

**UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL  
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PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA**

**PEDRO ARTHUR DE ALBUQUERQUE NUNES**

**ESTABILIDADE PRODUTIVA DO SISTEMA E AUTOSSUFICIÊNCIA EM AZEVÉM  
ANUAL (*Lolium multiflorum* Lam.) DE UM SISTEMA INTEGRADO SOJA-  
BOVINOS DE CORTE**

**Porto Alegre (RS), Brasil**

**Março, 2020**

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ANUAL (*Lolium multiflorum* Lam.) DE UM SISTEMA INTEGRADO SOJA-  
BOVINOS DE CORTE**

Tese apresentada como requisito para obtenção do Grau de Doutor em Zootecnia, na Faculdade de Agronomia, da Universidade Federal do Rio Grande do Sul.

**Orientador:** Paulo César de Faccio Carvalho

**Coorientador:** Amélie C. M. Gaudin

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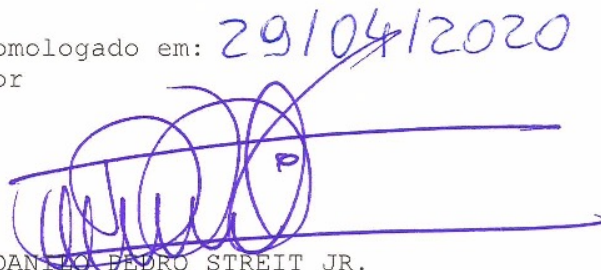
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Orientador

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Por



DANILO PEDRO STREIT JR.  
Coordenador do Programa de  
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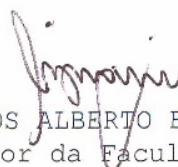
Jerome Bindelle  
Liège University



Caitlin Peterson  
Mad Agriculture



Christian Bredemeier  
UFRGS



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**MUITO OBRIGADO!**

# ESTABILIDADE PRODUTIVA DO SISTEMA E AUTOSSUFICIÊNCIA EM AZEVÉM ANUAL (*Lolium multiflorum* Lam.) DE UM SISTEMA INTEGRADO SOJA-BOVINOS DE CORTE<sup>1</sup>

Autor: Pedro Arthur de Albuquerque Nunes

Orientador: Paulo César de Faccio Carvalho

**Resumo:** O objetivo desta tese foi investigar como a estabilidade produtiva dos sistemas agrícolas é afetada pela integração lavoura-pecuária sob intensidades de pastejo contrastantes. No capítulo II, utilizou-se uma base de dados de 16 anos de um sistema integrado de produção de soja-bovinos de corte para medir os impactos do pastejo em diferentes intensidades sobre: 1) estabilidade produtiva, 2) risco de fracasso e probabilidade de altos rendimentos, e 3) mínimo e máximo potencial produtivo para indicadores de produção vegetal, animal e de sistema quando submetidos a um gradiente de condições ambientais. Os tratamentos consistiram em quatro intensidades de pastejo (intensa, moderada, moderada-leve e leve), definidas por alturas de manejo do pasto (10, 20, 30 e 40 cm, respectivamente) sob pastoreio contínuo, e um controle sem pastejo. O delineamento experimental foi o de blocos completos casualizados. A produção total de proteínas digestíveis e a lucratividade apresentaram maior estabilidade quando os pastos foram manejados sob intensidades moderadas, enquanto a intensificação excessiva ou a ausência de pastejo reduziram a estabilidade destas variáveis. O pastejo não prejudicou a produtividade de soja subsequente, mas reduziu a probabilidade de fracasso em anos desfavoráveis. Maiores alturas de manejo beneficiaram a estabilidade da produção de pasto, mas reduziram a lucratividade do sistema. O pastejo intenso também gerou produções de forragem mais estáveis, porém reduzidas, e ganhos de peso vivo menos estáveis. No capítulo III, foi avaliada a eficácia do estabelecimento do azevém anual (*Lolium multiflorum* Lam.) por ressemeadura natural quando submetido a diferentes intensidades de pastejo em um sistema integrado de produção de soja-bovinos de corte. A estrutura dos tratamentos foi um fatorial de cinco tratamentos de pastejo (10 cm de altura, ou pastejo intenso; 20 cm de altura, ou pastejo moderado; 30 cm de altura, ou pastejo moderado-leve; 40 cm de altura, ou pastejo leve; e um controle sem pastejo) e dois níveis de ressemeadura [ressemeadura natural (SS) e ressemeadura natural com adição de sementes (SS + Add)]. O delineamento experimental foi em blocos completos casualizados com parcelas subdivididas e medidas repetidas no tempo. As avaliações foram realizadas em 2017 e 2018, correspondendo à produção de sementes de 2016 e 2017. O pastejo intenso não foi uma alternativa sustentável para o estabelecimento do azevém por ressemeadura natural. Foram verificadas plantas mais pesadas, mas menor densidade populacional durante o estabelecimento como resultado deste manejo. A sementeira suplementar neste tratamento aumentou a densidade de plantas a valores comparáveis à intensidade de pastejo moderada, mas reduziu a massa individual, comprometendo a massa de forragem total. A combinação de densidade populacional e massa individual de plantas seguindo intensidades de pastejo moderadas foi suficiente para manter a massa de forragem comparável ao controle sem pastejo, independentemente do nível de ressemeadura.

**Palavras-chave:** intensidade de pastejo, intensificação sustentável, manejo do pasto, resiliência, ressemeadura natural, sistemas integrados de produção agropecuária

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<sup>1</sup> Tese de Doutorado em Zootecnia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (150 p.). Março, 2020.

## PRODUCTIVE STABILITY OF THE SYSTEM AND SELF-SUFFICIENCY IN ITALIAN RYEGRASS (*Lolium multiflorum* Lam.) OF AN INTEGRATED SOYBEAN-BEEF CATTLE SYSTEM<sup>2</sup>

Author: Pedro Arthur de Albuquerque Nunes

Advisor: Paulo César de Faccio Carvalho

**Abstract:** The aim of this thesis was to investigate how the productive stability of agricultural systems is affected by crop-livestock integration under contrasting grazing intensities. In Chapter II, we used a 16-year dataset from a long-term, no-till integrated soybean-beef cattle system in southern Brazil to measure the impacts of cover crop grazing at different intensities during the winter period on 1) yield stability, 2) downside risk and probability of high performance, and 3) minimum and maximum yield potentials of key crop, pasture, animal and whole-system outcomes in a range of environmental conditions experienced over time. The experimental design was a randomized complete block design with three replicates. Treatments consisted of four grazing intensities (intense, moderate, moderate-light and light) defined by contrasting sward heights (10, 20, 30 and 40 cm, respectively) under continuous stocking and an ungrazed control. Both human-digestible protein production and profitability presented greater stability when moderately grazed, while the over-intensification or the absence of grazing decreased system stability. Grazing did not affect subsequent soybean yields but reduced the chance of crop failure in unfavorable years. Taller sward heights benefited the stability of herbage production but reduced system profitability. Intense grazing was more stable producing less herbage but had less stable live weight gains. In Chapter III, we evaluated the effectiveness of Italian ryegrass (*Lolium multiflorum* Lam.) establishment by self-seeding and determined which grazing intensity requires the addition of seed to ensure a successful pasture establishment in the upcoming winter stocking period of an integrated soybean-beef cattle system. Treatment structure consisted of a factorial of five grazing treatments under continuous stocking (10 cm sward height, or intense grazing; 20 cm sward height, or moderate grazing; 30 cm sward height, or moderate-light grazing; 40 cm sward height, or light grazing; and an ungrazed control) and two reseeding levels [self-seeding only (SS) and self-seeding with addition of ryegrass seeds (SS+Add)]. The experimental design was a split-plot in a randomized complete block with repeated measures over time. Field samplings were performed during the winter stocking periods of 2017 and 2018, corresponding to Italian ryegrass seeds crops from 2016 and 2017. Intense grazing was not an effective strategy to ensure successful ryegrass establishment by self-seeding, resulting in lower plant population density. Addition of ryegrass seed increased the density of established plants to values comparable to those in moderate grazing intensities but reduced individual plant mass, compromising total herbage mass when plant size and population density were combined. The combination of individual plant mass and population density was sufficient to maintain herbage mass following moderate to light grazing intensities comparable to the ungrazed treatment, regardless of the reseeding level, positively affecting the performance and the resilience of integrated crop-livestock systems.

**Keywords:** environmental index, grazing intensity, integrated crop-livestock systems, pasture management, resilience, sustainable intensification

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<sup>2</sup> Doctoral thesis in Animal Science, Faculty of Agronomy, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil. (150 p.). March, 2020.



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### CAPÍTULO III Intense winter grazing impairs Italian ryegrass cover crop reestablishment by self-seeding in a no-till soybean-beef cattle system

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## LISTA DE ABREVIATURAS

|                               |  |
|-------------------------------|--|
| a.s.l.                        | above sea level  |
| ANOVA                         | analysis of variance                                     |
| BRL                           | Brazilian Real   |
| cm                            | centímetros/centimeters                                  |
| CV                            | coefficient of variation                                 |
| DM                            | dry matter   |
| e.g.                          | <i>exempli gratia</i> (por exemplo; latim)               |
| EI                            | Environmental Index                                      |
| FW                            | Finlay-Wilkinson (regression)                            |
| g                             | gramas/grams   |
| G10                           | intense grazing/10 cm sward height (treatment)           |
| G20                           | moderate grazing/20 cm sward height (treatment)          |
| G30                           | moderate-light grazing/30 cm sward height (treatment)    |
| G40                           | light grazing/40 cm sward height (treatment)             |
| ha                            | hectares   |
| HDP                           | human-digestible protein                                 |
| HSD                           | honest significant difference                            |
| i.e.                          | <i>id est</i> (isto é; latim)                            |
| ICLS                          | integrated crop-livestock system(s)                      |
| K                             | potassium  |
| K <sub>2</sub> O              | potassium oxide  |
| km                            | quilômetros/kilometers                                   |
| LW                            | live weight  |
| m                             | metros/meters  |
| Mg                            | megagramas/megagrams                                     |
| mm                            | milimeters   |
| MS                            | matéria seca   |
| N                             | nitrogen   |
| P                             | phosphorus   |
| P <sub>2</sub> O <sub>5</sub> | phosphorus pentoxide                                     |
| SE                            | standard error   |
| SEM                           | standard error of the mean                               |
| SIPA                          | sistema(s) integrado(s) de produção agropecuária         |
| SS                            | self-seeding only (treatment)                            |
| SS+Add                        | self-seeding with addition of ryegrass seeds (treatment) |
| UG                            | ungrazed cover crop (treatment)                          |
| USA                           | United States of America                                 |
| USD                           | American Dollar  |



## 1. CAPÍTULO I

## 1.1 INTRODUÇÃO

A intensificação da produção de alimentos ocorrida em muitas regiões do mundo nas últimas décadas deu-se, principalmente, a partir de modelos produtivos altamente especializados e amparados no uso irrestrito de insumos químicos e recursos não renováveis (Stoate et al., 2001; Tilman et al., 2001; 2002; Lemaire et al., 2014). Tal especialização em sistemas agrícolas comerciais – que trouxe consigo maior eficiência no uso do trabalho, cultivares agrícolas mais produtivas e economias de escala (Tilman et al., 2002) – gerou ganhos substanciais em produtividade para as principais commodities agrícolas. Os rendimentos da lavoura de soja [*Glycine max* (L.) Merr.], por exemplo, aumentaram em 90% nos Estados Unidos (USDA, 2019a) e 170% no Brasil (CONAB, 2019) nos últimos 40 anos (estes países, juntos, correspondem atualmente a aproximadamente dois terços da produção mundial desta commodity, USDA, 2019b).

Apesar dos inegáveis avanços gerados por este modelo de agricultura moderna, sistemas de produção altamente especializados e baseados na massiva utilização de insumos externos à propriedade geralmente apresentam custos ambientais proporcionalmente elevados (Stoate et al., 2001; Tilman et al., 2002; Foley et al., 2005). A busca incessante por produtividades cada vez mais altas trouxe consigo feitos colaterais, tais como a contaminação dos cursos d'água e lençóis freáticos (Verhoeven et al., 2006; Liu et al., 2010), concentrações crescentes de gases de efeito estufa (MacDonald & McBride, 2009; Vermeulen et al., 2012), degradação dos solos (Montgomery, 2007; Lal, 2015) e perdas de biodiversidade (Foley et al., 2005; Oliveira et al., 2017).

Ainda que o argumento de que a intensificação em áreas previamente cultivadas geraria o efeito “*land sparing*”, o que de fato se observa é o contínuo avanço de culturas agrícolas sobre ecossistemas até então preservados, como é o caso da conversão de áreas de pastagem natural para lavouras de soja no Bioma Pampa do Rio Grande do Sul, Brasil (Oliveira et al., 2017), que traz consigo incalculáveis perdas de ordem ambiental, econômica e cultural. No entanto, a intensificação da agricultura ainda é uma necessidade, uma vez que a população mundial atingirá a marca de 9,8 bilhões de pessoas de acordo com projeções recentes (UN, 2019), um incremento de 25% com relação aos números atuais (7,8 bilhões de pessoas). Em 2100, estima-se que os números cheguem a 10,9 bilhões de pessoas ao redor do mundo (UN, 2019).

Mais do que sistemas de produção capazes de produzir mais alimentos de maneira sustentável, a mudança antropogênica do clima global requer a busca de arranjos produtivos mais estáveis frente às crescentes oscilações ambientais a que estes sistemas estarão – ou já estão sendo – submetidos. De acordo com modelos climatológicos recentes, as próximas décadas experimentarão maior variabilidade – e consequentemente, incerteza – meteorológica, o que aumentará a frequência de eventos extremos, como severas secas e precipitações (Lobell & Field, 2007; Gornall et al., 2010; Osborne & Wheeler, 2013). Nesse contexto, agroecossistemas biologicamente simplificados (e.g., monocultivos) são mais vulneráveis aos eventos extremos proporcionados pelas mudanças climáticas vigentes (Lin, 2011; Gaudin et al., 2015; Peterson et al., 2018). Portanto, a busca por sistemas de produção altamente produtivos, mas que também se mantenham estáveis sob diferentes cenários climáticos se torna imperativa para assegurar o fornecimento de alimentos à crescente população mundial e sua segurança alimentar (Schmidhuber & Tubiello, 2007; Bullock et al., 2017; Knapp & van der Heijden, 2018).

Dessa forma, a reintegração dos componentes agrícola e animal em agroecossistemas biologicamente mais diversos tem retomado sua importância. Por serem capazes de conciliar elevadas produtividades com baixo impacto ambiental (quando bem manejados), os Sistemas Integrados de Produção Agropecuária (SIPA) se destacam como proposta que abrange boa parte das exigências contemporâneas para a produção de alimentos, sendo reconhecidos como via de intensificação sustentável rumo à segurança alimentar (Herrero et al., 2010). Dentre os principais benefícios atribuídos aos SIPA estão melhorias em atributos de qualidade do solo, ciclagem de nutrientes, produtividades dos componentes vegetal e animal, e performance econômica dos sistemas (Russelle et al., 2007; Carvalho et al., 2010; Bell & Moore, 2012; Moraes et al., 2014; Sulc & Franzluebbers, 2014; Carvalho et al., 2018a).

Em nível global, os SIPA representam uma das principais formas de uso da terra, ocupando cerca de 25 milhões de km<sup>2</sup> (Bell & Moore, 2012), produzindo aproximadamente a metade dos alimentos consumidos no mundo e alimentando quase 2 bilhões de pessoas nos países em desenvolvimento (Wright et al., 2011). São responsáveis por cerca de 50% da produção de cereais mundial e pela maior parte do alimento consumido pelos pobres (41% do milho, 86% do arroz, 66% do sorgo e 74% do milheto; Herrero et al., 2010), além de 75% do leite, 65% da carne bovina e 55%

da carne ovina consumida nos países em desenvolvimento (Herrero et al., 2010; Wright et al., 2011). No Brasil, estima-se que cerca de 11,5 milhões de hectares sejam cultivados em SIPA, especialmente nos estados do Mato Grosso do Sul (2,08 milhões ha<sup>-1</sup>), Mato Grosso (1,5 milhões ha<sup>-1</sup>) e Rio Grande do Sul (1,46 milhões ha<sup>-1</sup>) (Embrapa, 2016). No Estado do Rio Grande do Sul, destacam-se modelos de SIPA que integram a lavoura de soja ou milho em áreas de coxilha (terras altas) ou a lavoura arrozeira em áreas de várzea (terras baixas) com a pecuária, principalmente de corte. Nestes sistemas, destaca-se a utilização da aveia-preta (*Avena strigosa* Schreb.) e azevém anual (*Lolium multiflorum* Lam.) como principais espécies forrageiras, havendo ainda potencial para expansão da utilização dos SIPA em áreas sob cultivo conservacionista onde estas espécies forrageiras são empregadas como culturas de cobertura do solo, somente (Anghinoni et al., 2013; Moraes et al., 2014).

Quanto maior a diversidade biológica, quanto maior a escala de tempo com que diferentes arranjos de integração se repetem, e quanto menor o espaço entre os componentes da integração, maior é a possibilidade de ocorrência de sinergismos entre os compartimentos solo-planta-animal em SIPA (Anghinoni et al., 2013). No entanto, para melhor compreendermos a complexidade e os *trade-offs* entre os serviços ecossistêmicos gerados por estes sistemas, bem como determinar os fatores mais importantes para o sucesso dos mesmos, são necessários estudos amplos sobre bancos de dados de longo-prazo (Garrett et al., 2017).

