








Influence of summer crop residues on ¹⁵N present in organic matter fractions under two lowland soils

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ABSTRACT: The state of Rio Grande do Sul has about 20% of the total area as lowland soils, suitable for flooded rice (*Oryza sativa*). In order to mitigate damage caused by rice monoculture, new crops such as sorghum (*Sorghum bicolor*) and soybean (*Glycine max*) have been cultivated in these areas. With different qualities of crop residues, it is expected a change in soil organic matter (SOM) dynamics and consequently, nitrogen (N) availability. The objective of this study was to evaluate the influence of rice, soybean and sorghum crop residues on the N present in physical fractions of SOM of two lowland soils, using labeled ¹⁵N technique, under incubation for 180 days in aerobic condition and more 180 days in anaerobic condition. At 30, 180 and 360 days of incubation the remaining N of the plant residues and N destination from the residues in both soils were quantified in the physical fractions of SOM >250 μm, 250-53 μm and <53 μm. The soil with higher amount of clay+silt received a larger quantity of ¹⁵N from residues, while flooding of the soil after 180 days caused a loss of N added to the soil by the soybean and sorghum residues. In general, larger amounts of ¹⁵N were reported in the fraction <53 μm, associated with clay minerals, throughout the incubation period. These N losses should be considered in N fertilization for the following crops in rotation with flooded rice.

Key words: Physical fractionation, paddy soils, ¹⁵N isotope.

Influência de resíduos de culturas de verão sobre o ¹⁵N em frações da matéria orgânica de dois solos de terras baixas

RESUMO: O Rio Grande do Sul possui cerca de 20% da área total de solos de terras baixas, propícias para o cultivo do arroz (*Oryza sativa*) irrigado por inundação. Buscando mitigar danos ocasionados com o monocultivo de arroz, novas culturas, como o sorgo (*Sorghum bicolor*) e a soja (*Glycine max*), têm sido cultivadas nestas áreas. Com diferentes qualidades de resíduos culturais, espera-se uma alteração na dinâmica da matéria orgânica do solo (MOS) e, conseqüentemente, do nitrogênio (N). O objetivo deste estudo foi avaliar a influência de resíduos culturais de arroz, soja e sorgo na quantidade de N em frações físicas da MOS de dois solos de terras baixas, utilizando a técnica de marcação isotópica de ¹⁵N das culturas e uma incubação por um ciclo de 180 dias em condição aeróbica e mais 180 dias em condição anaeróbica. Aos 30, 180 e 360 dias de incubação foi quantificado o N remanescente dos resíduos vegetais e destino do N proveniente dos resíduos nos dois solos em frações físicas da MOS >250 μm, 250-53 μm e <53 μm. O solo com maior quantidade de argila+silt recebeu maior quantidade de ¹⁵N dos resíduos, enquanto que o alagamento do solo após 180 dias de incubação ocasionou uma perda do N adicionado ao solo pelos resíduos de soja e sorgo. De maneira geral, foram encontradas maiores quantidades de ¹⁵N na fração <53 μm, associado aos argilominerais. As perdas de N devem ser consideradas na adubação nitrogenada para as culturas seguintes nos sistemas de rotação de culturas com arroz irrigado.

Palavras-chave: Fracionamento físico, solos de várzea, isótopo ¹⁵N.

INTRODUCTION

In the state of Rio Grande do Sul (RS), lowland soils, with about 20% of the total area, cover 71% of rice (*Oryza sativa* L.) production in Brazil (CONAB, 2017). Rice production with flooding is predominant in these areas (SOSBAI, 2016). However, the rice monoculture for long time favors soil degradation, increasing pathogens population, and weed infestations (MARCHESAN et al., 2004). In these cases, crop rotation with sorghum (*Sorghum*

bicolor) or soybean (*Glycine max*) is recommended to breakdown the pests and diseases cycles (SILVA & PARFITT, 2004). Compared to rice residues, soybean and sorghum cultivation in these environments will result in the addition of crop residues with distinct chemical composition e.g. C/N ratio and the soluble organic fraction content. In this context, REDIN et al. (2014) verified that grasses produce about three times more root biomass than legumes. Additionally, reported the presence of larger amounts of cellulose, hemicellulose and lignin for sorghum residues than

soybean residues. Therefore, the quality of crop residues in rotation influences the decomposition and availability of nutrients in the soil (CHIVENGE et al., 2011; AITA & GIACOMINI, 2003). In short term, it is expected that the addition of these residues with different characteristics will cause changes in the intensity of mineralization-immobilization process and, in the long term, alter the compartments of organic N in the soil.

