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Highlights:

- The increased P, K, and some exchangeable cations, were ascribed to the green manure.
- The low C and N contents are presumably a product of microbial immobilization.
- The tendency of lower incidence of RHBV was shown with green manures.
- The highest yield was 8.36 $t.ha^{-1}$ with the CanE - F100 treatment.

Abstract: Green manuring is an environmentally friendly technology aimed at providing nutrients to plants, enhancing soil fertility, mitigating soil degradation, controlling weeds and pests, and decreasing reliance on inorganic fertilizers. However, it requires dissemination and support to be adopted, especially in the poorest agricultural communities in Latin America. The study was conducted at the El Porvenir INIA in San Martín, Perú; it assessed two treatment sets: (1) green manure *Crotalaria juncea* (CroJ), *Canavalia ensiforme* (CanE), no green manure; and (2) nitrogen fertilizer dose (FN75, FN100). It was arranged in a split-plot design with four replications. During the experiment we detected an important fluctuation in soil parameters, however, it is the diminished levels of soil carbon and nitrogen, which were presumably the outcomes of microorganism processes. Otherwise, we observed that CanE significantly reduced the diseased tillers by "White Leaf Virus" (RHBV) by 2.82% compared to the control. The superior outcomes were achieved through CanE, and the highest yield was 8.36 $t.ha^{-1}$ with the CanE - FN100 treatment. Additionally, the nutritional quality of rice was not altered by green manures or chemical nitrogen fertilization doses tested.
Keywords: Split plot; legume green manures; soil fertility; RHBV; regenerative agriculture.

INTRODUCTION

Rice (*Oryza sativ*a L.) is cultivated in over 95 countries worldwide, serving as a staple for over half of the world's population, rice plays an important role in various countries by significantly contributing to dietary needs, providing approximately 35–80% of consumed calories (Bautista y Counce, 2020). Moreover, among prevalent cereal grains, it stands out for its exceptional characteristics, boasting the highest net protein utilization and digestible energy levels (Xie et al., 2022).

In Perú, rice is one of the main crops, with a production of approximately 3,027.41 thousand tons in 2022 (INEI, 2023). However, due to the increase in prices of chemical fertilizers caused by the Russo-Ukrainian conflict, the rice cultivation area decreased by 47.2% during the February 2022 campaign (Comexperu, 2023), and for 2023 rice production exhibited a variation of -5.7%, experiencing a decline of 24.3% compared to 2021 and a 9.7% decrease compared to 2022 (INEI, 2023).

For the San Martín region, the cultivated area in 2021 was 108,431 hectares, producing 867,364 tons and being the main crop in the region (Gobierno Regional de San Martín, 2021). However, farmers dedicated to this cultivation commonly face soil fertility issues, flooding, and phytopathological problems (Shelley et al., 2016). This could lead to an increase in the product's price, jeopardizing the basic food basket and nutrition of Peruvian families as consequences. In light of this scenario, there is a need to assess and promote economically viable and environmentally friendly alternatives that can substitute or reduce the need for chemical fertilizers.

An alternative to chemical fertilizers is the use of organic amendments, which have the advantage of improving soil structure, porosity, water retention, buffering capacity, microbial biomass, organic matter, respiration rate, total carbon, and nitrogen content in soils (Assefa and Tadesse, 2019). Among different forms of organic soil fertilization, the planting and incorporating of certain fast-growing plants with the ability to enhance soil fertility through nitrogen and organic matter incorporation are known as green manures (Tanveer et al., 2019).

The advantages of green manures over other organic fertilizers include increased soil coverage, protection against erosion, reduced weed infestation, and decreased pests and diseases, ultimately enhancing crop quality and yield, reducing the use of pesticides and herbicides, preventing erosion, and improving soil fertility (Minh et al., 2023).

Peru witnessed the technological benefits of the green revolution, which consisted of the modernization of agriculture on a global scale, carried out by incorporating technological innovations in production as a way to guarantee food safety, and, as a subsequent effect, the expulsion of small rural workers from the countryside. Besides, inputs used in soil preparation and crop maintenance, such as fertilizers and pesticides, can be extremely harmful to human health and the environment. Currently, the maintenance of soil fertility and the sustainability of agricultural production are quality parameters for agriculture, and green manure can play an important role in this regard as it shows versatile impacts such as improving properties (Saini and Yadav, 2019).

Some studies demonstrated that the use of green manures applied to rice-cultivated soils resulted in modifications to microbial abundance, composition, and enzymatic activity; they also observed an increase in chlorophyll content, panicle number, yield, and crude protein content in rice cultivation (Wang et al., 2022). Others found that the use of green manures in rice cultivation improved physical soil characteristics (Ahmed et al., 2020). increased dissolved organic matter content in soils (Gao et al., 2018), reduced soil erosion, improved various soil properties, and even decreased methane emissions.

Keeping the soil surface permanently covered by plant materials in the vegetative phase or as mulch is, indeed, the most recommended management to protect and conserve the soil that directly influences the production of various crops (Nolla et al., 2019). In this regard, the use of green manures as a strategy to improve soil fertility and control pests and diseases could help address these problems, ultimately enhancing yields and reducing production costs in rice cultivation, with the contribution of the use of legumes as a source to biologically fix atmospheric nitrogen, transforming it into nitrogenous compounds contributing to the maintenance of soil quality and ensuring the sustainability of production systems (Meirelles et al., 2023).