A presente tese de doutorado é parte de uma pesquisa de longa duração iniciada em 2001, abordando um modelo de produção que integra a lavoura de soja, no verão, e a produção de bovinos de corte, no período de inverno, investigando o efeito de intensidades de pastejo contrastantes sobre aspectos produtivos e ecológicos destes sistemas. No Capítulo I, contextualiza-se a proposta do trabalho a partir de revisão bibliográfica, culminando na apresentação das hipóteses e objetivos dos estudos que serão apresentados. No Capítulo II, será apresentado um estudo acerca da estabilidade produtiva de variáveis de produção vegetal, animal e do sistema integrado como um todo ao longo de 16 anos do experimento acima referido, desde sua implementação. No Capítulo III, será apresentado um estudo acerca do efeito das diferentes intensidades de pastejo sobre o sucesso da ressemeadura natural e o estabelecimento da pastagem de azevém no ano subsequente.

## 1.2 REVISÃO BIBLIOGRÁFICA

### 1.2.1 *O desafio de alimentar 10 bilhões de pessoas em um mundo de crescentes incertezas climáticas e a busca por sistemas produtivos mais resilientes e sustentáveis*

A intensificação da produção de alimentos ocorrida em muitas regiões do mundo nas últimas décadas deu-se, principalmente, a partir de modelos produtivos altamente especializados e amparados no uso irrestrito de insumos químicos e recursos não renováveis – e.g., fertilizantes sintéticos, pesticidas, irrigação artificial e combustíveis fósseis (Stoate et al., 2001; Tilman et al., 2001; 2002; Lemaire et al., 2014). Este modelo de agricultura industrial pós 2ª Guerra Mundial seguiu o caminho da simplificação biológica, apoiando-se sobre os enunciados de Liebig (1840) de que o conceito de ciclagem de nutrientes a partir da utilização das fezes de animais poderia ser substituída pela utilização de fertilizantes sintéticos, o que facilitaria as práticas agrícolas (Kirschenmann, 2007) comparativamente àquelas que eram práticas comuns desde a domesticação das plantas e dos animais no período Neolítico (8-10 mil anos atrás, Halstead, 1996; Bogaard et al., 2013). Resultado disso foi a especialização da produção de commodities agrícolas de alto valor agregado e o abandono das policulturas e sistemas mistos, que integravam animais e suas excretas à produção de múltiplos cultivos agrícolas (Entz et al., 2005; Altieri et al., 2015).

Tal especialização em sistemas agrícolas comerciais – que trouxe consigo maior eficiência no uso do trabalho, cultivares agrícolas mais produtivas e economias de escala (Tilman et al., 2002) – gerou ganhos substanciais em produtividade para as principais commodities agrícolas. Os rendimentos da lavoura de soja [*Glycine max* (L.) Merr.], por exemplo, aumentaram em 90% nos Estados Unidos (USDA, 2019a) e 170% no Brasil (CONAB, 2019) nos últimos 40 anos (estes países, juntos, correspondem atualmente a aproximadamente dois terços da produção mundial desta commodity, USDA, 2019b). Outros benefícios do processo de intensificação experimentado pela agricultura nas últimas décadas foram o aumento da oferta de alimentos per capita a nível mundial, a redução do preço destes alimentos, a redução da fome e, supostamente, o efeito “*land sparing*”, quando a expansão de áreas agrícolas sobre áreas naturais é evitada a partir da intensificação em áreas previamente cultivadas (Tilman et al., 2002).

Apesar dos inegáveis avanços gerados por este modelo de agricultura moderna, sistemas de produção altamente especializados e baseados na massiva utilização de insumos externos à propriedade geralmente apresentam custos ambientais proporcionalmente elevados (Stoate et al., 2001; Tilman et al., 2002; Foley et al., 2005). A busca incessante por produtividades cada vez mais altas trouxe consigo feitos colaterais, tais como a contaminação dos cursos d'água e lençóis freáticos (Verhoeven et al., 2006; Liu et al., 2010), concentrações crescentes de gases de efeito estufa (MacDonald & McBride, 2009; Vermeulen et al., 2012), degradação dos solos (Montgomery, 2007; Lal, 2015) e perdas de biodiversidade (Foley et al., 2005; Oliveira et al., 2017). Mais do que isso, a crescente demanda por alimentos e culturas bioenergéticas a nível global acelerou a mudança no uso das terras, transformando ecossistemas naturais em áreas agrícolas cultivadas (Alexander et al., 2015). Ainda que o argumento de que a intensificação em áreas previamente cultivadas geraria o efeito “*land sparing*” (supracitado), o que de fato se observa é o contínuo avanço de culturas agrícolas sobre ecossistemas até então preservados, como é o caso da conversão de áreas de pastagem natural para lavouras de soja no Bioma Pampa do Rio Grande do Sul, Brasil (Oliveira et al., 2017), que traz consigo incalculáveis perdas de ordem ambiental, econômica e cultural.

No entanto, a intensificação da agricultura ainda é uma necessidade. A Organização das Nações Unidas para Alimentação e Agricultura (*Food and Agriculture Organization, FAO*) estimou que há uma necessidade em aumentar a produção de alimentos em 90% via intensificação (“maximização da produção primária por unidade de área”) e apenas 10% via expansão das áreas de cultivo para atender a demanda populacional global até 2050 (FAO, 2010), quando a população, de acordo com estimativas recentes, aproximar-se-á 9,8 bilhões de pessoas (UN, 2019), um incremento de 25% com relação aos números atuais (7,8 bilhões de pessoas). Em 2100, estima-se que os números cheguem a 10,9 bilhões de pessoas ao redor do mundo (UN, 2019). Por este motivo, o impacto ambiental dos sistemas agropecuários deverá ser reduzido, de forma que a intensificação destes sistemas ocorra de forma sustentável (“sem comprometer a capacidade do sistema em se manter produtivo”). A “intensificação sustentável”, ou “intensificação ecológica”, como também é chamada, tem em seu cerne o uso de mecanismos biológicos capazes de substituir intervenções químicas e físicas ou interagir positivamente com elas, exercendo o mesmo papel,

mas reduzindo-se ao máximo os custos externos, sobretudo os ambientais (Doré et al., 2011).

O objetivo da agricultura sustentável é maximizar os benefícios advindos da agricultura na produção de alimentos e fibras e na promoção de serviços ecossistêmicos e saúde à população, o que será obtido com incrementos na produtividade das culturas agrícolas, maiores eficiências de utilização dos nutrientes e da água, práticas de manejo ecológicas, uso racional de pesticidas e mudanças em alguns conceitos na produção pecuária (Tilman et al., 2002). Segundo Stavi et al. (2016), altas produtividades e baixo impacto ambiental são características conflitantes, portanto é crucial encontrar atividades agrícolas que não agridam o meio ambiente, mas que, ao mesmo tempo, alcancem patamares toleráveis de intensificação. Ainda, de acordo com Lemaire et al. (2014), os modelos produtivos atuais não são mais aceitos por parcela da sociedade, de modo que a ciência agrônômica precisa urgentemente superar a aparente contradição entre a necessidade de aumentar a produtividade dos sistemas agropecuários e, ao mesmo tempo, prevenir a degradação e promover a restauração do meio ambiente.

Mais do que sistemas de produção capazes de produzir mais alimentos de maneira sustentável, a mudança antropogênica do clima global requer a busca de arranjos produtivos mais estáveis frente às crescentes oscilações ambientais a que estes sistemas estarão – ou já estão sendo – submetidos. De acordo com modelos climatológicos recentes, as próximas décadas experimentarão maior variabilidade – e consequentemente, incerteza – meteorológica, o que aumentará a frequência de eventos extremos, como severas secas e precipitações (Lobell & Field, 2007; Gornall et al., 2010; Osborne & Wheeler, 2013). Embora ainda não se saiba quantificar de maneira robusta quais serão os efeitos disto para a produtividade da agricultura mundial, sabe-se que a variabilidade dos rendimentos dos principais cultivos agrícolas a nível mundial (*i.e.*, trigo, arroz, milho, soja, cevada e sorgo) está intimamente relacionada com a variabilidade meteorológica (~30% da variabilidade dos rendimentos explicada pela temperatura e precipitação), com efeitos negativos das mudanças climáticas já detectados por alguns estudos (Lobell & Field, 2007; Gornall et al., 2010; Osborne & Wheeler, 2013).

Nesse contexto, agroecossistemas biologicamente simplificados (e.g., monocultivos) são mais vulneráveis aos eventos extremos proporcionados pelas mudanças climáticas vigentes (Lin, 2011; Gaudin et al., 2015; Peterson et al., 2018).

Portanto, a busca por sistemas de produção altamente produtivos, mas que também se mantenham estáveis sob diferentes cenários climáticos – não somente em condições normais, mas também sob condições não usuais, sejam elas extremamente favoráveis ou de stress – se torna imperativa para assegurar o fornecimento de alimentos à crescente população mundial e sua segurança alimentar (Schmidhuber & Tubiello, 2007; Bullock et al., 2017; Knapp & van der Heijden, 2018).

*Estabilidade* possui uma série de significados nas disciplinas de Ecologia e Estatística, englobando os conceitos de *resistência*, *resiliência*, *variabilidade*, entre outros (Harrison, 1979; Lehman & Tilman, 2000; Peterson et al., 2018). Para alguns autores, *estabilidade* é a capacidade de um sistema retornar ao estado original após um distúrbio (Holling, 1973; Harrison, 1979), o que para outros é a definição de *resiliência* (Webster et al., 1975), ou ainda *elasticidade* (Orians, 1974). *Estabilidade* também pode ser definida como o quanto uma determinada variável flutua no decorrer do tempo, ou ainda *estabilidade temporal* (Tilman, 1999). Para Pimm (1991), esta é a definição de *variabilidade*. Este conceito se assemelha à definição utilizada por Lightfoot et al. (1987) e Raun et al. (1993), de que um *sistema estável* é aquele que oscila menos em resposta a mudanças ambientais. Outra medida da estabilidade de um sistema é a habilidade deste sistema em permanecer no mesmo estado quando submetido a distúrbios, também chamado *resistência* (Webster et al., 1975; Pimm, 1991), ou *inércia* (Orians, 1974). Diante desta multiplicidade de significados, torna-se de fundamental importância que o tipo de estabilidade (*i.e.*, conceito ou definição) seja claramente especificado e postulado em estudos futuros, a fim de evitar confusão na interpretação dos resultados da pesquisa (Orians, 1974; Harrison, 1979).

Várias métricas têm sido propostas para medir a estabilidade temporal do rendimento de cultivos agrícolas (Nielsen & Vigil, 2018; Li et al., 2019), dentre as quais 1) o desvio padrão, que é a variabilidade absoluta dos rendimentos ao longo dos anos (Knapp & van der Heijden, 2018; Nielsen & Vigil, 2018); 2) o coeficiente de variação, que mede a variabilidade ao longo dos anos com relação à média das produtividades neste mesmo intervalo de tempo (Temesgen et al., 2015; Knapp & van der Heijden, 2018; Nielsen & Vigil, 2018); 3) a amplitude de rendimentos, que é a máxima amplitude entre a mínima produtividade e a maior produtividade em uma série temporal (Temesgen et al., 2015; Nielsen & Vigil, 2018); e a análise de estabilidade de Finlay & Wilkinson (1963), que é a regressão linear dos rendimentos de uma determinada cultivar ou tratamento com relação à média de todas as cultivares/tratamentos



estudados em uma determinada região/ano experimental, gerando uma comparação entre o desempenho de determinada cultivar/tratamento ao longo de um gradiente ambiental, também chamado de Índice Ambiental (Finlay & Wilkinson, 1963; Raun et al., 1993; Nielsen & Vigil, 2018; Williams et al., 2016, 2018).

Até o momento, a maioria dos estudos sobre a estabilidade de ecossistemas agrícolas comparou o efeito de práticas de cultivo (*e.g.*, agricultura conservacionista ou orgânica vs. cultivo convencional do solo) (Knapp & van der Heijden, 2018; Nielsen & Vigil, 2018; Williams et al., 2018; Li et al., 2019) ou da diversidade de plantas (*e.g.*, incremento no número de espécies no espaço e no tempo) sobre atributos de estabilidade (Tilman et al., 2006; Gaudin et al., 2015; Isbell et al., 2015; Craven et al., 2018; Nielsen & Vigil, 2018). No entanto, nunca o efeito da adição de um novo nível trófico (*i.e.*, animais em pastejo) sobre a estabilidade dos sistemas agrícolas foi objeto de estudo com o mesmo nível de detalhamento, o que será feito no Capítulo II do presente documento.

### *1.2.2 Sistemas Integrados de Produção Agropecuária: reconciliando produtividade e sustentabilidade a partir da reintegração entre cultivos agrícolas e animais*

A associação entre cultivos agrícolas e animais não é uma ideia nova, uma vez que data dos primórdios da domesticação de plantas e animais, ainda no período Neolítico (8-10 milênios atrás; Halstead, 1996; Bogaard et al., 2013). Naquele período, os primeiros agricultores costumavam utilizar subprodutos da lavoura, como resíduos vegetais, para alimentar os animais em criação para a produção de couro, lã, carne, leite e produtos associados. Em contrapartida, fertilizavam as áreas cultivadas com esterco, visando incrementar a produtividade das lavouras, além de utilizar os animais como força de tração (Halstead, 1996; Bogaard et al., 2013). Conforme já foi citado, a industrialização dos sistemas agrícolas levou ao desacoplamento (ou desintegração; Hilimire, 2011) deste contínuo agricultura-pecuária, passando estes sistemas a serem não mais baseados em tecnologias de processos, como a ciclagem de nutrientes, mas puramente em tecnologias de insumos, gerando as problemáticas ambientais também já citadas neste texto.

Dessa forma, a reintegração dos componentes agrícola e animal em agroecossistemas biologicamente mais diversos tem retomado sua importância. Por serem capazes de conciliar elevadas produtividades com baixo impacto ambiental

(quando bem manejados, vide subitem 1.2.3, a seguir), os Sistemas Integrados de Produção Agropecuária, ou SIPA (outroza chamados sistemas de Integração Lavoura-Pecuária, ou ILP, nomenclatura ainda aceitável sob o linguajar técnico; Carvalho et al., 2014) se destacam como proposta que abrange boa parte das exigências contemporâneas para a produção de alimentos, sendo reconhecidos como via de intensificação sustentável rumo à segurança alimentar (Herrero et al., 2010).

Os SIPA, nos quais atividades agrícolas multifuncionais (e.g., grãos, madeira, produção animal) são simultaneamente ou sequencialmente produzidos a nível de campo, propriedade rural ou território, são sistemas planejados para explorar sinergismos oriundos das interações entre os compartimentos solo-planta-animal (Anghinoni et al., 2013; Moraes et al., 2014). Encobertas pela palavra planejamento estão as duas dimensões fundamentais dos arranjos produtivos em SIPA: espaço e tempo. Este planejamento espaço-temporal do uso das áreas visando favorecer interações sinérgicas, aliado ao bom manejo dos pastos (vide subitem 1.2.3) é o que diferencia um “SIPA verdadeiro” de uma simples sucessão de culturas (Moraes et al., 2018). Nestes sistemas, o grau de interações sinérgicas é dependente do quão complexo é o sistema com relação à diversificação, temporalidade e espacialização. Quanto maior a diversidade, incluindo a diversidade de espécies e categorias animais, quanto maior a escala de tempo com que diferentes arranjos de integração se repetem, e quanto menor o espaço entre os componentes da integração, maior é a possibilidade de ocorrência de sinergismos (Anghinoni et al., 2013). Quando o resultado destas interações é superior à soma das contribuições individuais de cada componente do sistema, diz-se que há a ocorrência de propriedades emergentes, que são características intrínsecas de sistemas complexos (Carvalho et al., 2018c).

Bonaudo et al. (2014) consideraram *resiliência*, *produtividade*, *eficiência* e *autossuficiência* como propriedades emergentes em SIPA, devido à natureza complexa destas características. A partir destes autores, Carvalho et al. (2018c) sumarizaram alguns resultados obtidos em SIPA que consideraram evidências da ocorrência de propriedades emergentes de acordo com a classificação de Bonaudo et al. (2014). Tais evidências podem ser encontradas na Figura 1. No entanto, a temática das propriedades emergentes ainda carece ser investigada com mais atenção em estudos futuros.

Na Ásia, sistemas integrados constituem a espinha dorsal da agricultura em pequenas propriedades, onde a diversidade de sistemas de cultivo é tão grande

quanto a variedade de cultivos e espécies existentes. Exemplo disso são sistemas de produção integrando aves aquáticas e peixes com arroz irrigado (Devendra & Thomas, 2002). Outros exemplos de SIPA são a integração de ovinos em pastejo entre parreiras em áreas de vitivinicultura da Nova Zelândia (Niles et al., 2018), a utilização de trigo duplo-propósito na porção sul dos *Great Plains*, nos Estados Unidos (Sulc & Franzluebbbers, 2014), e o pastejo de culturas de cobertura de inverno, principalmente a aveia-preta (*Avena strigosa* Schreb.) e o azevém anual (*Lolium multiflorum* Lam.) pastejados por bovinos de corte, no intervalo entre cultivos de verão, principalmente a soja, no sul do Brasil (Oliveira et al., 2013).



