RICE STRAW INCORPORATED JUST BEFORE SOIL FLOODING INCREASES ACETIC ACID FORMATION AND DECREASES AVAILABLE NITROGEN\(^{(1)}\)

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SUMMARY

Incorporation of rice straw into the soil just before flooding for water-seeded rice can immobilize mineral nitrogen (N) and lead to the production of acetic acid harmful to the rice seedlings, which negatively affects grain yield. This study aimed to evaluate the formation of organic acids and variation in pH and to quantify the mineral N concentration in the soil as a function of different times of incorporation of rice straw or of ashes from burning the straw before flooding. The experiment was carried out in a greenhouse using an Inceptisol (Typic Haplaquept) soil. The treatments were as follows: control (no straw or ash); incorporation of ashes from previous straw burning; rice straw incorporated to drained soil 60 days before flooding; straw incorporated 30 days before flooding; straw incorporated 15 days before flooding and straw incorporated on the day of flooding. Experimental units were plastic buckets with 6.0 kg of soil. The buckets remained flooded throughout the trial period without rice plants. Soil samples were collected every seven days, beginning one day before flooding until the 13th week of flooding for determination of mineral N- ammonium (NH\(_4^+\)) and nitrate (NO\(_3^-\)). Soil solution pH and concentration of organic acids (acetic, propionic and butyric) were determined.

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RESUMO: A INCORPORAÇÃO DA PALHA DO ARROZ PRÓXIMO AO ALAGAMENTO DO SOLO AUMENTA A FORMAÇÃO DE ÁCIDO ACÉTICO E DIMINUI A DISPONIBILIDADE DE NITROGÊNIO

A incorporação da palha do arroz em data próxima ao alagamento do solo para a semeadura do arroz, em sistema de cultivo pré-germinado, provoca a imobilização do nitrogênio (N) mineral e favorece a produção de ácido acético em níveis tóxicos às plântulas dessa cultura, interferindo negativamente na produção de grãos. Este estudo teve por objetivos avaliar a formação de ácidos orgânicos e as variações no pH da solução do solo e quantificar a concentração de N mineral no solo, em razão de diferentes épocas de incorporação da palha do arroz ou da cinza da queima da palha, antes do alagamento do solo. O experimento foi conduzido em casa de vegetação, utilizando-se um Cambissolo Háplico. Os tratamentos foram: testemunha (sem palha nem cinza); incorporação da palha no ultimado 15 dias; palha incorporada no dia do alagamento. As unidades experimentais foram balde plásticos com 6,0 kg de solo. A partir do alagamento, os balde permaneceram inundados durante todo o período experimental, sem a presença de plantas de arroz. As amostras de solo foram coletadas imediatamente antes do alagamento e a cada sete dias após, até a 13ª semana para a determinação do N mineral - amônio (NH₄⁺) e nitrato (NO₃⁻). O pH da solução do solo e a concentração de ácidos orgânicos (acético, propiónico e butírico) foram determinados. Todo o nitrato existente no solo, antes do alagamento, foi perdido antes de duas semanas de alagamento, em todos os tratamentos. Houve um comportamento sigmoidal na formação de NH₄⁺ em todos os tratamentos, ou seja, a concentração de amônio começou a aumentar rapidamente; após o alagamento decresceu levemente; e voltou a aumentar novamente. Aos 91 dias de alagamento, a concentração de NH₄⁺ no solo era 56 mg kg⁻¹ no tratamento testemunha, 72 mg kg⁻¹ no tratamento 60 dias de incorporação da palha, 73 mg kg⁻¹ no tratamento 60 dias e 53 mg kg⁻¹ quando a cinza foi incorporada ao solo. Essa concentração de amônio no solo corresponde a 84, 108, 110 e 80 kg ha⁻¹ N-NH₄⁺, respectivamente. Quando a palha foi incorporada no dia do alagamento ou 15 dias antes, a concentração de amônio era 28 e 54 mg kg⁻¹, equivalente a um acúmulo de 42 e 81 kg ha⁻¹ N-NH₄⁺, respectivamente. Houve a formação de ácido acético, que alcançou nível tóxico às plântulas (7,2 mmol L⁻¹) no 15º dia após o alagamento, apenas no tratamento com a palha incorporada ao solo no dia do alagamento. O pH da solução do solo de todos os tratamentos aumentou após o alagamento, sendo mais rápido nos tratamentos em que a palha foi incorporada ao solo, seguido pela cinza e depois pela testemunha. Aos 60 dias de alagamento, todavia, o pH alcançou 6,5 em todos os tratamentos, exceto na testemunha que atingiu pH 6,3. A incorporação da palha do arroz deve...
INTRODUCTION