Globally, there is a lot of research on the potential benefits of green manures in terms of improving soil fertility and increasing rice yields. However, it is necessary to extend knowledge of sustainable techniques to farmers in the San Martin region as an alternative to improve the productivity and sustainability of rice cultivation because it allows a reduction in production costs, nitrogen fertilizers, and days, without altering performance. With the application of green manures, the profitability of the crop is improved and agribusiness increases with the economic use of organic natural resources.

This study aims to evaluate the effect of applying green manures prepared from legumes such as Canavalia (*C. ensiforme* L.) and Crotalaria (*C. juncea* L.) to improve soil fertility, partially reduce the use of nitrogenous chemical fertilizers, and increase rice crop yield in plots located in the Juan Guerra district, San Martín province, San Martín region.

MATERIALS AND METHODS

Study Area

The experiment was conducted in the fields of the National Rice Program at the El Porvenir Agricultural Experimental Station belonging to the “Instituto Nacional de Innovación Agraria”- INIA (S: 6°35'50", W: 76°19'30", altitude 219 masl) in Juan Guerra district, province and region of San Martin, Peru (Figure 1), during the dry season from July 2022 to January 2023. The San Martin region experiences maximum temperatures ranging from 35.6 to 36°C and minimum from 12.1°C to 18°C. Additionally, the estimated annual precipitation for San Martin province was approximately 1213 mm. The soil samples from the plots were sent to “Laboratorio de Suelos, Agua y Foliares” (LABSAF) at El Porvenir Agricultural Experimental Station for soil characterization analysis.

The initial soil conditions were pH 7.11, electrical conductivity (EC) 0.14 ds.m^{-1}, cation exchange capacity (CEC) 23 cmol^{+}.kg^{-1}, organic matter (OM) 3.75%, total nitrogen (TN) 0.2%, available phosphorus (P) 17.56 mg.kg^{-1}, potassium (K) 212.23 mg.kg^{-1}, and a clayey texture, of a soil classified as a Vertisol.

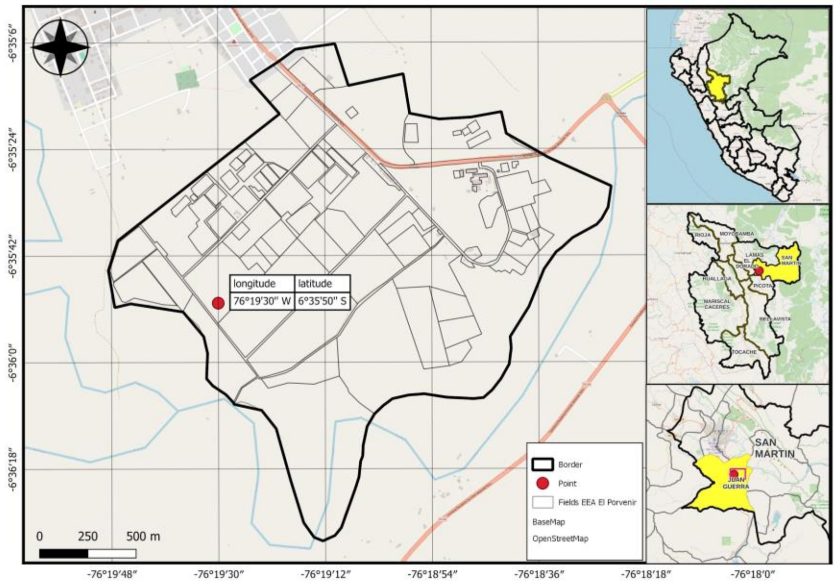

Figure 1. Field experiment area and location in Peru.

Botanical Material

INIA 507 "La Conquista" rice variety was acquired from the National Rice Program - INIA at El Porvenir Agricultural Experimental Station. This variety corresponds to the PNA 2394-F2-EP4-6-6-AM-VC1 lineage obtained through individual pedigree selection. This variety was distributed in 2006 to producers in Peru because it presents resistance to bacteria called *Burkholderia glumae*, an important pathogen in the cultivation of rice (*O. sativa*), being the main causal agent of the disease called bacterial panicle blight (BPB) (Kumar et al., 2023), where many producers registered reductions of more than 70% in their field yields (Galvis and Carrillo, 2015).

C. ensiformis (L.), is a plant shrub of Central American origin, which belongs to the Fabaceae family. It is widely cultivated in tropical and subtropical regions around the world, and it is an annual or biannual herbaceous legume, very rustic, low, with growth erect and determined with a slow onset, reaching 1.2 m in height. It is widely cultivated in tropical countries as a green manure. It also is resistant to variations in environmental conditions, insects, and microorganisms (Silva, 2012). Alternatively, *C. juncea* (L.) is a legume species, fast-growing, with high competition with weeds, and high production of plant biomass, which makes it a significant plant to include in crop studies as green manure. It has been cultivated since the early days of agriculture for its fiber, initially in India. It is widely planted in many areas of the tropics and subtropics. It is among the legumes with the best potential for green cultivation (Ferraço et al., 2019). Figure 2 and Figure 3 depict photographs of *C. ensiformis* and *C. juncea* plants prior and during their incorporation.

Figure 2. Plot photography with green manures before incorporation (from left to right CroJ, Control, CanE).

Figure 3. Plot photography green manures incorporation to the soil.