Figura 1 – Evidências de propriedades emergentes em Sistemas Integrados de Produção Agropecuária, adaptado de Carvalho et al. (2018c).

Em nível global, os SIPA representam uma das principais formas de uso da terra, ocupando cerca de 25 milhões de km<sup>2</sup> (Bell & Moore, 2012), produzindo aproximadamente a metade dos alimentos consumidos no mundo e alimentando quase 2 bilhões de pessoas nos países em desenvolvimento, metade das quais são pobres (Wright et al., 2011). São responsáveis por cerca de 50% da produção de cereais mundial e pela maior parte do alimento consumido pelos pobres (41% do milho, 86% do arroz, 66% do sorgo e 74% do milheto; Herrero et al., 2010), além de 75% do leite, 65% da carne bovina e 55% da carne ovina consumida nos países em desenvolvimento (Herrero et al., 2010; Wright et al., 2011). No Brasil, estima-se que cerca de 11,5 milhões de hectares sejam cultivados em SIPA, especialmente nos

estados do Mato Grosso do Sul (2,08 milhões ha<sup>-1</sup>), Mato Grosso (1,5 milhões ha<sup>-1</sup>) e Rio Grande do Sul (1,46 milhões ha<sup>-1</sup>), o que significa que 5,5% das áreas sob uso agropecuário estão em integração, um crescimento de aproximadamente 600% com relação a 2005, quando apenas 1,87 milhões ha<sup>-1</sup> eram cultivados sob integração em todo o Brasil (Embrapa, 2016). Uma extensa e detalhada descrição sobre o histórico e a evolução dos SIPA no Brasil pode ser encontrada em Moraes et al. (2018).

Dentre os principais benefícios atribuídos aos SIPA, de acordo com diversos trabalhos científicos e revisões de literatura sobre o assunto realizados em diversas regiões do mundo, estão as melhorias em atributos de qualidade do solo, ciclagem de nutrientes, produtividades dos componentes vegetal e animal, e performance econômica dos sistemas (Russelle et al., 2007; Carvalho et al., 2010; Bell & Moore, 2012; Moraes et al., 2014; Sulc & Franzluebbers, 2014; Carvalho et al., 2018a). Conforme Carvalho et al. (2011), o animal em pastejo é o agente dinâmico dentro destes sistemas, que continuamente introduz variabilidade ao sistema por meio da desfolha, do pisoteio e da distribuição de dejetos, catalisando processos tais como a ciclagem de nutrientes, e suas ações podem ser tanto positivas quanto negativas, dependendo do manejo empregado (principalmente a intensidade de pastejo). O solo é o compartimento que captura as consequências de todas as ações (positivas ou negativas), incluindo as ações dos animais, as rotações de culturas, as adições (e retiradas) de resíduos, as fertilizações (ou ausência de fertilizações), os impactos ocasionados por maquinários, etc, operando como a “memória do sistema” (Anghinoni et al., 2013). As plantas, por sua vez, reagem a estas informações, sinalizando a direção das consequências (positivas ou negativas).

### *1.2.3 O manejo do pastoreio como elemento chave para o sucesso: efeitos de diferentes intensidades de pastejo em Sistemas Integrados de Produção Agropecuária*

Apesar da pesquisa apontar o contrário, ainda existem barreiras à adoção dos SIPA na região sul do Brasil, principalmente vinculadas aos supostos impactos negativos ocasionados pela presença de animais em pastejo em áreas de agricultura conservacionista (*i.e.*, sob sistema de semeadura direta, ou sistema de plantio direto). Nestes sistemas, o pastejo seria considerado prejudicial, pois os animais consomem a biomassa das culturas que serviriam de cobertura para o solo, restando quantidades de palha supostamente insuficientes para a proteção e a melhoria dos atributos físico-

químicos do solo. Ainda é comum, nos dias de hoje, deparar-se com a ideia de que “quanto mais palha, melhor”, ou que “maiores quantidades de palha gerarão maiores produtividades”, ignorando as melhorias proporcionadas pelos animais em pastejo. Outra preocupação bastante recorrente é o persistente paradigma da compactação do solo por ação do pisoteio dos animais, que supostamente prejudicaria o desenvolvimento e os rendimentos das culturas de grãos subsequentes (Anghinoni et al., 2013; Martins et al., 2015; Carvalho et al., 2018a).

Outras barreiras à adoção dos SIPA foram listadas em estudo realizado na região do “cinturão do milho”, nos Estados Unidos, as quais podem ser encontradas também no Brasil. São elas: 1) a tradição das monoculturas, que se tornou uma regra na atual geração de agricultores e empresas agrícolas; 2) a maior facilidade de manejo de sistemas especializados com relação a sistemas diversos e as economias de escala, favorecidas por programas governamentais; 3) maior necessidade de gerenciamento, conhecimento e trabalho em sistemas de produção diversificados; 4) a falta de valorização e entendimento por parte de produtores rurais do conceito de sistema, *i.e.*, a performance dos componentes individuais de um sistema de produção é mais valorizada do que a performance do sistema como um todo; e 5) incentivos limitados a sistemas de produção diversificados e comprometidos com a temática ambiental (Sulc & Tracy, 2007).

Para alguns autores, a utilização de culturas de cobertura para fins de pastoreio bovino pode limitar consideravelmente a quantidade de biomassa destinada à cobertura do solo, comprometendo a atividade agropecuária (Nicoloso et al., 2006). Segundo estes autores, a adição anual de palha ao solo no sistema de plantio direto não deveria ser menor que 8 Mg ha<sup>-1</sup> de matéria seca (Lovato et al., 2004; Nicoloso et al., 2006). Bayer et al. (2006) estimou que a quantidade de MS anual a ser adicionada para manter ou aumentar os níveis de carbono em solos sob sistema de plantio direto no sul do Brasil é ainda maior, da ordem de 10 a 12 Mg MS ha<sup>-1</sup>. No entanto, a pesquisa em SIPA consolidados, bem manejados e com longo histórico de adoção tem demonstrado que olhar para estes sistemas considerando somente a biomassa residual do pasto ao final do período de pastejo, sem considerar toda a dinâmica e complexidade adicionada pelos animais, é um erro grosseiro (Carvalho et al., 2018a).

Considerando exclusivamente a biomassa residual ao final do período de pastejo (“palha” ou “palhada”), pastos mistos de azevém anual e aveia-preta manejados a 20, 30 e 40 cm de altura contribuíram com 30, 40 e 50% daquela

quantidade preconizada por Bayer et al. 2006), respectivamente. Portanto, olhando-se somente para a biomassa residual, o sistema integrado, mesmo sob intensidades de pastejo moderadas a leves, estaria aquém do potencial de um sistema conservacionista em termos de adição de carbono no solo (Souza Filho et al., 2019). No entanto, estudos recentes em um protocolo experimental de longa-duração situado no município de São Miguel das Missões, RS, Brasil, demonstraram que sob as intensidades de pastejo supracitadas, o acúmulo total de biomassa que ocorre no decorrer do período de pastejo (ou produção total de MS, Figura 2) é superior a áreas sem pastejo. Isto porque a palhada ao final do inverno não reflete toda a dinâmica que ocorre naquele sistema com a presença do animal (Carvalho et al., 2018a; Nunes et al., 2019; Souza Filho et al., 2019). Somada a esta produção de pasto, que pode facilmente ultrapassar as 8 Mg MS ha<sup>-1</sup> (e inclusive ultrapassar 10 Mg MS ha<sup>-1</sup> em adequadas condições climáticas e de fertilização; dados não publicados), há ainda a produção de biomassa aérea da cultura subsequente, que foi estimada por Assmann et al. (2014) para a cultura da soja em 5,2 Mg MS ha<sup>-1</sup>, o que significa que os requerimentos para adição de MS e acúmulo de carbono no solo são supridos quando os pastos são adequadamente manejados.

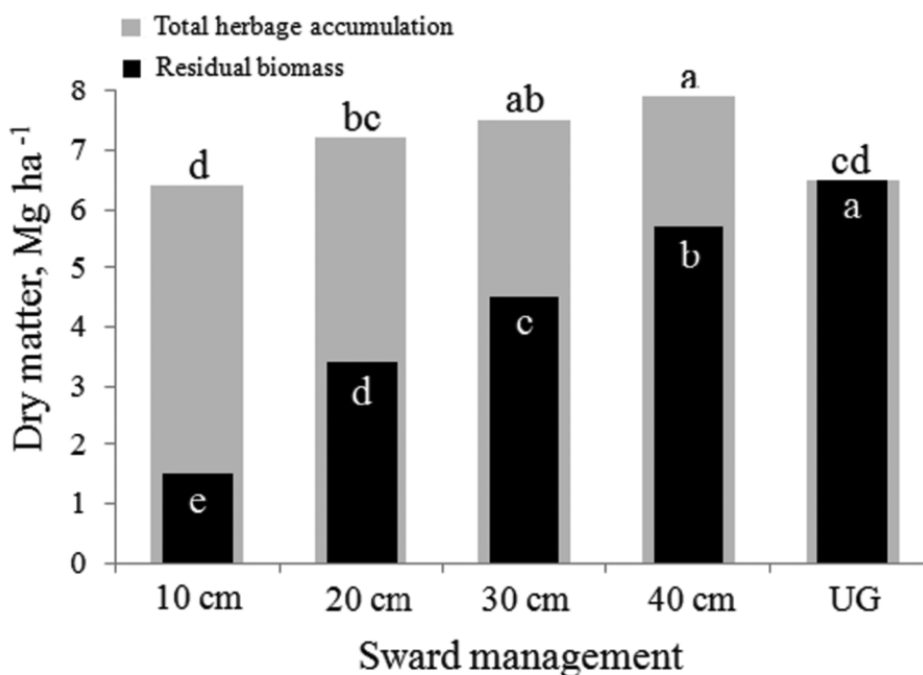


Figura 2 – Acúmulo total de matéria seca (ou produção total de pasto, Mg MS ha<sup>-1</sup>, barras cinzas) e biomassa residual do pasto (Mg MS ha<sup>-1</sup>, barras pretas) ao final do período de pastejo por bovinos em pastos mistos de aveia-preta

(*Avena strigosa* Schreb.) e azevém anual (*Lolium multiflorum* Lam.) em um Sistema Integrado de Produção Agropecuária sob diferentes intensidades de pastejo (pastos manejados entre 10 e 40 cm de altura ou sem pastejo - UG), retirado de Carvalho et al. (2018a). Letras diferentes significam diferenças significativas entre os tratamentos para cada variável de acordo com o teste de Tukey a 5% de significância.

Assmann et al. (2014) verificaram, sob as mesmas condições experimentais, que diferentes intensidades de pastejo afetaram as adições de carbono e nitrogênio ao solo, de modo que pastos manejados a 20, 30 e 40 cm de altura resultaram em incrementos nos estoques de C e N similares ao controle sem pastejo 10 anos após a implantação dos tratamentos. Já em situação de pastejo intenso (i.e., 10 cm de altura), ocorreram perdas de N da ordem de 1,17 Mg ha<sup>-1</sup> devido à degradação da matéria orgânica do solo. Como resultado do menor aporte de resíduos, o acúmulo de C no solo foi 30% menor quando comparado às demais intensidades de pastejo, incluindo o controle sem pastejo. Resultados similares foram encontrados por outros autores (Souza et al., 2009; Silva et al., 2014), e foram atribuídos ao menor acúmulo de MS nestas áreas e à exposição do solo por ausência de plantas de cobertura, que culminam em altas temperaturas no solo, acelerando a atividade microbiana e as taxas de degradação da matéria orgânica (Souza et al., 2010).

Com relação à compactação do solo, a literatura mostra resultados que variam desde a redução (Franzluebbers & Stuedemann, 2008), ausência de efeitos (Bell et al., 2011; Kunrath et al., 2015; Peterson et al., 2019), até incrementos nos rendimentos das culturas subsequentes (Tracy & Zhang, 2008; Carvalho et al., 2018a,b). Contudo, quando práticas conservacionistas (e.g., sistema de plantio direto) e o bom manejo dos pastos são empregados (i.e., assumindo a utilização de intensidades de pastejo adequadas e não havendo superpastejo, especialmente em anos com elevada precipitação), os efeitos sobre atributos físicos do solo – tais como adensamento – ficam restritos às camadas superficiais do solo e são facilmente revertidos pelas raízes das culturas (Bell et al., 2011), não afetando o desenvolvimento e a produtividade das culturas subsequentes (Flores et al., 2007; Conte et al., 2011).

Carvalho et al. (2018a) em recente revisão bibliográfica verificaram que o pastejo em intensidades moderadas não somente não prejudica a produtividade dos cultivos subsequentes, mas as favorece (Figura 3). Os autores constataram

rendimentos 3,4, 4,7, 10,4 e 10,8% superiores para a soja, o feijão, o arroz irrigado e o milho, respectivamente, quando em sequência a áreas pastejadas na região subtropical do Brasil. Adicionalmente, apesar de requererem um maior grau de instrução e capacidade gerencial, a diversificação das atividades dentro da propriedade pode funcionar como estratégia de mitigação às oscilações de mercado e do clima, reduzindo o risco da atividade agropecuária como um todo (Bell & Moore, 2012; Ryschawy et al., 2012) além de aumentar a eficiência do uso da terra e a rentabilidade da propriedade rural (Oliveira et al., 2013) com mínimo impacto ambiental (Souza Filho et al., 2019).

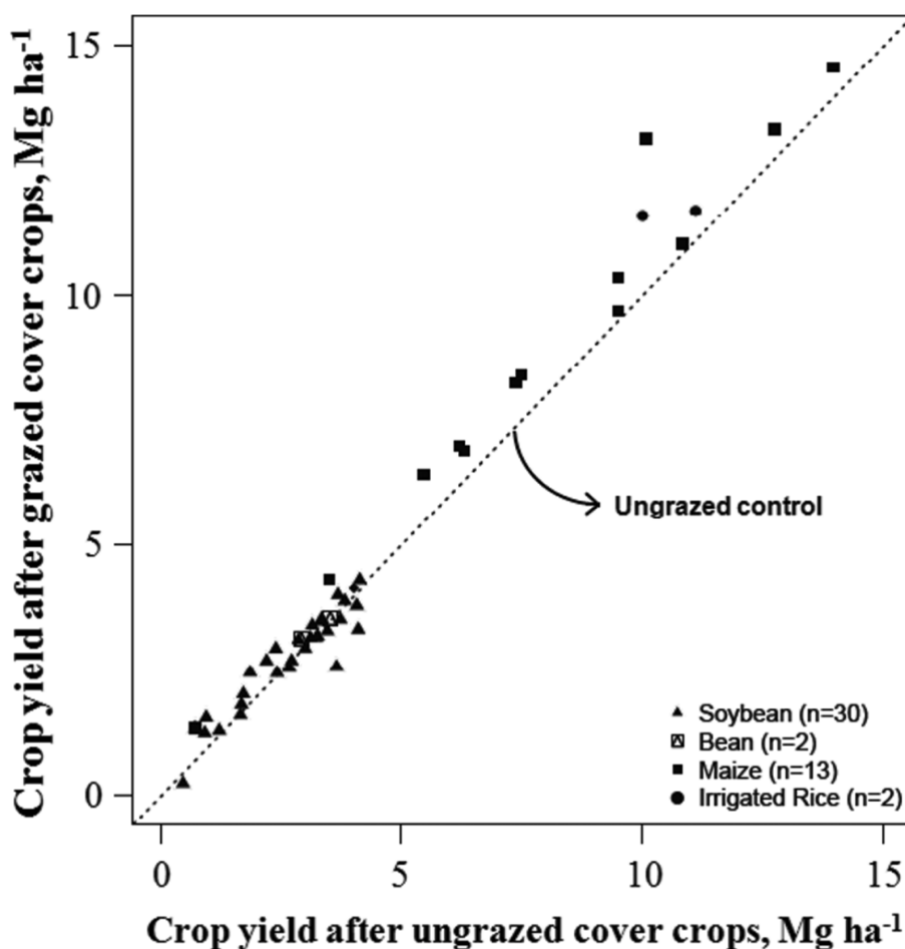


Figura 3 – Produtividade das culturas verão [soja, *Glycine max* (L.) Merrill; feijão, *Phaseolus vulgaris* L.; milho, *Zea mays* L.; e arroz irrigado, *Oriza sativa* L.] em áreas pastejadas sob intensidades moderadas (eixo y) comparativamente a áreas não pastejadas (eixo x) no período de inverno, retirado de Carvalho et al. (2018a), adaptado de Carvalho et al. (2018b).



#### 1.2.4 A ressemeadura natural do azevém anual (*Lolium multiflorum* Lam.) como elemento gerador de eficiência e economicidade em Sistemas Integrados de Produção Agropecuária

Originário da Europa, Ásia e norte da África, o gênero *Lolium* spp. compreende duas espécies de larga distribuição no mundo: o azevém perene (*Lolium perenne* L.), espécie amplamente difundida nas zonas temperadas ao redor do globo, mas praticamente inexistente no Brasil; e o azevém anual (*Lolium multiflorum* Lam.), segunda forrageira hibernal mais cultivada no país, seguindo espécies do gênero *Avena* (Carvalho et al., 2020). Trata-se de uma espécie amplamente utilizada por produtores de gado de corte e leite no sul do Brasil, devido à sua alta produção de forragem, capacidade de rebrote, tolerância ao pastejo e ao excesso de umidade, suporte a altas lotações, além de apresentar alto valor nutritivo e boa aceitação pelo gado (Carámbula, 1977).

