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Impacto de la rotación de cultivos sobre la nutrición nitrogenada y el rendimiento del arroz en Uruguay

Guillermo Santiago Fabini Ameixeiras

Magíster en Ciencias Agrarias

Opción Ciencias del Suelo

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Resumen

En los sistemas arroceros irrigados del Cono Sur, el arroz ha rotado históricamente con pasturas. En Uruguay los promedios nacionales superan las 8 t ha⁻¹ con dosis de fertilización menores a 100 kg N ha⁻¹. Sin embargo, la intensificación agrícola ha

acortado las fases forrajeras e incrementado la frecuencia del arroz y la soja, lo que podría comprometer la sostenibilidad del sistema. Este estudio comparó tres rotaciones contrastantes: arroz continuo (CR), arroz-soja (RS) y arroz-pastura (RP), distinguiendo entre el primer (R1PP) y segundo arroz (R2PP) después de pastura. Se evaluaron rendimiento, absorción de N y eficiencia en el uso del nitrógeno (EUN) con y sin fertilización, integrando además curvas de dilución crítica y resinas de intercambio catiónico (RIC) para estudiar la dinámica y disponibilidad de N a lo largo del ciclo. Con 100 kg N ha⁻¹, R1PP y RS alcanzaron rendimientos cercanos a 12,8 t ha⁻¹, con lo que superaron en un 28 % a R2PP y CR. R1PP y RS también presentaron las mayores absorciones de N (130 kg ha⁻¹), un 35,6 % superior a CR y R2PP. Sin fertilización, R1PP mantuvo rendimientos altos (11 t ha⁻¹), lo que produce bajos indicadores de eficiencia por unidad de fertilizante aplicado, excepto en el factor parcial de productividad (PFP). Por el contrario, RS (que obtuvo bajo rendimiento sin fertilizar) mostró la mayor respuesta relativa a la fertilización, con niveles de rendimiento y absorción equivalentes a R1PP, siendo el más eficiente en el uso de fertilizante. Las curvas de dilución reflejaron estos patrones: CR permaneció por debajo del nivel crítico durante todo el ciclo, mientras que RS y R1PP se mantuvieron cercanos o por encima del umbral, lo que indica un buen estatus nutricional. Las RIC captaron un pico de disponibilidad de NH₄⁺ hacia los 33 días después de la siembra, pero subestimaron la absorción real por parte del cultivo. Considerando todos los indicadores, se puede concluir que el arroz sembrado sobre arroz genera deficiencias más allá de las nutricionales o las generadas por los factores reductores. En cambio, el legado de las pasturas por sí solo llega a cubrir esas deficiencias y superar el cultivo sobre arroz fertilizado en todos los indicadores medidos.

Palabras clave: rotaciones arroceras, eficiencia de uso de nitrógeno, nutrición nitrogenada, rendimiento en arroz, curvas de dilución

Impact of Crop Rotation on Nitrogen Nutrition and Yield of Irrigated Rice in

Uruguay

Summary

In irrigated rice systems of the Southern Cone, rice has historically been rotated with pastures. In Uruguay, national yield averages exceed 8 t ha⁻¹ with fertilization rates below 100 kg N ha⁻¹. However, agricultural intensification has shortened pasture phases and increased the frequency of rice and soybean, potentially compromising the sustainability of the system. This study compared three contrasting rotations: continuous rice (CR), rice-soybean (RS) and rice-pasture (RP), distinguishing between the first (R1PP) and second (R2PP) rice crop after pasture. Yield, N uptake and nitrogen use efficiency (NUE) were evaluated with and without fertilization. Additionally, critical dilution curves and cation exchange resins (CER) were used to study N dynamics and availability throughout the crop cycle. With 100 kg N ha⁻¹, R1PP and RS reached yields close to 12.8 t ha⁻¹, exceeding R2PP and CR by 28%. R1PP and RS also showed the highest N uptake (130 kg ha⁻¹), 35.6% higher than CR and R2PP. Without fertilization, R1PP maintained high yields (11 t ha⁻¹), which in fertilized plots results in low efficiency indicators per unit of fertilizer, except for the partial factor productivity (PFP). In contrast, RS (which showed low yield without fertilization) exhibited the highest relative response to fertilization, achieving yield and N uptake equivalent to R1PP, and was the most efficient in fertilizer use. Dilution curves reflected these patterns: CR remained below the critical level throughout the cycle, while RS and R1PP remained close to or above the threshold, indicating good N status. CER detected a peak in NH₄⁺ availability around 33 days after sowing but underestimated the actual crop uptake. Considering all indicators, it can be concluded that rice grown on rice generates limitations beyond nutritional or phytopathological issues. In contrast, the legacy of pastures alone can offset these limitations and outperform fertilized rice-on-rice in all measured indicators.

Keywords: rice rotations, nitrogen use efficiency, nitrogen nutrition, rice yield, dilution curves

1. Introducción

El nitrógeno (N) es ampliamente reconocido como el nutriente más limitante en los sistemas arroceros, por lo que su manejo eficiente constituye un componente clave para avanzar en la intensificación sostenible de la agricultura (Fageria y Carvalho, 2014; Kumar et al., 2018; Oyange et al., 2019). Los fertilizantes se aplican para compensar los déficits de fertilidad natural del suelo en cuanto a requerimientos de N y así alcanzar altos rendimientos. La fertilización en arroz representa más del 14 % del uso global de fertilizantes (Bijay-Singh y Singh, 2017) y presenta la menor eficiencia de uso del nitrógeno (EUN) entre los cereales. Se estima que entre el 50 % y el 70 % del N aplicado se pierde, especialmente en condiciones de riego, donde la volatilización constituye la principal vía de pérdida (Cameron et al., 2013; Rose et al., 2022; Zhang et al., 2015, 2018). Estas ineficiencias generan serias preocupaciones, particularmente en sistemas de alto rendimiento que aplican entre 120 y 200 kg N ha⁻¹, debido a los costos ambientales y económicos asociados con las pérdidas de N.

1.1. Eficiencia en el uso del nitrógeno y manejo del sistema

Diversas estrategias se han implementado para mejorar la EUN en arroz, incluyendo la optimización del momento y la división de las aplicaciones (Ishfaq et al., 2020), métodos de colocación (Sharma, 2021), mejoras en el manejo del agua (Wang Zhang et al., 2016) y el uso de fertilizantes más eficientes (Fageria y Carvalho, 2014; Lee, 2021). Sin embargo, la recuperación del N aplicado por el cultivo suele mantenerse en torno al 50 % (Rose et al., 2022). Lograr una mejor EUN depende no solo de los métodos de aplicación, sino también de comprender cómo el cultivo absorbe N a lo largo del tiempo y cuánto puede proveer el suelo mediante mineralización. Esto es particularmente crítico en sistemas arroceros inundados y diversificados, donde las condiciones anaeróbicas limitan la mineralización y modifican los procesos microbianos, lo que ocasiona pérdidas a través de emisiones de N₂, N₂O y NH₃ (Kögel-Knabner et al., 2010; Sahrawat, 2004; Wei et al., 2021).

La absorción de N en arroz generalmente sigue una curva de dilución, donde la concentración de N en los tejidos disminuye a medida que se acumula biomasa. El modelo de dilución crítica de N (Lemaire et al., 1984) ofrece un marco práctico para evaluar las relaciones entre la demanda de N del cultivo y la oferta disponible. Cuando estas curvas están bien calibradas, proporcionan información sobre si el crecimiento del arroz está limitado en N a lo largo del ciclo y ayudan a identificar estrategias de manejo para optimizar el uso de insumos (Ata-Ul-Karim et al., 2016, 2017; Justes et al., 1994). Sin embargo, existe información limitada sobre cómo se comportan estas curvas en sistemas arroceros inundados bajo diferentes rotaciones y niveles de fertilización.

La rotación de cultivos desempeña un papel fundamental en la capacidad del suelo para suministrar nitrógeno, especialmente a través de su influencia en la dinámica de la materia orgánica. Las rotaciones que incluyen pasturas anuales o perennes mejoran el contenido de carbono orgánico del suelo, la biomasa microbiana y la estructura del suelo, lo que contribuye a una mayor capacidad de mineralización de N (Angus et al., 2006; Ernst et al., 2018; Pravia et al., 2019). Estos beneficios son importantes no solo para sostener el rendimiento, sino también para reducir la dependencia de los fertilizantes. Las rotaciones también mejoran la capacidad de amortiguación, la retención de agua y el ciclo de nutrientes, lo que a su vez aumenta la resiliencia del sistema y su salud general (Castillo et al., 2023; Yang et al., 2022). Sin embargo, la mayoría de los enfoques de manejo de nutrientes siguen centrados principalmente en la aplicación de fertilizantes, a menudo pasando por alto los efectos residuales de la rotación. Es necesario adoptar una perspectiva más integrada que considere tanto la demanda del cultivo como el historial de rotación para optimizar la eficiencia de los fertilizantes y mantener la fertilidad del suelo a largo plazo en el camino hacia una intensificación sostenible.

Medir el suministro de N del suelo vía mineralización en condiciones inundadas sigue siendo un desafío metodológico. Las resinas de intercambio iónico (RII), especialmente las resinas de intercambio catiónico (RIC), pueden ser herramientas útiles para estimar el N disponible para la planta al simular la absorción radicular (Dobermann et al., 1998; Pratt, 1951). Aunque las resinas aniónicas son

menos efectivas en ambientes anaeróbicos donde el amonio (NH_4^+) es la forma dominante de N, las RIC han mostrado éxito en la cuantificación de la disponibilidad de NH_4^+ en suelos arroceros inundados (Ding y Sartaj, 2016; Savant y De Datta, 1982).

1.2. El arroz en Uruguay

En Uruguay, el arroz irrigado generalmente se siembra en seco, se maneja en seco hasta V4 y luego se fertiliza con N antes de la inundación (Pittelkow et al., 2016). Aunque las dosis de N rondan en promedio los 80 kg N ha⁻¹ (menores que en muchos sistemas), los rendimientos nacionales promedian 9 Mg ha⁻¹ (DIEA Estadísticas Agropecuarias, 2023), entre los más altos del mundo. Este nivel de productividad está estrechamente relacionado con el uso extensivo de rotaciones arroz-pastura, que representan cerca de la mitad del área arrocera y se sabe que mejoran la calidad del suelo, la dinámica de nutrientes y la sostenibilidad del sistema (Baethgen et al., 2020; Castillo et al., 2021; Macedo et al., 2021; Ribas et al., 2021). Las contribuciones ya mencionadas que hacen las pasturas a los sistemas pueden reemplazar parcialmente los aportes de N sintético y, por ende, mejora la EUN. Sin embargo, la intensificación agrícola en América del Sur ha acortado las fases de pastura e incrementado la frecuencia de cultivos, a la vez que se incorpora soja (*Glycine max*) en los sistemas tradicionales. Este cambio ha incrementado los requerimientos de fertilización y modificado la dinámica de EUN, erosionando algunos beneficios aportados por las rotaciones agrícolas-ganaderas de larga data (Castillo et al., 2023; Macedo et al., 2022; Molina y Terra, 2023). Mientras que los rendimientos aumentaron un 45 %, el uso de N fertilizante creció un 88 %, lo que redujo la productividad parcial del factor de 124 kg a 96 kg de grano por kilo de N (Castillo et al., 2021; Tseng et al., 2021).

Para comprender y abordar mejor estos desafíos emergentes, esta tesis se centra en dos líneas complementarias de investigación basadas en datos de un ensayo de campo de largo plazo. La primera analiza cómo varían la EUN y las respuestas de rendimiento entre diferentes rotaciones basadas en arroz (con pastura, soja o arroz continuo) y distintos niveles de fertilización, buscando identificar caminos hacia una

intensificación más sostenible. La segunda explora la dinámica de absorción de N y el suministro de N del suelo mediante curvas críticas de dilución de N y mediciones con RIC para evaluar el N mineralizado y absorbido bajo condiciones de inundación. En conjunto, estos estudios buscan profundizar la comprensión de las interacciones suelo-cultivo en torno al N y contribuir a estrategias de manejo más eficientes y sostenibles en sistemas arroceros templados de alto rendimiento.

1.3. Objetivos

De manera general, el objetivo de este trabajo fue evaluar el efecto de distintas rotaciones de cultivo sobre el rendimiento, la absorción de nitrógeno y la eficiencia en el uso del nitrógeno en arroz, particularmente a través de la comparación de los indicadores de eficiencia de uso del N (NUE) en sus distintas componentes bajo los distintos sistemas de rotación y fertilización, caracterizando la oferta de N del suelo bajo diferentes rotaciones (continuo arroz, arroz-soja, arroz-pastura) mediante indicadores como resinas de intercambio catiónico y la absorción de N por parte del cultivo de arroz, apoyándose en las curvas críticas de dilución para determinar diferencias en la dinámica de absorción de N.

2. Rotation system and nitrogen fertilization shape rice yield and nitrogen use efficiency in temperate South America

Fabini, G.; Terra, J. A.; Grahmann, K.; Castillo, J.; Gonzalez, P.

CORE IDEAS

Without N fertilization, seeding rice after perennial pastures or soy results in higher yields compared to rice after rice

Rice after perennial pastures obtained highest yields and were not outperformed by rice sown after rice with the highest N dose

N doses above 100 kg ha⁻¹ did not have higher yields nor impacted any other measure taken from rice sown after rice

2.1. Abstract

Rice rotation systems involving pastures, livestock and/or other crops are common in temperate South America, sustaining high yields with low nitrogen fertilizer inputs. However, the legacy effects of perennial pastures on irrigated rice yield and nitrogen use efficiency (NUE) and their interaction with nitrogen fertilization, particularly compared with more intensive systems, remain poorly understood. We evaluated the effects of three rotations systems and four nitrogen fertilizer rates (0, 50, 100 and 150 kg N ha⁻¹) on rice yield and NUE over two years in a long-term experiment in Uruguay. The rotations were continuous rice (CR), rice-soybean (RS) and rice-perennial pasture (RPP), which included two rice phases (R₁PP and R₂PP). NUE indices included partial factor productivity (PFP), agronomic efficiency (AE), apparent N recovery (RE_{fertN}) and utilization efficiency (NUtE). Most rice N uptake across sequences was soil-derived, particularly in R₁PP (82%), where yield (11.05 Mg ha⁻¹) and N uptake (147 kg ha⁻¹) without fertilizer were 38% and 55% higher, respectively, than in CR and R₂PP. At the optimal fertilization rate (100 kg N ha⁻¹), R₁PP and RS yielded 12.86 Mg ha⁻¹ and absorbed 177 kg N ha⁻¹, representing increases of 27% and 42% compared to CR and R₂PP. R₁PP and RS showed the highest PFP, while RS also exhibited the greatest AE and RE_{fertN}. Further research on rice nitrogen uptake and soil mineralization pathways is needed to better

understand responses. While RPP reduces fertilizer dependence allowing high yield, its benefits are mostly limited to R₁PP, positioning soybeans as a promising alternative between R₁PP and R₂PP.

Keywords: agricultural efficiency, partial factor of productivity, nitrogen fertilization, sustainable intensification, residual nitrogen

2.2. Introduction

Nitrogen (N) is the most yield-limiting nutrient in rice production (Fageria & Carvalho, 2014; Kumar et al., 2018; Oyange et al., 2019). Fertilizers are applied to enhance natural soil fertility and fulfill the nitrogen requirements of a high-yielding crop. However, not all applied nitrogen translates into increased yield due to several inefficiencies. It is estimated that, globally, 70% of the N applied to rice is lost (Udvardi et al., 2021), making it the cereal with the lowest NUE (Zhang et al., 2015).

Enhancing rice NUE implies boosting nitrogen absorption by the crop while simultaneously minimizing potential losses to the environment (Choudhury & Kennedy, 2005; Cameron et al., 2013; Bowles et al., 2018). There are several categories of NUE indices grouped by denominator, some of which are based on fertilizer data, while others are grounded in soil, plant, or the production system data (Congreves et al., 2021). The choice of appropriated NUE indexes is important for accurately interpreting and drawing conclusions from a cropping system analysis. A common fertilizer-based NUE index is the recovery efficiency of fertilizer N (REfertN), which measures the proportion of applied N uptaken by the crop (Congreves et al., 2021). This index widely varies between regions depending mostly on agricultural practices. For example, REfertN can be limited to 20% when high N fertilizer rates are not split and irrigation management is deficient (Bijay-Singh & Singh, 2017). This may occur in certain regions of China, where N rates near 200 kg ha⁻¹ are commonly used (Chen et al., 2015), exceeding crop absorption and leading to significant losses through volatilization, leaching and denitrification. While low nitrogen doses can result in high REfertN by mining natural soil N, it is possible to achieve a REfertN of 70% under optimal conditions while preserving soil nitrogen content (Bijay-Singh & Singh, 2017). Significant research efforts have been done on

enhancing NUE in rice cropping, exploring different strategies through crop breeding (Chen et al., 2015), irrigation water management (Wang, Zhang et al., 2016; Santiago-Arenas et al., 2021), nutrient management (Li et al., 2018A; Li et al., 2018B; Wang et al., 2021) and crop rotation (Hu et al., 2018; Denardin et al., 2020; Castillo et al., 2023).

Integrated crop-livestock systems are useful to sustain productivity, preserve soil quality, enhance ecosystem services, bolster resilience, optimize profits and mitigate climate change (Ernst et al., 2018; Grahmann et al., 2020; Landers et al., 2020, Carvalho et al., 2021; Yang et al., 2022). Rice-pasture rotations are usual in temperate South America, contributing to boosting yields, improving eco-efficiency and reducing the environmental footprint (Pittelkow et al., 2016; Castillo et al., 2021; Macedo et al., 2022). In the current century, rice productivity has risen from 5.7 to 8.4 Mg ha⁻¹ in Southern Brazil (IRGA, 2023) and from 6.2 to 9.0 Mg ha⁻¹ in Uruguay (DIEA Estadísticas Agropecuarias, 2023). In this context, the high productivity and efficient use of nitrogen in rice rotating with pastures is remarkable (Denardin et al., 2020; Castillo et al., 2021). In these systems, rice is typically drill seeded and nitrogen fertilization is commonly split, with about 75% applied at crop tillering on dry soil just prior flooding and the remaining 25% at panicle initiation under flooded conditions. Adoption of integrated rice-livestock systems in Southern Brazil, just grazing annual winter pastures, enhanced soil biochemical functioning and nutrient cycling, increasing the NUE and reducing the need of nitrogen fertilizer compared with continuous rice cropping (Carlos et al., 2020; Denardin et al., 2020; Denardin et al., 2025). A country-level analysis of prevalent rice-livestock systems supported by perennial pastures in Uruguay found NUE values of 66% for rice, 13% for livestock, and 23% for the overall system, with slightly positive nitrogen balances in both subsystems (Castillo et al., 2021).

However, there are concerns regarding the emerging intensification and specialization processes in rice-livestock agroecosystems leading to a decline in their associated benefits and an increase in inputs, particularly nitrogen (Carvalho et al., 2021; Castillo et al., 2021; Castillo et al., 2023). In Uruguay, these systems were gradually intensified during this century by reducing the pasture phase, increasing

the frequency of rice and, recently, including soybeans in the rotation (Macedo et al., 2022, Molina & Terra, 2023). During this period, rice yield increased by 45%, but nitrogen fertilization rate, identified as one of the main factors explained the yield gap (Tseng et al., 2021), escalated by 88%, rising from 50 kg N ha⁻¹ to 94 kg N ha⁻¹ (Castillo et al., 2021; Molina & Terra, 2023). Thus, the partial factor productivity for N fertilizer at the national level, relatively stable during decades of yield increase (Pittelkow et al., 2016), dropped from 124 to 96 kg of grain per kg of N applied. Although rice yield is higher when rotated with soybeans compared to continuous rice (Ribas et al., 2021; Denardin et al., 2022; Macedo et al., 2022), it does not differ from that in rotation with perennial pastures and may be associated with negative N balances in the system (Castillo et al., 2023).

Improving NUE in rice cropping is a global challenge, and several NUE indices have proven to be robust measures to compare and rank different cropping practices and inputs at a short-term basis regarding their sustainability. However, the understanding of rice yield responses to nitrogen fertilization within contrasting rice rotation systems -combining perennial pastures, cover crops and other crops- remains limited. Long-term experiments are key sites that provide critical insights into the enduring effects of cropping sequence design and nutrient management on productivity, eco-efficiency and soil quality (Johnston & Poulton, 2018). We hypothesize that yield responses to nitrogen fertilization are higher in more intensive cropping systems, particularly in rice-rice sequences compared to rice following pastures or soybeans, while NUE and overall yield are lower. Thus, the objective was to determine the relative and interactive effects of three rice-based rotation systems and four N fertilization rates on rice yield and N use efficiency indicators in a long-term field experiment. Our results may provide sustainable rotation intensification pathways and N fertilizer inputs management to enhance rice productivity and NUE in high-yielding rice systems integrating pastures and other crops in temperate regions.