Soil type also has influence on the N dynamics along with the residue's quality. Clayey soils often contain more amounts of soil organic matter (SOM) (KLEBER et al., 2007) and microbial biomass (SIX et al., 2006). This is because clayey soils tend to have lower C transformation rates than sandy soils due to higher organic-complexation by promoting physical protection against microbial attack (CHIVENGE et al., 2011). Therefore, it is assumed that crop rotation, depending on soil characteristics, changes the SOM dynamic and consequently, the soil organic N content. The SOM and the organic N can be studied by fractionating in order to isolate fractions of different chemical nature and/or susceptibility to decomposition. Among the different methods, the physical fractionation (densimetric, granulometric or adaptation of both) have been used to understand the SOM dynamics when associated with the soil mineral fraction, and could also be used as a strategy to understand the organic N dynamics (ROSCOE & MACHADO, 2002; CHRISTENSEN, 2001).

Although a number of different procedures may be used, one is known for some operational simplicity, used by BALESSENT et al. (1991) with adaptations of DIOCHON et al. (2016) that allows to isolate three very contrasting fractions: >250 μm , called coarse sand fraction (CSF) primarily consists of organic residues in the earlier stages of decomposition (CHRISTENSEN, 2001); 53-250 μm , fine sand fraction (FSF) consists the partially decomposed plant material; and <53 μm , called silt+clay fraction (SCF), which is the most stable SOM fraction being constituted by organo-complexes with silt and clay particles (CHRISTENSEN, 1996).

In crop rotation systems with flooded rice, it is essential to consider the crop residues decomposition in aerobic and anaerobic cycles. According to findings of LUCE et al. (2014), the relationship between quality of residues and its interaction with soil mineral fraction, it is expected to be higher initial increment of N in the soil with high quality residues, such as soybean, stabilized as SOM fractions associated with smaller particles.

However, as this fraction also contains mineral N adsorbed, it is possible that this form of N is more easily lost due to anaerobic conditions in a next flooding cycle for rice cultivation.

This study aimed to evaluate the N present in the physical fractions of SOM after addition of rice, sorghum, and soybeans residues, using labeled ^{15}N in two soils with different textures.

MATERIALS AND METHODS

In this study, samples were used from an incubation experiment carried out at greenhouse of the Department of Soil Science at Federal University of Santa Maria (UFSM). We evaluated six treatments formed by the combination of three residues (root + shoot) plants (rice, sorghum, and soybeans) enriched with ^{15}N isotope and two soil types (Planossolo Háplico Eutrófico gleissólico - SXe_{glei} and Planossolo Háplico Eutrófico arenico - SXe_{aren}) (EMBRAPA, 2013). Both soils collected up to 0 to 20 cm for experiment and differ in their particle size distribution: SXe_{glei} with 173, 287 and 540 g kg^{-1} of clay, silt, and sand, respectively, having 1.8 times more silt+clay than SXe_{aren} with 99, 155 and 746 g kg^{-1} of clay, silt, and sand, respectively. A factorial experiment (three types of residues \times two types of soils) with complete randomized design (CRD) was used with three replicates per treatment. A control treatment (soil without addition of residues) was also installed to calculate the isotopic abundance.

The experimental units were mounted in PVC tubes with 50 mm diameter and 140 mm height. Each cylinder was individually wrapped with tissue type "voile" at the bottom in order to allow water percolation and filled with 235.6 g of soil, resulting in a layer of 10 cm and medium density 1.15 g cm^{-3} . In all treatments, 1.38 g (7 Mg ha^{-1}) of shoot (stems + leaves) and 0.39 g (2 Mg ha^{-1}) of root residues were added. The N contents measured in residues were 9.95, 6.7, and 16 g kg^{-1} while calculated C/N was 41.2, 61.3, and 26.8 for rice, sorghum, and soybean, respectively.