Devido à sua capacidade de ressemeadura natural, tende a se restabelecer na área quando do início de um novo período favorável. Desde que manejado em intensidades de pastejo de moderadas a leves, assegura produção de sementes e ressemeadura natural todos os anos. Em contrapartida, quando excessivamente pastejado, há necessidade de retirar o gado para diferimento e emissão das estruturas reprodutivas, prática que não assegura ressemeadura natural tão eficiente quanto o potencial desta forrageira permite (Barth Neto et al., 2014). Esta prática é, no entanto, bastante utilizada por produtores que, equivocadamente, objetivam utilizar ao máximo o pasto com excesso de animais em pastejo. Para se obter ressemeadura natural satisfatória são necessárias densidades entre 885 e 5650 perfilhos com espigas por m<sup>2</sup> (Bartholomew & Williams, 2009) de modo a assegurar a emergência de ~500 plântulas por m<sup>2</sup> no ano seguinte (Evers & Nelson, 1994; 2000).

A maior produção de biomassa do azevém anual se concentra nos meses de agosto e setembro, sendo que o potencial de produção depende do manejo do pasto e da adubação. Sua utilização pode se estender até o florescimento, levando em consideração a queda no valor nutricional da forragem neste estágio (Carvalho et al., 2020). Após a deiscência, as sementes de azevém anual têm capacidade de permanecer viáveis até a estação favorável seguinte (outono). Esta característica pode ser aproveitada pelo produtor a fim de evitar uma nova semeadura a cada ano, desde que o manejo do pasto seja feito de forma adequada durante o período de pastejo anterior, ou seja, de maneira a permitir o florescimento e a formação adequada

das sementes (Barth Neto et al., 2014). Em intensidades de pastejo moderadas ou leves, o período de pastejo pode ser estendido até o final do ciclo da planta, imediatamente antes da semeadura da cultura de verão, sem prejudicar o estabelecimento no ano seguinte.

Além da intensidade de pastejo, a cultura de verão utilizada na safra anterior também afeta o estabelecimento do azevém anual por ressemeadura natural no inverno subsequente. Áreas cultivadas com milho apresentaram menores densidades de perfilhos e massa de forragem ao final do estabelecimento quando comparadas às áreas de soja. Em sistemas sucedendo a soja, as plantas de azevém têm mais nitrogênio à disposição, favorecendo seu estabelecimento (Barth Neto et al., 2014).

Apesar dos estudos de Barth Neto et al. (2014) no sul do Brasil, pouco se sabe sobre a efetividade da ressemeadura natural do azevém anual quando submetido a intensidades de pastejo contrastantes, incluindo elevadas intensidades de pastejo ou a ausência do mesmo. Esta temática será estudada em profundidade no Capítulo III deste documento.

### 1.3 HIPÓTESES

Os capítulos seguintes foram desenvolvidos a partir das seguintes hipóteses: (1) O incremento da complexidade e da diversidade funcional gerados pelo animal em pastejo em sistemas integrados de produção agropecuária (SIPA) aumenta a produtividade do sistema e reduz sua vulnerabilidade (i.e., aumenta sua estabilidade) frente a oscilações ao longo do tempo, comparativamente a sistemas puramente agrícolas (Capítulo II); (2) a efetividade da ressemeadura natural do azevém anual (*Lolium multiflorum* Lam.) é afetada pela intensidade de pastejo em SIPA, de modo que há um limite a partir do qual será necessária a adição de sementes (ou semeadura suplementar) para assegurar o sucesso do estabelecimento da pastagem no inverno seguinte (Capítulo III); e (3) o uso de intensidades de pastejo moderadas é suficiente para garantir um adequado estabelecimento dos pastos de azevém anual por ressemeadura natural, representando um incremento em resiliência e autossuficiência para o SIPA (Capítulo III).

### 1.4 OBJETIVOS

Os objetivos dos estudos apresentados a seguir foram: (1) Avaliar a produtividade e a estabilidade de um sistema agrícola (componentes vegetais, animais e sistêmicos) quando submetido a intensidades de pastejo contrastantes (compondo um sistema integrado de produção agropecuária, SIPA) ou à ausência de pastejo (sistema puramente agrícola), ao longo de um gradiente de condições ambientais, a partir de um banco de dados de um experimento de longa duração (Capítulo II); (2) Avaliar o sucesso do estabelecimento do azevém anual (*Lolium multiflorum* Lam.) por ressemeadura natural após ser submetido a diferentes intensidades de pastejo em SIPA (Capítulo III); (3) Determinar a partir de qual intensidade de pastejo será necessária a adição de sementes (ou semeadura suplementar) para garantir o pleno estabelecimento de uma pastagem de azevém anual no período de pastejo subsequente em um SIPA (Capítulo III).

## **2. CAPÍTULO II**

**Livestock integration improves long-term stability of yields and profitability of soybean systems<sup>3</sup>**

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<sup>3</sup> Manuscrito elaborado conforme as normas do periódico *Scientific Reports* (Apêndice 4).

# Livestock integration improves long-term stability of yields and profitability of soybean systems

Pedro A. de A. Nunes<sup>1,\*</sup>, Emilio A. Laca<sup>2</sup>, Paulo C. de F. Carvalho<sup>1</sup>, Meng Li<sup>2</sup>, William de Souza Filho<sup>1</sup>, Taise R. Kunrath<sup>1</sup>, Amanda P. Martins<sup>3</sup> and Amélie C. M. Gaudin<sup>2</sup>

<sup>1</sup>Department of Forage Plants and Agrometeorology, Federal University of Rio Grande do Sul, Porto Alegre, RS 91540-000, Brazil

<sup>2</sup>Department of Plant Sciences, University of California - Davis, Davis, CA 95616, USA

<sup>3</sup>Department of Soil Science, Federal University of Rio Grande do Sul, Porto Alegre, RS 91540-000, Brazil

\*Corresponding author: pedro\_nuness@hotmail.com

## ABSTRACT

Climate models project greater weather variability over the coming decades. High yielding systems that can maintain stable crop yields under variable environmental scenarios are critical to enhance food security. However, the effect of adding a new trophic level (i.e. herbivores) on the long-term stability of agricultural systems is not well understood. We used a 16-year dataset from an integrated soybean-beef cattle experiment to measure the impacts of grazing on the stability of key crop, pasture, animal and whole-system outcomes. Treatments consisted of four grazing intensities (10, 20, 30 and 40 cm sward height) on mixed black oat (*Avena strigosa* Schreb.) and Italian ryegrass (*Lolium multiflorum* Lam.) pastures and an ungrazed control. We found that stability of both human-digestible protein production and profitability increased at moderate grazing intensities, while over-intensification or absence of

grazing decreased system stability. Grazing did not affect subsequent soybean yields but reduced the chance of crop failure in unfavorable years. At both lighter and heavier grazing intensities, tradeoffs occurred between the stability of herbage production and animal live weight gains. We show that ecological intensification of specialized soybean systems using livestock integration can increase system stability and profitability, but the probability of win-win outcomes depends on management.

## **Introduction**

Intensification of highly specialized crop production systems over the last decades has led to major productivity gains. Soybean yields, for instance, increased by 90% in the United States [1] and 170% in Brazil [2] in the past 40 years and these two countries alone account for two thirds of global soybean production [3]. However, climate models forecast greater weather variability and uncertainty over the coming decades, with more frequent severe droughts and heavy rainfall events, which are projected to negatively impact crop productivity [4-6].

Biologically simplified agroecosystems are more vulnerable to the extreme weather events expected to be more frequent with global climate change [7-10]. Moreover, world population is projected to increase by 25% and reach 9.8 billion people by 2050 [11]. Developing and adopting high yielding sustainable production systems able to maintain crop yields under different weather scenarios is therefore critical to maintain global food security in an increasingly challenging production environment [12-14].

Stability has multiple meanings in ecology and statistics and encompasses concepts like resistance and resilience [9, 15-17]. In this study, we considered the concept of stability as related to variability and defined a stable system as one that changes least in response to environmental changes [18]. Management approaches that promote biodiversity (e.g. organic agriculture and crop rotation diversity) and conservation practices (e.g. permanent soil cover

and reduced disturbance) have been shown to enhance yield stability [8, 10, 14, 17, 19-28]. Besides income diversification, more biodiverse systems can stabilize agroecosystem productivity through cross-scale mechanisms ranging from redundancy and facilitation in plant communities [16, 28], to creating habitat for natural enemies to promote pest suppression [7]. Conservation practices, in turn, can improve properties related to soil health and crop yield stability, such as soil organic matter and water retention [24, 25]. However, the effects of increasing system diversity and ecological complexity by adding a trophic level (i.e., grazing animals) on the long-term stability of no-till cropping systems have not yet been studied with the same level of detail.

The interconnectedness of crops and domestic animals dates back to the beginning of agriculture [29, 30] and remains the backbone of smallholder systems [31-33], where farmers use crop byproducts as livestock fodder and harness services provided by animals (e.g., nutrient recycling and weed control) to reduce input needs and enhance crop yields. However, industrialization has led to decoupling of crop and livestock production systems, resulting in poor nutrient cycling between agricultural operations [34-38], conversion of endangered native ecosystems to croplands and underutilization of ecosystem services provided by livestock for sustainable crop production and biodiversity conservation [39-41].

Re-integrating animal and crop components to form more diverse agroecosystems is increasingly proposed as a strategy to reconcile high levels of food production with maintenance of fundamental ecosystem services underlying sustainability [32]. Integrated crop-livestock systems (ICLS), where crops and livestock are simultaneously or sequentially produced on site, are designed to harness complementarities and synergies from soil-plant-animal interactions across spatiotemporal scales. ICLS include production systems such as duck/fish-rice integration in Asian smallholdings [31], sheep integration into New Zealand vineyards [42] and grazing of winter cover crops in large-scale integrated beef cattle-soybean

systems in Brazil [43]. About half of the world's food is already produced in these systems, supporting nearly two billion people in developing countries and making ICLS crucial to global food security [32, 33].

Various reports have reviewed the benefits and tradeoffs of crop-livestock integration for soil quality, nutrient cycling, crop and animal production and farm economic performance in a wide range of systems and regions of the world [44-49]. Yet, impact on production stability remains unclear. Although ICLS require intensive knowledge and a higher degree of managerial capacity, livestock integration increases land-use efficiency and farm profitability while providing opportunities to bolster ecological mechanisms underlying resilience [43]. Income diversification can reduce risks from uncontrolled variability in climate and market fluctuations, as annual returns from crop and livestock commodities are often uncorrelated [46, 50]. At the field scale, self-regulating processes such as greater nutrient cycling [45], higher microbial functional diversity [51] and improved soil structure [52] and organic matter [53] in grazed systems are suggested to increase systems' biophysical buffering capacity to less optimal environmental conditions, in ways that still require better understanding [9, 52]. Livestock production within ICLS also takes advantage of crop residues and grasses inedible to humans to produce high-quality food (e.g., beef and milk byproducts), thus reducing market competition for human-edible feed resources [54]. However, if we aim to use crop-livestock integration as a tool for sustainable intensification, it is imperative to assess its contribution to not only system productivity but also stability over the long-term.

The primary goal of this study was to evaluate long-term yields and stability of ICLS yields and profitability compared to non-integrated systems under a range of environmental conditions and test their potential as a strategy for sustainable intensification. We hypothesized that increased biodiversity and ecological complexity created by crop-livestock integration in no-till systems improve yields while decreasing vulnerability of system yields



and profitability to weather variation. We tested this hypothesis using a 16-year dataset from a long-term, no-till integrated soybean-beef cattle system in southern Brazil and measured the impacts of cover crop grazing at different intensities during the winter period on probability of high and low performance [8, 10], minimum and maximum yield potentials and stability of key crop, pasture, animal and whole-system outcomes using established metrics of stability [14, 19-25]. Our results provide insight into the long-term stability of subtropical soybean systems performance and the potential of livestock integration to build up sustainability and resilience in agriculture.

## **Methods**

### **Site description and experimental design**

The experiment was established at Espinilho Farm, in the municipality of São Miguel das Missões, Rio Grande do Sul State, southern Brazil (28° 56' 14" S, 54° 20' 52" W, 465 m above sea level) in 2001. The region has a warm, humid subtropical climate (Cfa, Köppen classification system) with an average annual temperature of 20.5 °C and average annual precipitation of 1934 mm [55]. Temperature and precipitation during the experimental period analyzed here (2001-2016) were collected by a weather station located at the experimental site (Supplementary Fig. S1). Missing weather data points were estimated using linear regression with values from the nearest meteorological station as predictor (National Institute of Meteorology, Cruz Alta, 78 km from the study site 28° 36' 12" S, 53° 40' 25" W, 427 m a.s.l.). The soil in the experimental site is an Oxisol (Rhodic Hapludox) [56], with clayey texture (540, 270 and 190 g kg<sup>-1</sup> of clay, silt and sand, respectively) and a deep, well drained profile.

The area has been managed as no-till soybean [*Glycine max* (L.) Merr.] cropland since 1993. In 2001, 22 hectares of land began to be managed as an integrated soybean-beef cattle

annual rotation with a mixture of black oat (*Avena strigosa* Schreb.) and Italian ryegrass (*Lolium multiflorum* Lam.) pastures grazed during the winter between soybean crops. Soybean was direct-seeded after the animals were removed from the experimental area, typically in November, and harvested after  $142 \pm 11$  days. After soybean harvest (April-May), experimental plots were drill-seeded with black oats into the volunteer ryegrass sward from the previous winter, immediately followed by broadcast seeding of ryegrass to ensure successful establishment for both species in all treatments.

The experiment was established as a randomized complete block design with three replicates. Treatments consisted of four grazing intensities (intense, moderate, moderate-light and light) defined by contrasting sward heights under continuous stocking (10, 20, 30 and 40 cm, respectively) and an ungrazed control with the same pasture species used as winter cover crops. Plot areas were 0.1 ha for the ungrazed treatment and ranged from 0.8 to 3.6 ha for grazed treatments. Plots differed in area to reduce the number of animals required to maintain the target treatment heights, especially for shorter swards.

Fertilization rates and soybean cultivars changed according to recommendations over the years but were the same for all plots. An average of  $160 \text{ kg ha}^{-1}$  urea (46% N) was applied yearly, split into two equal winter applications during the stocking period: 1) when pasture reached V3-V4 growth stage (i.e., plants with 3 to 4 fully expanded leaves on the main stem) and 2) just before animals entered the experimental plots, approximately 1 month after the first application. From 2001 to 2011, P and K (on average,  $60 \text{ kg ha}^{-1} \text{ P}_2\text{O}_5$  and  $70 \text{ kg ha}^{-1} \text{ K}_2\text{O}$ ) were applied at soybean sowing. From 2012 to 2016, P and K (on average,  $45 \text{ kg ha}^{-1} \text{ P}_2\text{O}_5$  and  $60 \text{ kg ha}^{-1} \text{ K}_2\text{O}$ ) were applied at pasture sowing to take advantage of the improved nutrient recycling provided by the grazing animals for primary production. The exact amount of fertilizer applied each year was based on standard recommendations [57] and soil analysis.

Grazing usually started in June-July, when average sward height reached  $24 \pm 4$  cm (or  $1485 \pm 379$  kg ha<sup>-1</sup> of dry matter) and lasted  $124 \pm 16$  days. To ensure that treatments remained close to their nominal targets (Supplementary Fig. S2), sward height was measured at 100 random points per plot every 15 days with a sward stick [58]. Three tester animals remained permanently in the plots over the stocking period and put-and-take animals were added or removed to adjust sward heights [59]. Average stocking rates used to maintain target sward heights throughout the stocking period were 376, 651, 948 and 1331 kg of live weight ha<sup>-1</sup> for light to intense grazing. Experimental animals were crossbred Angus x Hereford x Nelore steers with initial body weight of  $210 \pm 23$  kg and 12 months of age on average. Steers were weighed at the beginning and at the end of the stocking period after 12 hours of fasting.

### **Long-term data collection and variables studied**

We assessed five key indicators of crop, pasture, animal and whole-system performance: 1) soybean grain yield; 2) total herbage production; 3) animal live weight gain; 4) human-digestible protein (HDP) production; and 5) profitability. “Year” in all analyses refers to the year when soybeans were sown. Specific years were removed from the analysis when data for one or more treatments were missing for a variable. Years 2001, 2003 and 2008 were excluded from the analyses of soybean yield, protein production and income. Years 2001, 2004, 2005, 2006, 2007 and 2012 were excluded from the analyses of herbage yield. Year 2012 was excluded from the analysis of animal production, protein production and income.

Soybean yield (kg of grains ha<sup>-1</sup>) was determined at full grain maturity at 13% moisture content. Total herbage production (kg of dry matter ha<sup>-1</sup>) was calculated as the sum of pasture herbage mass on the first grazing day and the daily herbage accumulation rates over the whole stocking period. Daily herbage accumulation rates were estimated every 28 days using grazing exclusion cages [60], following a standard protocol described by Nunes et

al. [61]. Steer live weight gain ( $\text{kg of live weight ha}^{-1}$ ) was calculated as the product of number of animals per hectare, average daily gain ( $\text{kg of live weight steer}^{-1} \text{ day}^{-1}$ ) of the tester animals and number of grazing days of the stocking period.