2.3. Materials and Methods

2.3.1. Site Description

The study was conducted in selected plots within a long-term experiment (LTE) at the Paso de la Laguna experimental unit of the National Institute for Agricultural Research (INIA) in Treinta y Tres, Uruguay (33° 16' 23" S; 54° 10' 24" W). The LTE was established in 2012 in a 12 ha field that had a 30-year history of irrigated rice cropping under reduced tillage, rotating with 2-3 years of perennial mixture of temperate grasses and legumes. The soil at the site is a Typic Argialboll according to the USDA-NRCS Soil Taxonomy (Durán et al., 2006) and as a Mollic Planosol in the World Reference Base for Soil Resources (Schad et al., 2015). The initial soil test at the site (0-15 cm depth) indicated a pH of 5.7, a CEC of 0.02 cmol kg⁻¹, 14 g kg⁻¹ of organic C and 190, 510 and 300 g kg⁻¹ of sand, silt and clay, respectively. The climate is mesothermic humid, mean annual rainfall is 1,360 ± 315 mm and average temperature is 22.3 ± 0.85 °C in summer and 11.5 ± 0.82 °C in winter (INIA, 2025; Table 1 Appendix).

2.3.2. Treatments and Experimental Design

The LTE established in 2012 was aimed to assess the sustainability of six rice-based no-till rotation systems with different frequency and proportion of rice, other grain crops, cover crops and perennial pastures in the sequences (Macedo et al., 2021). The LTE consisted in a randomized complete block (RCB) design using sixty plots of 20 x 60 m containing all phases of the six rotations coexisting in time and three synchronic replications in space (Patterson, 1964).

Although the LTE platform evaluated six rotation systems, our study focused on rice-phase plots from three of these systems over two high-yielding growing seasons (2019-2020 and 2020-2021), after all sequences had stabilized in 2018. The three selected rotations were: a) continuous rice (CR, 1 yr rotation): rice–Egyptian clover (*Trifolium alexandrinum* L.) in winter; b) rice-soybean (RS, 2 years rotation): rice–ryegrass (*Lolium multiflorum* Lam.) in winter–soybean [*Glycine max* (L.) Merr.]–Egyptian clover in winter, and; c) rice-perennial pasture (RPP, 5 years

rotation): rice–ryegrass–rice, followed by 3.5 years of a perennial pasture mixture of tall fescue (*Festuca arundinacea* Schreb.), white clover (*Trifolium repens* L.), and birdsfoot trefoil (*Lotus corniculatus* L.) for grazing (Table 2). Since the RPP includes two rice phases (one following perennial pastures (R1PP) and another immediately after a ryegrass cover crop (R2PP) between them) four rice-phase sequences were evaluated in our study.

A factorial arrangement of four rice-phase rotations within the LTE and four levels of nitrogen fertilization in rice were assessed over two cropping seasons using a RCB split-plot design with three replications. Within each season, all replicated plots in the rice phase for CR (3 plots), RS (3 plots) and RPP (6 plots) were partially split into four subplots, each 4 m wide by 16 m long, to apply the nitrogen treatment rates. Urea was broadcasted to the rice at rates of 50 kg N ha⁻¹ (N50), 100 kg N ha⁻¹ (N100) and 150 kg N ha⁻¹ (N150), along with a control treatment with no nitrogen added (N0). Each N dose was split 75% at tillering initiation (V4) on dry soil immediately before flooding and 25% at panicle initiation (R0) on flooded soil, respectively. No physical separation of sub-plots was used as shown in 15N isotope studies by Castillo (personal communication, 2018).

Table 1. Selected rice-based crop rotations (CR: Continuous Rice; RS: Rice-Soy; RPP: Rice-Perennial Pasture) for the seasons 2019-2020 and 2020-2021 and their seasonal sequences. SS (Summer-Spring); AW (Autumn-Winter); Ta (*Trifolium alexandrinum*); Lm (*Lolium multiflorum*); SS (Summer-Spring); AW (Autumn-Winter); CR (Continuous Rice); RS (Rice-Soy); RPP (Rice-Perennial Pasture).

Rotation	Phase											
	1		2		3		4		5			
	SS	AW	SS	AW	SS	AW	SS	AW	SS	AW		
CR	Rice	Ta										
RS	Rice	Lm	Soy	Ta								
RPP	Rice	Lm	Rice	----- Pasture ----- -----								

2.3.4. Crop Management and Agricultural Practices

An indica rice variety (INIA Merín) was no-till seeded into dry soil at a rate of 140 kg ha⁻¹ in 0.17 m rows during mid-October of both years, using a 2.55 m working width no-till drill. Seeding dates were October 25, 2019, and October 15, 2020, close to the optimal for the variety. Cover crops were sown the previous fall on rice or soybean straw immediately after harvest and were not fertilized or grazed. Perennial pastures were established three years prior, annually fertilized and rotationally grazed by sheep between March to December. Pastures and cover crops were terminated five weeks before rice seeding using a mixture of glyphosate and dicamba herbicides for follow management. The biomass accumulated by annual ryegrass and Egyptian clover cover crops preceding rice in R2PP, CR and RS were 1.53, 2.16 and 1.82 Mg DM ha⁻¹, respectively. Mean annual biomass productivity of the perennial pasture preceding rice in R1PP was 5.84 Mg DM ha⁻¹ yr⁻¹.

Management practices, including fertilization, pesticides applications, water management and other practices followed INIA technical recommendations for farmers. Broadcast applications of phosphorus (P) and potassium (K) fertilizers were made immediately before rice seeding, based on soil test results for each rotation and critical crop levels (Hernández et al., 2013; Deambrosi et al., 2015). Previous annual N fertilization rates for rice (2012-2018), based on soil nitrogen mineralization potential, averaged 148 kg N ha⁻¹ for CR, 75 kg N ha⁻¹ for R1PP, 96 kg N ha⁻¹ for R2PP and 76 kg N ha⁻¹ for RS. However, fertilizer doses were adjusted to N treatments in the current study. No fungicides or insecticides were applied to the crop, although a mixture of thiamethoxam and tebuconazole was used for seed treatment. Rice was managed as a rainfed crop until the initial tillering stage (V4), when the first urea and postemergence herbicides were applied. After this, the rice plots were continuously flooded until physiological maturity and then drained 10 days before harvest. Irrigation was sourced from a nearby river to the crop 2-3 times a week via electric pump and PVC tubing to supplement rainfall and maintain a stable water layer of 10-15 cm. Irrigation water use throughout the rice cycle was approximately 9,000 m³ ha⁻¹ for all rotations. Equipment used for all agricultural operations resembles those used by farmers.

2.3.5. Field and sample procedures

Rice stands were determined in both seasons (2019/2020 and 2020/2021) by counting the number of plants in a 1 m section in four rows about 1 week after emergence in each subplot.

Rice plants were sampled at ground level accounting for all above-ground biomass, with the two central meters of each subplot reserved for yield determination. Immediately after physiological maturity, four 30 cm by 19 cm (one row) sub-samples were taken in each subplot to determine harvest index, dry matter and N absorption. Plants were stored at 4°C until processing in less than 48 hours. Samples were first cleaned with tap water and subsequently with distilled water to remove adhered soil. Cleaned biomass samples and a subsample of harvested grain and straw were dried at 65°C for 48 hours, weighted, and milled. The N content was determined using an element analyzer LECO CN628 and double-checked with Dumas (Shea & Watts, 1939) at the Comprehensive Analysis Laboratory (LAI) in INIA. Rice grain yield was determined during the first week of April in both years using an experimental plot combine, which harvested the two central meters in width and the full length of each subplot, totaling 32 m². Rice grain was weighed using a field digital scale and a digital grain moisture meter was used to correct rice yield to 13% using 3 subsamples.

2.3.6. NUE calculation methods

NUE can be measured in different ways according to the study's objective and nutrients of interest. For this study, treatments involve crop rotation management and variable N fertilization, and the focus was to assess rice N utilization and its impact on yield. Indices that relate to fertilizer input, plant N absorption and yield were selected as the most adequate as proposed by Congreves et al. (2021).

PF_P is the partial factor productivity of applied N input and represents the expression of yield (Y) per unit of N fertilizer (F) applied which is measured by dividing grain yield with the amount of fertilizer applied.

$$PF_P = Y / F$$

REfertN is the apparent crop recovery efficiency of applied nutrients. It is calculated from the difference between N absorption (whole crop, sum of straw and grain N) between the fertilized (U) and unfertilized crop (U0) related to N input.

$$\text{REfertN} = (\text{Uf} - \text{U0}) / \text{F}$$

AE is the agronomic efficiency, the surplus in yield due to fertilization. It is calculated from the difference in yield between fertilized (Y) and unfertilized crop (Y0), divided by N input (F).

$$\text{AE} = (\text{Y} - \text{Y0}) / \text{F}$$

Physiological efficiency (PE) relates to how much yield is produced and how much N was absorbed between the fertilized (Yf-Uf) and the unfertilized crop (Y0-U0)

$$\text{PE} = (\text{Yf} - \text{Y0}) / (\text{Uf} - \text{U0})$$

Nitrogen utilization efficiency (NUtE) is the relation between crop N (U) and the plants' ability to produce yield (Y).

$$\text{NUtE} = \text{Y} / \text{U}$$

NHI is the nitrogen harvest index and accounts for the fraction of the yield N relative to the whole biomass uptake. It allows to identify plants with better ability to translocate absorbed N to yield.

$$\text{NHI} = \text{Yield N} / \text{U}$$

2.3.7. Statistical analysis

Residual analysis was performed graphically using qq-plots and Shapiro-Wilk and Levene tests were used to evaluate normality and homoscedasticity. Data were analyzed through a randomized complete block design model with a split-plot treatment arrangement by season, as follows:

$$Y_{ijk} = \mu + \tau_i + \beta_j + \delta_{ij} + \gamma_k + \tau\gamma_{ik} + \varepsilon_{ijk}$$

where Y_{ijk} is the response variable of interest, μ is the overall mean, τ_i is the relative effect of the i-th crop rotation, β_j is the relative effect of the j-th block, δ_{ij}

is the main-plot error, γ_k is the relative effect of the k-th N doses level, and ε_{ijk} is the experimental error. The δ_{ij} and ε_{ijk} assumed a normal distribution with mean equal zero and variances equal σ_δ^2 and σ_ε^2 , respectively.

Analysis of variance for each trait was performed at 95% confidence level. When significant differences were found for any of the factors evaluated, a Tukey test was performed at 95% confidence level. All analyses were performed in the statistical software R (R Core Team, 2021), using the lme4 (Bates et al., 2015), lattice (Sarkar, 2008) and emmeans (Lenth & Lenth, 2018) packages. Cropping season was not considered as a random input and data were tested against each cropping cycle to check for interactions. Results did not affect the overall interpretation of the trends and therefore, data is presented averaging both seasons.

2.4. Results

2.4.1. Climatic conditions

Climatic conditions during the rice growing season (Oct.-Mar.) varied somewhat between both years, particularly during the sowing-emergence period and during flowering (Table 2 Annex). Above-average rainfall in October delayed seeding, germination and early growth stages during the 2019-2020 season, resulting in 35% lower stand compared to 2020-2021 (288 plants m⁻²). Despite the initial delays and the lower, but non-limiting, plant stand, conditions improved significantly after the V4 stage, with below-average precipitation and above-average temperatures and radiation until the end of the season. In contrast, the 2020-2021 season experienced favorable conditions for seeding, germination and vegetative growth, with sparse but mild rains, high temperatures and above-average radiation until flowering, when heavy rainfall occurred. Nevertheless, both seasons exhibited optimal temperatures and radiation for most of the cycle, as indicated by the national average yields (8.6 and 9.4 Mg ha⁻¹ in 2019-2020 and 2020-2021, respectively).

2.4.2. Rice yield

Crop rotation and N dose affected rice yield, total N uptake and nitrogen concentration in both grain and biomass, with a significant interaction between the

two factors (except for yield (Table 4)). A significant interaction between season and rotation was also observed for rice yield; however, the three-way interaction (season \times rotation \times N rate) was not significant. On average across both years, rice yield following perennial pastures (R₁PP: 12.23 Mg ha⁻¹) or a soybean-Egyptian clover sequence (RS: 11.83 Mg ha⁻¹) were significantly higher than after rice, regardless of whether the rice was grown in monoculture with a legume cover crop (CR: 9.33 Mg ha⁻¹) or as second crop of an integrated system with perennial pastures (R₂PP: 9.67 Mg ha⁻¹). However, while rice yields in R₁PP and RS increased by 12.9% in 2020–2021 compared to 2019–2020 (11.30 Mg ha⁻¹), no significant increase was observed in CR and R₂PP (2.6%), further widening the yield gap between the two groups while maintaining their relative rankings.

Table 2. ANOVA p-values for season, crop rotation, and N dose effect and their interactions for the variables grain yield, biomass N, grain N concentration, and total N absorbed by rice. Numbers in bold reveal a significant interaction between the variable and factor (p-value < 0.05).

	Variable			
	Yield	Biomass N	Grain N	Total N
Season	0.0652	0.2457	<.0001	<.0001
Rotation	<.0001	0.0031	<.0001	<.0001
N dose	<.0001	<.0001	<.0001	<.0001
Rot*N dose	0.198	0.0033	<.0001	<.0001
Season*Rot	0.0051	0.6996	0.721	0.1179
Season*N dose	0.0914	0.6189	0.006	0.9335
Season*Rot*N dose	0.8697	0.5011	0.0015	0.5621

Analysis by season revealed that rice yield was significantly affected by rotation phase and N rate in both years, but no interaction was found. In 2019-2020, rice yield following perennial pastures (R₁PP) or soybeans (RS) increased by 20.5% compared to both rotation phases after rice (9.38 Mg ha⁻¹), either CR or R₂PP (Figure 1). Similarly, in 2020-2021, rice productivity in R₁PP and RS was 32.6% higher than in sequences after rice (9.62 Mg ha⁻¹), whether under CR or R₂PP. No significant rice

yield differences were observed between R₁PP and RS or between R₂PP and CR in either year.

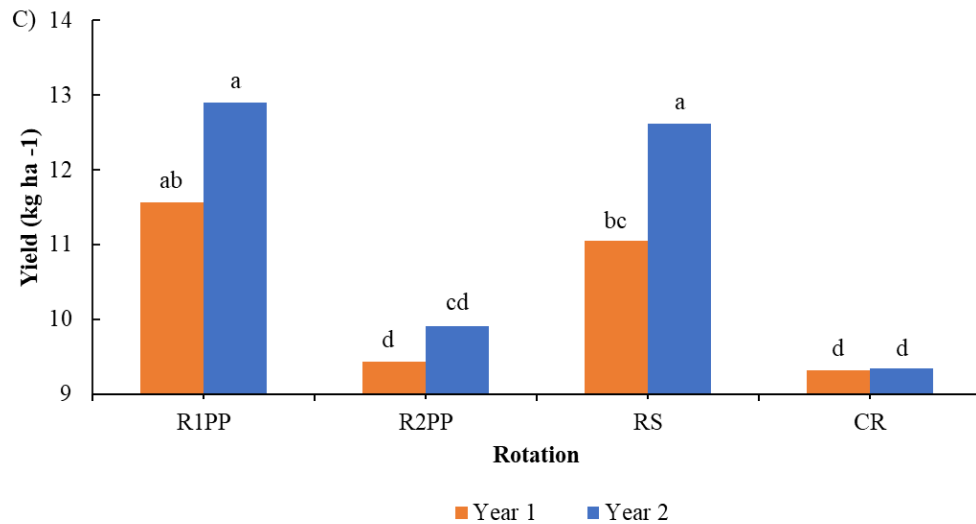


Figure 1. Effect of four crop rotation systems: continuous rice (CR), rice soy (RS), first rice after perennial pasture (R₁PP), and the second rice after pasture (R₂PP) and two growing seasons (2019-2020 and 2020-2021) on rice yield in a long-term experiment in Uruguay. Different letters indicate statistically significant differences in LSD (0.05).

Rice yield without N application (0 kg N ha⁻¹) was relatively high overall, averaging 9.32 Mg ha⁻¹ (Figure 2). The highest rice yield without N fertilizer was found in R₁PP (11.05 Mg ha⁻¹), which was 38% greater than the mean yield of CR and R₂PP. Although RS yielded 7.7% lower than R₁PP without N fertilizer, it was still 27.3% higher than the mean yield of CR and R₂PP. Even with the application of 150 kg N ha⁻¹, yields in CR (10.82 Mg ha⁻¹) and R₂PP (10.55 Mg ha⁻¹) did not match the productivity of R₁PP without N. Rice productivity without N fertilizer, expressed as a percentage of the maximum yield obtained with N in each rotation phase, were 85% in R₁PP, 78% in RS and 75% in both CR and R₂PP.

Overall years, the highest rice productivity across all rotations phases was achieved with applications of 100 kg N ha⁻¹, with no further yield increases observed at higher rates (Figure 2). At the optimal N dose, the mean rice yield in R₁PP and RS (12.86 Mg ha⁻¹) was 27.7% higher than the corresponding in CR and R₂PP. Like the

overall rotation effect, no significant yield differences were observed between R₁PP and RS or between R₂PP and CR at the optimal N rate.

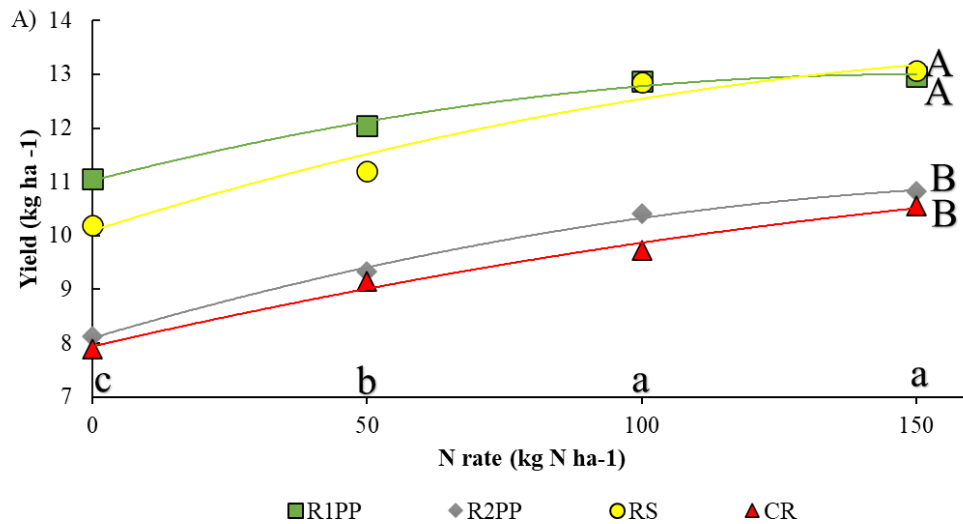


Figure 2. Effect of four crop rotation systems: continuous rice (CR), rice soy (RS), first rice after perennial pasture (R₁PP) and the second rice after pasture (R₂PP) and four N fertilizer rates (0, 50, 100 and 150 kg N ha⁻¹) on rice yield averaged for two growing seasons (2019-2020 and 2020-2021) in a long term-experiment in Uruguay. Different letters indicate statistically significant differences in LSD (0.05). Lowercase letters at the bottom indicate differences between N doses. Capital letters on the right indicate differences between rotations.

2.4.3. Nitrogen uptake in biomass and grain

There was a significant interaction effect between crop rotation and N dose on total absorbed N at rice physiological maturity (Table 4). Crop N absorption in R₁PP was consistently higher than in both rice-after-rice scenarios (CR and R₂PP) for all N doses (Figure 3a). Rice in R₁PP and managed without N fertilizer absorbed 54.7% more N than CR and R₂PP (mean: 94.9 kg N ha⁻¹) and had a 22.3% trend compared with RS (119.3 kg N ha⁻¹). As N rates increased, rice N absorption rose across all rotations phases, but the differences between R₁PP or RS, compared to CR or R₂PP, became more pronounced. Total absorbed N responded to fertilizer up to 100 kg N ha⁻¹ in R₁PP, R₂PP and CR, and up to 150 kg N ha⁻¹ in RS. On average, at the

optimal yield N rate, R₁PP and RS absorbed 35.6% more nitrogen than CR and R₂PP (130 kg ha⁻¹).