Field Experiment

The experiment was arranged in a split-plot design with 2 factors and 4 blocks, Figure 4 displays the rice paddies that were utilized in this study, blocking was made joining two horizontal rice paddies, and for factors each block was divided into two sections. For Factor 1, nitrogen fertilization dosage (main plot), a reference dosage of 180 kg of N per hectare (391 kg of urea) was used. The tested fertilization dosages included: 100% (FN100) and 75% (FN75) of the reference dosage. For Factor 2, a type of green manure (subplot), was employed: *C. juncea* (CroJ), *C. ensiformis* (CanE), and no green manure (Control).

Figure 4: Rice field distribution

The green manures were planted after plowing. CanE followed a spacing of 40 x 40 cm, with 3 seeds per hole, while CroJ had a spacing of 30 x 40 cm, with 10 seeds per hole. During the pre-flowering stage, the plants were incorporated using a harrow, and the green manures were left to decompose for 98 days. Rice planting commenced in October using rice seeds from the INIA 507 "La Conquista" variety and was conducted in two stages. In the first, rice seedbeds were prepared, and in the second stage, the seedlings were transplanted to the definitive field.

Crop Management and Evaluation

The preparation of seedbeds involved scattering rice seeds in a pool adjacent to the study area, and the agronomic management of the seedbeds followed local agricultural practices until the plants reached 30 days of growth. For sowing, seeds of pre-germinated rice seeds were employed, then seedbeds were fertilized using urea (200 $kg.ha^{-1}$).

To establish the definitive field, the seedlings were transplanted, placing 4 plants per hill at a spacing of 25 x 25 cm. Agronomic management adhered to local agricultural practices. Fertilization was made using diammonium phosphate, potassium chloride, and magnesium sulfate as phosphorus, potassium, and magnesium sources in doses of 150, 150, and 25 $kg.ha^{-}$

^{1} respectively, also boron was applied in a dose of 25 kg.ha^{-1} and nitrogen was applied in the water layer using urea, following the corresponding nitrogen dosages of 180 kg of N. ha^{-1} (391 kg of urea) and 135 kg of N. ha^{-1} (293.5 kg of urea), divided into two applications at 40 and 55 days after planting, respectively. For the evaluation of rice crop parameters, 10 random samples of 1 m^2 each were taken from the central part of each subplot. The assessed rice parameters included: white leaf virus (RHBV), number of tillers per square meter (NTM), panicle length (PL), number of panicles per square meter (NPM), plant height (PH), panicle fertility (PF), yield (Yield), and paddy grain (PG). The harvest was conducted 140 days after planting. Evaluations on rice parameters were made following the Standard Rice Evaluation System (Rosero, 1983).

Soil Physicochemical Analysis

For soil analysis, composite soil samples were collected at three experiment stages. The initial sampling occurred before the sowing of green manures, the second sampling occurred after the decomposition of green manures and before rice sowing, and the final sampling was conducted post-rice harvest. The methods employed for soil characterization were: EPA 9045D (pH), ISO 11265 Soil Quality (Electrical conductivity), ISO 11261 Soil Quality (Nitrogen), EPA 6020 B (Potassium), NOM-021-RECNAT-2000 AS-09. (Texture), NOM-021-RECNAT-2000 AS-07 (Organic Matter), NOM-021-RECNAT-2000 AS-10 (Phosphorus), EPA 9081 (Cation exchange capacity).

Paddy Grain Proximate Analysis

To determine the amount of dry matter (DM) and nitrogen (N) incorporated by the green manures, plant samples were taken from the central part of each subplot, representing plants grown in a 1 m^2 area. Subsequently, dry weight was determined by weighing oven-dried samples at 60 °C after 72 hours and nitrogen content was determined by the Kjeldhal method.

A composite mixture was taken to “La Molina Calidad Total Laboratorios - UNALM” for rice grain proximate analysis in Lima, Peru. The proximate analysis methods were: NTP 205.006:2017 (1:2018) (Fat), AOAC 945.38(C) Chapter 32, Page 47, 21st Edition 2019 (Ash), NTP 205.003:1980 (Reviewed 2011) (Fiber), NTP 205.005:2018 (Protein), and the digestible carbohydrates were calculated by difference.

Statistical Analysis

Data analysis was performed using the R statistical computing language and environment, version 4.2.1 (R Core Team, 2023), along with the dplyr package (Wickham et al., 2023) and agricolae package (de Mendiburu, 2023). The collected data were processed through ANOVA, and for mean comparison, the Fisher's LSD test was employed. In both tests, a significance level of $p < 0.05$ was considered.

RESULTS

Soil Physicochemical Analysis

In the soil parameter values before installation, pH was neutral (7.11), and not saline because of its low electrical conductivity (1.46 $dS.m^{-1}$), the organic matter (3.75 %), and N content (0.2 %) were medium. The available P was high (17.56 $mg.Kg^{-1}$) and K was medium (212.23 $mg.Kg^{-1}$), CEC (23 $cmol^{+}.Kg^{-1}$) was high and the texture was clayey.
After the incorporation of green manure, the average soil pH was basic (7.6) but similar to the control, and a significant elevation in the electrical conductivity was reported in this stage, the organic matter content was medium as before, and the CEC tended to show a higher value in the treatments with green manure (Figure 5).