We adopted human-digestible protein (HDP) as a metric to account for added production from the livestock component when comparing integrated to non-integrated systems. Livestock contributes to supplying human protein demand as much or more than crop production [54, 62] and do so by converting proteins from non-edible (grass) into edible forms. HDP is not intended as a comprehensive nutritional analysis; rather, it is an unbiased indicator of whole-system food production [63]. Total HDP production ( $\text{kg ha}^{-1}$ ) was calculated as the sum of protein from human-edible sources (i.e., animal and crop components of the system) multiplied by protein digestibility of the products (beef and soybeans) [62]. We estimated the protein content of a 350 kg live weight steer at the end of the stocking period as 19% of its body weight, based on National Research Council's equations [64]. Soybean protein content was assumed to be 35% for a grain moisture content of 13% [65].

We used gross profit ( $\text{USD ha}^{-1}$ ) as a metric of profitability, calculated as the difference between direct production costs (e.g., seed, fertilizer, weed control, labor) and revenues from animal and grain sales, using yearly market sale prices of beef cattle and soybean grains on November and April, respectively [66], converted from Brazilian Reals (BRL) to U.S. Dollars (USD) using the exchange rates of the respective months [67]. We used steer live weight gain to calculate income from livestock, assuming similar price per unit mass of beef at purchase and sale. Annual costs of soybean production were obtained from Brazil's National Supply Company for the study region [68]. Soybean costs were considered the same for all treatments, given similar crop management across experimental units.

### Statistical analysis

All statistical analyses were performed in R (version 3.6.1) [69]. Long-term crop, pasture, animal and whole-system mean yields were analyzed using the *lme4* package for mixed linear models [70] with treatments as fixed effects and years, blocks and plots within blocks as random effects ( $y \sim \text{factor}(\text{year}) * \text{treatment} + (1|\text{block}/\text{plot})$ ). Yield trends over the 16 years were analyzed using linear mixed-effects models with treatments and years as fixed effects and blocks and plots within blocks as random effects ( $y \sim \text{year} * \text{treatment} + (1|\text{block}/\text{plot})$ ). Analysis of variance (ANOVA, Supplementary Tables S1 and S2) was performed and when significant effects were detected, treatment means were compared with Tukey HSD test at 95% confidence level using the *emmeans* [71] and *lmerTest* [72] packages. Residuals of all analyses were visually checked for homogeneity of variance and normality was tested with quantile-quantile plots using the R *car* package [73]. When the residuals were not homogeneous or the distribution was not normal, data were log or square root transformed as appropriate.

### Yield stability analysis

We assessed stability of production (soybean yield, total herbage production, animal live weight gain, human-digestible protein) and profitability using four different metrics of stability: 1) yield range, which is the maximum amplitude between minimum and maximum yield values in a time series [19, 20]; 2) coefficient of variation and 3) standard deviation [13, 19, 20]; and 4) Finlay and Wilkinson's stability metric (FW) derived from the linear regression of treatment yield on the mean yield of the location/year, or Environmental Index (EI) [19, 21-24]. Regression of detrended yield on EI, also called adaptability analysis [22], can assess stability or treatment-specific effect across a range of environments [24]. Based on

regression of detrended yield on EI, stable systems are those with smaller slope (less sensitive to changes in environment).

Yield range was calculated as the difference between the highest and the lowest yields for each variable over the experimental period. Coefficient of variation, standard deviation and FW regressions were calculated using detrended data. Detrending removed long-term linear trends potentially generated by treatments in order to only consider variability of the residuals around the mean of each treatment due to transient environmental conditions. Data were detrended by removing treatment effects and treatment-specific linear temporal trends using the residuals of the linear model  $y \sim \text{year} * \text{treatment}$ . The overall average of the response variable was added to the residuals to get intuitively more understandable values (addition of the same constant to all values does not affect relevant statistical results).

Detrended data were analyzed as a function of the Environmental Index (EI) for each year and treatment with the following model:  $\text{detrended } y \sim \text{EI} * \text{treatment}$ . EI was calculated as the average yield of all treatments for each year, so that the highest and lowest EI indicated the year of highest and lowest system performance respectively. FW regression slopes were calculated and compared using simultaneous general linear tests with the R *multcomp* package [74].

Yield range, coefficient of variation and standard deviation were analyzed as a function of treatment and block ( $y \sim \text{treatment} + \text{block}$ ) and when significant differences were detected, treatment means were compared with Tukey HSD test at 95% confidence level ( $\alpha = 0.05$ ) using the R *agricolae* package [75].

Treatments were ranked from the lowest (i.e., greatest stability, rank #1) to the highest value (i.e., lowest stability, rank #5) for each stability metric regardless of the statistical significance. The overall stability of each system output was ranked based on mean stability rank for the four stability metrics, such that treatments with higher overall ranks indicated

higher stability of yield or profitability.

### **Minimum and maximum yield potentials**

Minimum and maximum yield potentials were calculated based on predicted responses for the smallest and largest observed EI values for each studied indicator [19, 24, 25]. Treatment effects on minimum and maximum yield potentials were tested with Tukey HSD test at 95% confidence level through the equation  $HSD = q\sqrt{2SE}$ , where  $HSD$  is Tukey's honest significant difference,  $q$  is the studentized range statistic obtained using the 'qtukey' function from R *stats* package [69], and  $SE$  is the standard error of the mean for the studied variable.

### **Downside risk and probability of high performance**

To determine the probability of extreme yield events over the given range of environmental conditions (EI), we modelled probability distributions of each treatment's detrended data using the 'density' function in R (Supplementary Code S1, adapted from Gaudin et al. [8]). Treatment distributions were compared to a randomized distribution created by bootstrapping data and ignoring treatment effects. Downside risk and probabilities of high performances were defined as estimated probabilities of achieving results below the 10<sup>th</sup> percentile and above the 90<sup>th</sup> percentile, respectively, for each of the studied indicators. 5,000 randomizations were sufficient to stabilize the p-values for every system output. Treatment effects on the downside risk or probability of high performance were identified when observed results were significantly different from the randomized distribution at the 95% confidence level beyond the determined percentiles.

## Results

### Mean yields and trends

Soybean yields were not affected by winter grazing of cover crops, regardless of the grazing intensity ( $p = 0.375$ , Table 1). Total herbage production increased with increasing sward height ( $p < 0.001$ , Table 1) but remained low in the ungrazed treatment. Steers' live weight gain per unit area increased with grazing intensity ( $p < 0.001$ , Table 1). Addition of cattle to the system increased total human-digestible protein production by up to 13% ( $p = 0.065$ , Table 1). Profitability in the two highest grazing intensities was 30% greater than in the two lowest ones, and 127% greater than in the ungrazed treatment ( $p < 0.001$ , Table 1).

All variables, except for total herbage production, presented an increasing linear trend over time (Supplementary Fig. S3, Supplementary Table S2). None of the linear trends were significantly affected by treatments, as indicated by the absence of treatment by year interactions, except for profitability, which increased at lower rates in the ungrazed treatment ( $p = 0.044$ , Supplementary Table S2). When year was included as a factor (categorical variable) in the model, there was a significant treatment by year interaction for total herbage production and live weight gain (Supplementary Table S1). However, we were unable to detect a clear pattern in the interactions.

### Yield stability

Soybean yield was the most stable when the pasture phase was managed at moderate grazing intensities (G20 and G30) according to the overall stability rank (Table 1). Ungrazed (UG) and lightly grazed (G40) treatments were more sensitive to the environmental gradient than more intensively grazed treatments (FW slopes  $> 1$ , Fig. 1a, Table 1), indicating lower stability. The ungrazed treatment presented the narrowest yield range but was ranked worst in



all the other stability metrics, making it the least favorable to soybean yield stability (Table 1). Intense (G10) and light grazing (G40) were similar and intermediate in overall stability.

Conversely, total herbage production was the least stable under moderate grazing intensities, with G30 and G20 ranking fifth and fourth, respectively, in all stability metrics (Table 1). Both treatments were more responsive to changes in Environmental Index (Fig. 1b). The UG control presented the most stable herbage production over the years, ranking first in FW slopes and yield range, and second in CV and standard deviation, followed by G10 and G40 (Table 1).

Increasing grazing intensity reduced the overall stability of live weight gain (Table 1). Light grazing (G40) ranked first for all stability metrics for live weight gain and, along with G30, was significantly more stable than G10 and G20 to the environmental gradient (Fig. 1c, Table 1).

Both human-digestible protein (HDP) production and profitability showed greater stability when pastures were moderately grazed (G30), while either over-intensification or the absence of grazing decreased system stability (Table 1). The UG control had a 26% and 107% higher CV for HDP production and profitability, respectively, than the grazed treatments (Table 1). FW slopes for HDP production followed the same trends as soybean yields, with greater slopes ( $>1$ ) for the G40 and UG treatments (Fig. 1d, Table 1). Profitability trended together with live weight gains, with lower FW slopes for G30 and G40 and greater slopes for G10 and G20 (Fig. 1e, Table 1).

### **Downside risk and minimum yield potentials**

The absence of grazing (UG) significantly 1) increased the downside risk for soybean yield ( $\alpha = 0.01$ , Fig. 2a) without significantly impacting the minimum yield potential (Table 2); 2) increased risks of obtaining low HDP production ( $\alpha = 0.01$ , Fig. 2d); and 3) had the lowest

minimum profitability (19.25 USD ha<sup>-1</sup>). G10 and G20 were ~30 times more profitable than the ungrazed control (600.20 and 501.97 USD ha<sup>-1</sup>,  $p < 0.05$ , Table 2) in the harshest environmental conditions, but changes in downside risk were not detected (Fig. 2e). Despite minimum HDP production not being significantly different among treatments, it was 55% greater in grazed treatments compared to UG and up to 69% greater than UG at the highest grazing intensity.

Conversely, UG presented a lower risk of low herbage production ( $\alpha = 0.01$ , Fig. 2b) despite no statistical differences in minimum yield potential (Table 2). G20 and G30 had higher probability of low herbage production (18 and 19%, respectively), but were not different from the random distribution ( $\alpha = 0.05$ , Fig. 2b). G20 probability of low live weight gain was significantly higher ( $\alpha = 0.05$ ). G40 presented significantly lower downside risk for live weight gain ( $\alpha = 0.01$ , Fig. 2c) but also a significantly lower minimum live weight gain potential ( $\alpha = 0.05$ , Table 2). No differences in probability of low performance were detected for soybean yield

### **Probability of high performance and maximum yield potentials**

Treatment effect on the probability of high performance was larger for live weight gains than for the other variables. High (G10) and moderate (G20) grazing intensities significantly increased the chance of obtaining live weight gains above the 90<sup>th</sup> percentile ( $\alpha = 0.01$  and  $\alpha = 0.05$ , respectively, Fig. 2c). Conversely, moderate-light (G30) and light (G40) grazing intensities reduced the chance of high live weight gains ( $\alpha = 0.05$  and  $\alpha = 0.01$ , respectively, Fig. 2c) and maximum yield potentials relative to G10 and G20 (Table 2).

We observed greater maximum profitability potential in G10 and G20 than in the UG control (1416.75 average vs. 882.82, a 60% increase,  $p < 0.05$ , Table 2), but probability of high performance was not affected (Fig. 2e). No changes in probability of high performance

were detected for soybean yield, total herbage production and HDP production. Likewise, maximum yield potentials were not statistically different between treatments (Table 2), despite the important difference in pasture dry matter production from G10 and UG to moderate to light grazing intensities (G20, G30 and G40) that ranged from 1445.87 kg DM ha<sup>-1</sup> (G20 vs. UG) to 2300.98 kg DM ha<sup>-1</sup> (G30 vs. G10).

## Discussion

Integrated crop-livestock systems (ICLS) are proposed as one possible strategy towards the sustainable intensification of food systems [32, 47-49]. In a context of climate change and increased environmental pressure, stability of agricultural systems performance – not just performance *per se* – needs to be evaluated to prioritize management strategies with the greatest adaptive gains. Mining data from long-term trials provides opportunities to comprehensively assess the performance and stability of key crop, pasture, animal and whole-system indicators when livestock is integrated into specialized cropping systems.

Grazing did not impair soybean grain yields regardless of grazing intensity, but moderate grazing intensities favored soybean long term yield stability (Fig. 3a). Our analysis of soybean yields supports previous studies showing that grazing is not detrimental to crop productivity [52, 76, 77]. The impact of livestock on subsequent crop yields has long been a concern, mainly due to potential soil compaction caused by animal trampling, consumption of cover crop biomass and nutrient export when animals are removed from the system [47, 76]. Results from literature on ICLS have shown everything from decreases in subsequent crop yield [78], to no effect [52, 76, 77] and even increases [47, 49, 79]. In our systems, grazing is combined with low disturbance (i.e., no-till) which may help mitigate potential negative impacts such as soil compaction. When conservation agricultural practices are used and grazing is well-managed (i.e., assuming no overgrazing or abnormally wet years), effects on

soil physical attributes such as increased soil density have been shown to be transient, restricted to soil surface, and of limited impacts on yields [76].

Our study provides the first evidence of grazing-induced long-term yield stability in no-till soybean systems where crops and livestock were integrated under moderate grazing intensity (Fig. 3a). Furthermore, our risk analysis has shown that the absence of grazing increases the risk of yielding below the 10<sup>th</sup> percentile in unfavorable years (Fig. 2a), despite greater litter amounts covering soil in ungrazed plots [61]. The underlying processes of increased yield stability with moderate grazing may be associated with increased biological diversity and ecological interactions created by livestock integration. Properties associated with the maintenance of soil functions and crop stability such as soil aggregation [45], microbial diversity [51, 80] and ratios of beneficial over detrimental soil nematodes [80] were shown to be improved by moderate grazing in previous studies at this experimental site and may have provided better growing conditions for the soybean crop in stressful years. Moderate grazing intensities enhance root growth, exudation and turnover which, combined with manure deposition, can directly benefit soil aggregation and microbial activity and diversity [45]. This in turn can lead to greater soil physical stabilization, organic matter accumulation [81, 82] and nutrient cycling [82]. These soil health benefits, including more biodiverse soil communities, may be particularly relevant to maintain soil functioning under stress as shown in other systems [83, 84] and potential core mechanisms underlying crop yield stability.

Total herbage production increased with increasing sward height but remained low in the ungrazed treatment, and was the least stable under moderate grazing intensities, demonstrating a possible trade-off between yield and stability in forage crops (Fig. 3b). No grazing (UG) and heavy grazing (G10) treatments were more stable but produced significantly less forage over the years (Table 1). Moderate grazing intensities created more

responsive forage growth to better environmental conditions and along with light grazing (G40) were able to produce ~11 tons DM ha<sup>-1</sup>, while G10 and UG reached less than 10 tons DM ha<sup>-1</sup> even in the best environment (Table 2). Lower stocking rates in G40 and UG favored the maintenance of target sward heights (and consequently leaf area index) in dry years, so that daily herbage accumulation rates in these treatments were less affected by poor environmental conditions. These results support long established plant-herbivore models [85] and the existence of two stable steady-states between vegetation growth and animal consumption in grazing lands: a low-productivity stable equilibrium at low plant biomass (G10), and a high-productivity stable equilibrium at high plant biomass (somewhere between G40 and UG). Moderate grazing (G20 and G30) provided a mid-range unstable state at which pasture growth is high, but herbage mass and accumulation rates are more easily affected by disturbances (e.g., weather fluctuations, fertilization or grazing itself), thus requiring more frequent adjustments of stocking rate to keep sward heights close to the nominal targets [85].

In the absence of grazing, forage yields presented a lower risk of low production in unfavorable years (Fig. 2). Keeping a dense layer of residual biomass on the soil surface in no-till systems (during winter as cover crop/pasture and after winter, as straw) improves soil water retention [52] and protects soil from erosion [86] and weed outbreak [87] with potential benefits to crops in rotation. For this reason, crop-livestock integration is seen by many farmers as detrimental to no-till systems. However, prior research at this site showed no direct impacts of greater litter mass on crop yields in the ungrazed system [52]. On the other hand, the greater herbage production under moderate to light grazing intensities and the reduced probability of low forage yields in the ungrazed system found in our study may help explain the increased soil carbon stocks found by previous authors in areas managed under these approaches compared to intensely grazed areas after a decade of crop-livestock integration at this site [53].

The linear increase of live weight gains per unit area (Table 1) with grazing intensity is consistent with previous studies and can be attributed to increased stocking rate required to keep pasture at target sward heights [88]. Constraints in animal dry matter intake when forage allowances are limiting could result in a quadratic response of live weight gain, with greater gains associated with moderate grazing intensities [89, 90]. The shortest sward height used in our study in fact limits the intake [91] and consequently the individual live weight gains [88, 91], but it was not restrictive enough to show the quadratic pattern when results were expressed on a per area basis because greater stocking rate compensated the decrease in individual performance.