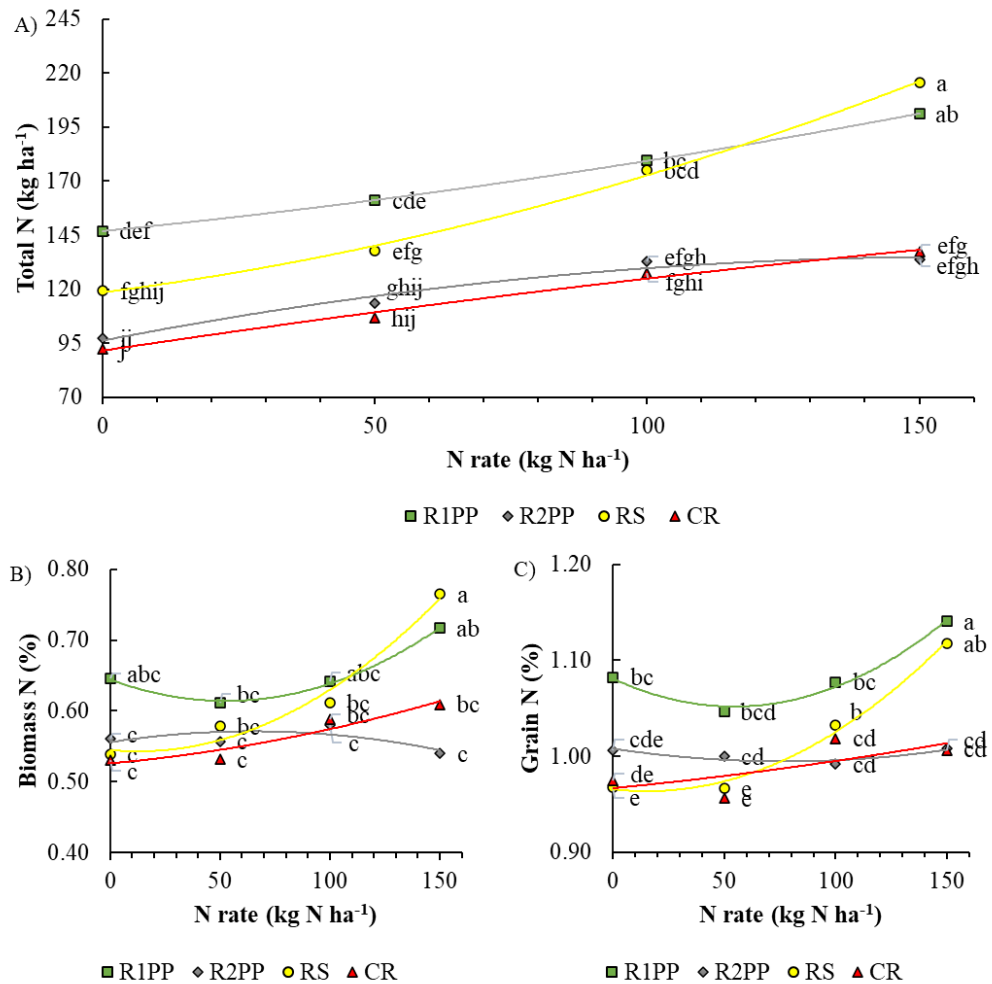


Figure 3. Effect of four crop rotation systems: continuous rice (CR), rice soy (RS), first rice after perennial pasture (R₁PP) and the second rice after pasture (R₂PP) and four N fertilizer rates (0, 50, 100 and 150 kg N ha⁻¹) on: A) total N absorbed by rice, B) biomass N concentration (%) and C) in grain N concentration (%) averaged for two growing seasons (2019-2020 and 2020-2021) in a long-term experiment in Uruguay. Different letters indicate statistically significant differences in LSD (0.05).

Nitrogen concentration (%) in straw biomass and grain was affected by the interaction of rotation and N dose (Table 4, Figures 3b and 3c). Although grain N concentration in R₁PP generally exceeded that in R₂PP and CR across all N doses,

significant differences were primarily observed at the highest N rate. No significant response to nitrogen fertilization was detected on grain or biomass N concentration for R₂PP and CR. In contrast, fertilizer application increased N concentration in R₁PP grain and in both grain and straw of RS, particularly with 150 kg N ha⁻¹. Despite a significant three-way interaction among rotation, N rate and season on grain N concentration, values observed in the first season were higher than those in the second.

2.4.4. NUE indices

Most nitrogen use efficiency indices were influenced by both crop rotation and N dose (Table 3). A significant interaction between rotation and N dose was observed for NUE, PFP and NHI. The IUE was affected by rotation and N rate, but not by their interaction. The REfertN and PE responded to crop rotation, but not to N dose. The AE was primarily impacted by N rate, although season also played a role. No significant interactions were found between season and rotation, season and N rate, or for the three-way interaction.

Table 3 ANOVA p-values for season, crop rotation, N dose effect and their interactions, for the indices NUE (nitrogen utilization efficiency), PFP (partial fertilizer partition), REfertN (recovery efficiency of fertilizer. Numbers followed by asterisks indicate significance levels: * $p < 0.05$; ** $p < 0.01$; * $p < 0.001$; NS, not significant.**

Index						
	NUE	PFP	REfertN	PE	AE	NHI
Season	***	NS	NS	NS	*	NS
Rotation	***	***	**	*	NS	***
N dose	***	***	NS	NS	*	***
Rot*N dose	***	***	NS	NS	NS	*
Season*Rot	NS	NS	NS	NS	NS	NS
Season*N dose	NS	NS	NS	NS	NS	NS
Season*Rot*N dose	NS	NS	NS	NS	NS	NS

Rice grown in R₁PP and RS consistently exhibited higher PFP than in CR and R₂PP at fertilization rates up to 100 kg N ha⁻¹ (Figure 4a). At this dose, corresponding to the maximum yield response, each kilogram of N applied in R₁PP and RS produced, on average, 28 kg more grain than in CR and R₂PP (100.7 kg kg⁻¹). As expected, increasing N fertilizer rate from 50 to 150 kg N ha⁻¹ led to diminishing returns in PFP across all rotations. No significant differences in PFP were observed between R₁PP and RS, or between CR and R₂PP, at equivalent N rates.

The AE was affected by N dose and season, but no significant interactions were observed (Table 3). Regardless of rotation (with the exception of RS at 100 kg N ha⁻¹), increasing N doses resulted in a reduction in AE (Figure 4b), with no significant differences between the 50 and 100 kg N ha⁻¹ rates (21.8 kg kg⁻¹). At the N dose corresponding to the maximum yield response (100 kg N ha⁻¹), AE in RS was 46% higher than in CR and R₁PP (18.2 kg kg⁻¹).

The NUtE was affected by crop rotation, N dose and their interaction (Table 3). On average, NUtE decreased by 9.2% across all rotations as N rates increased from 0

to 100 kg N ha⁻¹ (Figure 4c). At the N dose corresponding to the maximum yield response, R₁PP showed a 9.1% lower NUtE than CR and R₂PP (79.1 kg kg⁻¹).

The REfertN was affected by rotation, but not by N dose or their interactions (Table 3). On average, across all N rates, RS recovered 52% of the applied N fertilizer, while the remaining rotations recovered only 31% (Figure 4d). At the N dose corresponding to the optimum yield response (100 kg N ha⁻¹), REfertN was 56% in RS, compared to an average of 34% in the other rotations.

The NHI was significantly affected by rotation, N dose and their interaction (Table 3). However, no differences in NHI were observed between rotations at 0 and 100 kg N ha⁻¹ (Figure 4e). Rice in CR and R₂PP exhibited similar NHI across all N doses, whereas, in RS and R₁PP, the proportion of N in the grain decreased from 71.5% to 61% as fertilization rates increased from 0 to 150 kg N ha⁻¹. At the maximum N rate, R₂PP had the highest NHI (72%), while RS had the lowest (60%).

The PE was significantly impacted by crop rotation (Table 3), but neither N rate nor their interactions were significant. Overall, rice in R₁PP and RS had 34% lower PE compared to R₂PP and CR (Figure 4f). However, at a fertilization rate of 100 kg N ha⁻¹, PE in RS was 40% lower than in R₂PP (80 kg kg⁻¹), with no significant differences observed between other treatments. The lowest PE was recorded in RS at 150 kg N ha⁻¹ (32 kg kg⁻¹), while the highest PE occurred in CR at 50 kg N ha (90 kg kg⁻¹).

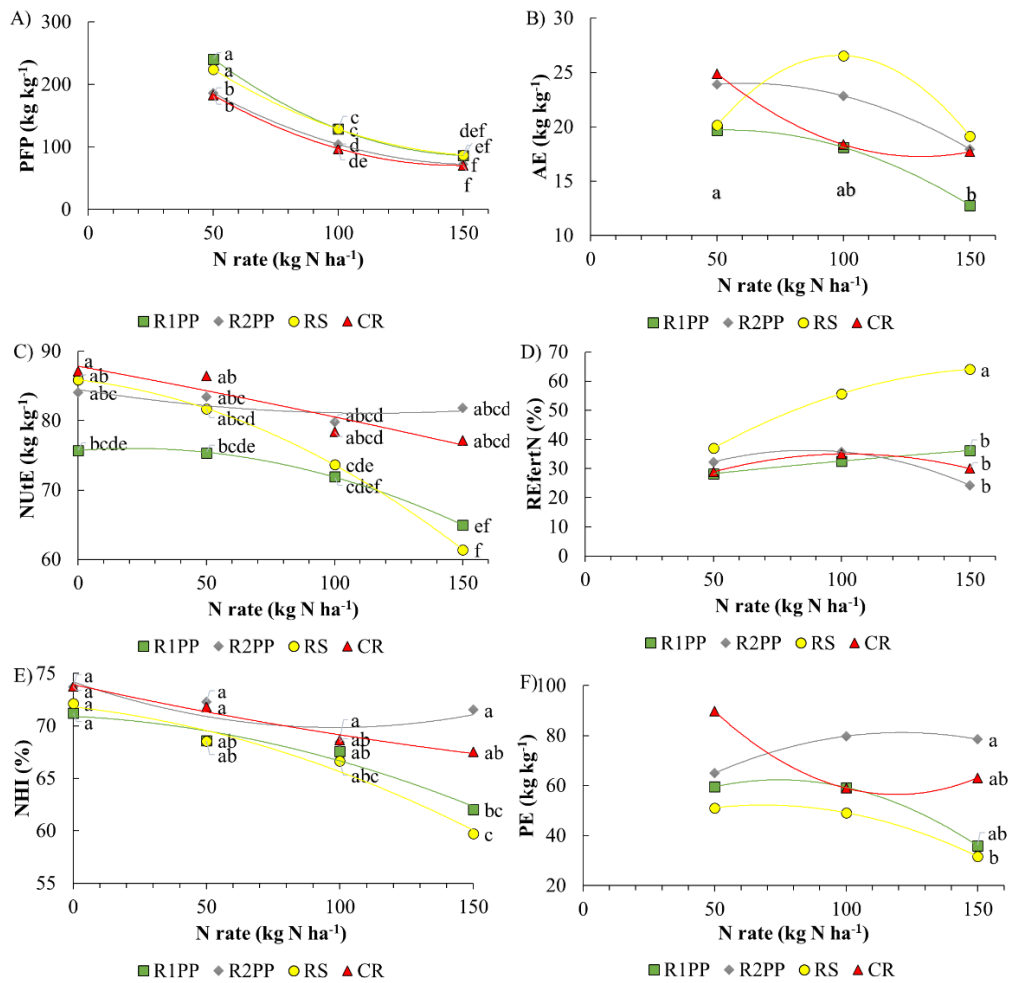


Figure 4. Effect of four crop rotation systems: continuous rice (CR), rice soy (RS), first rice after perennial pasture (R₁PP) and the second rice after pasture (R₂PP) and four N fertilizer rates (0, 50, 100 and 150 kg N ha⁻¹) on: A) partial-factor of N productivity (kg kg⁻¹), B) agronomic efficiency (kg kg⁻¹), C) N utilization efficiency (kg kg⁻¹), D) REfertN (%) E) N harvest index (%) and F), physiological efficiency (kg kg⁻¹) in rice averaged over two growing seasons (2019-2020 and 2020-2021) in a long-term experiment in Uruguay. Different letters indicate statistically significant differences in LSD (0.05). When no significant interaction was detected, letters correspond to main effects only (horizontal: N fertilizer rate, vertical: crop rotation)

In summary, N uptake, N translocation and rice yield were strongly influenced by crop rotation and nitrogen fertilizer rates. Most crop responses differed between two distinct rotation-phase groups categorized based on the immediately preceding

summer crop. The first group included situations where rice was sown after a previous rice crop, either continuously with a legume cover crop (CR) or as a second rice crop in rotation with perennial pastures (R₂PP). The second group consisted of rice sown after a perennial pasture mix (R₁PP) or following a soybean–Egyptian clover sequence (RS). Overall, rice in R₁PP and RS showed higher yields (27%), N absorption (42%) and N concentrations. However, both groups showed similar yield responses to nitrogen fertilization up to 100 kg N ha⁻¹, despite rice in R₁PP and RS accumulated N in grain and plant until a dose of 150 kg N ha⁻¹. As a result, most NUE indices were affected by crop rotation, nitrogen rate and their interactions. Specifically, at the nitrogen rate for optimum yield, R₁PP and RS exhibited the highest PFP, with RS also showing the highest AE and REferN, while R₂PP had the greatest PE. No significant differences between rotations phases were observed in NutE or NHI.

2.6. Discussion

This study assessed the effects of rotation system and nitrogen fertilization on irrigated rice responses in an LTE, with the aim of improving system design and optimizing the nutrient management in high-yielding environments. Evidence in rainfed crops shown that shifting from integrated crop-livestock systems to continuous annual cropping (through the replacement of pastures and reducing crop diversification) can lead to lower yields and increased nitrogen input requirements related with declines in soil quality and other ecosystems services (Rubio et al., 2022; Carvalho et al., 2021; Ernst et al., 2018). However, the impacts of rotation intensification and their interaction with nitrogen fertilization management on productivity and NUE have rarely been evaluated in rice-pastures systems (Denardin et al., 2025; Castillo et al., 2023). Assessing rice responses to nitrogen under different intensification pathways is relevant due to agroecosystems' impact on nitrogen cycling and management and the nutrient role as a key yield-limiting factor in rice (Tseng et al., 2021; Denardin et al., 2019; Fageria & Carvalho, 2014). High mean rice productivity was obtained at our site (10.77 Mg ha⁻¹), favored by an adapted high-yielding variety, adjusted management and optimal radiation and

temperature conditions, as expected in temperate regions (Chen et al., 2023; Ribas et al., 2021; Zhang et al., 2015). In this context, we found that rice yield and N uptake were significantly affected by crop rotation and nitrogen fertilizer rate. The discussion will focus on a) rice responses to rotations without nitrogen fertilizer, b) rice responses to nitrogen application across rotations and c) NUE across rotations at the optimal N rate.

2.6.1. Rice responses to rotation sequence

Through the analysis of rotation system sequences without nitrogen fertilization, we showed that rice nitrogen uptake and grain yield were significantly higher when grown after perennial pastures compared to rice-cover crops or even soybean-cover crop sequences. Grazed pastures in integrated systems contribute to soil carbon sequestration (Macedo et al., 2022), biological nitrogen fixation (Peoples & Baldock, 2001) and nitrogen cycling (Carlos et al., 2020; Denardin et al., 2020), thereby raising rice productivity (Meus et al., 2024; Castillo et al., 2021; Carmona et al., 2016). Hence, in our study, R₁PP without nitrogen fertilizer achieved 86% of the yield obtained at the optimal nitrogen rate, highlighting the substantial legacy effects of perennial pastures on soil quality and crop productivity (Castillo et al., 2021; Franzluebbers et al., 2014). Conversely, rice-rice sequences, particularly continuous rice systems, often lead to soil degradation, reduced nitrogen availability and yield stagnating (Ribas et al., 2021; Carlos et al., 2020; Maguire et al., 2020). In between, the positive effects of soybean as a preceding crop on rice yield and N dynamics (compared to continuous rice systems) were reported in a similar field-scale experiment in Brazil (Denardin et al., 2025; Denardin et al., 2022), in farmers' fields (Ribas et al., 2021), and during the initial years of our LTE (Macedo et al., 2022). At the beginning of our study, soil organic carbon at the 0-15 cm depth in RPP (15.9 g kg⁻¹) was 12% higher than in the other rotations, due to sequestration rates of 0.60 Mg C ha⁻¹ yr⁻¹, during the first eight years of the LTE (Macedo et al., 2022). Although CR and RS systems maintained the initial LTE levels of soil carbon and total nitrogen, labile forms of C and N declined in RS, indicating faster turnover and

a negative nitrogen balance in rice systems that include soybean (Castillo et al., 2023; Macedo et al., 2021).

Although the use of no-till and cover crops (particularly legumes) can enhance rice yield and soil quality in continuous rice systems (Yang et al., 2024; Weinert et al., 2023; Carlos et al., 2022; Carlos et al., 2021; Denardin et al., 2019), in our study the CR, with an Egyptian clover winter cover, did not match the performance of R₁PP or RS. Surprisingly, the beneficial effect of pastures on rice yield and nitrogen uptake was observed only in R₁PP, but was not evident in R₂PP, which performed similar to CR. High amounts of dissolved organic carbon released from residues in rice-rice sequences (used as a primary energy source by soil microorganisms) can lead to substantial nitrogen immobilization, which may partly explain nutrient dynamics in this complex wetting and drying environment (Gmach et al., 2021; Magill et al., 2000; Lundquist et al., 1999). Additionally, less diversified cropping systems negatively affect soil physicochemical properties (Zhou et al., 2014), reduce the diversity and functionality of soil microbial communities (Jiang et al., 2016) and experience considerable variation in soil conditions when transitioning from dry to flooded phases during the year (Tarlera et al., 2016). Although Maguire et al. (2020) did not find differences in soil microbial diversity between continuous rice and rice-pasture systems, Martínez (2023a) reported that the LTE bacterial/archaea communities were influenced by the frequency of rice in the rotation, and the fungal communities were more influenced by the previous crop. Moreover, Ghiazza et al. (2023) found that rotation systems in the LTE affected the structure of rhizospheric and endophytic diazotrophic communities associated with rice roots that might impact nitrogen availability or plant growth.

2.6.2. Rice responses to nitrogen fertilizer

Despite differences in soil nitrogen supply capacity and crop productivity among rotations (as reflected in the unfertilized plots) rice yield increased consistently across all rotation sequences up to 100 kg N ha⁻¹, showing no interaction and keeping the yield gap between them. The fact that rice without nitrogen fertilizer yielded, on average, 81% of that receiving the optimal rate suggests a high inherent

soil capacity at the site to meet the crop's nitrogen requirements, as likely in Mollisols under no-till systems including legumes (Yuexuan et al., 2025; Yao et al., 2025; Weinert et al., 2023; Eswaran & Reich, 2005). Therefore, the rice crop in all rotations sequences was positioned relatively high on the nitrogen response curve, nearing a threshold beyond which added fertilizer resulted in diminishing yield gains and reduced NUE (Dobermann, 2007; Roberts, 2007).

At the optimum nitrogen fertilization rate at the site, the crop absorbed on average 154 kg N ha⁻¹ and yielded 11.47 Mg ha⁻¹, reaching approximately 80% of the regional yield potential (Ribas et al., 2021; Meus et al., 2022; Carracelas et al., 2023). In other studies, nitrogen uptake has ranged from 84 kg N ha⁻¹ with an application of 100 kg N ha⁻¹ (Wang, Zhang et al., 2016) to 182 kg N ha⁻¹ with 225 kg N ha⁻¹ applied in high nitrogen-efficiency rice cultivars (Hameed et al., 2019). Although the grain nitrogen concentration (1.03%) observed in our test was below the typical range of 1.1-1.2% reported for rice (Omara et al., 2019; Fageria, 2014), total nitrogen absorption was slightly higher (Dempsey et al., 2017; Wang, Shi et al., 2016; Hameed et al., 2019), likely due to the high overall yield. This dilution effect is usual in high-yielding systems, leading to lower grain nitrogen concentrations and relatively stable absorption, which impacts NUE (Justes et al., 1994; Ata-Ul-Karim et al., 2016; Song et al., 2020).

Rice crop responses on our site indicate that nitrogen was a yield-limiting factor across all systems and that the yield benefits of rotation did not reduce fertilizer rate needs. However, R₁PP was 6-12% less dependent on fertilizer than other rotation sequences, as indicated by the ratio between nitrogen inputs at N₁₀₀ (fertilizer + soil estimated from crop uptake at N₀) and crop nitrogen uptake at N₀ as proposed by Quemada & Lassaletta (2024). These authors reported that a legume predecessor reduced fertilizer dependence in wheat and barley by 15% compared with the same-species predecessor; however, their coefficients were higher than those observed in our rice study, which ranged from 0.40 in R₁PP to 0.52 in CR. Similarly, using the N supply–demand ratio calculated from nitrogen removal in the N₀ and N₁₀₀ plots, as proposed by Tamagno et al. (2024), R₁PP showed a lower nitrogen fertilizer demand (0.14) compared with CR and R₂PP (0.22) and the RS

(0.26). Denardin et al. (2025) reported a lower proportion of fertilizer-derived nitrogen accumulated in rice under integrated rice-soybean-livestock (24%) and continuous rice-livestock (46%) systems compared with continuous rice (74%) in Brazil. According to these studies, sites with higher soil N supply or a low N supply–demand ratio as R₁PP should require less nitrogen fertilizer, which was not the case in our experiment.

This unpredicted outcome suggests that other factors in addition to soil N supply—such as poor synchronization between N availability and crop demand, or extra yield-limiting factors (e.g., soil properties constraints, or crop diseases)—may have affected CR and R₂PP responses. Rice straw may build up phenolic oxidation compounds, which can slow down nitrogen mineralization through polyphenol-induced stabilization (Zhang et al., 2015). Also, it raises dissolved carbon levels, which may promote nitrogen temporal immobilization by enhancing microbial activity (Aumtong et al., 2023; Yu et al., 2020). Additionally, rice–rice sequences may degrade soil structure (potentially affecting the early stages of rice growth before flooding) by reducing aggregate stability and microporosity that impacts gas exchange and microbial activity (Quintero et al., 2009). Finally, since no fungicides were applied in our test due to the use of a rice blast-resistant cultivar, higher stem rot severity (caused by *Nakataea oryzae*) may have affected the rice–rice sequences, as reported by Martínez (2023b) during the initial years of the LTE. The fact that neither yield nor crop N uptake in the CR and R₂PP increased beyond 100 kg N ha⁻¹ (maintaining the yield gap with R₁PP and RS) indicates that other limiting factors existed for these sequences.

