UNIVERSIDADE FEDERAL DO PARANÁ

RUBIA LUCIANE DOMINSCHEK LIMA DE LAI

WEED DYNAMICS IN INTEGRATED CROP-LIVESTOCK SYSTEMS IN SUBTROPICAL BRAZIL

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Tese apresentada ao Programa de Pós-Graduação em Agronomia, Área de concentração em Produção Vegetal, Setor de Ciências Agrárias, como parte das exigências para a obtenção do título de Doutora em Ciências.

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"You must be the change you wish to see in the world."

Mahatma Gandhi

RESUMO

Foi demonstrado que os Sistemas Integrados de Produção Agropecuária (SIPA), baseados na agricultura de conservação, alteram as comunidades de plantas daninhas e melhoram a produção agropecuária em geral. No entanto, o efeito dos SIPA na infestação de plantas daninhas pode variar de acordo com o arranjo do SIPA e práticas manejo, especialmente na fase pastagem. Além disso, ainda existe uma lacuna no conhecimento a respeito das plantas daninhas em SIPA. A presente tese teve como objetivo examinar a dinâmica de plantas daninhas em SIPA no Brasil Subtropical, em diferentes escalas espaço-temporais de integração de culturas e pecuária, e fertilização de pastagens. O capítulo 1 investigou o impacto de um SIPA, que inclui uma pastagem tropical perene por um período de três anos antes do cultivo de milho, na infestação de plantas daninhas e na produção de milho. Dois sistemas foram comparados, um SIPA e um sistema de rotação baseado em culturas agrícolas, em um experimento de SIPA de quatro anos, onde não há controle químico de plantas daninhas, em Pinhais, PR. Foram avaliadas as condições pré-experimentais da comunidade de plantas daninhas, o banco de sementes, e a ocorrência e interferência de plantas daninhas na quarta safra de verão do experimento. A estratégia de incluir uma pastagem tropical por três anos antes da safra de verão proporcionou redução na infestação e interferência de plantas daninhas no milho. Este arranjo de SIPA altera a composição do banco de sementes no curto prazo, selecionando as plantas daninhas folha larga sobre as gramíneas. O capítulo 2 teve como objetivo avaliar o impacto da adubação nitrogenada na cultura de cobertura hibernal sob pastejo no banco de sementes de plantas daninhas, e o efeito da palhada na flora emergente na safra de verão (feijoeiro), em um experimento de 10 anos do SIPA, em Guarapuava, PR. Para isso, foram testadas quatro doses de adubação nitrogenada (0, 75, 150 e 225 kg N ha^{-1}) e presença / ausência de resíduo no feijoeiro comum - safra 2016/17. Foram avaliados o banco de sementes e a flora emergente ao longo do ciclo do feijoeiro. O tamanho do banco de sementes de plantas daninhas diminuiu fortemente na maior dose de fertilização nitrogenada da cultura de cobertura hibernal sob pastejo. A densidade de plantas daninhas foi afetada pela adubação nitrogenada no inverno e presença de resíduo no início do ciclo da cultura do feijoeiro. O resíduo da cultura de cobertura hibernal sob pastejo é um fator importante para o controle de plantas daninhas em SIPA. O capítulo 3 teve como objetivo avaliar o impacto de um cultivo de arroz tradicional e 4 arranjos de SIPA em terras baixas sobre o banco de sementes de plantas daninhas, em protocolo experimental de 4 anos, localizado em Cristal, RS. O banco de sementes foi avaliado no quarto ano experimental, em três profundidades de solo. Em uma escala temporal de médio prazo, a diversificação em terras baixas por meio de SIPA não afeta o tamanho do banco de sementes de plantas daninhas. Arranjos de SIPA em terras baixas que compreendem a integração de culturas de verão com pastagens hibernais diminuem a proporção de espécies ciperáceas na camada mais superficial do banco de sementes do solo. A diminuição do banco de sementes de arroz vermelho é mais pronunciada nos arranjos de SIPA em terras baixas que contemplam diferentes culturas de verão em rotação integradas a pastagens hibernais.

Palavras-chave: integração lavoura-pecuária. controle cultural de plantas daninhas. banco de sementes de plantas daninhas.

ABSTRACT

Integrated crop-livestock systems (ICLS), based on conservation agriculture, have been shown to alter weed communities and enhance overall crop production. However, the effect of the ICLS on weed infestation may vary depending on the ICLS design and management practices, especially in the pasture phase. Additionally, there is still a gap in knowledge regarding weeds in ICLS. The present thesis aimed to examined weed dynamics in ICLS in Subtropical Brazil, in different spatial-temporal scales of crop and livestock integration and pasture fertilization. Chapter1 investigated the impact of an ICLS, which includes a perennial tropical pasture for a period of three years before maize cultivation, on weed infestation and maize yield. Two systems were compared, an ICLS and a crop-based rotation system, in a 4-year non-chemical weed control ICLS experiment, in Pinhais, PR. It was assessed the pre-experimental condition of weed community, the weed seedbank, and the occurrence and interference of weeds in the fourth experimental summer season. The strategy to include a tropical grassland for three years before summer crop provided reduction on weed infestation and weed interference in maize. This integrated crop-livestock system design changes the seedbank composition in the short-term, selecting broadleaf weeds over grasses. Chapter 2 aimed to assess the impact of nitrogen fertilization in winter grazing cover crop on the weed seedbank and the effect of residue on the emerged flora in the summer crop (common bean), in a 10-year ICLS experiment, in Guarapuava, PR. To do so, four nitrogen fertilization rates (0, 75, 150 and 225 kg N ha^{-1}) and the presence/absence of residue on the 2016/17 common bean crop were tested. The weed seedbank and the emerged flora throughout the common bean growing season were assessed. Weed seedbank size strongly decreased in the highest nitrogen fertilization rate of the winter grazing cover crop. Weed density was affected by nitrogen fertilization in winter and presence of residue in common bean early growing season. Grazing cover crop residue is an important factor to weed control in ICLS. Chapter 3 aimed to assess the impact of a traditional paddy field and four lowland ICLS on the weed seedbank, in 4-year ICLS experiment, in Cristal, RS. The weed seedbank was assessed in the fourth experimental year, at three soil depths. In a mid-term temporal scale, the diversification of paddy field through ICLS do not affect weed seedbank size. 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.

Keywords: crop–livestock integration. cultural weed control. weed seedbank.

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LIST OF ABBREVIATIONS

CHAPTER I

A	–	Abundance
asl	–	Above sea level
CBRS	–	Crop-based rotation system
DAE	–	Days after emergency
D	–	Density
DM	–	Dry matter
F	–	Frequency
IVI	–	Importance value index
ISA	–	Indicator Species Analysis
IV	–	Indicator Value
ICLS	–	Integrated crop-livestock systems
RA	–	Relative abundance
RD	–	Relative density
RF	–	Relative frequency
RI	–	Relative importance
SC	–	Sørensen´s similarity coefficient

CHAPTER II

A	–	Abundance
CA	–	Conservation agriculture
DAE	–	Days after emergency
D	–	Density
DM	–	Dry matter
F	–	Frequency
IVI	–	Importance value index
ICLS	–	Integrated crop-livestock systems
H	–	Shannon’s diversity index
J	–	Evenness index
NFR	–	Nitrogen fertilization rates

RA	–	Relative abundance
RD	–	Relative density
RF	–	Relative frequency
RI	–	Relative importance
S	–	Species richness index

CHAPTER III

asl	–	Above sea level
ICLS	–	Integrated crop-livestock systems
H	–	Shannon's diversity index
J	–	Evenness index
S	–	Species richness index
T1	–	Treatment 1 - rice monocropping
T2	–	Treatment 2 - rice-beef cattle integration
T3	–	Treatment 3 - soybean-rice–beef cattle integration
T4	–	Treatment 4 - Sudan grass-soybean-maize-rice-beef cattle integration
T5	–	Treatment 5 - rice-beef cattle integration in cultivated and natural grassland

SUMMARY

GENERAL INTRODUCTION

The integrated crop-livestock systems (ICLS) are defined by the Food and Agriculture Organization of the United Nations (FAO, 2010) as an intentional integration of crop and livestock that can be on-farm as well as on an area-wide basis that may involve some specialization. Successful designs involve the intentional integration that reflects a synergic relationship among the components of crops, livestock and/or trees. The integration can comprise different spatial-temporal scales and cropping rotations (Carvalho et al 2014).

In the context of a growing demand for food in the next few decades associated to environmental insecurity, the ICLS were recognized by FAO (2010) as a promising alternative to the dichotomy of production and conservation, since many aspects of the ICLS are considered important features to the modern concept of sustainable intensified agricultural production (Moraes et al., 2014a).

For instance, weed management will play an important role in determining whether the future food production requirements are achieved in a sustainable way, in a challenging scenario of a rising number of herbicide-resistant weeds (Westwood et al., 2017). Problems associated with simplified weed management (reliance on herbicides) motivate efforts for diversified strategies (Haring and Flessner, 2018), e.g. changing cropping systems (Petit et al., 2015). Thus, ICLS could be an alternative to improve weed control, considering that diversity and complexity are inherent characteristics of this systems (Anghinoni, et al., 2013).

However, the effect of the ICLS on weed infestation may vary depending on the ICLS design and management practices, especially in the pasture phase (Pelissari et al., 2011). Actually, few studies have explored weed dynamics in ICLS. Until 2013, Moraes et al. (2014b) identified that less than 5% of the studies concerning ICLS have focused on weeds. The few studies found by then have focused on the application of herbicides to reduce pasture competition, in ICLS designs of crops intercropped with tropical forages.

More recently, Lustosa et al. (2016); Shuster et al. (2016); Schuster et al. (2018) and Schuster et al. (2019) have studied the response of weed community in ICLS under Subtropical conditions. They observed that weed pressure in ICLS is related to grazing intensity in winter pasture. In fact, Carvalho et al. (2018) highlighted that grazing management is vital in determining the success or failure of ICLS.

Considering that: (1) ICLS can represent an alternative weed management strategy; (2) the response of weed community in ICLS still need to be better understood, regarding different ICLS compositions, especially in Subtropical conditions; (3) the pasture phase is an important factor regulating weed infestation in ICLS; the present thesis aimed to examined weed dynamics in ICLS in Subtropical Brazil, in different spatial-temporal scales of crop and livestock integration (Chapter I and Chapter II) and pasture fertilization (Chapter II).

Chapter I approached a different ICLS design from the usual observed in the Brazilian Subtropics. It discussed how the inclusion of a perennial tropical grassland for a period of three years rotated to maize cultivation impacts weed community and crop yield in an herbicide-free experiment.

Chapter II evaluated the impact of nitrogen fertilization in winter grazing cover crop on weed dynamics in an ICLS, in the most common design observed in Southern Brazil that integrates livestock grazing a winter cover crop to summer crops in rotation.

Finally, chapter III presented how the diversification of a very intensive and specialized production system, irrigated rice (traditional paddy field), through ICLS can impact important weed species for rice crop in the seedbank.

CHAPTER I

CAN AN INTEGRATED CROP-LIVESTOCK SYSTEM BE A NON-CHEMICAL WEED MANAGEMENT TOOL?[1]

[1] This manuscript is presented according to the *Crop Protection* guidelines.

CAN AN INTEGRATED CROP-LIVESTOCK SYSTEM BE A NON-CHEMICAL WEED MANAGEMENT TOOL?

HIGHLIGHTS

1. Two cropping systems were compared in a non-chemical weed control experiment.
2. The integrated crop-livestock system reduced early season weed infestation in a maize crop.
3. Weed seedbank composition was affected by the integrated crop-livestock system.
4. In the integrated crop-livestock system weeds did not interfere with maize yield.

ABSTRACT

Integrated crop-livestock systems (ICLS) with diversified crop rotations have been shown to alter weed communities and enhance overall crop production. This study investigated the impact of an ICLS, which includes a perennial tropical pasture for a period of three years before maize cultivation, on weed infestation and maize yield. Two systems were compared, an ICLS and a crop-based rotation system (CBRS), in randomized complete block design with three replications, in Southern Brazil. It was assessed the pre-experimental condition of weed community, the germinable weed seedbank and the occurrence of weeds in the fourth experimental summer season. To estimate weed interference on maize crop, it was measured grain yield in a weed-free and weedy condition. There was no difference in the germinable weed seedbank size for both systems, although in terms of composition, it was observed a higher proportion of broadleaf than grassy weeds in the ICLS. Regarding the initial weed infestation, both systems presented lower occurrence of weeds, however in ICLS the pasture-maize rotation had strongly decreased weed infestation. The suppressive effect of ICLS was more visible during first 45 days after maize emergence, which corresponded to an average decrease of 40%. The ICLS had the highest maize grain yield compared to CBRS under weedy condition. CBRS was affected by weedy condition, whereas there was no significant weed interference on maize grain yield in the ICLS. The strategy to include a tropical grassland for three years before summer crop provided reduction on weed infestation and weed interference in maize. This integrated crop-livestock system design changes the seedbank composition in the short-term, selecting broadleaf weeds over grasses.

Keywords: ley farming; cultural weed control; non-chemical weed control; grassland-crop rotation.

1.1 INTRODUCTION

Weeds compete with crops and reduce their yield and quality (Zimdahl, 2007). Considering the major crops grown worldwide, about 34% of yield losses are due to weed interference. These crop yield losses are usually higher than the ones caused by other pests (Jabran et al., 2015). Currently, most cropping management systems have relied on herbicides for weed control (Bajwa, 2014).

The overuse of herbicides has led to a global exponential increase in herbicide-resistant weeds, including species with resistance to multiple sites of action (HEAP, 2019). Additionally, human health and environmental risks are concerns with herbicide usage (Jabran et al., 2015). Considering all herbicides issues and that most weed studies focus on chemical control (Harker and O'Donovan, 2013), there is a need to develop non-chemical strategies for integrated weed management (Abouziena and Haggag, 2016).

To ensure weed control with less or no herbicide use, changes in cropping systems may be an alternative weed management strategy, e.g. diversified crop rotations (Petit et al., 2015). Integrated crop-livestock systems (ICLS) meet this proposal because diversity and complexity are inherent characteristics of them, providing both agricultural production and environmental quality (Lemaire et al., 2014). In fact, ICLS are recognized by FAO (2010) as an alternative to sustainable intensification of food production.