Our analysis showed a clear trade-off between yields and stability of live weight gains (Fig. 3c). Live weight gains were generally greater, but less stable at higher grazing intensities. Although pasture growth is less stable and requires more frequent stocking rate adjustments under moderate grazing intensities, more intense stocking rate adjustments are required at the extremities of the grazing intensity gradient. In other words, the closer to a stable state, the stronger the push (i.e., addition or removal of animals) in the opposite direction required to shift states will be [85]. In our case, this was translated as a strong removal of animals from the plots when swards got too short to allow pasture regrowth in higher grazing intensities, which probably resulted in less stable live weight gains. Besides being less stable, literature also shows that higher grazing intensities lead to greater greenhouse gas emissions, especially methane [91]. Thus, to sustainably intensify ICLS, a ‘conciliatory stocking rate’ [88, 92] able to achieve high animal yields and overall system stability while keeping low environmental footprint should be pursued.

Intensification of ruminant production in the last decades has increased protein production per area of land use, but primarily as a result of increased use of feed concentrates and human-edible nutrients in developed countries [37, 54]. However, addressing the ability

of a system to sustainably increase food production must consider the quality of food produced for human nutrition as well as the ability of this system to produce food from human inedible resources [54]. Grazing at moderate to light intensities increased HDP production and stability, while over-intensification and absence of grazing increased system vulnerability to environmental oscillations (Fig. 3d). Ungrazed cover crops represented a risk to food production in unfavorable years (Fig. 2d), since low soybean protein yields are not buffered by livestock protein yields as in integrated systems. By comprising protein from both crop and animal components of the system, our HDP analysis can be used as a measure of land-use efficiency [62]. Despite lacking statistical significance, grazing improved land-use efficiency by up to 13% due to the contribution of grass-based beef, an animal-derived protein of higher quality in human nutrition metrics than plant derived proteins [54].

The greater profitability of integrated systems, particularly in heavier grazing intensities (G10 and G20, Fig. 3e, Table 1), was similar to results from a previous study at this site [43] but differs in the magnitude of the results. This difference might be explained by the rise in cattle prices seen in the latter years of our dataset [66]. We attribute the significantly higher growth rates of gross profits in the grazed treatments compared to UG over the years (Supplementary Fig. S3, Supplementary Table S2) to this same cattle price increase. Our analysis did not include potential differences between cattle purchase and sale prices and cost of cattle parasite control due to a lack of reliable information, which is a limitation of our economic analysis. However, we aimed to evaluate treatment stability to the environmental gradient rather than to present a full economic analysis as already done elsewhere [43]. Costs related to pasture management (e.g., cost of seeds, fertilizer and operations) were the same for all treatments and therefore did not influence our results. The decrease in stability of whole-system profits with the over-intensification or the absence of grazing was consistent with HDP production, with G30 being the most stable treatment (Fig.

3e, Table 1). The significantly lower minimum profitability potential in UG could represent a riskier farm portfolio, while animal production in grazed treatments provide a mean to smoothing farm incomes in poor crop production years (Table 2). We provide empirical evidence of the risk reduction value of ICLS exploiting the lack of correlation between crop and livestock markets, working as a buffer against climate and price fluctuations [46, 50].

In conclusion, our data suggests that moderate grazing intensities benefit whole system stability and soybean yields to environmental variability and confirm that grazing does not impair subsequent soybean yields. Instead, it reduces the chance of crop failure in unfavorable years. Our study supports previous literature suggesting that over-intensification is the least beneficial option for sustainable intensification of food production systems. Our results likely apply to other ICLS designs, but best pasture management remains paramount to achieve benefits and reduce potential tradeoffs. Our study also highlights the importance of long-term experimental protocols to understand complex temporal system responses such as yield stability and improve predictions and adaptation to climate change. Questions remain regarding what mechanisms are driving these results, especially for grazing-induced soybean yield stability, but intensification of ecological processes likely plays a pivotal role.

## **Data Availability**

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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### **Author Contributions**

P.A.A.N. and A.C.M.G. conceived the original idea of this study. P.C.F.C. conceived the long-term experiment. E.A.L., P.C.F.C. and A.C.M.G. supervised the project. P.A.A.N. wrote the manuscript with input from E.A.L., P.C.F.C., M.L. and A.C.M.G. P.A.A.N. and E.A.L. analyzed the data. P.A.A.N., E.A.L., P.C.F.C. and M.L. contributed to the interpretation of results. P.A.A.N. prepared tables and figures with input from E.A.L., M.L. and A.C.M.G. P.A.A.N., W.S.F., T.R.K. and A.P.M. performed field work over years and organized the long-term dataset. All authors contributed to reviewing and revising the manuscript.

### **Competing Interests**

The authors declare no competing interests.

## Figures and Tables

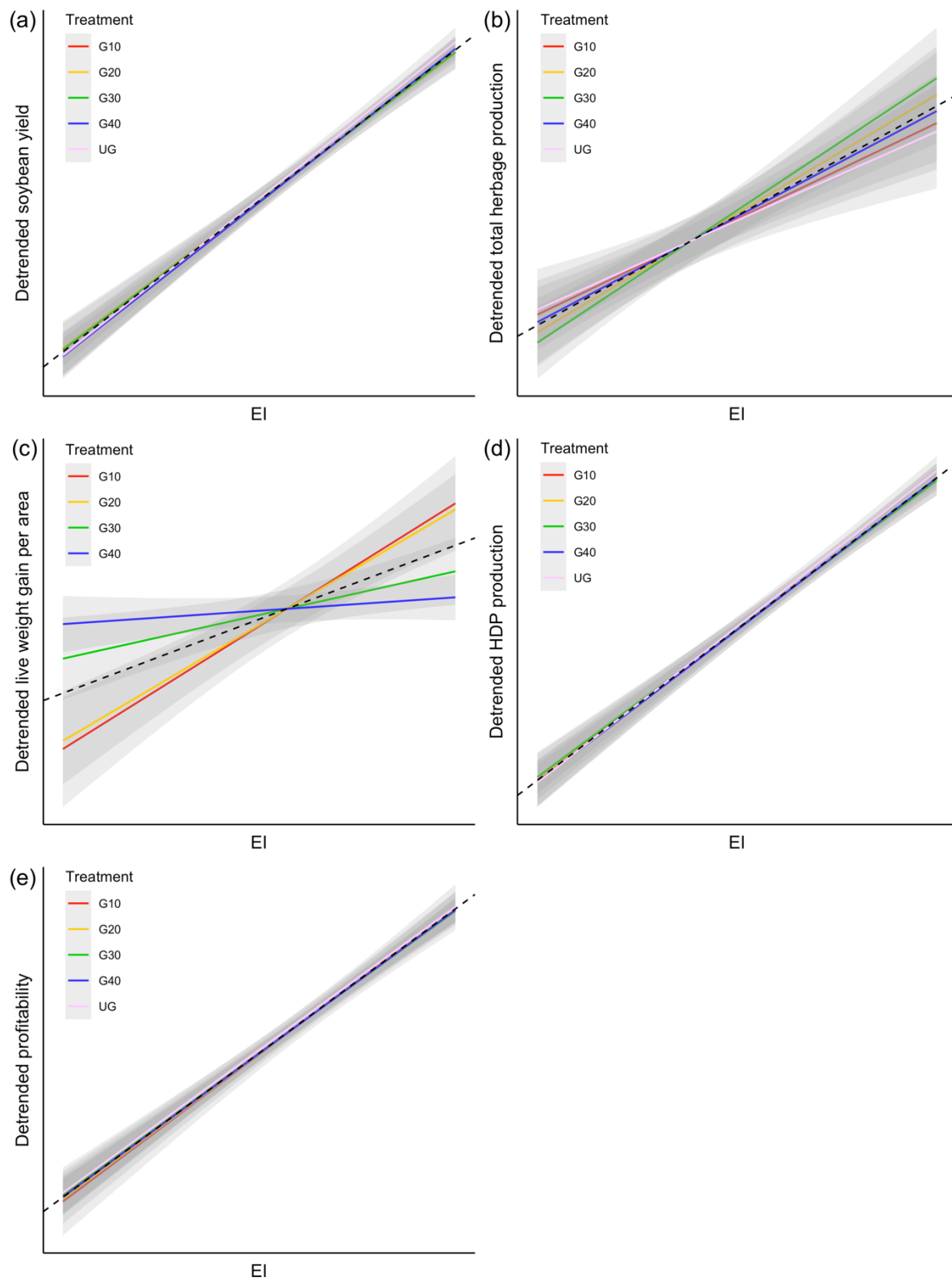


Figure 1. Yield stability of (a) soybean yield (kg grain ha<sup>-1</sup>), (b) total herbage production (kg dry matter ha<sup>-1</sup>), (c) animal live weight (LW) gain (kg LW ha<sup>-1</sup>), (d) human-digestible protein (HDP) production (kg HDP ha<sup>-1</sup>) and (e) profitability (USD ha<sup>-1</sup>) of soybean systems integrated with different levels of cattle grazing during the winter period. Environmental index (EI) was calculated as the yearly mean detrended yield. Dashed lines are the regression of detrended yields against the EI without treatment effects. G10: intense grazing (10 cm sward height); G20: moderate grazing (20 cm sward height); G30: moderate-light grazing (30 cm sward height); G40: light grazing (40 cm sward height); UG: ungrazed cover crop. Smaller slopes indicate greater yield stability.

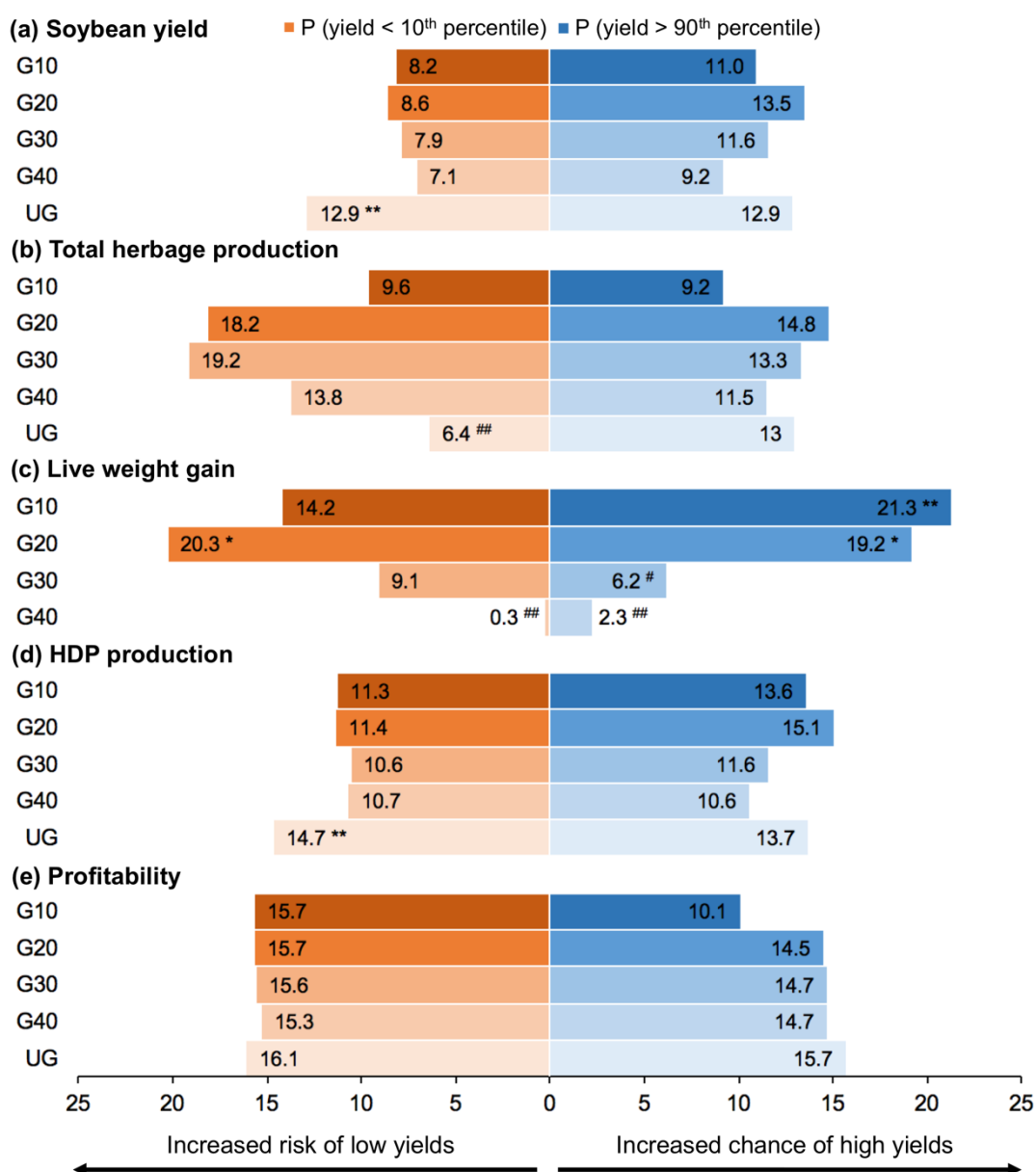


Figure 2. Effect of grazing intensity on the probability of obtaining high and low (a) soybean yield (kg grain ha<sup>-1</sup>), (b) total herbage production (kg dry matter ha<sup>-1</sup>), (c) animal live weight (LW) gain (kg LW ha<sup>-1</sup>), (d) human-digestible protein (HDP) production (kg HDP ha<sup>-1</sup>) and (e) profitability (USD ha<sup>-1</sup>) of soybean systems integrated with different levels of cattle grazing during the winter period in southern Brazil. Shown are the probabilities of yielding below the 10<sup>th</sup> percentile (orange bars) or above the 90<sup>th</sup> percentile (blue bars). Statistically significant treatment effect was identified for higher probability of high/low yields at the 95%

(\*) or 99% (\*\*) confidence level and for lower probability of high/low yields at the 95% (#) or 99% (##) confidence level. G10: intense grazing (10 cm sward height); G20: moderate grazing (20 cm sward height); G30: moderate-light grazing (30 cm sward height); G40: light grazing (40 cm sward height); UG: ungrazed cover crop.

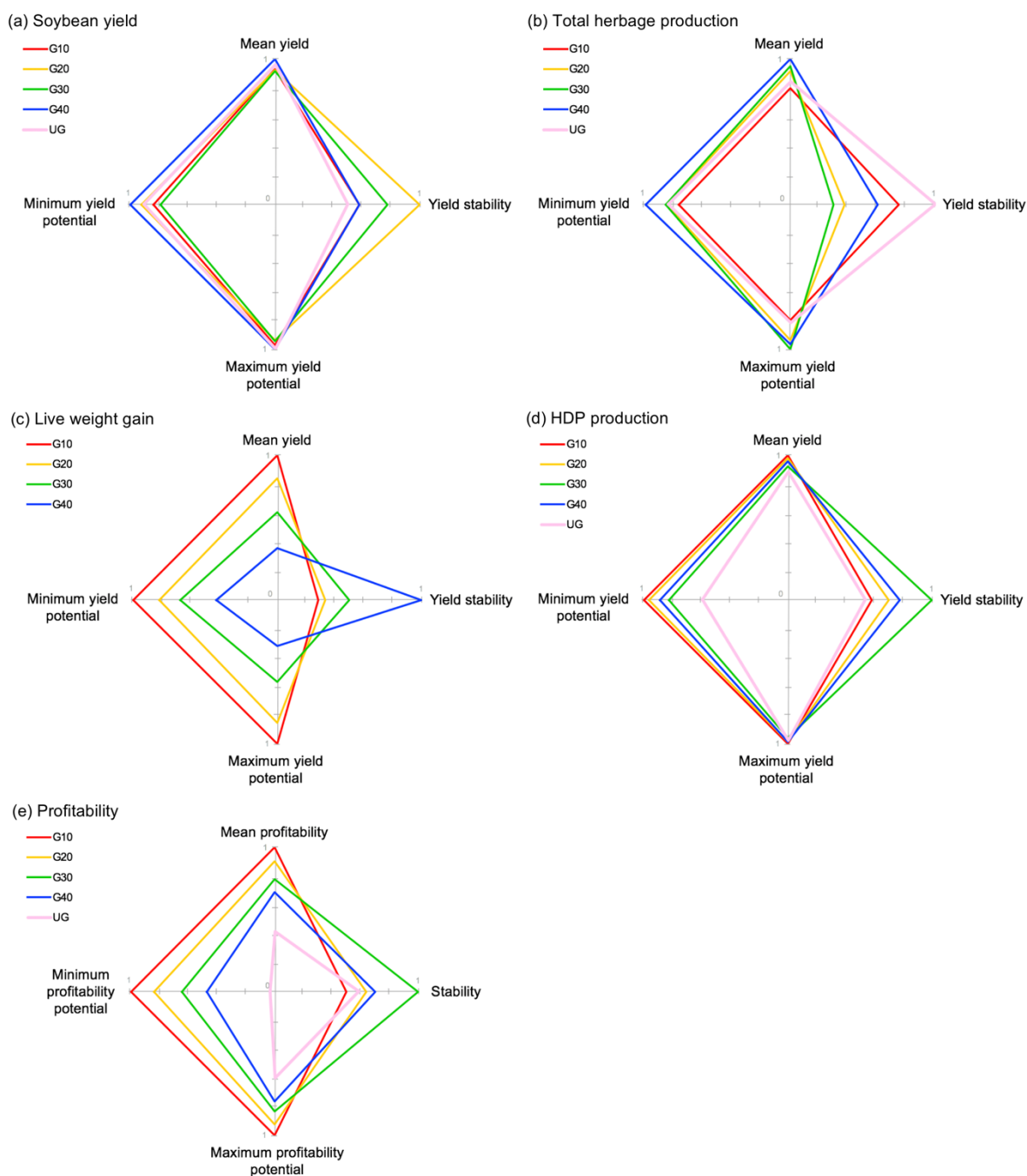


Figure 3. Tradeoffs between performance and stability of (a) soybean yield ( $\text{kg grain ha}^{-1}$ ), (b) total herbage production ( $\text{kg dry matter ha}^{-1}$ ), (c) animal live weight (LW) gain ( $\text{kg LW ha}^{-1}$ ), (d) human-digestible protein (HDP) production ( $\text{kg HDP ha}^{-1}$ ) and (e) profitability ( $\text{USD ha}^{-1}$ ) of soybean systems integrated with different levels of cattle grazing during the winter period in southern Brazil. Values represent standardized ratio to the maximum value for each metric. Yield stability is the average rank of four stability metrics (Table 1).

