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Integrated Crop-Livestock Systems in Paddy Fields: New Strategies for Flooded Rice Nutrition

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Core Ideas:

- Integrated crop-livestock system has different rice fertilization requirements;
- Rice in integrated crop-livestock system does not respond to P and K fertilization;
- Integrated crop-livestock system has higher eco-efficiency than conventional system;
- Nitrogen remains a limiting nutrient for rice development and yield.

Integrated Crop-Livestock Systems in Paddy Fields: New Strategies for Flooded Rice Nutrition

CEC, cation exchange capacity;

CS, conventional system;

ICLS, integrated crop-livestock systems;

RS, Rio Grande do Sul;

SOM, soil organic matter;

V%, base saturation.

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ABSTRACT

Integrated crop-livestock systems (ICLS) appear as a good alternative to increase nutrient-use efficiency of rice (*Oryza sativa* L.) through the improvement in nutrient cycling and soil chemical attributes in paddy fields. The objective of this study was to evaluate the impact of an ICLS on soil chemical attributes and on the fertilization requirement of N, P, and K by flooded rice in the Brazilian subtropical region. Nutritional status, yield and nutrient-use efficiency of flooded rice was evaluated by fertilization trials through rice response to different fertilization rates of N, P, and K. Soil chemical attributes were evaluated at the beginning of the experiment and 30 months later. Different fertilization rates were applied in two systems: Conventional system (CS), based on intensive tillage, rice monocropping and winter fallow; and ICLS, characterized by no-tillage and winter cattle grazing in annual ryegrass (*Lolium multiflorum* Lam.) pasture. Accumulation of N, P, and K in rice's shoot was greater under CS than ICLS at all fertilization levels. On the other hand, higher rice yields were observed under ICLS at almost every fertilization levels, suggesting higher nutrient-use efficiency than CS. In addition, rice yield was increased by 40% by fertilization of P and K under CS, while no response was observed under ICLS. These benefits were possibly related to greater nutrient cycling and greater synchronism between rice's nutrient uptake and nutrient release of the soil. Our results indicated that the adoption of ICLS ensures greater nutrient-use efficiency, becoming a system less dependent on external inputs.

INTRODUCTION

The requirements to increase global food production are well known and projected to double by 2050, when World's population will likely meet nine billion people (FAOSTAT, 2013). According to Alexandratos and Bruinsma (2003), the annual increase of corn, soybean, and rice production, which are three of the most important grain crops cultivated in the world, are insufficient to attend the rising demand by 2050, and must increase 67%, 55%, and 42%, respectively. This requirement will have to be achieved by using less land, water, labor, and chemicals (Khush, 2000).

Worldwide, rice corresponds to 29% of grain consumption, being the staple food of half of the population's diet in developing countries. Following a 35-year trend, increases in rice production are expected to come from greater yields, since rice acreage increased only 36.2% while its production increased 253% (Alexandratos and Bruinsma, 2003).

Outside of Asia, Brazil is the largest rice producer. In Brazil, rice is produced mainly in lowlands and floodplains in the subtropics (Southern region) (FAOSTAT, 2013). Rio Grande do Sul (RS) State produces 61% of Brazilian rice demand, where conventional rice cropping system includes yearly soil tillage and fallow periods. Traditionally, this system may or not be combined with extensive and inefficient livestock production. In addition, the cultivation system is still based on intense and frequent soil disturbance. According to Boeni et al. (2010), in all rice regions of RS State,

there was a predominance (71%) of areas with low soil organic matter (SOM) content ($\leq 2.5\%$). In addition, the predominance of sandy texture soils on these areas is detrimental to the rice yield performance, due to lower soil cation exchange capacity (CEC), nutrient availability (Denardin et al., 2018), and soil microbiological activity (Martins et al., 2017).

Soil disturbance has been pointed out as the main cause of soil degradation worldwide (Lal, 2015). Cassman et al. (1995) and Flinn and De Datta (1984) showed greater soil degradation in long-term experiments with soil disturbance in flooded rice cultivation systems due to lower pH, CEC, and nutrient-use efficiency. Soil degradation consequently decreases rice yield over time (Bado et al., 2010; Dobermann et al., 2000). Thus, current flooded rice cropping systems require higher fertilizer levels to sustain high yields (Boeni et al., 2010). On the other hand, soils managed under no-tillage favour SOM accumulation and nutrients in the topsoil (Denardin et al., 2019; Feng et al., 2006; Tang et al., 2007), as well as better soil microbiological attributes (Gao et al., 2004; Martins et al., 2017).

Although the adoption of no-tillage provides improvements in soil quality through increase of SOM content, rice yield under no-tillage requires 14 years to surpass conventional tillage system yields (Denardin et al., 2019). Thus, the adoption of this practice alone does not provide increases in rice crop yield, especially in the short term, and it is necessary to diversify the production system with other crops and/or activities (Huang et al., 2015; Denardin et al., 2019).

Carmona et al. (2016) found increases in yield and decreased rice response to fertilization under ICLS. The ICLS is based on rotation between grain crop under no-tillage and livestock under pasture cultivation (Carvalho et al., 2014). Pasture fertilization can optimize fertilizer use, determined by nutrient (re)cycling through grazing, improving consequently soil fertility (Carvalho et al., 2010; Assmann et al., 2017).

Our hypothesis is that ICLS, based on no-tillage and winter grazing, result in greater nutrient-use efficiency compared to the CS of flooded rice. The objective of the study was to evaluate the impact of an ICLS (under no-tillage combined with winter grazing) on soil chemical attributes and on the fertilization requirement of N, P, and K by flooded rice in the Brazilian subtropical region.

MATERIALS AND METHODS

Site description and historical characterization of the experimental area

An on-farm field trial was conducted in the 2015/2016 and 2016/2017 rice growing seasons at Corticeiras Farm, located in Cristal City, RS State, Brazil ($31^{\circ}37'13''\text{S}$, $52^{\circ}35'20''\text{W}$, 28 m above sea level). The regional climate is warm humid summer climate, classified as Cfa, according to Köppen (Kottek et al., 2006). The mean annual temperature is 18.3°C , and

the mean annual rainfall is 1522 mm (30 yr-normal) (Matzenauer et al., 2011). The soil is poorly drained, classified as Albaqualf (Soil Survey Staff, 2010), with sandy clay loam texture (24%, 23%, and 53% of clay, silt, and sand, respectively).

The experimental area has been cultivated with flooded rice followed by fallow since 1962. The last rice cropping season before the study was in 2009, followed by a fallow period until March 2013, when soil samples (0–10 cm soil layer) were taken for chemical characterization of the experimental site. In April 2013, the soil in the entire area was tilled with three heavy discs to incorporate lime applied at a rate of 4.5 Mg ha⁻¹ (with a total neutralization relative power of 70%), determined according to CQFS RS/SC (2004), to increase the 0–20 cm soil layer to pH 6.0.