Rice straw is the designation for the above ground parts of rice plants that remain in the field after harvest. The amount of straw produced by semi-dwarf rice cultivars is, on average, similar to the amount of grain (Costa, 2000; Knoblauch & Schiocchet, 2001). The recommended management procedure is incorporation of the straw into the soil instead of burning it (SOSBAI, 2010). In the State of Santa Catarina, southern Brazil, a state law restricts the practice of burning. However, incorporation of rice straw just before flooding for water-seeded rice can lead to the production of organic acids that are harmful to seedling development (Sousa & Bortolon, 2002; Schmidt et al., 2007) and immobilize soil mineral N (Broadbent & Nakashima, 1970; Serpa et al., 2009), which can affect negatively grain yield (Camargo et al., 1995).

Decomposition of organic matter (OM) in soil is performed by a group of bacteria, fungi, and actinomycetes that are very active in aerated soils but practically inactive under a flooded environment. Decomposition of OM in flooded soils is slower than in aerated soils (Becker et al., 1994; Sousa, et al., 2000). As a result, immobilization of N is less than in oxygenated soils (Buressh, 2008). In the State of California, USA, burning of rice straw was a common practice before being restricted in the 1990s (Linquist et al., 2006). Mutters (2009) observed that the transition from straw burning to incorporation coincided with a decrease in rice yield in that state. In Santa Catarina, many rice farmers do not incorporate the straw, due to operational difficulties and due to field observations related to better establishment and early development of seedlings when the straw is burned. However, burning is considered harmful to soil OM and to the air by releasing CO₂ and other gases to the atmosphere. In addition to N and C, rice straw contains other elements in significant quantities, such as P, K and S, which may be lost at least to a certain extent during the combustion process.

In Santa Catarina, research has not been conducted to characterize the problem of burning or incorporation of rice straw. Therefore, there are no strategies for management of rice crop residues.

This study aimed to evaluate the formation of organic acids and variations in pH and to quantify mineral N in the soil as a function of different times of rice straw or ash incorporation before flooding.

MATERIALS AND METHODS

The experiment was carried out in a greenhouse at the Universidade do Estado de Santa Catarina (Santa Catarina State University) in Lages, SC, Brazil, from September 2009 to February 2010. The soil was collected from the surface (0-15 cm) of an Inceptisol (Typic Hapludult) from an irrigated rice area in the Alto Vale do Itajaí (Upper Itajaí Valley), Santa Catarina, Brazil. Soil characteristics were: pH (H₂O) = 4.8; P = 2.0 mg kg⁻¹, K = 60 mg kg⁻¹, organic matter (OM) = 20.0 g kg⁻¹, Al = 1.7 cmol⁺ dm⁻³, Ca = 2.0 cmol⁺ dm⁻³, Mg = 0.8 cmol⁺ dm⁻³ and clay = 340 g dm⁻³.

The experiment consisted of six treatments: control, without incorporation of straw or ashes; ash from rice straw burning incorporated in the soil on the day of flooding; straw incorporated into the drained soil at 60, 30 and 15 days before flooding, respectively, and straw incorporated on the day of flooding. The amount of straw used was equivalent to 18 t ha⁻¹ (dry weight), corresponding to 50.0 g of straw in each pot. Before incorporation, the rice straw was cut into approximately 5.0-cm-length pieces.

Experimental units consisted of pots with 6.0 kg of dry soil, which was sieved in a 1.0 cm mesh. After the incubation period, the pots were flooded with distilled water to make mud. The pots then remained flooded throughout the experimental period at a water depth of 5.0 cm. There were no rice plants in the pots. For determination of mineral N (N-NH₄⁺ and N-NO₃⁻), soil samples were collected just before flooding and every seven days from the 1st week to the 13th week of flooding. Samples were collected with an iron soil probe, 12.0 cm long and 1.0 cm thick. After each sampling, the flooded soil (mud) was homogenized manually and approximately 10 g of subsamples was used for N extraction. The remaining soil was used for moisture determination. Ammonium and nitrate were extracted from the soil with 1.0 mol L⁻¹ KCl and analyzed by the semi-micro-Kjeldahl method (Tedesco et al., 1995).

