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Nitrogen fertilization as a weed management tool in an integrated crop-livestock system

Abstract – The objective of this work was to evaluate the effect of nitrogen fertilization rates applied to winter forage crops on weed emergence and seed bank patterns, as well as bean crop yield in the summer. The experiment is part of a long-term protocol under an integrated crop-livestock system, with data collected in the 10th year to capture the legacy effect of management. The applied nitrogen fertilization rates were 0, 75, 150, and 225 kg ha^{-1} N. The residual mass of the forage crops (ryegrass and black oat) was quantified before the summer crop (common bean) was planted. The seed bank was collected from 0 to 5.0 cm soil depth in October 2016, and the emerged plants were quantified at 15, 30, 45, and 60 days after bean emergence. The amount of residual biomass was 60% higher with the application of 225 kg ha^{-1} N than without nitrogen fertilization, resulting in a 65% reduction in emerged weeds and an almost threefold smaller seed bank. A reduction in weed infestation was observed up to 30 days after bean emergence. Integrated weed management should consider nitrogen fertilization of winter forage crops as a tool to reduce weed infestation in crop-livestock integration.

Index terms: crop-livestock integration, crop weed control, weed seed bank.

Adubação nitrogenada como ferramenta de manejo de plantas daninhas na integração lavoura-pecuária

Resumo – O objetivo deste trabalho foi avaliar o efeito das taxas de fertilização nitrogenada aplicadas em culturas de cobertura pastejada no inverno sobre a emergência de plantas daninhas e os padrões do banco de sementes, bem como a produção da cultura de feijão no verão. O experimento é parte de um protocolo de longo prazo sob sistema de integração lavoura-pecuária, com dados coletados no 10º ano para capturar o efeito de legado do manejo. As taxas de fertilização nitrogenada aplicadas foram de 0, 75, 150 e 225 kg ha^{-1} de N. A massa residual da cobertura pastejada (azevém e aveia-preta) foi quantificada antes do plantio da cultura de verão (feijão-comum). O banco de sementes do solo foi coletado à profundidade de 0 a 0,5 cm, em outubro de 2016, e as plantas emergidas foram quantificas aos 15, 30, 45 e 60 dias após a emergência do feijão. A quantidade de biomassa residual foi 60% maior com 225 kg ha^{-1} de N do que sem a adubação nitrogenada, tendo resultado na redução de 65% de plantas daninhas emergidas e em um banco de sementes quase três vezes menor. A redução da infestação de plantas daninhas foi observada até 30 dias após a emergência do feijão. O manejo integrado de plantas daninhas deve considerar a adubação nitrogenada das culturas de cobertura de pastagem de inverno como ferramenta para reduzir a infestação de plantas daninhas na integração lavoura-pecuária.

Termos para indexação: integração lavoura-agropecuária, controle cultural de plantas daninhas, banco de sementes de plantas daninhas.

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Introduction

Integrated crop-livestock systems (ICLSs) are sustainable agricultural approaches that combine crop production and livestock farming in the same area through rotation, succession, or intercropping systems. In the Brazilian subtropics, ICLSs are characterized by a within-farm integration of winter-grazed forage crops and summer grain crops under conservation agriculture (Carvalho et al., 2018). These systems were indicated by the Food and Agriculture Organization of the United Nations as a promising alternative to the dichotomy of production and conservation, since many aspects of the ICLS are considered important to the modern concept of sustainable intensified agricultural production (Carvalho et al., 2024). An ICLS may, for example, improve weed control depending on management practices, especially in the pasture phase (Pelissari et al., 2011).

Although from 1994 to 2013, less than 5% of the studies about ICLSs focused on weeds according to the systematic review of Moraes et al. (2014), currently, several researches have evaluated the effect of the grazing intensity of forage crops on the weed community (Lustosa et al., 2016; Schuster et al., 2016, 2019). These works mostly linked the potential suppressive effect of the ICLS on weeds to the biomass production of the winter forage crop.