After soil pH correction, five production systems involving the cultivation of flooded rice were established in an area of 18 hectares. However, for the present study only two of the five systems were evaluated: 1) CS, which is the dominant system in lowlands of Southern Brazil, based on intensive soil disturbance, rice monocropping and winter fallow period; and 2) ICLS, which is characterized by no-tillage adoption and integration between crop and livestock, with annual ryegrass pasture grazing during the winter season. Before the present study, there were two pasture seasons in the ICLS (winter 2013 and 2014) and two rice growing seasons in both systems (2013/2014 and 2014/2015 cropping seasons).

Treatments description and experimental design

In the 2015/2016 cropping season five different levels of NPK fertilization were applied in both systems (CS and ICLS) based on expected yield response to fertilization of flooded rice, according to SOSBAI (2014). Fertilization levels corresponded to different fertilizer rates (in kg ha⁻¹ of N-P₂O₅-K₂O), according to SOSBAI (2014): (i) no-fertilizer application; (ii) low expected yield response to fertilization (60-20-20); (iii) medium expected yield response to fertilization (90-30-35); (iv) high expected yield response to fertilization (120-40-50), and (v) very high expected yield response to fertilization (150-50-65), respectively. Fertilizer sources used were urea (46% N), single superphosphate (18% P₂O₅), and potassium chloride (60% K₂O). The experimental design was conducted as a split plot randomized complete block design with four replications. The experimental subplot size was 5 m x 5 m.

Due to the results obtained in the first cropping season, in the second cropping season (2016/2017), another experiment was carried out to separate the effects of P and K from N fertilization. For this, two treatments were designed to evaluate the rice response to the presence or absence of P and K supply (150-70-120 and 150-00-00, in kg ha⁻¹ of N-P₂O₅-K₂O, respectively), fertilized with the recommended N rate (150 kg N ha⁻¹). In addition, four treatments were

established providing different N rates, being: (i) 0-70-120; (ii) 50-70-120; (iii) 100-70-120 (iv) 150-70-120, in kg ha⁻¹ of N-P₂O₅-K₂O, respectively. All the fertilizer sources used were the same in both production systems, as well as the same experimental design and subplot size.

The N rates were split in two applications: 66% applied at V₃ phenological stage, immediately before flooding, and 33% applied at V₈ phenological stage. The rice stages were determined according to Counce et al. (2000). The IRGA 430 rice cultivar was used in the first cropping season, and the IRGA 424 in the second cropping season, seeded at a density of 100 kg ha⁻¹ in a 17 cm row spacing. The rice planting and harvest occurred in November 2015 and April 2016, respectively, in the 2015/2016 growing season, and in October 2016 and March 2017, in 2016/2017 growing season. Agronomic management for weed, insects, and diseases control was done according to the technical recommendations (SOSBAI, 2014).

Neutered male steers (Angus) weighing around 200 kg were used for grazing in ICLS in both winter seasons (2015 and 2016). Average adopted pasture height was 15 cm, and average stocking rate was 831±114 kg live weight ha⁻¹, simulating cattle fattening or finishing systems during 62±15 days. The cattle's diet was forage-based with only mineral salt being provided. Continuous grazing was adopted (with a minimum of three test steers), and annual ryegrass was re-sowed at a density of 30 kg ha⁻¹ using BRS Ponteio cultivar. The ryegrass fertilization was done according to CQFS RS/SC (2004) recommendations, with 110-110-100, 130-130-130, 120-120-120, and 120-120-120 kg ha⁻¹ of N-P₂O₅-K₂O in 2013, 2014, 2015, and 2016 winter seasons, respectively. The rice fertilization in the 2013/2014 and 2014/2015 summer seasons was according to SOSBAI recommendation (2014), representing 150-70-120 kg ha⁻¹ of N-P₂O₅-K₂O, respectively.

Soil analyses

The soil was sampled in March 2013, prior to the installation of the experiment. Soil samples (up to 10 cm soil layer) were composed by six sub-samples per plot. Georeferenced samples were collected in a sample grid of 2,025 m². In October 2015, before the first rice crop season, soil samples were collected at the same georeferenced points.

Soil samples were carried in plastic bags and transported to the Federal University of Rio Grande do Sul's Soil Fertility Research Laboratory. The samples were dried in a forced air circulation oven at 50°C, ground, sieved in 2 mm mesh, and analyzed. The soil properties used for the soil fertility evaluation were: pH in water, SOM content (wet combustion), available P and K (extracted by Mehlich-1), exchangeable Ca and Mg (extracted by 1.0 mol L⁻¹ KCl), CEC_{pH 7.0}, and base saturation (V%). All analyses were performed following the methodology described by Tedesco et al. (1995). The exchangeable Al³⁺ was determined by titration with 0.0125 mol L⁻¹ NaOH solution; Ca²⁺ and Mg²⁺ by atomic absorption spectrometry; K by flame photometry; and P by photolorimetry. The potential acidity (H+Al) was obtained through the equation proposed by Kaminski et al. (2001). The sum of bases was determined by the sum of Ca, Mg, and K. The CEC_{pH 7.0} was calculated by sum of bases + (H+Al); the V% was calculated using the relation: V% = 100 × sum of bases/CEC_{pH 7.0} (CQFS-RS/SC 2016).

Plant analyses

In the first cropping season (2015/2016), rice tissue samples were collected to evaluate the dynamics of absorption of different nutrients. The first plant sampling occurred at R₄ rice stage (Counce et al., 2000) to evaluate rice's nutritional status, nutrient accumulation, and shoot dry matter. For crop nutritional status evaluation, 50 rice flag leaves were collected per experimental unit. For nutrient accumulation and shoot dry matter evaluation, whole rice plants were cut from the ground level in 0.15 m² per plot at the beginning of flowering. Samples were oven-dried (forced air at 65°C), weighed, ground, and sieved ($\emptyset = 0.5$ mm). Nitrogen, P and K content in plant tissue were analysed after chemical digestion (H₂O₂ + H₂SO₄), according to Tedesco et al. (1995). Nutrient accumulation was calculated using data obtained from the whole plant, according to Eq. 1.

$$NA = SNC \times SDMP \quad (\text{Eq. 1})$$

where NA = nutrient accumulation (in kg ha⁻¹); SNC = shoot nutrient content (in kg Mg⁻¹) and SDMP = shoot dry matter production (in Mg ha⁻¹).

The second plant sampling took place at R₇-R₈ rice stage (Counce et al., 2000) in the 2015/2016 and 2016/2017 cropping seasons to determine grain yield. Harvests were performed by hand with plants sampled in 1.02 m² per plot. Samples were threshed, cleaned, and weighed. Grain moisture was determined and adjusted to 130 g kg⁻¹.

The N, P, and K use efficiency was obtained by determining the amount of grain produced per each kg of nutrient accumulated in rice shoot dry matter, according to Eq. 2.

$$NUE = GY / NA \quad (\text{Eq. 2})$$

where NUE = Nutrient-use efficiency (in kg grain kg nutrient⁻¹); GY = grain yield (in kg ha⁻¹) and NA = Nutrient accumulation (in kg ha⁻¹).

