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***ANIMAL INCLUSION IN PURELY AGRICULTURAL SYSTEMS AND
ANTICIPATION OF FERTILIZATION IN PRODUCTIVITY, RESOURCE USE
EFFICIENCY AND PLANT NUTRIENT STATUS***

Porto Alegre (RS), Brazil

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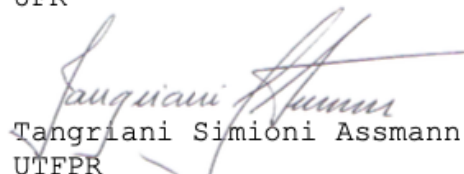
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Animal inclusion in purely agricultural systems and anticipation of fertilization in production, resource use efficiency and plant nutrient status¹

Author: Gustavo Duarte Farias

Advisor: Carolina Bremm

Co-advisor: Paulo Cesar de Faccio Carvalho

Abstract:

The aim of this thesis was to show opportunities and challenges in the integration of soybean crops and sheep (Chapter I); analyze the production dynamics of the system (Chapter II) and, of nutrients together with the nutritional status of Italian ryegrass and soybean plants (Chapter III), both as a result of systems grazed by sheep (ICLS) or non-grazed (cropping system) under different fertilization strategies (system or crop fertilization) in southern Brazil. Well-managed integrated crop-livestock systems can act as an interesting strategy for sustainable intensification, improving food production and security for a better future. In addition, small ruminants can play an important role as part of the integrated systems, mainly by the short time of gestation, high prolificacy, low turnoff age and could be a good alternative to integrated crop-livestock system in small farms. However, re-design the systems on the farm and landscape level, convince producers to use the ICLS based on the argument that the animal is beneficial to the cropland, and not the opposite, and generate more research about this topic can be presented as challenges. Also, in chapter II and III we argue that fertilization practices in ICLS must follow the same integrated approach. To test this, we compared a conventional crop fertilization strategy *versus* a system fertilization approach applied to two production systems being a conventional cropping system and ICLS. The experimental design was completely randomized blocks in a factorial 2 x 2 with four replicates. Results of chapter II demonstrate greater daily herbage accumulation rate (24%; $P < 0.01$) and total herbage production (18%; $P < 0.05$) in the system fertilization compared to conventional crop fertilization. Consequently, system fertilization allowed for greater stocking rates in the pasture phase (17%; $P < 0.05$). The ICLS presented greater equivalent soybean yield ($P < 0.001$), energy production ($P < 0.01$), and system productivity ($P < 0.05$) compared to the cropping system, regardless of fertilization strategies. Soybean yield was not affected by fertilization strategies or grazing. In addition, chapter III shows that Italian ryegrass P content was greater ($P < 0.001$) in system fertilization, regardless of days after Italian ryegrass sowing. For ICLS the content of P in Italian ryegrass was greater after 63 days compared to cropping system ($P < 0.05$). System fertilization presented, on average, 12% greater K content in Italian ryegrass compared to crop fertilization during stocking period ($P < 0.01$). Regarding the animal effect, we observed 14% greater K content, on average, in ICLS when compared to cropping system ($P < 0.01$). For all treatments, the ryegrass data were situated above the reference model %P and %K - %N relationship, indicating that at similar %N plant have a higher %P or %K as expected for their maximum biomass production. The soybean crop presented no effect of grazing, fertilization strategy or its interaction ($P > 0.05$) on P and K contents. In conclusion, the adoption of integrated systems seems to be a necessity for us to have sustainable productive systems. In addition, the results suggest that system fertilization is an evolution to crop fertilization.

Keywords: Integrated crop-livestock system, sheep, mixed system, system fertilization, sustainable intensification

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Inclusão animal em sistemas puramente agrícolas e antecipação da fertilização na produção, eficiência de uso de recursos e status de nutrientes nas plantas.

Autor: Gustavo Duarte Farias

Orientador: Carolina Bremm

Co-orientador: Paulo Cesar de Faccio Carvalho

Resumo:

O objetivo desta tese foi mostrar oportunidades e desafios na integração de lavouras e ovinos (Capítulo I); analisar a dinâmica de produção do sistema (Capítulo II) e, de nutrientes em conjunto com o estado nutricional das plantas de azevém e soja (Capítulo III), ambos como resultado de sistema pastejado por ovinos (SIPA) ou não (sistema de cultivo) sob diferentes estratégias de fertilização (fertilização de sistema ou de cultura) no sul do Brasil. Sistemas de integração lavoura-pecuária bem administrados podem atuar como uma estratégia interessante para a intensificação sustentável, melhorando a produção de alimentos e a segurança para um futuro melhor. Além disso, pequenos ruminantes podem desempenhar um papel importante como parte dos sistemas integrados, principalmente pelo curto tempo de gestação, alta prolificidade, baixa idade de abate e podem ser uma boa alternativa ao sistema de integração lavoura-pecuária em pequenas propriedades. No entanto, redesenhar os sistemas no nível da fazenda e da paisagem, convencer os produtores a usarem o SIPA com base no argumento de que o animal é benéfico para a lavoura, e não o contrário, e gerar conhecimento com pesquisas de longo prazo sobre este tópico pode ser apresentado como desafios. Além disso, nos capítulos II e III, argumentamos que as práticas de fertilização em SIPA devem seguir a mesma abordagem integrada. Para testar isso, comparamos uma estratégia convencional de fertilização de cultivo versus uma abordagem de fertilização de sistema aplicada a dois sistemas de produção, sendo um sistema de cultivo convencional e SIPA. O delineamento experimental foi em blocos casualizados com fatorial 2 x 2 e quatro repetições. Os resultados do capítulo II demonstram maior taxa de acúmulo diário de forragem (24%; $P < 0,01$) e produção total de forragem (18%; $P < 0,05$) no sistema de fertilização em comparação com a fertilização convencional. Conseqüentemente, a fertilização do sistema permitiu maiores taxas de lotação na fase pastagem (17%; $P < 0,05$). O SIPA apresentou maior rendimento equivalente de soja ($P < 0,001$), produção de energia ($P < 0,01$) e produtividade do sistema ($P < 0,05$) em relação ao sistema de cultivo, independentemente das estratégias de fertilização. A produtividade da soja não foi afetada pelas estratégias de fertilização ou pastejo. Além disso, o capítulo III mostra que o teor de P do azevém foi maior ($P < 0,001$) na fertilização de sistema, independentemente dos dias após a semeadura do azevém. Para SIPA, o conteúdo de P no azevém foi maior após 63 dias da semeadura em comparação com o sistema de cultivo ($P < 0,05$). A fertilização do sistema apresentou, em média, 12% a mais de teor de K no azevém em relação à fertilização da cultura durante o período de estocagem ($P < 0,01$). Em relação ao efeito animal, observamos teor de K 14% maior, em média, no SIPA quando comparado ao sistema de cultivo ($P < 0,01$). Para todos os tratamentos, os valores observados na pastagem de azevém estão situados acima do modelo de referência para a relação de %P e %K com %N, indicando que em %N semelhante a planta apresenta %P e %K superior, conforme esperado para sua produção máxima de biomassa. A cultura da soja não apresentou efeito do pastejo, estratégia de fertilização ou sua interação ($P > 0,05$) sobre os teores de P e K. Em conclusão, a adoção de sistemas integrados parece ser uma necessidade para obtenção de sistemas produtivos sustentáveis. Além disso, os resultados sugerem que a fertilização de sistema é uma evolução para a fertilização da cultura.

Palavras-chave: Integração lavoura-pecuária, sistemas mistos, ciclagem de nutrientes, pastejo animal, adubação de sistema

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1. CHAPTER I

Thesis introduction

1.1. Thesis introduction

The social pressure for productive systems to be in tune with nature, that is, producing food without harming the environment or preferably production systems that produce food with environmental benefits, is growing. This has redirected production systems to reintegration between crops and animals in the called integrated crop-livestock system. Animal grazing in these systems has the function, besides the additional food production (meat, milk, and/or wool), to enhance the recycling of nutrients (Franzluebbers et al., 2012; Lemaire et al., 2015). This has a crucial role in maintaining the nutrient circulating between soil-plant-animal for a longer time, which can result in greater production per unit of nutrient in addition to protecting these from possible losses. This is a concern because many nutrients are finite resources and with potential negative environmental impact (Galembeck et al., 2019). In this sense, the possibility of managing the application of fertilizer with the same holistic thinking of integration emerges, that is, enhancing the use of inputs through recycling. Thus, the anticipation of fertilizer in the system fertilization (Assmann et al., 2017) is an attempt to, together with the inclusion of the animal, enhance the recycling of nutrients, increasing production in an environmentally sustainable way.

Based on what has been described, this thesis was divided into three chapters. First, the reader will find a review (Chapter II) characterizing the productive system of the Rio de la Plata region where some opportunities and challenges for the inclusion of sheep in previously purely agricultural systems were discussed. In Chapter III, the reader will verify the impact of the presence or absence of animal grazing and two fertilization strategies on plants and animal productivity temporally distributed on the system. In a third moment (Chapter IV) it will be shown how these productive systems impact the nutrient status of Italian ryegrass (*Lolium multiflorum*) and soybean (*Glycine max*) plants. Closing with some considerations/suggestions for future research to better understand the functioning of processes in more complex systems (Chapter V).

2. CHAPTER II

Opportunities and challenges for sheep integration into croplands in the Rio de la Plata region of South America. A review¹

¹ Manuscript prepared according to the *Small Ruminant Research* journal rules (Appendix 1).

Opportunities and challenges for sheep integration into croplands in the Rio de la Plata region of South America. A review

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Abstract

In addition to the large production of small ruminants, mainly sheep, on grassland ecosystems, Rio de la Plata region allows a singular opportunity to produce a variety of cash crops such as soybean, corn, rice, wheat, forest, orchard and temperate and tropical pastures over the year. However, in general, these cultures are cultivated separately. Thus, the cash crop areas remain just with an unproductive food cover crop in a part of the year, which can be a beneficial management for agronomic attributes, but with an inefficient farmland use. In this way, well-managed integrated crop-livestock systems can act as an interesting strategy for sustainable intensification, improving food production and security for a better future. Therefore, despite being a challenge in that zone, small ruminants can play a very important role as part of the integrated systems, mainly by the short time of gestation, high prolificacy, low turnoff age and could be a good alternative to small farms. Furthermore, animal grazing changes the agroecosystem improving soil physical, chemical and biological parameters. This review presents the implications and challenges of how sheep can be part of integrated systems and the benefits that well-managed pastures can bring to food production with environmental sustainability in Southern America ecosystems.

Keywords: crops, grazing management, Mercosur, sheep; integrated crop-livestock system.

2.1. Introduction

The Rio de la Plata grasslands region occupies approximately 700 thousand km² in South America (between latitudes 28° and 38° S and longitudes 50° W and 61° W) (Baeza and Paruelo, 2020; Soriano et al., 1992), comprising central-eastern Argentina (parts of Buenos Aires, Córdoba, Corrientes, Entre Ríos, La Pampa, Misiones, Santa Fé and San Luis provinces), the entire country of Uruguay and the extreme south of Brazil (Figure 1, Andrade et al., 2018). As one of the largest grassland ecosystems in the Americas (Soriano et al., 1992), climate in the region varies considerably over its extension, with mean annual temperature decreasing southward (from 20 °C to 13 °C) and precipitation decreasing from the northeast to the southwest (from 1800 mm to 400 mm, (Andrade et al., 2018). As a result of this climate gradient, the region is characterized by a huge plant diversity (about 3 to 4 thousand species), with different combinations of C3 and C4 grasses, herbs, shrubs and even forests in some sites, usually along riverbanks (Andrade et al., 2018; Overbeck et al., 2007; Soriano et al., 1992). Grassland species are dominant, extensive cattle ranching being the region's main economic activity since Iberian colonization (Modernel et al., 2018; Soriano et al., 1992).

The coexistence of these biodiverse grasslands with livestock over four centuries has ensured the provision of fundamental ecosystem services to society, such as biodiversity conservation, water purification and regulation, soil erosion control, and low-input, grassland-based meat (Modernel et al., 2018) and wool production (Pallarés et al., 2005). However, the emergence of an industrialized model of agriculture and the resulting economies of scale with high international grain prices contrasted with the low productivity and income in those areas (most times due to overgrazing; Carvalho and Batello, 2009), triggering an unprecedented expansion of croplands over indigenous ecosystems in the past few decades (Baeza and Paruelo, 2020; Gras, 2009; Modernel et al., 2018, 2016; Oliveira et al., 2017). As a consequence, livestock were taken to feedlots in areas where cropland expansion was more substantial or concentrated in marginal grassland areas not suitable for agriculture, where increased stocking rates aggravated the overgrazing problem (Modernel et al., 2016). Land-use change, and agricultural intensification have led to several ecosystem service losses in the Rio de la Plata region, such as decreasing soil organic carbon stocks and diversity of plants, birds and mammals, and increasing soil erosion (Modernel et al., 2016).

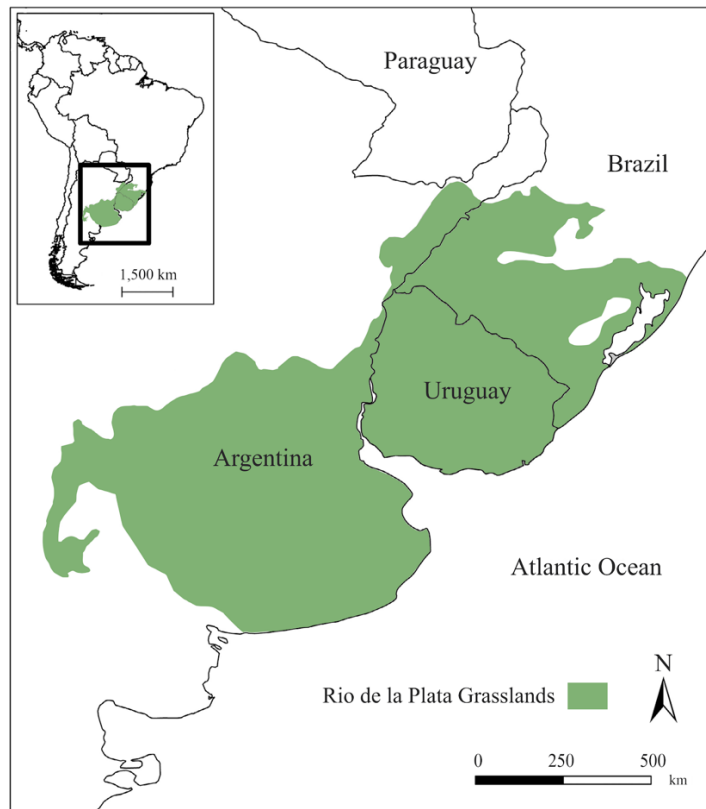


Figure 1 Rio de la Plata grasslands region, comprising central-eastern Argentina (parts of Buenos Aires, Córdoba, Corrientes, Entre Ríos, La Pampa, Misiones, Santa Fé and San Luis provinces), the entire country of Uruguay and the extreme south of Brazil.

Integrating crops and livestock under more biodiverse agroecosystems has been proposed as a strategy to reconcile high agricultural yields and the provision of ecosystem services underpinning sustainability in the region (Carvalho et al., 2021) and worldwide (Brewer and Gaudin, 2020; Herrero et al., 2010). Most studies about integrated crop-livestock systems (ICLS) in the Rio de la Plata region or worldwide have focused on cattle integration (Garrett et al., 2017; Peterson et al., 2020). However, sheep integration into annual cash crop-pasture rotations (Farias et al., 2020) and perennial systems (Niles et al., 2018) have shown to improve on-farm resource-use efficiency and profitability, besides providing ecological benefits. As livestock farms in Rio de la Plata region often raise both cattle and sheep in their grasslands (Modernel et al., 2016; Pallarés et al., 2005; Paparamborda, 2017), integrating sheep into existing cropping systems could help reduce competition for feed resources, improve farm profitability and diversify income sources. The easy adaptation of sheep to different climates, reliefs, and vegetation, along with their small, easy-to-manage body size and their reproductive specificities such as short gestation

period and prolificity (Benoit et al., 2019; Earle et al., 2017; Ripoll-Bosch et al., 2014) allow them to fit into a wide range of production systems, from smallholding systems to large commercial farms.

In this review, we discuss sheep integration into croplands as a strategy to regain ecosystem services lost during the agricultural intensification process and to move towards a sustainable, yet productive model of agriculture in the Rio de la Plata region. Based on published studies from the region and around the world, we outline the opportunities for sheep integration into existing specialized systems and the potential of ICLS for increasing resource-use efficiency and building more resilient farming systems through diversification of biological processes and income sources. We also discuss challenges faced by those considering the implementation of ICLS, and the need of paradigm shifting required to facilitate ICLS adoption by specialized farmers. Finally, we consider sheep integration as part of a wider farm and/or territory design framework where native grasslands and ICLS coexist and support each other in strategic moments, reducing the need for cropland expansion over native ecosystems.

2.2. Sheep production in the Rio de la Plata region of south America

The first animals arrived in Central America (i.e., the Antilles region) and rapidly disseminated southward in the following centuries (Rodero et al., 1992) until reaching the distant Patagonian grasslands in the 19th century (Martinic Beros, 1982). With the advance of World War I in the early 1900s, increased demand for meat and wool (the latter was the main sheep byproduct at that time; ARCO, 2020) raised market prices, and the sector experienced an important growth (Bofill, 1996). As a result, the countries of the Rio de la Plata region specialized their sheep industry in wool production focusing on the European market (Viana et al., 2010; Viana and de Souza, 2007; Viana and Silveira, 2009).

In the 1980s, the high stock of Australian wool, added to the beginning of synthetic fiber's commercialization, decreased wool market value in the Rio de la Plata region (Bofill, 1996; Nocchi, 2001). The agricultural expansion aggravated these factors in the 1990s, pushing ruminant livestock to marginal areas. As a result, productivity decrease reducing income margins and collaborated to a drastic decrease in sheep flock -74% (48 to 12.5 million sheep) from 1960 to 2002 in Argentina

(SENASA, 2020), -69% (12.8 to 3.9 million sheep) from 1974 to 2018 in southern Brazil (IBGE, 2020), and -40% (10.9 to 6.5 million sheep) from 2002 to 2019 in Uruguay (DIEA, 2020, 2010). The latest available census showed the regions corresponding to the Rio de la Plata region in Argentina, southern Brazil and Uruguay with approximately 4.7, 4 and 6.5 million sheep, respectively (15.2 million in total) (DIEA, 2020; IBGE, 2020; MAGP, 2020), which are mostly in mixed grazing systems with beef cattle.