In weed research under an integrated management approach, weed suppression through cover crops has been gaining more attention due to an increasing number of herbicide-resistant weeds (Osipitan et al., 2018). According to these same authors, important characteristics of cover crops for weed suppression include a high biomass productivity and a persistent residue. Among other effects, the presence of organic mulching (straw/cover crop residue) affects weed dynamics within an agroecosystem, either by acting as a physical barrier to weed emergence and promoting microclimatic changes or by the potential allelopathic effects of some species (Chauhan et al., 2012).

Nitrogen fertilization, especially under grazing conditions, positively influences the biomass production of grass cover crops, such as the winter cereals commonly used in ICLS designs in subtropical Brazil (Assmann et al., 2004). In fact, Blackshaw & Brandt (2008) highlighted that, when weed infestations consist of species known to be highly responsive to higher soil nitrogen levels, fertilizer management strategies should definitely favor crops over weeds.

Due to the lack of information on the ICLS regarding the response of weeds to other aspects of the winter forage crop besides grazing management, as well as to the relationship between the amount of biomass residue and the potential suppressive effect of weeds in this system, it was hypothesized that nitrogen fertilization of the winter forage crop reduces the weed seed bank in an ICLS under conservation agriculture.

The objective of this work was to evaluate the effect of nitrogen fertilization rates applied to winter forage crops on weed emergence and seed bank patterns, as well as bean crop yield in the summer.

Materials and Methods

The study, conducted in the 2016/2017 crop season, was part of a long-term ICLS experiment in the state of Paraná, Brazil (25°23'02"S, 51°29'43", at 1,100 m above sea level). The climate of the site is humid temperate according to Köppen's classification, 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 of the area was classified as a Latossolo Bruno distroférrico, according to Brazilian Soil Classification System (Santos et al., 2018), which is equivalent to an Oxisol.

The long-term experiment was established as an ICLS in the winter of 2006 up to 2017, being carried out in a randomized complete block design with three replicates. Previously, annual grain crops were cultivated in the area. The ICLS design consisted in the integration of a mixture of annual ryegrass (*Lolium multiflorum* Lam.) and black oat (*Avena strigosa* Schreb.) grazed by sheep in the winter season and of the grain crops maize (*Zea mays* L.) and common bean (*Phaseolus vulgaris* L.) in rotation over the years during the summer (Figure 1). The area of the experiment corresponded to 2.4 ha, being divided into paddocks (main plots) with an area of 0.2 ha each.

The treatments consisted of the following four nitrogen fertilization rates applied to the winter forage crops used for grazing: 0, 75, 150, and 225 kg ha^{-1} N.

Sowing both in winter (forage crop for grazing) and summer (grain crops) was performed in a no-tillage system two weeks after desiccation with the glyphosate herbicide, whose recommended rate is 2.5 kg ha^{-1}

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glyphosate. In the 2006 winter, the sown forage crop was annual ryegrass. Then, from 2007 to 2016, the winter cover crop was the mixture of annual ryegrass and black oat. Over the experimental years, the sowing date ranged from May to June, according to weather conditions, at a seeding rate of 60 and 20 kg seeds per hectare of black oat and annual ryegrass, respectively. At sowing time, 50 kg ha^{-1} P_2O_5 and 50 kg ha^{-1} K_2O were applied in the seed furrow. Nitrogen fertilization was carried out based on the treatment rates as topdressing urea in a single application at the beginning of tillering.

The FT Soberano, IPR Graúna, and IPR Tuiuiú common bean cultivars were sown in the summer season between the first and second week of December of 2006, 2008, and 2010 to 2016, respectively, in rows 40 cm apart, aiming to reach a seeding rate of 250,000 plants per hectare. The 30F53 maize hybrid was sown in October, in rows 80 cm apart, to reach a seeding rate around 65–75 thousand plants per hectare. The cultivars of both of these crops were chosen according to the regional recommendation and availability.