The integration of crop and livestock comprises different spatial-temporal scales. In the subtropical region of Brazil, ICLS are characterized by within-farm integration. The most usual design consists of the annual rotation of winter pastures for livestock and summer grain crops in a no-till system (Moraes et al., 2014). In this ICLS design, Schuster et al. (2016), Lustosa et al. (2016) and Schuster et al. (2019) demonstrated that weed infestation can be reduced according to the management of the winter pasture grazing intensity. Thus, the pasture phase is an important factor influencing weed community in an ICLS.

In this context, we hypothesize that an ICLS design that includes a tropical species for summer pasture could provide different selection pressure and stress affecting weed species during the summer. Therefore, the present study aimed to investigate the impact of an ICLS, which includes a perennial tropical grassland for a period of three years rotated to maize cultivation, on the weed community and maize yield in a herbicide-free experiment.

1.2 MATERIAL AND METHODS

1.2.1 Site and experimental design description

The study was developed in a long-term ICLS trial located on a 35-ha field at the Canguiri Experimental Farm of Federal University of Paraná, Brazil (25°24’02’’S, 49°07’12’’W, 890 m asl). This site has a humid subtropical climate (Cfb) according to the Köeppen classification system, with a yearly average precipitation of 1,400 mm, mean minimum temperature of 12.5 ºC and mean maximum temperature of 22.5 ºC. According to the WRB/FAO soil classification, the experimental area has Cambisols, Ferralsols and association of both groups, with small portions of Gleysols. There has been no pesticide usage in the experimental location since 1996. Maize had been cultivated in conventional tillage for 20 years before the experiment establishment.

The experiment was initiated in 2013. The treatments consisted of two systems under no-tillage: (1) integrated crop-livestock systems (ICLS), in a ley farming arrangement, where there was guineagrass cv. aries (*Megathyrsus maximus* (Jacq.) B. K. Simon & S. W. L. Jacobs cv. aries) for a period of three years followed by maize (*Zea mays* L.) cultivation for one year; and (2) crop-based rotation system (CBRS), with summer crops (sunflower [*Helianthus annuus* L.] and maize) in rotation with winter cover crop (mainly composed of black oat [*Avena strigosa* Schreb]). Rotations within the systems are summarized in Table 1. Treatments were organized in a randomized complete block design with three replications. The experimental units ranged from 0.3 to 0.55 ha for CBRS and from 1.5 to 2.2 ha for the ICLS. The experimental units in ICLS were larger to accommodate livestock grazing.

In the winter of 2013, black oat was established as a winter cover crop at a seeding rate of 60 kg ha^{-1} and fertilizer application of 100 kg ha^{-1} P_2O_5 in the seed furrow. During the following winters, the cover crop was sown at the same seeding rate and urea was topdressed at 300 kg ha^{-1}.

In the ICLS, for 2015 winter and 2015/2016 summer, grazing was forage-based in a continuous stocking system, according to the put-and-take method (Mott and Lucas, 1952) with Angus steers weighing approximately 150 ± 15 kg. The grazing method aimed to maintain sward heights at an average of 24 and 32 cm for black oat and guineagrass cv. aries, respectively.

Table 1

Temporal rotation plan for the crop-based rotation system (CBRS) and integrated crop-livestock system (ICLS) over the experimental years.

System	Winter 2013	Summer 2013/14	Winter 2014	Summer 2014/15	Winter 2015	Summer 2015/16	Winter 2016	Summer 2016/17
CBRS	Black oat	Maize / Sunflower	Black oat	Maize / Sunflower	Black oat	Maize / Sunflower	Black oat	Maize
ICLS	Black oat	guineagrass cv. aries	Black oat	guineagrass cv. aries	grazed Black oat	grazed guineagrass cv. aries	Black oat	Maize

During the summers of 2013/14, 2014/15 and 2015/16 half of the experimental units' area was sown with sunflower and the other half with corn, in rotation from a year to another.

At the end of the winter season (September/October) summer crops were established under no-tillage management without herbicide desiccation of the winter cover. Sunflower crops were sown in rows spaced 45 cm apart at a seeding rate of 4 seeds m^{-1}, with the cultivars Aguara 4 in 2013 and Aguara 6 in 2014 and 2015. Maize crops were sown in rows spaced 45 cm apart at a seeding rate of 5-6 seeds m^{-1}, resulting in ~ 110 thousand plants ha^{-1}, with the hybrids 2B655Hx, 30F53, 30F53VYHR, P2866H, in 2013, 2014, 2015 and 2016, respectively. No chemical control (herbicide) was used in the experiment to avoid interaction between systems and herbicide effects on weed community response. No other pesticide was used. Chemical analysis of soil collected before maize crop seeding in 2016 is presented in Table 2 with the concentrations of major elements in the 0 to 25 cm depth.

For the summer crops and perennial tropical forage (guineagrass cv. aries), 300 kg ha^{-1} and 200 kg ha^{-1} of natural phosphate and potassium chloride, respectively, were applied at the time of summer crop seeding. Urea fertilizer was topdressed at 300 kg ha^{-1}. Those fertilizer doses were the same in all summers.

Table 2

Chemical analysis of 0 to 25 cm soil depth, for the crop-based rotation system (CBRS) and integrated crop-livestock system (ICLS), in October 2016, prior to maize crop seeding.

System	pH $CaCl_2$	Al^{3+}	H+Al	Ca^{2+}	Mg^{2+}	K^+	SB	P	V
				$cmol_c\ dm^{-3}$				$mg\ dm^{-3}$	%
CBRS	5.13	0.03	7.07	3.9	2.8	0.2	6.9	4.7	49.4
ICLS	5.07	0.07	7.97	3.6	2.77	0.19	6.56	4.37	45.1

SB – Sum bases; V – Base saturation.

1.2.2 Pre-experimental condition of the weed community

Weed density and the percentage of soil area covered by weeds were assessed in summer of 2012 to characterize the weed infestation in the pre-experimental condition, considering the three experimental replicates. Weed species within quadrats of 50x50 cm were identified and counted. The percentage of soil area covered by each species was estimated, after visual practicing with a subdivided quadrat. The quadrats were placed at each 2-m interval along three 14-m transects, resulting in 21 subsamples. Transects were laid out randomly in the central area of each replicate. Plants were identified according to Kissmann and Groth (1997), Lorenzi (2008) and Lorenzi (2014) descriptions.

1.2.3 Botanical composition and amount of winter cover crop residue

Winter cover crop assessments were carried out prior to maize crop seeding in 2016. Species within quadrats of 50x50 cm were identified and the percentage of area covered by each species was estimated using the same sampling procedure described for the pre-experimental assessment, which resulted in 21 subsamples per experimental unit.

To estimate the amount of winter cover crop residue left above the soil surface at the beginning of the summer cropping season, six quadrats of 50x50 cm were randomly sampled from the central area of each experimental unit. The residue biomass samples were weighed after being dried at 65°C until reaching constant weight to estimate kg of residue dry matter (DM) ha^{-1}.

1.2.4 Seedbank sampling and seed tray maintenance

Seedbanks were sampled before the summer crop seeding in September 2016, which marked the beginning of the fourth year of the ICLS experiment described above. Soil samples were collected manually from the top 0 to 5cm layer, along three 28-m transects in each experimental unit using a steel 4-cm diameter probe. Transects were randomly laid out in the central area of each plot. Along the transect, two soil cores were collected at 4-m intervals and combined into one 42-core composite sample for each experimental unit.

According to Schuster el al. (2016), all soil samples were processed to remove stones and root fragments, then spread in 44x38 cm plastic trays and placed in a greenhouse for 12 months beginning in September 2016. Soil moisture was maintained in the trays using regular sub-irrigation. The seedling emergence method (Thompson et al., 1997) 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 0ºC, and the maximum temperature was 38ºC.

Emerged seedlings were periodically identified, counted and removed from the plastic trays. Seedling identification was conducted based on Kissmann and Groth (1997), Lorenzi (2008) and Lorenzi (2014) descriptions. To analyse the seedbank composition, plant species were grouped into grasses and broadleaf weeds, since no other monocotyledons families (such as Cyperaceae) were identified during seedbank evaluations.

1.2.5 Field weed sampling and emerged weed community composition in maize

In the 2016/2017 maize cropping season, weed seedling emergence was quantified at 15, 30, 45, 70 and 140 days after emergency (DAE) of the maize. The emerged weed seedlings were identified and counted within 50x50 cm quadrats. The same sampling method was used as described for the winter cover crop assessment, which resulted in 21 subsamples per experimental unit. Also, at 70 DAE, which corresponded to the same sampling time of the year as for the pre-experimental assessment, the percentage of soil area covered by each species within the quadrats was estimated.

In order to characterize the botanical composition of the weed community that emerged during the maize cropping cycle, the following phytosociological parameters were calculated, according to Adegas et al. (2010) formulas adapted from Mueller-Dombois and Ellenberg (1974):

$$\text{Frequency (F)} = \frac{\text{number of quadradts containing the species}}{\text{total number of quadrats}}$$

$$\text{Relative frequency (RF)} = \frac{\text{frequency of the species}}{\text{sum of the frequencies from all species}} \times 100$$

$$\text{Density (D)} = \frac{\text{number of individuals per species}}{\text{total number of quadrats}}$$

$$\text{Relative density (RD)} = \frac{\text{density of the species}}{\text{sum of the densities from all species}} \times 100$$

$$\text{Abundance (A)} = \frac{\text{number of individuals per species}}{\text{number of quadrats containing the species}}$$

$$\text{Relative abundance (RA)} = \frac{\text{adundance of the species}}{\text{sum of the abundances from all species}} \times 100$$

$$\text{Importance value index (IVI)} = \text{RF} + \text{RD} + \text{RA}$$

$$\text{Relative importance (RI)} = \frac{\text{IVI of the species}}{\text{sum of the IVI from all species}} \times 100$$

Systems were compared by the Sørensen´s (1972) similarity coefficient (SC) using the formula:

$$SC = \frac{2 \times a}{(b + c)}$$

Where a represents the number of plant species common to system 1 and 2; b represents total number of species present in system 1; c represents total number of species present in system 2. The purpose of this calculation was to estimate the current degree of weed similarity between the two cropping systems.

Indicator Species Analysis (ISA, Dufrene and Legendre, 1997) was used to identify and test the weed species showing strongest differences between the two systems. This method combines information on the species frequency in each system (presence or absence in the quadrats), which is called specificity, and the species abundance in each system (density of a species in the quadrats of each system), is called fidelity. Thus, we calculate the Indicator Value (IV) of an i species in relation to a j type of site:

$$\text{IV}ij = \text{Specificity}ij \times \text{Fidelity}ij \times 100$$

Where IVij is the Indicator Value of an i species in relation to a j type of site, Specificityij is the proportion of sites of type j with species i, and Fidelityij is the proportion of the abundance of species i that are in a j type of site. It returns IV for each species in each system varying between 0 (species absent from all quadrats of that system) and 100 (species is present with highest abundance in all quadrats of the system, thus ‘perfect indication’).

1.2.6 Maize grain yield and weed index

To evaluate the effect of weed interference in the maize crop, a weed free check treatment was added within each experimental unit during the 2016 summer cropping season. The weed free subplot was accomplished by a weekly hand hoeing. For this analysis, the rest of the plot area, where there was no weed control, was identified as “weedy”. A weed index value was calculated for each experimental unit according to the following formula:

$$\frac{(yield\ in\ weed\ free\ check - yield\ in\ weedy\ treatment)}{yield\ in\ weed\ free\ check} \times 100$$

This index indicates percent reduction in crop yield in different treatments compared to the weed free check.

To estimate grain yield, all maize cobs from three 3-m lines (about 4 m^2) were manually sampled and processed in the laboratory. The grain yield was calculated in t ha^{-1} and data were reported at 13 % moisture content.

1.2.7. Data Analysis

The 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. 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. The IV values for each species in each system (ISA) were tested for statistical significance using a randomization technique (4999 permutations of the quadrats' allocations to system).

1.3 RESULTS

1.3.1 Pre-experimental weed infestation

By the summer of 2012, all three replicates had 100% of the soil covered by weeds and weed density average was 66 ± 2 plants m^{-2} (Fig. 1). These results contrast with weed cover and density observed in 2016 at 70 days after maize emergence for both systems, that was on average 75% and 40% lower, respectively ($p<0.05$).

Thirty species of weeds were identified in the pre-experimental condition (Table A.1).

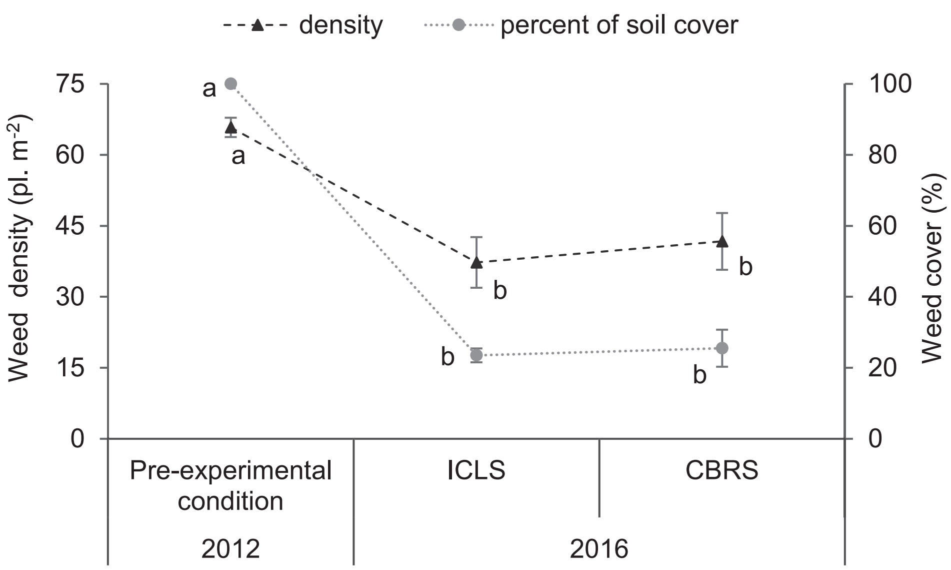

Fig. 1. Weed seedling emergence density (plants m^{-2}) and percentage of soil area covered by weeds (%) observed in the pre-experimental condition in 2012, and in 2016 at 70 DAE in the integrated crop livestock system (ICLS) and crop-based rotation system (CBRS). Points represent means, and error bars represent standard error of the mean. Same lower-case letters regarding the same dependent variable do not differ by Tukey test a 5% level of significance.