Sheep production is an activity that has a social and economic potential impact and due to its versatility could be of greater importance mainly to small farms and low-income rural areas. FAO (2018) estimates 300 million smallholders in the world producing food and income-dependent on small ruminants. In this way, it is essential that those systems could deal with uncertain events. Thus, Benoit et al. (2020) warn of two primary factors to sheep-farm resilience that is, fertility and animal nutrition. Fertility impact can be reduced using the shorter gestational time (5 months) allowing several lambing periods over the years. Also, the author mentions that none or controlled concentrate use is an essential strategy to reduce farm economic impact. Thus, it is necessary to focus on forage planning which allows forage offer and structure to animals over the year.

2.3. Integrated crop-livestock systems in the Rio de la Plata region

ICLS combines crop and animal production across multiple scales [e.g. at the farm level, through seasonal pasture-crop rotations or intercropping with forage species (Moraes et al., 2014), or at the territorial level by the exchange of livestock waste and forage resources between farms (Moraine et al., 2017)]. In the Rio de la Plata region, ICLS can include yearly rotations of summer cash crops (e.g., soybean, maize and rice) and winter annual pastures (Alves et al., 2019a; Carlos et al., 2020; Kunrath et al., 2020), variable periods of crop rotation succeeded by periods of grazing on perennial pastures [e.g., three years of continuous crop rotations followed by three years of perennial pasture (Salvo et al., 2010), or eight years of summer crop rotations with grazing of crop residues and weeds in winter followed by four years of perennial pasture (Fernández et al., 2011)], grazing of dual-purpose crops such as wheat (Bartmeyer et al., 2011) and grazing of understory vegetation in systems with trees, such as silvopastoral (Pontes et al., 2018), vineyards (Niles et al., 2018) and orchard (ARCO, 2020b). However, the range of possible combinations is as large as the

number of domestic plant and animal species multiplied by unlimited spatiotemporal designs.

Even though research proves ICLS is a necessary way to sustainable intensification with a range of possibilities to integrate crops, pastures and ruminants (Carvalho et al., 2018a), achieving synergy between agricultural production and environmental quality (Lemaire et al., 2015b), the total integrated area is still small in this region. For example, South Brazil presents only 13% of the cropland cultivated area as ICLS (Embrapa, 2016).

2.4. Crop production in the Rio de la Plata region: opportunities and challenges for crop-livestock integration

Rio de la Planta region allows a singular opportunity to produce a variety of cash crops. The arrival of Spanish and Portuguese in the 16th century was followed by the introduction of old-world crops such as wheat, barley, oats, rice and many temperate vegetables and fruits (Schwerin, 2008). We present here the main cash crops in Brazil, Argentina and Uruguay and the opportunities and challenges to integrate animals and crops.

Currently, it is estimated that approximately 15 million hectares of southern Brazil are cultivated with different cash crops (CONAB, 2019), the main crops being the commodities soybean, maize, and rice in decreasing order of cultivated area. From these areas, 4.7 million hectares are cultivated with winter crops as wheat (CONAB, 2019). Also, although grain production as a soybean, rice and maize represents the biggest part of agricultural production, 915 thousand hectares is occupied by orchard as an olive trees, vineyards, apple trees, orange trees, pecans, peach trees, yerba mate and forestry on Rio Grande do Sul state (DPADR, 2019).

In Argentina, crops were introduced by European immigrants in the 19th century and even as occurred in Brazil, crops advanced under native grasslands displacing livestock and became a prevalent activity (Lavado and Taboada, 2009). According to Peiretti and Dumanski (2014), cultivated area doubled in Argentina from 1970 to 2011 and the grain production increased 4.5-fold times in the same period. The author attributes this, in part to conservationists no-till adoption which evolved from 0 in 1988 to 78% in 2011. The greatest amount of arable land of Argentina is cultivated with

soybean followed by maize and sunflower in the spring-summer period and over the autumn-winter mainly wheat and barley (Lavado and Taboada, 2009).

Uruguay, although there is a historical connection with livestock production, agriculture presented a large expansion at the expense of native grasslands (Ran et al., 2013). Soybean represents a large part of agriculture increase in this country, improving cultivated area from 80 to currently 917 thousand hectares (DIEA, 2020; Ran et al., 2013). Soybean crop is followed by rice and maize as main crops in the spring-summer period in Uruguay which has 140 and 117 thousand hectares respectively (DIEA, 2020). Over the autumn-winter period, 237 thousand hectares are occupied by wheat and 165 thousand hectares by barley. Also, 34 thousand hectares are cultivated with orchards, olive trees and vineyards which remain only with cover crop between the tree lines, requiring mowing for control. Thus, emerge opportunities mainly to small ruminant integration forward to sustainability by synergic interaction between system components, optimizing land use and outcomes over-time (Cubbage et al., 2012; Devendra, 2014; FAO, 2019a; Gonzales, 2016; Niles et al., 2018).

As mentioned, the native grasslands were pressured by the advance of agriculture in the Rio de la Plata region, being pushed into marginal areas of hard mechanization. Such areas are of lower quality and productive capacity. In addition, (Carvalho et al., 2017) points out that the herbage production of native grasslands is under influence of the year season, suffering a drastic reduction on herbage production in the autumn-winter period. This is the same period that the nutritional exigence of female sheep increases, mainly due to pregnancy or lactation (NRC, 2007). The ewes are photoperiod negative and the top of the fertility curve occurs in summer-autumn with daily light down (Ungerfeld, 2020). Thus, a decrease in the forage supply in subsequent seasons as occurs under native grasslands, can be harmful to animal production mainly in the last two months of pregnancy period, which corresponds to the higher demand for energy to fetus growth, uterus, and mammary gland development (NRC, 2007). Previous studies related consequences of ewe malnutrition over pregnancy as a decrease in maternal expression in ewe lowest progesterone decrease that is negatively correlated with milk production, low birth weight, and decreasing lambs survive probability (Dwyer et al., 2016, 2003; Freitas-de-Melo et al., 2017). Therefore, the largely agricultural area maintained just which high-quality herbage to cover crop over the winter-spring period is an opportunity to supply this gap in herbage offer to sheep production in an ICLS.

In this way, it is possible to achieve good performances of higher requirement animal categories under well-managed Italian ryegrass pastures, such as lactating ewes ($0.025 \text{ kg dia}^{-1}$) and their lambs ($0.240 \text{ kg dia}^{-1}$) (Savian et al., 2014) and finishing lambs ($0.129 \text{ kg day}^{-1}$) (Farias et al., 2020; Farinatti et al., 2006). Savian et al. (2014) point out that it is possible to produce around 500 kg of LW gain per hectare (ewes and their lambs) only in 4 months of the year with Italian ryegrass pastures (winter-spring) integrated with soybean. In this sheep production model, male lambs can be slaughtered at 5 months of age with more than 40 kg LW (November-December), and female lambs can be joined in the next breeding season at approximately 8 months of age and more than 40 kg LW (i.e. February); these lambs can go to a native grassland, for example, after the end of the Italian ryegrass season, which when well-managed present the greater production potential in that part of the year, from October to March (see Mezzalira et al., 2012).

However, although we presented several opportunities for animal inclusion on the cropping systems, there are some challenges that make farmers resistant to animal inclusion on the system. Integrated systems improve system complexity, and it requires multidisciplinary management to reap the benefits of synergism. Also, paradigms regarding possible soil damages by animal trampling and lowest residues after stocking period that could decrease crop development and/or production in succession or tree damages that could harm wood or non-wood production are challenges which research shows be possible to get around.

In this sense, soil compaction could lead to a decrease in soil water content for successive commercial harvest. Thus, Peterson et al. (2019) contrasted physiological variables of soybean plants in ICLS versus specialized continuous cropping system, which remain with pasture only as ground cover in winter. Authors observed greater efficiency in the use of sunlight and a reduction in the leaf area index at the end of the crop cycle in non-grazed areas. These factors point to a faster physiological maturation and an earlier senescence in relation to the plots with the previous animal presence, which had a maturation time two weeks slower. Despite this, there was no difference in the soybean grain yield of the two systems. These results show that the insertion of the animal component in agricultural systems in southern Brazil can alter the phenology of plants, but without altering the productivity of the subsequent crop (Kunrath et al., 2020, 2015). In addition, Farias et al. (2020) show that there is a decrease (-49%) of herbage residual after stocking period in ICLS compared to

cropping systems. However, the same authors found similar ($P > 0.05$) soybean yield between integrated or cropping systems. On the other hand, authors have reported increases in grain production to maize followed by grazed oats and Italian ryegrass pasture (ICLS) compared to non-grazed systems (Assmann et al., 2003; Sartor et al., 2018a).

Furthermore, the tree inclusion on the system or the animal inclusion on the previously forest or orchard specialized system, adds one more complexity level as challenge. Trampling seedlings, breaking branches, and chewing leaves, bark, and branches are mentioned as possible animals damage to forest/orchard crops (Fedrigo et al., 2018; Porfírio-da-Silva et al., 2012) which may compromise the success of the system (Porfírio-da-Silva, 2009). Garret et al 2004 suggest that the inclusion of animals in the year of establishment of the trees should be avoided. In this sense, Varella (1997) evaluated the animal inclusion on forest establishment (*Eucalyptus saligna*) concluding which 182 and 154 cm is the minimum trees height to start grazing by cattle and sheep, respectively, to avoid several damages.

According to Bernardi et al. (2014), animal grazing experience on the forest environment, pasture structure (grazing intensity), and tree leaf palatability are some factors that have influence under possible tree damages that occurred in livestock-forest integration. Gonzales (2016) evaluated sheep and goat selectivity under two grazing intensities into forest integration. Results showed similar sheep selectivity in both grazing intensities and no tree preference. However, when goats were evaluated, in the higher grazing intensity there was high tree bark intake which was easily controlled by conditioning to aversion created using lithium chloride (LiCl).

In orchard trees, pastures as cover crop are maintained between tree lines to protect the soil of nutrient leaching and preserve their physical properties. However, control is necessary and for this is frequently mown and/or applied herbicide. The amount of controls is variable according to region and season, but Lanauskas et al. (2014) related to need 5 – 7 mowing by season to control perennial grasses between the apple tree lines, representing a high cost to farmers (Niles et al., 2018). Thus, the sheep grazing could be used to do this control (Figure 2).

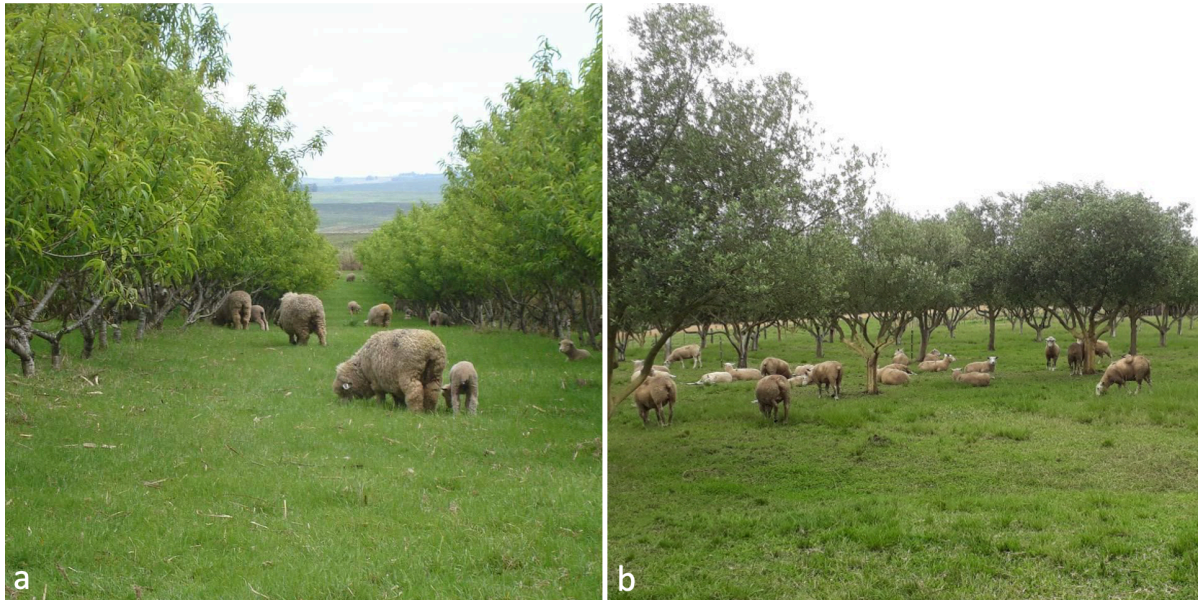


Figure 2 Sheep grazing the understory vegetation of (a) peach orchards in the municipality of Bagé and (b) olive orchards in the municipality of Cachoeira do Sul, both in Rio Grande do Sul State, Brazil. Photo courtesies: (a) Thomaz Z. Mercio and (b) Tales Altoé

In addition, integrated systems provide thermal comfort to the animals (Vieira Junior et al., 2019), reduce the severity of low temperatures in the pasture by microclimate control (Feldhake, 2002), and contribute to mitigate the emission of greenhouse gases (Torres et al., 2017). Thus, integrating sheep and trees constitutes productive diversification and is in line with the logic of sustainable intensification. However, to get these systems improvements is required that system design and diversity is assembled to capture trade-offs between life functions (Dumont et al., 2020).

2.5. Grazing management in integrated crop-livestock systems

The main objectives that drive grazing management strategies are optimal levels of livestock productivity and economic returns. More recently, environmental services are also desired outputs. However, grazing intensity is the most impactful parameter, and neither continuous nor rotational stocking method can compensate if improper grazing pressure is used (Briske et al., 2008).

Carvalho, (2013) suggested a new grazing management strategy based on animal ingestive behavior, named “Rotatinuous” stocking. This new concept aims to minimize grazing time, allowing animals to select optimal bites in leaves, and

consequently maximize intake rate. The goal is to offer plants in an optimal structure built by moderated grazing intensities, regardless of the stocking method. In rotational stocking, the ideal pre-grazing sward canopy height is the one that offers the highest dry matter intake per unit of grazing time. The post-grazing canopy height should not be less than 40% of the initial sward canopy height (Fonseca et al., 2013; Mezzalana et al., 2014).

Also, maximizing intake rate by adjusting grazing intensity generates a cascade effect in productive, economic and environmental indicators, which means greater herbage intake (Savian et al., 2020), live weight gain (Schons et al., 2021) and carcass production (Savian et al., 2021), and lower methane emissions per area, per kg of herbage intake (Savian et al., 2018) and per kg of carcass production (Savian et al., 2021) of sheep grazing Italian ryegrass pastures managed under rotational stocking, that is, pre- and post-grazing sward height of 18 and 11 cm, respectively. In addition, this grazing management presents greater herbage production when compared with traditional rotational stocking, mainly by the high percentage of residual leaf mass in the post-grazing (Schons et al., 2021), which consequently results also in a lower herbage cost (Savian et al., 2021).

In pastures managed under continuous stocking method, the average sward canopy height should be between the optimal ones in rotational stocking. Farias et al. (2020), using this grazing management strategy but now in continuous stocking (Italian ryegrass height of 15 cm) found satisfactory sheep production over the pasture phase (324 kg LW ha⁻¹) of an ICLS without impair soybean productivity in the crop phase (2875 kg ha⁻¹) compared to non-grazed system (2898 kg ha⁻¹). Also, evaluating an integrated sheep-rice system in Uruguay, Bermudéz et al. (2009) reported that the individual finishing lamb performance was significantly affected (-92%, from 97 to 8 g sheep day⁻¹) by increased grazing intensity in 167%. In addition, the authors related decrease in carcass quality (carcass finishing and boned leg weight) from low to high grazing intensity. Moreover, moderate grazing intensity positively impacts on weed management in the pasture and crop phase. Schuster et al. (2018) confirmed the effectiveness of higher forage allowance for sheep (20 kg DM 100 kg LW⁻¹) on reducing the bank seed size and the emergency of weed flora in subsequent crops (maize and soybean).

Also, de Souza Filho et al. (2019) found out that the moderate grazing intensity that provides optimal animal performance values in ICLS has the potential to reach 13-

14% of the mitigation target for CH₄ emissions from the agricultural sector if adopted at large-scale. The same positive response to animal production and CH₄ intensity mitigation is confirmed under moderate grazing intensity in native grasslands (Cezimbra et al., 2021). Although the mentioned studies worked with cattle, the same logical follow to sheep as shown by Savian et al. (2018) and Zubieta et al. (2021).

Thus, grazing management leads to significant impacts on other components of the ICLS. Therefore, we suggest that both in an ICLS as in any other grazing ecosystem, the pasture should be managed under moderate grazing intensity, offering to the animals, over the grazing period, an optimal sward structure that will maximize their forage intake per unit of grazing time (Carvalho, 2013).

2.6. Implications of sheep integration for soils and nutrient cycling

Livestock is the nutrient recycling component on the ICLS (Carvalho et al., 2010). As observed by Alves et al. (2019), more than 95% of phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) exportation from an ICLS area occurred during the harvest of summer grain crops, while less than 5% of nutrients were exported in the meat of grazing sheep in a yearly rotation of soybean and maize succeeded by winter pastures. In other words, most of the nutrients ingested by sheep (~90%) return to the soil in dung and urine (Haynes and Williams, 1993), increasing nutrient dynamics in grazed compared to non-grazed areas.

For instance, Arnuti et al. (2020) showed that the greatest portion of P and K release (~63%) from sheep dung occurred during the stocking period of an integrated soybean-sheep system mainly as a result of nutrient cycling from Italian ryegrass swards grazed in the vegetative stage, when plants had greater nutrient contents and higher portions of those nutrients in the labile fraction compared to later stages of development (i.e., post-flowering). Although most of the nutrient recycling happened within the pasture phase of ICLS, nutrient release from dung extended beyond animal removal from the area and stabilized during the succeeding crop phase, 200 days after the start of grazing in the previous winter (Arnuti et al., 2020a). About 65% of P and 100% of K returned to the soil in this period, reinforcing the role played by the animal in improving nutrient recycling but, more importantly, it showed that crop nutrition are under influence of the nutrients applied during the previous pasture establishment for a large part of its production period. Moreover, since the greatest portion of nutrients

were recycled during the vegetative stage of sward, initiatives able to promote longer vegetative periods (e.g., reaching pre-grazing targets earlier in the season through anticipated pasture sowing) and/or shifting the focus of fertilization from the crops to the pastures could substantially improve system resource-use efficiency and performance (i.e., system fertilization; Farias et al., 2020).