In the 2016/2017 crop season, the period in which the present study was carried out, the forage crop (mixture of annual ryegrass and black oat) was grown from May to November, whereas the IPR Tuiuiú common bean cultivar was cultivated from December to March. Other cultural practices were performed according to the technical recommendations for each crop (CTSBF, 2012).

Grazing was forage-based in a continuous stocking system, with a variable number of Ile de France lambs (two to five per paddock) according to the put-and-take method (Allen et al., 2011). The grazing method aimed to maintain sward heights at an average of 14 cm, and the grazing period lasted around four months over the experimental years, beginning in July–August and ending in November.

To estimate the amount of the winter cover crop residue (straw) left above soil surface at the beginning of the summer cropping season, five quadrats of 0.50×0.50 m 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 a constant weight, in order to estimate the kilogram of residue dry matter per hectare.

The seed banks were sampled before the seeding of the summer crop in October 2016, which marked the tenth year of the ICLS experiment described previously. Using a steel 4.8 cm diameter probe, soil samples were collected manually from the top 0.0 to 5.0 cm layer, along three 28 m transects in each experimental unit that were randomly laid out in the central area of each plot. Along each transect, two soil cores were collected at 4.0 m intervals and combined into one 42 core composite sample (14 samples per transect) for each experimental unit.

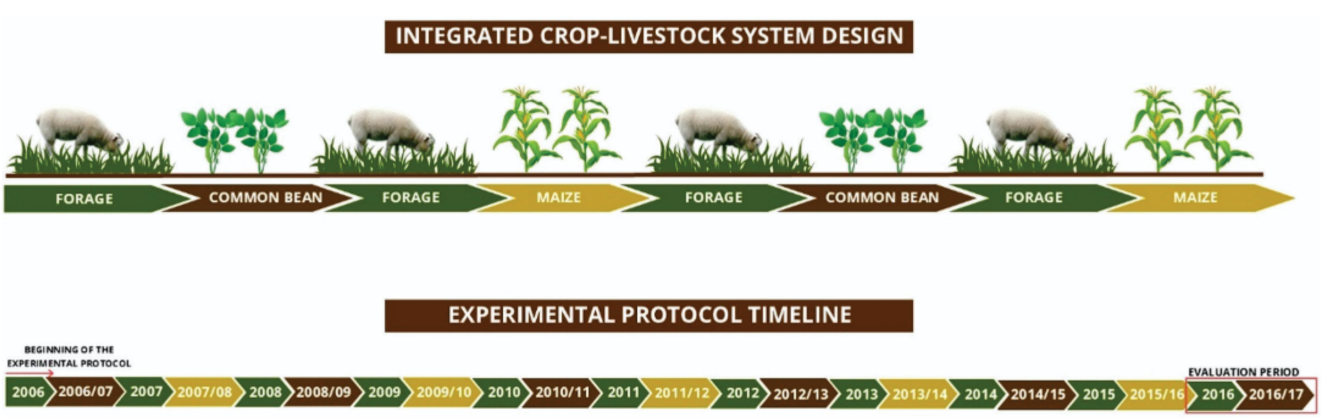

Figure 1. Schematic representation of the integrated crop-livestock system (ICLS) in which the experiment was carried out, showing crop succession over the years. The experimental protocol began in 2006, with the rotation of forage, common bean (*Phaseolus vulgaris*), and maize (*Zea mays*), represented by the colors green, brown, and yellow, respectively. The evaluation period corresponds to the 2016 and 2016/2017 crop years, highlighted in red in the timeline.

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 0.44×0.38 m plastic trays and placed in a greenhouse for 12 months beginning in November 2016. Soil moisture was maintained in the trays using regular sub-irrigation. The seedling emergence method was used to quantify the germinable seeds in the soil seed bank (Thompson et al., 1997), not accounting for dead or dormant seeds (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. The seedling identification was conducted based on the descriptions of Kissmann & Groth (1997) and Lorenzi (2014). To analyze seed bank composition, the species richness index was calculated by counting the number of different species per experimental unit.