The soils were kept under free drainage system up to 180 days with irrigation using distilled water every two weeks simulating a rain of 20 mm. After 180 days, the cylinders were sealed and soils were maintained under flooding conditions until 360 days. Three cylinders from each treatment were sampled at 30, 180 and 360 days of incubation. At each evaluation date the remaining residues and roots were collected from the soil surface and separated manually from the soil. Then, the residues and the

soil were dried in an oven at 60 °C for dry matter determination and subsequently finely ground and analyzed for total N content by an elemental analyzer (Flash EA 1112, Thermo Finnigan, Milan, Italy) and ^{15}N isotope through an isotope ratio mass spectrometer (DELTA V Advantage, Thermo Fisher Scientific, Bremen, Germany) coupled with the elemental analyzer.

Another subsample was carried out for soil particle size by physical fractionation described BALESIDENT et al. (1991) with adaptations according to DIOCHON et al. (2016). Three fractions were isolated: >250 μm , called coarse sand (CSF) mainly consisted of organic residues in the earlier stages of decomposition; 53-250 μm , called fine sand (FSF) that consisted of primarily materials at an intermediate stage of decomposition; and <53 μm , called silt+clay fraction (SCF) which corresponded to organic matter associated with the minerals; however, due to the aqueous method, in the later fraction (<53 μm) would also be mineral N, but not strongly protected in the soil. Fractions were dried at 60°C, weighed and homogenized, and then milled in a mortar to determine the total N content and ^{15}N isotopic abundance, as previously mentioned for residues and soil.

From the results, ^{15}N was calculated from remaining residues (shoot + roots) and soil from each sampling date using following equation:

$$^{15}\text{Nrem} \% = \frac{^{15}\text{Nrem}}{^{15}\text{Nad}} \times 100$$

Where, $^{15}\text{Nrem}\%$ represents the percentage of ^{15}N remaining in the residues and the soil, $^{15}\text{Nrem}$ is the amount of ^{15}N (mg kg^{-1}) remaining in residues and in soil, and Nad the amount of ^{15}N added with crop residues (mg kg^{-1}). The percentage of ^{15}N residue arising in each fraction was obtained by the following equation:

$$\%^{15}\text{Nfr} = \frac{^{15}\text{Nfr}}{^{15}\text{Nrem soil}} \times 100$$

Where, $\%^{15}\text{Nfr}$ is the percentage of ^{15}N calculated for each fraction, ^{15}Nfr is the quantity of ^{15}N in each fraction (mg kg^{-1}), and $^{15}\text{Nrem soil}$ is the amount of ^{15}N in the soil (mg kg^{-1}).

Normality assumptions were checked for errors and homogeneity of residual variance through the *Kolmogorov-Smirnov* and *Levene*, respectively. The analysis of variance (ANOVA) considering 2×3 factors (soils and crop residues) from the data ^{15}N remaining crop residues, soil, CSF, FSF and SCF fraction at each evaluation time (30, 180 and 360 days

after ^{15}N application) was performed. Means were compared using the Tukey test at 5% significance level using Microsoft Office Excel application and Sisvar software (FERREIRA, 2014).

RESULTS AND DISCUSSION

All variables met the errors normality assumptions and homogeneity of residual variance by the *Kolmogorov-Smirnov* test ($p\text{-value} \geq 0.370$) and *Levene* ($p\text{-values} \geq 0.193$), respectively, lending credibility to the results for ANOVA and comparisons means using the Tukey test.

The amount of remaining ^{15}N in crop residue (shoot + roots) decreased throughout the incubation period and was significantly influenced only by the type of residue in all evaluations, with no soil type effect (Table 1). Though, LUCE et al. (2014) have reported effects of two soils having different textures on remaining ^{15}N . Residues had a greater mineralization in the sandy soil, while type of soil had no influence on remaining ^{15}N . Absence of effect of soil on the N release from residues may be due to the fact that, in this research, the residues were deposited on the soil surface instead of incorporation, showing that little contact between residues and soil reduces or cancels their influence on the decomposition of residues (CARVALHO et al., 2008).