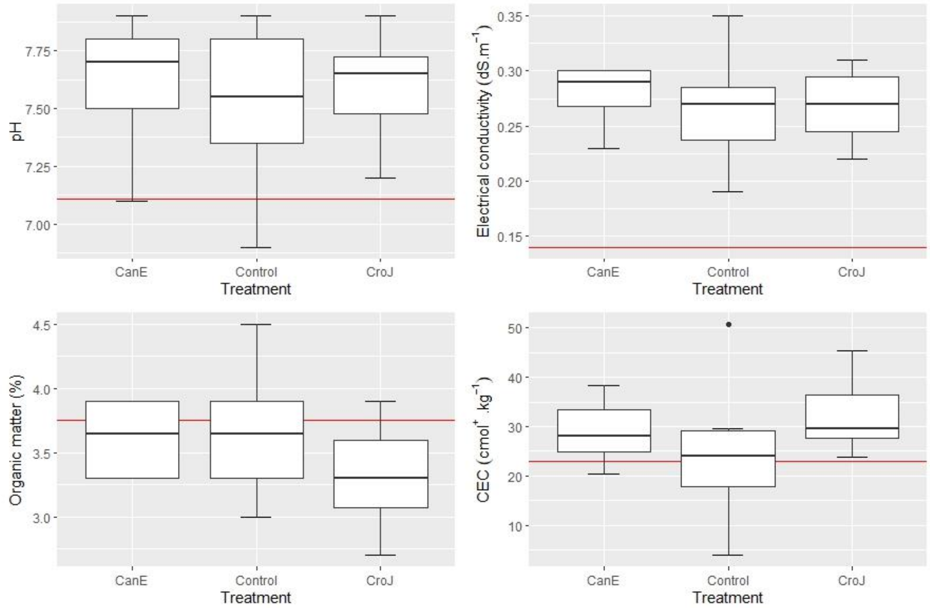

Figure 5. Values of soil pH, electrical conductivity, organic matter, and CEC between treatments after green manure incorporation. *The red line indicates the initial value.

In the evaluation of macronutrients, the available P and K increased concerning the initial value; however, the N content decreased; the results were significant for these last nutrients (Figure 6).

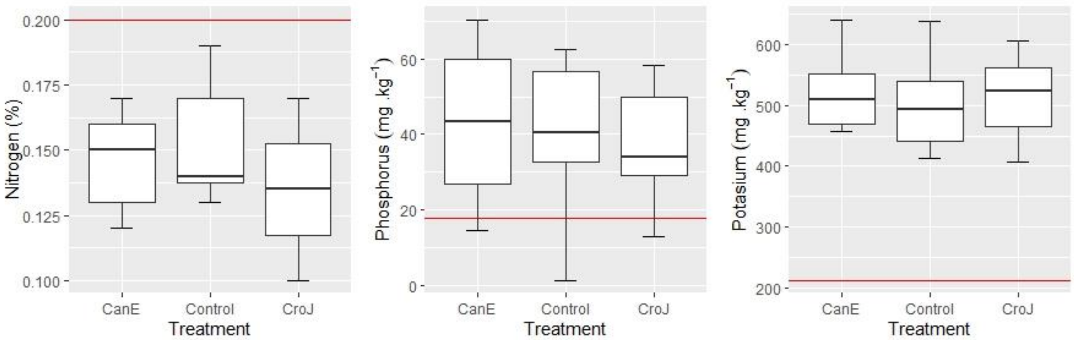

Figure 6. Values of soil macronutrients (N, P, K) between treatments after green manure incorporation. *The red line indicates the initial value.

At the end of the experiment, post-harvest physicochemical analysis showed a basic soil pH with significantly higher values and a tendency to increase EC; the OM percent was medium and exhibited a tendency to decrease (2.1 - 3.0 %), and the CEC showed lower significant values (Figure 7).