2.6.3. Nitrogen use efficiency

Globally, NUE in rice is lower than in other cereals (Zhang et al., 2015), with average yields of 4.7 Mg ha⁻¹ (FAO) and nitrogen fertilization rates usually exceeding 100 kg N ha⁻¹, reaching up to 250 kg N ha⁻¹ in high-input production systems (Maaz et al., 2021; Wang, Zhang et al., 2016). A review of 46 studies on site-specific nitrogen management in continuous rice or rice-upland crop systems conducted in Asia and Africa reported an average yield of 6.92 Mg ha⁻¹ with a mean

nitrogen application rate of 120 kg N ha⁻¹, resulting in PFP and AE values of 53 and 17 kg kg⁻¹, respectively (Chivenge et al., 2021). In contrast, our study achieved high rice yield with relatively low nitrogen rates, particularly in the R₁PP and RS sequences (12.86 Mg ha⁻¹), resulting in some NUE indices that differed from those reported by Chivenge et al. (2021) and other authors. For example, Wang, Zhang et al. (2016) reported yields of 9 Mg ha⁻¹ in transplanted rice in China with nitrogen applications of 200 kg ha⁻¹, yielding 22% less while applying twice the nitrogen compared to our study, resulting in lower PFP and AE. In Thailand, Santiago-Arenas et al. (2021) applied similar N rates in direct-seeded rice, but yields were 60% lower than those in our study, thereby reducing NUE indices. Conversely, Rose et al. (2022) reported higher yields (exceeding 14 Mg ha⁻¹) in comparable cropping systems with slightly higher nitrogen doses (120-180 kg N ha⁻¹). However, their unfertilized plots yielded significantly less (5.4 Mg ha⁻¹), which increased REfertN and AE, while maintaining a similar PFP. Finally, in an on-farm trial conducted in Brazil on a degraded Albaqualf, Denardin et al. (2020) reported yields, nitrogen responses, crop N uptake and NUE indices (PFP, AE, and NutE) in an integrated continuous rice-livestock system that were comparable to those observed in CR and R₂PP. Subsequently, in the same trial, it was found that integrating rice with livestock grazing of winter annual ryegrass (whether in continuous rice or rice–soybean rotation systems) improved productivity, nitrogen response and some NUE indices compared with continuous rice (Denardin et al., 2025).

The overall NUE responses in our study as fertilization increased were somewhat expected, given that the soil was capable of supplying most of the rice crop's nitrogen requirements and yield was already high and near a plateau (Hou et al., 2019). The higher PFP observed in R₁PP and RS compared with CR and R₂PP at N₁₀₀ were explained by their greater yields, but values were at least twice higher than the values of 50-55 kg kg⁻¹ reported by Chen et al. (2023) and Chivenge et al. (2021), and above the optimal range of 40-80 kg kg⁻¹ proposed by Dobermann (2007). Although high PFP values can be associated with soil nitrogen mining, we believe this was not the case in our study, given the productivity, the soil organic C and N content, the nitrogen balances and the positive nitrogen surplus observed across the

entire rotation's phases over nine years in the LTE (Castillo et al., 2023; Macedo et al., 2022). On the other hand, the AE of all rotation sequences at our site was slightly higher than the values of 15-17 kg kg⁻¹ reported in some reviews, but remained below those recommended for well-managed system, except in RS (Chen et al., 2023; Chivenge et al., 2021; Hameed et al., 2019). Similar to Denardin et al. (2025), the RS sequence achieved a higher REfertN compared to the other rotation sequences, which remained below the recommended threshold of 50% for well-managed systems (Uvardi et al., 2021). Although NHI has been positively associated with grain yield (Fageria, 2014), values across all rotation sequences were similar and the 68% observed at N₁₀₀ falls within the upper range reported by Gweyi-Onyango et al. (2021) and Fageria et al. (2008). The high NUtE values of CR and R₂PP sequences at N₁₀₀ could suggest a nitrogen deficiency (Padhan et al., 2023; Che et al., 2015; Dobermann, 2007). However, this was likely not the case in R₁PP and RS, given their high yields and nitrogen uptake at N₀, and the absence of further yield increases beyond N₁₀₀ despite increased nitrogen uptake. Finally, PE at N₁₀₀ in all sequences (mean: 60 kg kg⁻¹) exceeded the 33.8 kg kg⁻¹ reported by Che et al. (2015) in China and fell within the range proposed by Dobermann (2007), except for R₂PP, which showed a slightly higher value.

In summary, the differential NUE responses observed in our study compared to others may be mostly attributed to the region's high yield potential (Carracelas et al., 2023), the soil's capacity to meet most of the rice N demand due to its inherent quality and good state of conservation (Macedo et al., 2022), and the LTE rotations evaluated, all of which used no-till, included legumes and followed best management practices regardless of intensity. Nevertheless, there remains potential to further improve yield and NUE through refined rotation design, particularly in integrated systems, as suggested by Denardin et al. (2025) and Castillo et al. (2023).

2.6.4. Limitations and future directions

From a decision-making perspective, our results suggest that rotation sequences including either perennial pastures or soybean as preceding crops are key in achieving high rice yields with reduced nitrogen fertilizer dependence and

improved NUE, compared to rice-rice sequences. However, there are some limitations to this study.

First, the LTE was managed under strict no-till, which contrasts with current production systems where tillage intensity varies among farms and cropping systems, even within the same rotation. In temperate regions, continuous rice systems under no-till may experience reduced yields and lower NUE compared to minimum tillage, due to soil compaction and surface stubble accumulation, which hinder crop establishment and slow soil nitrogen dynamics (Vitali et al., 2024). On the other hand, conservation tillage can minimize positive effects of rotation sequences on rice yields (Yao et al., 2025).

Secondly, this study focused on rice agronomic responses and NUE in cropping systems, without addressing environmental impacts associated with nitrogen management (such as nutrient balances, soil and water quality, or greenhouse gas emissions), which could lead to alternative interpretations (Castillo et al., 2023, Macedo et al., 2022).

Third, not all rotation sequences from the LTE were included in this study, only the most contrasting ones. Given the positive impacts of both perennial pastures and soybeans on rice yield and NUE, integrating soybean into rice–pasture systems seems to be a promising approach for enhancing overall system performance (Macedo et al., 2022, Denardin et al., 2025). Future research should explore alternative scenarios and sequences involving rice, grazed pastures, cover crops and soybeans to optimize NUE and better understand nitrogen dynamics and rice responses within these systems.

Finally, the interpretation of results, particularly their comparison with values reported in other studies should be approached with caution, as many of those studies were based on regions characterized by highly intensive continuous rice systems, smaller field scales, different yield potentials and distinct soil and crop management practices (Chen et al., 2023; Chivenge et al., 2021).

Our findings contribute to a better understanding of the intensification and potential decoupling of rice–pasture rotation systems with respect to rice yield and NUE, offering key insights and alternative strategies for rotation design and nitrogen

management in high-yielding environments of temperate South America. In times where more integrated, diverse and resilient cropping systems are promoted for sustainability (Liu et al., 2022), understanding the impacts of rice-pastures intensification and eventual decoupling (particularly in regions where such systems have been developed for decades (Carvalho et al., 2021; Castillo et al., 2021)) is strategic. In this context, we demonstrated that transitioning from a rice–pasture rotation to continuous rice cropping (even when incorporating a legume cover crop and increasing nitrogen fertilizer rates) resulted in reduced rice yield, lower PFP and diminished soil nitrogen-supplying capacity, thereby increasing dependence on nitrogen fertilizers.

2.7. Conclusions

This study demonstrated that the careful design of rice crop rotations, either including perennial mixed pastures or a soybean-legume cover sequence preceding rice, can enable the sustainable intensification of rice production systems, allowing high yields and improved NUE. Perennial pastures had positive legacy effects on subsequent rice, with soil supplying up to 82% of its nitrogen requirements and increasing grain yield by 32%, nitrogen uptake by 41% and PFP by 32% compared to a continuous rice-Egyptian clover rotation. However, these benefits were not observed in the second rice crop of the RPP rotation, which performed similarly to continuous rice. As a result, a 35% nitrogen uptake gap and a 24% yield gap emerged between the first and second rice crops within the same rotation, which could not be corrected by fertilization. This reduced the overall rotation yield and suggested limitations beyond soil nitrogen supply in the second rice phase of RPP. Meanwhile, rice rotated with soybeans and Egyptian clover yielded and responded to nitrogen fertilizer similarly to rice following perennial pastures, reaching 90% of the regional yield potential and showing greater AE and RE_{fertN} , but also a trend to higher dependency on nitrogen fertilizer inputs.

The identification of two contrasting rotation clusters, rice sown after rice and rice following perennial pastures or soybeans, reflected patterns that go beyond the scope of this study. Further research is needed to uncover the mechanisms driving

these differences. Future studies should focus on comprehensive microbiome analysis to better understand the underlying factors contributing to improved NUE in integrated and diversified cropping systems.

Our data suggest that for rice–livestock systems in the high-yielding temperate region of South America, maintaining perennial mixed pastures, avoiding rice-rice sequences, and integrating soybeans and legume cover crops into existing systems are key strategies to enhance rice productivity, reduce nitrogen fertilizer dependence, and improve NUE.

2.8. References

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2.8. Appendixes

Table 1. Twenty-year (2001-2021) monthly average weather data for the experimental station Paso de la Laguna at the National Institute for Agricultural Research (INIA) Treinta y Tres, Uruguay.

	Solar radiation	Penman - Monteith ET	Heliophany	Precipitation	Maximum temperature	Minimum temperature	Medium temperature
	MJ day ⁻¹	m ² mm month ⁻¹	%	mm month ⁻¹	C°	C°	C°
Jan	21.5	158.1	60	115	29.7	16.9	23.3
Feb	19.1	124	57	143	28.9	17.2	22.8
Mar	15.8	101	55	117	27.1	15.2	20.6
Apr	12.2	64.5	54	127.6	24.1	12	17.8
May	8.8	36.2	46	134.4	20	8.6	14.1
Jun	7.2	25.3	41	115.5	17.2	6	11.6
Jul	7.9	30.2	44	94.8	16.6	5.6	10.7
Aug	10	46.1	45	132.7	18.2	7.1	12.8
Sep	13	67.6	45	142	19.7	8.9	14.4
Oct	16.8	99.9	50	128.8	22.5	11	16.7
Nov	20.5	129.9	58	89	25.7	12.9	19.4
Dec	21.8	154.8	59	106.8	28.3	15	21.7

Table 2. Radiation (cal cm² day⁻¹), rainfall (mm month⁻¹) and average temperatures (°C) summary for experimental research station Paso de la Laguna” at the National Institute for Agricultural Research (INIA) Treinta y Tres, Uruguay, for two growing seasons (2019-2020 and 2020-2021) and 20 year’s average 2001-2021.

	2019-2020			2020-2021			Average 2001-2021		
	Rad	PP	Temp	Rad	PP	Temp	Rad	PP	Temp
	MJ m ² day ⁻¹	mm month ⁻¹	°C	Cal cm ² dia ⁻¹	mm month ⁻¹	°C	Cal cm ² dia ⁻¹	mm month ⁻¹	°C
Oct	16.6	360.6	17.2	1919	48.1	16.8	1677	99.9	16.7
Nov	22.5	49.2	21.1	2363	81.5	20.0	2048	129.8	19.3
Dec	23.9	79.0	22.3	2494	41.1	22.0	2184	154.7	21.7
Jan	24.0	79.4	23.4	2285	206.5	23.2	2152	158.1	23.3
Feb	23.3	28.0	22.9	1903	249.5	21.8	1910	123.9	22.8
Mar	18.0	47.3	22.9	1605	88.6	20.7	1582	100.9	20.6
Apr	13.8	69.2	17.5	1233	180.3	19.4	1227	64.4	17.8

3. Rice-Pasture Rotations Enhance Rice Nitrogen Uptake and Soil Nitrogen Supply While Reducing Fertilizer Dependence in Uruguay.

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CORE IDEAS

Rice after perennial pastures obtained highest yields and were not outperformed by rice sown after rice with the highest N dose

Rice after pastures showed least reliability on nitrogen fertilizer, while rice sown after soy had the biggest response to fertilization

Diverse antecedent generates better nutrition status and indicators than rice sown directly after rice

3.1. Abstract

Rice yields are primarily nitrogen-limited under irrigated production systems worldwide. Crop rotations that enhance the soil's nitrogen-supplying capacity are key to reducing dependence on nitrogen fertilizers inputs. This study evaluated the effects of crop rotation on soil nitrogen supply, rice nitrogen uptake and yield across three systems: rice-perennial pasture (RPP), rice-soybean (RS) and continuous rice (CR), within a long-term experiment in Uruguay. Soil nitrogen supply capacity (SNSC) and rice nitrogen demand were estimated based on nitrogen uptake in unfertilized and fertilized (100 kg N ha⁻¹) plots, respectively. Crop biomass, nitrogen uptake, soil nitrogen mineralization using ion exchange resins (IER), nitrogen dilution curves and grain yield were measured across two growing seasons. Nitrogen uptake in unfertilized plots was a more reliable indicator of SNSC than mineralization estimated by IER. RPP achieved the highest yield (12.86 Mg ha⁻¹), SNSC (147 kg N ha⁻¹), and nitrogen uptake (180 kg N ha⁻¹), along with a better fit to the critical nitrogen dilution curve and lower fertilizer dependence, with soil supplying 82% of the crop's nitrogen demand. Conversely, CR had the lowest yield (9.73 Mg ha⁻¹), SNSC (92 kg N ha⁻¹) and nitrogen uptake (127 kg N ha⁻¹), with a

poorer fit to the critical dilution curve and higher fertilizer dependence (soil supplying 72% of crop demand). RS had similar yield (12.85 Mg ha^{-1}), nitrogen uptake (175 kg N ha^{-1}) and dilution curve as RPP, but lower SNSC (119 N ha^{-1}), indicating higher fertilizer reliance. These findings highlight the role of perennial mixed pastures in integrated rice–livestock systems in enhancing soil nitrogen cycling and supply, while reducing the need for nitrogen fertilizers in temperate climates of South America.

Keywords: rice-pasture rotations, cation exchange resins, soil mineralization, native nitrogen, nitrogen dilution curves

3.2. Introduction

Rice cropping is highly dependent on nitrogen inputs, accounting for 20% of global nitrogen (N) fertilizer use (Rao et al., 2017). More than 70% of rice is produced in irrigated systems under flooded conditions. Nitrogen fertilizer rates in high yielding systems range from 120 to 200 N kg ha^{-1} , but it is estimated that 50% to 70% of this nitrogen is lost (Cameron et al., 2013; Prasad & Singh Shivay, 2015; Rose et al., 2022). Most N losses occur through volatilization (Zhang et al., 2018), making rice the least nitrogen use-efficient cereal (Udvardi et al., 2021; Zhang et al 2015), with the crop rarely absorbing more than 50% of the applied N fertilizer (Rose et al., 2022).

Rice rotation systems including pastures for livestock, as well as other crops like soybeans, are common in temperate South America, enabling high yields with low nitrogen fertilizer inputs (Denardin et al., 2025; Castillo et al., 2023; Ribas et al., 2021). Rice in the region is typically direct-drilled and fertilized with 80–120 kg N ha^{-1} , with 70% of the nitrogen broadcasted at tillering on dry soil immediately before flooding, and the remaining 30% at panicle initiation (Meus et al., 2024; Castillo et al., 2021; Ribas et al., 2021). In Uruguay, where rice is mostly rotated with pastures and occasionally other crops, the farmers yield from 2021 to 2025 averaged 9.25 Mg ha^{-1} with an application of 80–90 kg N ha^{-1} (Molina & Terra, 2023), approximately half the nitrogen fertilizer inputs used in high-yielding systems in China (Zhang et al., 2018). Rice yield following grazed pastures or soybean-cover crop sequences is

significantly higher than after rice, and nitrogen use efficiency (NUE) is also greater (Denardin et al., 2025; Castillo et al., 2023; Macedo et al., 2022; Ribas et al., 2021). Castillo et al. (2023), analyzing the entire system and considering all nitrogen inputs in a long-term experiment, found that rice-pasture rotation had an almost neutral nitrogen balance (-6 kg N ha^{-1}), a slightly positive nitrogen surplus ($+13 \text{ kg N ha}^{-1}$) and a NUE of 54%. Pastures contribute to soil carbon sequestration (Macedo et al., 2022), biological nitrogen fixation (Peoples & Baldock, 2001) and enhanced nitrogen cycling (Carlos et al., 2020; Denardin et al., 2020), thereby reducing rice dependence on nitrogen fertilizer inputs (Denardin et al., 2025; Castillo et al., 2023). Soils following a period of grazed pastures have a greater capacity to supply indigenous nitrogen than soils under continuous rice, potentially providing 70–80% of the total nitrogen required by high-yielding rice crops (Denardin et al., 2025; Castillo et al., 2021). Considering attainable potential yields of 11.5 Mg ha^{-1} in the region (Carracelas et al., 2023) and typical grain and straw nitrogen concentrations of 1.1 and 0.6%, respectively (Omara et al., 2019; Fageria & Carvalho, 2014), crop nitrogen uptake is expected to reach approximately 158 kg N ha^{-1} , of which up to 125 kg N ha^{-1} may be indigenous nitrogen.

Control plots without nitrogen fertilizer application serve as valuable proxies for assessing the indigenous nitrogen supply capacity of soils, which originates from soil organic matter mineralization, biological nitrogen fixation and residual sources such as atmospheric deposition and irrigation water (Quemada & Lassaletta, 2024; Tamagno et al., 2024). Comparing crop yield and nitrogen uptake between unfertilized and fertilized treatments allows estimating the contribution of soil-derived nitrogen to crop nutrition and evaluating the efficiency of fertilizer applications (Cassman et al., 2002; Dobermann, 2007). This approach is relevant in integrated rice-livestock systems, where biological processes and organic matter inputs can enhance the soil's N-supplying capacity, reducing dependence on external nitrogen inputs while improving productivity.

Soil organic matter mineralization dynamics are key to determine soil N availability for the rice crop. Under flooded conditions, organic matter does not mineralize as fast as in dry conditions due to the lack of oxygen resulting in an

accumulation of organic matter (Sahrawat, 2004). Without oxygen, microbial communities turn to alternative electron donors such as NO_3^- , Mn^{4+} , Fe^{3+} and SO_4^{2-} , and emit N_2 , N_2O , H_2S , CH_4 and NH_3 (which is lost due to volatilization), explaining the low efficiency of nitrogen fertilizers in irrigated systems (Kögel-Knabner et al., 2010; Wei et al., 2021). Also, mineralization rates depend on several abiotic factors such as carbon-nitrogen ratio, texture, temperature and redox potential, among others (Yeasmin et al., 2012; Kader et al., 2013). The N compounds found in organic matter get released during mineralization in inorganic forms, mostly NO_3^- (Zhang et al., 2017b), but, without oxygen, NH_4^+ is the main source of N for rice (Lee, 2021). The N is rapidly absorbed during the early stages of crop growth, resulting in high N concentrations at low biomass levels (Justes et al., 1994). Over time, biomass accumulation outpaces N uptake, causing a dilution effect. The concept of critical N concentration (cN), introduced by Lemaire et al. (1984), links N concentration to aboveground biomass and is expressed by the equation $cN = aW^{-b}$, where W represents biomass and a and b are empirical parameters. Research has focused on determining the minimum N concentration required for optimal biomass production (Justes et al., 1994; Lemaire et al., 1984, 2007, 2008), as well as its relationship with grain yield (Ata-Ul-Karim et al., 2016, 2017).

Ion exchange resins (IER) have been used to measure nutrient availability since 1951 (Pratt) and 1955 (Amer et al.). Resins absorb nutrients present in soil solution in exchange with their own. Usually made in the form of small plastic porous beads, the IER polymer material is permanently attached with a fixed ion and a counterion to balance charges and maintain electrical neutrality (Rom & Haas, 2008). This counterion (which may vary for different products) is then replaced by nutrients in the soil solution. Depending on the nutrient of interest, either cation exchange resins (CER) or anion exchange resins are selected. Dobermann et al. (1998) estimated that the usefulness of anion exchange resins may be reduced since anion activity on flooded soils is limited due to the lack of oxygen. This is especially relevant when trying to measure N, which in flooded soils tends to be almost exclusively in the form of NH_4^+ , a cation (Savant & De Datta, 1982; Kögel-Knabner et al., 2010).

NH_4^+ removal from aqueous solution has been done as a water treatment solution with high levels of success (Ding & Sartaj, 2016).

We hypothesized that soils under rice-perennial pasture rotation systems can supply a substantial proportion of the rice crop's nitrogen demand in high-yielding environments, thereby reducing reliance on nitrogen fertilizer inputs compared to more intensive continuous cropping systems.

This study assesses soil N mineralization, N uptake and biomass accumulation over two irrigated rice growing seasons, with and without fertilization, across contrasting rice-based rotations. Additionally, it assesses the suitability of ion exchange resins (IER) for measuring soil nitrogen dynamics under flooded conditions. The CER were selected to absorb inorganic N, providing valuable insights into N mineralization dynamics over two cropping cycles.

3.3. Materials and Methods

3.3.1. Experimental Site

The study was carried out in selected plots from a long-term experiment (LTE) at the Paso de la Laguna' experimental station of the National Institute for Agricultural Research (INIA), located in Treinta y Tres, Uruguay (33° 16' 23" S; 54° 10' 24" W). Established in 2012, the LTE evaluates the sustainability of six no-till rice-based rotation systems with varying intensity and diversity levels, defined by the frequency of rice, perennial pastures, cover crops and other crops (Macedo et al., 2021). The LTE was established as a randomized complete block (RCB) design using 60 plots (20 × 60 m), with all phases of the six rotation systems simultaneously represented and replicated three times in space (Patterson, 1964). The climate is mesothermic humid, with a mean annual precipitation of 1350 mm, an average summer temperature of 22.4 °C and a winter average of 11.7 °C (INIA, 2025; Table 1 Appendixes). Soil at the site is classified as a Typic Argialboll according to the USDA Soil Taxonomy (2006) and as Mollic Planosol under the World Reference Base for Soil Resources (Schad et al., 2015). Initial soil test in the surface layer indicated a pH of 5.7, cation exchange capacity of 0.02 cmol kg⁻¹, 14.2 g kg⁻¹ of

organic C, and a texture composition of 190, 510 and 300 g kg⁻¹ of sand, silt and clay, respectively (Table 4 Appendixes).

