

# Diversification of traditional paddy field impacts target species in weed seedbank<sup>1</sup>

## Diversificação de arrozal tradicional impacta espécies alvo no banco de sementes de plantas daninhas

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**ABSTRACT** - Weed competition is a challenge to rice monocropping growers in the context of herbicide resistant weeds and difficult weeds to manage using chemical control. Diversifying crop rotations through integrated crop-livestock systems (ICLS) can be an alternative to face this challenge. As weed seedbank reflects management practices, this study aimed to assess the impact of a traditional paddy field and four lowland ICLS on the weed seedbank, in a long-term ICLS experiment located in Cristal, RS, Brazil. Treatments consisted of five cropping systems: T1 – rice monocropping; T2 – rice-beef cattle integration; T3 – soybean-rice–beef cattle integration; T4 – Sudan grass-soybean-maize-rice-beef cattle integration; and T5 - rice-beef cattle integration in cultivated and natural grassland. The seedbank was assessed in the fourth experimental year, Oct-2016, at three soil depths (0-5; 5-10 and 10-20 cm). In a mid-term temporal scale, the diversification of paddy field through ICLS did not affect weed seedbank size. However, seeds in T1 were equally distributed along the soil profile, in contrast to the other cropping systems, where weed seeds accumulated in the 0-5 cm depth. Lowland ICLS designs that comprises the integration of summer crops with grazing winter cover crops decrease the proportion of Cyperaceae weed species in the topsoil seedbank. The depletion of weedy rice seedbank is more pronounced in lowland ICLS designs that integrates different summer crop in rotation with grazing cover crops.

**Key words:** Rice-based cropping system. Flooded rice. Crop-livestock integration.

**RESUMO** - A competição de plantas daninhas é um desafio para os produtores de arroz em monocultivo no contexto de espécies resistentes a herbicidas e plantas daninhas difíceis de manejar usando o controle químico. Diversificar a rotação de culturas por meio de sistemas integrados de produção agropecuária (SIPA) pode ser uma alternativa para enfrentar esse desafio. Como o banco de sementes de plantas daninhas reflete práticas de manejo, este estudo teve como objetivo avaliar o impacto de um arrozal tradicional e quatro SIPA em terras baixas sobre o banco de sementes de plantas daninhas, em um experimento de longa duração localizado em Cristal, RS, Brasil. Os tratamentos consistiram em cinco sistemas de cultivo: T1 - monocultivo de arroz; T2 - integração arroz-pecuária; T3 - integração soja-arroz-pecuária; T4 – integração soja-milho-arroz-pecuária; e T5 - integração arroz-pecuária, em pastagens cultivadas e campo nativo. O banco de sementes foi avaliado no quarto ano experimental, outubro de 2016, em três profundidades do solo (0-5; 5-10 e 10-20 cm). No médio prazo, a diversificação do arrozal por meio de SIPA não afetou o tamanho do banco de sementes. No entanto, em T1 as sementes estavam distribuídas ao longo do perfil do solo, em contraste com os demais sistemas de cultivo, onde as sementes de plantas daninhas se acumularam na profundidade de 0-5 cm. Arranjos de SIPA que compreendem a integração de culturas de verão e pastagens hibernais diminuem a proporção de plantas daninhas ciperáceas na camada superficial do banco de sementes do solo. A diminuição do banco de sementes de arroz daninho é mais pronunciada nos arranjos de SIPA em terras baixas que integram diferentes culturas de verão em rotação com pastagens hibernais.

**Palavras-chave:** Sistema de cultivo de arroz. Arroz irrigado por inundação. Integração lavoura-pecuária.

DOI: 10.5935/1806-6690.20220030

Editor-in-Chief: Alek Sandro Dutra - alekdutra@ufc.br

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Received for publication 23/06/2020; approved on 04/03/2022

<sup>1</sup>Part of the first author's thesis, presented to Postgraduate Program in Agronomy, UFPR, Curitiba, Paraná. This study was funded by the Coordination for the Improvement of Higher Education Personnel (CAPES)

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## INTRODUCTION

Brazil is the largest producer of rice regarding the Mercosur (Southern Common Market), accounting for 78% of the economic bloc production (average from 2009/10 to 2017/18) (SOCIEDADE SUL-BRASILEIRA DE ARROZ IRRIGADO, 2018). Most of the Brazilian production of rice is located in Southern States lowlands (65~70%), corresponding to more than one million hectares. These areas are characterized by flooded rice fields, which present high yields, around 7 Mg ha<sup>-1</sup> (COMPANHIA NACIONAL DE ABASTECIMENTO, 2019).