1.3.2 Winter cover crop residue

There was no difference in the amount of winter cover crop residue or in the composition of the residue (i.e., proportion of black oat and annual ryegrass [*Lolium multiflorum* Lam.]) between the two management systems. The average of residue dry matter left on the soil surface prior to no-tillage maize sowing was 9.0 ± 0.9 t ha^{-1}.

Black oat, as the seeded cover crop, was the most frequent species (98%) and represented an average of almost 70% of the soil area covered. The other two most frequent species in the cover crop residue were annual ryegrass and wild radish (*Raphanus raphanistrum* L.), with 37 and 43% frequency, respectively, and each represented about 15% of the soil coverage. Other plant species in the cover crop had low frequency (<10%) and when present in the cover crop, they rarely represented more than 10% of the soil area covered.

1.3.3 Germinable weed seedbank

No difference was observed between the two management systems in size of the germinable weed seedbank, with an average across systems of 4,100 ± 1,500 seeds m^{-2}. However, the systems affected the weed seedbank composition. There was a higher proportion of broadleaf weeds ($p<0.05$) in the ICLS, which represented 90% of the total seeds in the weed seedbank, whereas in the crop-based rotation system broadleaf weeds represented 60% of seeds present (Fig. 2).

The most numerous species in the ICLS seedbank were *Oxalis corniculata*, *Gnaphalium spicatum*, *R. raphanistrum* and *Richardia brasiliensis*. Together they represented almost 65% of the total germinable weed seedbank. In the CBRS, grasses represented about 40% of weed seeds in the upper 5 cm of soil. *Megathyrsus maximus*, *Eleusine indica*, *Digitaria horizontalis* and *Urochloa plantaginea* were the most numerous species, listed in descending order of amount of seeds in the weed seedbank of CBRS.

A total of 32 species were identified in the weed seedbank, of which 27 species were common to both systems. There were 31 and 28 identified species in the CBRS and ICLS, respectively. Those species were distributed in 17 families. The most representative families in number of species were Asteraceae and Poaceae (all identified species in the weed seedbank assessment are presented in Table A.1.)

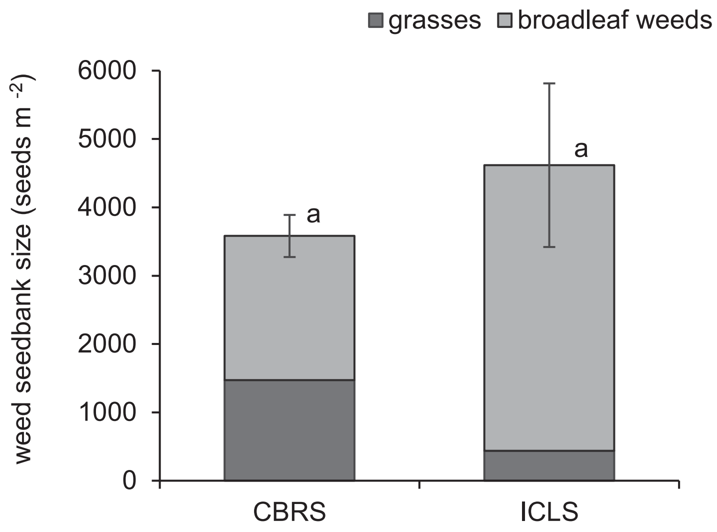

Fig. 2. Weed seedbank size (seeds m^{-2}) observed in the integrated crop livestock system (ICLS) and crop-based rotation system (CBRS), prior to maize crop seeding. The amount of seeds was aggregated into grasses and broadleaf weed categories. Columns represent means, and error bars represent standard error of the mean. Same lower-case letters do not differ by Tukey test a 5% level of significance.

1.3.4 Weed seedling emergence in maize crop

Weed seedling emergence density throughout the maize cropping period was lower in ICLS during the first 45 DAE compared with CBRS ($p<0.01$, Fig. 3). The suppressive effect of the tropical grassland-maize rotation represented 45, 40 and 35% reduction in weed emergence density at 15, 30 and 45 DAE, respectively.

Weed density did not differ between systems at 70 DAE. A smaller difference between systems for weed density was observed by the end of the maize cropping cycle (140 DAE), when there was an average of 33 and 22 plants m^{-2} for the CBRS and ICLS, respectively.

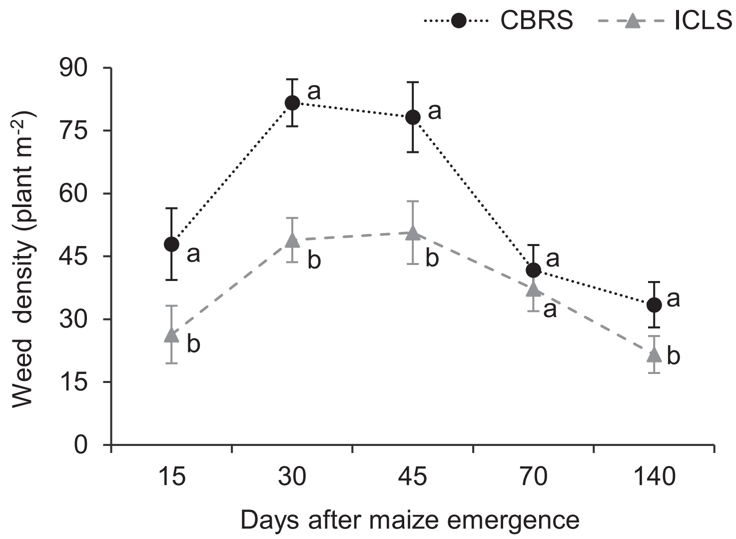

Fig. 3. Weed seedling emergence density (plants m^{-2}) over the maize crop period of the integrated crop livestock system (ICLS) and crop-based rotation system (CBRS) in 2016/2017 summer. Points represent means, and error bars represent standard error of the mean. Lower-case letters compared systems at each evaluation date. Same letters do not differ by Tukey test a 5% level of significance.

1.3.5 Composition of emerged weeds in maize crop

Throughout the seedling emergence assessments, 34 weed species were identified, belonging to 17 plant families. The similarity index comparing both systems was 0.98. Of the 34 species found in the experiment, 33 were common to both systems. Although the similarity index was high, the importance of each species differed within the systems. For example, annual ryegrass (*L.multiflorum)* was the most important species in the CBRS up to 45 DAE, due to its high frequency and density. For the same period, wild radish (*R. raphanistrum*) was the most important species in the ILCS, also for being the most frequent and with higher relative density (Fig.4).

During the maize growing season, the most important species identified in both systems, according to relative importance, were: *Lolium multiflorum*, *Alternanthera philoxeroides*, *Raphanus raphanistrum*, *Eleusine indica*, *Megathyrsus maximus*, *Cyperus esculentus*, *Cynodon sp.*, *Digitaria horizontalis*, *Bidens pilosa*, *Oxalis corniculata*, *Richardia brasiliensis* and *Ageratum conyzoides* (Fig.4).

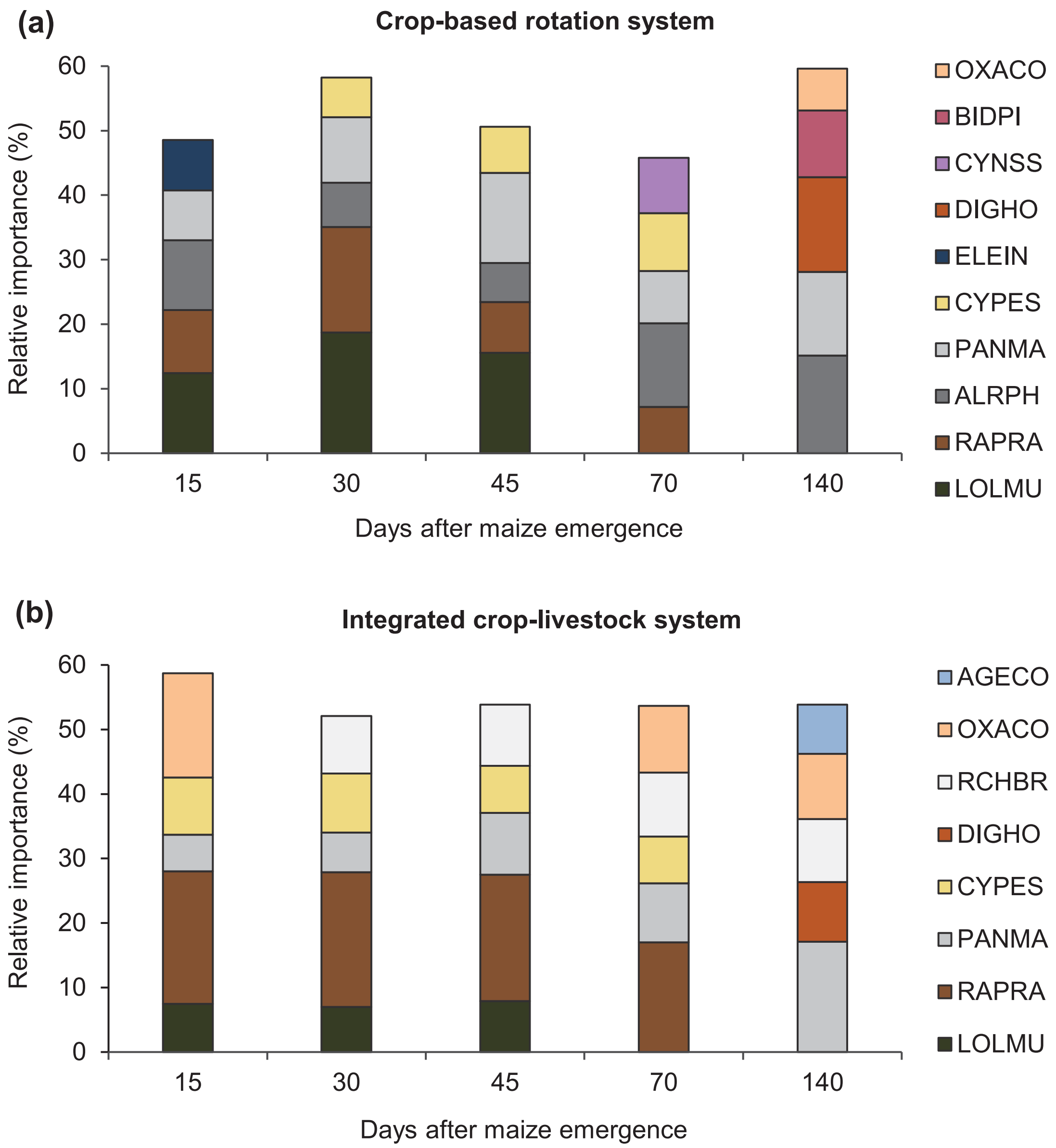

Fig. 4. Relative importance (RI) of the five most important weed species identified in the crop-based rotation system (a) and integrated crop-livestock system (b), according to the evaluations over the maize cropping cycle in the 2016/2017 summer. Species are presented in EPPO code (EPPO, 2019): *Loluim multiflorum* Lam. (LOLMU), *Alternanthera philoxeroides* (Mart.) Griseb. (ALRPH), *Raphanus raphanistrum* L. (RAPRA), *Eleusine indica* (L) Gaertn. (ELEIN), *Megathyrsus maximus (Jacq.) B.K.Simon & S.W.L.Jacobs*. (PANMA), *Cyperus esculentus* L. (CYPES), *Cynodon* sp. (CYNSS), *Digitaria horizontalis* Willd. (DIGHO), *Bidens pilosa* L. (BIDPI), *Oxalis corniculata* L. (OXACO), *Richardia brasiliensis* Gomes (RCHBR), *Ageratum conyzoides* L. (AGECO).

According to the Indicator Species Analysis (Table 3), CBRS had fewer but strong indicator species: hairy beggartick (*B. pilosa*) and gallant sodier (*G. parviflora*), both from Asteraceae family. In the ICLS, there were more indicator species, although presenting lower indicator values: *Ipomoea* sp., *Oxalis corniculata*, *Richardia brasiliensis*, *Artemisia verlotiorum*, *Rumex obtusifolius* and Oxalis *latifolia*.

Table 3

Indicator Species Analysis of the crop-based rotation system (CBRS) and integrated crop-livestock system (ICLS) in 2016.

Species*	EPPO Code	Systems		p-value
		CBRS	ICLS	
Indicators of CBRS				
Bidens pilosa	BIDPI	69.3	4.9	0.001
Galinsoga parviflora	GASPA	28.6	0	0.001
Indicators of ICLS				
Ipomoea sp.	IPOSS	0.7	15.3	0.021
Oxalis corniculata	OXACO	0	14.2	0.006
Richardia brasiliensis	RCHBR	0.3	12.2	0.032
Artemisia verlotorum	ARTVE	0	9.5	0.034
Rumex obtusifolius	RUMOB	0	9.5	0.028
Oxalis latifolia	OXALA	0	7.9	0.004

*Only species with p< 0.05 are shown.

1.3.6 Maize grain yield

There was a significant systems x weed coexistence interaction for maize crop yield. Considering the weedy treatment, the ICLS had the highest grain yield ($p < 0.05$), with an average of 12.8 ± 0.4 t ha^{-1} versus 10.4 ± 1.2 t h ha^{-1} in the CBRS (Fig.5).

Weed interference reduced grain yield in the CBRS by 20% compared with the weed-free check. In the ICLS no significant difference was observed due to weed interference, with average grain yield of 13.5 ± 1.2 t ha^{-1}.