| Indicator  | Treatment | Mean yield | Stability parameters |                              |                    |            | Overall rank |
|--|-----------|------------|----------------------|------------------------------|--------------------|------------|--------------|
|  |           |            | Yield range          | Coefficient of variation (%) | Standard deviation | FW slope   |              |
| Soybean yield<br>(kg grain ha <sup>-1</sup> )                        | G10       | 2882.58    | 4020.60 (3)          | 40 (3)                       | 1157.72 (4)        | 0.99 (2)   | 3            |
|  | G20       | 2857.27    | 3993.37 (2)          | 39 (2)                       | 1127.47 (2)        | 0.98 (1)   | 1.8          |
|  | G30       | 2835.15    | 4026.83 (4)          | 40 (3)                       | 1115.64 (1)        | 0.98 (1)   | 2.3          |
|  | G40       | 3086.35    | 4030.53 (5)          | 38 (1)                       | 1157.51 (3)        | 1.02 (3)   | 3            |
|  | UG        | 2974.50    | 3797.03 (1)          | 46 (4)                       | 1279.53 (5)        | 1.04 (4)   | 3.5          |
| Total herbage<br>production<br>(kg DM ha <sup>-1</sup> )             | G10       | 6493.02 b  | 5382.73 (2)          | 26 (3)                       | 1678.63 (1)        | 0.87 (2)   | 2            |
|  | G20       | 7447.46 ab | 6445.77 (4)          | 28 (4)                       | 2119.21 (4)        | 1.08 (4)   | 4            |
|  | G30       | 7735.80 a  | 7023.67 (5)          | 29 (5)                       | 2202.73 (5)        | 1.21 (5)   | 5            |
|  | G40       | 8118.69 a  | 6074.67 (3)          | 24 (1)                       | 1949.07 (3)        | 0.96 (3)   | 2.5          |
|  | UG        | 6859.84 ab | 5079.05 (1)          | 25 (2)                       | 1733.51 (2)        | 0.81 (1)   | 1.5          |
| Live weight gain<br>(kg LW ha <sup>-1</sup> )                        | G10       | 509.92 a   | 348.23 a (4)         | 18 (2)                       | 93.75 a (4)        | 1.66 a (4) | 3.5          |
|  | G20       | 428.41 b   | 270.57 ab (3)        | 19 (3)                       | 80.35 ab (3)       | 1.57 a (3) | 3            |
|  | G30       | 310.83 c   | 212.27 ab (2)        | 18 (2)                       | 56.23 bc (2)       | 0.59 b (2) | 2            |
|  | G40       | 183.16 d   | 135.67 b (1)         | 16 (1)                       | 28.74 c (1)        | 0.18 b (1) | 1            |
| Human-digestible<br>protein production<br>(kg HDP ha <sup>-1</sup> ) | G10       | 780.55     | 1121.66 (5)          | 36 (1)                       | 320.14 (4)         | 0.99 (2)   | 3            |
|  | G20       | 765.09     | 1111.16 (4)          | 36 (1)                       | 314.16 (3)         | 0.99 (2)   | 2.5          |
|  | G30       | 720.54     | 1095.90 (3)          | 37 (2)                       | 304.94 (1)         | 0.98 (1)   | 1.8          |
|  | G40       | 749.94     | 1094.92 (2)          | 37 (2)                       | 313.69 (2)         | 1.00 (3)   | 2.3          |
|  | UG        | 692.29     | 1036.59 (1)          | 46 (3)                       | 349.31 (5)         | 1.03 (4)   | 3.3          |
| Profitability<br>(USD ha <sup>-1</sup> )                             | G10       | 1068.22 a  | 1612.92 (5)          | 39 b (1)                     | 431.11 (5)         | 1.02 (3)   | 3.5          |
|  | G20       | 963.93 a   | 1367.79 (4)          | 40 b (2)                     | 399.27 (3)         | 1.00 (2)   | 2.8          |
|  | G30       | 832.58 b   | 1331.59 (2)          | 44 b (3)                     | 388.40 (1)         | 0.99 (1)   | 1.8          |
|  | G40       | 735.40 b   | 1340.48 (3)          | 50 b (4)                     | 392.49 (2)         | 0.99 (1)   | 2.5          |
|  | UG        | 446.56 c   | 1329.84 (1)          | 89 a (5)                     | 426.04 (4)         | 1.00 (2)   | 3            |



Table 1. Mean yields and stability parameters of a long-term (2001-2016) soybean system integrated with livestock at different grazing intensities or left ungrazed in the winter period. G10: intense grazing (10 cm sward height); G20: moderate grazing (20 cm sward height); G30: moderate-light grazing (30 cm sward height); G40: light grazing (40 cm sward height); UG: ungrazed cover crop. FW slope represents the Finlay and Wilkinson regression slope. Numbers in parentheses rank the treatments for each variable within each column. Different letters in the column represent significant differences among treatments according to the Tukey test ( $\alpha = 0.05$ ).

| Indicator  | Treatment | Minimum yield potential | Maximum yield potential |
|--|-----------|-------------------------|-------------------------|
| Soybean yield<br>(kg grain ha <sup>-1</sup> )                        | G10       | 509.43                  | 4925.05                 |
|  | G20       | 558.09                  | 4746.89                 |
|  | G30       | 479.18                  | 4784.52                 |
|  | G40       | 605.47                  | 5049.36                 |
|  | UG        | 548.67                  | 5026.09                 |
| Total herbage production<br>(kg DM ha <sup>-1</sup> )                | G10       | 4798.73                 | 9271.93                 |
|  | G20       | 5347.92                 | 10891.05                |
|  | G30       | 5396.34                 | 11572.91                |
|  | G40       | 6252.24                 | 11179.98                |
|  | UG        | 5287.69                 | 9445.18                 |
| Live weight gain per area<br>(kg LW ha <sup>-1</sup> )               | G10       | 399.79 a                | 593.43 a                |
|  | G20       | 327.30 ab               | 507.20 a                |
|  | G30       | 270.06 b                | 338.66 b                |
|  | G40       | 170.32 c                | 190.26 c                |
| Human-digestible protein<br>production<br>(kg HDP ha <sup>-1</sup> ) | G10       | 234.54                  | 1267.77                 |
|  | G20       | 225.82                  | 1250.97                 |
|  | G30       | 193.21                  | 1211.53                 |
|  | G40       | 207.71                  | 1254.28                 |
|  | UG        | 139.02                  | 1241.88                 |
| Profitability<br>(USD ha <sup>-1</sup> )                             | G10       | 600.20 a                | 1471.79 a               |
|  | G20       | 501.97 a                | 1361.71 a               |
|  | G30       | 384.99 ab               | 1223.57 ab              |
|  | G40       | 284.13 ab               | 1129.56 ab              |
|  | UG        | 19.25 b                 | 882.82 b                |

Table 2. Minimum and maximum yield potentials of a long-term (2001-2016) soybean system integrated with livestock at different grazing intensities or left ungrazed in the winter period. G10: intense grazing (10 cm sward height); G20: moderate grazing (20 cm sward height); G30: moderate-light grazing (30 cm sward height); G40: light grazing (40 cm sward height); UG: ungrazed cover crop. Different letters in the column represent significant differences among treatments according to the Tukey test ( $\alpha = 0.05$ ).

### **3. CAPÍTULO III**

**Intense winter grazing impairs Italian ryegrass cover crop reestablishment by self-seeding in a no-till soybean-beef cattle system<sup>4</sup>**

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<sup>4</sup> Manuscrito elaborado conforme as normas do periódico *Grass and Forage Science* (Apêndice 5).

## **Intense winter grazing impairs Italian ryegrass cover crop reestablishment by self-seeding in a no-till soybean-beef cattle system**

Short running title: Italian ryegrass self-seeding in ICLS

Pedro Arthur de Albuquerque Nunes<sup>1,\*</sup>, Emilio Andrés Laca<sup>2</sup>, Taise Robinson Kunrath<sup>1</sup>, William de Souza Filho<sup>1</sup>, Amanda Posselt Martins<sup>3</sup>, Paulo César de Faccio Carvalho<sup>1</sup>

<sup>1</sup>Department of Forage Plants and Agrometeorology, Federal University of Rio Grande do Sul, Porto Alegre, RS 91540-000, Brazil

<sup>2</sup>Department of Plant Sciences, University of California - Davis, Davis, CA 95616, USA

<sup>3</sup>Department of Soil Science, Federal University of Rio Grande do Sul, Porto Alegre, RS 91540-000, Brazil

\*Corresponding author: pedro\_nuness@hotmail.com (P. A. A. Nunes)

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### **Abstract**

We evaluated the effectiveness of Italian ryegrass (*Lolium multiflorum* Lam.) establishment by self-seeding and determined the need of additional seeding as a function of grazing intensity to ensure a successful pasture reestablishment in the stocking period of an annual integrated soybean-beef cattle system in southern Brazil. Treatment structure consisted of a factorial of five grazing treatments under continuous stocking (intense, moderate, moderate-light and light grazing, defined by sward heights of 10, 20, 30 and 40 cm, and an ungrazed

control) and two reseeding levels, self-seeding only (SS) and self-seeding with addition of ryegrass seeds (SS+Add). The experimental design was split-plot in a randomized complete block with repeated measures over time. Field measurements were performed during the winter stocking periods of 2017 and 2018, corresponding to Italian ryegrass seed crops from 2016 and 2017. Intense grazing was not an effective strategy to ensure successful ryegrass establishment by self-seeding in the following year, resulting in lower plant population density only partially compensated by larger individual plants than in the other treatments. Addition of ryegrass seed in this treatment increased the density of established plants to values comparable to those in moderate grazing intensities but reduced individual plant mass, compromising total herbage mass when plant size and population density were combined. The combination of individual plant mass and population density was sufficient to maintain herbage mass following moderate to light grazing intensities comparable to the ungrazed cover crop, regardless of the reseeding level, positively affecting integrated crop-livestock system's performance and resilience.

**Keywords:** annual ryegrass, integrated crop-livestock systems, mixed systems, natural reseeding, pasture management, sward height

## **Introduction**

Agriculture changed from diverse, polycultural agroecosystems inherited from the Neolithic period (Halstead, 1996; Bogaard et al., 2013) to biologically simplified, input-driven monospecific cropping systems (Altieri et al., 2015) in many regions of the world over the last decades. Highly productive, specialized production systems with heavy reliance on external inputs often present proportionally high environmental costs (Tilman, Cassman, Matson, Naylor, & Polasky, 2002; Liu et al., 2010). Climate change and its consequent increased frequency of extreme weather events such as floods and droughts will raise the frequency of crop losses (Lobell & Field, 2007; IPCC, 2014) just as the global human population is expected to surpass 9.8 billion people by 2050 (UN, 2019). Thus, a transition towards environmentally friendly, resilient production systems is imperative for future food security (Altieri et al., 2015; Bullock et al., 2017).

Crops and livestock can be integrated when crops and animals are simultaneously or sequentially produced within farms through seasonal pasture-crop rotations or intercropping with forage species (de Moraes et al., 2014), or within regions by exchange of resources such as livestock waste and forage (Moraine, Duru, & Therond, 2017). Such integrated crop-

livestock systems (ICLS) are increasingly proposed as a strategy to reconcile high levels of food production with the maintenance of fundamental ecosystem services underlying sustainability (Herrero et al., 2010; Lemaire, Franzluebbers, Carvalho, & Dedieu, 2014). Specialized systems such as monoculture cropping and feedlots contaminate water resources (Verhoeven, Arheimer, Yin, & Hefting, 2006; Liu et al., 2010) and release large quantities of greenhouse gases (MacDonald & McBride, 2009; Gerber et al., 2013). Conversely, ICLS are planned to harness complementarities and synergies from soil-plant-animal interactions, increasing system self-sufficiency and reducing exportation of pollutants (Bonaudo et al., 2014; de Moraes et al., 2014; de Souza Filho et al., 2019).

For this reason, commercial-scale ICLS are regaining attention globally (Garrett et al., 2017) even as mixed systems remain dominant in most traditional smallholder systems in developing countries (Herrero et al., 2010). However, there are still concerns about supposed negative impacts of livestock integration into specialized cropping systems. One of these concerns in traditional soybean/maize - winter pasture rotations of the Brazilian subtropical region is that grazing could impair the self-seeding ability of forage and cover crops such as Italian ryegrass (*Lolium multiflorum* Lam.).

The establishment of Italian ryegrass swards by self-seeding is a common practice in no-till systems of southern Brazil, as well as other regions of the world, such as Argentina, Uruguay, Paraguay (Barth Neto et al., 2014) and USA (Evers & Nelson, 2000). Self-seeding reduces production costs and extends the grazing period by advancing its starting date (Evers & Nelson, 2000). In addition, a system that does not require reseeding requires less importation of energy and seed and has a lower environmental footprint. However, successful establishment of Italian ryegrass by self-seeding depends on the production of enough seeds and the establishment of sufficient seedlings from the soil seed bank (Bartholomew & Williams, 2009). In case of mismanagement, pasture establishment can be delayed or fail due to low seedling number and/or poor vigor (Evers & Nelson, 1994). This would demand a new seeding operation every year, since Italian ryegrass establishment by self-seeding depends on annual soil seed bank replacement, which in turn depends on the production of enough mature reproductive tillers at the end of the season (Barth Neto et al., 2014).

Many authors report that the stocking period must be finished early in the spring to prevent the consumption of reproductive tillers, and therefore the success of self-seeding (Young, Chilcote, & Youngberg, 1996; Evers & Nelson, 2000; Bartholomew & Williams, 2009). Approximately 500 seedlings m<sup>-2</sup> are required for satisfactory ryegrass stands (Evers & Nelson, 1994). To this end, densities between 885 and 5650 seed heads m<sup>-2</sup> are required

(Bartholomew & Williams, 2009). Early removal of grazing has been the standard management practice for Italian ryegrass pastures in subtropical Brazil. However, reducing the number of grazing days is costly in terms of animal production and weakens the advantage of self-seeding to extend the grazing season (Barth Neto et al., 2014).

Despite the importance of Italian ryegrass as a winter forage, little is known about the effects of grazing intensity on its self-seeding and reestablishment ability. Barth Neto et al. (2014) reported that both continuous and rotational grazing with moderate grazing intensity ensured a successful pasture establishment by self-seeding, even when animals grazed up to the end of the grass production cycle in late spring. However, their study compared only moderate vs. low grazing intensity and did not reflect the high grazing intensity most commonly used by ranchers in southern Brazil. Moreover, the authors did not compare grazed vs. ungrazed areas or the effects of supplementary seeding.

We aimed to 1) evaluate the success of Italian ryegrass establishment by self-seeding following a range of grazing intensities in an annual integrated soybean-beef cattle system, and 2) investigate how grazing intensity affects system resilience by determining when the addition of seeds is needed to ensure the success of pasture establishment in the following winter stocking period. We hypothesized that 1) density of ryegrass plants established by self-seeding is inversely proportional to grazing intensity in the previous grazing season; reductions of density are compensated by increases in plant size up to a threshold beyond which supplementary seeding is necessary to ensure forage production potential is achieved, and 2) the use of adequate grazing intensities is enough to ensure a successful pasture establishment by self-seeding, representing an important gain to ICLS self-sufficiency.

## **Materials and Methods**

### *Site description and experimental design*

The study was part of a long-term experiment established at Espinilho Farm, in the municipality of São Miguel das Missões, Rio Grande do Sul State, southern Brazil (28° 56' 14" S, 54° 20' 52" W, 465 m above sea level) in 2001. The region has a warm, humid subtropical climate (Cfa, Köppen classification system) with an average annual temperature of 20.5 °C and average annual precipitation of 1934 mm (Embrapa, 2012). Temperature and precipitation during the experimental period (Figure 1) were collected at the nearest meteorological station (National Institute of Meteorology, Cruz Alta, 78 km from the study site, 28° 36' 12" S, 53° 40' 25" W, 427 m a.s.l.). The soil in the experimental site is an Oxisol

(Rhodic Hapludox, USDA Soil Survey Staff, 1999), with clayey texture (540, 270 and 190 g kg<sup>-1</sup> of clay, silt and sand in the 0-20 cm layer) and a deep, well drained profile.