Weed competition is one of the biggest challenges to maintain high yields in rice cropping systems (BRIM-DEFOREST *et al.*, 2017), considering that several weed species from Brazilian paddy fields have been reported as herbicide-resistant: *Echinochloa* spp., *Sagittaria montevidensis* Cham. & Schltld., *Fimbristylis miliacea* (L.) Vahl., *Cyperus difformis* L. and *Cyperus iria* L. (SOCIEDADE SUL-BRASILEIRA DE ARROZ IRRIGADO, 2018). Additionally, rice growers face the difficulty to manage a feral species (*Oryza sativa* L.), called weedy or wild rice, which occurs frequently and is widely distributed around regions of rice cultivation (ULGUIM *et al.*, 2018).

In this context and considering that most traditional paddy fields in Southern Brazil are rice monocropping (rice in summer and fallow in winter), crop rotation could be an effective weed management alternative. Selection pressure is diversified by changing patterns of disturbance, forcing well-established weeds, associated to practices from a single crop, to face different competitive conditions (SCHUSTER *et al.*, 2020).

Integrated crop-livestock systems (ICLS) could be an alternative to enhance weed control through cropping diversification in rice monocropping systems and at the same time optimize area utilization (MORAES *et al.*, 2014). Among other aspects, ICLS have been reported to reduce weed infestation (weed seedbank and emerged flora) in highlands by Lustosa *et al.* (2016) and Schuster *et al.* (2016, 2018). However, the effect of ICLS on weed communities in lowlands has not been fully understood.

Furthermore, ICLS comprises different designs, integrating crop and livestock in several possible temporal and spatial scales (CARVALHO *et al.*, 2014), that may result in different effects on weeds (NICHOLS *et al.*, 2015).

As weed seedbanks reflect past weed populations and management practices and are the source of weed infestations to come (SCHUSTER *et al.*, 2016), it was hypothesized that different levels of diversification of traditional paddy fields through ICLS would promote changes in weed community of rice crop. Therefore, this

study aimed to assess the impact of a traditional paddy field and four lowland ICLS on the weed seedbank.

## MATERIAL AND METHODS

The study was carried out in the fourth year of a long-term ICLS experiment located on an 18-ha field at the Corticeiras Farm, in Cristal County, Rio Grande do Sul State, Brazil (31° 37' 13" S; 52° 35' 20" W; 28 m asl). The climate is a warm humid summer climate (Cfa), according to the Köppen classification, with a yearly average temperature and precipitation of 18.3 °C and 1,522 mm, respectively. The site is characterized by a flat relief similar to the most part of paddy fields in the Brazilian subtropics. The soil is a poorly drained Albaqualf (SOIL SURVEY STAFF, 2010) with a sandy clay loam texture (24%; 23% and 53% clay, silt and sand, respectively).

The experimental area has been cultivated since the 1960s, alternating rice cropping with fallow periods. After the last rice cropping, in 2009, the area remained fallow until the trial establishment. The pre-experimental chemical characterization of the soil is available in Martins *et al.* (2017). Due to high acidity levels, the soil in the entire area was tilled with three heavy discs to incorporate lime applied at a rate of 4.5 Mg ha<sup>-1</sup>, immediately before the experiment establishment, in 2013 Autumn.

Treatments consisted of five lowland rice-based cropping systems, also called paddy-farming systems, with different combinations of soil tillage (conventional tillage and no-till), vegetation diversity (both in time and in space) and grazing season (summer and/or winter), distributed in a randomized block design with three replicates. Among systems, the utilized summer crops were: rice (*Oryza sativa* L.), soybean [*Glycine max* (L.) Merrill], maize (*Zea mays* L.), grazing Sudan grass [*Sorghum sudanense* (Piper) Stapf] and natural grassland - native pasture species established by natural seedling (commonly termed as 'succession field'). Winter grazing cover crops were annual ryegrass (*Lolium multiflorum* Lam.), sole or mixed to birdsfoot trefoil (*Lotus corniculatus* L. cv. São Gabriel) and white clover (*Trifolium repens* L.). The T1 treatment - rice monocropping - represents the dominant system used in Southern Brazil, under conventional tillage. The other treatments comprise different ICLS designs, under no-till: T2 - rice-beef cattle integration; T3 - soybean-rice-beef cattle integration; T4 - Sudan grass-soybean-maize-rice-beef cattle integration; T5 - rice-beef cattle integration in cultivated and natural grassland. All cropping sequences are summarized in Table 1. Plots ranged from 0.8 to 0.9 ha for T1, and from 1 to 1.5 ha for the other treatments. The experimental units in ICLS were larger to accommodate livestock grazing.