3.3.2. Treatments and Experimental Design

We selected 9 rice-phase plots from three rotation systems in the LTE during two growing seasons (2019–2020 and 2020–2021), corresponding to the rice–pasture (RPP), rice–soybean (RS) and continuous rice (CR) systems (Table 1). The RPP rotation follows a 5-year sequence comprising a perennial pasture mixture of tall fescue (*Festuca arundinacea* Schreb.), white clover (*Trifolium repens* L.) and birdsfoot trefoil (*Lotus corniculatus* L.) grazed for three years, followed by two years of rice, with annual ryegrass (*Lolium multiflorum* Lam.) used as a cover crop between rice crops; only the first rice crop after pastures was selected for our study. The RS rotation was a 2-year sequence of soybean (*Glycine max* [L.] Merr.) and Egyptian clover (*Trifolium alexandrinum* L.) as a cover crop, followed by rice and an annual ryegrass cover. The CR system consisted of a continuous sequence of rice and Egyptian clover cover crop.

A factorial arrangement of three selected rice-phase rotations and two nitrogen fertilization levels was evaluated over two cropping seasons using a split-plot randomized complete block (RCB) design with three replications. Within each season, each 20 × 60 m plot corresponding to the rice phase of CR, RS and the first rice of RPP (three plots per rotation) was partially split into two subplots (4 m × 16 m): one receiving 100 kg N ha⁻¹ as urea (N100) and the other receiving no nitrogen fertilizer (N0). Urea in the fertilized treatment was broadcast in two split applications: 75 kg N ha⁻¹ at tillering initiation (V4) on dry soil immediately before flooding and 25 kg N ha⁻¹ at panicle initiation (R0) on flooded soil. No physical separation was used between subplots.

Table 1. Crop rotation systems and their phase sequences evaluated during the 2019–2020 and 2020–2021 seasons in a long-term experiment with a complete statistical design in Uruguay. SS: Spring-Summer; AW: Autumn-Winter; Ta: *Trifolium alexandrinum*; Lm: *Lolium multiflorum*.

Rotation	Sequence phases									
	1		2		3		4		5	
	AW	SS	AW	SS	AW	SS	AW	SS	AW	SS
Continuous rice	Ta	Rice								
Rice-soybean	Lm	Soy	Ta	Rice						
Rice-pasture	----- Mixed perennial pasture -----						Rice 1	Lm	Rice 2	

3.3.3. Crop Management and Agricultural Practices

The rice crop (cv. INIA Merín) was no-till seeded into dry soil at a rate of 140 kg ha⁻¹ in 0.17 m rows on October 25, 2019 and October 15, 2020. Egyptian clover cover crops preceding rice in the CR and RS systems were sown in late April, immediately after rice and soybean harvest, respectively. In the RPP system, perennial pastures preceding rice were established 3.5 years earlier, fertilized annually and rotationally grazed by sheep from March to December. Pastures and cover crops were terminated with a mix of herbicides in September, five weeks before rice seeding. Egyptian clover accumulated 2.16 and 1.82 Mg DM ha⁻¹ of biomass in CR and RS, respectively. In contrast, perennial pastures in RPP produced an average biomass of 5.84 Mg DM ha⁻¹ yr⁻¹.

Crop management practices, including fertilization, pesticides applications, irrigation and other operations, followed INIA extension guidelines for rice producers. Phosphorus (P) and potassium (K) fertilizers were broadcast immediately before rice seeding, based on soil test results and crop critical levels. From 2012 to 2019, annual nitrogen fertilization rates for rice averaged 75 kg N ha⁻¹ yr⁻¹ for RPP, 76 kg N ha⁻¹ yr⁻¹ for RS and 148 kg N ha⁻¹ yr⁻¹ for CR, based on soil test and N

balances calculations. No fungicides or insecticides were applied, except for seed treatment. Post-emergence herbicides and the first urea dose were applied at the initial tillering stage (V4) immediately before flooding. Irrigation water, sourced from a river, totaled approximately 9,000 m³ ha⁻¹, supplementing rainfall to maintain a 10–15 cm water layer for 15 weeks, from crop tillering to physiological maturity, one week before harvest. Agricultural operations were carried out using equipment similar to that used in commercial rice production.

3.3.4. Data collection and determinations

3.3.4.1. Field and sample procedures

Crop dry matter accumulation, N uptake and soil mineral N were determined every 15 days from flooding until ripening, using three subsamples per plot. Dry matter was estimated by aboveground biomass samples, which were also analyzed for nitrogen concentration to calculate crop N uptake. Soil N mineralization was estimated using two complementary approaches: (a) punctual soil cores to measure instantaneous inorganic N content at 0-15 cm depth and (b) ion-exchange resins (IERS), which absorb inorganic N over 15-day intervals, providing an estimate of accumulated inorganic N. The IERS were deployed every 15 days across seven sampling periods over 105 days. With repeated measures, this resulted in 21 observations per plot (Figure 1).

As three subsamples were removed for each measurement period, removal started from the front to the back of the plot to avoid trampling and to obtain undisturbed samples if possible. Two central meters of each plot remain unsampled for grain harvest at the end of the season (Figure 1). For safety reasons, we sampled in the following order: soil, IERS and above ground biomass.

Since rice primarily absorbs nitrogen as NH₄⁺, and this is the dominant inorganic form during the flooded stage (Reddy et al., 1984), this part of the study focused on determining NH₄⁺ concentrations. Accordingly, it was essential to select IERS capable of capturing NH₄⁺ (Table 3 of appendixes) and to process soil and water samples using methods that allowed accurate measurement of NH₄⁺.

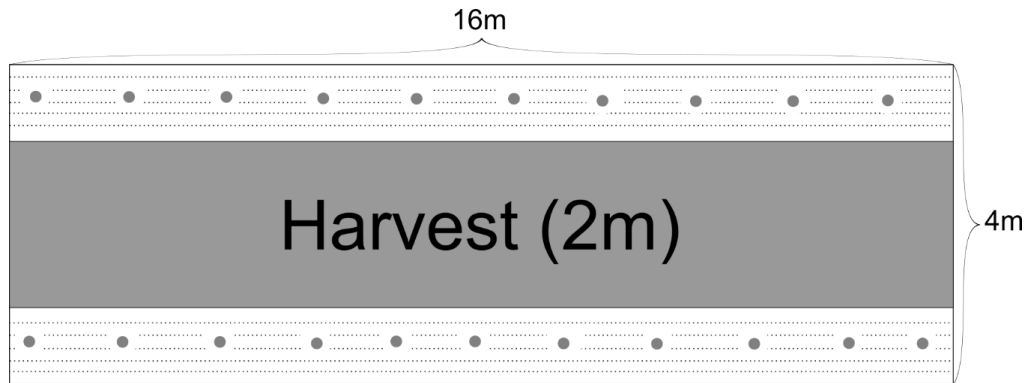


Figure 1. Plot distribution of sampling sites and area for harvest. ● Point represent pvc pipes positions along the cycle and dotted lines represent crop rows.

3.3.4.2. IER installation and processing

Cylindrical holes were made using a soil auger to install the IER bags before flooding, at a depth of 10 cm and a radius of 1 cm. The holes were located between crop rows, spaced 1.5 m apart (Figure 1)

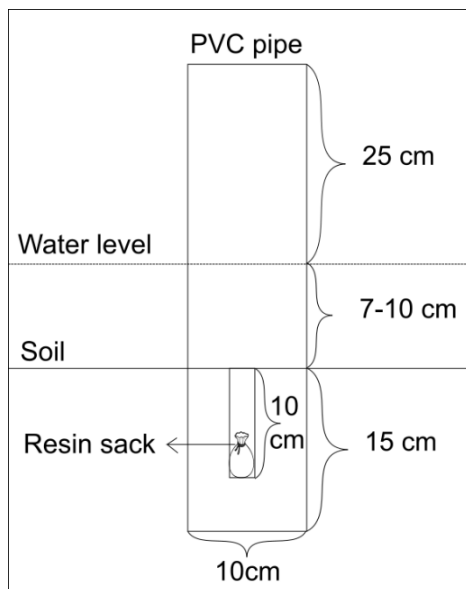


Figure 2. Scheme of IER bags and PVC pipe placement in each rice experimental unit.

Purolite® SSTC60 cationic exchange IERs were used to measure soil N mineralization, capable of retaining NH_4^+ . Under controlled laboratory conditions, IERs were capable of absorbing 100% of the NH_4^+ 10M solution of 200ml after the

first extraction with KCl 2M. Organza bags (5x5 cm) were filled with 20 g of IER and installed in the previously prepared holes. To avoid direct contact with the soil, a PVC pipe of 11 mm diameter was installed 15 cm deep delimiting a soil portion of 4861 cm³ (accounting the entire hole) as shown in figure 2. After 15 days, IER bags were removed and placed in sterile plastic jars in a fridge (5°C) until processing the next day.

IERs were cleaned with deionized water three times and placed in a new sterile plastic container filled with 200 ml of KCl 2M. The containers were placed into a shaker at 180 RPM and the extracted solution was placed in Eppendorf tubes to be analyzed by absorbance method in an auto microplate reader (UT-2100C, MRC Laboratory Equipment, Israel). Unfortunately data from season 2020-2021 of the first sampling date (18 DAF) was lost due to shaker malfunctioning.

3.3.4.3. Soil sampling and processing

Soil samples were taken annually at the beginning of the experiment, immediately prior to seeding, from each rotation sequence plot. Ten sampling cores to a depth of 15 cm were collected and composited in each plot, one fraction was dried at 55°C and ground to a 2-mm sieve and analyzed for soil organic carbon and total nitrogen by dry combustion (LECO/Dumas). Additional fresh subsamples were analyzed for anaerobic nitrogen mineralization potential (Keeney, 1982).

During the rice growing cycle, additional soil samples were taken next to IER location using an auger with 1 cm radius to a depth of 15 cm and a horizontal spacing of 15-20 cm. After sampling, soil was stored in the fridge at 5 °C and processed shortly after according to Rothamsted potassium chloride extraction of soil for the determination of NH₄⁺ and NO₃⁻ N. The extract was analyzed by an absorbance method in the auto microplate reader UT-2100C.

3.3.4.4. Plant sampling and processing

Biomass samples were taken next to the IER position and stored in the fridge until processing the next day. Biomass samples were washed with tap water and then with distilled water. Washed samples were dried at 55 °C for 48 hours and then weighed for dry matter. A sample portion is grounded and sent to INIA La

Estanzuela's lab to analyze N concentrations by NIRS (near-infrared spectrometry) with a Perten analyzer (DA7250) and results were paired with dry combustion method (Dumas) for greater precision using LECO elemental analyzer results (CHN 626). Total N uptake was estimated using measured N concentrations and dry biomass data. These values were subsequently used to calculate N uptake using. The differences between each sampling date expressed as the amount of N absorbed during 15 days at each stage.

3.3.4.5. Harvest samples

The day before harvest, four plant samples, each from a 30 cm section of the sown row, were collected alongside the biomass samples to evaluate biomass accumulation, harvest index, final N uptake in grain and straw and yield components. Yield was determined by harvesting the two central meters of the plot with an experimental combine (Figure 1). The harvest index was calculated by splitting grain and straw from plant samples and then used to estimate total biomass from yield data. From that, a sub-sample of grain and straw was taken to determine N uptake by Dumas method in INIA La Estanzuela's lab.

One of the goals of this study was to assess the indigenous nitrogen supply capacity and rice fertilizer dependence across contrasting rotation sequences. To this end, we used nitrogen fertilizer inputs and crop nitrogen uptake and allocation, with and without fertilization, to calculate rice fertilizer dependence as proposed by Quemada and Lassaleta (2024), and the relative nitrogen supply–demand ratio as described by Tamagano et al. (2024). Fertilizer dependence was calculated as the proportion of nitrogen fertilizer input relative to the total nitrogen input, comprising both fertilizer and indigenous nitrogen supply (estimated from N uptake in plots without N fertilizer): $[N_{100} / (N_{100} + N_{uptakeN100})] \times 100$. The relative nitrogen supply-demand ratio was calculated as the ratio of grain nitrogen removal in unfertilized plots (N_0) to that in fertilized plots (N_{100}): $[1 - (N \text{ removal}_{N0} / N \text{ removal}_{N100})]$.

3.3.5. Statistical analysis

Residual analysis was performed graphically using qq-plots and Shapiro-Wilk and Levene tests were used to evaluate normality and homoscedasticity. Data were analyzed through a randomized complete block design model with a split-plot treatment arrangement by season, as follows:

$$Y_{ijkl} = \mu + \tau_i + \lambda_l + \beta_j + \tau\lambda_{il} + \gamma\lambda_{kl} + \delta_{ij} + \gamma_k + \tau\gamma_{ik} + \gamma\tau\lambda_{ikl} + \varepsilon_{ijkl}$$

where Y_{ijkl} is the response variable of interest, μ is the overall mean, τ_i is the relative effect of the i -th crop rotation, β_j is the relative effect of the j -th block, δ_{ij} is the main-plot error, γ_k is the relative effect of the k -th N doses level and ε_{ijkl} is the experimental error. The δ_{ij} and ε_{ijkl} assumed a normal distribution with mean equal zero and variances equal σ_δ^2 and σ_ε^2 , respectively.

Analysis of variance for each trait was performed at 95% confidence level. When significant differences were found for any of the factors evaluated, a Tukey test was performed at 95% confidence level. All analyzes were performed in the statistical software R (R Core Team, 2021), using the lme4 (Bates et al., 2015), lattice (Sarkar, 2008) and emmeans (Lenth & Lenth, 2018) packages. Two cropping seasons were not considered as a random input, and data were tested against each cropping cycle to check for interactions.

3.4. Results

3.4.1. Climatic conditions

Climatic conditions were generally favorable for rice cultivation in both years, with some variations, particularly during emergence and flowering (Table 2, Appendixes). In 2019-2020, above-average rainfall in October delayed crop emergence and reduced plant stand by 35% compared to 2020–2021 (288 plants m⁻²), although it remained within the recommended range. However, temperature and radiation were favorable from the tillering stage (post-flooding) through to physiological maturity. In contrast, the 2020-2021 season presented optimal conditions for crop establishment until the reproductive period, although radiation

during the ripening period was less favorable due to rainfall. Despite minor inconvenients, both seasons had near-optimal conditions overall, as reflected in the high mean rice yield recorded in the experiment (10.77 Mg ha⁻¹).

3.4.2. Inorganic soil nitrogen

Soil in RPP (0-15 cm depth) contained 18% more organic carbon (16.6 g C kg⁻¹) and exhibited 39% higher anaerobic nitrogen mineralization potential (26.5 mg N-NH₄⁺ kg⁻¹) compared to CR and RS (data not shown), which was associated with a greater capacity to meet rice nitrogen demand in this system.

On the other hand, soil inorganic NH₄⁺ concentrations during the rice growing season were significantly affected by N fertilization, sampling date and their interaction, but not by rotations (Table 1). Significant differences between fertilized and unfertilized plots were observed only at the first two sampling dates after flooding (3 and 18 DAF), following the first urea application of 75 kg ha⁻¹ at the rice tillering stage. Beyond 18 DAF, no significant differences in soil NH₄⁺ were detected between fertilization treatments (Figure 3). The figure combines data from both years, as their trends were consistent: fertilized plots showed higher soil NH₄⁺ concentrations initially, with differences diminishing thereafter as the season advanced.

Table 2. ANOVA p-values for the effects of season, crop rotation, N dose, sampling date and their interactions on NH₄⁺ absorbed by ion-exchange resins (Resin N) and NH₄⁺ present in the soil solution (Soil N) across two growing seasons (2019–2020 and 2020–2021) in a long-term rice rotation experiment in Uruguay. Bolded values indicate statistically significant effects (p < 0.05).

Model variables	Resin NH ₄ ⁺	Soil NH ₄ ⁺
Year	<.0001	<.0001
Rotation	0.043	NS
N dose	<.0001	0.0004
Date	<.0001	<.0001
Year*rotation	NS	NS
Year*N dose	NS	NS
Year*date	<.0001	<.0001
Rotation*N dose	NS	NS
Rotation*date	NS	0.038
N dose*date	<.0001	0.0012
Year*date*N dose	0.0017	NS
Year*rotation*N dose	NS	NS
Year*rotation*date	0.0021	0.0045
Rotation*N dose*date	NS	NS
Year*rotation*N dose*date	NS	NS

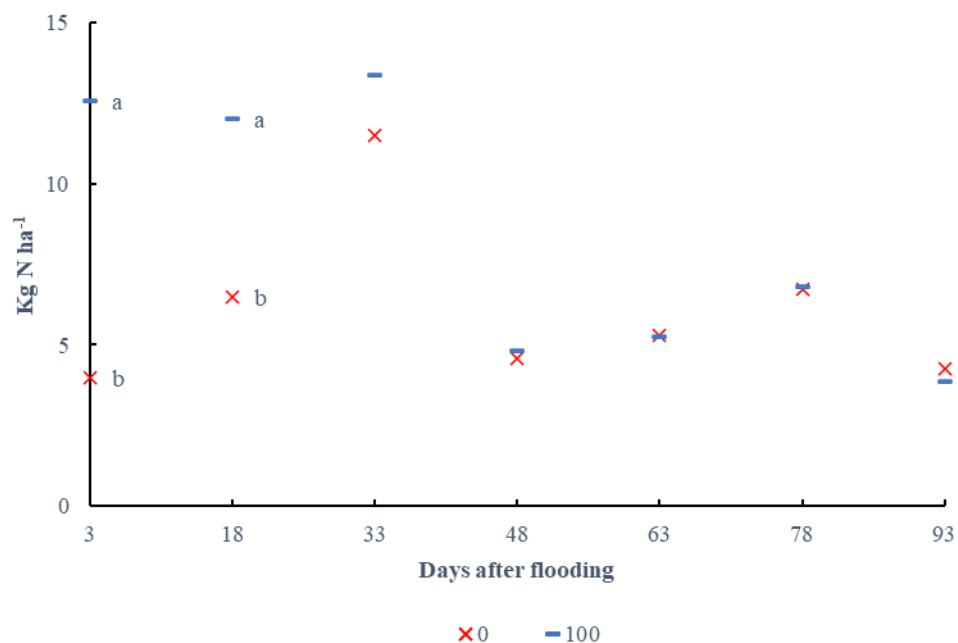


Figure 3. Effect of nitrogen fertilization on soil solution NH_4^+ content (kg N ha^{-1}) over seven sampling dates from 3 to 93 days after rice flooding (DAF), averaged across two growing seasons (2019–2020 and 2020–2021) in a long-term rotation experiment in Uruguay. Different letters indicate statistically significant differences in LSD (0.05).

3.4.3. Nitrogen captured by IERs

The amount of NH_4^+ captured by IERs over 15-day intervals was affected by several factors (Table 2). Higher N absorption was observed during the early measurement periods (Figure 1 Annex). The most notable effect was a three-way interaction between season, N dose and sampling date. In general, NH_4^+ captured by IERs was greater in the 2019-2020 season than in 2020-2021. Within the same season and sampling date, fertilized plots typically showed higher N levels than unfertilized ones during the initial sampling periods; however, from the third sampling date onward, no significant differences were observed in 2019–2020. In contrast, the 2020–2021 season showed lower NH_4^+ levels overall and no clear pattern between fertilization treatments. In both seasons, NH_4^+ values were higher at early sampling dates and decreased toward the last two (93 and 108 DAF). When

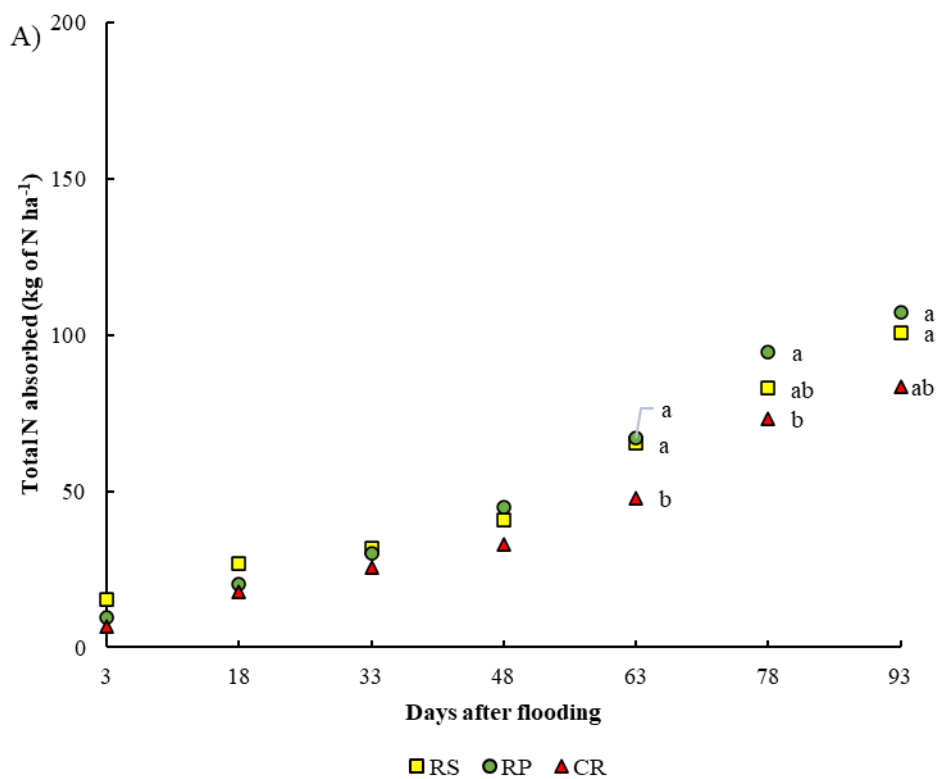
analyzed by rotation, the continuous rice (CR) system showed higher inorganic N capture by IERs compared to RS, with both being similar to RPP.