Statistical analyses

Statistical analyses were performed using linear models (PROC GLM) of the Statistical Analysis System (SAS Institute, 1997). Data was assessed using the Shapiro-Wilk normality test ($p < 0.05$), Levene's homogeneity of variance test ($p < 0.05$), and analysis of variance at $p < 0.05$, and, when significant, means were compared using Tukey's test ($p < 0.05$).

RESULTS

Soil fertility attributes

The short-term (30 months) adoption of flooded rice cultivation increased the available K, exchangeable Ca and Mg, and V% levels in both systems in 71.2%, 62.8%, 27.3%, and 29.8%, respectively (**Table 1**). The SOM content, pH in water, and CEC did not change along time. The ICLS improved the available P content from 9.9 to 26.1 mg dm⁻³, while CS did not alter its available P content.

Nutritional status and nutrient accumulation

The different input rates of N, P, and K fertilizer did not affect ($p = 0.94$) the nutritional status of rice, measured in the flag leaf (**Figure 1**), and the two rice production systems also remained

similar to each other. The N, P, and K flag leaf content showed averages of 26.4 , 3.1, and 17.0 g N, P, and K kg⁻¹, respectively.

Despite no differences in nutrient content measured in rice flag leaf (**Figure 1**), nutrient accumulation in the whole plant at R₄ stage was significantly different among the production systems and fertilization levels (**Table 2**). For N, fertilization levels' effect was only significant under CS, where the highest response was obtained at the low fertilization level, achieving an increase of 65% as compared to no fertilizer addition. However, N content at the low fertilization level did not differ from medium and high fertilization levels. On the other hand, at the very high level of fertilization, there was a decrease in N accumulation, becoming similar to the no-fertilizer treatment. In ICLS, only medium and very high fertilization levels showed higher N accumulation than the no-fertilizer treatment. Higher N accumulation was observed in CS than in ICLS at low and high fertilization levels, with 52 and 33 kg N ha⁻¹, respectively (**Table 2**).

Different from ICLS, the P accumulation in shoot dry matter in CS showed responses depending on different fertilization levels. At the low fertilization level the P accumulation was 45% higher than the no-fertilizer treatment. In the low and very high fertilization levels, the P accumulation in rice shoot dry matter was in average 11 kg P ha⁻¹ higher in CS as compared to ICLS. Regarding K accumulation, regardless of fertilization levels, the CS K accumulation was 23 kg K ha⁻¹ higher than the ICLS (**Table 2**).

Rice shoot dry matter and grain yield

The rice shoot dry matter was not affected by fertilization levels in the ICLS, presenting an average of 10.8 Mg ha⁻¹ (**Figure 2**). In contrast, the increase in fertilization levels under CS resulted in an increase of rice shoot dry matter. In this system, the very high fertilization level resulted in a production approximately 50% higher than the control (no-fertilizer). The low, medium, and high fertilization levels showed a similar shoot biomass, not differing from the other fertilization levels. Differences between the production systems were observed only in the very high fertilization level, resulting in an increase of 0.5 Mg ha⁻¹ in CS when compared to ICLS (**Figure 2**).

Increases in rice yield due to fertilization levels were observed in both production systems (**Figure 3**). In the CS, all fertilizer rates were higher than the no-fertilizer treatment, and the highest yield (9.7 Mg ha⁻¹) was obtained in the very high fertilization level (3.2 Mg ha⁻¹ higher than the treatment without fertilization). The low and medium fertilization levels showed intermediate values of 8.5 and 8.7 Mg ha⁻¹, respectively.

On the other hand, the grain yield of flooded rice under ICLS did not show response to fertilizer levels (**Figure 3**). In such system, the low fertilization level yielded 8.5 Mg ha⁻¹, which was not different from the control (7.9 Mg ha⁻¹). The medium and the high fertilization levels presented intermediate grain yields (9.8 and 9.9 Mg ha⁻¹, respectively) compared to low and very high fertilization levels, and similar between themselves. The very high fertilization level showed the highest yield (10.1 Mg ha⁻¹) and was significantly higher than both the control (no-fertilizer) and low

fertilization level. Finally, the ICLS showed higher yield comparing to the CS in most of the fertilization levels, except for the low and very high fertilizer rates. In the no-fertilizer, medium and high fertilization levels, the ICLS yielded 1.4, 1.1, and 1.6 Mg ha⁻¹ more than CS, respectively (**Figure 3**).

Regarding the second cropping season evaluated (2016/2017), the P and K supply did not affect rice yield in the ICLS, which showed an average yield of 10.5 Mg ha⁻¹ (**Figure 4**). On the other hand, rice yield in CS was increased by almost 40% by P and K fertilization. The rice yield was higher in ICLS than in CS both with and without P and K fertilization. While without P and K fertilization the yield difference between ICLS and CS was 4 Mg ha⁻¹, with P and K such difference was 1.1 Mg ha⁻¹ (**Figure 4**).

Differently from P and K, the N rates affected rice yield in both systems (**Table 3**). On average of the production systems, the 150 kg N ha⁻¹ rate increased rice yield by 1.7 Mg ha⁻¹. The 50 and 100 kg N ha⁻¹ rates presented intermediate rice yields of 8.8 and 9.4 Mg ha⁻¹, respectively, and did not differ from the other N rates. Although both systems showed response to N rates, the ICLS had a higher yield than CS at all N rates, with a mean yield that was 1.4 Mg ha⁻¹ higher than CS (**Table 3**).

Nutrient-use efficiency

Only the N efficiency was affected by fertilization levels and production systems (**Table 4**). The highest efficiency was found in the absence of fertilization, with 90.9 kg of grain produced per kg of N accumulated (kg grain kg N⁻¹). The lowest efficiency was found in the treatment with medium fertilization level (69.5 kg grain kg N⁻¹), while the other fertilization levels showed similar efficiencies (77.8 kg grain kg N⁻¹). On average, ICLS was more efficient than CS, yielding more 23 kg grain kg N⁻¹. In the same way, the ICLS yielded 30% and 27% more grains than the CS with the same amount of accumulated P and K in the shoot biomass, respectively.

The relationship established between flooded rice grain yield and nutrient accumulation in shoot dry matter shows that, regardless of the nutrient (N, P, or K), with the same amount of nutrient accumulated there are always higher yields in the ICLS than in the CS (**Figure 5**). The correlation between these two variables was similar between the systems only considering the N accumulated, with R² of 0.81 for CS and 0.79 for ICLS. For P and K, only CS presents a R² of 0.88 for both nutrients. The ICLS has a lower relation between accumulated P and K and grain yield, with R² of 0.49 and 0.34 for P and K, respectively.