At 30 days the amount of remaining ^{15}N was higher in the rice residues (77.0%) and almost half of ^{15}N was no longer in soybean and sorghum residues (Table 1). This result should be directly related to the difference in chemical composition (quality) of crop residues. COPPENS (2006) relates the quick N release after adding straw to the soil by releasing soluble and readily degradable substances containing N. According REDIN et al. (2014), the high content of soluble fraction in soybean residues, particularly in leaves, should have favored the microbial decomposing activity. The effect of the quality of the residues was also observed after 180 days of incubation, and the remaining ^{15}N amount in the soybean residues, was approximately half (26, 0%) in the average of the two soils than that observed in the rice and sorghum residues (Table 1). After 360 days of incubation, the highest quantity of ^{15}N remaining was quantified for sorghum residues. According to REDIN et al. (2014), this fact is possibly related to a predominance of more recalcitrant fractions with high levels of lignin and insoluble complex which remained in the grass residue, particularly in stems, prohibiting its decomposition in the later stages than rice residues.

Regarding the amount of ^{15}N transferred to the soil from the residues, there was isolated effect of

Table 1 - ^{15}N remaining in the rice, sorghum, and soybean residues added to the soil Planossolo Háplico Eutrófico gleissólico (SXE_{glei}) and Planossolo Háplico Eutrófico arênico (SXE_{aren}) at 30, 180, and 360 days of incubation.

Soil	Rice	Sorghum	Soybean	Average
-----% of added ^{15}N -----				
-----30 days-----				
SXE_{aren}	77.0	51.6	54.6	61.0
SXE_{glei}	72.6	46.6	45.6	54.9
Average	74.8a	49.1b	50.0b	
CV (%)	-----12.7-----			
-----180 days-----				
SXE_{aren}	52.2	42.5	26.5	40.4
SXE_{glei}	50.3	53.6	25.5	43.2
Average	51.3a	48.0a	26.0b	
CV (%)	-----12.4-----			
-----360 days-----				
SXE_{aren}	3.3	20.1	6.7	10.0
SXE_{glei}	4.0	22.1	9.1	11.7
Average	3.6b	21.1a	7.9b	
CV (%)	-----28.4-----			

Lower-case letters in horizontal line show mean difference among crop residues while upper-case letters in vertical columns show mean difference between soils, compared by Tukey test ($P < 0.05$ of error probability).

crop residues after 30 days of incubation with the soybean crop residue showed higher amount of ^{15}N in soil (Table 2). The result approves the findings obtained by LUCE et al. (2014), which showed the chemical quality of residues is an important factor in the N release to the soil at the initial stage of the crop residues decomposition. At 180 days of incubation there was great effect of soil and crop residues, and the soil with higher amounts of clay (SXE_{glei}) and soybean was one with the highest amount of ^{15}N (Table 2).

At 360 days of incubation, there was interaction between soil and residues. Rice residues provided significantly higher amounts of ^{15}N in the soil (SXE_{glei}) compared to soybean and sorghum; however, the residue did not differ in the sandy soil (SXE_{aren}). It is possible that this soil treatment with soybean, whose decomposition was more intense in the first 180 days of incubation, the N from residues have been converted to nitrate and; therefore, lost for denitrification during the flooding period, even if the residues continued to be decomposed during this period. Flooded soil condition, in the absence of oxygen, bacteria utilize inorganic compounds oxidized of N instead of O_2 as a final electron acceptor (MOREIRA & SIQUEIRA, 2006). The nitrate is the N form which serves as the electron

receiver replacing oxygen (PONNAMPERUMA, 1972), favoring denitrification to N_2O or N_2 , escaping to the atmosphere being volatile in nature.

It is also possible to observe that treatment with rice residues, which had decomposed least in the first 180 days, followed releasing N in flooded conditions. However, in the anaerobic cycle, the N may have be kept in the ammonium form, without significant losses in the flooding period, which may explain the N amount increased in the SXE_{glei} soil than the same soil with soybean at the end of the 360 days of incubation (Table 2). The O_2 absence during flooded conditions stops the nitrification process, favoring the NH_4^+ accumulation in flooded soils that can be retained in the soil CEC, absorbed by plants, immobilized biomass, and/or suffer nitrification under aerobic regions (MOREIRA & SIQUEIRA, 2006).