Shannon's diversity index and the evenness of the seed bank were estimated according to Kent & Coker (1992). For each species, global relative abundance (GRA), considering all seed bank samples, was calculated, as follows: GRA = (number of counted seeds of the species ÷ total number of seeds found in all seed bank samples) ×100.

In the 2016/2017 common bean growing season, weed emergence was quantified at 15, 30, 45, and 60 days after emergence (DAE) of the common bean crop. For this, five permanent quadrats of 0.50×0.50 m were randomly set in each subplot within the field experiment. Then, at each evaluation date, seedlings were identified, counted, and removed from the permanent quadrats.

With the collected data, the following phytosociological parameters were calculated considering all quadrats at each evaluation date, as described by Dominschek et al. (2019):

Frequency = number of quadrats containing the species ÷ total number of quadrats

Relative frequency = (frequency of the species ÷ sum of the frequencies of all species) ×100

Density = number of individuals per species ÷ total number of quadrats

Relative density = (density of the species ÷ sum of the densities of all species) ×100

Abundance = number of individuals per species ÷ number of quadrats containing the species

Relative abundance = (abundance of the species ÷ sum of the abundances of all species) ×100

Importance value index = relative frequency + relative density + relative abundance

Relative importance = (importance value index of the species ÷ sum of the importance value index of all species) ×100

To estimate the grain yield of the common bean crop, all pods of plants within an area of 4.8 m^2 in each plot were manually sampled and processed in a laboratory. Grain yield was calculated in kg ha^{-1}, and data were reported at 13% moisture content.

The data were analyzed in the R, version 3.4.0, software (R Core Team, 2017). The homogeneity of variances and the normal distribution of residuals (normality assumption) were verified. In highly skewed distributions, the dependent variable was transformed according to the box-cox test (square root or logarithm transformation) to meet the assumptions of inferential statistics. Each evaluated attribute was subjected to the analysis of variance by the F-test with fitted linear models, using the lm function. When significant, means were compared by Tukey's test, at 5% probability, and common bean yield was compared at 10% probability.

Results and Discussion

Nitrogen fertilization of the winter forage crops annual ryegrass and black oat positively influenced the amount of residue biomass left on soil surface prior to the sowing of common bean under no-tillage (Figure 2 A). The residue biomass obtained with 225 kg ha^{-1} N was, on average, 60% higher than that with the rates of 0 and 75 kg ha^{-1} N (3,056 kg ha^{-1} vs. 1,892 kg ha^{-1}).

Other authors also found a positive effect of nitrogen fertilization on winter forage crops. In a three-year field research, Cornelius & Bradley (2017) reported that annual ryegrass reduced weed emergence in 50% compared with the non-treated control, attributing this suppressive effect, as well as that of cereal rye (*Secale cereal* L.) and winter wheat (*Triticum aestivum* L.), to the faster emergence, quicker growth, and greater ground-cover percentage of these forage crops due to nitrogen fertilization, especially under grazing conditions (Assmann et al., 2004; Pellegrini et al., 2010).

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The potential response of pasture production to nitrogen is well known across scientific studies, as the one conducted by Assmann et al. (2004) in an ICLS field experiment also in Midwestern Paraná. The authors found that the same winter cover crops (annual ryegrass and black oat) showed a linear responsive potential to nitrogen up to 300 kg ha^{-1} N under grazing conditions. For the rate of 200 kg ha^{-1} N, close to the maximum one used here, there was an increment of 25% in pasture total dry matter production and of 74% in the amount of residue at the end of the grazing period compared with the treatment without nitrogen fertilization, similarly to the observed in the present work.

