (23.63 g), while the lowestwas observed in green gram (12.03 g). As for root fresh weight, dhaincha showed the highestone(21.47 g), followed by sunn hemp (12.17 g), rice bean (7.97 g), white cowpea (5.90 g), and the lowest was observed in green gram (3.93 g) Figure 1(a)). It was observed that there were no significant differences among GM varieties for shoot and root dry weight (g $plant^{-1}$) at the ($P<0.05$) level (Figure 1(b)).The shoot dry weight of dhaincha was the highest at 42.27 g, followed by rice bean at 37.77 g, sunn hemp at 18.63 g, white cowpea at 14.63 g, and lab lab bean at 5.17 g. In terms of root dry weight (g $plant^{-1}$), dhaincha had the highest at 11.70 g, followed by sunn hemp at 5.27 g, rice bean at 2.87 g, white cowpea at 1.50 g, and green gram at 0.50 g. These findings suggest that dhaincha, sunn hemp, and rice bean are superior to the ten GMs due to their different growthcharacteristics. The amount and type of added organic materials also influence soil properties.

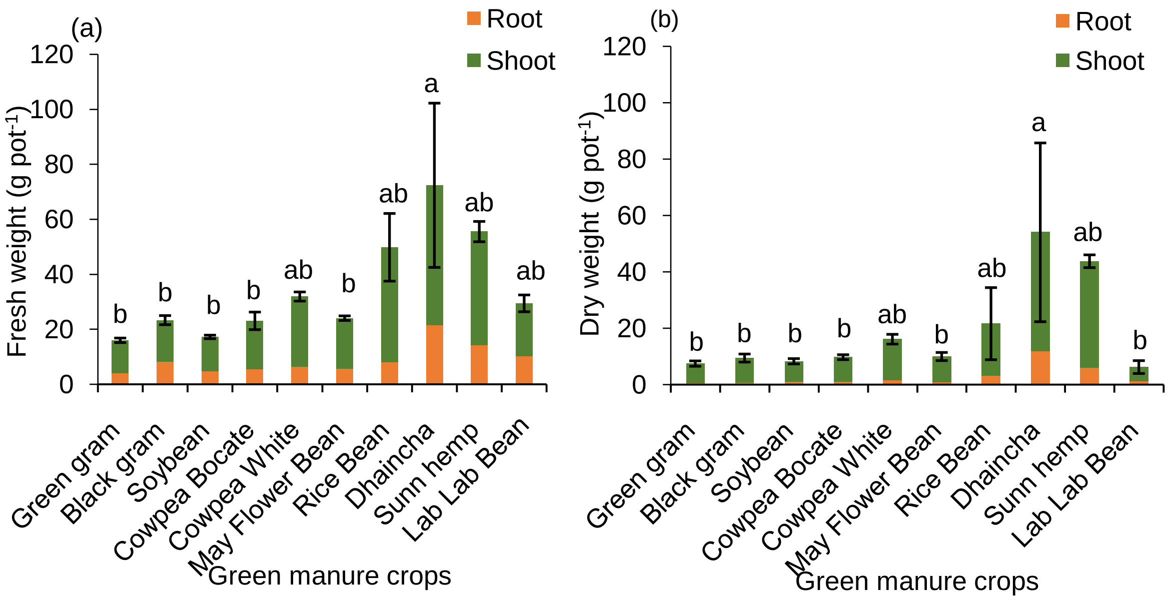

Fig. 1. (a) Fresh weight (g pot^{-1}) and (b) Dry weight (g pot^{-1}) of GMs at the flowering stage

4.3Analysis of the chemicalcomposition of green manure

The chemical composition of various GMvarieties was analyzed and compared based on their moisture content (%), total nitrogen (N) content (%), total phosphorus (P_2O_5) content (%), total potassium (K_2O) content (%), and organic carbon content (%). This analysis helped in selecting the superior GM varieties for crop cultivation, based on their chemical composition.