Organic acids (acetic, propionic, and butyric) and pH were determined in the soil solution. The soil solution was extracted from the soil by a PVC pipe 5.0 cm in diameter and 6.0 cm long, wrapped at both ends with a 150-mesh nylon screen. The pipes were introduced into the soil and the top remained 1.0 cm below the soil surface. Soil solution samples were collected at 3, 6, 10, 15, 18, 23 and 29 days of flooding. After sampling, pH was determined. The soil solution was acidified with 1.0 mol L⁻¹ sulfuric acid to reach...
pH = 3.0, then placed in plastic vials and stored in the refrigerator until determinations of acids.

Measurements of organic acids were performed in the laboratory of the Itajaí Experimental Station/Epagri in Itajaí, Santa Catarina, in the following manner. Before injection of the solution into the chromatograph, 1.5 mL of sample were transferred to Eppendorf tubes and centrifuged at 13,000 rpm. Then, part was transferred to suitable containers for analysis by chromatography. Determination of organic acids was performed using high performance liquid chromatography (HPLC) - Shimadzu LC10-VP, equipped with a quaternary gradient pump, an autosampler with thermostated (10°C) sample holder, a column oven (40°C) and a UV absorption detector (set to 210 nm). The system is managed by a workstation equipped with dedicated software. The column used was Aminex–HPX-87H (BioRad). The mobile phase was 5 mmol L\(^{-1}\) H\(_2\)SO\(_4\), isocratic mode, with a flow of 0.6 mL min\(^{-1}\). The volume of sample injection was 20 uL. The pH of the soil solution was determined at 10, 14, 17, 22, 30 and 60 days of flooding. A completely randomized experimental design was used, with four replications.

RESULTS AND DISCUSSION

Ammonium (N-NH\(_4^+\)) concentration in the control treatment without straw or ash began to increase on the day of flooding, reaching values near 40 mg kg\(^{-1}\) N-NH\(_4^+\) on the 28th day of flooding. It stabilized for about one month and then increased again, reaching 56 mg kg\(^{-1}\) on the 91st day (Figure 1). This sigmoidal behavior in N release during mineralization of native OM in wetland soils was also observed by other authors (Savant & De Datta, 1980; Becker et al., 1994; Vahl, 1999, Li et al., 2003). These authors reported that the formation of ammonium in flooded soils usually occurs in two phases: in the first phase, the more labile fraction of OM is mineralized, followed by a slow phase when the more recalcitrant fraction is mineralized. Considering a 15-cm soil layer, where more than 95 % of rice roots are present (Espinal, 1997) on the 91st day of flooding, the soil would accumulate about 86 kg ha\(^{-1}\) of N-NH\(_4^+\) in the control treatment. All treatments with straw or ash incorporation showed sigmoidal accumulation of ammonium in the soil, similar to the control treatment (Figure 1). However, the amount of ammonium accumulated had different behavior in the straw treatments compared to the control (Figure 2).

Up to 70 days of flooding, the highest concentrations of ammonium occurred in the control soil, ash incorporated soil, and soils with incorporation of straw at 60 and 30 days before flooding, reaching an average
of 43 mg kg⁻¹ N-NH₄ (Figure 1). However, after 70 days of flooding, treatments incorporating straw at 60 and 30 days before flooding continued with a high rate of ammonium accumulation, while in the control and ash incorporated treatments, the intensity of N accumulation began to decrease (Figure 2).

Considering the 15-cm soil layer, the amount of ammonium accumulated after 91 days of flooding was equivalent to approximately 110 kg ha⁻¹ of N-NH₄⁺ in the treatments with straw incorporated at 60 and 30 days before flooding, and 84 and 80 kg ha⁻¹ of N-NH₄⁺ in the control and ash treatments, respectively.

In the treatments where straw was incorporated on the day of flooding or 15 days before, ammonium formation was similar up to 63 days of flooding, reaching 17 and 19 mg kg⁻¹ of N-NH₄⁺ (Figure 1). After that, there was an increase in ammonium concentration in the treatment where the straw was incorporated 15 days before flooding. The increase was higher than the treatment where the straw was incorporated on the day of flooding, reaching 54 and 28 mg kg⁻¹ N-NH₄⁺, respectively (Figure 1). Those values were equivalent to 81 and 42 kg ha⁻¹ of N-NH₄⁺, respectively. Considering the differences in ammonium concentration when straw was incorporated at 60 or 30 days before flooding with straw incorporated on the day of flooding, there was a difference in favor of straw which was incorporated earlier of approximately 68 kg ha⁻¹ of N-NH₄⁺.

Figure 2 shows the concentration of N-NH₄⁺ in the soil in treatments with straw or ash incorporated after subtracting the values of the control treatment. The negative values of ammonium concentration, therefore, correspond to lower concentration than the control.