When comparing the soils, the soil with higher amount of clay+silt provided better N preservation from released by the residues at 180 and 360 days of incubation (Table 2), which should be attributed to its clay reactivity characteristics such as the adsorption and retention of elements capacity (KLEBER et al., 2007). The SOM can be, physically and chemically stabilized by adsorption on the surface of clay minerals (TISDALL & OADES, 1982).

Table 2 - Total ^{15}N in the Planossolo Háplico Eutrófico gleissólico (SXE_{glei}) and Planossolo Háplico Eutrófico arênico (SXE_{aren}) coming from rice, sorghum, and soybean residues at 30, 180, and 360 days of incubation.

Soil	Rice	Sorghum	Soybean	Average
-----% of added ^{15}N -----				
-----30 days-----				
SXE_{aren}	7.8	10.4	13.0	10.4
SXE_{glei}	7.1	16.7	23.3	15.7
Average	7.5b	13.6ab	18.2a	
CV (%)	-----41.9-----			
-----180 days-----				
SXE_{aren}	16.8	15.8	24.4	19.0B
SXE_{glei}	23.4	22.2	37.6	27.7A
Average	20.1b	19.0b	31.0a	
CV (%)	-----20.5-----			
-----360 days-----				
SXE_{aren}	34.6aB	31.2aB	33.5aA	33.1
SXE_{glei}	56.1aA	49.6bA	31.6bA	45.8
Average	45.4	40.4	32.5	
CV (%)	-----19.7-----			

Lower-case letters in horizontal line show mean difference among crop residues while upper-case letters in vertical columns show mean difference between soils compared by Tukey test ($P < 0.05$ of error probability).

Several different types of functional groups of organic substances can react with soil minerals, forming organo-mineral complexes of various degrees of stability against biological and chemical occurrence (ALLISON, 1973; CANELLAS et al., 2008). In this regard, the physical protection may also result in the encapsulation of the SOM in soil aggregates or its coating particles of silt and clay (SKJEMSTAD et al., 1993). Thus, the physical protection consists in forming a barrier that prevents or hinders access of the SOM decomposing microorganisms,

In general, there was added ^{15}N to the soil in three size fractions during the incubation period (Table 3). At 30 days, the soybean residues had the highest contributions to ^{15}N in the CSF and SCF, with no difference between the crop residues on the FSF. The highest amount of ^{15}N reported in soybean CSF in the first 30 days of incubation may be associated with increased degradation of the more soluble carbon (and hence organic N). For REINERTSEN et al. (1984), CHRISTENSEN (1985) and AITA & GIACOMINI (2003), the high initial rate of decomposition of crop residues is due to the easy way as organic

compounds, especially carbohydrates water-soluble fraction, are used as an energy source of microbial population. According to CHIVENGE et al., (2011), the decomposition rate of crop residues and the formation of organic material fractions is mediated by microorganisms, succeeding the mineralization, being controlled by the quality of the crop residues (C/N ratio of the residue) and soil texture.

High quantities of N in CSF were obtained in the sandy soil and are in accordance with findings of LUCE et al., (2014), larger amounts of N in CSF were quantified in sandy soil than in the clay, where occurs most frequent microbial attack on organic material and hence, a larger amount of this fraction is displayed on sandy soil. This is because the CSF includes particulate matter partially decomposed vegetable residues along with microbial by-products that are great sources of N to soil microorganisms (GREGORICH et al., 2006).

The CSF showed increased values of added ^{15}N for both soils under incubation, with an interaction between soil and residue at 180 days of incubation (Table 3). According ROSCOE & MACHADO (2002), CSF is only a small part of the total mass of the soil, but can store a significant portion of the total C and N of soil. According to these authors, it is possible to enlighten the constant ^{15}N increase over the incubation period in which the ^{15}N contained in the plant material is quantified in this fraction of coarser materials of SOM.