After analyzing the plant parts, cowpea (Bocate) was found to have the highest moisture content, at 85.13%, while sunn hemp had the lowest moisture content, at 57.64%. However, there were no significant differences in moisture content among the different GM varieties (as shown in Table 3).
The percentage of total nitrogen was notdifferent between GM varieties, but the highest amounts were found in sunn hemp (2.52%), dhaincha (2.42%), and rice bean (2.02%) respectively, while the lowest percentage was observed in green gram (0.87%). Similarly, higher amounts of total P_2O_5 were found in dhaincha (1.39%), sunn hemp (1.01%), rice bean, and mayflower bean (0.84%), while soybean had the lowest amount (0.21%) among GM varieties. The percentage of total K_2O in GMs was highest in cowpea (Bocate) (6.09%), followed by white cowpea (5.95%), black gram (4.24%), and soybean (3.47%), while the lowest amount was observed in sunn hemp (1.42%). The highest composition of organic carbon was found in dhaincha (63.38%), while the lowest amount was in white cowpea (37.99%), and they were not significantly different among GM crops. The lower C:N ratio was observed in sun hemp (20.27), rice bean (25.10), and dhaincha (26.19), which were superior for mineralization activity among GM crops. These results suggest that GM amendments of the soil significantly increased the organic matter contents of thesoil and enhanced soil nutrient dynamics in terms of organic carbon, nitrogen, total carbohydrates, and organo-minerals contents [23]. The application of high-quality GM, such as legumes with low lignin and low C:N ratios, can provide nutrients more efficiently by releasing nutrients quickly to the plant [24]; [25]. Finally, Parton et al. [26] reported that the C:N ratio of plant materials used is an important consideration parameter to predict the dynamic patterns of nutrient release and organic C mineralization rate.

Table 3. Chemical composition of green manures

No.	Treatments	Moisture (%)	Total N (%)	Total P_2O_5 (%)	Total K_2O (%)	Organic carbon (%)	C:N
1	Green gram	77.00	0.87	0.26	2.39	58.00	66.67
2	Black gram	81.72	1.17	0.24	4.24	58.00	49.57
3	Soybean	78.38	0.95	0.21	3.47	55.48	58.40
4	Cowpea Bocate	85.13	1.25	0.41	6.09	50.75	40.60
5	White cowpea	80.40	1.13	0.24	5.95	37.99	33.62
6	May Flower Bean	62.98	1.79	0.84	1.69	53.17	29.70
7	Rice Bean	60.67	2.02	0.84	1.46	50.71	25.10
8	Sunn hemp	65.51	2.42	1.39	2.39	63.38	22.06
9	Dhaincha	57.64	2.52	1.01	1.42	51.09	25.10
10	Lab Lab Bean	81.01	1.75	0.40	1.63	53.14	30.37

N = nitrogen, P_2O_5 = phosphorus, K_2O = potassium, C = organic carbon

4.4 N mineralization from green manure incorporated into the soil

The experiment analyzed the mineralizable nitrogen from GMs incorporated soil for four different intervals: 0 weeks, 5 weeks, 10 weeks, and 15 weeks after sowing. The results showed a gradual increase in N mineralization from 0 weeks to 10 weeks, followed by a decrease from 10 weeks to 15 weeks in all varieties except for cowpea (Bocate). Sunn hemp, dhaincha, and rice bean demonstrated higher N mineralizable followed by white cowpea variety and mayflower bean in all tested weeks (Figure 2). The rate of N mineralization varied among different GMs incorporated into the soil, which mainly depended on the C:N ratio, the microbe activity, soil moisture, soil temperature, and aeration. This experiment was conducted under controlled soil moisture conditions to maintain field capacity by watering the pot with an equal amount, and the location and aeration were kept constant. Therefore, the different rates of N mineralization observed from different GM varieties may be attributed to their varying decomposition rate in the soil, which is influenced by their C:N ratio and plant characteristics, such as leaves, stems, and root structures. The lower C:N ratio of the GM can generate a higher decomposition rate and N mineralization. Additionally, soil and environmental factors, such as moisture, temperature, light, relative humidity, and soil aeration, also played a crucial role. In a tropical rice-based system, nitrogen accumulation by GM legumes was affected by water regime, soil fertility, photo-period, inoculation, and growth duration [27].