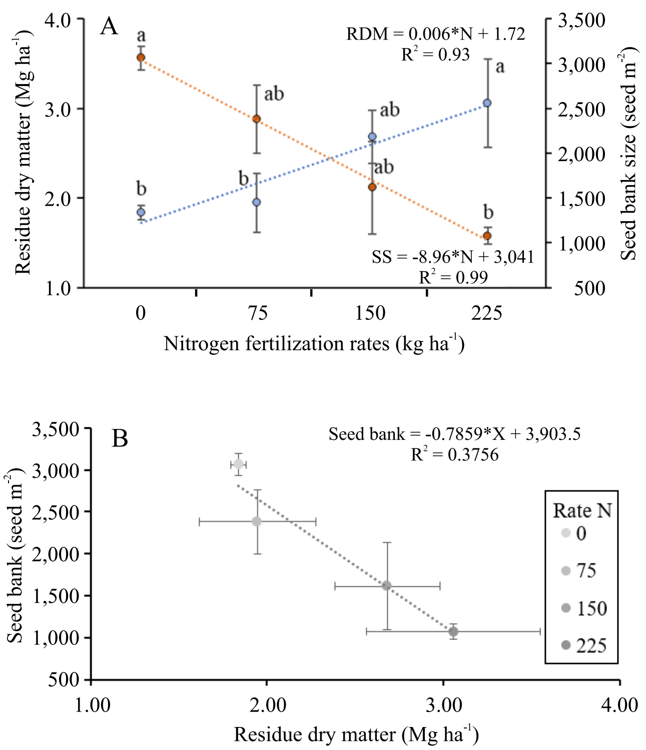

Figure 2. Dry matter residue (RDM) of the forage cover crops black oat (*Avena strigosa*) and ryegrass (*Lolium multiflorum*) and weed seed bank size prior to common bean (*Phaseolus vulgaris*) crop seeding according to the nitrogen fertilization rates (0, 75, 150, and 225 kg ha^{-1} N) applied to the winter forage cover crops (A), as well as the relationship between residual dry matter and seed bank size (B). Points represent means, and error bars represent the standard error of the mean. Equal lowercase letters regarding the same dependent variable do not differ by Tukey's test, at 5% probability.

Seed bank size (number of seeds per square meter) was also affected by nitrogen fertilization in winter. The size of the seed bank was almost three times lower for the rate of 225 kg ha^{-1} N compared with that of 0 kg ha^{-1} N, representing an average of 1,076 in contrast to 3,060 seeds per square meter, respectively, i.e., a ~ 65% reduction. However, the size of the weed seed bank did not differ at the intermediate nitrogen rates of 75 and 150 kg ha^{-1} N.

The highest nitrogen fertilization rate of the winter forage crop resulted in a higher biomass residue (straw) and a smaller weed seed bank, reducing 0.78 weed seeds per square meter in the soil for every 1.0 kg ha^{-1} of residue dry matter (Figure 2 B). Therefore, the amount of residue left on soil surface is an important factor for regulating weed dynamics in the ICLS.

Kelton et al. (2011) concluded that the inclusion of high-residue cover crops in a conservation tillage system can reduce weed seeds within the upper 7.6 cm of the soil seed bank. In recent studies, the amount of straw from the different sward heights (grazing intensities) provided a physical barrier to weed emergence in ICLSs under moderate grazing intensities (moderate forage allowance), resulting in a lower weed pressure, i.e., lower weed emergence (Lustosa et al., 2016; Schuster et al., 2016, 2019) and smaller weed seed bank (Schuster et al., 2016).

For the weed seed bank community, the richness index, Shannon's diversity index, and the evenness index were similar among nitrogen fertilization rates (Table 1). In terms of the composition of the weed community, 66 species were identified, although only

Table 1. Richness index, Shannon's diversity index, and evenness index of the weed seed bank community according to the nitrogen fertilization rates applied to winter cover crops under grazing conditions.

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

[(1)]Means do not differ by the F-test, at 5% probability.

12 presented more than 1% global relative abundance considering all samples (Table 2); together, these 12 species represented almost 85% of the seed bank. In agroecosystems, the seed bank is mainly composed of a few weedy species (Haring & Flessner, 2018), as observed in the research of Maqsood et al. (2018), who found that 4 species contributed about 70% to the total weed seed bank.