In this sense, studies show that it is possible to anticipate N (Assmann et al., 2003) and P and K (Farias et al., 2020) fertilization from crop to pasture establishment could improve or keep similar crop productivity compared to fertilization in the crop phase. This new fertilization approach named system fertilization (T. S. Assmann et al., 2017; Bernardon et al., 2020a) is according to nutrient fluxes and appropriate temporal and spatial dynamics (see Farias et al., 2020) in order to take advantage of the nutrient cycling made by the animals which allow greater forage production in the pasture phase and, consequently, greater animal production, generating extra income in the activity without affecting the crop yield in succession. Sartor et al. (2018a) applying this new fertilization approach found a similar production of maize grains when N was applied all in the pasture (225 kg ha^{-1}) compared to the same N application made only in the maize crop. Increased productivity as a result of grazing sheep and N anticipation was also observed in the bean crop (Andreolla et al., 2014), with the residual effect of N fertilization in the pasture for the crop rotation. In addition to the increase in forage production with the anticipation of N fertilization, Bernardon et al. (2020) show that the nutrient concentration in the plant tissue remained above the dilution curve proposed by Lemaire (1997) when N was applied in the pasture establishment. On the other hand, when the pasture was N dependent on N carryover from crop fertilization, the pasture presented N status below dilution curve suggesting N deficiency. The dilution curve delimits the optimal condition of the nutrient in the plant tissue for maximum production.

Tropical and subtropical soils have problems with acidity, such as low pH and high Al saturation (von Uexküll and Mutert, 1995). Thus, in many cases, the use of acidity correctives becomes necessary. Due to the low solubility of the limestone, its superficial application in areas maintained under no-tillage has resulted in correction of only the first centimeters of the soil (dos Santos et al., 2018). However, recent studies have shown that the insertion of grazing animals into cropping systems can potentialize the effects of subsurface correction, with increased pH and decreased

aluminum saturation (Martins et al., 2014), promoting a better environment for root development of crops.

The key factors that should be considered in ICLS, with sheep and crops, is the grazing management and crop rotation. In an ICLS, when sheep are managed under low grazing intensities in the pasture phase, and this is combined with crop rotation in the crop phase, it was more efficient in nutrient use (Alves et al., 2019a). Hence, managing pastures under low grazing intensity, favors the increase of carbon (C) and nitrogen (N) stocks on soil (Alves et al., 2020), this is important, since more systems that accumulate C and N in the soil are being sought, reducing greenhouse gas emissions and consequently global warming (Ribeiro et al., 2019; Sá et al., 2017).

One of the factors that limits the widespread use of ICLS in Southern America is the potential physical limitation that can occur in soil imposed by grazing animals, such as soil compaction and decrease in water infiltration rate (Batista et al., 2019; Hunt et al., 2016). However, recent studies have shown that these effects are momentary and limited to the topsoil, and that at well-managed pastures the soil regenerates after an annual crop cycle (Ambus et al., 2018), causing no damage to crop production (Peterson et al., 2020, 2019) and system energy (pasture + sheep + soybean) production (Farias et al., 2020).

In addition to improvements in nutrient availability and absence of physical damage capable of compromising crop productivity, ICLS promotes improvements in soil biological properties (Moraes et al., 2014). The adoption of ICLS, in relation to specialized continuous cropping systems, promotes positive responses in the microbial community of the soil, such as an increase in microbial biomass, in addition to increasing the population of arbuscular mycorrhizal fungi (Sekaran et al., 2021), which are extremely important for the C accumulation and stabilization in the soil (Veloso et al., 2020).

Although studies with ICLS have evolved in recent years, studies with sheep exploiting its interference in soil properties and nutrient cycling are still scarce. Therefore, studies that introduce sheep into continuous cropping systems should be performed, mainly in long-term protocols. Thus, we will have more accurate results related to the inclusion of the animal component and its changes in the soil system, mainly involving different arrangements of small ruminants and cultures (e.g. cash crops and trees), and types of soil and climatic zones.

2.7. Sheep and crop integration as part of farm design for future food production

As previously seen, agriculture and livestock were disconnected and evolved to simplified systems over the last decades, which have influenced efficiency, resilience and ecosystems services (Klasen et al., 2016). Also, this process led to equipment, farm structure and farmer's activities specialization making it difficult for the animal to return to the system or even greater diversification of cultures. However, the currently high pressure to be environmentally friendly (e.g. decrease and/or to be more efficient in the use of non-renewable resources) at the same time which productivity is kept or improved has led to agricultural restructuring to achieve this by biodiversity benefits (Franzluebbers et al., 2014; Moraine et al., 2017; Peyraud et al., 2014; Pocard-Chapuis et al., 2014; Tracy and Zhang, 2008). Thus, the integration of the components already mentioned in this review, can be arranged in different ways to reach improvement productivities and ecological benefits. Figure 3 shows farm design possibilities not to follow as models but to exemplify some possibilities.

For example, native grasslands could be the core of the farm (see Jaurena et al., 2021), which is common in the Rio de la Plata region, and the other crops rotate in spring-summer such as soybean, maize or rice with cover crop over the autumn-winter such as mixed Italian ryegrass and white/red clover, which will be used for livestock - including just sheep, cattle or these mixed species; the last model is more common in this region - feed in the moment of the year that the native pastures presents low productivity if not improved with fertilization and/or inclusion of winter species (Figure 3a). In this way, cultivated pasture use can be an ally to present a fair offer of forage throughout the year, where the goal is to not have pasture gaps. Thus, it allows farm intensification of sustainable ways where livestock is benefited with high quality of low-cost food (cover crop) and the system will be benefited from nutrient cycling provided by animals under well-managed pastures (Alves et al., 2019a; Farias et al., 2020). For this, a part of the livestock herd could be moved from native to annual pastures in succession to soybean/maize/rice (ICLS).

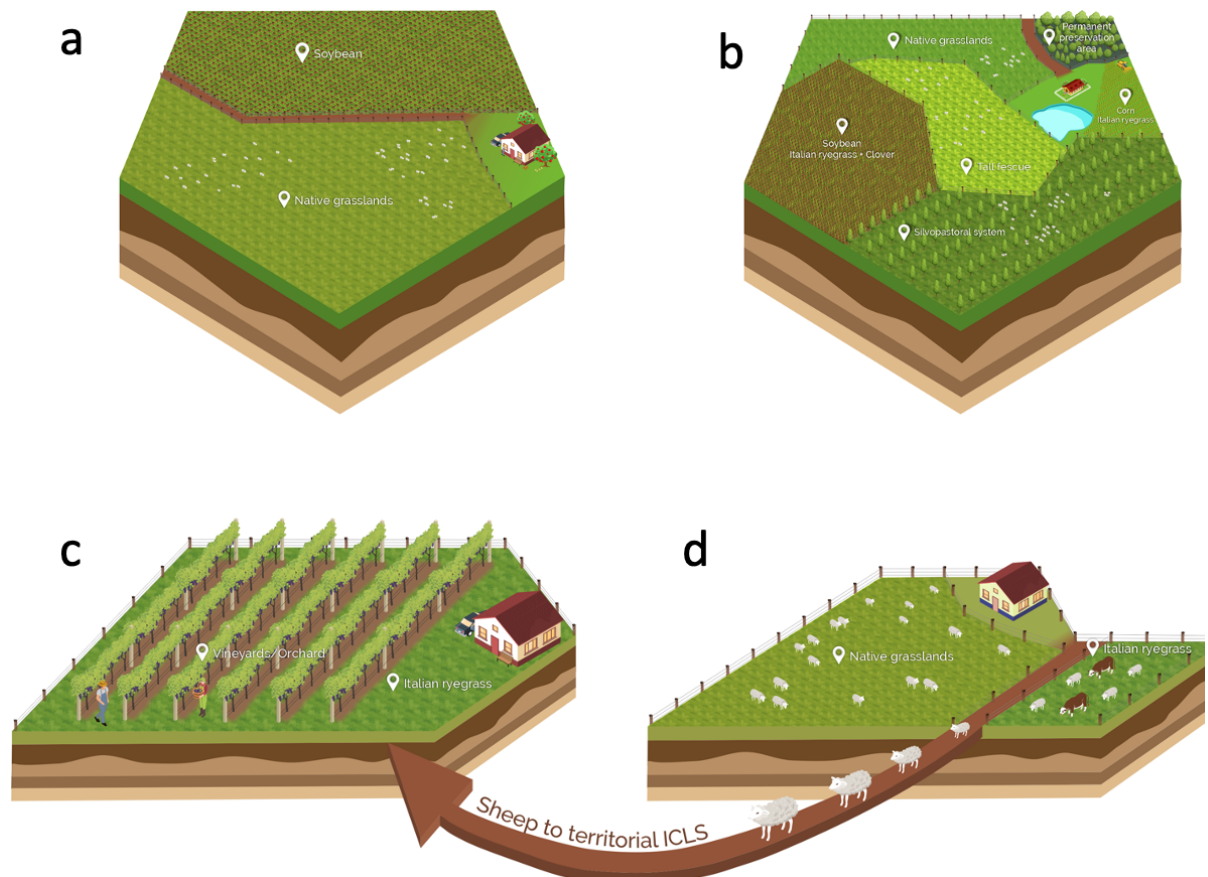


Figure 3 Schematic representation of different levels of system intensification. (a) representing a system with low level of crop diversity presenting biggest part of natural pastures and a little area of crop production; (b) system with high level of crop diversity; (c and d) two farms with low crop diversification but with territorial integration between them.

Figure 3b shows which system complexity level increases with forest component inclusion and one more in silvopastoral (forest-animal integration) or agro-pastoral system (crop-livestock-forest integration). Thus, ecosystem services as habitat for biodiversity, biomass for materials and energy, and environmental recreation can be reached with tree system inclusion (Felton et al., 2020). This scenario creates a range of possibilities of grass and legume forage species condition to use in space and time throughout the year, such as C4 (e.g. sorghum, pearl millet, Panicum, Tifton and alfalfa) and C3 (e.g. Italian ryegrass, oat, wheat, tall fescue, cocksfoot, white clover, and red clover). For instance, the C3 pastures – sown mixed in a specific area of the farm or over-sown on the native grasslands (winter) – play an essential role which is the provision of feed to the animals in a moment of the year that the native pastures present low productivity. Furthermore, C4 cultivated pastures sown in a separate area or between tree lines, can help to potentialize and at the same time

conserve the native grasslands, mainly by the reduction of stocking rate in those native areas, which are in most cases overgrazed (Modernel et al., 2016). More complex systems when well-managed and planned could be more stable over the time, mainly in the face of a drought for example. Nunes (2020) concluded that the ICLS presents greater productive and economic stability than non-integrated systems. Also, designing systems with diverse plant species is pivotal to the ruminants select a diet in benefit to their nutrition, health and welfare, and mitigate negative environmental impacts (Distel et al., 2020). In addition to that, botanically diverse pastures result in better meat quality (Dawson et al., 2011), which is consequently beneficial to the health of humans (Provenza et al., 2019).

On the other hand, systems can be integrated at the landscape level (territorial ICLS, Figure 3c) wherewith neighboring agreement mutual benefits to their orchards (e.g. cover crop control and nutrient cycling) and sheep production (e.g. high-quality sheep feed) can be reached (Garrett et al., 2020a; Niles et al., 2018). Thus, it is possible to build several forms of production systems. However, easy implantation and management added to enhancement of the synergy between components, maximizing land use must be prioritized to arrangement definition. Also, evaluate farm resources (i.g. equipment and workforce), technical practices and their recent evolution are essential examples which should be considered to plan farm design (Moraine et al., 2017). Although the integration among forages, animals and trees such as orchards or wood plantations are almost nonexistent in this region, this model could be an interesting way to improve the efficient use of areas and productivity of the region.

In addition to integrating cash crops and animals in space and time, the mixed grazing should be considered on farm planning. Although cattle grazing is predominant in the ICLS of South America, integration between multi-species such as sheep, cattle and/or horses, which is common in the native grasslands of the Rio de la Plata region, should be an important way to improve the sustainability of livestock farms (Martin et al., 2020). The purpose of herbivores species combination in the same area is the grazing standard complementarity exploiting trophic resources from different ecological niches which under well-management lead to agro ecological benefits, efficient pasture use and consequently higher animal and system production (Cuchillo Hilario et al., 2017; Martin et al., 2016). This result is associated with different foraging behavior according to animal body size, which possibly an ecological benefit to coexistence (Laca et al., 2010).

2.8. Conclusions

The integration between pastures, animals and cash crops in the Rio de la Plata region is a necessity to re-design the production systems and to improve productivity, and economic and environmental sustainability. However, just integrating the systems is not enough. The ICLS only works well when the grazing management is well done. Therefore, in this review we present opportunities, challenges and strategies for sheep integration into croplands in the Rio de la Plata region of South America: Opportunities) this region can produce a variety of cash crops and pastures over the year, cropland areas that are left fallow, that is, unused for part of the year can be used with ruminants, small orchard farmers can diversify and increase income introducing sheep, wood plantations when planned to that can be a profitable silvopastoral system, and native grasslands can benefit from integrated systems, where cultivated pastures integrated with cash crops in a part of the farm can help reduce overgrazing on native pastures and diversify the cultures and income; challenges) re-design the systems on the farm and landscape level, convince producers to use the ICLS based on the argument that the animal is beneficial to the cropland, and not the opposite, and generate more research about this topic (e.g. sheep integration into orchards); and strategies) plan the system according to the market and the interests of the producer, manage the pastures well with moderate grazing intensity (e.g. 'Rotatinuous' stocking), and have a well-prepared forage planning.

Finally, this review highlights the importance of the ICLS to diversify food production and income. In this way, we encourage the researchers from research centers and universities to make more attention and develop research -with robust experimental design- about integrated systems in the Rio de la Plata region to generate solid scientific knowledge that could be used by the farmers in the future.

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Hypotheses

The following chapters were developed based on the following hypotheses: (1) We hypothesized that ICLS with system fertilization results in greater herbage and animal production compared to crop fertilization, without affecting crop grain yield (Chapter III). (2) In chapter IV we have two hypotheses: (i) system fertilization in ICLS has a positive effect in nutrient content of Italian ryegrass swards over the pasture phase and ensures nutrient carryover for soybean crop in succession, and (ii) carryover of P and K from crop fertilization in a grazed or non-grazed system is not enough to supply nutrition status of Italian ryegrass over the pasture phase compared to system fertilization.

Objectives

The objectives of the studies presented below were: (1) to evaluate the effect of cropping system (soybean and non-grazed Italian ryegrass cover crop) or ICLS (soybean and sheep grazing Italian ryegrass cover crop), and two fertilization strategies (system fertilization or crop fertilization) on herbage and animal production, soybean grain yield, total system production and system productivity in terms of use of resources (inputs). (2) In chapter IV, the goal was to evaluate nutrient dynamics and nutrition status of Italian ryegrass and soybean plants as a result of different fertilization approaches (system or crop fertilization) and animal effect (ICLS or non-grazed cropping system).

3. CHAPTER III

Integrated crop-livestock system with system fertilization approach improves food production and resource-use efficiency in agricultural lands²

² Manuscript prepared and published (<https://doi.org/10.1007/s13593-020-00643-2>) according to the *Agronomy for Sustainable Development* rules (Appendix 2).

Integrated crop-livestock system with system fertilization approach improves food production and resource-use efficiency in agricultural lands

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Abstract

Integrated crop-livestock systems (ICLS) can be an alternative to increase the productivity of agroecosystems by enhancing nutrient cycling via grazing animals. Despite the holistic approach that bears the designing of ICLS, fertilization practices are proceeded in a conventional crop basis, disregarding nutrient fluxes at the appropriate spatial and temporal dynamics. We argue that fertilization practices in ICLS must follow the same integrated approach. To test this, we compared a conventional crop fertilization strategy *versus* a system fertilization approach applied to two production systems being a conventional cropping system and ICLS. The conventional cropping system consisted of a soybean crop succeeded by a non-grazed Italian ryegrass cover crop. The ICLS model consisted of a soybean-Italian ryegrass rotation grazed by sheep. In the conventional crop fertilization strategy phosphorus and potassium were applied at soybean sowing and nitrogen at the Italian ryegrass establishment. The system fertilization consisted of the application of all nutrients during the Italian ryegrass establishment. Accordingly, treatments were fertilization strategies in a factorial framework with production systems randomly distributed in a complete block design with four replicates. Results indicated for the first time greater daily herbage

accumulation rate (24%; $P < 0.01$) and total herbage production (18%; $P < 0.05$) in the system fertilization compared to conventional crop fertilization. Consequently, system fertilization allowed for greater stocking rates in the pasture phase (17%; $P < 0.05$). The ICLS presented greater equivalent soybean yield ($P < 0.001$), energy production ($P < 0.01$), and system productivity ($P < 0.05$) compared to the cropping system, regardless of fertilization strategies. Soybean yield was not affected by fertilization strategies or grazing. In conclusion, the adoption of system fertilization strategy and crop-livestock integration enhance the production without jeopardizing soybean grain yields, so that land use is optimized by a greater energy production per unit of nutrient applied.

Keywords: Cropping systems, Grazing, Mixed crop-livestock systems, Nutrient cycling, Soybean, Crop fertilization.

3.1. Introduction

Human population and income have been increasing in the last decades and, simultaneously, the global requirement for animal source food is expected to rise soon (Mottet et al. 2017). Thus, production systems that supply large amounts of food to global markets will need to increase their production. In the current scenario, there is an increasing social and political pressure to preserve natural ecosystems, added to increasing urbanization, and the specialized commercial agroecosystem models with high use of non-renewable resources. These facts pose barriers to the expansion of agricultural frontiers to increase food, fiber, and energy production per unit of area and input (Lemaire et al., 2015b). Specialized commercial agroecosystems such as the cropping system, although using conservation precepts (e.g. no-till), have low complexity and diversification, making it difficult to increase food production.