The SCF is the portion that best quantified ^{15}N in both soils and higher relative proportion among the fractions (Table 3). According to LUCE et al. (2014), the addition of crop residues stimulates microbial activity which may result in an initial increase in the amounts of ^{15}N microbial biomass and mineral forms, which will be quantified in the SCF (BALESDENT et al., 1991). This behavior is evidenced in BLANCO-MOURE et al. (2016), who reported that stable organic fraction constituted the majority of soil organic carbon (80%) and were not significantly affected by soil management. In this paper, the authors emphasized the role of clay in chemical stabilization through complex between minerals and organic substances and the physical protection of the main C preservation mechanism in soils.

Between soils, the SXE_{glei} presented more ^{15}N addition for rice and sorghum residues after 180 days of incubation and rice residues at 360 days of incubation (Table 3). These data confirm LUCE et al., (2014), which postulates that the organic matter fractionation elucidated the fate of N derived

Table 3 - ^{15}N added from different crop residues to Planossolo Háplico Eutrófico gleissólico (SXE_{glei}) and Planossolo Háplico Eutrófico arenico (SXE_{aren}) in coarse sand (CSF), fine sand (FSF), and silt + clay (SCF) fractions at 30, 180, and 360 days after incubation.

Solo	-----Coarse sand (CSF)-----				-----Fine sand (FSF)-----				-----Silt + clay (SCF)-----			
	Rice	Sorghum	Soybean	Average	Rice	Sorghum	Soybean	Average	Rice	Sorghum	Soybean	Average
	-----% of added ^{15}N -----											
	-----30 days-----											
SXE_{aren}	1.1	2.8	4.6	2,8A	0.3	0.4	0.3	0.3	6.4	7.5	8.6	7.5
SXE_{glei}	0.8	1.5	3.3	1,9B	0.2	0.4	0.8	0.5	5.0	10.2	12.1	9.1
Average	1.0b	2.2b	3.9a	2.4	0.2	0.4	0.5		5.7b	8.9ab	10.4a	
CV (%)	-----36.3-----				-----41.9-----				-----27.2-----			
	-----180 days-----											
SXE_{aren}	1.6bA	4.1aA	4.9aA	3.5	1.6	1.5	3.2	2.1A	11.5aB	6.9bB	10.5aA	9.6
SXE_{glei}	0.9bB	2.9aA	0.2bB	1.3	1.3	1.5	2.3	1.7B	14.8aA	9.9bA	6.9bB	10.5
Average	1.3	3.5	2.5	2.4	1.5b	1.5b	2.7a	1.9	13.1	8.4	8.7	
CV (%)	-----33.9-----				-----19.7-----				-----20.5-----			
	-----360 days-----											
SXE_{aren}	8.7	7.5	7.7	8.0	11.5	5.8	4.1	7.1	23.7aB	18.4abA	12.4bA	18.2
SXE_{glei}	6.2	6.5	4.6	5.8	7.0	6.5	2.5	5.3	35.6aA	13.8bA	18.6bA	22.7
Average	7.5	7.0	6.1	6.9	9.2a	6.1ab	3.3b		29.7	16.1	15.5	
CV (%)	-----38.5-----				-----36.9-----				-----21.1-----			

Lower-case letters in horizontal line show mean difference among crop residues while upper-case letters in vertical columns show mean difference between soils compared by Tukey test ($P < 0.05$ of error probability). Without letters indicates that analysis of variance was not significant by F-test ($P < 0.05$ of error probability).

from plant residues decomposition and element association with mineral soil matrix. Considering the data obtained in this study and in order that the nitrogen fertilization is based on the amount of soil organic matter, care must be taken when adding crop residues with high chemical quality in lowland soil in rice rotation with species of upland, because the N mineralized can be lost to the environment after the new flooding cycle for irrigated rice.

CONCLUSION

In the first 180 days of incubation, there was higher amount of ^{15}N in soil derived from soybean crop residue decomposition. However, at the end of the 360 days of incubation, the highest amount of ^{15}N added was verified at treatments with rice crop residues application. The soil with highest silt plus clay content presented greater N retention capacity and the SOM fraction associated with smaller particle size was the main pool of the N derived from crop residues after 360 days of incubation.

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DECLARATION OF CONFLICTING OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

CSP, LSS, and SJG conceived and designed experiments. CSP, BC, LRN, and MFD performed the experiments and carried out the lab analyses. ACF performed statistical analyses of experimental data. CSP, LSS and SJG prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

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