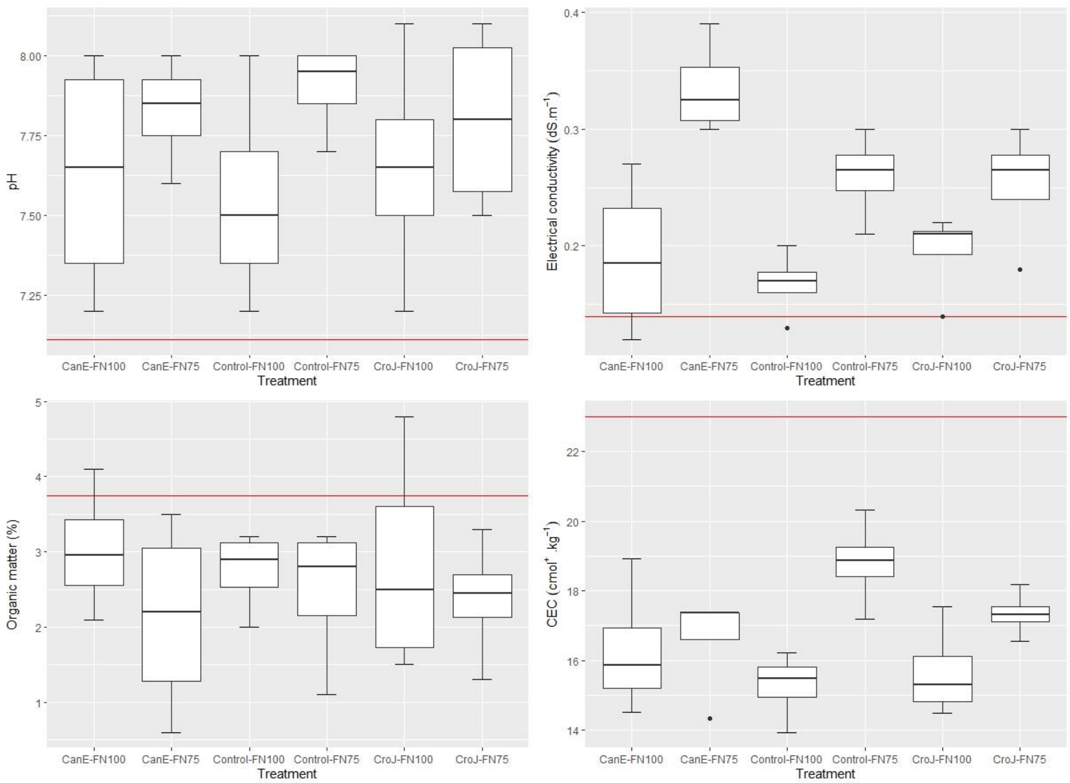

Figure 7. Values post-harvest of soil pH, electrical conductivity, organic matter, and CEC between treatments. *The red line indicates the initial value.

The N content remained similar to values after the incorporation of green manure and the available P decreased compared to the values reported after this incorporation; the available K tended to increase and were higher than the beginning (Figure 8).

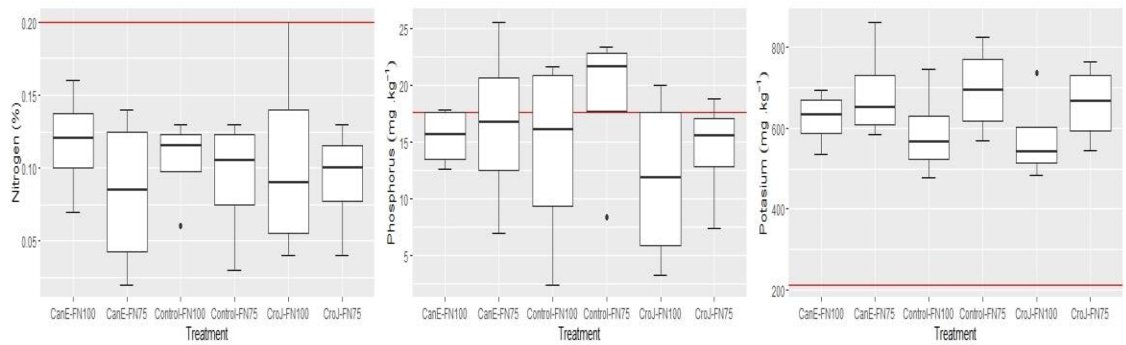

Figure 8. Values of soil macronutrients (N, P, K) post-harvest. *The red line indicates the initial value.

Agronomic Parameters

The Green Manure analysis showed that the average DM of *C. ensiformis* (CanE) was 1.85 $t.ha^{-1}$ and the N content in vegetal tissue was 2.02% contributing with 37.41 kg of $N.ha^{-1}$. Similarly, the average DM of *C. juncea* (CroJ) incorporated into the soil was 3.59 $t.ha^{-1}$, and the N content in plant tissue was 3.61% contributing to 129.35 kg of $N.ha^{-1}$.

About Rice Agronomic Performance, the parameters showed that the tillers affected by RHBV did not reveal significant differences for the subplot effect, main treatment, or interaction ($p < 0.05$). Concerning the use of green manures, the treatment with the highest RHBV incidence was the Control treatment, with an affectation of 7.25% of tillers, followed by CroJ treatment with 6.75%, and CanE treatment with 5.25%. On the other hand, regarding fertilizer dosage, there is a 1.33% higher incidence of the disease in plots with lower fertilizer dosage.

The Analysis of Variance ($p < 0.05$) for the parameters NTM, PL, NPM, PH, Yield, and PG did not show significant differences for the subplot effect, main treatment, or interaction. However, it is important to highlight that, despite the lack of significance, the FN75 treatment exhibited better results than the Control for NTM and NPM parameters, with improvements of 5.00% and 1.88%, respectively. Similarly, relating to the effect of green manures, it can be observed that NTM for CanE and CroJ treatments had 5.93% and 5.57% more tillers than the Control treatment. Likewise, for the NPM parameter, CanE and CroJ treatments increased the number of panicles by 4.15% and 2.50% compared to the Control. Similarly, the yield for CanE and CroJ treatments was 6.04% and 4.96% higher than the Control. In addition, CanE and CroJ treatments were 3.19% and 3.60% more than the Control for the PG parameter. This aligns with a study that concluded that green manures can partially substitute the use of chemical fertilizers without affecting rice production or soil fertility (Chen et al., 2020). Furthermore, another experiment reported that green manures can substitute up to 40% of N fertilization without impacting rice production (Xie et al. 2016). We noted that the plot treated with CanE and 100% of the recommended nitrogen dosage attained the highest grain yield, reaching 8,355.75 $kg.ha^{-1}$. One possible explanation for our results is that applying green manures allowed for biological N fixation in legume roots, resulting in a continuous nutrient supply in the soil solution, ultimately increasing nutrient contribution to rice plants Muntasir et al. (2001).

