Green manuring and fertilization on rice (*Oryza sativa* **L.): A peruvian Amazon study**

Abonos verdes y fertilización en arroz (*Oryza sativa* **L.): un estudio en la Amazonía peruana**

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Abstract

The study was conducted in Juan Guerra district, province and region of San Martin, Peru; it assessed two treatment sets: (1) nitrogen fertilizer dose (FN75, FN100); (2) green manure *Crotalaria juncea* (CroJ), *Canavalia ensiformis* (CanE), and without green manure. It was arranged in a split-plot design with four replications. During the experiment, we observed an important fluctuation in soil parameters. Notably, there was a decrease in soil carbon and nitrogen levels, likely attributed to microorganism metabolism. On the other hand, we observed that CanE significantly reduced the diseased tillers through "White Leaf Virus" (RHBV) by 2.82% compared to the control, and significant panicle fertility was achieved by CroJ (91.88%). No significant differences were obtained in yields during this first campaign; however, the highest reported yield was 8.36 t ha⁻¹ with the CanE - FN100 treatment. Additionally, the nutritional quality of the rice was not affected by either green manuring or the application of chemical nitrogen fertilization. These findings allow deeper studies to consider strategic alternatives to reducing dependency on inorganic fertilizers among the poorest communities.

Keywords

split-plot • legumes • soil fertility • RHBV • regenerative agriculture

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Resumen

El estudio se realizó en el distrito de Juan Guerra, provincia y región de San Martín, Perú; se evaluaron dos conjuntos de tratamientos: (1) dosis de fertilizante nitrogenado (FN75, FN100); (2) abono verde *Crotalaria juncea* (CroJ), *Canavalia ensiformis* (CanE), y sin abono verde. Se dispuso en un diseño de parcela dividida con cuatro repeticiones. Durante el experimento observamos una fluctuación importante en los parámetros del suelo. Notablemente, hubo un decremento en los niveles de carbono y nitrógeno del suelo, comúnmente atribuidos al metabolismo microbiano. Por otra parte, observamos que CanE redujo significativamente los macollos enfermos por el "Virus de la Hoja Blanca" (RHBV) en un 2,82% en comparación con el control, y CroJ logró una fertilidad de panícula significativa (91,88%). No se obtuvieron diferencias significativas en los rendimientos durante esta primera campaña; sin embargo, el mayor rendimiento reportado fue 8,36 t ha⁻¹ con el tratamiento CanE - FN100. Además, la calidad nutricional del arroz no se vio alterada por los abonos verdes o la fertilización química nitrogenada. Estos alcances permiten ahondar en los estudios para considerar alternativas estratégicas para disminuir la dependencia de los fertilizantes inorgánicos por las comunidades más pobres.

Palabras clave

parcela dividida • leguminosas • fertilidad del suelo • RHBV • agricultura regenerativa

INTRODUCTION

Rice (*Oryza sativa* 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 (9). Moreover, among prevalent cereal grains, it stands out for its exceptional characteristics, boasting the highest net protein utilization and digestible energy levels (64).

In Perú, rice is one of the main crops, with a production of approximately 3,027.41 tons in 2022 (28). However, due to the increase in prices of chemical fertilizers, the cultivation area decreased by 47.2% during the February 2022 campaign (17), and for 2023 production exhibited a variation of -5.7%, experiencing a decline of 24.3% compared to 2021 and a 9.7% decrease compared to 2022 (28). Rice production in Peru is primarily located in the Coast and Amazon regions. Until 2016, over 55% of the national supply came from the Coast; however, since then, the majority has shifted to the Amazon. In 2022, rice production in the Amazon accounted for 50.5% of the national production, with approximately 87% coming from the regions of San Martín, Amazonas, Cajamarca, and Huánuco, with San Martín being the main producing region in Peru, yielding 877 thousand tons in 2022 (41). Nevertheless, farmers dedicated to this cultivation commonly face soil fertility issues, flooding, and phytopathological problems (55). In addition, the most common forms of land tenure are ownership and leased, in both situations the farmer must hire workers and this may minimize productivity. Besides, the leased have higher technical and allocative inefficiency costs (59). 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 (40). Some studies demonstrated that green manures applied to rice-cultivated soils modified microbial abundance and composition, enzymatic activity, chlorophyll content, panicle number, yield, and crude protein content in rice cultivation (61). Others found improved physical soil characteristics (2), and increased dissolved organic matter content in soils (24). Moreover, depending on the nutrient and the fertilization dose, differences can be obtained in the edible part of the crop (21). Keeping the soil surface permanently covered by plants in the vegetative phase or as mulch is the most recommended management to protect and conserve the soil that directly influences the production of various crops (46). In the sense of increasingly using strategies more affordable, safer, and low-impact approach to crop growth, the organic amendments stand out as an alternative as part of a broader sustainable crop management strategy (23). However, it is necessary to extend knowledge of sustainable