**Table 1** - Temporal rotation plan (crop succession) for the five studied lowland rice-based cropping systems (CS – T1, T2, T3, T4 and T5) from the experiment establishment (2013) to 2016/2017 summer

CS	Crop succession							
	winter	summer	winter	summer	winter	summer	winter	summer
	2013	2013/2014	2014	2014/2015	2015	2015/2016	2016	2016/2017
T1	fallow period <sup>1</sup>	Irrigated rice	fallow period	Irrigated rice	fallow period	Irrigated rice	fallow period	Irrigated rice
T2	Annual ryegrass <sup>2</sup>	Irrigated rice						
T3	Annual ryegrass <sup>2</sup>	Soybean	Annual ryegrass <sup>2</sup>	Irrigated rice	Annual ryegrass <sup>2</sup>	Soybean	Annual ryegrass <sup>2</sup>	Irrigated rice
T4	Annual ryegrass + white clover <sup>2</sup>	Sudan grass	Annual ryegrass + white clover <sup>2</sup>	Soybean	Annual ryegrass + white clover <sup>2</sup>	Maize	Annual ryegrass + white clover <sup>2</sup>	Irrigated rice
T5	Annual ryegrass + white clover + birdsfoot trefoil <sup>2</sup>	natural grassland <sup>3</sup>	Annual ryegrass + white clover + birdsfoot trefoil <sup>2</sup>	natural grassland <sup>2</sup>	Annual ryegrass + white clover + birdsfoot trefoil <sup>2</sup>	natural grassland <sup>2</sup>	Annual ryegrass + white clover + birdsfoot trefoil <sup>2</sup>	natural grassland <sup>2</sup>

<sup>1</sup> Spontaneous vegetation. <sup>2</sup> Grazed by bovine steers. <sup>3</sup> In the first summer season of this treatment, grazing was not performed because natural grassland (succession field) was not well-established yet. CS – Cropping systems. Adapted from Martins *et al.* (2017)

For the winter cover crops, sowing was performed in April at 30; 3 and 6 kg ha<sup>-1</sup> seed rates for annual ryegrass, white clover and birdsfoot trefoil, respectively. At the end of winter season, all plots were desiccated with glyphosate, except for T5. During summer, rice and Sudan grass were sown around October-November in rows spaced 17 cm apart at a density of 80 and 30 kg kg ha<sup>-1</sup>, respectively. Maize was sown in October and soybean in November, in rows spaced 70 and 45 cm apart, respectively. Seeding rate was variable according to maize and soybean cultivars. Legume seed inoculation was performed as recommended and agronomic management was conducted according to the technical recommendations for each crop (i.e. the use of herbicides, insecticides, and fungicides). Fertilizations rates are presented in Table 2. Seeding and harvest dates are summarized in Table 3.

For grazing, neutered male steers (Angus) approximately 10 months old and weighing about 200 kg were used to simulate cattle fattening or finishing system. During the grazing cycle, the cattle feeding was forage based, and mineral salt was furnished. A continuous grazing system was adopted, according to the put-and-take method, aiming to maintain sward heights at an average of 15 cm for winter grazing cover crops and 50 cm for Sudan grass. Grazing period lasted 3-4 months over the experimental years, beginning in June-July ending in November, varying according to cropping sequence (Table 3).

Seedbanks were sampled prior to the return of rice crop in all treatments, in October 2016, which marked the experiment fourth year. Soil samples were collected manually at three soil depths (0-5; 5-10 and 10-20 cm), along three 25-m transects in each experimental unit using a steel 3.7-cm diameter probe. Transects were randomly laid out in the central area of each plot. Along the transect, two

soil cores were collected at 5-m intervals and combined into one 30-core composite sample for each experimental unit.

Seed tray maintenance was conducted according to Schuster *et al.* (2016). All soil samples were processed to remove stones and root fragments, then spread in 44 x 38 cm plastic trays and placed in a greenhouse for 12 months beginning in November 2016. Soil moisture was maintained in the trays using regular sub-irrigation. The seedling emergence method was used to quantify the germinable seeds (not accounting for dead or dormant seeds) in the soil seedbank (MA *et al.*, 2014). The lowest temperature during the 12-month germination period was 0 °C, and the maximum temperature was 38 °C.

Emerged seedlings were periodically identified, counted, and removed from the plastic trays. Seedling identification was conducted based on Lorenzi (2014) Descriptions. At this early growth stage, some seedlings, especially from the Cyperaceae family, could not be identified to species, so they were all classified according to genus: *Cyperus*, *Fimbristylis*, *Eleocharis*, *Polygonum* and *Sagittaria*. When species could not be identified, seedlings were transplanted to plant vase to grow until identification was possible. Total counts for all species were summed to calculate weed seedbank size (number seed m<sup>-2</sup>).

To analyze the seedbank composition, species richness index (*S*) was calculated by counting the number of different species per experimental unit. Shannon's diversity index (*H*) and evenness of the seedbank (*J*) were estimated as described in Schuster *et al.* (2019):

$$H = -\sum_{i=1}^S \left( \frac{n_i}{N} \right) \times \left( \log \frac{n_i}{N} \right) \quad (1)$$

$$j = \frac{H}{\log(S)} \quad (2)$$

Where  $N$  is the total number of individuals per experimental unit,  $ni$  refers to the number of individuals per species per experimental unit, and  $S$  describes the total number of species.