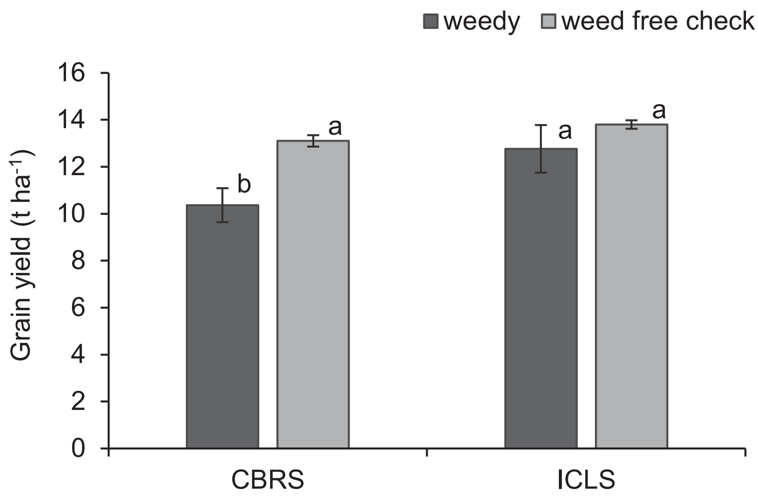

Fig.5. Effect of systems (ICLS, CBSR) and weed coexistence (weedy and weed free check treatment) on maize grain yield (t ha^{-1}). Columns represent means, and error bars represent standard error of the mean. Columns with the same letters do not differ based on Tukey's Honest Significant Difference (p>0.05).

1.4 DISCUSSION

1.4.1 Weed infestation

Winter cover crop characterization (botanical composition and amount of residue) did not vary significantly between the two systems, i.e. cover crop residue was not an additional source of variation for the response of the main factor (systems). In other words, it can be assumed the differences in summer weed community between systems was not due to effects of residue.

Considering the decrease in weed infestation over the course of the experiment in both systems compared with the initial pre-experimental condition, the presence of a thick organic mulch (high amounts of residue DM) contributed to weed suppression (Osipitan et al., 2018) (Fig.1). Therefore, winter cover crop residue was an important non-chemical strategy for managing weeds in both systems (Buchanan et al., 2016; Wallace et al., 2018), among other factors (e.g. higher density seeding rates). The surface residue suppresses weeds due to physical effects preempting space and resources and reducing light transmittance and soil temperatures; as well as possible chemical effects arising from allelochemicals released by the residue left on the surface (Oliveira Jr et al., 2014).

The decrease of weed occurrence within ICLS compared with CBSR was mainly due to the presence of guineagrass cv. aries in the system rotation. Tropical perennial grasses, such as guineagrass cv. aries, are strong competitors for natural resources primarily due to rapid expansion of the foliar canopy during the growing season, C_4 photosynthetic pathway, and intense root growth (Zimdahl, 2007). Considering guineagrass cv. aries as a crop, its competitive ability relies on efficiently occupying soil area (Constantin, 2011). Thus, tropical grasslands have a competitive advantage compared with annual crops. However, the soil coverage achieved by the grassland depends on grazing management (Pelissari et al., 2013), thus the weed suppressive effect seen in this study is related to moderate grazing intensity in the ICLS, according to findings of Schuster et al. (2016).

Concenço et al. (2011) also identified two mechanisms related to the lower infestation and delayed emergence of seedlings in systems that integrate crops and livestock: allelopathic issues and the direct presence and action of livestock at the area. Grazing (Popay and Field, 1996) and trampling (Marchezan et al., 2003), could both reduce production of new seeds and vegetative propagules from weed species, as well as forcing quiescent seeds to dormancy and later loss of their viability.

We expected to observe differences between the systems in size of the germinable weed seedbank (Fig.2), considering the lower weed infestation in the ICLS and according to results of Schuster et al. (2016). However, they observed a smaller weed seedbank size after 15 years in ICLS, in contrast to the current work that is in the first ICLS rotation cycle.

The incipient response of weed seedbank to the ICLS resulted in a higher proportion of broadleaf/grass weeds. It is possible that grazing animals preferentially selected grassy weeds during grazing, resulting in lower rates of grassy weed seed rain. Another possible explanation for this response is that there was a higher contribution of broadleaf weed seed rains, as many weed species belonging to this category produce huge amounts of seeds. *R. brasiliensis* and *R. raphanistrum* were important weed species in the maize cropping cycle and were indicator species for the ICLS. They have the potential to produce more than 10000 and 70000 seeds plant^{-1}, respectively (Constantin, 2011). They were among the most representative species in the ICLS seedbank. Leon and Wright (2018) also pointed out that grazing can increase the number prostrate species in the weed seedbank, such as *R. brasiliensis.*

The Asteraceae e Poaceae families had with greater numbers of identified species, considering both weed seedbank and weed emergence in the maize crop assessments. Those plant families are the most important ones in terms of number of weed species in Brazil (Lorenzi, 2008). Annual ryegrass and wild radish were among the most important species found in both systems up to 45 DAE (Fig.4), corresponding to the period when the maize crop was most susceptible to weed interference. Those species are typically found in winter to spring in South Brazil (Lorenzi, 2008), and thus, their degree of importance decreased after 45 DAE.

There were four species (*Nothoscordum inodorum*, *Cyperus esculentus*, *Cynodon* sp., *Pennisetum clandestinum*) that occurred during the maize growing season that were not identified in the germinable weed seedbank. That is because these species reproduce mainly (if not only) through vegetative structures.

A very high similarity in weed species occurrence was observed in the two management systems (0.98 similarity index). Even though the most important species during the maize cycle were the same, they differed in degree of importance, with more prominence of grasses in CBRS and more broadleaf weeds in the ICLS.

1.4.2 Weed interference

The reduction in weed infestation in the ICLS reflected positively in maize grain yield. The weed suppressive effect of ICLS was more evident during first 45 DAE (Fig.3), the interval that comprises, in most edaphoclimatic conditions, the period when the maize crop is more sensitive to weed interference (Kozlowski, 2002). After 70 DAE a minor difference between systems was observed, however, at this stage the maize crop had achieved canopy closure, improving the crop competition over weeds. Additionally, by this point in the season, weed interference does not significantly affect grain yield (Kozlowski, 2002).

Weed interference did not affect grain yield in the ICLS (Fig. 5), i.e. no significant difference was observed in grain yield between the weed-free and maize coexisting with weed treatments. This was related to the lower occurrence of weeds in that system (average of 40% during the first 45 DAE).

Introducing a perennial tropical grassland for three year before maize cultivation proved to be an efficient non-chemical strategy to manage weeds, which could provide economic and environmental benefits due to no herbicide application. Furthermore, another potential environmental benefit relies on the coexistence of weeds and crops without crop yield losses, since weeds contribute to the functioning of agroecosystems (Petit et al., 2015).

Finally, the overall decrease on weed infestation compared to the initial condition and good maize yields for both systems (> 10 t ha-1) highlight what is possible to achieve without herbicide use when combining several cultural weed management strategies: adequate cover crop residue management, rapid growing cultivars, adequate mineral nutrition and high-density seeding rates.

1.5 CONCLUSION

Introducing a tropical grassland for three years prior to maize cultivation provides reduction in both weed infestation and weed interference with maize yield compared to a crop-based rotation system. This integrated crop-livestock system design changes the seedbank composition in the short-term, selecting broadleaf weeds over grasses.

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1.7 APPENDIX

Table A.1

Species identified in all weed assessments carried out in this study, organized according to plant family, with indication of when each one occurred.

EPPO Code	Species	Family	2012	2016			
			summer emergence	weed seedbank		summer emergence	
			pre-experimental condition	CBRS	ICLS	CBRS	ICLS
ALLNE	*Nothoscordum inodorum* (Aiton) G. Nicholson	Alliaceaea				x	x
ALRPH	*Alternanthera philoxeroides* (Mart.) Griseb.	Amaranthaceae	x	x		x	x
AMARE	*Amaranthus retroflexus* L.	Amaranthaceae		x	x		
APULE	*Apium leptophyllum* (Pers.) F. Muell. Ex Benth.	Apiaceae	x				
AGECO	*Ageratum conyzoides* L.	Asteraceae	x	x		x	x
ARTVE	*Artemisia verlotorum* Lamotte	Asteraceae	x	x		x	x
BIDPI	*Bidens pilosa* L.	Asteraceae	x	x	x	x	x
CNDSS	*Conyza* spp.	Asteraceae	x	x	x	x	x
GASPA	*Galinsoga parviflora* Cav.	Asteraceae	x	x	x	x	x
GNAPU	*Gnaphaluim spicatum* Lam.	Asteraceae	x	x	x	x	x
HRYBR	*Hypochaeris brasiliensis* (Less.) Benth. & Hook. f. ex Griseb.	Asteraceae	x	x	x		
SENBR	*Senecio brasiliensis* Less.	Asteraceae	x	x	x	x	x
SONOL	*Sonchus oleraceus* L.	Asteraceae	x	x	x		
TAGMI	*Tagetes minuta* L.	Asteraceae	x				
TAROF	*Taraxacum officinale* F. H. Wigg.	Asteraceae	x				
XANST	*Xanthium strumarium* L.	Asteraceae	x			x	x
RAPRA	*Raphanus raphanistrum* L.	Brassicaceaea	x	x	x	x	x
SILGA	*Silene gallica* L.	Caryophyllaceae		x	x		x
STEME	*Stellaria media* (L.) Vill.	Caryophyllaceae		x	x		
COMBE	*Commelina benghalensis* L.	Commelinaceae	x				
IPOSS	*Ipomea* spp.	Convolvulaceae	x	x	x	x	x
CYPES	*Cyperus esculentus* L.	Cyperaceae	x			x	x
EPHHL	*Euphorbia heterophylla* L.	Euphorbiaceae	x	x	x	x	x
TRFPR	*Trifolium pratense* L.	Fabaceae		x	x	x	x
VICSA	*Vicia sativa* L.	Fabaceae		x	x	x	x
SISFA*	*Sisyrinchium fasciculatum* Klatt	Iridaceae		x	x		
STAAR	*Stachys arvensis* L.	Lamiaceaea		x	x		
SIDRH	*Sida rhombifolia* L.	Malvaceae	x	x	x	x	x

Continued next page

Table 1 (continued)

OXACO	*Oxalis corniculata* L.	Oxalidaceae		x	x	x	x
OXALA	*Oxalis latifolia* Kunth	Oxalidaceae				x	x
PLATO	*Plantago tomentosa* Lam.	Plantaginaceae	x	x	x	x	x
BRADC	*Brachiaria decumbens* Stapf.	Poaceae	x				
CYNSS	*Cynodon* sp.	Poaceae	x			x	x
DIGHO	*Digitaria horizontalis* Willd.	Poaceae	x	x	x	x	x
ELEIN	*Eleusine indica* (L.) Gaertn.	Poaceae	x	x	x	x	x
LOLMU	*Lolium multiflorum* Lam.	Poaceae		x	x	x	x
PANMA	*Megathyrsus maximus* (Jacq.) B.K.Simon & S.W.L.Jacobs.	Poaceae		x	x	x	x
PESCL	*Pennisetum clandestinum* Hochst. Ex Chiov.	Poaceae				x	x
BRAPL	*Urochloa plantaginea* (Link) Hitch.	Poaceae		x	x	x	x
RUMOB	*Rumes obtusifolius* L.	Polygonaceae	x	x		x	x
ANGAR	*Anagallis arvensi*s L.	Primulaceae			x		
RCHBR	*Richardia brasiliensis* Gomes	Rubiaceae	x	x	x	x	x
BOILF	*Spermacoce latifola* Aubl.	Rubiaceae	x				
SOLAM	*Solanum americanum* Mill.	Solanaceae	x	x	x	x	x
SOLSI	*Solanum sisymbriilfolium* Lam.	Solanaceae	x				

CHAPTER II

NITROGEN FERTILIZATION IN WINTER GRAZING COVER CROP AS A WEED MANAGEMENT TOOL IN AN INTEGRATED CROP-LIVESTOCK SYSTEM [1]

[1] This manuscript is presented according to the *Planta Daninha* Journal guidelines

NITROGEN FERTILIZATION IN WINTER GRAZING COVER CROP AS A WEED MANAGEMENT TOOL IN AN INTEGRATED CROP-LIVESTOCK SYSTEM

HIGHLIGHTS

1. Weed seedbank size strongly decreased in high nitrogen fertilization rate of the winter grazing cover crop (225 kg N ha^{-1}).
2. Weed density was affected by nitrogen fertilization in winter and presence of residue in common bean early growing season.
3. Grazing cover crop residue is an important factor to weed control in integrated crop-livestock systems under subtropical conditions.

ABSTRACT

In Subtropical Brazil, integrated crop-livestock systems (ICLS) are based on conservation agriculture and most designs comprises the integration of winter grazing cover crops and summer grain crops. One of ICLS benefits is the potential to suppress weeds, mainly due to the management of grazing cover crop and its residue. The present study aimed to assess the impact of nitrogen fertilization in winter grazing cover crop on the weed seedbank and the effect of residue on the emerged flora in the summer crop (common bean), in a 10-year ICLS experiment. To do so, four nitrogen fertilization rates in winter pasture (NFR – 0, 75, 150 and 225 kg N ha^{-1}) and the presence/absence of residue on the 2016/17 common bean crop were tested in a split-plot design with three repetitions, in Guarapuava, Paraná State. The germinable seedbank and the merged flora at 15, 30, 45 and 60 days after common bean emergence (DAE) were assessed. After 10 years of the ICLS, the seedbank in 0 NFR was almost three times bigger than the 225 NFR, mainly due to the amount of residue biomass that was 60% superior in 225 NFR compared to 0 NFR. The main effects of treatments on weed density were observed in common bean early growing season (15 and 30 DAE). The composition of weed community was not affected by the NFR. The nitrogen fertilization management of winter grazing cover crops is an important factor regulating the occurrence of weeds in ICLS.

Keywords: crop–livestock integration. cultural weed control. weed seedbank.