techniques to farmers in the San Martin region as an alternative because it allows a reduction in production costs, nitrogen (N) fertilizers, and days, without altering performance. With the application of green manures, the profitability of the crop and agribusiness increases with the economic use of organic natural resources.

This study evaluated the effect of applying green manures prepared from Canavalia (*Canavalia ensiformis* L.) and Crotalaria (*Crotalaria juncea* L.) to improve soil fertility, partially reduce the use of N fertilizers, increase rice crop growth parameters, yield, and rice grain nutritional quality in plots located in Juan Guerra district, San Martín province.

Materials and methods

Study area

The experiment was conducted in the fields of the National Rice Program at the El Porvenir Agricultural Experimental Station - "Instituto Nacional de Innovación Agraria" (INIA) (S: 6°35'50'', W: 76°19'30'', altitude 219 masl) in Juan Guerra district, San Martín province and region, Peru (figure 1A), 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, the estimated annual precipitation was approximately 1213 mm.

Figure 1. Geographical location of the rice paddies (A), and distribution of blocks, main plots, and subplots in the rice fields (B).

Figura 1. Ubicación geográfica de las parcelas de arroz (A), y distribución de los bloques, parcelas principales y subparcelas en el campo de arroz (B).

 The initial sampling showed the following soil conditions: pH 7.11, electrical conductivity (EC) 0.14 ds m⁻¹, cation exchange capacity (CEC) 23 cmol⁺kg⁻¹, organic matter (OM) 37.5g kg⁻¹, total N 2.0 g kg⁻¹, available P 17.56 mg kg⁻¹, and K 212.23 mg kg⁻¹, and a clayey texture, of a soil classified as a Vertisol.

Botanical material

Rice seed, INIA 507 "La Conquista'' variety, was acquired from the National Rice Program (INIA) at El Porvenir Agricultural Experimental Station. It corresponds to the PNA 2394-F2-EP4-6-6-AM-VC1 lineage obtained through individual pedigree selection for the resistance to *Burkholderia glumae*, the main causal agent of bacterial panicle blight (BPB) (36). Alternatively, *C. ensiformis* L. is widely cultivated in tropical and subtropical regions, and it is an annual or biannual herbaceous legume, very rustic, low, growth erect, and determined with a slow onset, reaching 1.2 m in height. It is resistant to variations in environmental conditions, insects, and microorganisms (56). *C. juncea* L. is a fast-growing legume, with high competition with weeds, and plant biomass production.

Field experiment

The experimental field, approximately $15,554$ m² in size, was arranged in a split-plot design with 2 factors and 4 blocks. The blocks I $(3,541 \text{ m}^2)$, II $(4,279 \text{ m}^2)$, III $(3,262 \text{ m}^2)$, and IV $(5,472 \text{ m}^2)$ were divided into main plots by a partition wall. Additionally, the main plots Block1-FN100 (2,068 m²), Block1-FN75 (1,473 m²), Block2-FN100 (1,862 m²), Block2-FN75 $(2,417 \text{ m}^2)$, Block3-FN100 $(1,557 \text{ m}^2)$, Block3-FN75 $(1,705 \text{ m}^2)$, Block4-FN100 $(3,574 \text{ m}^2)$, and Block4-FN75 $(1,898 \text{ m}^2)$ were subdivided into 3 subplots of approximately the same size (figure 1B, page 3). Therefore, the evaluated factors were: Factor 1, N fertilization dosage (main plot), with a reference dosage of 180 kg of N per hectare (391 kg of urea). The tested fertilization dosages included 100% (FN100) and 75% (FN75) of the reference dosage, this fertilization dosages were split into two applications of 50% of the dose at 40 and 55 days after sowing, during the tillering stage; and Factor 2, a type of green manure (subplot), which included *C. juncea* (CroJ), *C. ensiformis* (CanE), and without green manure (Control).