The effects of nitrogen fertilization of the winter forage crops on weed emergence differed over DAE of the common bean crop (Table 3). At 15 and 60 DAE, there was significant reduction in weed seedling density, probably because of the suppressive effect of the residue of the winter forage crop (Chauhan et al., 2012; Osipitan et al., 2018). At the beginning of common bean emergence at 15 DAE, weed density at the rate of 225 kg ha^{-1} N was 98% lower than with 0 kg ha^{-1} N due to the positive suppressive effect of the residue. Furthermore, the main effects of treatments were observed at 15 DAE, the interval that comprises the critical weed-free period for the common bean crop under Brazilian conditions (Parreira et al., 2012). However, at 45 and 60 DAE, when the common bean crop had already reached canopy closure, no difference was detected. These last evaluations presented an overall low weed emergence.

The composition of the emerged weed community also did not vary significantly among nitrogen fertilization rates (Table 4). Eighteen species were identified, of which the following 7 presented a

Table 2. Species identified in the weed seed bank that presented more than 1% global relative abundance (GRA), as well as their respective families and group of flowering plants.

Scientific name	Family	Group	GRA (%)[1]
Gamochaeta purpurea (L.) Cabrera (Syn. *Gnaphalium spicatum* Lam.)	Asteraceae	Dicot	26.3
Richardia brasiliensis Gomez	Rubiaceae	Dicot	12.9
Lolium multiflorum Lam.	Poaceae	Monocot	10.8
Veronica arvensis L.	Plantaginaceae	Dicot	7.8
Digitaria horizontalis Willd.	Poaceae	Monocot	6.8
Borreria latifolia (Aubl.) K.Schum. (Syn. *Spermacoce latifolia* Aubl.)	Rubiaceae	Dicot	6.5
Commelina benghalensis L.	Commelinaceae	Monocot	3.2
Hypochaeris brasiliensis Griseb.	Asteraceae	Dicot	2.8
Cyperus spp.	Cyperaceae	Monocot	2.6
Sisyrinchium fasciculatum Klatt	Iridaceae	Monocot	2.1
Stellaria media (L.) Vill.	Caryophyllaceae	Dicot	1.5
Brachiaria plantaginea Hitchc.	Poaceae	Monocot	1.3

[1]Calculated considering all seed bank samples.

Table 3. Density of weed seedling emergence throughout the common bean (*Phaseolus vulgaris*) growing season at 15, 30, 45, and 60 days after crop emergence[1].

Nitrogen fertilization (kg ha^{-1} N)	Number of plants			
	15 DAE	30 DAE	45 DAE	60 DAE
0	328.8aA	33.9aB	2.7aB	8.5aB
75	29.6bA	9.3aA	18.4aA	8.8aA
150	38.4bA	19.7aA	23.5aA	4.3aA
225	6.8bA	2.9aA	7.5aA	4.2aA

[1]Equal lowercase letters in each column do not differ by Tukey's test, at 5% probability, whereas equal uppercase letters, in each column, at each evaluation date (15, 30, 45, and 60 days after emergence), also do not differ by Tukey's test, 5% probability.

relative importance above 5% in at least one of the four evaluation dates: *L. multiflorum*, *Urochloa plantaginea* (Link) R.D.Webster, *Commelina benghalensis* L., *Digitaria horizontalis* Willd., *Borreria latifolia* (Aubl.) K.Schum, *Richardia brasiliensis* Gomez, and *Euphorbia heterophylla* L. These species were also among the most abundant in the weed seed bank, except for *C. benghalensis*, which also spreads by vegetative reproduction.

At the highest nitrogen fertilization rate of 225 kg N ha^{-1}, a lower weed pressure was observed (weed seed bank and weed emergence in the summer), likely due to the effect of a higher amount of residue and also to an adequate nutrition of the winter forage crops, influencing the competitiveness of the whole system. Additionally, in this ICLS long-term experiment, there was a residual effect of winter fertilization on summer crop yields, as also observed in maize by Müller (2015). These findings are an indicative that better nutritional conditions enhance crop capacity to compete with weeds. The forage crops themselves compete with weeds emerged in winter and early spring, since they remain in the field at least from July to November. However, the direct effect of animal grazing on weeds is also factor to be considered in ICLS and should be further explored in future studies.