ADUBAÇÃO NITROGENADA DA CULTURA DE COBERTURA HIBERNAL SOB PASTEJO COMO UMA FERRAMENTA AO MANEJO DE PLANTAS DANINHAS EM UM SISTEMA INTEGRADO DE PRODUÇÃO AGROPECUÁRIA

RESUMO

No Brasil subtropical, os sistemas integrados de produção agropecuária (SIPA) estão baseados na agricultura de conservação e a maior parte dos arranjos compreende a integração de culturas de cobertura hibernais sob pastejo e culturas graníferas de verão. Um dos benefícios dos SIPA é o potencial de suprimir plantas daninhas, principalmente devido ao manejo da cultura de cobertura hibernal sob pastejo e sua palhada residual. O presente estudo objetivou avaliar o impacto da adubação nitrogenada da cultura de cobertura hibernal sob pastejo sobre o banco de sementes de plantas daninhas e o efeito da palhada sobre a flora emergente na cultura de verão (feijoeiro), em um experimento de SIPA de 10 anos. Para isso, foram testadas quatro doses de adubação nitrogenada n pastagem de inverno (0, 75, 150 e 225 kg N ha^{-1}) e a presença/ausência de resíduo na cultura do feijoeiro em 2016/2017 em um arranjo de parcelas subdivididas com 3 repetições, em Guarapuava, Estado do Paraná. Foram avaliados o banco de sementes de plantas daninhas e a flora emergente na cultura do feijoeiro aos 15, 30, 45 e 60 dias após emergência (DAE). Após 10 anos do SIPA, o banco de sementes de plantas daninhas na dose 0 N foi quase três vezes maior que a dose 225 N, principalmente devido a quantidade de biomassa de palhada que foi 60% superior na dose 225 N comparada a dose 0 N. Os principais efeitos dos tratamentos sobre a densidade de plantas daninhas foram observados no início do ciclo do feijoeiro (15 e 30 DAE). A composição da comunidade de plantas daninhas não foi afetada pelas doses de N. O manejo da adubação nitrogenada da cultura de cobertura hibernal sob pastejo é um importante fator regulador da ocorrência de plantas daninhas em SIPA.

Palavras-chave: integração lavoura-pecuária. Controle cultural de plantas daninhas. Banco de sementes de plantas daninhas.

2.1 INTRODUCTION

The integrated crop-livestock systems (ICLS) in the Brazilian subtropics are characterized by within-farm integration of winter grazing cover crops and summer grain crops under conservation agriculture (CA) (Moraes et al., 2014a). They were indicated by the Food and Agriculture Organization of the United Nations (FAO, 2010) as a promising alternative to the dichotomy of production and conservation, since many aspects of the ICLS are considered important features to the modern concept of sustainable intensified agricultural production (Moraes et al., 2014a). One of these aspects is that ICLS may improve weed control, although depending on management practices, especially in the pasture phase (Pelissari et al., 2011).

Moraes et al. (2014b) identified in their systematic review, covering the years from 1994 to 2013, that less than 5% of the studies concerning ICLS have focused on weeds. Recently, Lustosa et al. (2016); Shuster et al. (2016); Schuster et al. (2018) and Schuster et al. (2019) have studied the response of weed community to grazing intensity of the winter cover crops. They mostly linked the potential suppressive effect of the ICLS on weeds to biomass production of the winter grazing cover crop.

In weed research, under an integrated management approach, weed suppression by using cover crops is gaining more attention in the context of an increasing number of herbicide-resistant weeds, according to Osipitan et al. (2018) review. In the same study, they identified that important characteristics of cover crops for weed suppression are high biomass productivity and persistent residue (Osipitan et al., 2018). Among other effects, the presence of an organic mulching (straw / cover crop residue) affects weed dynamics within an agroecosystem, either by acting as a physical barrier to weed emergence, promoting microclimatic changes or to potential allelopathic effects of some species (Chauhan et al., 2012).

Biomass production of grass cover crops, such as the winter cereals commonly used in ICLS designs in subtropical Brazil, is positively influenced by nitrogen fertilization, especially under grazing conditions (Assmann et al., 2004). In fact, Blackshaw and Brandt (2008) highlighted that fertilizer management strategies that favor crops over weeds deserve greater attention, even when weed infestations consist of species known to be highly responsive to higher soil N levels.

Considering that there is lack of information on ICLS regarding the response of weeds to other aspects of the winter cover crop than grazing management; and that the amount of residue biomass is related to the potential weed suppressive effect of the ICLS, it was hypothesized that nitrogen fertilization in the winter grazing cover crop influences weed pressure (weed seedbank and weed flora) in ICLS under CA. Therefore, the present study aimed to assess the impact of nitrogen fertilization in winter grazing cover crop on the weed seedbank and the effect of residue on the emerged flora in the summer crop (common bean).

2.2 MATERIAL AND METHODS

The present work was carried out in a long-term ICLS experiment located at the Midwest State University of Paraná (UNICENTRO) Experimental Field - *Campus* CEDETEG - in Guarapuava, Paraná State, Brazil (25º 33’ 36” S latitude, 51º 27’ 39” W longitude and 1,100 m altitude). The site has a humid temperate climate according to the Köeppen classification system, characterized by a well-distributed rainfall throughout the year, with an average annual temperature and precipitation of 17.2 °C and 1,925 mm, respectively (IAPAR, 2020). The soil is an Oxisol, classified as *Latossolo Bruno Distroférrico* according to the Brazilian Agricultural Research Corporation (EMBRAPA, 2007).

The experiment was stablished as an ICLS in the winter of 2006, in a randomized complete block design with three replications. Previously, annual grain crops were cultivated in the area. The ICLS design consists in the integration of cover crops grazed by sheep in winter season and grain crops - maize and common bean - in rotation during summer season. The total area of the experiment corresponded to 3.5 hectares, being divided in pickets (main plots) with an area of 0.2 ha each.

The present study refers to the season of 2016/2017. It was set in a split-plot design with two factors. The whole-plot factor (main plots) consisted of four nitrogen fertilization rates (NFR) in winter grazing cover crop: 0, 75, 150 and 225 kg N ha^{-1}. The split-plot factor was stablished only in 2016/2017 summer cropping season and consisted in presence or absence of winter residue on the soil surface of common bean crop, aiming to assess the effect of residue biomass on weed emergence.

As conservation agriculture (CA) is one of the ILCS' pillars (Moraes et al., 2014a) both winter (grazing cover crop) and summer (grain crops) sowing were performed in a no-tillage system, approximately two weeks after desiccation with glyphosate herbicide.

In 2006 winter, the sown cover crop was annual ryegrass (*Lolium multiflorum* Lam.*)*. From 2007 to 2016 the winter grazing cover crop was the mixture of annual ryegrass and black oat (*Avena strigose* Schreb.). Over the experimental years, sowing date ranged from May to June, according to weather conditions, at a seeding rate of 60 and 20 kg seeds ha^{-1} of black oat and annual ryegrass, respectively. At sowing time, 50 kg P_2O_5 ha^{-1} and 50 kg K_2O ha^{-1} were applied in the seed furrow. The nitrogen fertilization was done based on the treatments rates, topdressing urea in a single application at the beginning of tillering.

Grazing was forage-based in a continuous stocking system, according to the put-and-take method (Mott and Lucas, 1952) with lambs of the *Ile de France* breed. The grazing method aimed to maintain sward heights at an average of 14 cm. Grazing period lasted around 4 months over the experimental years, beginning in July-August ending in November.

For summer seasons, common bean was sown between the first and second week of December, in rows 40 cm apart, aiming to reach a seeding rate of 250,000 plants ha^{-1}, with the cultivars FT Soberano, IPR Graúna and IPR Tuiuiú in 2006, 2008 and 2010 till 2016, respectively. Maize was sown in October, in rows 80 cm apart, aiming to reach a seeding rate around 65-75 thousand plants ha^{-1}, with the 30F53 hybrid. Cultivars for both common bean and maize were chosen according to the regional recommendation and availability. Other cultural practices were performed according to the technical recommendations for each crop.

To estimate the amount of winter grazing cover crop residue left above the soil surface at the beginning of the summer cropping season (straw), five quadrats of 50x50 cm were randomly sampled from the central area of each experimental unit (main plots), prior to winter desiccation. The samples of residue biomass were weighed after being dried at 65°C until reaching constant weight to estimate kg of residue dry matter (DM) ha^{-1}.

Seedbanks were sampled before the summer crop seeding in October 2016, which marked the tenth year of the ICLS experiment described above. Soil samples were collected manually from the top 0 to 5cm layer, along three 28-m transects in each experimental unit using a steel 4.8-cm diameter probe. Transects were randomly laid out in the central area of each plot. Along the transect, two soil cores were collected at 4-m intervals and combined into one 42-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 44x38 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 (Thompson et al., 1997) 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 0ºC, and the maximum temperature was 38ºC.

Emerged seedlings were periodically identified, counted and removed from the plastic trays. Seedling identification was conducted based on Kissmann and Groth (1997), Lorenzi (2008) and Lorenzi (2014) descriptions. 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 the evenness of the seedbank (J) were estimated as described in Schuster et al (2019), according to Kent and Coker (1992):

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

$$J = \frac{H}{\log{(S)}}$$

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.

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

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

In the 2016/2017 common bean growing season, weed emergence was quantified at 15, 30, 45 and 60 days after common bean emergence (DAE). Five permanent quadrats of 50x50 cm were randomly set in each subplot. At each evaluation dates, seedlings were identified, counted and removed from the permanent quadrats.

With the collected data, phytosociological parameters were calculated considering all quadrats at each evaluation date, according to Adegas et al. (2010) formulas adapted from Mueller-Dombois and Ellenberg (1974):

$$\text{Frequency (F)} = \frac{\text{number of quadradts containing the species}}{\text{total number of quadrats}}$$

$$\text{Relative frequency (RF)} = \frac{\text{frequency of the species}}{\text{sum of the frequencies from all species}} \times 100$$

$$\text{Density (D)} = \frac{\text{number of individuals per species}}{\text{total number of quadrats}}$$

$$\text{Relative density (RD)} = \frac{\text{density of the species}}{\text{sum of the densities from all species}} \times 100$$

$$\text{Abundance (A)} = \frac{\text{number of individuals per species}}{\text{number of quadrats containing the species}}$$

$$\text{Relative abundance (RA)} = \frac{\text{adundance of the species}}{\text{sum of the abundances from all species}} \times 100$$

$$\text{Importance value index (IVI)} = \text{RF} + \text{RD} + \text{RA}$$

$$\text{Relative importance (RI)} = \frac{\text{IVI of the species}}{\text{sum of the IVI from all species}} \times 100$$

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.

2.3 RESULTS AND DISCUSSION

Nitrogen fertilization in winter grazing cover crop positively influenced the amount of residue biomass left on the soil surface prior to no-tillage common bean sowing ($p<0.05$). The residue production in 225 NFR was, on average, 60% higher than the 0 and 75 NFR, although not differing from the 150 NFR (Figure 1). When there is no or less N fertilization (0 – 75 NFR) in the ICLS, the residue production was around 1,900 kg DM ha^{-1}, in contrast to 3,000 kg DM ha^{-1} in the highest fertilization rate.

The potential response of pasture production to N is well-known across scientific studies, and it was confirmed by Assmann et al. (2004) conducting an ICLS field experiment also in Midwestern Parana. They observed a linear N responsive potential of the same winter pasture (annual ryegrass and black oat), up to 300 kg N ha^{-1}. For instance, the 200 kg N ha^{-1} rate (close to the present study maximum rate) presented and increment of 25% in pasture total dry matter production and 74% in amount of residue at the end of grazing period compared to no nitrogen application.

This result points to the seedbank size (number of seeds m^{-2}) that was also affected by the nitrogen fertilization in winter ($p<0.05$). The seedbank in 0 NFR was almost three times bigger than the 225 NFR, an average of 3,060 in contrast to 1,076 seeds m^{-2}, respectively (Figure 1). Nevertheless, both 0 and 225 did not differ from the intermediate nitrogen rates of 75 and 150 kg N ha^{-1}.

Considering that the highest nitrogen fertilization rate in winter grazing cover crop provided more residue biomass (straw) and a smaller weed seedbank was observed, the amount of residue left in the soil surface is an important factor regulating weed dynamics in ICLS. Kelton et al. (2011) findings indicated that the inclusion of high residue cover crops into a conservation tillage system can reduce weed seeds within the upper 7.6 cm of the soil seedbank. In recent studies on ICLS regarding the grazing effect on weeds demonstrated that in moderate grazing intensities (moderate forage allowance) there is less weed pressure, i.e. fewer weed emergence (Lustosa et al., 2016; Shuster et al., 2016; Schuster et al. 2018; Schuster et al., 2019) and smaller weed seedbank (Shuster et al. 2016; Schuster et al. 2018). They attributed this response to amount of straw resulted from the different sward heights (grazing intensities) that provides a physical barrier to weed emergence.

Regarding weed emergence, the effects of N fertilization in winter and presence/absence of residue were different over the common bean cropping cycle (Table 1, Figure 2). At 15 DAE, there was no significant effect of the residue on the weed seedling density, when a suppressive effect of the winter cover crop residue was expected (Chauhan et al., 2012; Osipitan et al., 2018). Especially in the 0 NFR, a high weed density was observed where there was residue. However, 73% of the emerged seedlings in this treatment were annual ryegrass, the cover crop species. As the spikelets of annual ryegrass, which contain the seeds, remain in the residue from one season to another, the residue itself contributed to seedlings emergence, and thus, no different was observed in the presence or absence of straw.

Weed density was affected by the different nitrogen fertilization rates in winter at 15 DAE ($p<0.05$). The 0 NFR presented an average of 208 pl. m^{-2}, more than 17 pl. m^{-2} observed in the 225 NFR, although not differing from 75 and 150 NFR. This same effect of the nitrogen fertilization was observed at 30 DAE ($p<0.05$).

The positive suppressive effect of the residue was observed at 30 DAE ($p<0.01$). This effect was more pronounced in the 0 and 75 NFR, where weed emergence was, on average, 55% and 30% lower in the areas with residue, respectively.

At 45 DAE, when the common bean crop has already reached canopy closure, no difference was observed regarding both studied factors. At 60 DAE, there was a difference in weed density according to the presence or absence of residue ($p<0.05$), an average of 6 and 15 pl. m^{-2}, respectively. Despite the difference, this last evaluation presented an overall low weed emergence.

Throughout the weed seedling emergence assessments, the main effects of treatments were observed at 15 and 30 DAE, the interval that comprises the critical weed free period (CWFP) for the common bean crop in Brazilian Subtropical conditions, according to Kozlowski et al. (2002). The suppressive effect of residue was more visible in the conditions of low nitrogen fertilization rates in winter (0 and 75 kg N ha^{-1}), since there was a higher incidence of weeds in these conditions.