**Table 2** - Fertilization rates of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O (kg ha<sup>-1</sup>) in winter and summer for the five-lowland rice-based cropping systems (CS – T1, T2, T3, T4 and T5), over the experimental years (from 2013 to 2016)

CS	N - P <sub>2</sub> O <sub>5</sub> - K <sub>2</sub> O (kg ha <sup>-1</sup> )						
	winter	summer	winter	summer	winter	summer	winter
	2013	2013/2014	2014	2014/2015	2015	2015/2016	2016
T1	0-0-0	150-70-120	0-0-0	160-70-115	0-0-0	150-70-120	0-0-0
T2	110-110-110	150-70-120	130-130-130	160-70-115	130-130-130	150-70-120	130-130-130
T3	110-110-110	20-110-120	130-130-130	160-70-115	130-130-130	0-105-80	130-130-130
T4	110-130-130	130-80-120	130-130-130	30-140-150	130-130-130	130-70-120	130-130-130
T5	110-130-130	130-80-120	130-130-130	130-90-90	130-130-130	130-70-40	130-130-130

Adapted from Denardin *et al.* (2018)

**Table 3** - Seeding date, beginning and end of grazing period, and harvest date of the five studied lowland rice-based cropping systems (T1, T2, T3, T4 and T5), over the experimental years (from 2013 to 2016)

Season	Activity	Cropping systems					
		T1	T2	T3	T4	T5	
Winter 2013	Seeding date	-	9-Apr	9-Apr	9-Apr	9-Apr	
	grazing period	beginning	-	2-Jul	2-Jul	2-Jul	
		end	-	2-Oct	5-Nov	5-Nov	15-Dec
Summer 2013/2014	Seeding date	18-Oct	18-Oct	26-Nov	27-Nov	-	
	grazing period	beginning	-	-	-	-	
		end	-	-	-	-	-
	harvest date	28-Mar	28-Mar	28-Mar	-	-	
Winter 2014	Seeding date	-	10-Jun	20-Apr	20-Apr	20-Apr	
	grazing period	beginning	-	23-Aug	12-Jun	24-Jun	24-Jun
		end	-	3-Oct	3-Oct	11-Nov	11-Nov
Summer 2014/2015	Seeding date	15-Nov	15-Nov	15-Nov	24-Nov	-	
	grazing period	beginning	-	-	-	-	
		end	-	-	-	-	-
	harvest date	4-Apr	4-Apr	4-Apr	21-Apr	-	
Winter 2015	Seeding date	-	2-Apr	2-Apr	2-Apr	2-Apr	
	grazing period	beginning	-	14-Aug	24-Jun	17-Jun	24-Jun
		end	-	6-Oct	12-Sep	24-Oct	25-Nov
Summer 2015/2016	Seeding date	23-Nov	23-Nov	24-Nov	30-Nov	-	
	grazing period	beginning	-	-	-	-	26-Nov
		end	-	-	-	-	22-Mar
	harvest date	4-Apr	4-Apr	20-Apr	10-May	-	
Winter 2016	Seeding date	-	19-Apr	22-Apr	-	23-Mar	
	grazing period	beginning	-	4-Jul	4-Jul	-	16-Jun
		end	-	29-Sep	29-Sep	29-Sep	29-Sep

Adapted from Denardin *et al.* (2018)

For each species, a global relative abundance considering all seedbank samples was calculated:

$$\text{global relative abundance} = \frac{\text{number of accounted seeds of the species}}{\text{total number of seeds found in all seedbank samples}} \times 100$$

To assess the contribution of each identified species in the seedbank of the different treatments and depths, the relative abundance of each experimental unit was calculated:

$$\text{relative abundance} = \frac{\text{number of accounted seeds of the species}}{\text{total number of seeds found in the experimental unit}} \times 100$$

Data analyses were performed in R software, version 3.4.0 (R CORE TEAM, 2017). Homogeneity of variance and the normal distribution of residuals (normality assumption) were verified. In highly skewed distributions, the dependent variable was transformed according to the boxcox test (square root or logarithm transformation) to meet the assumptions of inferential statistics. Each evaluated attribute was submitted to analysis of variance by the F test with fitted linear models (“lm” function). When significant ( $p < 0.05$ ), means were compared by Tukey test at 5% probability.