Integrated crop-livestock systems (ICLS) are a sustainable intensification alternative to specialized commercial agroecosystems. Hendrickson et al. (2008) defined sustainability as an approach to producing food and fiber which is profitable, and with resources-use efficiency on the farm. Thus, diverse agricultural production systems such as ICLS might ensure productive conditions in the future and enhance environmental quality. Grazing animals uncouple nutrients and return a large portion to the system via urine and dung (Haynes and Williams 1993). Hence, grazing management is a key factor affecting nutrient dynamics in grazed systems. Sound grazing management increases belowground biomass (López-Mársico et al. 2015), soil fauna, microbial diversity, and the functionality of these populations (Davinic et al., 2013). These are important factors affecting nutrient cycling and increasing C and N stocks (Silva et al., 2014). Furthermore, ruminants are able to upscale human-inedible materials, such as grasses, into highly nutritious animal source food, such as meat and milk (Mottet et al., 2017). The integration of livestock into

cropping systems has positive effects on the agroecosystem, minimizing environmental impacts due to synergisms between system components, with the benefit of increasing the food production per unit of land without converting natural habitats.

The knowledge of plant nutrient requirements and the use of inorganic fertilizer allows an increase in crop production. Annually, fertilizer demand is growing 1.4, 2.2, and 2.6 percent for N, P, and K, respectively (FAO 2015). Therefore, there is a growing concern about the limited availability of mined fertilizers and the potential for contamination of water bodies. Boring et al. (2018) pointed out an increase in soybean and corn yields with phosphorus and potassium application on poor soils, but these responses have been irregular in soil with high nutrient levels. Currently, fertilizer recommendations target to meet crop needs and to increase soil nutrient levels above critical thresholds. Conserving the nutrients is key for agroecosystem success, and the grazing animal play a crucial function to nutrient cycling and can affect positively subsequent crops yields when managed under moderate grazing intensities (Sartor et al. 2018). Thus, a new approach of fertilization emerges - the system fertilization -which is based on the conceptual framework that fertilizer must be applied in the system phase that presents lower nutrient extraction and higher nutrient cycling capacity to maximize total system production (T. S. Assmann et al., 2017). This new approach considers all benefits of well-managed grazing during the pasture phase, including the reduced amounts of nutrients extracted by livestock and accelerated nutrient cycling returned to the soil via excreta (Haynes and Williams 1993). However, there is a lack of research evaluating the effects of system fertilization with phosphorus (P_2O_5) and potassium (K_2O) in ICLS and cropping systems.

The present study pairs a detailed analysis of system production dynamics of ICLS and cropping system under different fertilization strategies in Southern Brazil (Fig. 1). We hypothesized that ICLS with system fertilization (on pasture phase) results in greater herbage and animal production compared to conventional crop fertilization (on crop phase), without affecting crop grain yield. The objectives of this study were to evaluate the effect of cropping system (soybean monoculture and non-grazed Italian ryegrass cover crop) or ICLS (soybean monoculture and sheep grazing Italian ryegrass cover crop), and two fertilization strategies (system fertilization or crop fertilization) on herbage and animal production, soybean grain yield, total system production and system productivity in terms of use of resources (inputs).

3.2. Materials and methods

3.2.1. Site, climate, and soil description

The experiment was conducted in 2017 and 2018 at the Experimental Agronomic Station of the Federal University of Rio Grande do Sul (EEA-UFRGS), in Eldorado do Sul, Rio Grande do Sul, southern Brazil (latitude 30°05'S, longitude 51°39'W and 46 m of altitude).

The climate of the site is subtropical humid. Daily mean data on air temperature and rainfall were obtained from a nearby (~1 km) meteorological station. Average air temperatures were 19.8 and 19.2°C in 2017 and 2018, respectively, and annual rainfall was 1510 and 1214 mm in 2017 and 2018, respectively (Fig. 2).

The soil at the experimental site was classified as an Acrisol. At the beginning of the experimental protocol (2017), the soil diagnostic surface (0-10 cm) presented 17 g kg⁻¹ of organic carbon, pH (H₂O) of 3.9, 1.1 cmol dm⁻³ Ca, 0.5 cmol dm⁻³ Mg; 15% of base saturation (V%), 49% of Al saturation, and available phosphorus and potassium (extracted by Mehlich 1) of 94 and 97 mg dm⁻³, respectively. Based on the soil chemical analysis, 7.5 Mg ha⁻¹ of dolomitic limestone [CaMg(CO₃)₂] with a total neutralization power of 72% was applied to raise soil pH to 6.0.

3.2.2. Experimental design and treatments

The experimental design was a randomized complete block in a factorial 2 x 2 with four replicates. Factors included two no-till production systems: (i) soybean in crop phase and sheep grazing Italian ryegrass (*Lolium multiflorum*) cover crop in the pasture phase, consisting of an integrated crop-livestock system – ICLS, and (ii) soybean in crop phase and non-grazed Italian ryegrass as cover crop in the pasture phase, consisting of a cropping system only; and two periods of phosphorus (P₂O₅) and potassium (K₂O) fertilization: (i) conventional crop fertilization, with the fertilizer applied in the soybean sowing, and (ii) system fertilization, with the fertilizer applied in the pasture establishment (Fig. 3). The P₂O₅ and K₂O fertilization rates were calculated for a soybean grain production of 4.0 Mg ha⁻¹. Nitrogen fertilization (150 kg N ha⁻¹) in the form of urea was performed once in all treatments on Italian ryegrass establishment. The experimental area was 4.4 ha, divided into 16 experimental units (paddocks), ranging between 0.23 and 0.32 ha each being large enough to avoid nutrient transfer between the experimental units.

3.2.3. Pasture phase

In both years, 2017 and 2018, the stocking period started in June and finished in October, totalizing 125 and 120 days of grazing, respectively. After soybean harvest, Italian ryegrass was sown (25 kg of viable pure seeds per ha). In ICLS treatments, the continuous stocking method with three tester sheep per paddock and a variable number of 'put-and-take' sheep were used to maintain

the targeted sward canopy height of 15 cm. This grazing management strategy was defined to offer to the animal an optimal sward canopy structure to maximize herbage intake per unit of eating time (“Rotatinuous” stocking; Carvalho (2013)).

3.2.3.1. Sward measurements

To maintain the desired sward canopy height, 150 random points per paddock were measured weekly with a sward stick. Herbage mass (kg DM ha^{-1}) was measured in all paddocks prior to the beginning of the stocking period and every 28 days (subperiod). For this, six random forage samples (0.25 m^2) per paddock were clipped at ground level. Daily herbage accumulation rate was evaluated by through the use of four grazing exclusion cages per paddock. At the beginning of each stocking period, herbage mass was determined by clipping at ground level (0.25 m^2) at four random places and cages were allocated nearby. The cages places were chosen by similarity with herbage mass cut. Approximately 28 days after, the herbage mass inside cages was cut at ground level as previously mentioned. Then, herbage samples were oven-dried at 55°C for 72 h and weighed for assessment of dry matter (DM) content. Daily herbage accumulation rate ($\text{kg DM ha}^{-1} \text{ day}^{-1}$) was calculated by the difference between the DM of the sampling dates divided by the period (days) between cuts. This process was performed in each subperiod.

Total herbage production (kg DM ha^{-1}) was calculated by the sum of herbage production in each subperiod [daily herbage accumulation rate ($\text{kg DM ha}^{-1} \text{ day}^{-1}$) multiplied by the number of days of each subperiod], and the initial herbage mass (evaluated one day before starting the stocking period). Finally, at the end of the stocking period, residual herbage mass (kg DM ha^{-1}) was estimated following the same methodology used to measure herbage mass.

3.2.3.2. Animal measurements

The study was approved and carried out in strict accordance with the recommendations of the Ethical Review Committee on the Use of Animals of the Federal University of Rio Grande do Sul, Brazil (project no 34358).

The animals were 11-month-old Corriedale castrated sheep, weighing $24.5 \pm 0.3 \text{ kg}$ and $29.8 \pm 0.6 \text{ kg}$ of live weight (LW) at the beginning of the stocking period in 2017 and 2018, respectively. Sheep were weighed after fasting from solids and liquids for approximately 12 h. Average daily gain (ADG, $\text{g animal}^{-1} \text{ day}^{-1}$) was calculated as the difference between final and initial LW of tester animals, divided by the number of days in each subperiod. Whenever necessary to put or take sheep to keep the target sward canopy management, these sheep were weighed, and their weights and time spent in the paddock were considered to the stocking rate calculation. The stocking rate ($\text{kg LW ha}^{-1} \text{ day}^{-1}$) was calculated by the sum of average LW of testers and put-and-

take animals, multiplied by the number of days that the animals remained in the paddock, expressed per unit area. The LW gain per hectare was obtained by the sum of sheep LW gain in each subperiod. For that, stocking rate (in number of animals per ha) was multiplied by the ADG of the tester sheep and by the number of days of the subperiod.

3.2.4. Crop phase

3.2.4.1. Crop management

In both years, after the stocking period, the Italian ryegrass was desiccated with glyphosate (3 L ha⁻¹) and saflufenacil (100 g ha⁻¹). Soybean seeds (*Glycine max*) were treated with insecticide and fungicide, inoculated and sown in rows spaced 0.45 m apart at a density of 36 seeds m⁻², under no-tillage. The management was performed as recommended (specific product). Pest control in soybean crop was weekly monitored and the use of herbicides, insecticides and fungicides was conducted according to the technical recommendations. Soybean harvest occurred every April.

3.2.4.2 Crop measurements

Six areas per paddock were randomly chosen to measure the soybean grain yield (kg ha⁻¹) in the phenological stage R8. In each area, six two-linear-meter (0.9 m² per sample) of soybean plants were clipped at ground level and the grains were harvested, weighted and had their humidity measured. The soybean yield was estimated by multiplying the grain weight by ten thousand and dividing by the sample area (0.9 m²) and then multiplied by a correction factor to obtain soybean yield adjusted to 13% of humidity.

3.2.5. System production and resource-use efficiency

The system production was assessed in two ways, by calculating the equivalent soybean (kg ha⁻¹) and equivalent energy (Gj ha⁻¹) produced in each system phase. The sum of commercial prices of sheep and soybean sales in September and April respectively was divided by soybean sale prices to be expressed as equivalent soybean yields (kg ha⁻¹). Product sale prices were obtained from the Management Planning Division of Rio Grande do Sul state, Brazil (Emater/Ascar), converted into US\$ by Central Bank of Brazil and used to calculus. System production in equivalent energy production (GJ ha⁻¹) was obtained multiplying pasture phase production (total herbage production and sheep LW gain) and crop phase production (soybean yield) by their caloric values. The caloric values used were: 18.05 MJ kg⁻¹ for above-ground biomass (Fuksa et al. 2013), 13.1 MJ kg⁻¹ for meat sheep carcass (Silva et al., 2005), and 15.05 MJ kg⁻¹ for soybean grain (Alimaghani et al., 2017). The meat equivalent energy was measured multiplying LW gain by equivalent carcass [44.1% of LW; Carvalho et al. (2006)], multiplying by the equivalent energy. The system

productivity was obtained by system production, in equivalent energy production (Gj ha^{-1}), divided by inputs (kg of N, P_2O_5 and K_2O) applied in the system. A system that presented greater productivity compared to other was considered more efficient in the use of resources.

3.2.6 Data analysis

The assumptions of the analysis of variance (ANOVA) were achieved (normality by Shapiro test ($P > 0.05$), variance homogeneity by Bartlett test ($P > 0.05$), and visual residual analysis). The ANOVA was run using a mixed model by LMER function of package lme4 in R Studio software (v.3.6.0). The production system (grazed vs. non-grazed), fertilization strategy effect (crop fertilization vs. system fertilization), and their interaction were considered fixed effects. Random effects included block, subperiod, and year. The subperiod effect was included in the model for response variables evaluated every 28 days. For animal performance, fertilization strategies were considered fixed effect, and block, subperiod and year, as random effects. Animal performance per area included fertilization strategies as fixed effect and block and year as random effects. For herbage production, soybean yield, system production and productivity, fertilization strategy and their interaction were fixed effects and random effects were block and year.

3.3. Results and discussion

Pasture variables presented no interaction ($P > 0.05$) between fertilization strategies and production systems (Table 1). An important factor to assign the results to the effects of treatments is the pasture baseline. In that regard, initial herbage mass did not differ ($P = 0.55$) between treatments. Average sward canopy height during the pasture phase was greater ($P < 0.01$) for the cropping system (non-grazed) than for the ICLS (grazed) treatments. This result was expected due to free plant growth in the absence of grazing, leading to faster internode elongation and early flowering, compared to grazed areas that extended the plant vegetative growing period (Rocha et al., 2004). However, the sward canopy height between ICLS treatments with different fertilization strategies was similar (~ 16 cm; $P = 0.85$) and close to the target of moderate grazing intensity proposed in this study. Since herbage mass and sward canopy height are linearly related (Kunrath et al., 2020), herbage mass in our study did not differ ($P > 0.05$) between fertilization strategies (Table 1).

The pasture results show that sheep were kept in similar grazing conditions, so average daily gain (ADG) was similar ($P = 0.21$) between treatments (Table 1). Assuming that herbage intake was similar as a consequence of successful sward canopy height control, the only difference in ADG would come from herbage chemical quality. Therefore, despite possible differences in nutrient composition that were not studied here, the similarity for ADG regardless of the fertilization

strategy suggests that sward structure prevails over herbage chemical quality. Results are in agreement with (Carvalho et al., 2018b), who argued grazing intensity as a major factor influencing animal performance in ICLS via sward canopy height, which affects the bite mass and, consequently, the herbage intake.

Well-managed pastures kept sufficient leaves after being grazed and stimulate the regrowth of new tillers that were previously shaded, increasing the productivity of the entire plant community (Lemaire, 2001). This process can explain the greater daily herbage accumulation rate (+27%; $P < 0.01$) and total herbage production (+20%; $P < 0.05$) obtained in the ICLS compared to the cropping system. Nunes et al. (2019) observed similar results when evaluating the herbage accumulation in ICLS. They found higher daily herbage accumulation rate and total herbage production under moderate to light grazing intensities (20 to 40 cm sward height) compared to non-grazed areas of mixed black oat (*Avena strigosa*) and Italian ryegrass pastures.

The system fertilization approach promoted greater herbage accumulation rate (+24%; $P < 0.01$) and total herbage production (+18%; $P < 0.05$) compared to the conventional crop fertilization (Table 1). According to Lemaire et al. (2019), N supply increases P demand by plants. This could explain our results, being that, when N, P, and K were applied in system fertilization, the N increase P and K demand which in this system the plant had easy availability compared to crop fertilization. In addition, Grant et al. (2001) suggested that plants subjected to low soil temperatures have a greater requirement for the more easily obtainable nutrient. In our experimental site, the lower temperatures occur during the pasture phase and the system fertilization strategy provide soluble P and K. The increase in total herbage production resulted in a greater stocking rate (+17%; $P < 0.05$) to keep the targeted sward canopy height at system fertilization compared to crop fertilization (Table 1). However, this difference was not enough to impact LW gain per area ($P > 0.05$) even though system fertilization presented ~9% greater compared to crop fertilization.

The residual herbage mass presented no interaction between fertilization strategies and production systems ($P = 0.98$; Table 1), and no difference was found for the fertilization strategies ($P > 0.05$). Results reaffirm the successful sward canopy height control up to the end of the stocking period. A key factor affecting agroecosystem sustainability is the presence of crop residues on the soil. These residues allow soil protection from direct rainfall impact, avoid compaction due to machinery traffic and animal trampling, water, and wind erosion, and improve soil organic matter developing better conditions for plants to grow. Considering the comparison between ICLS and cropping system, the presence of grazing animals has an obvious consequence on decreasing average herbage mass and residual herbage mass ($P < 0.01$) in the ICLS compared to the cropping system (Table 1). The residual herbage mass is an important variable of connection between pasture and crop phases in no-till systems (Kunrath et al., 2020). However, although the ICLS presented

lower residual herbage mass (2882 kg DM ha⁻¹), no effect ($P = 0.88$) was found in the soybean grain yield compared to the cropping system (5620 kg DM ha⁻¹).

Grazing decreases residual herbage mass to crop in succession, making farmers resist to the idea of including animals in cropping systems. However, ICLS have benefits that sometimes are not easy to notice in the short-term. According to (Carvalho et al., 2018c), animal contributions to system resilience are more evident over the long term. Grazing stimulates greater root production, increasing exudation of root organic compounds that promote the increase in microbial biomass (Davinic et al., 2013). Also, livestock excreta (urine and dung) improve litter quality and grazing might increase 1.5-fold the carbon exudation from grazed plants (Hamilton et al., 2008). This process increases the rhizospheric decomposer community resulting in a 5-fold rhizospheric daily net mineralization rate. Furthermore, Peterson et al. (2019) evaluating a 16-year experiment, pointed out that beef cattle managed under moderate grazing intensity (2500-4000 kg residual DM ha⁻¹) in the pasture phase of an ICLS does not affect soybean yield, despite the lower water content in the soil when compared to non-grazed areas (6000-8000 kg residual DM ha⁻¹). Thus, the soybean plants, sensitive to abiotic factors as rain-fed conditions, kept your production even in lower soil water condition compared to the cropping system, in long term under ICLS could improve grain yield (Carvalho et al., 2018b).

3.3.1 System production and resource-use efficiency

Diversity and trophic complexity in agroecosystems are important factors in conservation agriculture, affecting system sustainability over-time. These systems increase the production from an existing agricultural land reducing risks and environmental impact by the diversification and complexity (Carvalho et al., 2018c) that are inherent properties of natural agroecosystems. In this study, we contrasted for the first time ICLS and cropping system using a system fertilization approach compared to the conventional crop fertilization. Animal grazing (ICLS) and system fertilization affect positively herbage production without decreasing soybean yield. This is an important result since soybean is a summer crop with high demand on soil fertility and highly responsive to grain yield with fertilizer application. This shows that fertilizers applied in the pasture phase were kept on soil and were easily obtained by soybean plants. In addition, the system fertilization strategy potentially improves the efficiency of crop sowing operations by decreasing the time spent with reloading the planter with fertilizer (T. S. Assmann et al., 2017). Carvalho et al. (2018a) analyzed the impact of introducing grazing to cover crops in rotation with grain crops and found out that grazing cover crops improved the yield of the following grain crops by 3.4, 4.7, 10.4 and 10.8% on average to soybean, bean, irrigated rice and maize, respectively. The authors argue

that reports indicating the superiority of crop yield of non-grazed areas compared to grazed areas are rare, and commonly associated with the use of inappropriate grazing intensity.