In highest nitrogen fertilization rate (225 kg N ha^{-1}), lower weed pressure was observed (weed seedbank and weed emergence in summer), not only considering the effect of higher amount of residue but also the fact that an adequate nutrition of the winter grazing cover crop influence the competitiveness of the whole system.

For instance, the grazing cover crop itself compete with weeds emerged in winter and early spring, since it remains in the field at least from July to November. In Cornelius and Bradley (2017) 3-year field research on cover crops, annual ryegrass reduced winter annual weed emergence in 50% relative to the nontreated control. They attributed this suppressive effect of annual ryegrass, among other winter cover crops (cereal rye and winter wheat), to some characteristics of which faster emergence and growth, and greater percent of ground cover are positively influenced by nitrogen fertilization, especially under grazing condition (Assmann et al. 2004; Pellegrini et al., 2010).

Additionally, in this ICLS long-term experiment, there is a residual effect of the winter fertilization in summer crops yields, according to Müller (2015) and Pacentchuk (2016) researches on maize and common bean, respectively. Thus, better nutritional conditions enhance the crops capacity to compete with weeds.

Finally, in terms of the weed community composition, sixty-six species were identified in the seedbank assessment, although only twelve presented more than 1% of global relative abundance (considering all samples) (Table 2). Together these twelve species represented almost 85% of the seedbank. In agroecosystems, the seedbank is mainly composed by few weedy species (Haring and Flessner, 2018), e.g., in Maqsood et al. (2018) research, four species contributed about 70% of total weed seedbank. There was no difference among NFR regarding richness, Shannon's diversity index and evenness index (Table 3).

The emerged weed community composition also did not significantly vary among treatments. Eighteen species were identified, of which seven presented a relative importance above 5% at least in one of the four evaluation dates: *Lolium multiflorum*, *Urochloa plantaginea*, *Commelina benghalensis*, *Digitaria horizontalis*, *Borreria latifolia*, *Richardia brasiliensis*, *Euphorbia heterophylla* (Table 4). These species were also among the most abundant in the weed seedbank, except for *C. benghalensis*, that also spreads by vegetative reproduction.

Considering that nitrogen regulates aspects related to the system competitiveness, e.g. growth rate and biomass production, in this study nitrogen fertilization in winter was not characterized as a strong ecological filter to the weed community, such as herbicide application and cropping sequence for example.

In resume, under a weed management perspective, applying 225 kg N ha^{-1} in winter grazing cover crop provides less weed pressure in ICLS. Additionally, grazing cover crop residue plays an important role when considering an integrated weed management in ICLS under subtropical conditions.

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2.5 FIGURES AND TABLES

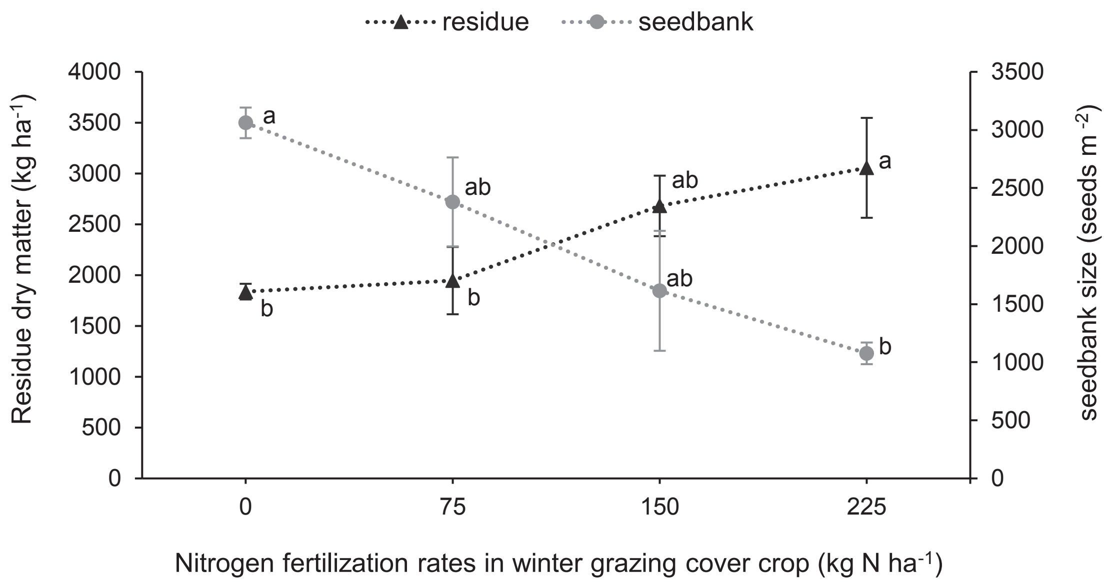

Figure 1. Amount of grazing cover crop (*Avena strigosa* and *Lolium multiflorum*) residue dry matter (kg ha^{-1}) and weed seedbank size (seeds m^{-2}) prior to common bean (*Phaseolus vulgaris*) crop seeding, according to the nitrogen fertilization rates in winter grazing cover crop (0, 75, 150 and 225 kg N ha^{-1}). Points represent means, and error bars represent standard error of the mean. Same lower-case letters regarding the same dependent variable do not differ by Tukey test a 5% level of significance.

Table 1. Resume from the statistical analysis of the weed seedling emergence density throughout the common bean (*Phaseolus vulgaris*) growing season – 15, 30, 45 and 60 days after emergence (DAE), according to Tukey test.

Nitrogen fertilization rate in winter (kg N ha^{-1})	Analysis of weed seedling emergence density							
	15 DAE		30 DAE		45 DAE		65 DAE	
	without[1]	with	without	with	without	with	without	with
0	Aa[2]	Aa	Aa	Ba	Aa	Aa	Aa	Ba
75	Aab	Aab	Aab	Bab	Aa	Aa	Aa	Ba
150	Aab	Aab	Aab	Bab	Aa	Aa	Aa	Ba
225	Ab	Ab	Ab	Bb	Aa	Aa	Aa	Ba

[1] Absence (without) and presence (with) of grazing winter cover crop residue.

[2] The same lower-case letters in a column do not differ by Tukey test a 5% level of significance. The same capital letters in a line, at each evaluation date (15, 30, 45 and 60 DAE), do not differ by Tukey test a 5% level of significance.

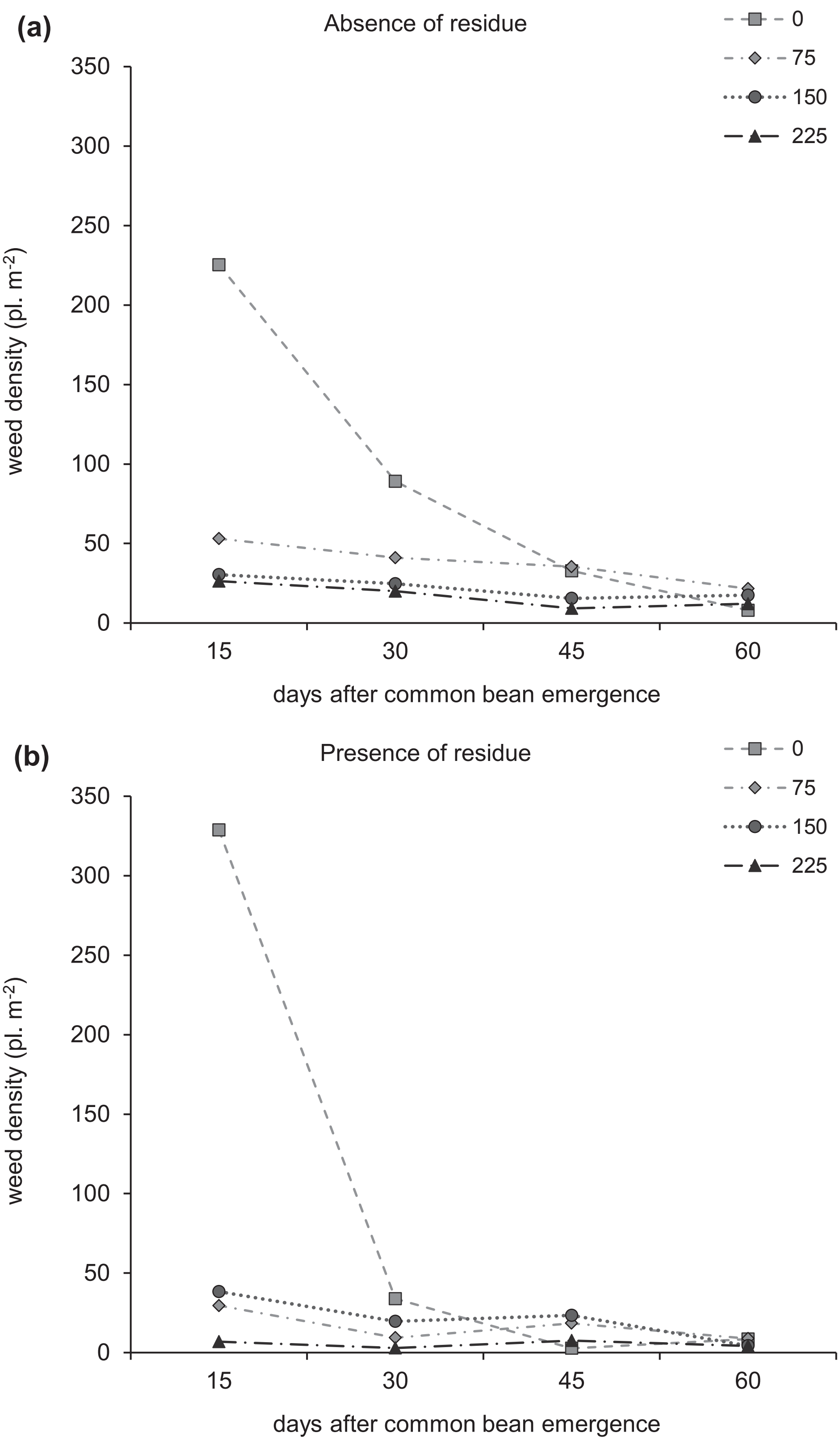

Figure 2. Weed seedling emergence density (plants m^{-2}) over the common bean (*Phaseolus vulgaris*) growing season in 2016/2017 summer - 15, 30, 45 and 60 days after emergence (DAE), according to the nitrogen fertilization rates in winter grazing cover crop (0, 75, 150 and 225 kg N ha^{-1}) in the absence (a) and presence (b) of residue on the soil surface.

Table 2. Species identified in the weed seedbank that presented more than 1% of global relative abundance (GRA)*, and their respective families and groups of flowering plants.

Scientific name	Authority	Family	Group	GRA (%)
Gamochaeta purpurea *(syn. Gnaphalium spicatum)*	(Linnaeus) Cabrera	Asteraceae	dicot	26.3
Richardia brasiliensis	(Moquin-Tandon) Gomes	Rubiaceae	dicot	12.9
Lolium multiflorum	Lamarck	Poaceae	monocot	10.8
Veronica arvensis	Linnaeus	Plantaginaceae	dicot	7.8
Digitaria horizontalis	Willdenow	Poaceae	monocot	6.8
Borreria latifolia *(syn. Spermacoce latifolia)*	(Aublet) Schumacher	Rubiaceae	dicot	6.5
Commelina benghalensis	Linnaeus	Commelinaceae	monocot	3.2
Hypochaeris brasiliensis	Grisebach	Asteraceae	dicot	2.8
Cyperus spp.	-	Cyperaceae	monocot	2.6
Sisyrinchium fasciculatum	Klatt	Iridaceae	monocot	2.1
Stellaria media	(Linnaeus) Villars	Caryophyllaceae	dicot	1.5
Urochloa plantaginea	(Link) Hitchcock	Poaceae	monocot	1.3

*Global relative abundance calculated considering all seedbank samples.

Table 3. Richness (S), Shannon's diversity index (H) and Evenness index (J) of the weed seedbank community according to the nitrogen fertilization rates (NFR) in winter grazing cover crop (0, 75, 150 and 225 kg N ha^{-1}).

Nitrogen fertilization rate in winter (kg N ha^{-1})	Richness (S)	Shannon's diversity index (H)	Evenness index (E)
0	20 ± 4 ^{ns}	2.21 ± 0.21 ^{ns}	0.75 ± 0.04 ^{ns}
75	22 ± 2 ^{ns}	2.29 ± 0.16 ^{ns}	0.75 ± 0.03 ^{ns}
150	23 ± 3 ^{ns}	2.38 ± 0.25 ^{ns}	0.76 ± 0.05 ^{ns}
225	19 ± 3 ^{ns}	2.00 ± 0.28 ^{ns}	0.69 ± 0.07 ^{ns}
average	21	2.22	0.74

^{ns} Means do not differ by the F-test ($p > 0.05$).