DISCUSSION

The traditional paddy field system in the Brazilian subtropics is based on intensive soil disturbance (SOSBAI, 2018), which results in high levels of soil degradation (Boeni et al., 2010). Alternatives like the long-term adoption of no-tillage have arisen as important tools to improve soil quality in the paddy fields (Denardin et al., 2019). However, we observed improvements in soil chemical attributes under ICLS in the short-term. After 30 months of ICLS adoption, higher soil P content was observed under ICLS compared to CS (**Table 1**). While tendencies of higher SOM content under ICLS (0.4%) compared to CS (0.2%) could already be noted, no statistically significant differences could be observed. Thus, soils under ICLS and CS would both be classified as having low SOM content (<2.5%), very high P content (> 12 mg dm⁻³) and high K content (91 – 180 mg dm⁻³), according to soil chemical fertility for flooded rice cultivation (CQFS RS/SC, 2016). Therefore, theoretically, the same yield response to fertilization would be expected (SOSBAI, 2018) from both systems.

Despite the similarity regarding soil chemical analysis for both systems, which would indicate the same fertilizer requirement, the ICLS showed higher yields in almost all evaluated fertilizations rates (**Figure 3**, **Figure 4**, and **Table 3**), and consequently higher nutrient-use efficiency than CS (**Table 4**). Our results are in agreement with Dalal et al. (2011) and Martins et al. (2014) who found higher nutrient-use efficiency through no-tillage and ICLS adoption, respectively. Greater nutrient-use efficiency was also identified in the ICLS yield compared to CS when no fertilizer was provided (**Figure 3**). Also, the nutrient-use efficiency in ICLS was observed in similar yields under the medium fertilization level compared to high and very high fertilization levels (**Figure 3**), saving up to 60 kg N ha⁻¹, 20 kg P₂O₅ ha⁻¹ and 30 kg K₂O ha⁻¹ in the flooded rice cultivation.

Rice yield under ICLS did not respond to P and K fertilization (**Figure 4**), which may be related to nutrient cycling (Assmann et al., 2017) that was favoured by the large amount of animal and plant residues under ICLS (Schoenau and Campbell, 1996; Díaz-Zorita et al., 2002; Bayer et al., 2006; and Thomas et al., 2007). Therefore, as the animal exports a minimum amount of nutrients through meat (Whitehead, 2000), the replenishment of P and K exported by rice grains can be performed on successive pastures. This may be a more efficient alternative of fertilization with P and K for rice cultivation under ICLS.

Different from that observed for P and K fertilization, rice yield still responds to N fertilization in both production systems (**Table 3**). However, ICLS had a yield 17% higher (1.4 Mg ha⁻¹) than CS, possibly due to higher SOM labile fraction in this system, as observed by Martins et al. (2017). According to Denardin et al. (2019), the N deficiency remains as the main limitation of rice yield with adoption of no-tillage alone. Normally, N is the most limiting nutrient in systems with high residue amounts, and this is due to the greater advantage and capacity of the soil microorganisms for N uptake compared with plant roots across any concentration range (Geisseler et al., 2010; Kuzyakov and Xu, 2013; Liu et al., 2016).

In the present study, 66% of N fertilizer was applied in V₃ rice stage, aiming to stimulate tillering (plant growth). Under CS, N addition is mostly converted to plant growth, while N dynamics may be different under ICLS due to the greater residue accumulation with a high C/N ratio on the soil surface, which can favour great N immobilization by soil microorganisms (Bijay-Singh et al., 2008;

Gómez-Rey et al., 2012; Kuzyakov and Xu, 2013). As a result, N added can be initially less available to plant growth leading to lower shoot dry matter production under ICLS (**Figure 2**). This explains why we observed higher rice yield but lower shoot dry matter under ICLS, resulting in higher nutrient-use efficiency compared to CS.

Higher yields observed under ICLS are also possibly related to greater synchronism between N uptake by rice and N released from soil and microorganisms (as immobilized N becomes available for rice after death of microorganisms) compared to CS (Kuzyakov and Xu, 2013; Liu et al., 2016). Short immobilization of mineral N by microorganisms is an important adaptation of ecosystems, and the allocation of N within soil microorganisms protects against losses, such as N leaching or denitrification (Kuzyakov and Xu, 2013). Consequently, the ICLS is less susceptible to N losses and rice in ICLS is more efficient in converting N to grain in relation to CS.

The results of flag leaf analysis, which is widely used as a reference for the evaluation of rice nutritional status, showed in both systems an adequate nutritional status for N (range for 23-28 g N kg⁻¹) (**Figure 1**) and above adequate for P and K (> 2.5 g P kg⁻¹, > 14 g K kg⁻¹, respectively) (SOSBAI, 2018). This fact is probably due to the P and K luxury consumption by rice (i.e. when plant continues to uptake nutrients without reflecting in higher yield) (Dobermann et al., 1996; Zou et al., 2001; Burgos et al., 2006), especially under high content of these nutrients in the soil. According to Dobermann and Fairhurst (2000), the relationship between grain yield and nutrient uptake that reveals an optimal internal nutrient-use efficiency is 68, 385, and 69 kg grain yielded per kg of N, P, and K accumulated in the rice shoot biomass, respectively. Considering N, rice cultivated under CS showed an optimal internal nutrient-use efficiency, with 67.3 kg grain kg⁻¹ N. Rice cultivated under ICLS, however, was more efficient, yielding 90.0 kg grain per kg N accumulated in shoot biomass (**Table 4**).

Both systems were inefficient in the use of P and K, yielding 235.5 and 307.4 kg grain per kg P and 41.2 and 52.5 kg grain per kg K accumulated in shoot biomass in CS and ICLS, respectively (Dobermann and Fairhurst, 2000) (**Table 4**). However, ICLS was more efficient than CS, yielding 30% and 27% more grain, with the same amount of accumulated P and K in the shoot biomass, respectively. Nitrogen uptake is directly related to plant biomass production (Marschner, 2011), and consequently to uptake of other nutrients, such as P and K. Thus, the higher availability of initial N for rice under CS possibly resulted in higher biomass production and P and K uptake, showing lower nutrient-use efficiency than ICLS (**Table 3**). Therefore, in addition to the higher N use efficiency in ICLS, this system also contributes to decrease the luxury consumption of other nutrients, increasing the P and K use efficiency. In this sense, the adoption of ICLS appears as a new and appropriate strategy to increase the nutrient-use efficiency in paddy fields of the Brazilian subtropical region.

Systems that receive more fertilization and produce more biomass did not guarantee greater rice yield. Thus, efforts for a better understanding of the processes regarding nutrient cycling under conservation management systems in rice cultivation, such as ICLS, seems to be necessary to develop new standards of fertilization, aiming to find more efficient systems regarding nutrient use and with less dependence on external inputs.

CONCLUSIONS

Integrated crop-livestock system and CS have different yield responses to fertilization levels, even with the same interpretation of soil fertility levels. Flooded rice under ICLS yields more grain while requiring lower fertilizer application than CS. Integrated crop-livestock system has higher nutrient-use efficiency compared to CS, demonstrated by the greater use efficiency of nutrients, yielding a higher amount of grain with the same amount of nutrient uptake. Rice yield under ICLS does not respond to P and K fertilization supply, suggesting an important contribution of nutrient cycling to plant nutrition. However, N remains a limiting nutrient for development and production of flooded rice.

Efforts for a better understanding of the processes regarding nutrient cycling under ICLS seems to be necessary to develop new standards of fertilization, aiming to find more efficient systems regarding nutrient use and with less dependence on external inputs.