## RESULTS AND DISCUSSION

Seedbank size did not differ among treatments. On average,  $50 \pm 10$  thousands of seeds were found in the 0-20 cm depth of the soil, considering all five cropping systems (Figure 1a). In a classical Brazilian research relating weed seedbank and different

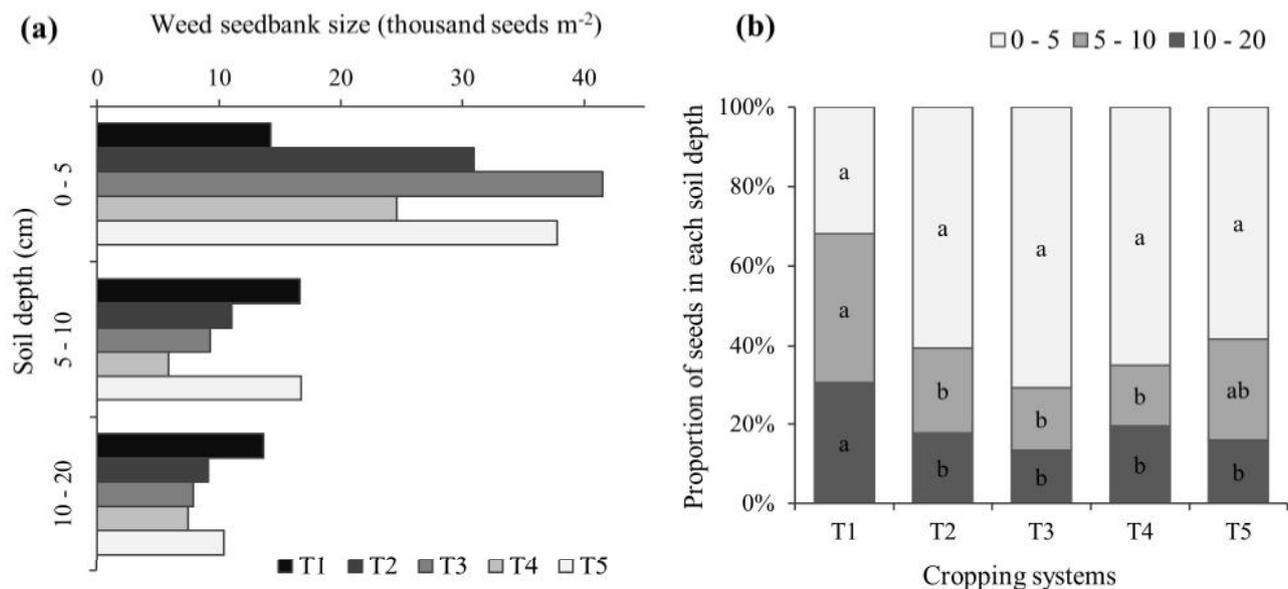
agroecosystems, Carmona (1995) identified that lowlands presented higher amounts of seeds, due to high water availability and constant soil perturbation that favor weeds infestations. However, the distribution of seeds along the soil profile were different ( $p < 0.01$ ) and varied according to systems (significant interaction treatments x soil depth) (Figure 1b).

In the traditional rice-based cropping system, seeds were equally distributed at the different soil depths. Proportionally, 32%; 37% and 31% of seeds were at 0-5; 5-10 and 10-20 cm, respectively. The mechanical manipulation of the soil provided the inversion of soil layers, i.e. tillage redistributes seeds throughout the soil profile (NICHOLS *et al.*, 2015; SINGH; BHULLAR; CHAUHAN, 2015).

In contrast, soils from the other cropping systems presented most seeds concentrated in the 0-5 cm, which represented, on average,  $64 \pm 5.5\%$  of the total amount of seeds found in the soil profile (0-20 cm). In no-till systems, such as T2, T3, T4, T5, there is minimal soil disturbance and seeds infiltrate the soil via slow processes, which results in the accumulation of seeds near the soil surface (CHAUHAN; SINGH; MAHAJAN, 2012; NICHOLS *et al.*, 2015).

In terms of composition, a total of 61 species were identified, although only 19 presented more than 1% of global relative abundance (Table 4). Eleven of these species are monocotyledonous weeds (monocots), of which five belong to the Cyperaceae and four to the Poaceae families.

**Figure 1** - (a) Weed seedbank size (thousand seeds  $m^{-2}$ ) according to treatments (T1, T2, T3, T4 and T5) and soil depths (0-5; 5-10, 10-20 cm); and (b) relative distribution (percentage) of seeds in each soil depth for each treatment



(a,b) Columns represent means. (b) Lower-case letters compare soil depths in each treatment. Same letters do not differ significantly by the Tukey test ( $p > 0.05$ )

In all treatments, monocots were predominant among the five most numerous species composing the weed seedbank (Table 5 and 6). The proportion of

monocots seeds in the seedbank did not vary among cropping systems, which was  $81\% \pm 16\%$  on average (Figure 2).