Table 4. Species identified in the weed seedling emergence, in 2016/2017 common bean growing season, their respective families and relative importance (RI) at each evaluating date -15, 30, 45 and 60 days after common bean (*Phaseolus vulgaris*) emergence.

scientific name	Authority	Family	RI (%)			
			15*	30	45	60
Bidens pilosa	Linnaeus	Asteraceae	2.2	1.6	2.7	1.7
Borreria latifolia (syn. *Spermacoce latifolia*)	(Aublet) Schumacher	Rubiaceae	3.1	10.3	7.6	8.3
Urochloa plantaginea	(Link) Hitchcock	Poaceae	30.1	13.3	19.0	7.2
Chamaesyce hyssopifolia	(Linnaeus) Small	Euphorbiaceae	-	1.7	0.7	-
Commelina benghalensis	Linnaeus	Commelinaceae	9.0	14.9	21.5	20.0
Desmodium sp.	-	Fabaceae	-	-	1.0	-
Digitaria horizontalis	Willdenow	Poaceae	10.7	15.7	10.7	8.6
Eleusine indica	(Linnaeus) Gärtner	Poaceae	-	1.3	-	-
Conysa spp.	-	Asteraceae	-	-	0.7	-
Euphorbia heterophylla	Linnaeus	Euphorbiaceae	5.6	3.2	7.6	2.9
Galinsoga Parviflora	Cavanilles	Asteraceae	0.4	-	-	-
Gamochaeta purpurea (syn. *Gnaphaluim spicatum*)	(Linnaeus) Cabrera	Asteraceae	0.4	-	-	-
Ipomea spp.	-	Convolvulaceae	4.0	1.5	4.5	4.2
Lolium multiflorum	Lamarck	Poaceae	28.5	24.9	13.7	41.9
Richardia brasiliensis	(Moquin-Tandon) Gomes	Rubiaceae	4.7	7.8	7.1	5.3
Senecio brasiliensis	(Spreng.) Less.	Asteraceae	0.4	-	-	-
Sida rhombifolia	Linnaeus	Malvaceae	1.0	1.2	1.5	-
Sonchus oleraceus	Linnaeus	Asteraceae	-	2.5	1.7	-

*Days after common bean crop emergence (DAE)

CHAPTER III

DIVERSIFICATION OF LOWLAND RICE CROPPING THROUGH INTEGRATED-CROP LIVESTOCK SYSTEMS IMPACTS TARGET SPECIES IN WEED SEEDBANK

[1] This manuscript is presented according to the *Pesquisa Agropecuária Brasileira* Journal guidelines

DIVERSIFICATION OF LOWLAND RICE CROPPING THROUGH INTEGRATED-CROP LIVESTOCK SYSTEMS IMPACTS TARGET SPECIES IN WEED SEEDBANK

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 in rice crop. Diversifying crop rotations through integrated crop-livestock systems (ICLS) can be an alternative to face this challenge. As weed seedbank reflects management's 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 germinable seedbank was assessed in the fourth experimental year, Oct-2016, at three soil depths (0-5, 5-10 and 10-20 cm). 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. In a mid-term temporal scale, the diversification of paddy field through ICLS do not affect weed seedbank size. Lowland ICLS designs that comprises the integration of summer crops with grazing winter cover crops decrease the proportion of Cyperaceae weed species (*Fimbristylis* spp., *Eleocharis* spp., *Cyperus* spp. and *Bulbostylis capillaris*) 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.

Keywords: Rice-based cropping system. irrigated rice. crop-livestock integration.

3.1 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) (SOSBAI, 2018). The majority of Brazilian production of rice is located in Southern States lowlands (65~70%), corresponding to more than one million hectares. These areas are characterized by irrigated rice fields, which present high yields, around 7 Mg ha-1 (CONAB, 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 weeds species from Brazilian paddy fields have been reported as herbicide-resistant: *Echinochloa* spp., *Sagittaria montevidensis* Cham. & Schltdl., *Fimbristylis miliacea* (L.) Vahl., *Cyperus difformis* L. and *Cyperus iria* L. (SOSBAI, 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 the 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-stablished weeds, associated to practices from a single crop, to face different competitive conditions (Koochek et al., 2009).

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); Shuster et al. (2016); Schuster et al. (2018) and Schuster et al. (2019). 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 (Moraes 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 (Chauhan and

Johnson, 2010), 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.

3.2 MATERIAL AND METHODS

The study was carried out in the fourth year of a long-term ICLS experiment located on a 18-ha filed 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 ºC 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^{-1}, 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.) Merril], 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. The other treatments comprise different ICLS designs: 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, diversification and tillage aspects of each system are summarized in Table 1 and Table 2, respectively. 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.

For the winter cover crops, sowing was performed in April at 30, 3 and 6 kg ha^{-1} 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^{-1}, 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 3. Seeding and harvest dates are summarized in Table 1 APPENDIX.

For grazing, neutered male steers (Angus) approximately 10 months old and weighing approximately 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 (Mott and Lucas, 1952), 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 1 – APPENDIX).

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 15-

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 44x38 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 (Thompson et al., 1997) 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 0ºC, and the maximum temperature was 38ºC.

Emerged seedlings were periodically identified, counted and removed from the plastic trays. Seedling identification was conducted based on Kissmann and Groth (1997), Lorenzi (2008) and Lorenzi (2014) descriptions. At this early growth stage, some seedlings, especially from the Cyperacea 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 growth until identification was possible. Total counts for all species were summed to calculate weed seedbank size (number seed m^{-2}).

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), according to Kent and Coker (1992):

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

$$J = \frac{H}{\log{(S)}}$$

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.

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

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

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:

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

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.

3.3 RESULTS AND DISCUSSION

Seedbank size did not differ among treatments. On average, 50 ± 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 et al. 2015).

In contrast, soils from the other cropping systems presented most seeds concentrated in the 0-5 cm, which represented, on average, 64 ± 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 et al., 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 2 – APPENDIX). Eleven of these species are monocotyledonous weeds (monocots), of which five belong to the Cyperaceae and four to the Poaceae families. In all treatments, monocots were predominant among the five most numerous species composing the weed seedbank (Table 4 and 5). The proportion of monocots seeds in the seedbank did not vary among cropping systems, which was 81% ± 16% on average (Figure 2).

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 et al. (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 significant different. However, soil depth affected weed species, richness, with a higher number of species in the topsoil layer (0-5 cm) (Table 6). 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 4 and 5). 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 ± 3% and 8 ± 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 ± 8%) are due to soil disturbance and monocropping. Cyperaceae species are among the most important weeds in conventional rice cropping systems (Ulguim et al., 2018; SOSBAI, 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 et al., 2018). In this context, inversion of soil layers constantly feeds the soil seedbank at every tillage operation. In agreement to that, Singh et al. (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 (SOSBAI, 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 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 et al., 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.) account for 35% of seeds at the 0-5 cm soil depth (Table 5). As annual bluegrasss is a winter grassy weed, there is a lower number of seeds from summer weed species in this soil in T3.

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 ± 1 Mg ha^{-1}. As highlighted by Schweizer e 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.

3.4 CONCLUSION

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.

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3.6 TABLES

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

Lowland rice-based cropping systems	Treatment abbreviation	Crop succession							
		winter 2013	summer 2013/2014	winter 2014	summer 2014/2015	winter 2015	summer 2015/2016	winter 2016	summer 2016/2017
Rice monocropping	T1	fallow period[1]	Irrigated rice	fallow period	Irrigated rice	fallow period	Irrigated rice	fallow period	Irrigated rice
Rice-beef cattle integration	T2	Annual ryegrass[2]	Irrigated rice	Annual ryegrass[2]	Irrigated rice	Annual ryegrass[2]	Irrigated rice	Annual ryegrass[2]	Irrigated rice
Rice-soybean–beef cattle integration	T3	Annual ryegrass[2]	Soybean	Annual ryegrass[2]	Irrigated rice	Annual ryegrass[2]	Soybean	Annual ryegrass[2]	Irrigated rice
Rice-sudangrass-soyabean-maize-beef cattle integration	T4	Annual ryegrass + white clover[2]	Sudangrass	Annual ryegrass + white clover[2]	Soybean	Annual ryegrass + white clover[2]	Maize	Annual ryegrass + white clover[2]	Irrigated rice
Rice-beef cattle integration in cultivated and natural grassland	T5	Annual ryegrass + white clover + birdsfoot trefoil[2]	natural grassland[3]	Annual ryegrass + white clover + birdsfoot trefoil[2]	natural grassland[2]	Annual ryegrass + white clover + birdsfoot trefoil[2]	natural grassland[2]	Annual ryegrass + white clover + birdsfoot trefoil[2]	natural grassland[2]

[1] Spontaneous vegetation

[2] Grazed by bovine steers

[3] In the first summer season of this treatment, grazing was not performed because natural grassland (succession field) was not well-stablished yet.

Adapted from Martins et al. (2017).

Table 2. Soil tillage and rates of soil disturbance, spatial-temporal diversification and temporal frequency of rice crop in the five-lowland rice-based cropping systems (T1, T2, T3, T4 and T5).

Lowland rice-based cropping systems	Treatment abbreviation	Soil tillage	Soil disturbance	Spatial-temporal diversification	Temporal frequency of rice crop
Rice monocropping	T1	Disking	+ + + +	-	+ + +
Rice-beef cattle integration	T2	No-till	+ +	+	+ + +
Rice-soybean–beef cattle integration	T3	No-till	+ +	+ +	+ +
Rice-sudangrass-soyabean-maize-beef cattle integration	T4	No-till	+ +	+ + +	+
Rice-beef cattle integration in cultivated and natural grassland	T5	No-till	+	+ + + +	+

Adapted from Denardin et al. (2018).

Table 3. Fertilization rates of N-P_2O_5-K_2O (kg ha^{-1}) in winter and summer for the five-lowland rice-based cropping systems (T1, T2, T3, T4 and T5), over the experimental years (from 2013 to 2016).

CS	N - P2O5 - K20 (kg ha^{-1})						
	winter 2013	summer 2013/2014	winter 2014	summer 2014/2015	winter 2015	summer 2015/2016	winter 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

Abbreviations: CS – 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; T5 - rice-beef cattle integration in cultivated and natural grassland. Adapted from Denardin et al. (2018).

Table 4. Relative abundance of the five most numerous weed species in the soil seed bank 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.

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

Table 5. Relative abundance of the five most numerous weed species in the soil seed bank 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.

Scientific name	Family	Group of flowering plants	Relative abundance (%)			
			T2	T3	T4	T5
Fimbristylis spp.	Cyperaceae	monocot	8.5	8.8	4.2	13.1
Sagittaria spp.	Alismataceae	monocot	57.0	9.9	3.7	-
Urochloa plantaginea	Poaceae	monocot	-	-	64.9	-
Cyperus spp.	Cyperaceae	monocot	6.8	-	-	14.2
Echinochloa crus-galli	Poaceae	monocot	1.3	-	-	8.4
Eleusine indica	Poaceae	monocot	-	-	10.5	-
Eleocharis spp.	Cyperaceae	monocot	5.3	-	-	8.4
Ludwigia leptocarpa	Onagraceae	dicot	-	-	-	8.6
Ludwigia octovalvis	Onagraceae	dicot	-	21.6	-	-
Poa annua	Poaceae	monocot	-	34.3	-	-
Silene gallica	Caryophyllaceae	dicot	-	7.0	-	-
Sisyrinchium fasciculatum	Iridaceae	moncot	-	-	2.9	-

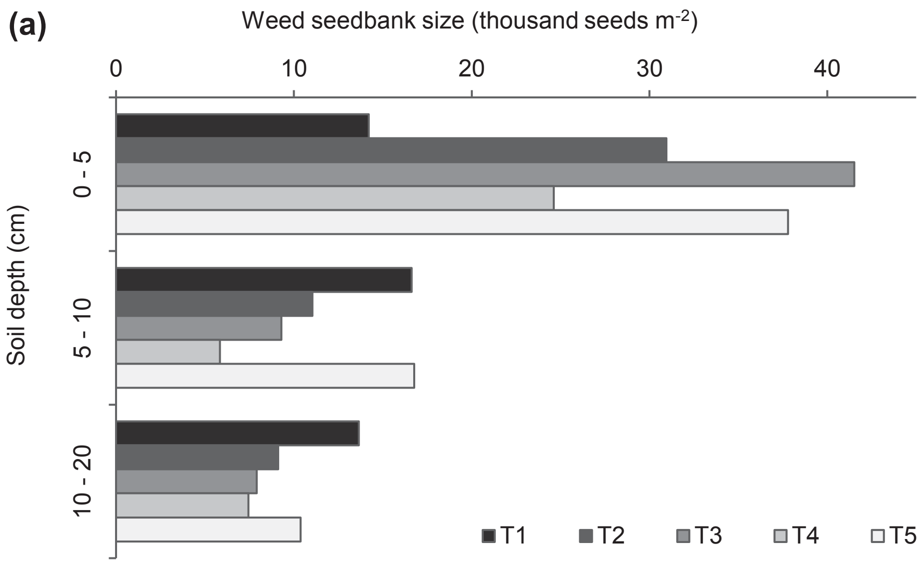
Note: Only the most abundant species of the 0-5 cm depth was presented for T2, T3, T4 and T5, considering that seeds in the soil of the ICLS treatments are concentrated in the topsoil layer. Abbreviations: T1 – rice monocropping, T2 – rice-beef cattle integration; T3 – soybean-rice–beef cattle integration; T4 – Sudan grass-soybean-maize-rice-beef cattle integration; T5 - rice-beef cattle integration in cultivated and natural grassland. Means in the same line followed by different letters differ by the Tukey ($p<0.05$).

Table 6. Richness (S), Shannon's diversity index (H) and evenness index (J) of the weed seedbank from the five-lowland rice-based cropping systems (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

Abbreviations: CS – 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; T5 - rice-beef cattle integration in cultivated and natural grassland. Means in the same line followed by different letters differ by the Tukey ($p<0.05$).

3.7 FIGURES



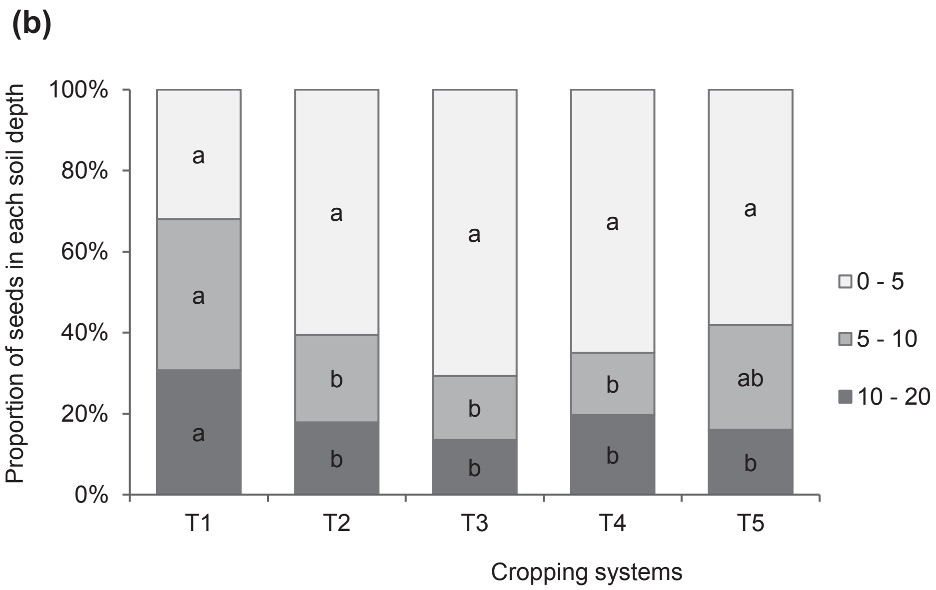


Figure 1. (a) Weed seedbank size (thousand seeds m^{-2}) according to treatments (T1, T2, T3, T4 and T5) and soil depths (0-5, 5-10, 10-20 cm); and (b) relative distribution (percentage) of seeds in each soil depth for each treatment. Columns represent means. Lower-case letters compare soil depths in each treatment. Same letters do not differ significantly by the Tukey test ($p > 0.05$). Abbreviations: T1 – rice monocropping, 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.