Table 1. Agronomic parameters in *Oryza sativa.*

Treatment		RHBV (%)	NTM (Tillers. m^{-2})	PL (cm)	NPM (Panicles .m^{-2})	Yield ($kg.ha^{-1}$)	PG ($kg.ha^{-1}$)	PH (cm)	PF (%)
Fertilizer Dose (FD)									
FN100		5.75 ± 2.67	245.71 ± 46.34	25.76 ± 0.85	256.42 ± 15.85	7926.00 ± 999.52	5056.70 ± 297.25	120 ± 0.07	90.5 ± 2.6
FN75		7.08 ± 2.71	258.00 ± 42.19	25.33 ± 0.36	261.25 ± 12.88	7720.78 ± 1169.52	4621.25 ± 810.65	116 ± 0.05	91.8 ± 1.8
Significance level		NS	NS	NS	NS	NS	NS	NS	NS
CV (%)		26.5	16.7	3.4	5.0	13.1	1.1	6.4	1.6
Green Manure (GM)									
CanE		5.25 ± 2.60	256.94 ± 28.50	25.62 ± 0.38	263.72 ± 10.79	8002.67 ± 692.86	4860.75 ± 481.83	120 ± 0.04	90.00 ± 3.2
Control		7.25 ± 2.43	242.56± 53.27	25.67 ± 1.04	253.22 ± 18.22	7546.60 ± 1336.10	4710.57 ± 781.00	118 ± 0.07	91.63 ± 1.6
CroJ		6.75 ± 3.01	256.06 ± 50.03	25.33± 0.72	259.56 ± 12.77	7920.90 ± 1164.04	4880.29 ± 780.05	117 ± 0.09	91.88 ± 1.6
Significance level		**.**	NS	NS	NS	NS	NS	NS	*****
CV (%)		25.0	16.4	2.5	5.9	10.2	19.2	4.1	1.6
Fertilizer Dose x Green Manure (FD: GM)									
CanE	**FN 100**	5.50 ± 3.70	243.38 ± 25.23	25.75 ± 0.36	267.69 ± 11.64	8355.74 ± 599.93	5130.00 ± 379.93	122 ± 0.03	88.5 ± 3.7
	FN 75	5.00 ± 1.41	270.50 ± 27.71	25.49 ± 0.40	259.75 ± 9.71	7649.61 ± 654.06	4591.50 ± 451.70	117 ± 0.02	91.5 ± 1.9
Control	**FN 100**	6.50 ± 2.08	227.75 ± 47.02	25.99 ± 1.25	248.94 ± 11.08	7693.71 ± 1527.44	5047.33 ± 355.78	121 ± 0.04	91.5 ± 1.3

	FN 75	8.00 ± 2.83	257.38 ± 61.85	25.36 ± 0.83	257.50 ± 24.56	7399.49 ± 1332.15	4458.00 ± 968.02	115 ± 0.08	91.75 ± 2.1
CroJ	**FN 100**	5.25 ± 2.63	266.00 ± 63.57	25.57 ± 0.92	267.13 ± 6.84	7728.55 ± 774.57	4968.33 ± 178.67	117 ± 0.12	91.50 ± 1.3
	FN 75	8.25 ± 2.87	246.13 ± 39.19	25.13 ± 0.49	252.00 ± 13.47	8113.24 ± 1569.40	4814.25 ± 1087.25	117 ± 0.06	92.25 ± 2.1
Significance level		NS	NS	NS	NS	NS	NS	NS	NS
CV (%)		25.0	16.4	2.5	5.9	10.2	19.2	4.1	1.6

RHBV: White leaf virus, NTM: Number of tillers per square meter, PL: Panicle length, PG: Paddy grain, PF: Panicle fertility.

The data in the table express the average and standard deviation (μ ± σ) of the evaluated parameters.

Those values with different letters in the same column indicate significant differences between the treatments. ($p < 0.05$).

"**" significant difference $p < 0.01$, "*" significant difference $p < 0.05$, "." significant difference $p < 0.1$, NS no significant difference

Rice Proximate Analysis

The effect of factors of nitrogen fertilization dosage, the type of green manure, and the interaction did not present significant differences in the nutritional analysis of fat, ash, fiber, carbohydrates, and protein content (Table 2).

Table 2. Paddy grain proximate analysis.

Treatment	Fat	Ash	Fiber	Carbohydrate	Protein
			Fertilizer dose (FD)		
FN75	2.05 ±0.3	4.94 ±0.6	9.36 ±0.5	75.15 ±3.8	7.22 ±0.3
FN100	2.15 ±0.2	4.98 ±0.3	9.48 ±0.5	73.87 ±0.4	7.35 ±0.3
			Green Manure (GM)		
Control	2.13 ±0.3	5.05 ±0.4	9.22 ±0.5	74.02 ±0.4	7.13 ±0.2
Canavalia	2.05 ±0.1	4.89 ±0.5	9.38 ±0.5	75.43 ±4.5	7.38 ±0.3
Crotalaria	2.13 ±0.3	4.96 ±0.5	9.63 ±0.5	73.98 ±0.6	7.3 ±0.5

Fertilizer dose x Green Manure (FD:GM)						
FN 75	**Control**	2 ±0.4	5.3 ±0.4	9.03 ±0.3	73.93 ±0.6	7.07 ±0.3
	Canavalia	2.08 ±0.1	4.7 ±0.6	9.45 ±0.5	77.03 ±6.4	7.4 ±0.3
	Crotalaria	2.08 ±0.4	4.9 ±0.8	9.53 ±0.5	74.2 ±0.7	7.15 ±0.4
FN 100	**Control**	2.27 ±0.1	4.8 ±0.2	9.4 ±0.6	74.1 ±0.2	7.2 ±0
	Canavalia	2.03 ±0.2	5.08 ±0.4	9.3 ±0.5	73.83 ±0.6	7.35 ±0.3
	Crotalaria	2.18 ±0.3	5.03 ±0.2	9.73 ±0.6	73.75 ±0.5	7.45 ±0.5