Although soybean yield in our study was not different ($P > 0.05$), when the LW gain of the ICLS is converted to equivalent soybean grains and added to soybean yield, the result represent an increase in 58% ($P < 0.001$) to ICLS compared to cropping system (Table 1). These results corroborate with data reported by (Carvalho et al., 2018c) who found 60% greater soybean grain equivalent in the ICLS compared to the cropping system in a long-term study.

The ICLS had greater energy production ($P < 0.05$) compared to the cropping system, regardless of fertilization strategy (Table 1), with no interaction between factors ($P > 0.05$). Greater energy production was attributed to two factors: greater herbage production added to animal production. The animal grazing is the key to this system due to the capacity to convert herbage in the highly nutritious human-edible food sources. Besides removing nutrients by intake and returning them via excretion, the grazing animal has the capacity to convert plant organic nutrients to inorganic nutrients during the digestion process (Haynes and Williams, 1993). These authors found 80% of inorganic P in the dung of animals that ingested plant material with 64% of inorganic P. Dung is a source of labile nutrients, which may increase microbial biomass (Hatch et al., 2000). This allows rapid access to nutrients by microorganisms and growing plants. Moreover, livestock makes a necessary and important contribution to global nutrition, contributing 17% of calories and 33% of protein (FAO, 2019b).

The need to increase food production to meet the demand of a growing population has led to an increase in the use of human-edible feed ingredients, such as soybeans and cereals, in the ruminant sector. This is a concern, since it increases the competition with the human population for a limited global supply of grain crops, adding to the already existing demand for grains by the monogastric animal production sector (Wilkinson and Lee, 2018). Southern Brazil has approximately 15 million ha of land under agricultural use (CONAB, 2019). From this area, only 1.95 million ha (13%) is integrated with livestock (Embrapa, 2016) and approximately 4.7 million ha (31% of total agricultural land) covered with winter cereal crops (CONAB, 2019), resulting in 44% of agricultural land used during the winter season. Thus, it is possible to explore 56% (~8.4 million ha) of agricultural land to food production in Southern Brazil. Considering the average animal performance from this study, the 8.4 million ha that are currently not used during the winter season in Southern Brazil could produce 1.2 billion kg of sheep carcass in well-managed pastures without using human-edible feed resources or expanding the agricultural area. Our study illustrates a fattening system typical of Southern Brazil where sheep are purchased early in the winter and sold for slaughter at the end of the stocking period. However, there are several ways to explore this kind

of integration in real farms, such as rearing females for herd replacement in full-cycle ranches where forage is scarce in the winter period.

In addition to increasing production and contributing to the global food supply, it is necessary to be more efficient in input use. Improvements in resource-use efficiency can be achieved through technology, animal health, management and feed crop varieties (FAO, 2019b). Thus, we investigated how the inclusion of grazing on cover crops (ICLS) and the application of a new conceptual model of fertilization (system fertilization) would affect system productivity in terms of energy production per unit of nutrient input and how efficient these systems could be in the use of these resources (Fig. 4). In this sense, the system productivity efficiency ($\text{Gj kg fertilizer}^{-1}$) did not present interaction between production system and fertilization strategy ($P > 0.05$). Moreover, even though the efficiency of nutrient use was not affected by fertilization strategies ($P = 0.07$), system fertilization was 12%, on average, more efficient in the use of N, P_2O_5 and K_2O compared to crop fertilization. In addition, ICLS presented 15 and 17% more efficient in the use of N and P_2O_5 compared to the cropping system ($P < 0.05$). Similar result was found for K_2O ($P < 0.05$), which produced 2.9 ± 0.08 and 2.2 ± 0.19 $\text{GJ kg K}_2\text{O}^{-1}$ for ICLS and cropping system, respectively.

Despite Brazilian farmer's perceptions that the integration of grazing animals into cropping systems is detrimental to crop production (Carvalho et al., 2018c), our results show the positive effects of well-managed grazing of cover crops in ICLS on increasing total energy produced per unit area and improving fertilizer use efficiency. According to FAO (2019), greater input use efficiency is a crucial strategy for decoupling growth in the livestock sector to environmental impact. It is important to highlight that the crop and livestock integration do not impair the production system, on the contrary, these integrated systems when well-managed are beneficial and important for the world food production in the future.

3.4. Conclusions

Our findings highlight for the first time that system fertilization strategy and integrated crop-livestock systems (ICLS) results in greater herbage production without affecting soybean yield. Sheep production makes these systems more productive and efficient in the use of resources through the production of high-quality food. Finally, we believe that the specialized systems as a cropping system, could be unsustainable in the near future, and the ICLS with well-managed pastures and system fertilization strategy in soils with high nutrient levels are a potential and necessary pathway to increase food production, improving the land use sustainability and productivity without increasing agriculture expansion and/or deforestation, which in our view should be considered as a climate-smart agriculture strategy.

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Conflict of interest The authors declare that they have no conflict of interest.

Author contribution Conceptualization, P.C.F.C., C.B., G.D.F., T.T. and A.P.M.; Formal analysis, G.D.F and C.B.; Investigation, G.D.F., L.P.D., J.V.S., and L.A.A.; Data Curation, G.D.F., C.B., J.V.S. and L.P.D., Writing - original draft, G.D.F., Writing – review and editing, G.D.F., J.V.S., P.C.F.C., J.C.B.D., C.B., T.T., L.A.A. and A.P.M.; Visualization, G.D.F., Project administration, G.D.F. and L.A.A., Supervision, C.B. and P.C.F.C.

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Figures and tables



Fig 1 **a** Italian ryegrass cover crop (non-grazed) in specialized system (cropping system). **b** Sheep grazing Italian ryegrass (*Lolium multiflorum*) under moderate grazing intensity (15 cm sward canopy height) in the integrated crop-livestock system (ICLS). **c** Soybean in the middle summer growing season (January).

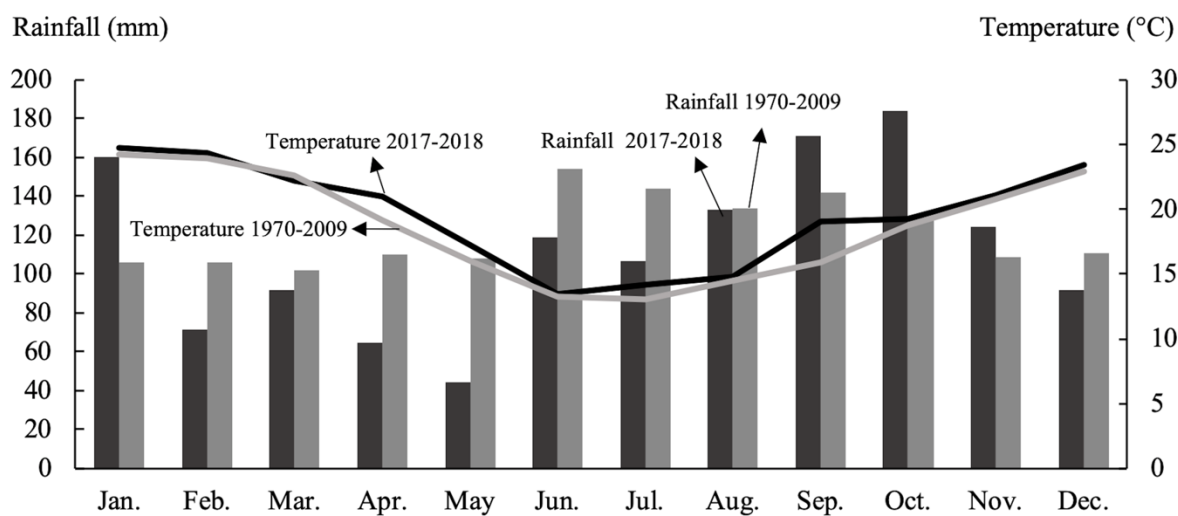


Fig 2 Annual average rainfall and mean air temperature at the Agronomy Experimental Station from Federal University of Rio Grande do Sul during the experimental period (2017 – 2018) and the long-term climatic means between 1970 and 2009.

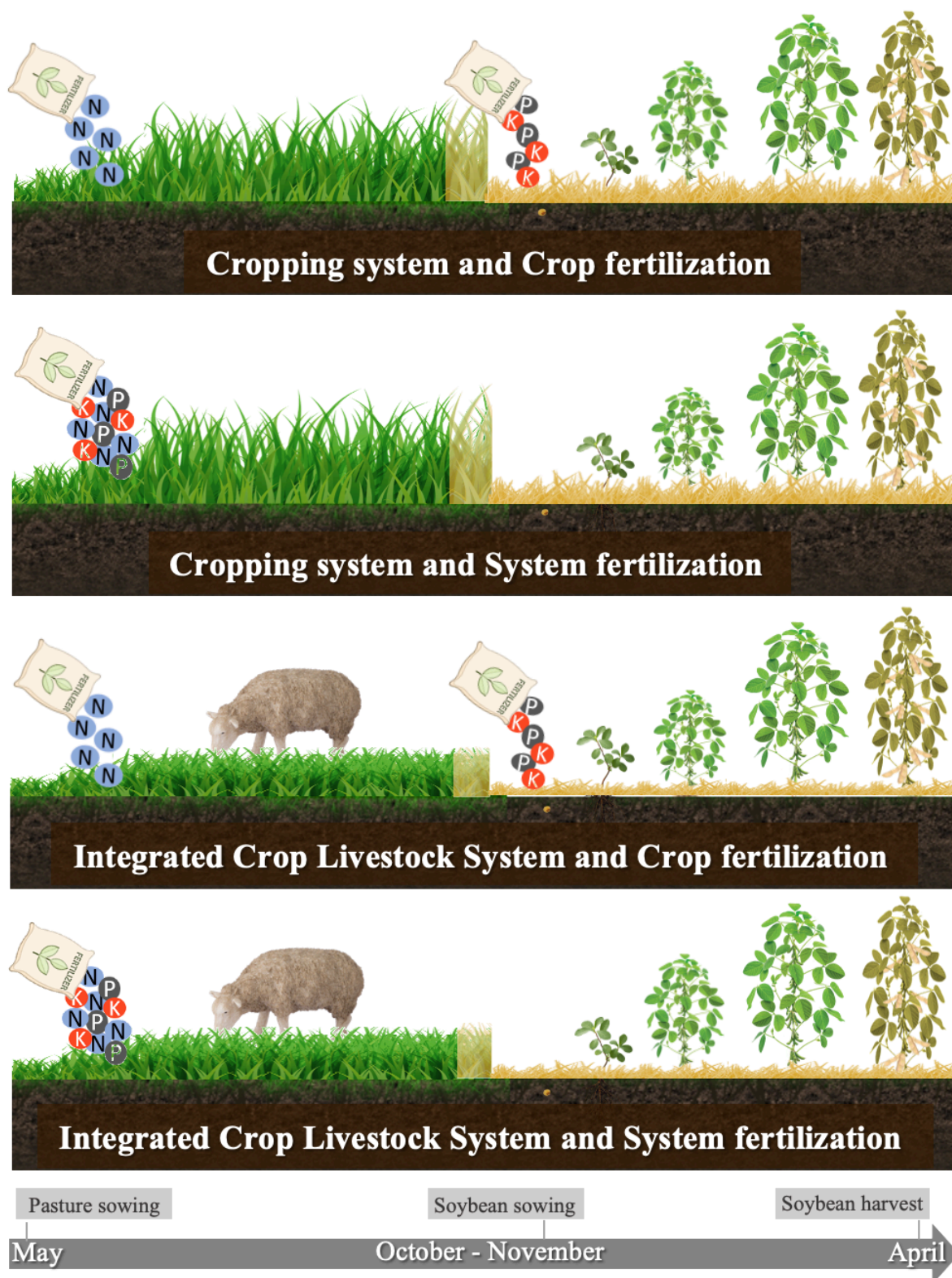


Fig 3 Schematic representation of the treatments: cropping system or integrated crop-livestock system (ICLS) with crop fertilization or system fertilization in southern Brazil.

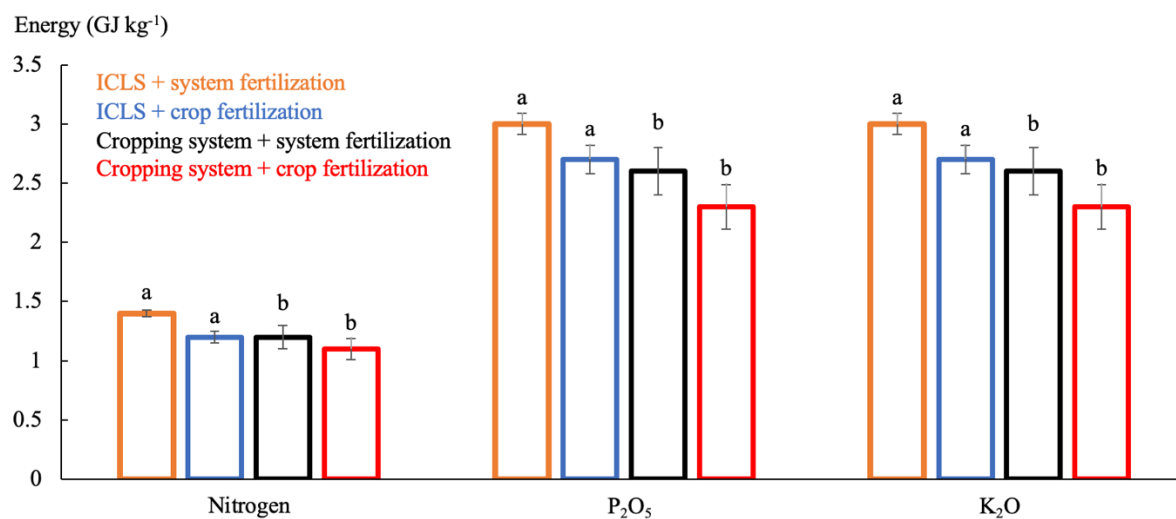


Fig 4 Energy produced by input (kilogram of Nitrogen, P₂O₅ and K₂O) applied (2017/2018 and 2018/2019) in an integrated crop-livestock system (ICLS) or cropping system with the system or crop fertilization in southern Brazil. The different letters are the significance level at 5% of the production system effect (ICLS versus cropping system).

Table 1 Characteristics and average production in the first two years of pasture and crop phases in an integrated crop-livestock system or cropping system with crop or system fertilization in southern Brazil.

Variables	ICLS		Cropping system		P_F	P_A	$P_{F \times A}$
	SF	CF	SF	CF			
<i>Herbage (pasture phase)</i>							
Sward canopy height (cm)	16.2 ± 0.3	16.3 ± 0.3	37.6 ± 2.3	37.9 ± 1.8	ns	***	ns
Initial herbage mass (kg DM ha ⁻¹)	1258 ± 103	1374 ± 87	1367 ± 149	1615 ± 152	ns	ns	ns
Herbage mass (kg DM ha ⁻¹)	2220 ± 114	2200 ± 131	3688 ± 309	4065 ± 271	ns	***	ns
Daily herbage accumulation rate (kg DM ha ⁻¹)	67.3 ± 4.2	57.7 ± 4.8	56.6 ± 7.2	42.2 ± 7.1	**	**	ns
Total herbage production (kg DM ha ⁻¹)	9395 ± 407	8061 ± 488	7897 ± 862	6629 ± 596	*	*	ns
Residual herbage mass (kg DM ha ⁻¹)	3002 ± 154	2763 ± 102	5735 ± 570	5504 ± 570	ns	***	ns
<i>Animal (pasture phase)</i>							
Average daily gain (g sheep ⁻¹ day ⁻¹)	123 ± 11.7	134 ± 11.5	-	-	ns	-	-
Stocking rate (kg LW ha ⁻¹)	872 ± 57.1	745 ± 52.0	-	-	*	-	-
Live weight gain (kg ha ⁻¹)	337 ± 9.1	310 ± 27.2	-	-	ns	-	-
<i>Soybean (crop phase)</i>							
Soybean yield (kg ha ⁻¹)	2730 ± 172	3019 ± 135	2920 ± 163	2877 ± 212	ns	ns	ns
<i>System production (pasture + crop phase)</i>							
Eq. soybean yield (kg ha ⁻¹)	4537 ± 140	4652 ± 186	2920 ± 163	2877 ± 212	ns	***	ns
Eq. energy production (GJ ha ⁻¹)	212.7 ± 6.1	192.1 ± 8.7	150.8 ± 25.1	162.9 ± 13.1	ns	**	ns

ICLS = integrated crop-livestock system; SF = system fertilization; CF = crop fertilization; DM = dry matter; LW = live weight. P_F = significance level for fertilization effect; P_A = significance level for animal effect (ICLS or cropping system); $P_{F \times A}$ = significance level for interaction between fertilization and animal effect (ICLS or cropping system); * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; ns = Not significant.

4. CHAPTER IV

**Can fertilization approaches in non- or integrated crop-livestock system
change nutrient status of plants?³**

³ Manuscript prepared according to the *European Journal of Agronomy* rules (Appendix 3).

Can fertilization approaches in non- or integrated crop-livestock system change nutrient status of plants?

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Abstract

Integrating crop with livestock has been proposed for increasing the whole agriculture system's productivity, but the management of crop fertilization to better take advantage of the animal's potential to recycle nutrients has been little studied. Thus, our study hypothesized that the anticipation of phosphorus and potassium fertilizers (system fertilization) in ICLS has a positive effect on the nutritional status of ryegrass pastures during the pasture phase and ensures the transfer of nutrients to the soybean crop in succession. For this purpose, we tested the influence of sheep grazing on ryegrass pasture (ICLS) as compared to non-grazed (cropping system) with the anticipation of phosphorus (P) and potassium (K) fertilization for the establishment of pasture (system fertilization) or traditional application of fertilizer in the establishment of grain culture (crop fertilization). The experimental design was completely randomized blocks in factorial 2 x 2 with four replicates. Results show that Italian ryegrass P content was greater ($P < 0.001$) in system fertilization, regardless of days after Italian ryegrass sowing. The content of P in Italian ryegrass was greater after 63 days in ICLS when compared to cropping system ($P < 0.05$). System fertilization presented, on average, 12% greater K content in Italian ryegrass compared to crop fertilization during stocking period ($P < 0.01$). Regarding the animal effect, we observed 14% greater K content, on average, in ICLS when compared to cropping system ($P < 0.01$). For all treatments,

the ryegrass data were situated above the reference model %P and %K - %N relationship, indicating that at similar %N, plants present higher %P or %K as expected for their maximum biomass production. The soybean crop presented no effect of grazing, fertilization strategy or its interaction ($P > 0.05$) on P and K contents. Our results highlight that ICLS and system fertilization strategy improves phosphorus and potassium nutrition status in Italian ryegrass plants over the pasture phase. In addition, soybean nutrition status is not affected by fertilization strategies or by animal effect (grazed or non-grazed pastures).