**Table 4** - Most abundant weed species identified in the weed seedbank, and their respective families, groups of flowering plants, with the indication of number of plots containing the species (N plots) and global relative abundance (calculated considering all seedbank samples)

Species	Family	Group of flowering plants	N samples	Global relative abundance (%)
<i>Sagittaria</i> spp.	Alismataceae	monocot	41	14.62
<i>Fimbristylis</i> spp.	Cyperaceae	monocot	44	12.86
<i>Eleocharis</i> spp.	Cyperaceae	monocot	43	9.51
<i>Ludwigia octovalvis</i> (Jacq.) P.H.Raven	Onagraceae	dicot	26	8.35
<i>Cyperus</i> spp.	Cyperaceae	monocot	43	7.85
<i>Urochloa plantaginea</i> (Link) R.D.Webster	Poaceae	monocot	28	7.30
<i>Poa annua</i> L.	Poaceae	monocot	28	6.47
<i>Ludwigia leptocarpa</i> (Nutt.) H. Hara	Onagraceae	dicot	19	3.94
<i>Echinochloa crus-galli</i> (L.) P.Beauv.	Poaceae	monocot	37	3.18
<i>Sisyrinchium fasciculatum</i> Klatt	Iridaceae	monocot	42	3.09
<i>Eclipta prostrata</i> (L.) L.	Asteraceae	dicot	35	2.16
<i>Stellaria media</i> (L.) Vill.	Caryophyllaceae	dicot	30	2.05
<i>Lepidium didymum</i> L.	Brassicaceae	dicot	40	1.96
<i>Eleusine indica</i> (L.) Gaertn	Poaceae	monocot	31	1.56
<i>Bulbostylis capillaris</i> (L.) C.B. Clarke.	Cyperaceae	monocot	17	1.27
<i>Silene gallica</i> L.	Caryophyllaceae	dicot	9	1.19
<i>Polygonum</i> sp.	Polygonaceae	dicot	16	1.04
<i>Veronica peregrina</i> L.	Plantaginaceae	dicot	23	1.02

Note: Weed species presenting more than 1% of global relative abundance were considered the most abundant ones

**Table 5** - Relative abundance of the five most numerous weed species in the soil seedbank of rice monocropping system (T1), according to soil depths (0-5; 5-10 and 10-20 cm), with indication of family and group of flowering plants

Species	Family	Group of flowering plants	Relative abundance (%)		
			0-5	5-10	10-20
<i>Sagittaria</i> spp.	Alismataceae	monocot	22.9	14.2	13.8
<i>Facelis retusa</i>	Asteraceae	dicot	7.6	-	-
<i>Fimbristylis</i> spp.	Cyperaceae	monocot	8.6	12.6	11.4
<i>Cyperus</i> spp.	Cyperaceae	monocot	15.7	12.1	15.9
<i>Eleocharis</i> spp.	Cyperaceae	monocot	-	-	17.7
<i>Sisyrinchium fasciculatum</i>	Iridaceae	monocot	-	-	5.9
<i>Setaria parviflora</i>	Poaceae	monocot	-	9.5	-
<i>Heteranthera reniformis</i>	Pontederiaceae	monocot	8.4	6.5	-

**Table 6** - Relative abundance of the five most numerous weed species in the soil seedbank of the integrated crop-livestock systems (T2, T3, T4 and T5) in the 0-5 cm depth, with indication of family and group of flowering plants

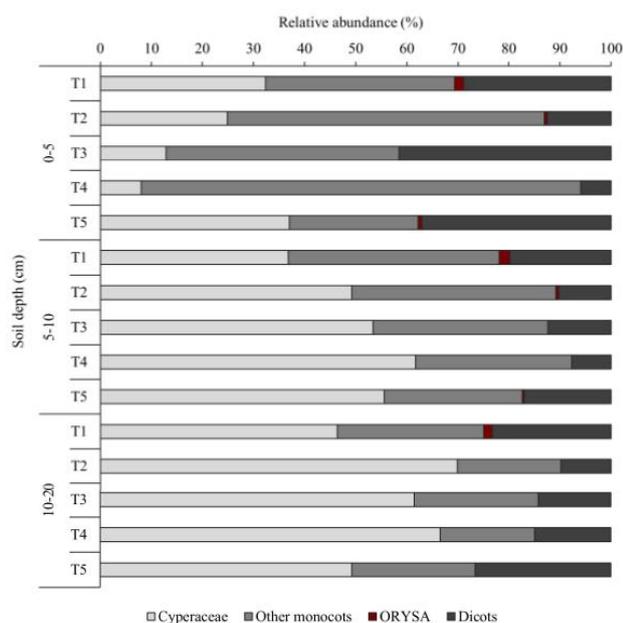
Species	Family	Group of flowering plants	Relative abundance (%)			
			T2	T3	T4	T5
<i>Fimbristylis</i> spp.	Cyperaceae	monocot	8.5	8.8	4.2	13.1