The obtained results show that nitrogen fertilization of the winter forage crops at 225 kg ha^{-1} N increased common bean yield in 22% in comparison with the treatment without fertilization (Figure 3). Despite this positive finding, nitrogen fertilization was not considered a strong ecological filter to the weed community, compared with herbicide application and cropping sequence, for example, since this nutrient regulates aspects related to the competitiveness of a system, such as growth rate and biomass production. However, other types and levels of fertilization of forage crops prior to the establishment of the summer crop should be evaluated, considering that recent studies have indicated cover crop mixtures as an option for a better weed control (Baraibar et al., 2018).

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

Scientific name	Family	Relative importance (%)			
		15 DAE	30 DAE	45 DAE	60 DAE
Bidens pilosa L.	Asteraceae	2.2	1.6	2.7	1.7
Borreria latifolia (Aubl.) K.Schum. (Syn. *Spermacoce latifolia* Aubl.)	Rubiaceae	3.1	10.3	7.6	8.3
Urochloa plantaginea (Link) R.D.Webster	Poaceae	30.1	13.3	19.0	7.2
Chamaesyce hyssopifolia (L.) Small	Euphorbiaceae	-	1.7	0.7	-
Commelina benghalensis L.	Commelinaceae	9.0	14.9	21.5	20.0
Desmodium sp.	Fabaceae	-	-	1.0	-
Digitaria horizontalis Willd.	Poaceae	10.7	15.7	10.7	8.6
Eleusine indica (L.) Gaertn.	Poaceae	-	1.3	-	-
Conysa spp.	Asteraceae	-	-	0.7	-
Euphorbia heterophylla L.	Euphorbiaceae	5.6	3.2	7.6	2.9
Galinsoga parviflora Cav.	Asteraceae	0.4	-	-	-
Gamochaeta purpurea (L.) Cabrera (Syn. *Gnaphaluim spicatum* Lam.)	Asteraceae	0.4	-	-	-
Ipomea spp.	Convolvulaceae	4.0	1.5	4.5	4.2
Lolium multiflorum Lam.	Poaceae	28.5	24.9	13.7	41.9
Richardia brasiliensis Gomez	Rubiaceae	4.7	7.8	7.1	5.3
Senecio brasiliensis (Spreng.) Less.	Asteraceae	0.4	-	-	-
Sida rhombifolia L.	Malvaceae	1.0	1.2	1.5	-
Sonchus oleraceus L.	Asteraceae	-	2.5	1.7	-

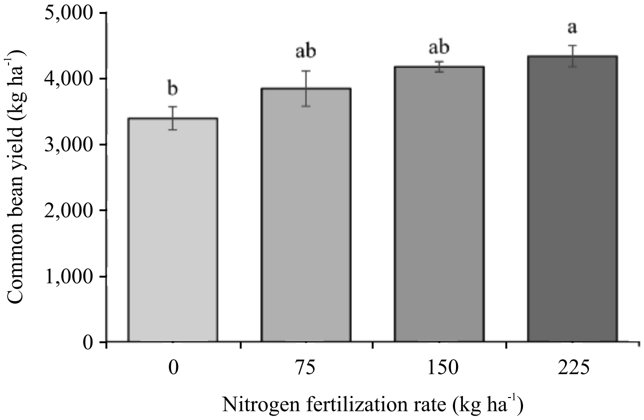

Figure 3. Common bean (*Phaseolus vulgaris*) yield in the summer of 2016/2017 according to the nitrogen fertilization rates (0, 75, 150 and 225 kg ha^{-1} N) applied to the winter forage crops. Equal lowercase letters do not differ by Tukey's test, at 10% probability.

Conclusion

Increasing nitrogen fertilization rates of the winter forage crops black oat (*Avena strigosa*) and ryegrass (*Lolium multiflorum*) results in a lower weed pressure and a higher common bean (*Phaseolus vulgaris*) yield in an integrated crop-livestock system.

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