DISCUSSION

Based on the identified variations in the physicochemical parameters of the soil, the pH increase, especially in the harvest phase, may occur from the organic anions present in carboxylic acids commonly found in plant residues resulting in net alkalinization (Rukshana et al., 2014). Also, in the soil, urea fertilizer transforms into ammonium carbonate, potentially leading to a transient elevation in local pH levels that in some cases could be detrimental. Increases in EC after the incorporation of green manure were observed in other studies (Peralta-Antonio et al., 2019). The rise in levels of available P and K, as well as soil exchangeable cations Mg and K, could be attributed to the incorporation of green manure, known for its richness in these nutrients, and then released into the soil (Bressani y Sosa, 1990; Choi et al., 2014; Adekiya et al., 2019; Barbosa et al., 2020). Tropical soils frequently exhibit low productivity due to the susceptibility of some of these soils to significant phosphate fixation, which hinders the availability of phosphorus to plants; soils prone to intense phosphate fixation, typically involving adsorption to oxides and clay minerals, and often necessitate exceptionally high applications of phosphate fertilization to mitigate the impact of phosphate fixation. The presence of organic manures substantially improved the available P in the soil, this heightened availability led to increased P uptake by the plants (Mrudhula et al., 2020).

The soil carbon and nitrogen content are expected to increase through the incorporation of green manure. However, as observed above, with the incorporation of green manure in an unstabilized form, i.e., with labile fractions of C and N, microbial activity in the soil likely increased, resulting in the immobilization of these elements. Consequently, the reduction of available oxygen may have stimulated denitrifying groups, leading to the subsequent loss of N in the form of N_2O (Carter et al., 2014). In previous studies, with the incorporation of green manure, the OM is depleted into substances containing aliphatic and hydrophilic groups, suggesting an accumulation of aromatic residues, which may be associated with a higher fraction of N directly bound to aromatic C. This N would be less bioavailable and could explain a decrease in soil N uptake by crops (Sharma et al., 2017).

OM stands out as a crucial and widely recognized agent for stabilizing aggregates in soil (Bissonnais, 1995), therefore generating electrical charges with which the CEC increased, however, the incorporation of green manure materials is known to be less efficient in increasing aggregate stability due to their lower resistance to decomposition and stabilization compared to other amendments such as farmyard manure and paddy straws (Bandyopadhyay et al., 2010). Nevertheless, inputs of OM remain essential across a broad spectrum of tropical cropping systems; tree leaves, litters, green and farmyard manures, as well as stover and roots from crop residues, sustain and manage short-term supply for soil fertility. These organic inputs and biological processes play a crucial role in maintaining soil fertility in tropical agricultural systems (Palm et al., 2001).

A trend of lower incidence of RHBV was shown with green manures. Sogata (*Tagosodes orizicolus*), is the main pest that affects production in rice plantations, with its bites it causes damage to the plants, and it is also the vector of the White Leaf Virus (RHBV), among the management methods is weed control (Rodríguez Delgado et al., 2018), and respect of that, green manure has been employed to aid in weed control by allelopathic effects, limiting the available space for weed growth and competing for essential resources such as water, light, oxygen, and nutrients, thereby suppressing the potential for reinfestations (Timossi et al., 2011; Recalde et al., 2015; Álvarez-Iglesias et al., 2018). Also, plants used for green manures can affect the community of predatory arthropods, for example, pollen of crotalaria species can feed some predatory arthropods (Venzon et al., 2006), and Canavalia species can create a more balanced ecosystem, promoting biodiversity and providing habitats for these beneficial predators (de Melo et al., 2019).

According to USDA, Canavalia rapidly develops and adapts to tropical conditions with high precipitation, temperatures, and long days. It can produce pesticidal, bactericidal, and fungicidal substances, and it can yield between 5500 and 7000 $kg.ha^{-1}$ of DM (4915-6250 $lb.ac^{-1}$), fixing approximately 187 to 230 kg of $N.ha^{-1}$ per year (167–205 lb $N.ac^{-1}$) (Sheahan, 2012a). Other authors report that applying 9.76 $t.ha^{-1}$ of Canavalia DM contributes 291.25 kg of $N.ha^{-1}$, representing an N concentration of 2.98% in plant tissue (Martín et al., 2007). In our study, N concentrations in Canavalia plant tissue are similar to those reported. However, the N contribution is much lower, as the planting density used was 40 $kg.ha^{-1}$, while Sheahan (2012a) recommends a planting density between 56 to 90 $kg.ha^{-1}$ (50-80 lbs.ac-1). About the use of Crotalaria as green manure, the USDA reports that can incorporate up to 5600 $kg.ha^{-1}$ (5000 $lb.ac^{-1}$) of DM and 134 kg of $N.ha^{-1}$ (120 $lb.ac^{-1}$) when planted at a density of 55 $kg.ha^{-1}$ (49 $lb.ac^{-1}$) to 65 $kg.ha^{-1}$ (58 $lb.ac^{-1}$) (Sheahan, 2012b). The results of this study indicate that the N incorporation into the soil was very similar to the values reported by the USDA. Also, green manure could serve as a replacement for nitrogen (N) and other essential plant nutrients, aiding in the retention of elevated chlorophyll levels in rice (Islam et al., 2019).