Keywords: mixed system, plant nutrition, Integrated system, nutrient content, system fertilization

4.1. Introduction

Food production requires large amounts of nutrients, being nitrogen (N), phosphorus (P) and potassium (K) the most frequently deficient on soils. The P limits food production in the world on arable land to around 40% (Divito and Sadras, 2014). FAO (2019) estimates an average annually increase of 1.3% in world synthetic fertilizer demand for N, P and K from 2016 to 2022. Although N is dependent of a large energy cost and greenhouse gas emission, is practically inexhaustible nutrient (Galembeck et al., 2019). However, P and K are a finite resource and to ensure food security and sustainability over time, fertilizers should be used with caution, conserving them within circular production, consumption and recovery cycles (Galembeck et al., 2019). Thus, agricultural systems with high potential of nutrient recycling are crucial for the future of food production.

Although the 7 and 3.5-fold increase in the use of N and P fertilizers, respectively, drove to the huge increase in crop production from the end of the Second World War until the end of the 20th century (Lemaire et al., 2019; Tilman et al., 2002), it in part led the production system to specialization in cropping system due reduction need for animals to fertilization areas with manure. Cropping system is characterized by the non-inclusion of animal grazing, that is, a system that has grain production in the summer and throughout the winter remains with pastures like oat and/or Italian ryegrass in southern Brazil only as a cover crop. Also, specialization was driven by the increasing technical complexity of production in a diversified system (Garrett et al., 2020). Specialized systems go in the opposite direction from nature regarding the sustainable functioning of a system in the long term. In this sense, FAO (2010) recognizes integrated crop-livestock systems (ICLS) as a sustainable manner to intensify production. Thus, ICLS is an alternative to the specialized system (cropping

system), in the direction of long-term sustainable systems with profitable food and fiber production (Bell et al., 2021; Kunrath et al., 2015; Nunes et al., 2019). For sustainable production, resource use-efficiency is essential in the farm, and ruminant capacity for recycling nutrients, exporting little amount of them is a crucial step (Farias et al., 2020; Alves et al., 2021; Hendrikson et al., 2008).

Based on animal capacity of nutrient cycling emerge a new approach of fertilization named system fertilization. In summary, the fertilization of crop is anticipated to the pasture phase to be performed in the system phase with less nutrient export and high nutrient recycling capacity (Assmann et al., 2017). Studies have highlighted improvements in system production when N is applied in anticipation as system fertilization approach performed in an ICLS (Assmann et al., 2003; Sartor et al., 2018). A recent study showed that system fertilization approach with P and K presented positive response for system production (Farias et al., 2020). However, there is a gap in knowledge regarding plant nutrition status when P and K are applied anticipated, that is, in the pasture phase of the ICLS.

In this way, we have two hypotheses: (i) system fertilization in ICLS has a positive effect in nutrient status of Italian ryegrass swards over the pasture phase and ensures nutrient carryover for soybean crop in succession, and (ii) carryover of P and K from crop fertilization in a grazed or non-grazed system is not enough to supply nutrition status of Italian ryegrass over the pasture phase compared to system fertilization. Thus, the aim of this study was to evaluate nutrient dynamics and nutrition status of Italian ryegrass and soybean plants as a result of different fertilization approaches (system or crop fertilization) and animal effect (ICLS or non-integrated system).

4.2. Materials and Methods

4.2.1. Study area characterization

The trial was carried out at the Experimental Agronomic Station of the Federal University of Rio Grande do Sul (EEA — UFRGS), in Rio Grande do Sul, Brazil (30°05'S, 51°39'W and 46 m a.s.l.). The region's climate is subtropical humid presenting over the experimental period an average annual air temperature of 19.8, 19.2 and 19.8 °C, and annual rainfall of 1510, 1214 and 964.8 mm in 2017, 2018 and 2019, respectively. Fig. 1 presents the monthly variations of air temperature and rainfall during experimental years and the long-term climatic means (from 1970 to 2009). Air

temperature and rainfall were obtained from a meteorological station located approximately 1 km from the experimental site (EEA — UFRGS).

The experimental area was a long-term protocol between 2003 and 2016 managed with a soybean-sheep integrated system under no-till and annually fertilized with nitrogen (N), phosphorus (P) and potassium (K). Experimental area soil is classified as a sandy clay loam Acrisol (FAO, 2006). Soil chemical condition, fertilizations and treatments prior to this experiment can be accessed in Alves et al. (2019).

In 2017 the experimental protocol was restructured and before starting the new protocol, soil chemical analysis was performed in the diagnostic layer soil (0-10 cm). Soil chemical analysis presented 1.7% of organic matter, 3.9 of pH (H₂O), 1.1 cmolc dm⁻³ of calcium, 0.5 cmolc dm⁻³ of magnesium, 15% of base saturation, 49% aluminum saturation, and 94 and 97 mg dm⁻³ of available P and K (extracted by Mehlich 1), respectively. This results in a P and K soil status above optimal condition according to CQFS-RS/SC (2016) needing only limestone application which was performed in a quantity of 7.5 Mg ha⁻¹ (PRNT 72%) to raise soil pH to around 6.0.

4.2.2. Experimental design and treatments

The experimental area presented a total of 4.4 ha, which was divided into 16 paddocks (experimental units) ranging between 0.23 and 0.32 ha each. The experiment was a randomized complete block design with a 2 × 2 factorial arrangement and four replicates, totaling 16 paddocks. The treatments were two production systems (soybean in the crop phase and grazing sheep in the pasture phase, that is, an integrated crop livestock system – ICLS, and soybean in the crop phase and non-grazed Italian ryegrass in the pasture phase, that is, a cropping system), and two periods of P and K fertilization (traditional crop fertilization, with all amount of fertilizer applied in the soybean during sowing, and system fertilization, with all amount of fertilizer applied in the pasture establishment). For more details see Farias et al. (2020). The amount of P and K fertilization were calculated based on soybean grain production of 4 Mg ha⁻¹ (CQFS-RS/SC, 2016). In all paddocks, Italian ryegrass was fertilized with 150 kg N ha⁻¹ (Lemaire, 1997b; Marino et al., 2004) once in phenological stage V3 (3 totally expanded leaves).

4.2.3. Sward management and sampling

Stocking period was started in June, in 2017 and 2018, and in July, in 2019, and finished in October in all years, totalizing, respectively, 125, 120 and 114 days of stocking period. Stocking period started when Italian ryegrass sward canopy reach at 15 cm, on average. Sheep were managed under continuous stocking method keeping sward canopy at 15 cm over the pasture phase. For this, sward canopy heights were weekly measured with a sward stick at 150 randomly samples per paddock. To maintain the sward canopy height target we used put-and-take sheep (Moot and Lucas, 1952); see detail in Farias et al. (2020).

In 2017 and 2018, herbage samples were clipped at the beginning of stocking period (31 days after Italian ryegrass sowing, DAS) and each ~30 days (subperiod). For that, six herbage samples of 0.25 m² per paddock were clipped at ground level. At the same time, four grazing exclusion cages were allocated per paddock in a similar sward canopy to calculate the herbage accumulation rate. These herbage samples were clipped at ground level and allocated in identified paper bags and dried at 55 °C in a forced-air oven until constant weight (approximately 72 hours). Afterward, samples were weighed obtaining the partially dry weight of each sample (kg MS 0.25m⁻²) that were used to estimate the amount of pasture per hectare by multiplying these values by 40,000 (number of samples of 0.25m² in one hectare, kg MS ha⁻¹). Herbage accumulation in each subperiod were a result of difference between sward canopy clipped inside grazing exclusion cages and the sward canopy clipped at beginning of each subperiod. These values were used to obtain Italian ryegrass biomass accumulated that was calculated as a sum of herbage mass sampled at beginning of stocking period with herbage accumulation in each subperiod.

In 2019, extra grazing exclusion cages were allocated to collect Italian ryegrass samples under free growth. The forage sampling started when the ryegrass reached 1 Mg ha⁻¹ (49 DAS) occurring every ~14 days until 90 DAS (before reproductive phenological stage). Samples were allocated in identified paper bags, and then were dried at 55 °C in a forced-air oven until constant weight (approximately 72 hours). After, these samples were weighted, grounded in a knife mill (1 mm sieve), and homogenized in a composite sample per paddock to chemical analysis.

4.2.4. *Crop management*

In the first week after the end of the stocking period, in October, the Italian ryegrass was desiccated with glyphosate herbicide (3 L ha⁻¹) and Saflufenacil (100 g

ha⁻¹) in 2017 and with glyphosate herbicide (3 L ha⁻¹) and 2,4D (1.5L ha⁻¹) in 2018 and 2019. Two days after Italian ryegrass desiccation, soybean seeds (*Glycine max* (L) Merr.) that received insecticide and fungicide treatment, and inoculation, were sown under no-tillage in a density of 255 thousand plants per hectare at 0.45 m of line-plant space. Pest control in soybean crop was weekly monitored following integrated pest management.

Soybean samples were collected in the phenological stages R2 and R4. For this, phenological stage was weekly monitored according to Fehr and Caviness (1977) description with randomly ten points by one-linear meter (0.45 m²) in each paddock. Due to soybean present undetermined growth, the sampling was performed when paddock's plants achieved 50% or more of the target phenological stage. Four samples per paddock were clipped at ground level over one-linear meter, allocated at identified paper bags and taken to a forced-air oven at 55 °C until constant weight to obtain partially dry matter. In sequence, samples were ground in a knife mill (1 mm sieve), homogenized and a subsample taken to the laboratory to chemical analysis.

4.2.5. *Nutrient measurement and nutritional status of the plants*

Plant tissue analyses were performed according to the method proposed by Tedesco et al. (1995). The P and K contents in Italian ryegrass and soybean plants were analyzed in 2017 and 2018. In 2019, the P, K and N contents of Italian ryegrass were analyzed. For that, to determine the N, P and K contents, firstly we performed acid digestion in 0.200 g of the plant sample to obtain digestion extract. The digestion extract was used to obtain N content by the kjeldahl method. The plant P content was obtained by spectrophotometry in an aliquot of the digestion extract after the addition of ammonium and aminonapholsulfonic acid. The plant K content was obtained by flame photometry after diluting the digestion extract, adjusting the sensitivity of the apparatus to the appropriate standards.

Nitrogen nutrition status of Italian ryegrass were evaluated using a reference value proposed by Lemaire (1997). For this, N critical value (N_c) was estimated by equation 1 where the coefficient 4.8 characterizes the maximum percentage of N at low dry matter (DM, Mg ha⁻¹), and the coefficient – 0.32 characterizes the temporal N dilution behavior during pasture growth. After, were calculated the nitrogen nutrition index (NNI) by division between N verified in the samples and critical N value according to equation 2.

$$N_c = 4.8 \times DM^{-0.32} \quad (\text{Eq. 1})$$

$$NNI = N_{\text{verif.}} / N_c \quad (\text{Eq. 2})$$

The nutrition status of P and K was assessed as the ratio of %P and %K with %N. This is due to the stoichiometric relationship between these nutrients. Thus, the models proposed by Duru and Théliier-Huché (1995) for P ($P_{\text{ref.}}$) and K ($K_{\text{ref.}}$) were used as reference to compare with our results (see Eq. 3 and 4).

$$P_{\text{ref.}} = 0.15 + 0.065 * N\% \quad (\text{Eq. 3})$$

$$K_{\text{ref.}} = 1.6 + 0.525 * N\% \quad (\text{Eq. 4})$$

These models clearly indicates that plant %P and %K cannot be used directly as diagnosis of the P and K nutrition of the crop as their values highly depend of that of plant %N as a consequence of (i) the value of biomass (DM) as shown in Eq. 1; and also of (ii) the level of N nutrition of the crop as attested by Eq. 2. So, Eq. 3 and Eq. 4 from Duru and Thellier-Huché (1995) are expected to represent the %P and %K corresponding to non-limiting P and K nutrition. Nevertheless, a high uncertainty remains whether these equations can be considered as “critical %P and %K” value in a large range of conditions. So, we used these equations only as reference for ranking the differences observed in %P and %K between the different treatments in relative terms.

4.2.6. Statistical analysis

4.2.6.1. *Ryegrass and soybean characterization in 2017 and 2018*

Data of dry matter accumulated and nutrient content (P and K) of Italian ryegrass sward were submitted to analysis of variance (ANOVA), considering 5% of significance level. Normality and homogeneity of variance by Shapiro-Wilk test ($P > 0.05$) and Bartlett test ($P > 0.05$), respectively, and visual residual analysis were checked. Analysis of variance was run using a mixed model by lmer function of package lme4 in R software (version 4.0.2), considering the fixed effects of animal effect (ICLS or cropping system), fertilization strategy (crop or system fertilization), days after Italian ryegrass sowing, and their interactions. Paddock was included in the model as repeated measure in time, since each experimental unit was measured at different periods (days after sowing). We tested different models with the inclusion of random effects for block and/or year. The best fit model was chose using the Akaike’s criterion (AIC). The final model for dry matter accumulated, and P and K content over ryegrass

phase included the block and year as random effects, as well as the repeated measure in time.

Data of soybean P and K content were averaged for the two phenological stages, R2 and R4, and submitted to ANOVA using a mixed model that included the animal effect, fertilization strategy and its interaction as fixed effects, and block and year as random effects. When differences between the studied effects were observed, means were compared by the Tukey test ($P < 0.05$), using the packages `emmeans` and `multcompView` of R software.

4.2.6.2. *Ryegrass nutrition status evaluated in year 2019*

Firstly, we evaluated the N nutrient status of ryegrass estimated by the NNI using Equations 1 and 2, previously specified. Thus, NNI was submitted to ANOVA using a mixed model that included the animal effect, fertilization strategy, days after Italian ryegrass sowing and their interactions as fixed effect. Paddock was considered random effect in the model, characterizing a repeated measured in time. Different models were tested, and the best fit model was chosen by minimizing AIC. When differences between means were observed, they were compared by the Tukey test ($P < 0.05$).

For Italian ryegrass %P and %K, we run linear and non-linear models (square root, asymptotic and weibull) using, respectively, the `lm` and `nls` functions in R software. %N was considered the independent variable in all models. We compared the linear and non-linear models for each treatment using the Akaike's criterion, and we choose the linear ($y = a + bx$) as the best fit model. The next step was to compare the linear models between treatments. For that, we considered a 95% of confidence interval for comparing the slope and the intercept between treatment models, using the `confint2` function of package `nlstools` of R software. When the slope and intercept of models were similar ($P > 0,05$), one single model was generated. We also compared the linear models of each treatment with the model proposed by Duru and Th  lier-Huch   (1995), using 95% of confidence interval.

4.5. Results

4.5.1. *Italian ryegrass biomass accumulated and P and K contents in Italian ryegrass and soybean plants*

Italian ryegrass biomass accumulated is presented in Fig. 2. Results show a growing accumulated biomass over days after Italian ryegrass sowing ($P < 0.001$) but without difference between treatments until 122 days after Italian ryegrass sowing. However, significant animal ($P = 0.006$) and fertilization effect ($P = 0.012$) were observed in 151 days after Italian ryegrass sowing, where grazed system (ICLS) and system fertilization presented greater biomass accumulated compared to non-grazed system (cropping system) and crop fertilization, respectively.

The Italian ryegrass P and K contents are shown in Fig. 3. The P content in Italian ryegrass presented no interaction between animal effect and fertilization strategy ($P = 0.14$). Similarly, no interaction between fertilization strategy and days after Italian ryegrass sowing was found ($P = 0.07$). Italian ryegrass P content was greater ($P < 0.001$) in system fertilization, regardless of days after Italian ryegrass sowing (Fig. 3a). We observed an interaction between animal effect and days after Italian ryegrass sowing for P content ($P < 0.05$). The content of P in Italian ryegrass was similar ($P > 0.05$) between grazed (ICLS) and non-grazed (cropping system) systems in the first two periods after sowing (31 and 63 days); however, after that (91, 122 and 151 days), ICLS presented greater ($P < 0.05$) P content compared to cropping system (Fig. 3b).

No interaction between animal effect and fertilization strategy ($P = 0.16$), neither between animal effect ($P = 0.07$) or fertilization strategy ($P = 0.60$) with days after ryegrass sowing were observed for K content in Italian ryegrass. System fertilization presented, on average, 12% greater K content in Italian ryegrass compared to crop fertilization during stocking period ($P < 0.01$; Fig. 3c). Regarding the animal effect, we observed 14% greater K content, on average, in ICLS when compared to cropping system ($P < 0.01$; Fig. 3d).

The soybean crop presented no effect of grazing, fertilization strategy or its interaction ($P > 0.05$) on P and K contents (Table 1).

4.5.2. N, P and K nutrition status of Italian ryegrass

No effect of fertilization strategy, animal effect or its interaction on N nutrition index of Italian ryegrass was observed ($P > 0.05$; Fig. 4). However, we observed significant effect of days after Italian ryegrass sowing on N nutrition index, independent of treatments, with lower value (0.73 ± 0.03) at 90 days when compared to 49 (0.94 ± 0.02), 62 (0.97 ± 0.03), and 76 days (0.86 ± 0.03) ($P < 0.001$, Fig. 4).

Figure 5 allows the analysis of the link of %P and %K of with %N according to the linear model of Duru and Thellier-Huché (1995), Eqs. 3 and 4. Italian ryegrass %P – %N and %K – %N relationships were adjusted to linear models. For all the four treatments, the data are situated above the Duru and Thellier-Huché line, indicating that at similar %N plant had higher %P or %K as expected for their maximum biomass production.

The ICLS with system fertilization presented 180% greater increase in the P content per unit of increased N content in the plant tissue compared to the cropping system with crop fertilization ($P < 0.05$; Fig. 5a). On the other hand, cropping system with system fertilization and ICLS with crop fertilization presented no difference between the other treatments. Herbage K content increases linearly with increase in the herbage N content, but without effect of fertilization strategy, animal effect nor its interaction ($P > 0.05$; Fig. 5b).