3.4.4. Crop N Uptake during the growing season

Rice N uptake up to 93 days after flooding (DAF), corresponding to the milk stage of the ripening stage, was significantly influenced by rotation, sampling date, N fertilization rate and their interactions (Table 3). On average, rice in RPP and RS exhibited greater N uptake at 93 DAF than in CR (24% higher), but the difference was 42% in fertilized plots and 28% in unfertilized plots. Similarly, total N uptake 93 DAF was 40% higher in fertilized plots compared to unfertilized ones (98 kg N ha^{-1}). Regarding N uptake rates, rice in RPP had the highest average ($19.1 \text{ kg N ha}^{-1}$ every 15 days), while in CR showed the lowest ($13.7 \text{ kg N ha}^{-1}$ every 15 days). Nitrogen fertilization significantly increased uptake rates, with fertilized plots averaging 41% higher values ($19.6 \text{ kg N ha}^{-1}$ every 15 days) than unfertilized plots ($13.9 \text{ kg N ha}^{-1}$ every 15 days). Although the interaction between rotation and N dose was not statistically significant, RPP and RS consistently showed higher uptake rates than CR. Notably, fertilized plots in RS exhibited high variability in N uptake during the crop cycle.

Table 3. ANOVA p-values for the effects of crop rotation, nitrogen dose, sampling date, and their interactions on total N uptake and N uptake rate in rice across two growing seasons (2019–2020 and 2020–2021) in a long-term experiment in Uruguay. Significant effects ($p < 0.05$) are shown in bold.

	Rotation	N dose	Date	Rotation *N dose	Rotation *date	N Dose*date	Rotation *N dose*date
Total N uptake	<.0001	<.0001	<.0001	0.0004	<.0001	<.0001	0.0156
N uptake rate	0.031	0.0012	<.0001	NS	0.045	0.0001	0.0007



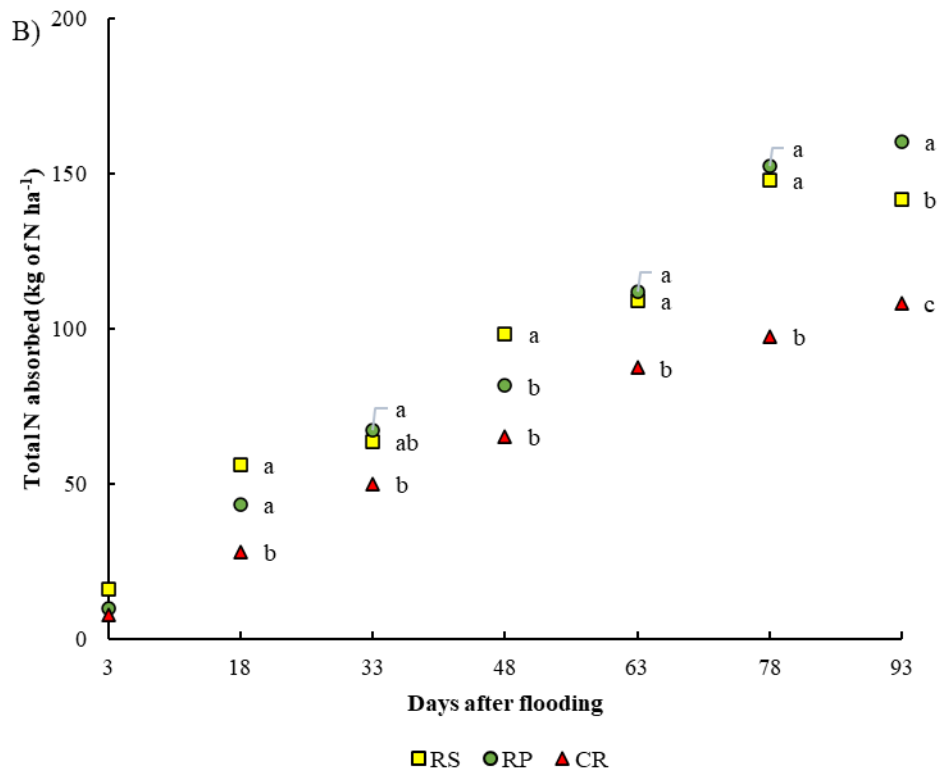


Figure 4. Effect of three crop rotation systems [continuous rice (CR), rice-soybean (RS) and rice-perennial pasture (RPP)] on accumulated N uptake by rice (kg N ha⁻¹ every 15 days) in (A) unfertilized plots and (B) fertilized plots receiving 100 kg N ha⁻¹, averaged over two growing seasons (2019–2020 and 2020–2021) in a long-term experiment in Uruguay. Different letters indicate statistically significant differences according to the LSD test ($p < 0.05$).

3.4.5. Dry Matter and N concentration

Dry matter accumulation up to 93 DAF was significantly affected by crop rotation and nitrogen fertilization rate, while their interaction was not significant (table 4). As expected for a cumulative variable, sampling date also had a significant effect, reflecting the continuous increase in biomass over time. On average, rice in the RS and RPP rotations accumulated 31% more biomass at 93 DAF compared to CR, which produced 11.58 Mg DM ha⁻¹ (table 4). Similarly, nitrogen fertilization at 100 kg N ha⁻¹ increased rice biomass accumulation by 47% relative to unfertilized plots, which averaged 11.89 Mg DM ha⁻¹, regardless of rotation.

Table 4. ANOVA p-values for the effects of crop rotation, nitrogen dose, and sampling date, and their interactions, on rice dry matter (DM) and plant nitrogen concentration across two growing seasons (2019-2020 and 2020-2021) in a long-term experiment in Uruguay. Significant effects ($p < 0.05$) are shown in bold.

	Rotation	N dose	Date	Rotation *N dose	Rotation *date	N Dose*d ate	Rotation*N dose*date
DM	<.0001	<.0001	<.0001	0.0503	<.0001	<.0001	0.0705
%N	0.3220	<.0001	<.0001	0.0517	0.0206	<.0001	0.9970

The interaction between crop rotation and nitrogen dose significantly influenced dry matter accumulation over time. In the early stages following flooding, no significant differences were detected among either rotations or fertilizer treatments.

However, differences associated with nitrogen fertilization emerged from 33 DAF onward, while effects of crop rotation became significant after 48 DAF. From these points, plots receiving 100 kg N ha^{-1} consistently accumulated greater dry matter than unfertilized plots and rice grown under the RS and RPP rotations outperformed that in the CR system

In contrast to dry matter accumulation, crop nitrogen concentrations exhibited the highest differences during the early sampling dates and tended to converge toward the later stages. Fertilized plots showed significantly higher plant nitrogen concentrations up to 48 DAF. However, beyond 3 DAF, crop rotation had no significant influence on crop nitrogen concentration. At this early stage, RS had the highest N concentration, whereas RPP exhibited the lowest values (table 5).

Table 5. Rice dry matter (DM) and nitrogen concentration (%N) as affected by crop rotation and nitrogen dose across sampling dates in two growing seasons (2019–2020 and 2020–2021) in a long-term experiment in Uruguay. Continuous Rice (CR); Rice-Soybean (RS); and Rice-Perennial Pasture (RPP).

Treatment	3	18	33	48	63	78	93
Dry matter (kg ha ⁻¹)							
Rotation							
RS	390	1356	2784	4647 a	7942 a	12765 a	14521 a
RP	267	1053	2589	4249 ab	7754 a	12547 a	14921 a
CR	198	759	2086	3404 b	5875 b	9537 b	11576 b
N dose							
0	284	884	1851 b	2983 b	5503 b	9267 b	11885 b
100	286	1227	3122 a	5218 a	8877 a	13966 a	15460 a
%N							
Rotation							
RS	3.93 a	3.07	1.65	1.45	1.06	0.89	0.84
RP	3.68 b	3.15	1.79	1.44	1.34	0.97	0.89
CR	3.78 ab	3.1	1.71	1.4	1.39	0.91	0.84
N dose							
0	3.60 b	2.57 b	1.57 b	1.32 b	1.08	0.9	0.82
100	3.96 a	3.64 a	1.88 a	1.54 a	1.15	0.94	0.88

Different letters indicate statistically significant differences according to the LSD test ($p < 0.05$).

Using dry matter production and nitrogen concentration data across the rice cycle, nitrogen dilution curves were constructed to support the interpretation of the results (Figure 3). Rice nitrogen status was affected by both, crop rotation and nitrogen application. The RPP and RS from 38 to 93 DAF maintained greater biomass accumulation than CR at comparable nitrogen concentrations and nitrogen fertilization rates. Similarly, fertilized plots had a better fit with the optimal dilution curve and higher biomass than unfertilized ones at similar N concentrations.

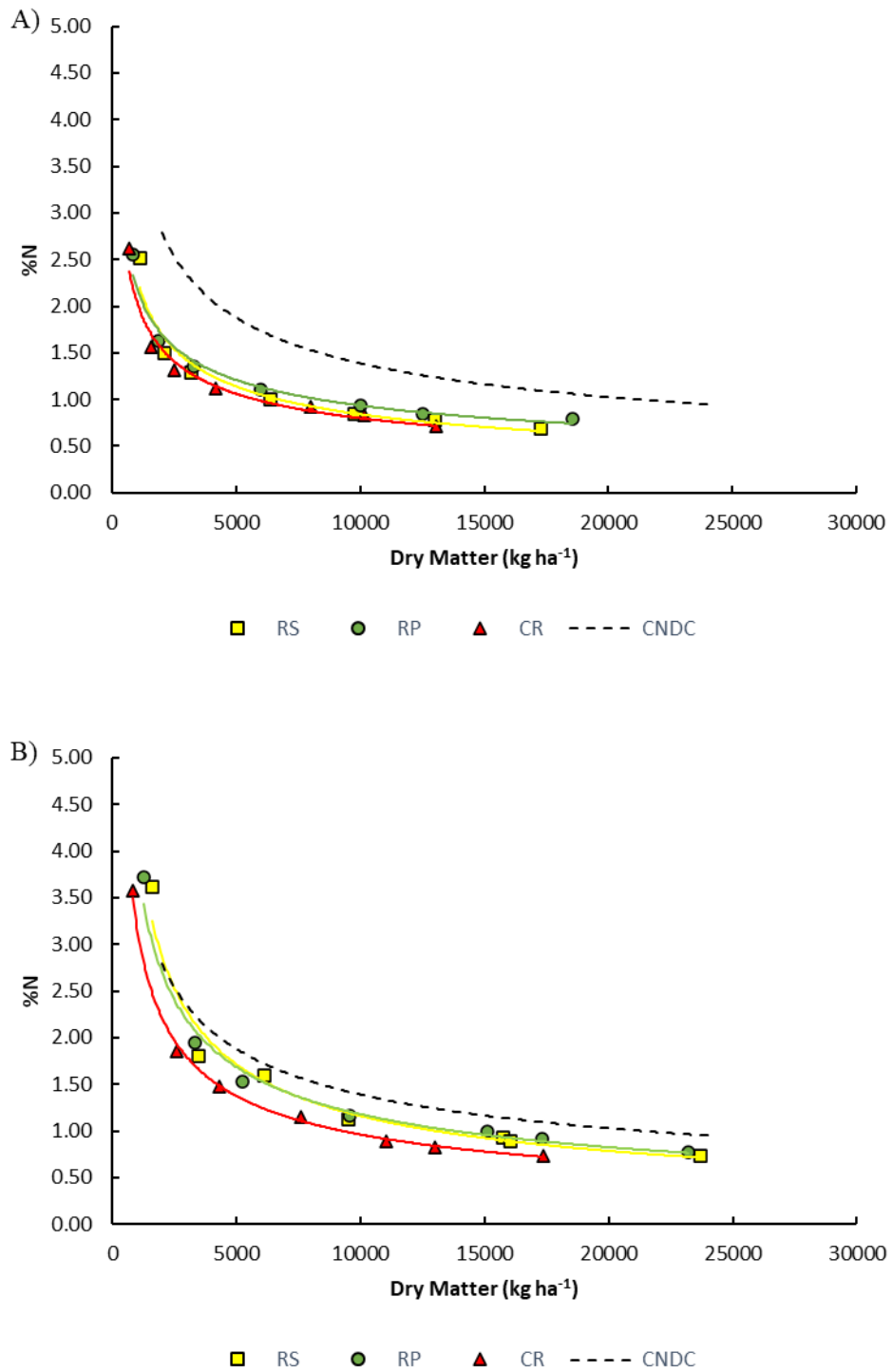


Figure 5. Effect of three rice rotation systems (continuous rice (CR), rice–soybean (RS) and rice–perennial pasture (RPP)) on the relationship between dry matter accumulation and nitrogen concentration (%N) in relation to the critical

nitrogen dilution curve (CNDC) established by Moreira et al. (2025), for (A) unfertilized plots and (B) fertilized with 100 kg N ha⁻¹. Data was averaged over two growing seasons (2019–2020 and 2020–2021) in a long-term experiment in Uruguay.

3.4.6. Rice yield and total crop N uptake

Rice grain yield, grain N content, and total crop N uptake at the end of the crop cycle were significantly affected by crop rotation and nitrogen fertilization, but not by their interaction (Table 6). On average, rice yield in RPP and RS was 33% higher than in CR (8.82 Mg ha⁻¹). This difference was consistent either in unfertilized treatments (10.63 vs. 7.90 Mg ha⁻¹) or fertilized with 100 kg N ha⁻¹ (12.86 vs. 9.73 Mg ha⁻¹) (figure 6). Even with nitrogen fertilization, rice yield in CR was 8% lower than in unfertilized RPP and RS plots. Rice yield responses to nitrogen fertilization were greater in RS (26%) and CR (23%) than in RPP (16%).

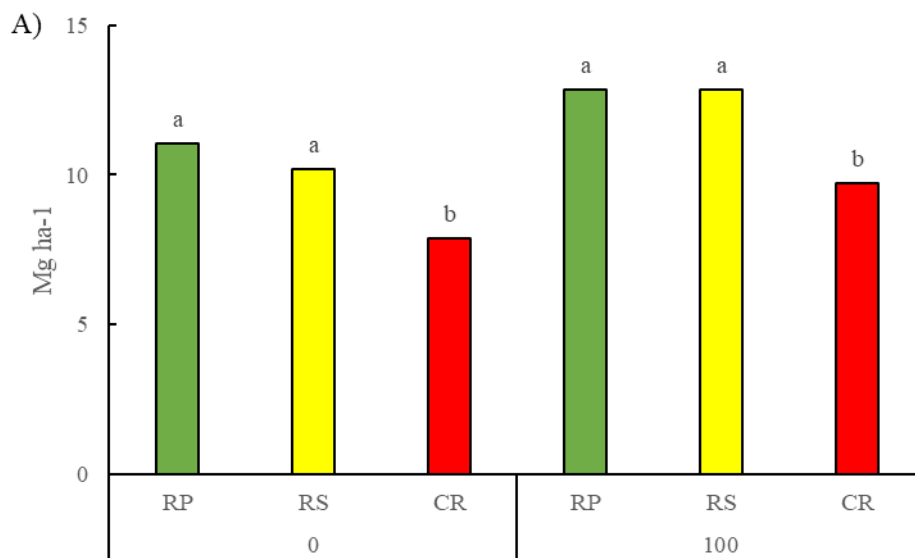
Table 6. ANOVA p-values for the effects of crop rotation, nitrogen dose and their interaction on rice yield, harvest index (HI), plant nitrogen concentration, grain nitrogen concentration, grain N content and total N uptake at harvest across two growing seasons (2019-2020 and 2020-2021) in a long-term experiment in Uruguay. Significant effects ($p < 0.05$) are shown in bold.

	Yield	HI	Plant %N	Grain %N	Grain N (kg ha ⁻¹)	Total N (kg ha ⁻¹)
Rotation	<.0001	NS	NS	NS	0.001	<.0001
N dose	<.0001	0.006	0.047	NS	<.0001	<.0001
N dose*rotation	NS	NS	NS	NS	NS	NS

Rice grain N content in RP and RS (106.9 kg of N ha⁻¹) was 38% higher than in CR (figure 6). On the other hand, fertilized plots had 26% greater grain N content compared to unfertilized plots (86.1 kg N ha⁻¹). Notably, grain N content in fertilized CR plots did not surpass that of unfertilized RPP plots, although it matched RS values (86 kg N ha⁻¹). Accordingly, the supply-to-demand ratio in RPP (0.14) was lower than in CR (0.22) and RS (0.26).

Finally, total nitrogen uptake at the end of the cycle in RPP and RS (155.2 kg N ha⁻¹) was 41% higher than in CR (figure 6). Fertilized plots absorbed 34% more nitrogen than unfertilized plots at the end of the season (119.5 kg N ha⁻¹). The highest N uptake (177.2 kg N ha⁻¹) was recorded in fertilized plots of RPP and RS, representing a 39% increase compared to CR at the same fertilizer rate. The indigenous nitrogen supply in the RPP system met 82% of the crop's total nitrogen uptake, compared to 73% in CR and 68% in RS. As a result, nitrogen fertilizer dependence in RPP was 5% and 12% lower than in RS (0.46) and CR (0.52), respectively.

Grain N concentrations were not affected by rotation although biomass N concentrations and harvest index were affected by N dose. Biomass N concentrations increased from 57% to 61% and harvest index from 55% to 60%.



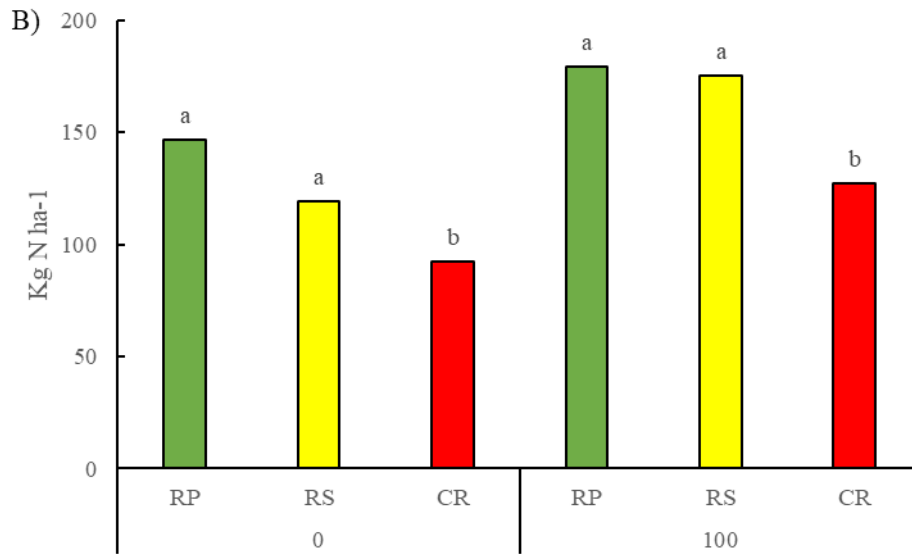


Figure 6. Effect of three crop rotation systems, continuous rice (CR), rice soy (RS), rice perennial pasture (RP) and fertilization (0 kg of N ha⁻¹ and 100 kg N ha⁻¹) on: A) Yield (Mg ha⁻¹) and B) Total N absorption at the end of the cycle (kg of N ha⁻¹) averaged for two growing seasons (2019-2020 and 2020-2021) in a long-term experiment in Uruguay. Different letters indicate statistically significant differences in LSD (0.05).

3.5. Discussion

A growing number of evidence indicates that integrated rice-livestock systems improve soil carbon sequestration, soil quality and nitrogen cycling (Macedo et al., 2022; Carvalho et al., 2021). These benefits enhance the soil's capacity to supply nitrogen to subsequent crops, reducing the need for fertilizer inputs compared to more intensive and simplified cropping systems (Denardin et al., 2025; Castillo et al., 2021; Carlos et al., 2020; Denardin et al., 2020). Although some studies have reported the benefits of rice-livestock integration on soil dynamics and crop performance, most were conducted on degraded soils previously under continuous rice, which were later converted to integrated systems with an annual winter pasture (Denardin et al., 2025; Denardin et al., 2022). The interactive effects of rotation diversification and nitrogen fertilization on soil nitrogen mineralization and rice nitrogen uptake have rarely been evaluated in stable rice-perennial pasture systems

on non-degraded soils (Castillo et al., 2023). A better understanding of these processes will facilitate the sustainable redesign and eventual intensification of such systems, preserving their high soil nitrogen supply capacity and low nitrogen fertilizer dependence, while sustaining high yields.