We found that the use of CanE and CroJ could increase whole grain rice yield by at least 4.96%, equivalent to an increase in production of 374.5 $kg.ha^{-1}$. Additionally, we observed that the use of CanE and CroJ could improve paddy grain yield by at least 3.19% compared to the control, equivalent to 150.18 kg more paddy grain produced. We obtained better results using Canavalia. However, in contrast to our experiment, another study observed that green manure prepared from Crotalaria yielded better results for rice production. Also, they mentioned that the use of green manure combined with N fertilizers increases grain yield by over 30% compared to conventional fertilization (Ramírez Gómez et al., 1998). Furthermore, in other experiments for Crotalaria, in accompaniment to nitrogen fertilization, they found a significant increase in grain yield compared to chemical fertilization alone up to 9% more (Islam et al., 2019).

In Peru, the overall rice production in 2023 amounted to 8.08 $t.ha^{-1}$, engaging over 70,000 producers. Nevertheless, in the province of San Martín and the district of Juan Guerra, the yield was approximately 7 $t.ha^{-1}$. Besides, according to the reported yields using the INIA 507 variety, another study (Mori, 2014) documented only a yield of 6.6 tons per hectare, surpassing the present experiment by 12 to 27%. Nonetheless, the present study reached 8.36 $t.ha^{-1}$ with the plot treated with CanE and 100% of the recommended nitrogen dosage. It is important to notice that green cover cropping involves cultivating with the primary aim of absorbing soil nitrogen during the winter months to mitigate leaching.

About proximate analysis, there were no notable distinctions detected between treatments utilizing chemical nitrogen fertilization and those employing green fertilizers. Consequently, it can be established that the nutritional quality of rice remains unaffected by the substitution of chemical fertilization. The fat content was around 2.1 %, which is similar to Indian rice (2.463%) and Philippine rice (2.783%) (Ibrahim et al., 2021), and resembles that observed in grains of brown rice (2.47%) (Wang et al., 2006). Concerning the protein content, it was 7.3% on average, close to the values of Mexican cultivars (7.1–11.0%) (Chávez-Murillo et al., 2011), the values observed in non-aromatic rice (6.97-7.17%) (Verma y Srivastav, 2017), and Brazilian variety with high amylose content and long grain (8.5%) (Monks et al., 201). However, another rice variety from the San Martín region ("La Esperanza") exhibited an elevated protein concentration ranging between 9% and 9.48% (Ríos-Ruiz et al., 2020). The established protein content range typically falls between 7% to 8% (Juliano, 1985); consequently, the findings in this study indicate a significantly higher protein content. The fiber had values of 9.4% on average, however, other studies report lower values like 2.4% (Monks et al., 2013). Regarding carbohydrates, values were presented at 74.5% on average, which is lower than what was found in other studies with non-aromatic rice (80.14-81.83%) (Verma and Srivastav, 2017). The rice carbohydrate accumulation is intricately linked to nitrogen nutrition. A substantial nitrogen application significantly reduced the starch content in varieties that respond to low nitrogen by exhibiting droopy characteristics. In contrast, the high-nitrogen-responsive variety, characterized by erect leaves, maintained a relatively high starch content even under heavy nitrogen fertilization. The elevated starch content under high nitrogen levels can serve as a biochemical indicator for the high nitrogen responsiveness of rice varieties (Yoshida and Ahn, 1968).

The technology of green manures not only contributes to environmental benefits but also leads to cost savings on expensive nitrogen fertilizers. A study reports that nitrogen fertilizing savings are among the direct benefits of using cover crops and green manures, besides other indirect costs, such as reduction in the use of pesticides (Sullivan, 2003). Several factors, including the establishment, management, and productivity of the subsequent cash crop, aversion to risk, and characteristics specific to the producer and the farm can influence the profitability of cover crops (Tack and Yu, 2021; Bergtold et al., 2019).

CONCLUSIONS

Throughout the experiment, we observed a significant variation in soil parameters. The elevation in concentrations of available P and K, along with some exchangeable cations, may be ascribed to the application of green manure. The low C and N contents are presumably a product of microbial immobilization, with the subsequent loss of these nutrients through respiration and denitrification processes, or accumulating in aromatic forms. The tendency of lower incidence of RHBV was shown with green fertilizers, through the control of the transmitting vectors, by 2.82% compared to the control treatment. Our findings indicate that employing CanE and CroJ could enhance the yield of whole grain rice by a minimum of 4.96%, and an improvement in paddy grain yield of at least 3.19% when compared to the control. The superior outcomes were achieved through the utilization of Canavalia, and the highest yield was 8.36 t.ha-1 with the CanE - F100 treatment. Finally, concerning the proximal analyses, it can be concluded that the nutritional quality of rice remains unaffected by the green manures or chemical nitrogen fertilization doses.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Supplementary Materials: Supplementary data to this article can be found online at:

https://drive.google.com/drive/u/1/folders/1lF7WswSggiHR0dRJa6SV-oL-pLS1RDM2

Figure 1. Field experiment area and location in Peru.

Figure 2. Field photography of different types of green manure before incorporation

Figure 3. Field photography of incorporation of green manure to the soil

Figure 4. Rice field distribution

Figure 5. Values of soil pH, electrical conductivity, organic matter, and CEC between treatments after green manure incorporation

Figure 6. Values of soil macronutrients (N, P, K) between treatments after green manure incorporation

Figure 7. Values Post Harvest of soil pH, electrical conductivity, organic matter, and CEC between treatments

Figure 8. Values of soil macronutrients (N, P, K) post-harvest

Table 1. Agronomic parameters in *Oriza sativa*

Table 2. Paddy grain proximate analysis