Crops management

The sowing of CanE green manure was done with a spacing of 40 x 40 cm with 3 seeds per hole, while for CroJ a spacing of 30 x 40 cm, with 10 seeds per hole was used. 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 began in October and was conducted in two stages; first rice seedbeds were prepared, and then the seedlings were transplanted to the definitive field.

Preparing the seedbeds involved spreading pre-germinated rice seeds (120 kg) in an adjacent pond (300 m²). Ten days after sowing, lambda-cyhalothrin and thiamethoxam $(0.3 \text{ L} \text{ ha}^{\text{-1}})$ were applied to control pests. The seedbeds were fertilized using urea (200 kg ha⁻¹) twelve days after sowing, and finally, fipronil (0.2 L ha⁻¹) was applied twenty days after sowing for pest control. Four seedlings were transplanted per hill at 25 x 25 cm. Fertilization used diammonium phosphate, potassium chloride, and magnesium sulfate as P, K, and Mg sources in doses of 150, 150, and 25 kg ha⁻¹ respectively. Also, B was applied in a dose of 25 kg ha-1.

Evaluation of the soil fertility

Composite soil samples were collected in three stages. The initial sampling occurred before the sowing of green manures; the second sampling fell out after green manure incorporation, and the final sampling was conducted post-rice harvest. For the soil analysis, the methods were: pH (EPA 9045D), Electrical conductivity (ISO 11265), N (ISO 11261), P (NOM-021-RECNAT-2000 AS-10), K (EPA 6020 B), Texture (NOM-021-RECNAT-2000 AS-09), Organic Matter (NOM-021-RECNAT-2000 AS-07), Cation exchange capacity (EPA 9081). The soil samples analysis was done at "Laboratorio de Suelos, Agua y Foliares" (LABSAF) at EEA El Porvenir (INIA).

Growth and yield parameters evaluation

For the rice evaluation, 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 infection percentage (RHBV), number of tillers per square meter (NTM), panicle length (PL), number of panicles per square meter (NPM), plant height (PH), panicle fertility percentage (PF), yield, and paddy grain (PG) or "unmilled rice" in kg ha⁻¹.

The harvest was conducted 140 days after planting. Evaluations were made following the Standard Evaluation System for Rice (29).

To determine the amount of dry matter (DM) and N incorporated by the green manures, plant samples were taken from the central part of each subplot, representing plants grown in a 1 m² area. Dry weight was determined by weighing oven-dried samples at 60°C after 72 hours and N content was determined by the Kjeldhal method. For rice grain nutritional quality analysis, a composite mixture was taken to "La Molina Calidad Total Laboratorios - UNALM" in Lima, standardized methods were followed (5, 30, 31, 32), and the digestible carbohydrates calculated by difference, *i.e.* 100 percent minus the sum in percent of fat, ash, fiber, and protein.

Statistical analysis

Data analysis was performed using the R statistical computing language and environment, version 4.2.1 (2023), along with the dplyr package (63), and agricolae package (19). The collected data were processed through a two-way ANOVA: green manure, N dose, and its interaction, 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

After green manure incorporation, the soil pH values were basic including the control, the organic matter content was medium as before. In the evaluation of macronutrients, the N content decreased, but the available P and K increased, and the CEC tended to show higher values with green manure. Nevertheless, the results were significant only for the N and K nutrients (figure 2).

* The red line indicates the value at the initial soil sampling $(p < 0.05)$. * La línea roja indica el valor en el muestreo de suelo inicial (p < 0,05).

The post-harvest physicochemical analysis showed that pH, N, K, and CEC values were statistically different compared to the initial sampling; it was found basic soil pH with significantly higher values, the OM percent was medium and exhibited a tendency to decrease (2.1 - 3.0 %), the N decreased in significant content, the available P showed similar values, the available K increased significantly, and the CEC showed meaningful lower values (figure 3).

*The red line indicates the value at the initial soil sampling $(p < 0.05)$. *La línea roja indica el valor en el muestreo de suelo inicial ($p < 0.05$).