This study assessed soil nitrogen mineralization and rice nitrogen uptake and yield across contrasting rotation systems and crop nitrogen fertilizer inputs in a LTE, aiming to understand how system design affects soil nitrogen supply capacity and crop nitrogen status over the growing season. In the context of two high-yielding years averaging 10.77 Mg ha⁻¹, we found that crop nitrogen uptake in unfertilized plots (relative to fertilized ones) was a more reliable indicator of soil nitrogen supply capacity across different rotation sequences than nitrogen mineralization estimated by IER and soil sampling. Thus, rice in the RPP system exhibited greater yield, biomass accumulation, crop nitrogen uptake, and a better-fitting nitrogen dilution curve throughout the growing season compared with CR. In this system, rice yielded 12.86 Mg ha⁻¹ with a nitrogen fertilizer input of 100 kg ha⁻¹, of which 82% of the crop's nitrogen demand was met by indigenous soil nitrogen. Overall, the results highlight the relevant role of perennial pastures in rice rotations in shaping soil nitrogen availability, crop nitrogen status, nitrogen uptake dynamics and low nitrogen fertilizer dependence in high-yielding rice systems.

3.5.1. Soil N mineralization

The IER method provided some insights into soil-available NH₄⁺; but, despite proper deployment, handling and processing, it failed to accurately estimate total soil nitrogen mineralization during the rice flooding period, as indicated by nitrogen uptake in crop tissues. The technique is known to require a large number of subsamples due to spatial variability, which likely exceed the three subsamples per sampling date used in our study (St. Luce et al., 2011). Additionally, potential drawbacks of the IER method include the risk of underestimating N fluxes due to IER saturation with other soil cations or poor contact with the soil solution (Noe, 2011; Buck et al., 2016). The highest amounts of captured N by IER bags, either in fertilized or unfertilized plots, were found between 18 and 33 DAF, similar to

incubation studies done by Thiyagarajan et al. (1997) and might be related to the particularities of rice cropping systems in Uruguay. During the off-season and the initial rainfed period of rice cultivation, the soil is rarely water-saturated, allowing for NO_3^- formation (Ali et al., 2007). Upon rice flooding, this N-NO_3^- is prone to denitrification losses (Cannavo et al., 2008; Cameron et al., 2013) and even dissimilatory reduction to NH_4^+ (Zhang et al., 2015). Also, N mineralized in the absence of oxygen is released in the form of NH_4^+ , which explains the higher N levels at the first couple of sample dates in our site (Reddy et al., 1984; Dobermann et al., 1994). In unfertilized plots, soil N was significantly lower in the first sampling dates (3 to 33 DAF). Considering that soil analysis provides snapshots of NH_4^+ , it was expected that fertilized plots exhibit higher NH_4^+ concentration. From 48 to 93 days after flooding (DAF), nitrogen concentrations in both resin and soil reached a low and stable plateau. Ion exchange resins (IERS) absorbed no more than 2.5 kg N ha^{-1} every 15 days, while soil NH_4^+ levels peaked at 7 kg N ha^{-1} available at any given time.

It is worth mentioning that the amount of N captured by IERS during 15 days was lower than the amount of inorganic N determined in soil samples. One of the explanations is that the absorption distance of CER was lower than was previously thought, although studies from Friedel et al. (2000) showed remarkable capabilities of IERS capturing NH_4^+ in incubated soil. According to Skogley and Doberman (1996), IER bags are suitable for “diffusion sensitive methods” as is the case for NH_4^+ capture. Another possible explanation is that the captured NH_4^+ was not that strongly attached to the CER beads (Rhom and Haas, 2008), since, if we chose IERS for affinity, it may have been more efficient to use K^+ based IERS instead of Na^+ based IERS, according to Qian and Schoenau (2002). Due to the high clay content of the Bt horizon at the site (beginning at 25 cm depth), nitrogen losses through leaching were considered negligible. According to Kader et al. (2013) and Li et al. (2021), high pH and low Fe contents may reduce the N mineralization potential during the cropping cycle. The soil at the site had lower Fe concentrations than those reported by Fageria et al. (2011) in their review of typical rice soils in Brazil. However, given the high yield and N uptake observed in unfertilized plots, this

hypothesis was discarded. The depth of the aerobic soil layer (Reddy et al., 1984) was not measured; however, it is generally shallow (typically no deeper than 0.1-1 cm (Dobermann et al., 1998)) and most mineral nitrogen is known to accumulate within the top 20 cm of soil (Ali et al., 2007).

Thus, we ruled out the possibility that ammonium was rapidly transformed into nitrate before being captured by the CER. If that had been the case, the use of mixed cation–anion IER beads would have been more appropriate. Contrary to initial concerns, laboratory results demonstrated a high absorption capacity of CERs, approaching 100% after the first extraction (Figure 2, Annex). Furthermore, findings by Ding and Sartaj (2016) confirm that CERs are capable of effectively retaining NH_4^+ in wastewater, even after multiple reuses. Therefore, the low nitrogen capture by IERs observed in our study may be more plausibly attributed to the complexity of soil-resin interactions, especially considering that plants were able to take up significantly greater amounts of nitrogen. Although not statistically significant, the mean values of nitrogen captured by IER were approximately 10% of the rice nitrogen uptake (Figure 2, Complementary Data).

3.5.2. Rice yield and N uptake

Rice yield in our study was high, averaging 10.77 Mg ha^{-1} with a mean nitrogen uptake of 140 kg N ha^{-1} , corresponding to 77% of the national yield potential (Carracelas et al., 2023). The higher yield observed in RPP and RS compared to CR were not unexpected, as similar results were reported in the LTE (Castillo et al., 2023; Macedo et al., 2022) and in other studies evaluating rice-based systems in the region (Denardin et al., 2025; Ribas et al., 2021; Denardin et al., 2019).

Overall, N uptake in the unfertilized (N0) plots relative to fertilized ones (N100) indicates a strong soil nitrogen supply capacity at our site (Quemada & Lassaletta, 2024; Tamagno et al., 2024). Particularly noteworthy were the yield (11.05 Mg ha^{-1}) and total crop nitrogen uptake (147 kg N ha^{-1}) in unfertilized RPP plots (equivalent to 86% of the yield and 82% of the nitrogen absorbed in fertilized plots) highlighting the high native nitrogen supply capacity of these systems (Castillo

et al., 2021). Therefore, rice yield in RPP with N100 (12.86 Mg ha⁻¹) reached 92% of the national yield potential (Carracelas et al., 2023), comparable to yields reported in high-yielding regions of California (Rehman et al., 2019), but with low fertilizer dependence and a favorable nitrogen supply–demand ratio. Curiously, total N uptake in RPP (180 kg N ha⁻¹) was 20% below the expected uptake for the yield obtained in RPP (Dobermann & Fairhurst, 2000). Beecher et al. (1994) reported significant differences between rotations and fertilized-unfertilized plots with similar yields (up to 12 Mg ha⁻¹) but higher N uptake (207 kg of N ha⁻¹). In a meta-analysis, Xu et al. (2021) found N absorptions of more than 250 kg of N ha⁻¹ in hybrid rice to reach similar yields with average fertilization rates of 144 kg ha⁻¹. These findings underscore the efficient use of indigenous and applied nitrogen in RPP, highlighting the potential to achieve high yield with reduced fertilizer inputs.

Some of our results in CR and RS were similar with those reported by Denardin et al. (2019, 2025) in a comparable study that integrated winter annual ryegrass grazing into continuous rice and rice-soybean systems. For example, total crop N uptake and fertilizer responses in CR were similar to the mentioned studies at comparable nitrogen rates, but, in our study, the soil supplied 73% of the crop's nitrogen demand, compared with 54% in continuous rice systems integrated with grazed annual ryegrass in Brazil. On the other hand, the supply demand ratio in the RS system (26%) matches with Denardin et al. (2025) in an integrated rice-soybeans with annual ryegrass (24%) revealing low fertilizer inputs demand in both, but with 19% higher yield at our site. The lower fertilizer dependence in CR and RS in our experiment, despite their cropping intensity, was likely due to the Egyptian clover cover crop preceding rice (as reported in legume-rice rotations under intensive systems (Yao et al., 2025)) and to the inherent quality and well-preserved condition of the Mollisol at the site.

While nitrogen uptake at 93 DAF (coinciding with the initiation of the milk stage) was lower than the values reported by Thiyagarajan et al. (1997), rice yields in our study were nearly twice as high. Similarly, nitrogen uptake at 33 DAF in RPP and RS with N100 (coinciding with panicle initiation) was 30% lower than the recommended value of 94 kg ha⁻¹ for this stage in high-yielding systems in California

(Rehman et al., 2019). No significant differences in nitrogen uptake rate were observed between rotations in unfertilized plots throughout the growing cycle, although accumulated nitrogen began to diverge from 63 DAF onward. In contrast, differences between rotations in fertilized plots emerged as early as 18 DAF, suggesting that the first measurable effects of nitrogen fertilization on crop uptake became evident approximately two weeks after application. The relative response to fertilization was greater for nitrogen uptake (34%) than for grain yield (22%), indicating potential inefficiencies in nitrogen utilization and/or a substantial contribution of indigenous nitrogen supply in RPP and RS.

3.5.3. Crop nitrogen dilution curves and INN

These curves are useful for evaluating the nutritional status of the crop, as they can reveal which treatments produced plants with a better nitrogen status (Lemaire et al., 2007; Ata-Ul-Karim et al., 2017; Wang et al., 2020).

Although significant differences in N concentration were only observed at the first sampling date, this was expected, as, early in the crop cycle, nitrogen concentrations vary widely due to large differences in biomass and N content (Justes et al., 1994). As the crop develops and nitrogen dilution progresses, concentrations tend to stabilize around 1% depending on the study (Ata-Ul-Karim et al., 2016; Song et al., 2020).

Instead of comparing treatments against critical curves that were developed under different conditions or genotypes (Sheehy et al., 1998; Ata Ul -Karim et al., 2017; Li et al., 2021), we opted for a straight comparison with a national CNDC (Moreira et al., 2024) and a point-by-point comparison of N concentration and biomass between treatments (table 5), since there was no significant interaction between them. This allowed us to evaluate relative differences in nitrogen status across treatments at each point in time.

Based on this information, both under 0N and 100N conditions, RS and RP consistently produced more biomass and had higher nitrogen concentrations than CR. This suggests that beyond yielding results, these rotations created a more favorable environment for plant development and biomass accumulation in unfertilized plots.

Moreover, they also provided a better context to capitalize on applied nitrogen, leading to even more marked differences under fertilized conditions. In cases where a comparison with critical dilution curves is desired, the Nitrogen Nutrition Index (NNI) could be a useful tool. Based on critical curves, NNI values close to 1 indicate the optimal balance between maximum biomass accumulation and minimal nitrogen concentration (Lemaire et al., 2008). This index could help identify, along each sampling date of the dilution curve, which treatments were more efficient in utilizing N.

3.5.4. Rotation and N management

No-till with residue retention (enhancing nitrogen recycling and reducing leaching (Yang et al., 2018; Hyun-Hwoi Ku et al., 2019; Ding et al., 2021)) along with Egyptian clover fixing biological N (Toukabri et al., 2022) in an undegraded Mollisol, may explain the soil N supply and high yields in unfertilized CR plots (7.9 Mg ha⁻¹) compared to other continuous rice systems (Che et al., 2015; Rong et al., 2021). Although nitrogen fertilizer increased CR yield and N uptake, it did not reach the values observed in RPP and RS. We speculate that rice straw contributes minimally to nitrogen supply in our CR system despite the high biomass, as only 22–26% of its nitrogen is mineralized over 105 days (Ghoneim, 2008). In contrast, a legume cover crop biomass of 2-3 Mg ha⁻¹ (terminated with herbicides and followed by rice sowing) may play a more significant role in nitrogen dynamics (Yao et al., 2025; Fontaine et al., 2003; Fageria et al., 2005). Legumes integrated into rice systems contribute to soil organic carbon buildup (Jian et al., 2020), which is closely linked to nitrogen supply (Peng et al., 2017; Zhang et al., 2017b) and may explain their maintenance levels in the LTE. Although soil organic C and NMP in CR were lower than in RPP, no differences in soil available nitrogen were found between rotations during the cycle, suggesting that lower yields and N uptake in CR may be due to factors beyond N supply, or to different NUE. Additionally, Buresh et al. (2021) reported that continuous rice systems can develop asymptomatic diseases as found by Martínez (2023) during the stabilization of the LTE.

Pastures in integrated systems are key to enhance environmental resilience (Franzluebbers et al., 2014) and soil quality while maintaining high crop productivity (Ernst et al., 2018; Pravia et al., 2019). Crop-livestock systems can reduce nitrogen fertilizer use, increase NUE and promote sustainable intensification (Deandrin et al., 2020; Castillo et al., 2021, 2023; Rovira et al., 2022). For example, mixed pastures with white clover as in RPP can fix up to 218 kg N ha⁻¹ (Enriquez-Hidalgo et al., 2016), contributing significantly to soil N pools. Mixed pastures including legumes also increase dry matter production by nearly 3 Mg ha⁻¹, and N fertilization mainly replaces biologically fixed N (Enriquez-Hidalgo et al., 2016). Higher N uptake in RP may be partly attributed to higher SOC and fixed N incorporated to soil N pool (Grahmann et al., 2020; Kumar et al., 2018). Studies have shown that mineralized N is lowest in monocultures and increases in rotations that include legumes such as soybean, white clover or alfalfa (Carpenter-Boggs et al., 2000). Mixed perennial pastures, particularly their roots, are key to increase SOC and total soil N as reported by Macedo et al. (2022) at the LTE site, which may be closely linked to greater N uptake in rice (Peng et al., 2017; Zhang et al., 2017a).

Sustaining high yields in continuous rice is expensive and difficult. Rotating rice with other crops like soybean or maize has proven effective to increase yields and sustainability (Goulart et al., 2020; Ribas et al., 2021, Denardin et al., 2025). Soybeans have been increasingly integrated into rice-based systems in temperate South America, either in continuous rice systems or in rice-pasture systems. Notably, RS showed similar yields and N uptake performance to RPP in our test. The main challenge in rice–soybean systems is combining irrigated rice with rainfed soybean. However, their overall profitability is comparable to continuous rice, as rice following soybean requires less nitrogen, and soybean has lower production costs despite lower gross income (Ribas et al., 2021).

Microbiome–rhizosphere interactions play a key role in plant nutrition (Cassmann & Dobermann, 2022). Rice-legume rotations have a particularly strong influence on soil microbial communities that regulate nitrogen mineralization, outperforming monoculture systems (Jiang et al., 2016). Soil organic carbon and potential mineralizable nitrogen are major drivers of N mineralization (Illarze et al.,

2018). Although CR is not strictly a monoculture, its exclusive use of rice as a summer crop (combined with annual flooding) may contribute to reduced N uptake (Ali et al., 2007). Crop rotation also alters soil properties; for example, Maguire et al. (2020) found that pH differences among rotations explained shifts in microbial activity and composition. Similarly, excessive N fertilization can lower soil pH and disrupt microbiome functions, particularly those involved in nitrification and biological N fixation (Cassmann & Dobermann, 2022).

3.6. Conclusions

The rice–perennial pasture rotation had positive effects on estimated soil nitrogen supply, crop N uptake, nutritional status and yield compared to simpler and more intensified systems over two growing seasons in a long-term experiment. This system exhibited the highest soil nitrogen supply capacity, meeting up to 82% of crop N uptake in optimally fertilized plots yielding 12.86 Mg ha⁻¹, indicating the lowest fertilizer dependence and the most favorable nitrogen supply–demand ratio. On the other hand, the continuous rice system even with a legume winter cover crop showed the lowest yield, crop N uptake, nutritional status and soil nitrogen supply capacity, although its performance was still superior to that of comparable rice-based systems worldwide. Finally, the rice-soybean system achieved high yield and nitrogen uptake comparable to the rice–pasture system, but with lower soil nitrogen supply capacity, indicating higher fertilizer dependence and a less favorable nitrogen supply-demand ratio. Nitrogen dilution curves support these findings, as rice following perennial pastures or soybean-Egyptian clover sequences remained closer to the critical N concentration throughout the crop cycle, either fertilized or unfertilized, suggesting that nutritional deficits resulting from the rotation sequence cannot be fully corrected by nitrogen fertilization only.

Although IER-accumulated and soil-available NH₄⁺ did not fully reflect plant N uptake, they provided valuable insights into nitrogen dynamics. A peak in N availability at panicle initiation 33 DAF was observed, likely due to NH₄⁺ accumulation and low crop uptake. As IERs capture less N than plants absorbed,

further research is needed to better understand N mineralization and improve synchronization between N supply and crop demand in rice systems.

Our findings provide further evidence of the key role of perennial pastures in sustaining high-yielding rice-based systems in the mesothermic, humid climates of South America, by enhancing rice productivity and soil nitrogen supply while reducing dependence on N fertilizers.

3.7. References

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3.8. Appendixes

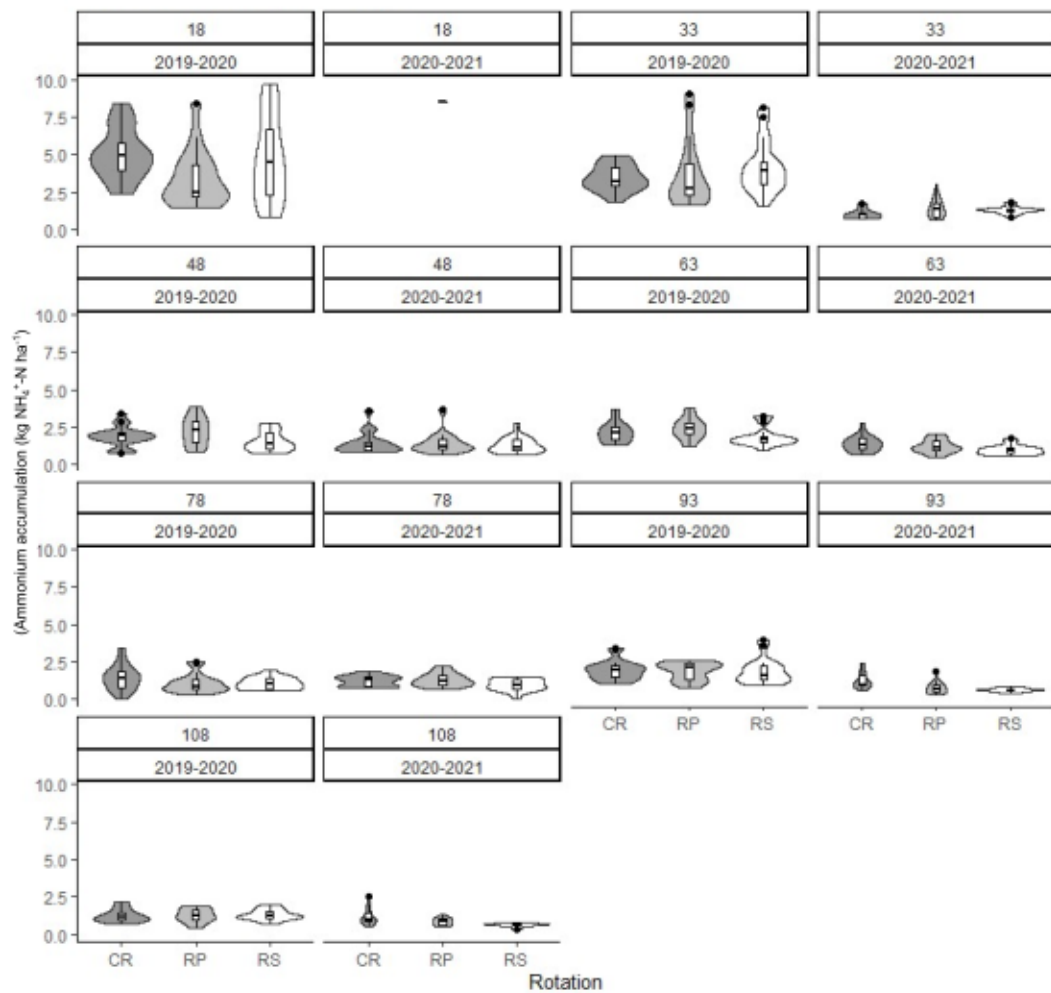


Figure 1. Effect of three crop rotation systems: continuous rice (CR), rice soy (RS), rice perennial pasture (RP) on NH₄⁺ captured by IER (kg of N ha⁻¹) for seven sampling dates starting 18 days after flooding until 108 days after flooding for two growing seasons (2019-2020 and 2020-2021) in a long-term experiment in Uruguay. Different letters indicate statistically significant differences in LSD (0.05).

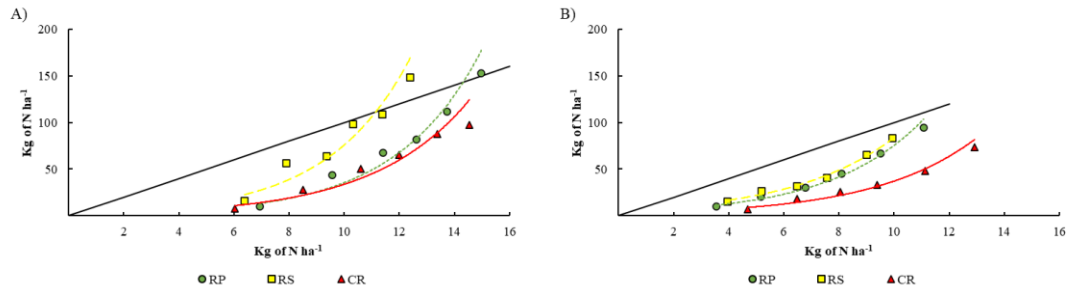


Figure 2: Relationship between N absorbed as measured IER and plant N uptake at 15-day intervals across three crop rotation systems, continuous rice (CR), rice-soybean (RS), and rice-perennial pasture (RP) during two growing seasons (2019–2020 and 2020–2021) in a long-term experiment in Uruguay.