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REFERENCES

- Alexandratos, N., and J. Bruinsma. 2003. World agriculture: towards 2015/2030: an FAO perspective. Land use policy. 20:375–380. doi:10.1016/S0264-8377(03)00047-4
- Assmann, J.M., A.P. Martins, I. Anghinoni, L.G.O. Denardin, G.H. Nichel, et al. 2017. Phosphorus and potassium cycling in a long-term no-till integrated soybean-beef cattle production system under different grazing intensities in subtropics. Nutr Cycl Agroecosys. 108:1–13. doi: 10.1007/s10705-016-9818-6
- Bado, B.V., A. Aw, and M. Ndiaye. 2010. Long-term effect of continuous cropping of irrigated rice on soil and yield trends in the Sahel of West Africa. Nutr Cycl Agroecosys. 88:133–141. doi: 10.1007/s10705-010-9355-7
- Bayer, C., L. Martin-Neto, J. Mielniczuk, A. Pavinato, and J. Dieckow. 2006. Carbon sequestration in

two Brazilian Cerrado soils under no-till. *Soil Till Res.* 86:237–245.

doi:10.1016/j.still.2005.02.023

Bijay-Singh, Y.H. Shan, S.E. Johnson-Beebout, Yadvinder-Singh, and R.J. Buresh. 2008. Crop Residue Management for Lowland Rice-Based Cropping Systems in Asia. *Adv Agron.* 98:117–199.

doi:10.1016/S0065-2113(08)00203-4

Boeni, M., I. Anghinoni, S.A. Genro Junior, and B.D. Osório Filho. 2010. Evolução da fertilidade dos solos cultivados com arroz irrigado no Rio Grande do Sul. Cachoeirinha: IRGA.

Burgos, N.R., R.J. Norman, D.R. Gealy, and H. Black. 2006. Competitive N uptake between rice and weedy rice. *Field Crop Res.* 99:96–105. doi:10.1016/j.fcr.2006.03.009

Carmona, F.C., I. Anghinoni, C.P. Mezzari, A.P. Martins, and P.C.F. Carvalho. 2016. Effectiveness of current fertilizer recommendations for irrigated rice in integrated crop-livestock systems. *Rev Bras Cienc Solo.* 40:1–8. doi:10.1590/18069657rbcs20140798

Carvalho, P.C.F., A. Moraes, L.S. Pontes, I. Anghinoni, R.M. Sulc, and C. Batello. 2014. Definições e terminologias para Sistema Integrado de Produção Agropecuária. *Revista Ciência Agronômica.* 5:1040–1046. doi:10.1590/S1806-66902014000500020

Carvalho, P.C.F., I. Anghinoni, A. Moraes, E.D. Souza, R.M. Sulc, et al. 2010. Managing grazing animals to achieve nutrient cycling and soil improvement in no-till integrated systems. *Nutr Cycl Agroecosys.* 88:259–273. doi:10.1007/s10705-010-9360-x

Cassman, K.G., S.K. De Datta, D.C. Olk, J.M. Alcantara, M.I. Samson, et al. 1995. Yield decline and the nitrogen economy of long-term experiments on continuous, irrigated rice systems in the tropics. In: Lal R, Stewart BA, editors. *Soil management: experimental basis for sustainability and environmental quality*. Boca Raton: Lewis/CRC. p. 181–222.

Counce, P., T.C. Keisling, and A.J. Mitchell. 2000. A uniform, objective, and adaptive system for expressing rice development. *Crop Sci.* 40:436–443.

CQFS RS/SC. 2004. Manual de adubação e de calagem para os estados do Rio Grande do Sul e Santa Catarina, Sociedade Brasileira de Ciência do Solo – Porto Alegre: Núcleo Regional Sul.

CQFS-RS/SC. 2016. Manual de calagem e adubação para os Estados do Rio Grande do Sul e Santa Catarina. Porto Alegre: SBCS-NRS/EMBRAPA-CNPT.

- Dalal, R.C., W. Wang, D.E. Allen, S. Reeves, and N.W. Menzies. 2011. Soil Nitrogen and Nitrogen-Use Efficiency under Long-Term No-till Practice. *Soil Sci. Soc. Am. J.* 75:2251–2261. doi:10.2136/sssaj2010.0398
- Denardin, L.G.O., F.C. Carmona, M.G. Veloso, A.P. Martins, T.F.S. Freitas et al. 2019. No-tillage increases irrigated rice yield through soil quality improvement along time. *Soil Till Res.* 186:64–69. doi:10.1016/j.still.2018.10.006.
- Denardin, L.G.O., A.P. Martins, F.S. Carlos, I. Anghinoni, F.G. Moojen et al. 2018. Geração do conhecimento. In: Carmona FC, Denardin LGO, Martins AP, Anghinoni I, Carvalho PCF, editors. *Sistemas Integrados de Produção Agropecuária em Terras Baixas*. 1st ed. Porto Alegre: UFRGS. p. 39–100.
- Dobermann, A., K.G. Cassman, P.C.S. Cruz, M. Adviento, and M.F. Pampolino. 1996. Fertilizer inputs, nutrient balance, and soil nutrient-supplying power in intensive, irrigated rice systems. II: Effective soil K-supplying capacity. *Nutr Cycl Agroecosys.* 46:11–21.
- Dobermann, A., D. Dawe, R.P. Roetter, and K.G. Cassman. 2000. Reversal of rice yield decline in a long-term continuous cropping experiment. *Agron J.* 92:633–643. doi:10.2134/agronj2000.924633x
- Dobermann, A., and T. Fairhurst. 2000. Rice: nutrient disorders & nutrient management, Handbook Series.
- FAO – FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. 2010. An international consultation on integrated crop-livestock systems for development: The way forward for sustainable production intensification. Rome: FAO.
- FAOSTAT - Food and Agriculture Organization of the United Nations Statistics. Production / Crops – Rice, paddy – World – 2013. <http://faostat3.fao.org/browse/Q/QC/E>. Accessed: 20 Mai 2018.
- Feng, Y., Y. Zou, R.J. Buresh, G. Xu, H. Ao, and S. Wang. 2006. Effects of no-tillage and direct broadcasting on soil physical and chemical properties and growth and yield formation in hybrid rice. *Acta Ecologica Sinica.* 32:1728–1736.
- Flinn, J.C., and S.K. De Datta. 1984. Trends in irrigated-rice yields under intensive cropping at Philippine research stations. *Field Crop Res.* 9:1–15. doi:10.1016/0378-4290(84)90002-9