Continuation Table 6

<i>Sagittaria</i> spp.	Alismataceae	monocot	57.0	9.9	3.7	-
<i>Urochloa plantaginea</i>	Poaceae	monocot	-	-	64.9	-
<i>Cyperus</i> spp.	Cyperaceae	monocot	6.8	-	-	14.2
<i>Echinochloa crus-galli</i>	Poaceae	monocot	1.3	-	-	8.4
<i>Eleusine indica</i>	Poaceae	monocot	-	-	10.5	-
<i>Eleocharis</i> spp.	Cyperaceae	monocot	5.3	-	-	8.4
<i>Ludwigia leptocarpa</i>	Onagraceae	dicot	-	-	-	8.6
<i>Ludwigia octovalvis</i>	Onagraceae	dicot	-	21.6	-	-
<i>Poa annua</i>	Poaceae	monocot	-	34.3	-	-
<i>Silene gallica</i>	Caryophyllaceae	dicot	-	7.0	-	-
<i>Sisyrinchium fasciculatum</i>	Iridaceae	monocot	-	-	2.9	-

Note: Only the most abundant species of the 0-5 cm depth was presented for T2, T3, T4 and T5, considering that seeds in the soil of the ICLS treatments are concentrated in the topsoil layer

**Figure 2** - Relative abundance of Cyperaceae weeds, other monocotyledonous weed species and dicotyledonous weeds in the soil seedbank according to treatments (T1, T2, T3, T4 and T5) and soil depths (0-5; 5-10 and 10-20 cm), with indication of presence of weedy rice (*Oryza sativa* L. – ORYSA)



Considering the species more frequently observed, i.e. present in more than 80% of all samples (N samples > 36), eight out of nine species were monocots. The Cyperaceae species *Fimbristylis* spp., *Eleocharis* spp. and *Cyperus* spp. were found in more than 95% of samples and presented high global relative abundance, 12.9%; 9.5% and 7.8%, respectively. This result agrees with Mesquita, Andrade and Pereira (2013) findings that observed a dominance of Cyperaceae species in soil seedbank of rice crop.

Richness considerably varied regarding treatments (coefficient of variation of 29.4%) and was not significantly different. However, soil depth affected weed species richness, with a higher number of species in the topsoil layer (0-5 cm) (Table 7). In spite of richness, diversity (H) and evenness (J) tend to decrease near the soil surface ( $p < 0.05$ ), considering that few species are dominant in the 0-5 cm layer (Table 5 and 6). T5 tended to be more even ( $p = 0.5075$ ), as it presents higher diversification (considering the number of species in the natural grassland) and low soil disturbance.

The proportion of weeds from the Cyperaceae family varied along the soil profile ( $p < 0.05$ ), and the effect of depths was different according to treatments (significant interaction treatment x depth). Seeds of Cyperaceae weeds were more equally distributed in all 0-20 cm soil layer in T1 and T5 cropping systems. In T2, T3 and T4 systems there were lower amounts of seeds from Cyperaceae weeds at the 0-5 cm depth, with a more pronounced effect in T3 and T4, where on average, Cyperaceae composed only  $13 \pm 3\%$  and  $8 \pm 1\%$  of the topsoil seedbank.

Species from the Cyperaceae family are part of the natural grassland in Subtropical Brazil (SOUZA *et al.*, 2019), being predominant plants in vegetation of many wetlands (MISHRA *et al.*, 2016). Additionally, in T5 cropping system there has been no chemical control since the experiment establishment. Thus, that explains the greater proportion of Cyperaceae seeds in T5 topsoil layer, in contrast to other ICLS systems.

On the other hand, in T1 the distribution of seeds along the soil profile and considerable relative abundance of Cyperaceae weeds at the 0-5 cm soil depth ( $32 \pm 8\%$ ) are due to soil disturbance and monocropping. Cyperaceae species are among the most important weeds in conventional rice cropping systems

**Table 7** - Richness (S), Shannon's diversity index (H) and evenness index (J) of the weed seedbank from the five-lowland rice-based cropping systems (CS – T1, T2, T3, T4 and T5) at 3 soil depths 0-5; 5-10 and 10-20 cm

CS	S			H			J		
	0-5	5-10	10-20	0-5	5-10	10-20	0-5	5-10	10-20
T1	24 ± 4 a	25 ± 3 b	24 ± 4 ab	2.02 b	2.25 ab	2.33 a	0.64 b	0.70 ab	0.74 a
T2	25 ± 1 a	19 ± 2 b	19 ± 1 ab	1.57 b	1.97 ab	1.87 a	0.49 b	0.68 ab	0.64 a
T3	22 ± 2 a	19 ± 4 b	19 ± 2 ab	1.56 b	2.08 ab	2.19 a	0.50 b	0.71 ab	0.74 a
T4	22 ± 2 a	14 ± 4 b	21 ± 2 ab	1.38 b	1.76 ab	2.24 a	0.45 b	0.68 ab	0.74 a
T5	25 ± 2 a	18 ± 3 b	18 ± 1 ab	2.35 b	1.97 ab	2.10 a	0.73 b	0.69 ab	0.72 a

Means in the same line followed by different letters differ by the Tukey ( $p < 0.05$ )

(SOCIEDADE SUL-BRASILEIRA DE ARROZ IRRIGADO, 2018; ULGUIM *et al.*, 2018), adapted to the paddy field. So, in the absence of different competitive environment (cropping diversification) and herbicides mode of action, they continue to thrive.