4.6. Discussion

Efficient nutrient management in agricultural systems started with knowledge of the nutrient content and nutrition status of the plants that are spatially and temporally distributed in the system. In agreement with our first hypothesis, we observed an increase in nutrient content of Italian ryegrass managed under system fertilization compared to crop fertilization (Fig. 3a and 3c). This shows that even in soil with built fertility, that is, soil P and K levels above the critical content (CQFS-RS/SC, 2016), the Italian ryegrass nutrient absorption is greater when fertilization strategy is applied with a system approach. The greater herbage accumulated in the last evaluated period was observed in system fertilization compared to crop fertilization (Fig. 2). These findings suggest that in crop fertilization, soil reserves after soybean harvest seems not to provide enough P and K to meet Italian ryegrass requirements for optimal growth compared to system fertilization.

These results are in agreement with Alves et al. (2021), who reported greater values of P and K contents in the Italian ryegrass swards managed under system fertilization strategy. Almeida et al. (2021) and Alves et al. (2019) show that the nutrients exportation by crop grains harvest represents above of 95% of total system nutrients exportation, such as P, K, Ca and Mg, greater values compared to the less than 5% exported by animal production in an ICLS. In this sense, harvesting grains could cause depletion of nutrients in the soil and, when fertilization is carried out based

on the potential for nutrient cycling within the productive system (system fertilization), nutrients should be replenished as soon as the largest export of nutrients from the system occurs (Assmann et al., 2017). Also, this replaced nutrient after greater extraction by crop harvest improves nutrient gradient which consequently can increase diffusion, the main process of P and K soil mobility to the plant root (Bucher et al., 2018; Meurer et al., 2018; Oliveira et al., 2004). Kellermeier et al. (2014) evaluating nutrient contents of shoots under different levels and combinations of N, P and K show greater P and K contents with increases in soil P and K availability. In addition, these authors found a positive correlation between shoot nutrient contents (P, Ca, Mg, Na, Mn, and K) and the number of lateral roots, suggesting that the number of lateral roots can determine overall uptake of these nutrients.

ICLS presented similar shoot P content until 63 days after Italian ryegrass sowing. However, after this period there was a greater P in ICLS compared to the cropping system (Fig 3b). Italian ryegrass could be classified as a grazing tolerant plant (Briske, 1996) with physiological and morphological mechanisms which enhance its growth after defoliation, such as enhancing nutrient absorption and reallocation. Also, grazing process can promote root exudate which have influence in soil microorganisms (López-Mársico et al., 2015; Sekaran et al., 2021), improving its biomass and enzymatic activity (Aldezabal et al., 2015; Xun et al., 2018). In this sense, Zhu et al. (2016) observed a positive effect of fertilization with manure on arbuscular mycorrhizal fungi community composition, and Ehteshami et al. (2018) showed greater dry matter yield and P content in sorghum plants when fertilized or with arbuscular mycorrhizal fungi seed application compared to non-fertilized treatment. These results highlight an indirect effect of grazing process in ICLS on plant nutrient content and suggest a greater amount of nutrient which will return to soil via animal manure and plant senescence.

Although forage plants are efficient in K extraction and recycling decreasing losses (Garcia et al., 2008), our results showed that in well-managed grazed pastures this capacity seems to be potentiated. This seems to be made clear when we observe the result of greater K content in ICLS compared to the non-grazed cropping system (Fig. 1d). According to Haynes and Williams (1993), grazing process improve K availability in soil surface, and this result can be attributed to high K recycling (90%) performed by animal grazing being greater part of K recycled from urine, in form readily available to plant uptake.

There is concern among farmers about the inclusion of grazing animals in agricultural areas, largely due to trampling and a reduction in the amount of pasture residues after the grazing period. However, previous research show that well-managed pastures do not cause damage to the soil. On the contrary, it brings positive benefits to the production system (Abdalla et al., 2018; Ambus et al., 2018; Bell et al., 2011; Franzluebbbers et al., 2012). On the other hand, the reduction of pasture residues seems to be compensated by animal manure and its ability to promote nutrient cycling (da Silva et al., 2014). Our results show that even with reduction in pasture residue by animal grazing in the ICLS treatment (see in Farias et al., 2020) and with the anticipation of fertilization for the pasture phase (system fertilization), the nutrient status of soybean did not differ from a purely agricultural system (Table 1). Thus, it is important to highlight here that although there is a reduction in the pasture residue, a management that ensure at least 2000 kg DM ha⁻¹ is considered enough for an ICLS work well in the long-term (Kunrath et al., 2015; Nunes et al., 2021).

The results of this study show that our second hypothesis was not confirmed. Considering the model proposed by (Duru and Thélier-Huché, 1995) as a reference, regardless of the fertilization strategy or the presence (ICLS) or not (cropping system) of grazing animals in the system, Italian ryegrass presented adequate nutrition status of P and K in all evaluated periods (Fig. 5). In this figure, temporal evolution is indicated by high %N values at the right part of the figure, corresponding to the beginning of the Italian ryegrass growth period, to the lowest %N at the left part of the figure, corresponding to increasing days after Italian ryegrass sowing. So, for all treatments %P and %K decline with %N decreasing as a result of the dilution associated with increase in biomass, as expected by Eqs. 3 and 4.

The anticipation of fertilization with P and K for the pasture phase (system fertilization) led to a higher %P per unit of %N in the plant tissue when in greater N status, which was potentiated in the grazed systems (ICLS, Fig. 5a). This response can be attributed to greater nutrient availability at the beginning of the evaluation period in ICLS with system fertilization; where N fertilization stimulates plant growth and the high P availability due to system fertilization led to plant better conditions and greater nutrients relationship compared to non-grazed cropping system with crop fertilization. However, the fast decrease represented by the high slope in the model of ICLS with system fertilization, achieving similar P when N is zero (intercept of the model) compared to other treatments suggests that this system performs better in a greater N

availability. This is supported by the N nutrition index result which presented a significant decrease at 90 days after Italian ryegrass sowing compared to the first three periods (42, 63 and 76 days, Fig. 4). Although previous studies have shown that N fertilization at doses of 100-150 kg ha⁻¹ has satisfactory N nutrition levels throughout the pasture phase (Lemaire, 1997; Marino et al., 2004), our results suggest that 150 kg N ha⁻¹ in a single application was not sufficient to maintain the N nutrition index in the Italian ryegrass plants after 76 days after Italian ryegrass sowing (Fig. 3). In that regard, it is important to study the effect of the amount and the splitting of N fertilization on the nutrition index of the swards, which is unknown in ICLSs managed under system fertilization.

Finally, the results of this study indicate no effect of grazing, fertilization strategy, or its interaction in the increment of K according to N increases. Considering that the model proposed by Duru and Théliier-Huché (1995) is an estimated plant K condition close to the critical level, our findings highlight that regardless the studied treatments, all of them are above the reference model, suggesting being in an optimal plant K nutrition status (Fig. 5b). Similar results were found by Bernardon et al. (2020) evaluating N levels, who noted the K plant condition above the estimated critical model regardless of N fertilization level. These results can be attributed to the ease of recycling of mineral K, which although fulfilling important functions in plant physiology such as turgor generation and cell expansion, it is not a structural component in the plant and can be quickly recycled from residues (Ragel et al., 2019). In this perspective, Arnuti et al. (2020) show that a higher part of K content in sheep dung was located in labile compartment, resulting in a faster K release until 60-80 days after exposition to environment. In agreement with that, Assmann et al. (2017) found a high rate of K release of dung and pasture without effect of grazing intensity. This faster K release is attributed to higher part of K be maintained in a water-soluble form (Weeda, 1977).

4.7. Conclusion

Our results highlight that ICLS and system fertilization strategy improves phosphorus and potassium nutrition status in Italian ryegrass plants over the pasture phase. In addition, soybean nutrition status is not affected by fertilization strategies or by animal effect (grazed or non-grazed pastures). Finally, we demonstrate for the first time that ICLS with well-managed pastures and system fertilization strategy, i.e. in the pasture phase, is a good way to promote high nutrition status in Italian ryegrass

pastures and soybean crop, which may indicate a reduction in the need for synthetic fertilizers in the long term.

4.8. References

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Figures and tables

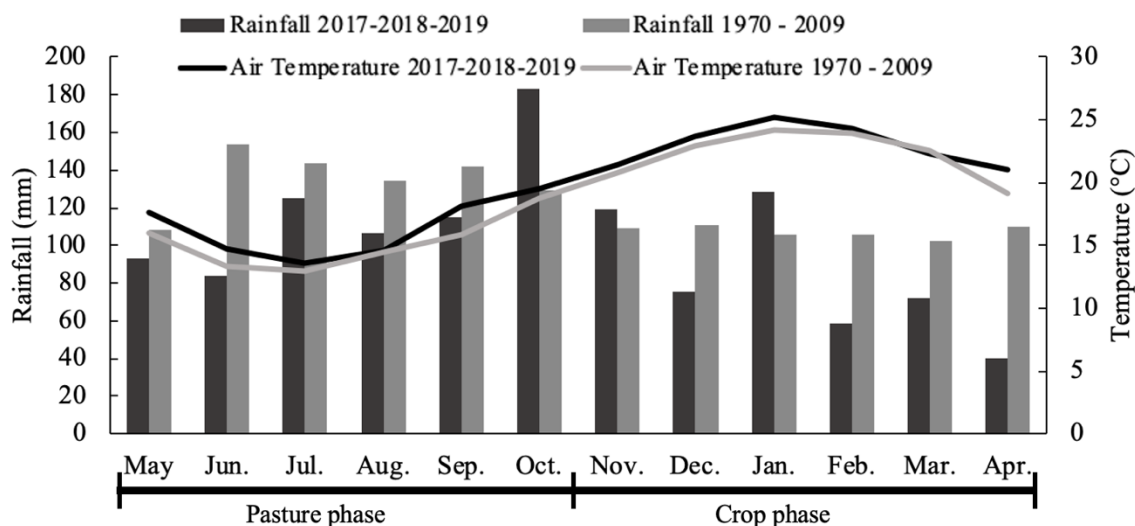


Figure 4 Annual average rainfall and air temperature at the Agronomy Experimental Station from Federal University of Rio Grande do Sul during the experimental period (2017 – 2018 – 2019) and the long-term climatic means between 1970 and 2009.

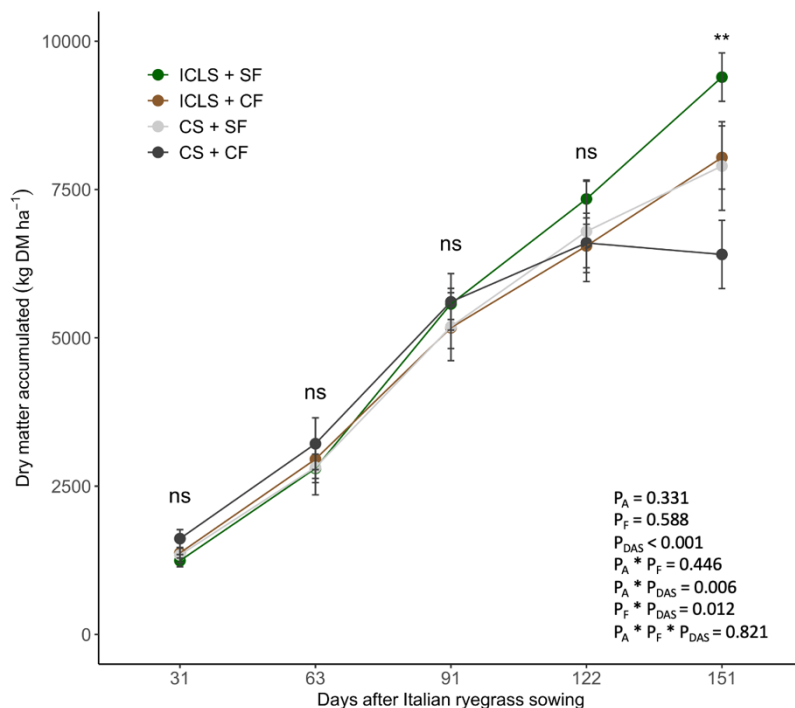


Figure 5 Average of dry matter accumulated in a Italian ryegrass over ryegrass phase 2017 and managed under integrated crop-livestock system (ICLS) or cropping system (CS) with system (SF) or crop fertilization (CF).

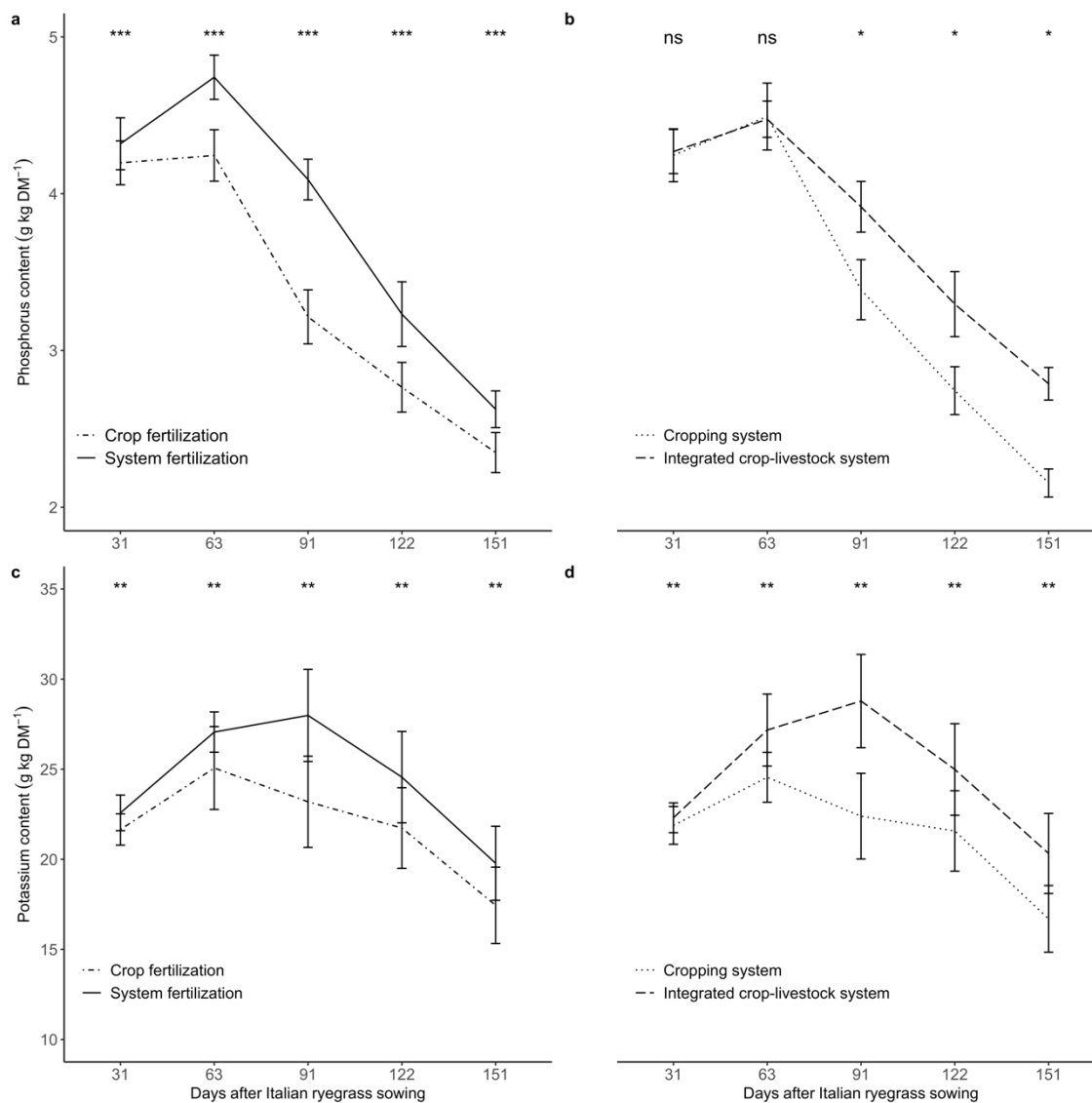


Figure 6 Average of nutrient content in Italian ryegrass plants during stocking period of 2017 and 2018. Phosphorus (a) and potassium (c) for system or crop fertilization and phosphorus (b) and potassium (d) content in the integrated crop-livestock system or cropping system. The significant level is represented by * = $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and ns = not significant. Bars represent the standard error of the mean.

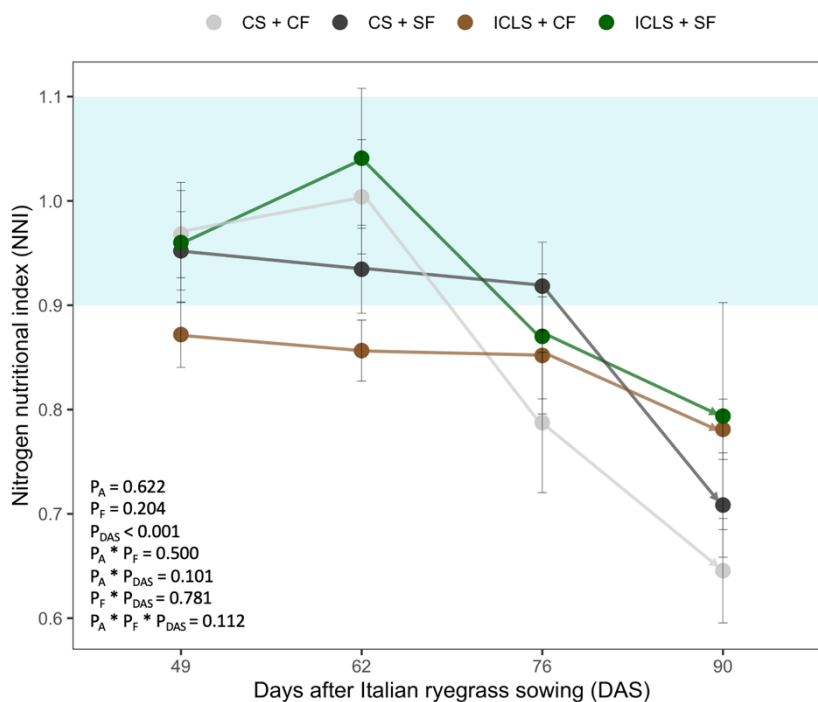


Figure 7 Relationship between nitrogen nutrition index and days after Italian ryegrass sowing of the pastures managed under integrated crop-livestock system (ICLS) or cropping system (CS) with system (SF) or crop fertilization (CF). Below the blue band indicates poor plant nutrient absorption, within the blue band characterizes the optimal plant nutrient absorption and above the blue band, luxury absorption condition of plants. P = p-value; A = animal effect; F = fertilization strategy; DAS = days after Italian ryegrass sowing.