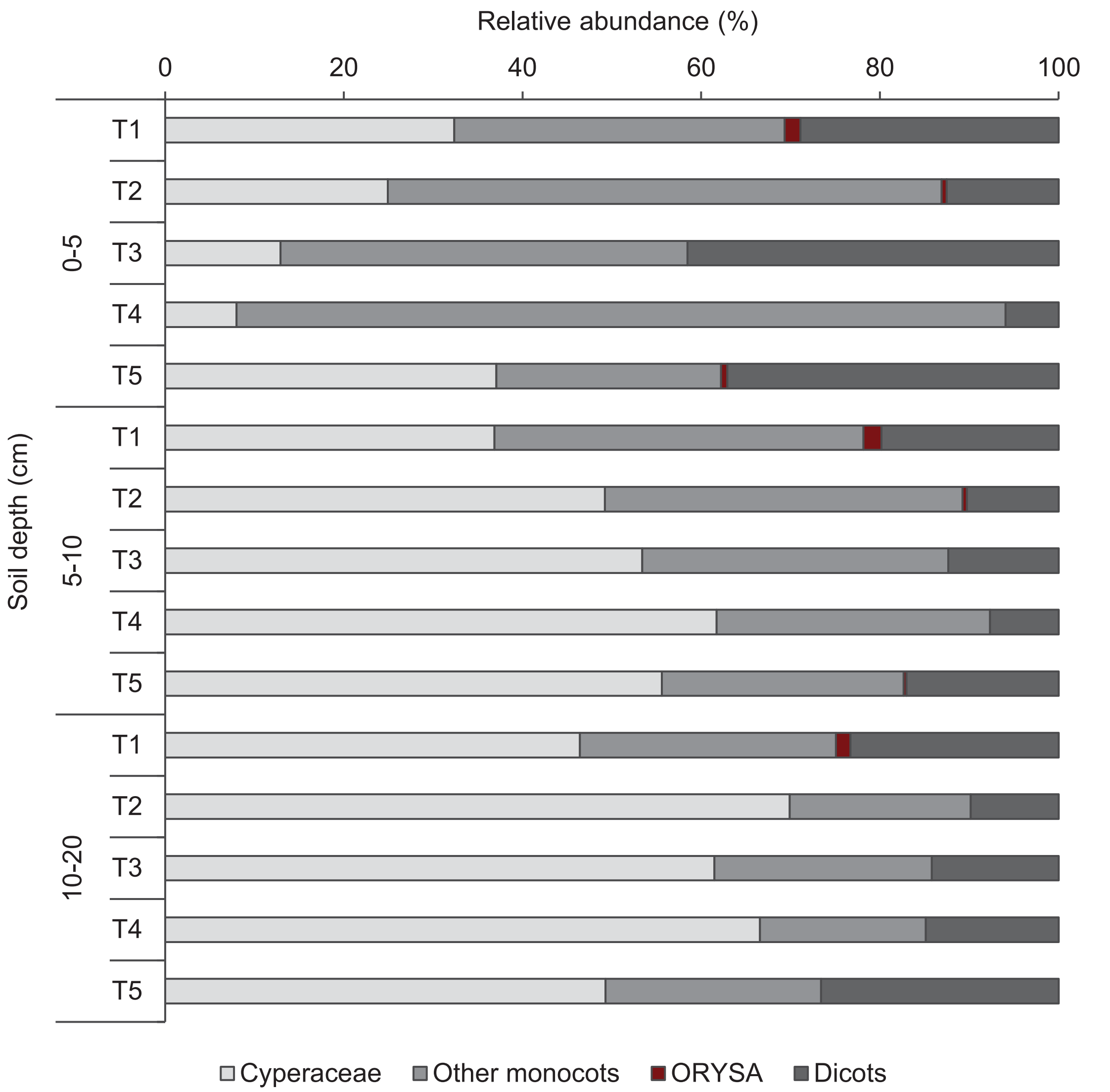


Figure 2. Relative abundance of Cyperaceae weeds, other monocotyledonous weed species and dicotyledonous weeds in the soil seedbank according to treatments (T1, T2, T3, T4 and T5) and soil depths (0-5, 5-10, 10-20 cm), with indication of presence of weedy rice (*Oryza sativa* L. – ORYSA). Abbreviations: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

3.8 APPENDIX

Table 1. 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	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

*T1 – rice monocropping, 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.

Table 2. Species identified the weed seedbank (all samples) and their respective EPPO Codes, families, groups of flowering plants, type of reproduction, life cycle, number of plots containing the species (N plots), global relative abundance (calculated considering all seedbank samples).

EPPO code	Scientific name	Authority	Family	Group of flowering plants	Reproduction	Life cycle	N sampless	Relative abundance (%)
1SAGG	*Sagittaria* spp.	-	Alismataceae	monocot	seeds / rhizomes / tubers	annual / perennial	41	14.62
1FIMG	*Fimbristylis* spp.	Vahl	Cyperaceae	monocot	seeds	annual / perennial	44	12.86
ELOSS	*Eleocharis* spp.	R. Brown	Cyperaceae	monocot	seeds / rhizomes	perennial	43	9.51
LUDOC	*Ludwigia octovalvis*	(Jacquin) Raven	Onagraceae	dicot	seeds	perennial	26	8.35
CYPSS	*Cyperus* spp.	Linnaeus	Cyperaceae	monocot	seeds / bulbs	annual / perennial	43	7.85
BRAPL	*Brachiaria plantaginea* (syn. *Urochloa plantaginea*)	(Link) Hitchcock	Poaceae	monocot	seeds	annual	28	7.30
POAAN	*Poa annua*	Linnaeus	Poaceae	monocot	seeds	annual	28	6.47
LUDLE	*Ludwigia leptocarpa*	(Nuttall) Hara	Onagraceae	dicot	seeds / stems	annual / perennial	19	3.94
ECHCG	*Echinochloa crus-galli*	(Linnaeus) Palisot de Beauvois	Poaceae	monocot	seeds	annual	37	3.18
SISSS	*Sisyrinchium fasciculatum*	Klatt	Iridaceae	monocot	seeds / bulbs	perennial	42	3.09
ECLAL	*Eclipta prostrata* (syn. *Eclipta alba*)	(Linnaeus) Linnaeus	Asteraceae	dicot	seeds	annual	35	2.16
STEME	*Stellaria media*	(Linnaeus) Villars	Caryophyllaceae	dicot	seeds	annual	30	2.05
COPDI	*Lepidium didymum* (syn. *Coronopus didymus*)	Linnaeus	Brassicaceae	dicot	seeds	annual	40	1.96
ELEIN	*Eleusine indica*	(Linnaeus) Gärtner	Poaceae	monocot	seeds	annual / perennial	31	1.56
BULCA	*Bulbostylis capillaris*	(Linnaeus) C.B.Clarke	Cyperaceae	monocot	seeds	annual	17	1.27
SILGA	*Silene gallica*	Linnaeus	Caryophyllaceae	dicot	seeds	annual	9	1.19
1POLG	*Polygonum sp.*	Linnaeus	Polygonaceae	dicot	seeds / stems / rhizomes	annual / perennial	16	1.04
VERPG	*Veronica peregrina*	Linnaeus	Plantaginaceae	dicot	seeds	annual	23	1.02

Continued next page

Table 1 (continued)

RCHBR	*Richardia brasiliensis*	(Moquin-Tandon) Gomes	Rubiaceae	dicot	seeds	annual	19	0.71
PANDI	*Panicum dichotomiflorum*	Michaux	Poaceae	monocot	seeds (mainly)	annual / perennial	25	0.59
FACAP	*Facelis retusa*	(Lamarck) Schultz Bipontinus	Asteraceae	dicot	seeds	annual	6	0.58
	(syn. *Facelis apiculata)*							
ORYSA	*Oryza sativa*	Linnaeus	Poaceae	monocot	seeds	annual	15	0.52
GNAPE	*Gamochaeta pensylvanica* (syn. *Gnaphalium pensylvanicum*)	(Willdenow) Cabrera	Asteraceae	dicot	seeds	annual	27	0.51
OXACB	*Oxalis debilis* var. *corymbosa*	(de Candolle) Lourteig	Oxalidaceae	dicot	seeds / bulbs	perennial	15	0.50
	(syn. *Oxalis corymbosa*)							
HETRE	*Heteranthera reniformis*	Ruiz & Pavón	Pontederiaceae	monocot	seeds (mainly)	perennial	14	0.44
PLUSA	*Pluchea sagittalis*	(Lamarck) Cabrera	Asteraceae	dicot	seeds	annual / perennial	27	0.43
SCFDU	*Scoparia dulcis*	Linnaeus	Plantaginaceae	dicot	seeds	annual	24	0.39
SETGE	*Setaria parviflora*	(Poiret) Kerguélen	Poaceae	monocot	seeds	annual	5	0.36
	(syn. *Setaria geniculata*)							
SOVSE	*Soliva sessilis*	Ruiz & Pavón	Asteraceae	dicot	seeds	annual	24	0.35
	(syn. *Soliva pterosperma*)							
1HRYG	*Hypochaeris chillensis*	(Kunth) Britton	Asteraceae	dicot	seeds / roots	annual / biennual	28	0.28
TRFRE	*Trifolium repens*	Linnaeus	Fabaceae	dicot	seeds / stolons	perennial	13	0.23
SINAR	*Sinapis arvensis*	Linnaeus	Brassicaceae	dicot	seeds	annual	14	0.22
CLLAS	*Centella asiatica*	(Linnaeus) Urban	Apiaceae	dicot	seeds / stolons / rhizomes	perennial	15	0.19
LOLMU	*Lolium multiflorum*	Lamarck	Poaceae	monocot	seeds	annual	13	0.15
HXYDE	*Hypoxis decumbens*	Linnaeus	Hypoxidaceae	monocot	seeds / vegetatively	perennial	9	0.13
ERIBO	*Erigeron bonariensis*	Linnaeus	Asteraceae	dicot	seeds	annual	8	0.11
	(syn. *Conyza bonariensis*)							
SOLAM	*Solanum americanum*	Miller	Solanaceae	dicot	seeds	annual	8	0.11

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Table 1 (continued)

AMARE	*Amaranthus retroflexus*	Linnaeus	Amaranthaceae	dicot	seeds	annual	8	0.08
PLATO	*Plantago tomentosa*	Lamarck	Plantaginaceae	dicot	seeds	perennial	7	0.06
MOLVE	*Mollugo verticillata*	Linnaeus	Molluginaceae	dicot	seeds	annual	5	0.05
DIGHO	*Digitaria horizontalis*	Willdenow	Poaceae	monocot	seeds	annual	9	0.05
CERGL	*Cerastium glomeratum*	Thuillier	Caryophyllaceae	dicot	seeds	annual	1	0.04
BOILF	*Borreria latifolia* (syn. *Spermacoce latifolia*)	(Aublet) Schumacher	Rubiaceae	dicot	seeds	annual	2	0.03
SIDRH	*Sida rhombifolia*	Linnaeus	Malvaceae	dicot	seeds	annual / perennial	4	0.02
PASUR	*Paspalum urvillei*	Steudel	Poaceae	monocot	seeds (mainly)	perennial	2	0.02
SONOL	*Sonchus oleraceus*	Linnaeus	Asteraceae	dicot	seeds	annual	5	0.02
AESSS	*Aeschynomene* sp.	-	Fabaceae	dicot	seeds	annual	4	0.02
VEBLI	*Verbena litoralis*	Kunth	Verbenaceae	dicot	seeds	annual / perennial	3	0.02
LOTCO	*Lotus corniculatus*	Linnaeus	Fabaceae	dicot	seeds / stolons / rhizomes	perennial	3	0.01
STAAR	*Stachys arvensis*	(Linnaeus) Linnaeus	Lamiaceae	dicot	seeds	annual	2	0.01
RUMOB	*Rumex obtusifolius*	Linnaeus	Polygonaceae	dicot	seeds / rhizomes	perennial	2	0.01
VEBBO	*Verbena bonariensis*	Linnaeus	Verbenaceae	dicot	seeds	perennial	2	0.01
non-identified								
1CYPF	*Cyperaceae-1*	-	Cyperaceae	monocot	seeds / bulbs	annual / perennial	40	2.88
-	*2-unknown*	-	-	-	-	-	9	0.14
1GRAF-3	*3-Poaceae weed*	-	Poaceae	monocot	-	-	3	0.14
1GRAF-1	*1-Poaceae weed*	-	Poaceae	monocot	-	-	2	0.06
-	*3-unknown*	-	-		-	-	5	0.05
1GRAF-2	*2-Poaceae weed*		Poaceae	monocot	-	-	3	0.01
-	*8-unknown*	-	-	-	-	-	1	0.01
-	*4-unknown*	-	-	-	-	-	1	0.01
-	*5-unknown*	-	-	-	-	-	1	0.01

FINAL CONSIDERATIONS

This thesis aimed to explored different ICLS designs and thus, contribute to the knowledge generation regarding ICLS and weeds. It is important to highlight that it was only possible to accomplish this thesis goals due to a cooperation of different Universities and research institutions. Cooperation is the main key to generate innovative technology about ICLS and their implications. Either if it is for an operational matter, such as maintaining long-term experimental protocols; or exploring data under a holistic view, demanding a transdisciplinary level of research organization, as pointed by Moraes et al. (2014b).

Complex environments, such as ICLS, requires detailed data collection and a detailed description of the systems management so weed dynamics can be approached. Detailed data are especially important for modelling purposes, a trend in weed research.

Regarding all thesis findings, I consider that many other aspects can still be explored, especially around weed seed bank data in ICLS. Briefly, some examples are: assessing the persistent soil seed bank; evaluating other vegetative propagules like tubers, rhizomes and stolons, rather than only seeds; exploring different weed traits that could be linked to different roles within the agroecosystems; seed predation in ICLS; and potential allelopathic effect of the winter cover crop residue.

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