The persistence of weed species in the soil seedbank, for instance seeds of Cyperaceae weeds, is influenced by tillage since it affects vertical seed distribution. The incorporation of seeds into deeper soil layers favors dormancy of several weed species, considering that light and alternating temperature regimes are the most important environmental factors triggering seed germination (HUMPHRIES; CHAUHAN; FLORENTINE, 2018). In this context, inversion of soil layers constantly feeds the soil seedbank at every tillage operation. In agreement to that, Singh, Bhullar and Chauhan (2015) comparing tillage systems in dry direct-seeded rice concluded that in conventional tillage a greater proportion of weeds seeds are expected to carry over to the next season.

This discussion leads to the results of occurrence of weedy rice (*Oryza sativa f. spontanea*) in the soil seedbank. This species is among the worst weeds in most rice growing areas, especially in traditional paddy fields in Southern Brazil (SOCIEDADE SUL-BRASILEIRA DE ARROZ IRRIGADO, 2018). Seeds of weedy rice were found in T1, T2 and T5 cropping systems. In the rice monocropping seeds were well distributed along the soil profile (as a result of tillage), accounting an average of 672 seeds  $m^{-2}$  in the 0-20 cm soil layer. Zhang *et al.* (2019) observed that burial depth positively affects weedy rice seed survival rate, favoring the persistence of this weed in the soil seedbank of traditional paddy fields.

The negative effect of a weedy rice seedbank was observed by Ulguim *et al.* (2018) in a phytosociological survey performed at the same experimental site, in 2016/2017 summer (right after seedbank sampling). The rice monocropping system (T1) presented the highest density of weedy rice comparing all systems. In another

study comparing different cultivation systems (tillage), also located in Rio Grande do Sul lowland, Ulguim *et al.* (2018) observed a higher density of weedy rice in conventional tillage and weedy rice stood out as the most important species, according to the importance value index.

Thus, the challenge of managing seedbanks under conventional tillage relies on the fact that deeply buried seeds can become the potential source of new infestation; and that, over the years, constant soil disturbance contributes to the persistence of some species in the soil seedbank, as for weedy rice.

In T2 and T5 seeds of weedy rice were mainly located in the 0-5 cm depth, where they are more likely to germinate but also more susceptible to desiccation, weather variation and predation (CHAUHAN; SINGH; MAHAJAN, 2012; NICHOLS *et al.*, 2015) that favors a greater depletion of viable seeds of weedy rice in shallow soil than in deep soil (ZHANG *et al.*, 2019).

Besides the effect of tillage, the depletion of weedy rice seedbank in T3 and T4 systems was due to cropping diversification, consequently, use of different herbicides modes of action. Chemical control acts as a strong management filter on weed community (RYAN *et al.*, 2010), and thus, the cropping systems with a more diverse cash crop design (considering that T5 is more diverse in number of species) resulted in better control of weedy rice (ULGUIM *et al.*, 2018). Finally, another important aspect common to all ICLS (T1, T2, T3 and T4) is the cultivation in winter season (there is fallow in T1) and the presence of residue (straw) previous to summer crop sowing. The latter is considered to be a main factor regulating weed community in highlands ICLS (SCHUSTER *et al.*, 2019).

Both T3 and T4 had a predominance of a grassy weed in the topsoil layer of the seedbank. In T3, annual bluegrass (*Poa annua* L.) accounted for 35% of seeds at the 0-5 cm soil depth (Table 5). As annual bluegrass is a winter grassy weed, there is a lower number of seeds from summer weed species in T3 soil.

In T4, marmeladegrass [*Urochloa plantaginea* (Link) Hitch.] represented 65% of all seeds in the topsoil layer. That was due to a bad weed management control in 2015/2016 maize crop, that resulted in a high incidence of marmeladegrass, estimated in  $6.9 \pm 1 \text{ Mg ha}^{-1}$ . As highlighted by Schweizer and Zimdahl (1984), in any cropping system, if weeds are neglected even for just one cropping season, soil seedbank can rebound rapidly. This expresses the importance of an adequate weed management, considering that some weed species can persist in the soil seedbank for years.

## CONCLUSIONS

1. In a mid-term temporal scale, the diversification of paddy field through ICLS do not affect weed seedbank size, considering the 0-20 cm soil layer;
2. Lowland ICLS designs that comprises the integration of summer crops with grazing winter cover crops decrease the proportion of Cyperaceae weed species in the topsoil seedbank;
3. The depletion of weedy rice seedbank is more pronounced in lowland ICLS designs that integrates different summer crop in rotation with grazing cover crops.

## ACKNOWLEDGEMENTS

The authors would like to thank the Corticeira Farm owners and all its staff, as well as all the research team of Federal University of Rio Grande do Sul (UFRGS) and their partners for conducting the lowland ICLS experiment. We also thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for scholarship support.

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