For the treatments where the straw was incorporated 60 or 30 days before flooding and where there was ash incorporation, the amount of ammonium in the soil was higher than the control from 7 to 21 days of flooding (Figure 2). This behavior is due to the decomposition of the more labile fraction of the OM added (Vahl, 1999; Li et al., 2003). After peak concentration from 14 to 21 days of flooding, there was a period of immobilization of N up to 49 days. After that period, the ammonium concentration started to increase again. However, after 70 days of flooding, the treatments with straw incorporated 60 or 30 days before flooding started to release ammonium more than in the control, while in the treatment with ash incorporation, ammonium release stabilized and started to decrease (Figure 2). Similar behavior in N dynamics with straw incorporation in flooded soils was related by Becker et al. (1994) in an experiment conducted in the Philippines.

When rice straw was incorporated on the day of flooding or 15 days before, the concentration of ammonium in the soil began to decrease right after flooding when compared to the control treatment and, at around 42 days of flooding, immobilization of N reached values over 21 mg kg⁻¹ of N-NH₄⁺ in both treatments (Figure 2). In these treatments, the dynamics of N immobilization had similar behavior up to the 63rd day. As of that time, the straw incorporated on the day of flooding kept immobilizing N while, for the straw incorporated 15 days before, immobilization of N stabilized and started to release the N immobilized. Considering a soil layer of 15 cm, the amount of immobilized N at 77 days of flooding was 47 and 26 kg ha⁻¹ of N-NH₄⁺ in the treatments with straw incorporated on the day of flooding or 15 days before, respectively.

More than 95 % of irrigated rice cultivars grown in Santa Catarina are late maturing cultivars (Noldin et al., 2009). One of the stages in which rice plants have high demand for N is the tillering stage (SOSBAI, 2010). In late maturing cultivars, this phase normally occurs from 30 to 80 days after sowing (DAS) (SOSBAI, 2010), which means that straw incorporated just before flooding can reduce the availability of N in a period of high demand for the nutrient. On the other hand, when straw was incorporated into the soil 30 or 60 days before flooding, the N mineralization rate was higher than the control up to 70 days of flooding.

In the reproductive stage, rice plants also have a high demand for N (SOSBAI, 2010). In late maturing cultivars this stage starts, on average, at 80 DAS. If straw is incorporated 30 days in advance of flooding or more, it will provide a greater amount of N in the reproductive stage of rice plants as compared to the soil that does not receive straw.

The N immobilization rate due to straw incorporation varies according to the increase in environmental temperature (Savant & De Datta, 1982). In this experiment carried out in a greenhouse, the inside temperature was higher than the outside. According to Ponnamperruma (1984), in hot areas, straw may be incorporated up to one month prior to flooding, while in cool areas, incorporation should be anticipated.

In this experiment, we added 60 g of straw per pot. Each pot had 6.0 kg of soil, i.e., we added 10 g of straw per kg of soil. N concentration in the straw was 6.0 mg kg⁻¹, which means we added 60 mg kg⁻¹ of N. In the treatments with straw incorporated 60 or 30 days before flooding, N concentration was 17 mg kg⁻¹ more than in the control at 91 days of flooding (Figure 2). This represents only 28.3 % of the amount of N contained in the rice straw. Russell et al. (2006) stated that rice straw incorporated into the soil in California, USA, took two years to decompose completely. Linquist et al. (2006), in the same region, recommend a reduction in the amount of N application of 20 kg ha⁻¹ N when straw is incorporated in the soil instead of burning.

In this experiment, one day before flooding, nitrate concentrations in the soil in the treatments that had
not received straw were greater than those with straw (Figure 3). This shows that straw caused immobilization of the available nitrate. In the treatment with straw on the day of flooding, nitrate concentrations were higher than in the control, followed by ash incorporation, with concentrations of 40, 33, and 32 mg kg\(^{-1}\) of N-NO\(_3^-\), respectively. When rice straw was incorporated 15, 30 or 60 days before flooding, nitrate concentrations in the soil were 11.8, 9.0 and 7.0 mg kg\(^{-1}\), respectively (Figure 3).

Immobilization of mineral N usually occurs when materials are added with a high C:N ratio (Ernani, 2008). The rice straw used in the experiment had a C:N ratio of 60:1. For non-irrigated land, this relationship is harmful to plants, since it may restrict N availability. In irrigated rice, the immobilization of N-NO\(_3^-\) can be beneficial to the rice plants because part of immobilized N-NO\(_3^-\) will be released as ammonium during decomposition of straw after flooding (Ponnamperuma, 1984).