- Gao, M., B. Zhou, C. Wei, D. Xie, and L. Zhang. Effect of tillage system on soil animal, microorganism and enzyme activity in paddy field. *Chinese Journal of Applied Ecology*. 15:1177–1181.
- Geisseler, D., W.R. Horwath, R.G. Joergensen, and B. Ludwig. 2010. Pathways of nitrogen utilization by soil microorganisms—a review. *Soil Biol Biochem*. 42:2058–2067. doi:10.1016/j.soilbio.2010.08.021
- Gómez-Rey, M.X., A. Couto-Vázquez, and S.J. González-Prieto. 2012. Nitrogen transformation rates and nutrient availability under conventional plough and conservation tillage. *Soil Till Res*. 124:144–152. doi:10.1016/j.still.2003.12.002
- Huang, M., X. Zhou, F. Cao, B. Xia, and Y. Zou. 2015. No-tillage effect on rice yield in China: a meta-analysis. *Field Crops Research*. 183:126–137. doi:10.1016/j.fcr.2015.07.022
- Khush, G.S. 2000. Strategies for increasing the yield potential of rice. *Stud Plant S*. 7:207–212. doi:10.1016/S0928-3420(00)80016-6
- Kottek, M., J. Grieser, C. Beck, B. Rudolf, and F. Rubel. 2006. World map of the Köppen-Geiger climate classification updated. *Meteorol Z*. 15:259–263. doi:10.1127/0941-2948/2006/0130
- Kuzyakov, Y., and X. Xu. 2013. Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. *New Phytol*. 198:656–669. doi:10.1111/nph.12235
- Lal, R. 2015. Restoring Soil Quality to Mitigate Soil Degradation. *Sustainability*. 7:5875–5895. doi:10.3390/su7055875
- Liu, Q., N. Qiao, X. Xu, X. Xin, J.Y. Han et al. 2016. Nitrogen acquisition by plants and microorganisms in a temperate grassland. *Sci Rep-UK*. 6:22642. doi:10.1038/srep22642
- Marschner, H. 2011. *Marschner's mineral nutrition of higher plants*. 3rd ed. Academic press.
- Martins, A.P., S.E.V.G.A. Costa, I. Anghinoni, T.R. Kunrath, F. Balerini et al. 2014. Soil acidification and basic cation use efficiency in an integrated no-till crop-livestock system under different grazing intensities. *Agric Ecosyst Environ*. 195:18–28. doi:10.1016/j.agee.2014.05.012
- Martins, A.P., L.G.O. Denardin, J.B.M. Borin, F.S. Carlos, T. Barros et al. 2017. Short-term Impacts on Soil-quality Assessment in Alternative Land Uses of Traditional Paddy Fields in Southern Brazil. *Land Degrad Dev*. 28:534–542. doi:10.1002/ldr.2640

- Matzenauer, R., B. Radin, I.R. Almeida. 2011. Atlas Climático: Rio Grande do Sul. Porto Alegre: Secretaria da Agricultura Pecuária e Agronegócio; Fundação Estadual de Pesquisa Agropecuária (FEPAGRO).
- SAS Institute SAS/STAT software. 1997. Changes and enhancements through release 1 6.12. Statistical Analysis System Institute, Cary.
- Schoenau, J.J., and C.A. Campbell. 1996. Impact of crop residues on nutrient availability in conservation tillage systems. *Can J Plant Sci.* 76:621–626. doi:10.4141/cjps96-111
- Soil Survey Staff. 2010. Keys to Soil Taxonomy. U.S. Department of Agriculture Natural Resources Conservation Service.
- SOSBAI. 2014. Arroz irrigado: Recomendações técnicas da pesquisa para o Sul do Brasil. Pelotas: SOSBAI.
- SOSBAI. 2016. Arroz irrigado: Recomendações técnicas da pesquisa para o Sul do Brasil. Bento Gonçalves: SOSBAI.
- SOSBAI. 2018. Arroz irrigado: Recomendações técnicas da pesquisa para o Sul do Brasil. Farroupilha: SOSBAI.
- Tang, X., J.A. Shao, M. Gao, C.F. Wei, D.T. Xie et al. 2007. Effects of conservational tillage on aggregate composition and organic carbon storage in purple paddy soil. *Chinese Journal of Applied Ecology.* 18:1027–1032.
- Tedesco, M.J., C. Gianello, C.A. Bissani, H. Bohnen, S.J. Volkweiss. 1995. Análises de solo, plantas e outros materiais. Porto Alegre: UFRGS.
- Thomas, G.A., R.C. Dalal, and J. Standley. 2007. No-till effects on organic matter, pH, cation exchange capacity and nutrient distribution in a Luvisol in the semi-arid subtropics. *Soil Till Res.* 94:295–304. doi:10.1016/j.still.2006.08.005
- Whitehead, D.C. 2000. Nutrient elements in grassland. Wallingford: CABI Publishing.
- Zou, C., Q. Daozhu, X. Minggang, S. Huaping, and W. Boren. 2001. Nitrogen, phosphorous and potassium uptake characteristics of rice and its relationship with grain yield. *Journal Nanj Agr Univ.* 25:6–10.

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Figure 1. Flag leaf nitrogen (A), phosphorus (B) and potassium (C) contents affected by fertilization levels in the 2015/2016 cropping season of different flooded rice production systems in the Brazilian subtropics. The range highlighted in gray corresponds to the crop adequate nutritional status, according to SOSBAI (2014). The “ns” indicates absence of statistical difference according to Tukey’s test ($p>0.05$). Error bars represent standard error of the mean ($n=3$).

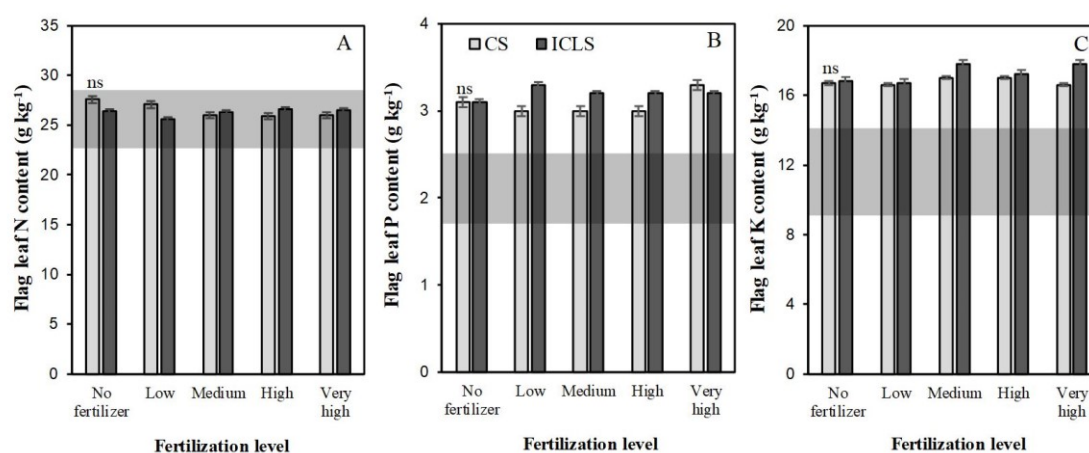


Figure 2. Flooded rice shoot dry matter affected by fertilization levels in the 2015/2016 cropping season of different production systems in the Brazilian subtropics. Tukey's test ($p < 0.05$): Different lowercase letters indicate differences among the fertilization levels within each flooded rice production system; Different uppercase letters indicate differences between flooded rice production systems within each fertilization level.