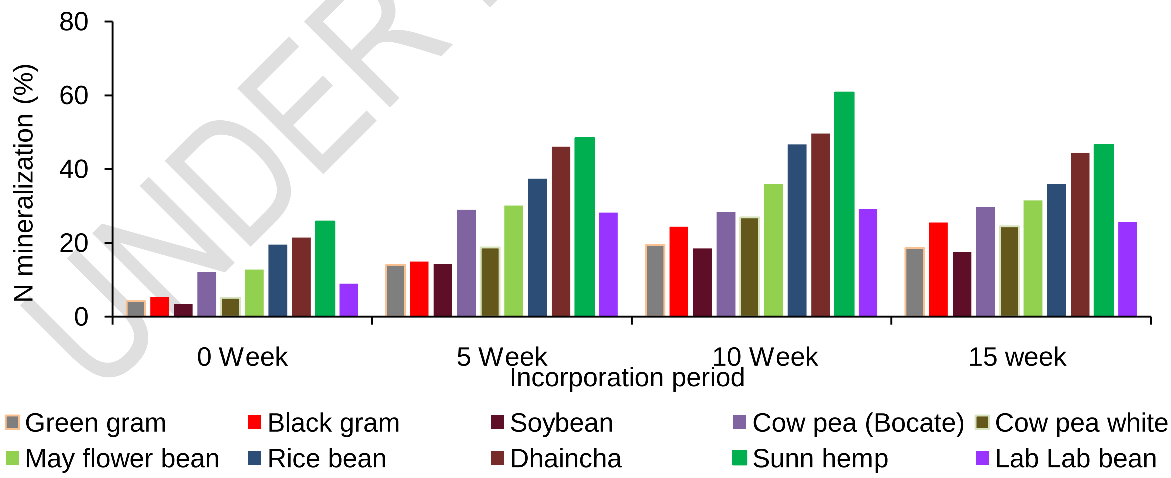

Fig. 2. Nitrogen mineralization (%) from theGMs incorporated into the soil

4.5 Applicable mineralized nitrogen

The mineralization pattern was analyzed at various durations of GM's incorporation, i.e., 0, 5, 10, and 15 weeks. The rate of mineralizable N was observed as original in the 0 week, which means it had pre-incorporated soil. The analysis was conducted immediately after GM's incorporation into the soil. Applicable mineralized nitrogen was calculated from mineralizable nitrogen percent multiplied by the biomass yield of GMs. The applicable mineralizable nitrogen increased from 5 to 10 weeks, which is when the highest application of GMs to the soil was observed, and then decreased in the 15 weeks. These results indicate that the applicable mineralized N decreased after 70 days (10 weeks) of GM's incorporation into the soil. This proves that nitrogen applied by decomposed GMs can be lost in various ways such as leaching, volatilization, and fixing by soil. The effects of GM application on N mineralization and soil nutrients depend on the GM crop quality due to their difference in chemical composition. The highest applicable mineralized nitrogen was absorbed in dhaincha, followed by sunn hemp, rice bean, white cowpea, and green gram while the lowest one was in lab lab beansin all tested weeks (Figure 3).

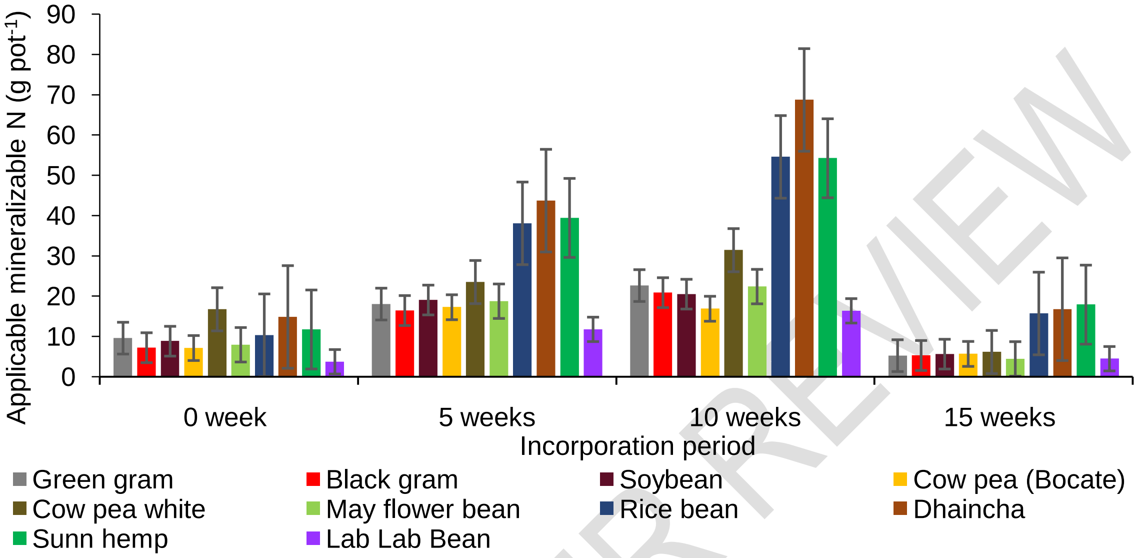

Fig. 3. Applicable mineralizable N (g pot^{-1}) from GMs incorporated into the soil

5. CONCLUSION

This study was conducted to assess the impact of growing green manure varieties and adding their biomass to the soil. The study aimed to investigate how green manure cropsaffect the soil's physicochemical properties and N mineralization. Growing high-quality green manures before crop cultivation can improve soil health and crop yield. This study identified three superior green manures;dhaincha, sunn hemp, and rice bean those are suitable for sandy loam and moderately acidic soils due to their higher biomass yield and nutrient content (N, P_2O_5, and K_2O). These GMs have a lower C:N ratio, meaning that they have a higher mineralizable N. Overall, incorporating green manures can improve the soil's physicochemical properties and ensure sustainable crop yields.

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