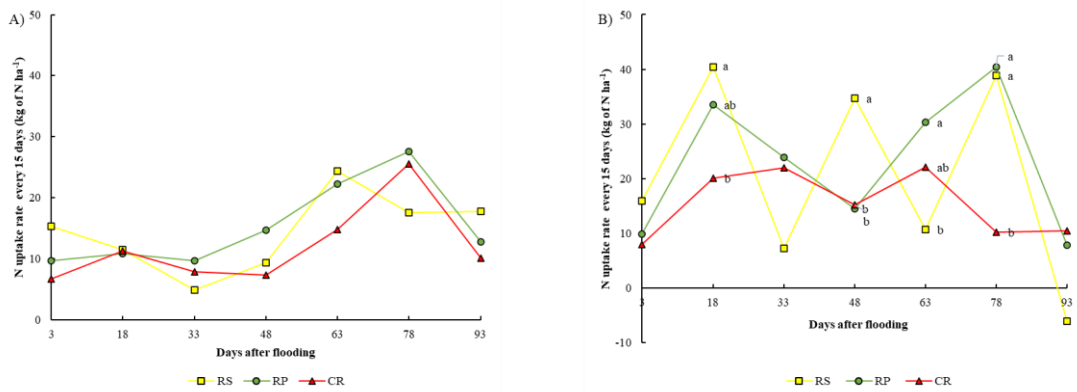


Figure 3. Effect of three crop rotation systems [continuous rice (CR), rice-soybean (RS) and rice-perennial pasture (RPP)] on N uptake rate by rice (kg N ha^{-1} every 15 days) in (A) unfertilized plots and (B) fertilized plots receiving 100 kg N ha^{-1} , averaged over two growing seasons (2019-2020 and 2020-2021) in a long-term experiment in Uruguay. Different letters indicate statistically significant differences according to the LSD test ($p < 0.05$).

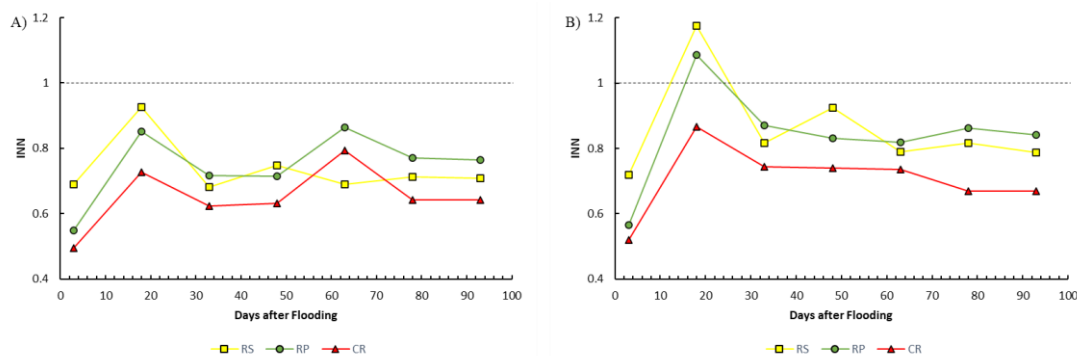


Figure 3. Effect of three rice rotation systems (continuous rice (CR), rice–soybean (RS) and rice–perennial pasture (RPP)) on INN in relation to the critical nitrogen dilution curve (CNDC) established by Moreira et al. (2025), for (A) unfertilized plots and (B) fertilized with 100 kg N ha⁻¹. Data was averaged over two growing seasons (2019–2020 and 2020–2021) in a long-term experiment in Uruguay.

Table 1. Radiation (cal cm² day⁻¹), rainfall (mm month⁻¹) and average temperatures (°C) summary for experimental research station Paso de la Laguna at the National Institute for Agricultural Research (INIA) Treinta y Tres, Uruguay, for two growing seasons (2019-2020 and 2020-2021) and for 20 year’s average 2001-2021.

	2019-2020			2020-2021			Average 2001-2021		
	Rad	PP	Temp	Rad	PP	Temp	Rad	PP	Temp
	MJ m ² day ⁻¹	mm mont h ⁻¹	°C	Cal cm ² dia ⁻¹	mm month -1	°C	Cal cm ² dia ⁻¹	mm month -1	°C
Oct	16,6	360.6	17.2	1919	48.1	16.8	1677	99.9	16.7
Nov	22,5	49.2	21.1	2363	81.5	20	2048	129.8	19.3
Dec	23,9	79	22.3	2494	41.1	22	2184	154.7	21.7
Jan	24	79.4	23.4	2285	206.5	23.2	2152	158.1	23.3
Feb	23,3	28	22.9	1903	249.5	21.8	1910	123.9	22.8
Mar	18	47.3	22.9	1605	88.6	20.7	1582	100.9	20.6
Apr	13,8	69.2	17.5	1233	180.3	19.4	1227	64.4	17.8

Table 2. Twenty-year (2001-2021) average monthly weather summary for the experimental station Paso de la Laguna at the National Institute for Agricultural Research (INIA) Treinta y Tres, Uruguay.

	Solar radiation	Penman - Monteith ET	Heliophany	Precipitation	Maximum temperature	Minimum temperature	Medium temperature
	MJ day ⁻¹	m ² mm month ⁻¹	%	mm month ⁻¹	C°	C°	C°
Jan	21.5	158.1	60	115	29.7	16.9	23.3
Feb	19.1	124	57	143	28.9	17.2	22.8
Mar	15.8	101	55	117	27.1	15.2	20.6
Apr	12.2	64.5	54	127.6	24.1	12	17.8
May	8.8	36.2	46	134.4	20	8.6	14.1
Jun	7.2	25.3	41	115.5	17.2	6	11.6
Jul	7.9	30.2	44	94.8	16.6	5.6	10.7
Aug	10	46.1	45	132.7	18.2	7.1	12.8
Sep	13	67.6	45	142	19.7	8.9	14.4
Oct	16.8	99.9	50	128.8	22.5	11	16.7
Nov	20.5	129.9	58	89	25.7	12.9	19.4
Dec	21.8	154.8	59	106.8	28.3	15	21.7

Table 3. IER testing with three different levels of NH₄⁺ solution concentration and a blank. Three successive extractions, average concentration for three repetitions and recovery rates respect of diluted concentration and blank recovery.

Concentration	Extraction	Average (3 repetitions)	Recovery rate %	Recovery rate-blank %	CV%
Blank	1	0.2			
	2	0			
	3	0			
2,5 M	1	2.6	104	95	6
	2	0.3			
	3	0.1			
5 M	1	5.3	106	101	3
	2	0.6			
	3	0.1			
10 M	1	11	110	107	7
	2	1.3			
	3	0.1			

Table 4. Initial soil analysis (0-15 and 15-30 cm depths) for the long-term rice-based rotations experiment established in 2012 in Uruguay. N: total nitrogen; SOC: soil organic carbon; T.A.: titratable acidity; CEC_{pH7}: cation exchange capacity at pH 7; T.B.: total bases; %Sat: base saturation percentage; EC: electrical conductivity.

	N	SOC	Ca	Mg	K	Na	T.A.	CEC _{pH7}	T. B.	% Sat
Depth (m)	%	%	meq/100g	meq/100g	meq/100g	meq/100g	meq/100g	meq/100g	meq/100g	Bases
0-0.15	0.14	1.42	6.99	2.84	0.25	0.35	2.12	12.54	10.43	83.47
0.15-0.3	0.08	0.72	8.89	3.73	0.23	0.77	0.70	14.32	13.62	94.99
	N-NO3	Cítric P	Cu	Fe	Mn	Zn	pH	S-SO4	EC	Carbo nates
	µg N/g	µg P/g	mg/k g	mg/k g	mg/k g	mg/k g	(H2O)	µg S/g	mmh os/cm	%Ca CO3
0-0.15	6.23	10.25	2.08	148.3 6	120.4 2	0.56	5.65	6.25	0.20	0.08
0.15-0.3	5.13	2.77	1.85	98.17	53.38	0.15	6.54	3.91	0.23	0.05

4. Resultados y discusión

Esta tesis examinó cómo la rotación y la fertilización con nitrógeno (N) interactúan para determinar el rendimiento, la absorción de N y la eficiencia en el uso del nitrógeno (EUN) en sistemas arroceros templados e irrigados en proceso de intensificación. El arroz sigue siendo el cereal con menor EUN internacionalmente, en gran parte porque una proporción significativa del N aplicado se pierde por volatilización, desnitrificación y otras vías típicas en ambientes inundados (Cameron et al., 2013; Rose et al., 2022; Zhang et al., 2015, 2018). En la región pampeana, los sistemas históricamente integrados arroz-ganadería han logrado altos rendimientos con aportes modestos de fertilizante, al mejorar la materia orgánica del suelo, la fijación biológica de N y el reciclaje de N (Carpenter-Boggs et al., 2000; Castillo et al., 2021, 2023; Macedo et al., 2022; Peoples y Baldock, 2001). A medida que los sistemas acortan la fase de pasturas, aumentan la frecuencia de arroz y se incorpora la soja, crece la preocupación de que la capacidad intrínseca del suelo para aportar N se deteriore, lo que genera un aumento en insumos de fertilizante (Carvalho et al., 2021; Macedo et al., 2022; Molina y Terra, 2023). Los dos estudios presentados en esta tesis abordan piezas complementarias de este rompecabezas: 1) cómo rotaciones contrastantes y dosis de N afectan el rendimiento y la EUN del sistema y 2) cómo el legado de rotación influye sobre la oferta de N durante el ciclo, el estatus de N del cultivo y la dilución de N en relación con una curva crítica de dilución de N (CCDN).

En ambos estudios, el primer arroz luego de pasturas (R1PP) o soja (RS) consistentemente obtuvo mayor rendimiento sin fertilización y mayor absorción de N que sistemas continuos o arroz-arroz (CR, R2PP en algunas comparaciones), lo que confirma un fuerte legado de rotación en el aporte biológico de N. R1PP alcanzó el 86 % del rendimiento con fertilización óptima (el porcentaje más alto del rendimiento máximo) sin N aplicado, lo que subraya la capacidad de las fases pastoriles para acumular MOS, fijar y reciclar N de origen biológico y mejorar la mineralización de N (Carlos et al., 2020; Castillo et al., 2021; Denardin et al., 2020; Franzluebbbers et al., 2014; Macedo et al., 2022). RS también superó a CR y R2PP, en

concordancia con trabajos regionales y resultados experimentales (Denardin et al., 2022, 2025; Macedo et al., 2022; Ribas et al., 2021). En contraste, CR y R2PP mostraron evidencia de un deterioro funcional del suelo que va más allá de la dinámica del N, lo que sugiere limitaciones biológicas y estructurales que afectan la productividad y la respuesta a la fertilización (Gmach et al., 2021; Lundquist et al., 1999; Magill et al., 2000; Maguire et al., 2020; Tarlera et al., 2016; Zhou et al., 2014). Estos efectos probablemente contribuyeron a los menores rendimientos sin fertilización observados en CR y R2PP.

Aunque las diferencias en la oferta de N sin fertilización entre rotaciones fueron significativas, las ganancias de rendimiento se estabilizaron en 100 kg N ha^{-1} en todos los sistemas y las parcelas sin fertilización alcanzaron, en promedio, el 81 % de ese rendimiento. Esto sugiere que el experimento se realizó en suelos ubicados en el tramo superior de la curva de respuesta al N, en concordancia con mollisoles bien estructurados bajo siembra directa y con influencia de leguminosas (Eswaran y Reich, 2005; Weinert et al., 2023; Yao et al., 2025; Yuexuan et al., 2025). No obstante, los sistemas con mayor oferta de N del suelo no requirieron proporcionalmente menos fertilizante para maximizar el rendimiento, lo que indica una asincronía entre la mineralización de N y la demanda del cultivo u otras limitaciones en rotaciones menos diversas. Estos hallazgos refuerzan la necesidad de considerar la capacidad biológica del suelo y la dinámica de nutrientes sensibles al potencial redox en sistemas de arroz inundado (Aumtong et al., 2023; Quintero et al., 2009; Martínez, 2023b; Yu et al., 2020; Zhang et al., 2025).

Los altos rendimientos con dosis modestas de N en este estudio resultaron en alta productividad parcial del fertilizante (PPF) y eficiencia agronómica (EA) promedio (en el rango bajo), incluso cuando la recuperación del fertilizante (ERfertN) permaneció por debajo del 50 % ideal (Chen et al., 2023; Chivenge et al., 2021; Dobermann, 2007). Aunque por debajo del umbral frecuentemente citado para sistemas bien manejados en cuanto a ERfertN (Uvardi et al., 2021), la combinación de alto rendimiento y la alta oferta de N del suelo sostuvo una PFP elevada, valores aproximadamente el doble de los resumidos por Chivenge et al. (2021) y por encima del rango de $40\text{-}80 \text{ kg grano kg}^{-1} \text{ N}$ sugerido por Dobermann (2007). Esto se explica

en parte por R1PP y RS superando las 11 Mg ha⁻¹ con 100 kg N ha⁻¹, con lo que sobrepasan muchos sistemas asiáticos de alta intensidad que aplican 120-200 kg N ha⁻¹ (Santiago-Arenas et al., 2021; Wang et al., 2016). Los valores altos de REfertN sugieren un suministro orgánico robusto de N que minimizó las necesidades de N externo sin agotar las reservas del suelo, lo cual es evidenciado por los balances de N positivos y los niveles sostenidos de MOS (Castillo et al., 2023; Macedo et al., 2022). RS mostró la mejor recuperación del fertilizante, mientras que CR y R2PP exhibieron mayor eficiencia de utilización de N (EUtN) a la dosis óptima, lo que sugiere respuestas fisiológicas compensatorias ante menor disponibilidad de N (Che et al., 2015; Dobermann, 2007; Padhan et al., 2023).

Los datos de resinas de intercambio catiónico (RIC) y de NH₄⁺ del suelo no capturaron completamente las diferencias entre tratamientos observadas en la absorción por las plantas, particularmente en las etapas tempranas posteriores al anegamiento. Las bolsas de RIC recuperaron cantidades limitadas de N (<2,5 kg N ha⁻¹ cada quince días), mucho menores que la absorción del cultivo, ilustrando las limitaciones conocidas de los enfoques basados en resinas en suelos inundados con flujos elevados y alta heterogeneidad espacial (Buck et al., 2016; St Luce et al., 2013; Skogley y Dobermann, 1996). La competencia con otros cationes y el contacto variable con la solución del suelo bajo condiciones anaeróbicas pueden influir en los resultados (Noe, 2011; Rhom, 2008; Qian y Schoenau, 2001; Savant y De Datta, 1982). Extracciones de laboratorio sugirieron una alta capacidad teórica de las resinas según la literatura (Ding y Sartaj, 2016) y nuestros propios ensayos en laboratorio, por lo que los valores bajos en campo probablemente reflejan dinámicas poco comprendidas del sistema suelo-agua-plantas más que fallas del método o del material, lo que subraya la importancia de integrar métricas basadas en plantas para el monitoreo de N. Los picos de N disponible detectados en suelo y resinas en los días 18-33 después de la siembra concuerdan con acumulación de NO₃⁻ preanegamiento seguida de desnitrificación y reducción disimilatoria de NO₃⁻ a NH₄⁺ después del anegamiento, en combinación con mineralización anaeróbica y fertilización previa (Ali et al., 2007; Cameron et al., 2013; Cannavo et al., 2008; Dobermann et al., 1994; Reddy, 1984; Zhang et al., 2015).

Las curvas de dilución de N y el índice de nutrición nitrogenada (NNI) aportaron información adicional sobre el estado nutricional del cultivo, con lo que complementaron los datos de EUN. La rotación influyó el contenido de N en tejido en relación con la biomasa acumulada. Los tratamientos que se aproximaron más a la CNDC establecida por Moreira et al. (2025) y, por tanto, con valores de NNI más cercanos a 1, no solo evidenciaron un mejor estatus nutricional teórico, sino que también produjeron mayores rendimientos finales, con lo que confirmaron la utilidad de curvas específicas por sitio y variedad (Li et al., 2022; Sheehy et al., 1998). A partir de los 38 días después del anegamiento, R1PP y RS mantuvieron mayor biomasa y concentración de N en tejido en comparación con CR, con y sin fertilizante, lo que indica mejor nutrición general y mayor capacidad para capitalizar el N aplicado. Esto respalda los hallazgos del estudio de EUN e indica que la rotación influye tanto en la disponibilidad basal de N como en la capacidad del cultivo para responder al fertilizante y destaca el potencial diagnóstico de las CNDC para guiar estrategias nutricionales.

En resumen, diversos mecanismos explican por qué RS y R1PP obtuvieron mayores rendimientos y mejores indicadores en general: i) Fijación biológica de N y retorno de fases con leguminosas (Denardin et al., 2020, 2025; Enriquez-Hidalgo et al., 2015; Peoples y Baldock, 2001); ii) acumulación de MOS y reservas de C lábil que regulan la mineralización-inmovilización (Franzluebbers et al., 2014; Gmach et al., 2021; Pravia et al., 2019); iii) diversidad microbiana y grupos funcionales (diazótrofos, descomponedores, nitrificadores/desnitrificadores) modulados por la frecuencia de rotación, el ciclo redox y la calidad de residuos (Dobermann et al., 2021; Ghiazza et al., 2023; Jiang et al., 2016; Tarlera et al., 2016); (iv) Condiciones físicas del suelo (estabilidad de agregados, porosidad, cambios de pH, cargas de residuos y su calidad, e inundación repetida) (Dobermann et al., 2021; Quintero et al., 2009; Maguire et al., 2020; Vitali et al., 2024) y v) interacciones con la sanidad vegetal que deprimen los rendimientos en sistemas arroz-arroz (Buresh et al., 2021; Martínez et al., 2023 a y b).

Desde una perspectiva de manejo, los resultados respaldan la inclusión intencional de pasturas perennes o leguminosas en las rotaciones para mantener altos

rendimientos y reducir el uso de fertilizante (Castillo et al., 2021, 2023; Denardin et al., 2025; Macedo et al., 2022). Cuando la intensificación acorta la duración de las pasturas, las estrategias compensatorias deben enfocarse explícitamente en los procesos de provisión de N del suelo: cultivos de cobertura, manejo estratégico de residuos y prácticas que mantengan la calidad de la MOS (Fontaine et al., 2003; Jian et al., 2020; Peng et al., 2017; Zhang et al., 2017), aunque parece que el efecto de las pasturas es irremplazable. Las tácticas de fertilización como el fraccionamiento o el uso de urea de liberación controlada siguen siendo relevantes (Cao et al., 2021; Chen et al., 2015; De Datta et al., 1991; Dempsey, 2016; Ishfaq et al., 2020), pero aumentar la eficiencia parece depender de su coordinación con herramientas basadas en plantas como el NNI y las CNDC. Combinarlas con indicadores diagnósticos del suelo (ejemplo: potencial de mineralización de N; Castillo et al., 2015) puede optimizar aún más el uso de N en tiempo real.

5. Conclusiones

Los resultados de ambos estudios destacan la importancia estratégica del diseño de las rotaciones en sistemas de arroz bajo riego para lograr una intensificación sostenible, con alto rendimiento y mayor eficiencia en el uso del nitrógeno (EUN). Las rotaciones que incluyeron pasturas mixtas perennes o una secuencia de soja y cobertura previas al arroz permitieron un mejor estado nutricional, mayor absorción de nitrógeno, mayor respuesta a la fertilización y rendimientos cercanos al potencial regional, en contraste con el arroz en sucesión continua (CR) o el segundo arroz después de pasturas (R2PP), que mostraron limitaciones agronómicas incluso con fertilización.

El legado de las pasturas perennes fue particularmente notable: el suelo aportó hasta el 82 % del nitrógeno absorbido por el arroz, lo cual aumentó el rendimiento en un 32 % y la eficiencia agronómica del fertilizante en un 41 %, en comparación con CR. No obstante, estos beneficios no se mantuvieron en R2PP, donde surgieron brechas significativas en rendimiento (24 %) y absorción de N (35 %) que no pudieron ser compensadas por la fertilización, lo que sugiere restricciones adicionales más allá del suministro de N del suelo.

Los índices de nutrición nitrogenada (NNI) y las curvas de dilución confirmaron que el efecto del cultivo antecesor tiene un impacto decisivo en el estado nutricional del arroz y que el agregado de fertilizante no corrige las deficiencias impuestas por rotaciones desfavorables. A pesar de que los indicadores de disponibilidad de N (NH_4^+ y resinas de intercambio catiónico) no captaron plenamente las diferencias observadas en las plantas.

En conjunto, los resultados sugieren que, para los sistemas arroz-ganadería en regiones templadas de alta productividad del Cono Sur, es clave evitar secuencias arroz-arroz, mantener pasturas mixtas perennes y diversificar las rotaciones incorporando soja y cultivos de cobertura leguminosos. Estas estrategias pueden reducir la dependencia de fertilizantes nitrogenados, mejorar la eficiencia del sistema y acercar los rendimientos al potencial regional. Estudios futuros deberían enfocarse en comprender los mecanismos edáficos y microbiológicos que subyacen a estas

diferencias, para optimizar el manejo del N y lograr una verdadera sincronía entre la oferta y la demanda para minimizar pérdidas.

6. Bibliografía

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