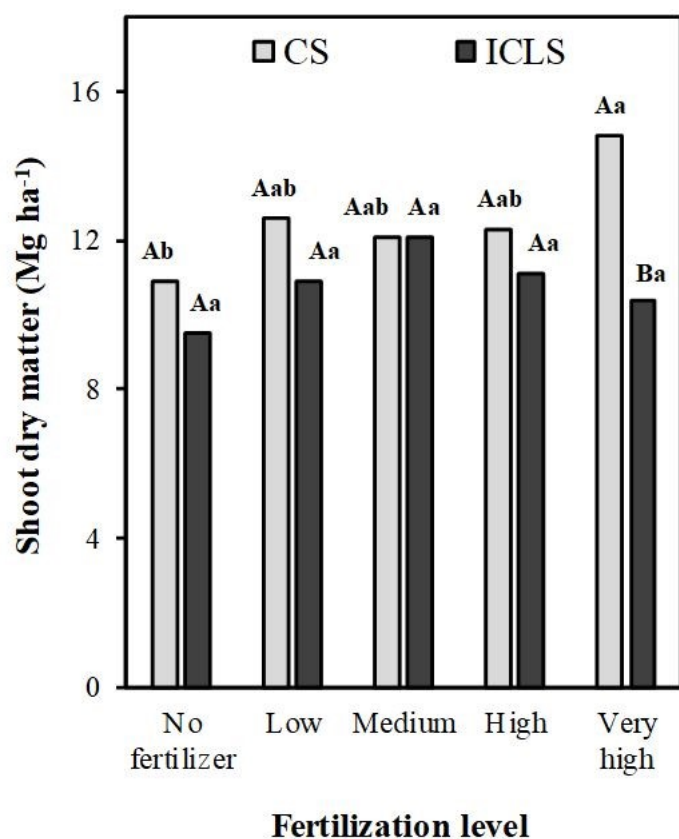


Figure 3. Flooded rice grain yield affected by fertilization levels in the 2015/2016 cropping season of different production systems in the Brazilian subtropics. Tukey's test ($p < 0.05$): Different lowercase letters indicate differences among the fertilization levels within each flooded rice production system; Different uppercase letters indicate differences between flooded rice production systems within each fertilization level.

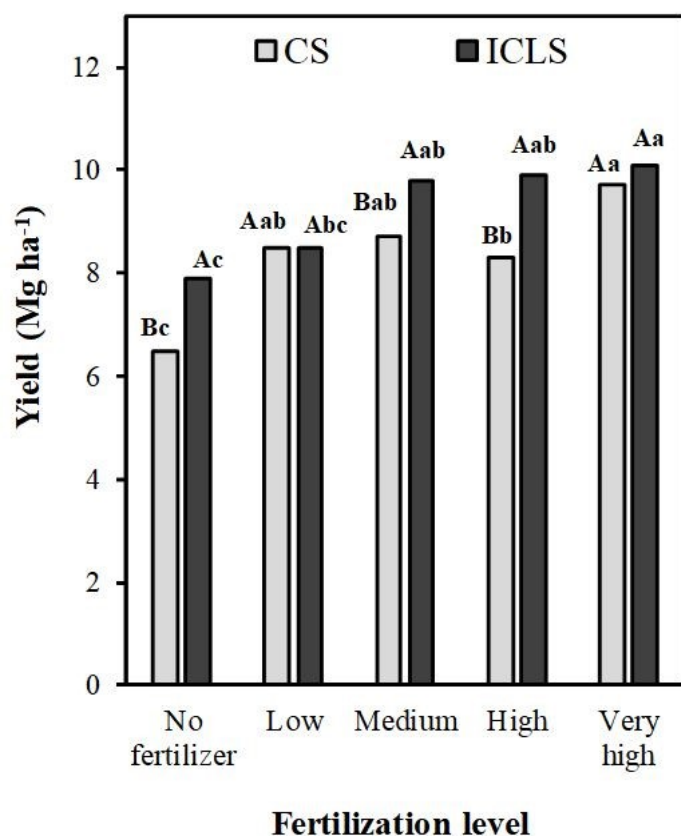


Figure 4. Flooded rice grain yield with or without P and K fertilization in the 2016/2017 cropping season in different production systems in the Brazilian subtropics. Tukey's test ($p < 0.05$): Different lowercase letters indicate differences between the P and K fertilization within each flooded rice production system; Different uppercase letters indicate differences between flooded rice production systems within each P and K fertilization.

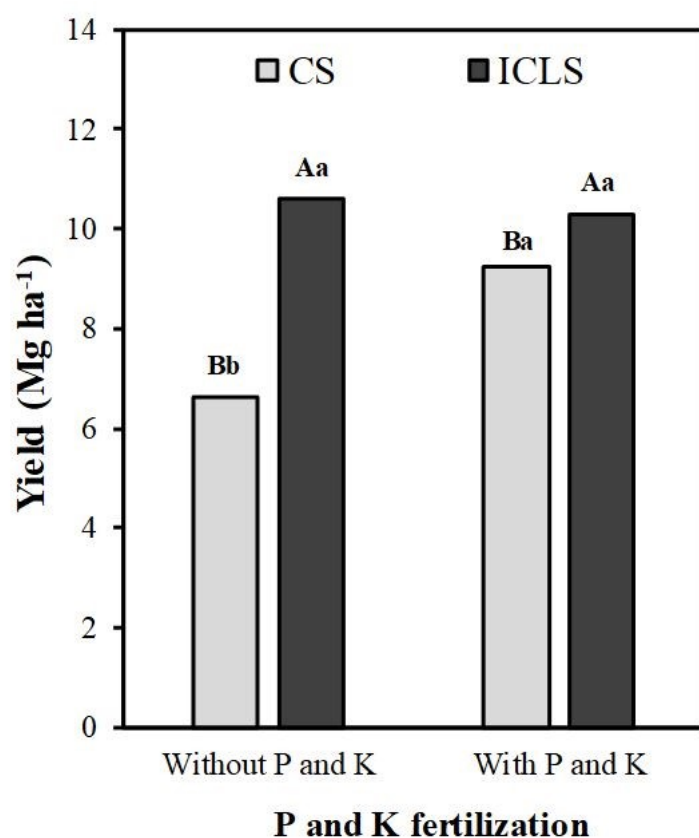
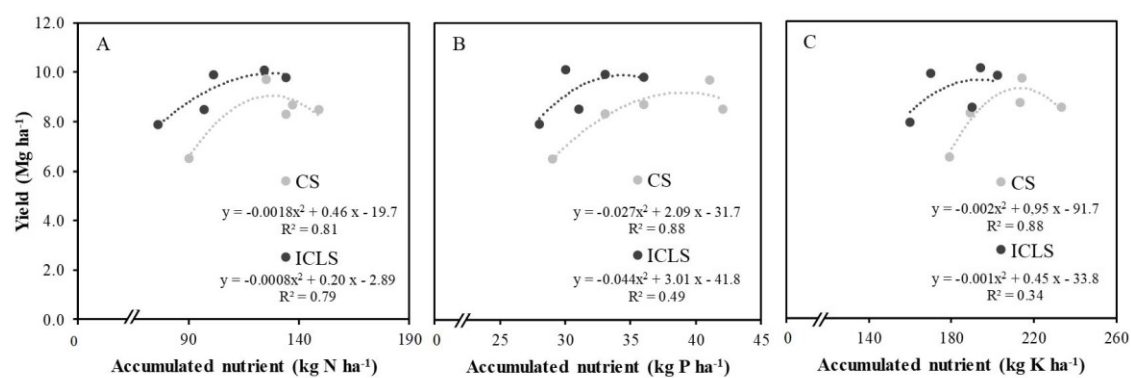


Figure 5. Relationship between flooded rice yield and N (A), P (B), and K (C) accumulated in shoot dry matter, affected by fertilization levels in the 2015/2016 cropping season in different production systems in the Brazilian subtropics.