Figure 3. Post-harvest soil parameters. **Figura 3.** Parámetros del suelo poscosecha.

Green manure and rice growth/yield parameters

The green manure analysis showed that the average DM of CanE was 1.85 t ha⁻¹ and the N content in vegetal tissue was 2.02% contributing to 37.41 kg of N ha⁻¹. Similarly, the average DM of 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. Parameters evaluated in *O. sativa* showed that the tillers affected by RHBV did not reveal significant differences for the fertilizer effect or interaction ($p < 0.05$), however, concerning green manures, the treatment with the lowest RHBV incidence was CanE. The Analysis of Variance ($p < 0.05$) for the parameters NTM, PL, NPM, PH, Yield, and PG did not show significant differences for the fertilizer effect, green manure treatment, or interaction. However, it is important to mention that, despite the lack of significance, the FN75 treatment exhibited better results than the Control for NTM and NPM parameters, with 5.00% and 1.88% increments, respectively. Similarly, relating to the effect of green manures, it could 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. We noted that the plot treated with CanE and 100% of the recommended N dosage attained the highest grain yield, reaching 8.355.75 kg ha⁻¹. Nevertheless, it was not statistically different(table 1, page 7).

Table 1. Agronomic parameters in *Oryza sativa*. **Tabla 1.** Parámetros agronómicos en *Oryza sativa*.

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.

RHBV: Virus de hoja blanca, NTM: Número de macollos por metro cuadrado, PL: Longitud de panícula, PG: Grano de arroz, PF: Fertilidad de panícula. Los datos de la tabla expresan el promedio y desviación estándar (μ ± σ) de los parámetros evaluados. Aquellos valores con letras diferentes en la misma columna indican diferencias significativas entre los tratamientos. (p < 0,05). "**" diferencia significativa p < 0,01, "*" diferencia significativa p < 0,05, "." diferencia significativa p < 0,1, NS no diferencia significativa.

Rice grain nutritional quality

The effect of factors of N 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, page 8).

Table 2. Rice grain nutritional quality. **Table 2.** Calidad nutricional del grano de arroz.

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

Los datos de la tabla expresan la media y la desviación estándar (μ ± σ) de los parámetros evaluados.

Discussion

Regarding the soil physicochemical analysis, in terms of pH, we can observe a significant increase in values, especially during the harvest phase, which might be attributed to organic anions in carboxylic acids commonly found in plant residues, resulting in a net alkalinization of soils (52). Also, urea transformation into ammonium carbonate potentially leads to a transient elevation in pH levels (50).

On the other hand, the soil C and N contents are expected to increase with the incorporation of green manure, because, the C: N ratios between 9.4 and 22.7 favor a mineralization process (15), and for CroJ and CanE are approximately 21.7 ± 0.5 and 14 ± 4 , respectively (11, 22). However, we observed a statistically significant decrease in N after green manure incorporation and rice post-harvest. Some explanations could be that N losses rise when soil mineral N concentrations are high when supply surpasses crop demand. Excess mineral N from decomposed green manures can be lost through leaching as nitrate (NO³⁻) and emitted as the greenhouse gas nitrous oxide (N₂O) (62). Labile fractions of C and N increase soil microbial activity, therefore, the reduction of available oxygen caused by this increase may stimulate denitrifying groups, leading to the subsequent loss of N in the form of N₂O (13). When the conditions for the mineralization of soil organic carbon are met this will lead to a high availability of easily available N and degradable C, these conditions provide hot moments for high N_2 O fluxes. The management of vegetable crop residues and soil type significantly influences N_2O and NH_3 emissions, fine-textured soils, such as this research, tend to produce higher N_2O but lower NH_3 emissions than coarse-textured soils. Also, incorporating crop residues by plowing increases N_2O emissions (45). In addition, mineral N from the mineralization of soil organic matter (SOM) and plant residues in combination with periods of bare soil or sparse plant growth and precipitation surplus provide drivers for leaching (26). In other ways, the recalcitrant fraction of organic matter may bind nitrogen to aromatic carbon, reducing availability (53). Therefore, mainly mineral N released by legumes was susceptible to loss processes like soil denitrification and leaching, with the reduction in residual green manure N in the soil and the increase in cumulative N loss (37).