All nitrate content in the soil before flooding, regardless of the initial concentration, was lost by denitrification in the first 14 days of flooding (Figure 3). Considering that the demand for N for rice plants grown in a water-seeded system starts from 20 to 30 DAS (SOSBAI, 2010), NO\(_3^-\) would have been completely lost before plant uptake. Denitrification occurs in flooded soils due to lack of oxygen. In anaerobic environments, microorganisms use NO\(_3^-\) as an electron acceptor during anaerobic decomposition of OM, and N is lost mainly as N\(_2\)O and N\(_2\) (Ponnamperuma, 1972, 1984; Patrick Jr. & Reddy, 1978).

The pH of the soil solution after flooding increased in all treatments (Figure 4). In the control there was a steady increase over time, reaching 4.7 on the 10\(^{th}\) day of flooding, 5.6 on the 22\(^{nd}\) day, and stabilizing around 6.3 at 60 days of flooding (Figure 4). In all treatments where straw was incorporated into the soil, regardless of the period of anticipation, the pH increases were faster than in treatments without straw, reaching values close to 6.0 in 10 days of flooding and stabilizing at pH 6.5 as of the 22\(^{nd}\) day (Figure 4). Ash incorporated into the soil increased the pH of the solution faster than in the control, but slower than in the treatments with incorporated straw (Figure 4).

When we did not have straw or ash incorporated into the soil, the pH of the soil solution increased slower and, moreover, did not reach the same pH.

The increase of pH in the soil solution after flooding occurs due to the processes of reduction of some compounds in the soil. Those reactions are as fast as the electron supply to the soil solution (Ponnamperuma, 1972). The electron source is soil OM and organic materials added to the soil (Ponnamperuma, 1972; Sousa et al, 2000). Thus, the intensity of soil reduction depends mainly on the soil type and amount of available OM. Environmental

![Figure 3. Temporal variation of N-NO\(_3^-\) in the soil after flooding as a function of the time of incorporation of rice straw preceding the soil flooding or incorporation of the ash from burned straw. Control: without straw or ash; Ash: straw incorporated on the day of flooding; 60 days before: straw incorporated into the soil 60 days before flooding; 30 days before: straw incorporated 30 days before flooding; 15 days before: straw incorporated 15 days before flooding; and Flooding day: straw incorporated on the same day as flooding.](image1)

![Figure 4. pH of the soil solution after flooding as a function of the time of incorporation of rice straw preceding the soil flooding or incorporation of the ash from burned straw. Control: without straw or ash; Ash: ash incorporated on the day of flooding; 60 days before: straw incorporated into the soil 60 days before flooding; 30 days before: straw incorporated 30 days before flooding; 15 days before: straw incorporated 15 days before flooding; and Flooding day: straw incorporated on the same day as flooding.](image2)
conditions, such as pH and temperature, may affect the activity of microorganisms. In this study, rice straw was the main source of electrons for reduction of the soil. Increasing the pH of acidic soils is very important for the rice crop since it favors N and P release and decreases the effect of potentially toxic elements and compounds such as Al, Fe, Mn, CO$_2$, organic acids, and H$_2$S (Sousa et al., 2000).

The incorporation of rice straw to the soil released acetic acid, but the presence of propionic and butyric acids was not detected. The presence of acetic acid was observed in all treatments with straw from 10 to 18 days of flooding, but it was not observed in the control and ash incorporation treatments (Figure 5). The maximum concentration of acetic acid in most treatments occurred at around 15 days of flooding. The highest concentration occurred when the straw was incorporated on the day of flooding, reaching 7.2 mmol L$^{-1}$ (Figure 5). This concentration is considered toxic to the roots of rice seedlings (Sousa & Bortolon, 2002).

The formation of volatile organic acids in flooded soil depends on several factors, but the temperature and pH of the soil solution are the most important. At high temperatures and near neutral pH, organic acids are produced in low quantities and have a short life. In contrast, at low temperature and low pH of the soil solution, the formation and stability of organic acids may be high (Ponnamperuma, 1972).

**CONCLUSIONS**

1. Incorporation of rice straw into the soil must be carried out a minimum of 30 days before flooding and sowing of rice in a water-seeded system. Otherwise, microorganisms that decompose the straw will cause the immobilization of N, resulting in the need for application of higher doses of N. Moreover, the fermentation of straw will produce acetic acid, which may be harmful to rice seedlings;

2. As of the period of flooding, the pH of the soil solution increases faster when the soil contains incorporated straw, as opposed to no straw.

**LITERATURE CITED**


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