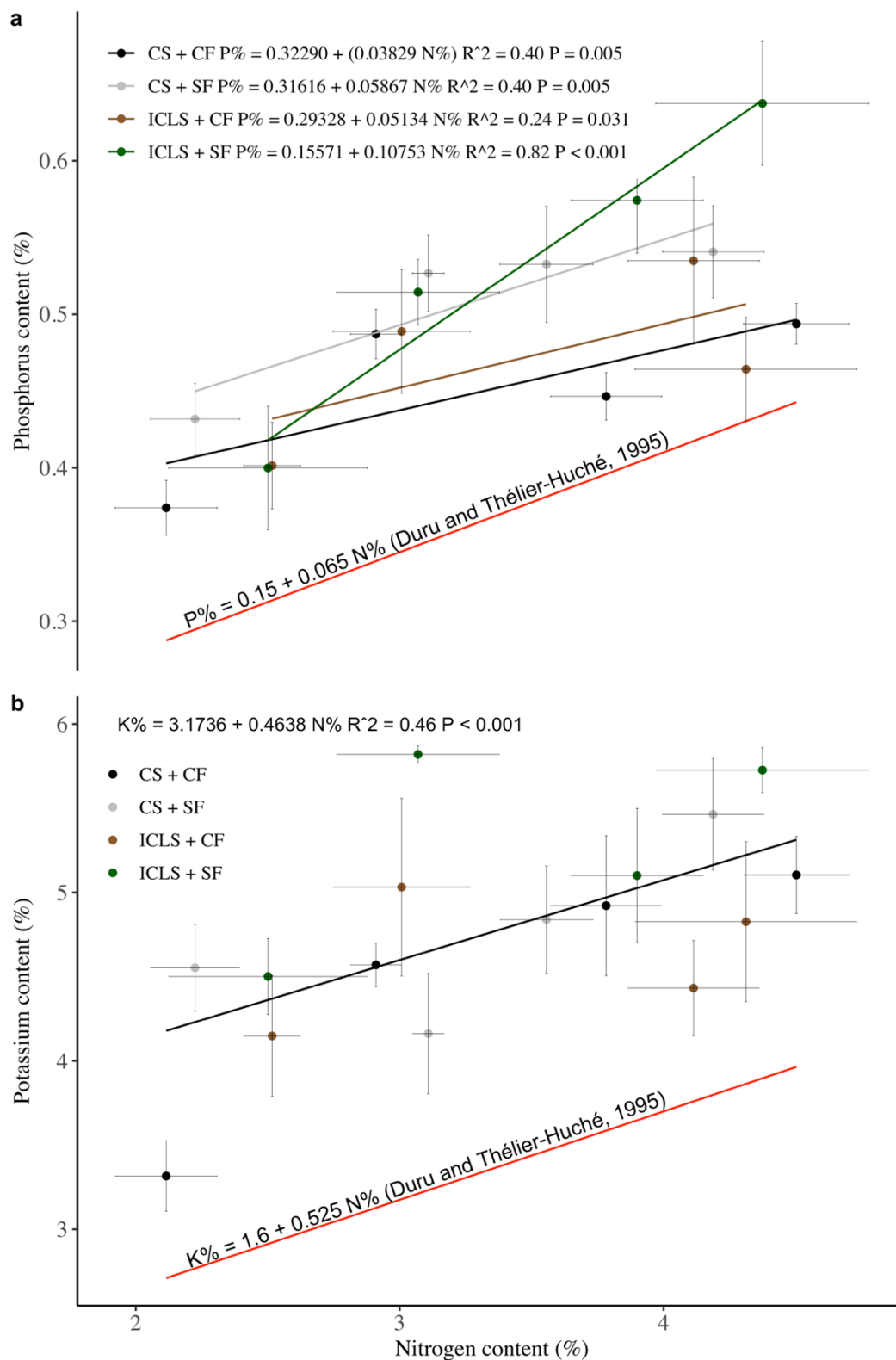


Figure 8 Nutrition index of Italian ryegrass pastures managed under integrated crop-livestock system (ICLS) or cropping system (CS) with system (SF) or crop fertilization (CF). (a) relationship between phosphorus nutrition index and days after Italian ryegrass sowing

Table 1 Average of phosphorus and potassium contents (g kg DM⁻¹) in soybean crop (phenological stages R2 and R4) in an integrated crop-livestock system (ICLS) or cropping system (CS) with system (SF) or crop fertilization (CF).

Variables	ICLS		Cropping system		P_F	P_A	$P_{F \times A}$
	SF	CF	SF	CF			
Phosphorus	3.1±0.1	3.2±0.1	3.0±0.1	3.1±0.1	0.323	0.352	0.603
Potassium	20.0±0.6	20.3±0.7	21.0±1.2	21.0±1.0	0.713	0.289	0.956

P_F = significance level for fertilization strategy (system or crop fertilization); P_A = significance level for animal effect (ICLS or cropping system); $P_{F \times A}$ = significance level for interaction between fertilization strategy and animal effect.

5. CHAPTER V

FINAL CONSIDERATIONS

In Chapter I we showed that sheep can be a flexible alternative that can be fitted in different systems in order to obtain the beneficial effects of the synergism between the soil-plant-animal-environment components. In this sense, the need to redesign production systems with a holistic vision is highlighted in order to capture the benefits of synergies. However, it is still necessary to evolve in the understanding of how the sheep can benefit the system and benefit from it. For that, it is important that research are developed using the sheep model or even the bovine/sheep mix as an animal model in order to better understand the interactions and potential productive and environmental benefits.

In Chapter II, it was possible to demonstrate that increasing the complexity of productive systems by reintegrating the animal component with adequate management of pasture and agricultural crops is a necessity to increase food production with greater efficiency in the use of resources, many of with potential to cause damage to the environment. In addition, adjusting the management of fertilizer P and K anticipating its application to the pasture phase in the same holistic approach that supports the inclusion of animal in the system, that is, the nutrient recycling is beneficial and necessary for productivity increase and efficiency in the use of finite resources such as P and K. In this sense, it is important to highlight that such benefits with the anticipation of fertilizer P and K were observed in soil with the availability of nutrients above the critical level established by the CQFS-RS/SC (2016).

A third study was carried out (Chapter III) in which it was discussed about a possible effect of different systems on the nutrient content in ryegrass and soybean plants. It was observed that even in soil with nutrient conditions above the critical level, the ryegrass plant concentrated a greater amount of nutrients in their tissues with the addition of more nutrients via inorganic fertilizer. Also, and perhaps most importantly, is that anticipating fertilization (system fertilization) in a holistic view of the system, in addition to responding with greater biomass production and maintaining a greater amount of nutrients protected against possible losses, it does not affect the nutrient content in the soybean crop in succession nor its productivity. These results show that the fertilization approach usually employed, which only targets a specific crop, can be incomplete and should be evolved to a holistic approach such as system fertilization.

In this way, we think that the results presented in this thesis are an advance in the knowledge of integrated crop-livestock systems and in the construction of the concept of system fertilization. Here, it has been demonstrated that it is possible to make production systems more efficient without losing productivity. On the other hand, we see the need for novel research, which evaluates the possibility of reducing the input of finite resources such as P and K and the capacity of resistance to maintain the productivity of the system when reducing, limiting, or not providing these nutrients. Also, determine N level and fertilization management to keep adequate nutritional conditions over all period of the pasture phase.

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APPENDIX

Appendix 1 – Rules for the preparation and submission of scientific papers to the journal *Small Ruminant Research*

Types of article

1. Original Research Papers (Regular Papers)
2. Review Articles
3. Short Communication
4. Technical Notes
5. Short Technical Notes
6. Book Reviews

Original Research Papers should report the results of original research. The material should not have been previously published elsewhere, except in a preliminary form.

Review Articles should cover subjects falling within the scope of the journal which are of active current interest. Reviews will often be invited, but submitted reviews will also be considered for publication. All reviews will be subject to the same peer review process as applies for original papers.

A *Short Communication* is a concise but complete description of a limited investigation, which will not be included in a later paper. Submission of short communications is not encouraged. Short Communications may result from a request to condense a regular paper, during the peer review process. Results and Discussion are merged. Short Communications should not exceed 3,000 words, including the words in figure and table captions, and references. The number of tables and figures should not exceed four.

A *Technical Note* is a report on a new method, technique or procedure falling within the scope of *Small Ruminant Research*. It may involve a new algorithm, computer program (e.g. for statistical analysis or for simulation), or testing method for example. The Technical Note should be used for information that cannot adequately incorporated into an Original Research Article, but that is of sufficient value to be brought to the attention of the readers of *Small Ruminant Research*. The note should describe the nature of the new method, technique or procedure and clarify how it differs from those currently in use. It should not exceed 4,000 words.

Short Technical Notes of approximately 500 words can be submitted by geneticists to report the existence of genes and mutations found in small ruminants.

Book Reviews will be included in the journal on a range of relevant books which are not more than 2 years old. Book reviews will be solicited. Unsolicited reviews will not usually be accepted, but suggestions for appropriate books for review may be sent to the Editor-in-Chief.

What is publishable: Papers on polymorphism studies will be considered only if they

contain significant new information and have direct relevance to those species described in the aims and scope of this journal. Submissions on studies involving single-nucleotide polymorphism (SNP) only, without linking them strongly and experimentally to production traits, are not encouraged. Manuscripts with quantitative RT-PCR without multiple normalizer gene products will be declined at preliminary review.

Geneticists can submit Short Technical Notes of approximately 500 words, which will include the name of the gene, the location of the mutation (the sequence has to be deposited), the description of the population (breed, location, significant characters), possibly the allele frequency, even in small population, and some additional relevant information, with no need to demonstrate significant association with phenotypic traits or discussion. Accumulation of such information may lead to design comprehensive association studies in sheep and goats.

Papers on the use of feeds in nutrition are publishable only if these feeds have more than local importance, which should be detailed in the introduction. In many studies of nutrition, the effect on animal performance of substituting a feed with another is investigated and the hypothesis is that no effect is anticipated. We recommend a power analysis to determine sample size before planning the study. If authors want to report that they have discovered no difference they should add confidence limits to the difference between the sample means: if the sample size is indeed too small, these limits will usually be too broad to be informative. If the authors' aim is to show no effect, then the usual rule for bioequivalence is that the 90%CI for the ratio between the two means needs to lie between 0.8 and 1.25.

Authors need to clearly state the experimental unit and degrees of freedom for the error term. With nutrition papers involving feeding animals in paddocks or pens with more than one animal, it is the number of paddocks or pens which determines the experimental units, not the number of animals in total, unless it is demonstrated that each animal takes independent foraging decisions.

Manuscripts that deal with the effects of plant secondary metabolites (PSMs) or plant extracts using in-vitro methods only are not published, unless if associated to a large-scale, long-term in vivo study. In studies with PSMs or plant extracts, advanced chemical analysis of the extracts should be documented. In vitro studies of the nutritional value of feeds are not in our scope unless they provide a background for in vivo studies in the same manuscript. Studies of the quality of semen, oocytes, embryos, following exposure to various materials (plant extracts, anti-oxidants, fatty acids and diluents) will be considered only if they are associated with in vivo evidence.

In the field of health, case reports presenting work in individual animals will not be considered. Only case reports presenting population medicine approaches will be considered for further evaluation on the condition that they have wide implications, well beyond their local interest, and good statistical evidence.

For products, we will consider studies on carcasses but not on the further processing of meat products for human food. Studies on the textile processing of fibres are also excluded. We will evaluate studies with milk as a whole entity, in the frame of a well-defined production system, and not as a generic commodity. Studies on the manufacture of "milk products" as mixtures of milk components or fractionated milk with non-milk ingredients will not be considered for publication.

Papers on production systems will be considered only if their results can be connected to concepts and knowledge published elsewhere and/or extend them to

scale up in genericity. Therefore, descriptive papers on production systems and local projects without connection to global development issues will generally not be considered. Special attention is given to the quality of methodological approaches and bibliographical references.

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If the work involves the use of human subjects, the author should ensure that the work described has been carried out in accordance with [The Code of Ethics of the World Medical Association](#) (Declaration of Helsinki) for experiments involving humans. The manuscript should be in line with the [Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals](#) and aim for the inclusion of representative human populations (sex, age and ethnicity) as per those recommendations. The terms [sex and gender](#) should be used correctly.

Authors should include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed.

All animal experiments should comply with the [ARRIVE guidelines](#) and should be carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, [EU Directive 2010/63/EU for animal experiments](#), or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and the authors should clearly indicate in the manuscript that such guidelines have been followed. The sex of animals must be indicated, and where appropriate, the influence (or association) of sex on the results of the study.

Unnecessary cruelty in animal experimentation is not acceptable to the Editors of *Small Ruminant Research*.

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RESEARCH ARTICLE



Integrated crop-livestock system with system fertilization approach improves food production and resource-use efficiency in agricultural lands

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Abstract

Integrated crop-livestock systems (ICLS) can be an alternative to increase the productivity of agroecosystems by enhancing nutrient cycling via grazing animals. Despite the holistic approach that bears the designing of ICLS, fertilization practices are proceeded in a conventional crop basis, disregarding nutrient fluxes at the appropriate spatial and temporal dynamics. We argue that fertilization practices in ICLS must follow the same integrated approach. To test this, we compared a conventional crop fertilization strategy versus a system fertilization approach applied to two production systems being a conventional cropping system and ICLS. The conventional cropping system consisted of a soybean crop succeeded by a non-grazed Italian ryegrass cover crop. The ICLS model consisted of a soybean-Italian ryegrass rotation grazed by sheep. In the conventional crop fertilization strategy phosphorus and potassium were applied at soybean sowing and nitrogen at the Italian ryegrass establishment. The system fertilization consisted of the application of all nutrients during the Italian ryegrass establishment. Accordingly, treatments were fertilization strategies in a factorial framework with production systems randomly distributed in a complete block design with four replicates. Results indicated for the first time greater daily herbage accumulation rate (24%; $P < 0.01$) and total herbage production (18%; $P < 0.05$) in the system fertilization compared with conventional crop fertilization. Consequently, system fertilization allowed for greater stocking rates in the pasture phase (17%; $P < 0.05$). The ICLS presented greater equivalent soybean yield ($P < 0.001$), energy production ($P < 0.01$), and system productivity ($P < 0.05$) compared with the cropping system, regardless of fertilization strategies. Soybean yield was not affected by fertilization strategies or grazing. In conclusion, the adoption of system fertilization strategy and crop-livestock integration enhance the production without jeopardizing soybean grain yields, so that land use is optimized by a greater energy production per unit of nutrient applied.

Keywords Cropping systems · Grazing · Mixed crop-livestock systems · Nutrient cycling · Soybean · Crop fertilization

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1 Introduction

Human population and income have been increasing in the last decades and, simultaneously, the global requirement for animal source food is expected to rise soon (Mottet et al. 2017). Thus, production systems that supply large amounts of food to global markets will need to increase their production. In the current scenario, there is an increasing social and political pressure to preserve natural ecosystems, added to increasing urbanization, and the specialized commercial agroecosystem models with high use of non-renewable resources. These facts pose barriers to the expansion of agricultural frontiers to increase food, fiber, and energy production per unit of area and input (Lemaire et al. 2015). Specialized

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Introduction

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VITA

Gustavo Duarte Farias, filho de Mara Nubia Duarte Farias e Fabio Luçardo Farias, nasceu no dia 08 de outubro de 1990, em Canguçu, Rio Grande do Sul, Brasil. Coursou a maior parte do Ensino Fundamental e Médio na Escola Gladi Machado Garcia, em Minas do Camaquã, onde concluiu seus estudos no ano de 2006. Após este período trabalhou em uma moro peças na cidade de Canguçu enquanto se preparava para o vestibular. Devido seu apreço a produção animal atribuído a ter desde muito jovem contato com a produção animal junto a propriedade da família, em 2009 prestou vestibular na Universidade Federal de Pelotas (UFPEL) ao qual foi aprovado para cursar Zootecnia. Assim, ingressou em setembro de 2009, na 3ª turma do curso de Zootecnia da UFPEL, onde imediatamente iniciou suas atividades como voluntário de iniciação científica no Grupo de Estudos em Comportamento dos Animais de Produção (GECAP) o qual posteriormente (2012) foi unido ao Grupo de estudo em pastagens e plantas forrageiras (GEPAF) da mesma Universidade emergindo o Núcleo Zootecnia de Precisão (ZOOPREC) sob supervisão da professora Dra Isabella Dias Barbosa Silveira. Formou-se Zootecnista em Agosto de 2014 e assumiu o posto de gerente em uma propriedade rural localizada na cidade de Cristal/RS. No entanto, seu vínculo com a ciência durante todo o período da graduação o levou a se inscrever e prestar as provas de avaliação do mestrado acadêmico na UFPEL. Foi aprovado e em setembro de 2015 ingressou no Mestrado em Zootecnia sob orientação do Dr Ricardo Zambarda Vaz, um dos líderes do Grupo de Estudos em Cadeias Produtivas de Ruminantes (GECAPPEC). Tornou-se Mestre em Zootecnia em março de 2017. Em abril do mesmo ano, iniciou seus estudos no curso de Doutorado em Zootecnia na UFRGS, sob orientação da Dra Carolina Bremm e co-orientação do Dr. Paulo Cesar de Faccio Carvalho. Esteve por quatro meses como aluno visitante no *North Florida Research and Education Center, University of Florida*, Estados Unidos da America sob orientação do pesquisador Dr. José Carlos Batista Dubeux. Tem interesse nas temáticas de sistemas integrados de produção agropecuária e comportamento ingestivo em diferentes ecossistemas pastoris. Até o momento da publicação deste documento, tem em seu currículo 12 artigos científicos e 1 texto em revista, 2 artigos científicos em tramitação e dezenas de resumos publicados em anais de congressos. Gustavo foi submetido à banca de defesa da Tese de Doutorado no dia 19 de março de 2021.