Tables

Table 1. Soil fertility (0-10 cm layer) after 30 months (March 2013 and October 2015) of the adoption of CS and ICLS production systems in paddy fields of a Brazilian subtropical region

| Production systems | SOM | pH | P | K | Ca | Mg | CEC | V% |
|--------------------|--------------------------|-------------------|---------------------------------|---------|--|-------|--------------------|-------|
| | % | | ----- mg kg ⁻¹ ----- | | ----- cmol _c kg ⁻¹ ----- | | | % |
| | ----- March 2013 ----- | | | | | | | |
| CS | 2.0 | 5.5 | 9.2Aa | 69.3 | 3.2 | 2.1 | 9.3 | 58.1 |
| ICLS | 1.8 | 5.4 | 9.9Ba | 76.6 | 3.7 | 2.3 | 11.0 | 56.1 |
| Average | 1.9 | 5.5 | 9.5 | 72.9 B | 3.5 B | 2.2 B | 10.2 | 57.1B |
| | ----- October 2015 ----- | | | | | | | |
| CS | 2.2 | 5.7 | 16.4Ab | 116.0 | 5.1 | 2.6 | 10.6 | 75.3 |
| ICLS | 2.2 | 5.5 | 26.1Aa | 133.6 | 6.2 | 3.0 | 12.4 | 72.9 |
| Average | 2.1 ^{ns} | 5.6 ^{ns} | 21.2 | 124.8 A | 5.7 A | 2.8 A | 11.5 ^{ns} | 74.1A |

Tukey's test ($p < 0.05$): Different lowercase letters indicate differences between the flooded rice production systems within each year; Different uppercase letters indicate differences between years within each flooded rice production systems. The "ns" indicates absence of statistical difference.

Table 2. Nitrogen, phosphorus, and potassium accumulation in shoot dry matter of flooded rice affected by fertilization levels in the 2015/2016 cropping season of different production systems in the Brazilian subtropics

| Production systems | Fertilization level | | | | | Average |
|--------------------|---|--------|--------|---------|-----------|---------|
| | No-fertilizer | Low | Medium | High | Very high | |
| | ----- Nitrogen (kg ha ⁻¹) ----- | | | | | |
| CS | 90 Ab | 149 Aa | 137 Aa | 134 Aa | 125 Aab | 127 |
| ICLS | 76 Ab | 97 Bab | 134 Aa | 101 Bab | 124 Aa | 106 |
| Average | 83 | 133 | 135 | 117 | 124 | |
| | ----- Phosphorus (kg ha ⁻¹) ----- | | | | | |
| CS | 29 Ab | 42 Aa | 36 Aab | 33 Aab | 41 Aab | 36 |
| ICLS | 28 A ^{ns} | 31 B | 36 A | 33 A | 30 B | 31 |
| Average | 29 | 36 | 36 | 33 | 36 | |
| | ----- Potassium (kg ha ⁻¹) ----- | | | | | |
| CS | 179 | 233 | 213 | 189 | 214 | 206 A |
| ICLS | 160 | 190 | 202 | 170 | 194 | 183 B |
| Average | 169 ^{ns} | 212 | 207 | 180 | 204 | |

Tukey's test ($p < 0.05$): Different lowercase letters indicate differences among the fertilization levels within each flooded rice production system; Different uppercase letters indicate differences between flooded rice production systems within each fertilization level. The "ns" indicates absence of statistical difference.

Table 3. Flooded rice grain yield affected by nitrogen supply levels in the 2016/2017 cropping season in different production systems in the Brazilian subtropics

| Production systems | Nitrogen supply (kg N ha ⁻¹) | | | | Average |
|--------------------|--|--------|--------|-------|---------|
| | 0 | 50 | 100 | 150 | |
| CS | 7.2 | 7.8 | 8.7 | 9.3 | 8.3 B |
| ICLS | 8.7 | 9.8 | 10.0 | 10.2 | 9.7 A |
| Average | 8.0 b | 8.8 ab | 9.4 ab | 9.7 a | |

Tukey's test ($p < 0.05$): Lowercase letters compare the nitrogen supply levels in each flooded rice production system; Uppercase letters compare flooded rice production systems in each nitrogen supply level. Different

lowercase letters indicate differences among the nitrogen supply levels within each flooded rice production system; Different uppercase letters indicate differences between flooded rice production systems within each nitrogen supply level.

Table 4. Flooded rice grain yield per each kg of N, P, and K accumulated in shoot dry matter in the 2015/2016 cropping season, affected by fertilization levels in different production systems in the Brazilian subtropics

| Production systems | Fertilization level | | | | | Average |
|--------------------|---|---------|--------|---------|-----------|---------|
| | No-fertilizer | Low | Medium | High | Very high | |
| | ----- Nitrogen (kg grain kg N ⁻¹) ----- | | | | | |
| CS | 74.3 | 58.8 | 63.5 | 61.7 | 77.9 | 67.3 B |
| ICLS | 107.5 | 91.3 | 75.5 | 92.2 | 84.9 | 90.3 A |
| Average | 90.9 a | 75.0 ab | 69.5 b | 77.0 ab | 81.4 ab | |
| | ----- Phosphorus (kg grain kg P ⁻¹) ----- | | | | | |
| CS | 229.2 | 207.2 | 248.8 | 247.2 | 244.8 | 235.5 B |
| ICLS | 286.9 | 299.3 | 286.3 | 306.9 | 357.5 | 307.4 A |
| Average | 258.1 ^{ns} | 253.3 | 267.6 | 277.1 | 301.2 | |
| | ----- Potassium (kg grain kg K ⁻¹) ----- | | | | | |
| CS | 36.9 | 36.8 | 41.6 | 44.5 | 46.2 | 41.2 B |
| ICLS | 51.3 | 48.0 | 50.9 | 59.3 | 52.8 | 52.5 A |
| Average | 44.1 ^{ns} | 42.4 | 46.2 | 51.9 | 49.5 | |

Tukey's test (p<0.05): Different lowercase letters indicate differences among the fertilization levels within each flooded rice production system; Different uppercase letters indicate differences between flooded rice production systems within each fertilization level. The "ns" indicates absence of statistical difference.