Organic matter is essential for stabilizing soil aggregates (35), in this sense, cover crops contribute to soil carbon stocks, v.g. they could increase their concentration by up to 12% $(1.11 \text{ Mg C} \text{ ha}^{-1})$ compared to a control treatment without cover crops (39). However, they are less effective in enhancing aggregate stability than farmyard manure and paddy straws due to their lower resistance to decomposition and stabilization (6). Also, non-conservationist cultivation practices can cause nutrient and C losses (25). In the present research, there was no significant difference in organic matter content, also in soils with high SOM, like this research, the existing organic matter already meets the nutrient requirements for grain crops, so additional organic matter does not significantly boost yields despite increasing SOM content. In addition, some authors reported that leguminous green manures did not increase grain crop yield significantly when SOM exceeded 3 g 100 $g⁻¹$, such as the value of initial sampling (38). Our results indicated that the green manures did not alter SOC, suggesting that it is not sensitive to short-term changes in soil quality. This finding highlights the need for a longer evaluation period to observe significant changes in SOM (14). Conversely, the increase in available K was statistically significant, this could be attributed to the high content of these and other nutrients in green manures, which are then released into the soil (1, 7, 16, 44, 57).

Sogata (*Tagosodes orizicolus*), is the main pest that affects rice production and transmits RHBV. A lower incidence of RHBV was shown with green manures because they control weeds, a strategy for plague management (51). Green manures have been employed by allelopathic effects, limiting the available space for weed growth and competing for essential resources such as water, light, oxygen, and nutrients, suppressing the potential for reinfestations (4, 49). Early studies showed that plants belonging to Crotalaria and Canavalia genera exhibited high predator diversity, and can create a more balanced ecosystem, promoting biodiversity and providing habitats for these beneficial predators (8, 18).

The PF (filled grains per panicle) was statistically significant for CroJ, which is an important factor for achieving good yields, and climatic conditions can be the reason for the formation of a higher number of grains (20).

The present research reached an 8.36 t ha⁻¹ yield with the plot treated with CanE and 100% N dosage. However, it was not significant in the experiment, it is important to expose that in Peru, rice production in 2023 amounted to 8.2 t ha⁻¹, while in the province of San Martín and the district of Juan Guerra, the yield was approximately 7 t ha⁻¹ (42). Besides, according to the reported yields using the INIA 507 variety, another study documented only a yield of 6.6 t ha⁻¹ (27).

There were no notable distinctions in proximate analysis between treatments utilizing chemical N 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 $(2.783%)$ (27) . Concerning the protein content, it was 7.3% on average, close to the values of Mexican cultivars (6.8%) (3), the values observed in non-aromatic rice (6.97-7.17%) (60), and Brazilian variety with high amylose content and long grain (8.5%) (43). However, another rice variety from the San Martín region ("La Esperanza") exhibited an elevated protein concentration ranging between 9% and 9.48% (50). The established protein content range typically falls between 7% and 8% (34), consequently, the findings indicate a significantly higher protein content. The fiber had values of 9.4% on average, however, other studies report lower values like 2.4% (43). Regarding carbohydrates, values were presented at 74.5% on average, which is lower than other studies with non-aromatic rice (80.14-81.83%) (60), about this, N fertilizer rates can influence the concentration of non-structural carbohydrates at the filling stage (12).

The technology of green manures contributes to environmental benefits and their long-term application has proven to be economically advantageous (54). Other experiments that combined with N fertilization, showed a significant 9% increase in grain yield compared to using only chemical fertilization (33). However, establishment, management, and productivity of the subsequent cash crop influence the profitability of cover crops. It is also important to state aversion to risk, and characteristics specific to the producer and the farm can affect profit (10, 58).

Conclusions

Green manure biomass incorporation influenced soil physicochemical properties. Particularly, soil pH, P, K, and CEC increased, while N and OM declined. A lower incidence of RHBV was shown with green manures, and CroJ achieved a significant PF. Nevertheless, no significant differences were obtained in yields during this campaign, the superior outcomes were achieved through CanE, and the highest yield was 8.36 t ha⁻¹ with the CanE - FN100 treatment. Concerning the proximal analysis, it can be concluded that the nutritional quality of rice remains unaffected by replacing chemical nitrogen fertilization with green manure fertilization.

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