The area has been managed as no-till soybean [*Glycine max* (L.) Merr.] cropland since 1993. In 2001, 22 hectares of land started being managed as an integrated soybean-beef cattle annual rotation with a mixture of black oat (*Avena strigosa* Schreb.) and Italian ryegrass (*Lolium multiflorum* Lam.) pastures grazed in the winter period. Soybean was direct-seeded after the animals were removed from the experimental area, typically in November. Each year after soybean harvest (between April-May), experimental plots were drill-seeded with black oats into the volunteer ryegrass sward from the previous winter, immediately followed by supplementary broadcast seeding of ryegrass to ensure the successful establishment for both species in all treatments. Starting in 2017, oats were no longer seeded and the winter stocking period was managed as monospecific pasture to study of the effectiveness of Italian ryegrass self-seeding under contrasting grazing intensities.

The experimental design was a split-plot in a randomized complete block with repeated measures over time. Treatment structure consisted of a factorial of five grazing treatments under continuous stocking [10 cm sward height, or intense grazing (G10); 20 cm sward height, or moderate grazing (G20); 30 cm sward height, or moderate-light grazing (G30); 40 cm sward height, or light grazing (G40); and ungrazed cover crops (UG)] and two reseeding levels [self-seeding only (SS) and self-seeding with addition of ryegrass seeds (SS+Add)]. Addition of seeds consisted of supplementary broadcast seeding with 40 kg of seeds ha<sup>-1</sup> in April 28th, 2017 and 30 kg of seeds ha<sup>-1</sup> in April 21st, 2018. Each of the three blocks was divided into large plots to which grazing treatments were assigned randomly once at the beginning of the experiment. Plot areas were 0.1 ha for the ungrazed treatment and ranged from 0.8 to 3.6 ha for grazed treatments. Two small subplots of 18 m<sup>2</sup> were randomly located within each large plot in each year; one was randomly assigned to self-seeding only and the other assigned to self-seeding with addition of seeds each year. Subplots assigned to self-seeding only were covered with an 18 m<sup>2</sup> (3 m x 6 m) plastic tarp moments before the addition of seeds to the experimental area (04/28/2017 and 04/21/2018) to avoid seed deposition during the seeding operations and removed immediately after it. This design has three levels of spatial grouping: blocks, large plots and subplots.

#### *Pasture management*

We evaluated pasture establishment in the winter stocking periods of 2017 and 2018, corresponding to Italian ryegrass seeds crops from 2016 and 2017. To ensure that grazing



treatments remained close to their nominal targets, sward height was measured at 100 random points per plot every 15 days with a sward stick (Barthram, 1985). Three tester animals remained permanently in the plots over the stocking period and put-and-take animals (Mott & Lucas, 1952) were used to adjust sward heights. Experimental animals were crossbred Angus x Hereford x Nelore steers with initial body weight of  $197 \pm 9$  kg and  $188 \pm 11$  kg in 2016 and 2017, respectively, and 10 months of age on average. In 2016, grazing started in July 27<sup>th</sup> when average sward height of grazed treatments reached  $30 \pm 5$  cm [or  $1806 \pm 416$  kg of dry matter (DM) ha<sup>-1</sup>] and lasted 99 days. In 2017, grazing begun in June 28<sup>th</sup> when average sward height of grazed treatments reached  $18 \pm 5$  cm (or  $959 \pm 314$  kg DM ha<sup>-1</sup>) and lasted 113 days. Average stocking rates used to maintain target sward heights were 1202, 887, 624 and 430 kg of live weight ha<sup>-1</sup>, average of 2016 and 2017 stocking periods.

In 2016, fertilization consisted of 54 kg ha<sup>-1</sup> N + 90 kg ha<sup>-1</sup> K<sub>2</sub>O broadcast on June 10<sup>th</sup>, and 60 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> + 48.5 kg ha<sup>-1</sup> N on July 12<sup>th</sup>. In 2017, 110 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> + 100 kg ha<sup>-1</sup> K<sub>2</sub>O were broadcast on April 29<sup>th</sup>, and 80 kg ha<sup>-1</sup> N on June 12<sup>th</sup>. In 2018, 85 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> + 85 kg ha<sup>-1</sup> K<sub>2</sub>O were broadcast on May 25<sup>th</sup>, and 80 kg ha<sup>-1</sup> N on June 14<sup>th</sup>. All fertilization management was done before animals accessed the experimental area. Fertilization dates followed resource availability on farm, and fertilization rates followed the standard recommendations based on yearly soil analysis (CQFS-RS/SC, 2016), but varied subtly according to availability of commercial fertilizers of various compositions.

### *Measurements*

Plots were sampled three times during ryegrass establishment but after addition of seed to the corresponding plots each year, on 05/14, 06/13 and 06/24 in 2017 and 05/21, 06/19 and 07/14 in 2018. On each date, six 0.1-m<sup>2</sup> quadrats were randomly placed in each subplot. In each quadrat, we counted the number of plants and clipped all plants to ground level and oven dried at 65 °C until constant weight. Total herbage dry mass (g DM m<sup>-2</sup>) was measured and average plant mass (g DM plant<sup>-1</sup>) was calculated as the ratio of total mass over number of plants.

### *Statistical analysis*

Data from the six quadrats in each subplot were averaged before statistical analyses. Averages were log transformed to achieve normality of residuals. All statistical analyses were performed in R (version 3.6.1, R Core Team, 2018). Data were analyzed using the *lme4* package for mixed linear models (Bates, Maechler, Bolker, & Walker, 2015). The model for

number of plants included treatment, sampling period and reseeding level as fixed effects, and subplots within years as random effects ( $y \sim \text{treatment} * \text{period} * \text{level} + (1|\text{year}/\text{subplot})$ ). The model for mass per plant and herbage mass included treatment, sampling period and reseeding level as fixed effects and subplot by years as random effects ( $y \sim \text{treatment} * \text{period} * \text{level} + (1|\text{year}:\text{subplot})$ ). We tested the effect of blocks and then removed it from the models because it was not significant. Analysis of variance (ANOVA) was performed and when significant effects were detected, treatment means were compared with the Tukey HSD test at 95% confidence level using the *emmeans* (Lenth, 2018) and *lmerTest* (Kuznetsova, Brockhoff, & Christensen, 2017) packages. Residuals of all analyses were visually checked for homogeneity of variance and normality was tested with quantile-quantile plots using the *Rcar* package (Fox & Weisberg, 2011).

## Results

All measurements were performed before grazing started during the evaluated years, thus, the plants measured were not directly affected by grazing. The only effects of grazing on the results occurred via effects in soil seed bank and other effects on the soil that persisted beyond the year when grazing occurred.

Plant population density (plants  $\text{m}^{-2}$ ) increased with decreasing grazing intensity for both reseeding treatments (Figure 2). Addition of seeds caused an increase in plant density relative to self-seeding that was more pronounced under higher grazing intensities, as revealed by the significant treatment:reseeding interaction ( $p = 0.0003$ , Figure 2).

Lower plant density in G10 compared to the other treatments was consistent over time up to the last sampling period ( $p < 0.0001$ , Table 1). The ungrazed treatment was the only one that presented significant self-thinning over time according to the treatment:period interaction ( $p < 0.0001$ ), with a reduction in plant density of more than 50% from period 1 to 3 (Table 1).

Average mass per plant was greater in G10 without addition of seed than in other treatments ( $p = 0.06$ , Figure 3), probably due to the corresponding lower population density. Addition of seed in this treatment reduced individual plant mass to values equivalent to the other treatments (Figure 3). Plants increased in mass from the first to the third sampling period in all treatments for both reseeding levels ( $p < 0.0001$ , Table 1).

Herbage mass was lower in G10 than other treatments during the whole establishment phase (94.39 g DM  $\text{m}^{-2}$  in G10 and 269.70 g DM  $\text{m}^{-2}$  on average in G20, G30, G40 and UG in the last sampling period,  $p < 0.0001$ , Figure 4). All treatments presented increasing herbage mass over time, regardless of the reseeding level ( $p < 0.001$ , Figure 4).

On average, ryegrass exhibited a typical inverse relationship between plant mass and population density, whereby reductions in plant density were compensated by increases in plant mass up to a threshold where increments in plant size were not sufficient to compensate for the lack of density, mainly in G10 treatment without addition of seed (Figure 5). Although the addition of seed in G10 resulted in plant densities very similar to G20, it also resulted in smaller plants than in G20, and consequently, in lower total herbage mass.

## **Discussion**

The importance of Italian ryegrass as a winter forage species in livestock systems of southern Brazil is well documented (Carvalho, dos Santos, Gonçalves, de Moraes, & Nabinger, 2010; Barth Neto et al., 2014; de Moraes et al., 2014), as well as management practices oriented to improve its utilization and animal performance (Amaral et al., 2013; da Silva, 2013; Savian et al., 2018). Yet, little is known about one of its main characteristics: its self-seeding ability under different uses in grazing systems, which has the potential to increase systems' self-sufficiency and resilience. This is especially true under complex arrangements such as integrated crop-livestock systems (ICLS), which are regarded as a promising way to produce food under the requirements of an increasingly demanding society, either in terms of food demand or environmental conservation (Herrero et al., 2010).

In this paper, we have shown that intense grazing (i.e., keeping swards as short as 10 cm height) is not an effective strategy to ensure a successful pasture establishment by self-seeding in the following stocking season. Addition of ryegrass seeds in G10 has the potential to increase the number of established plants to a value comparable to those observed in moderate grazing intensities (Figure 2) but results in smaller plants than G20, which ends compromising total herbage mass when individual plant mass and population density are combined (Figure 5). The greater individual plant mass resulting from low population density in G10 without addition of seed (Figure 3) was not sufficient to compensate for the lack of density (Figure 5). Consequently, G10 presented lower herbage mass than other treatments in all sampling periods, regardless of the reseeding level (Figure 4). On the other hand, the combination of plant population density and individual plant mass was sufficient to maintain herbage mass following moderate to light grazing intensities comparable to the ungrazed treatment during pasture establishment phase, regardless of the reseeding level (Figure 4 and 5).

This size/density compensation is well recognized in grassland science (Bircham & Hodgson, 1983; Davies, 1988; Chapman & Lemaire, 1993; Matthew, Lemaire, Sackville

Hamilton, & Hernández Garay, 1995). It is known that at low sward heights (or high grazing intensities) a higher population density of smaller tillers optimizes sward leaf-area index (LAI) and, conversely, at higher sward heights (or lower grazing intensities) a lower population density of larger tillers optimizes sward LAI, generally following the  $-3/2$  self-thinning rule (i.e., tiller size/density combinations would lie along a common  $-3/2$  slope on a logarithmic plot, Yoda, Kira, Ogawa, & Hozumi, 1963). This principle was also observed in undefoliated swards, except at low light levels (Kays & Harper, 1974; Lonsdale & Watkinson, 1982). However, the  $-3/2$  trajectory was defined for approximately constant canopy leaf areas, whereas swards with different LAI normally present slopes steeper than  $-3/2$  (Bircham & Hodgson, 1983; Matthew, Lemaire, Sackville Hamilton, & Hernández Garay, 1995).

Because our objective was to verify the effectiveness of self-seeding and this could be reached by studying only to the whole plant level, we didn't include tiller dynamics in our analysis. However, our results of plant density have shown a clear size-density compensation up to moderate grazing intensities (Figure 5) and followed the self-thinning rule when data were log transformed and plotted as a log-log chart, with a slope close to  $-3/2$  (around  $-1.7$  and  $-1.4$  for the years 2017 and 2018, respectively; Supporting information). Size-density compensation in areas succeeding intense grazing apparently followed a distinct pattern, showing stronger reduction of plant size with increasing plant population (Figure 5), which suggests the influence of other factors such as soil conditions or weed competition in G10 treatment. These factors were not measured in our study but deserve further consideration.

According to Evers & Nelson (1994; 2000), about 500 established seedlings  $m^{-2}$  are needed for successful Italian ryegrass establishment. The only treatment that was not able to reach this requirement with self-seeding was G10, and this was solved with the addition of seed (Figure 2). However, there seems to be a threshold at a higher plant density around 1000 plants  $m^{-2}$  below which increments in plant size are not sufficient to maintain total herbage mass even with supplementary seeding, as in the G10 treatment (Figure 5). In practical terms, this threshold can be used as a management indicator for the need for supplementary seeding, but managing pastures at 20 cm sward height or taller was shown to be more effective for maintaining herbage productivity in the establishment phase.

Similarly, Barth Neto et al. (2014) found no differences in herbage mass during the pasture establishment phase between moderate and light grazing intensities up to the last sampling period (just before the beginning of the stocking period), although our values of herbage mass were somewhat higher. Those authors reported  $1674 \text{ kg DM ha}^{-1}$  on average herbage mass for their low and moderate grazing intensity treatments following soybean crop.

In our study, herbage mass of moderate to light grazing treatments was 2517 kg DM ha<sup>-1</sup> on average in the last sampling period. This discrepancy might be explained by differences in soil type and fertility (a Rhodic Hapludox with 54% clay and between 3.2 and 4.0% organic matter in our case, Peterson et al., 2019; versus a Typic Paleudult with 15% clay and 2.0% organic matter in their experimental site) and environmental conditions (below-average precipitations and temperatures in May and June were reported in their study).

Poor pasture establishment as in G10 will delay the beginning of stocking period when criteria such as sward height or herbage mass are used as guidelines, consequently impairing system efficiency due to a reduction in the number of grazing days as well as animal performance on that area (Barth Neto et al., 2014). Moreover, poor stands present frequent bare soil patches, a situation that worsens over time due to intense animal trampling and overgrazing (Nunes et al., 2019). Such conditions boost weed infestation (Schuster et al., 2016), water runoff, soil erosion (Bonetti, Anghinoni, Gubiani, Cecagno, & de Moraes, 2019) and nutrient losses (Assmann et al., 2014), ultimately compromising profitability and sustainability of the system and surrounding areas in the long-term (Liu et al., 2010). On the other hand, ensuring a successful pasture establishment enhances system resilience to weather or market variability. Besides reducing costs associated with weed control (estimated to be 2 times higher in G10 compared to G20 treatment, Schuster et al., 2016) and supplementary seeding operations (Evers & Nelson, 2000), early pasture establishment can increase total system livestock yields.

Livestock performance depends on sward structure, but also on the length of the stocking period to improve total animal yield. Although recent studies have shown that managing pastures using sward structural parameters (i.e., sward height) is the most practical and efficient way to optimize animal performance (Carvalho, 2013; Savian et al., 2018), structural parameters such as sward height are directly related to herbage mass (Kunrath et al., 2020). Recommended pre-grazing sward height for Italian ryegrass pastures in order to maximize forage intake rates and animal performance is 18.5 cm (da Silva, 2013; Savian et al., 2018), which corresponds to approximately 1500 - 2000 kg DM ha<sup>-1</sup> (Carvalho, dos Santos, Gonçalves, de Moraes, & Nabinger, 2010; Savian, 2017). This herbage mass was reached in the second sampling period of our study and surpassed in our last sampling date by moderate to light grazing intensities and the ungrazed treatment. The G10 treatment, however, did not reach this herbage mass during the experimental period. In this scenario, ranchers have two options: to delay grazing start date, or to start grazing in these conditions, generating a cascade of effects that will increasingly compromise the sustainability of the ICLS.

Using a stocking density of three young steers  $\text{ha}^{-1}$  with an average daily gain of  $1.08 \text{ kg ha}^{-1}$  (moderate grazing intensity, Kunrath et al., 2020), local market prices of beef cattle at Brazilian currency during the study period ( $4.65 \text{ BRL kg}^{-1}$  body weight $^{-1}$ , Agrolink, 2019a), and converting these values to equivalent soybean sale prices ( $1.09 \text{ BRL kg}^{-1}$  soybean grain $^{-1}$ , Agrolink, 2019b) as in Carvalho et al. (2018), one extra month of grazing in these systems would represent the equivalent value of  $415 \text{ kg ha}^{-1}$  of soybean grains, revenue that could buffer crop losses due to an excessively dry summer or eventual drop in prices of this commodity (this is 40% above the yield of the driest year in that time series). According to these authors, grazing at moderate intensities (i.e. 20 cm sward height) increased ICLS resilience as it represented an addition of 60% to average crop yields when live weight gains  $\text{ha}^{-1}$  were converted to equivalent soybean  $\text{Mg ha}^{-1}$  over 14 years of study in southern Brazil.

### **Conclusion**

Intense grazing that keeps swards at 10 cm of height is not an effective strategy to ensure a successful Italian ryegrass establishment by self-seeding in the following year. In these conditions, greater individual plant mass resulting from low population density does not fully compensate for the lack of density, resulting in lower herbage mass by the end of the pasture establishment phase. The addition of ryegrass seed in these areas has the potential to increase the number of established plants comparable to moderate grazing intensities but results in smaller plants that, when combined with plant density, end compromising total herbage mass as well. On the other hand, at moderate grazing intensity (i.e., 20 cm sward height) self-seeding is enough to ensure a sufficient combination of plant density and individual plant mass able to maintain herbage mass comparable to the ungrazed cover crop, even with livestock grazing up to the end of grass production cycle, positively affecting ICLS performance and resilience. Our study suggests that there is a threshold around  $1000 \text{ plants m}^{-2}$  below which increments in plant size are not sufficient to maintain total herbage mass even with supplementary seeding. In practical terms, this threshold can be used as a management indicator for the need for supplementary seeding. This practice, however, is not as effective as managing pastures at 20 cm sward height or taller in the previous stocking period for maintaining herbage productivity in the establishment phase.

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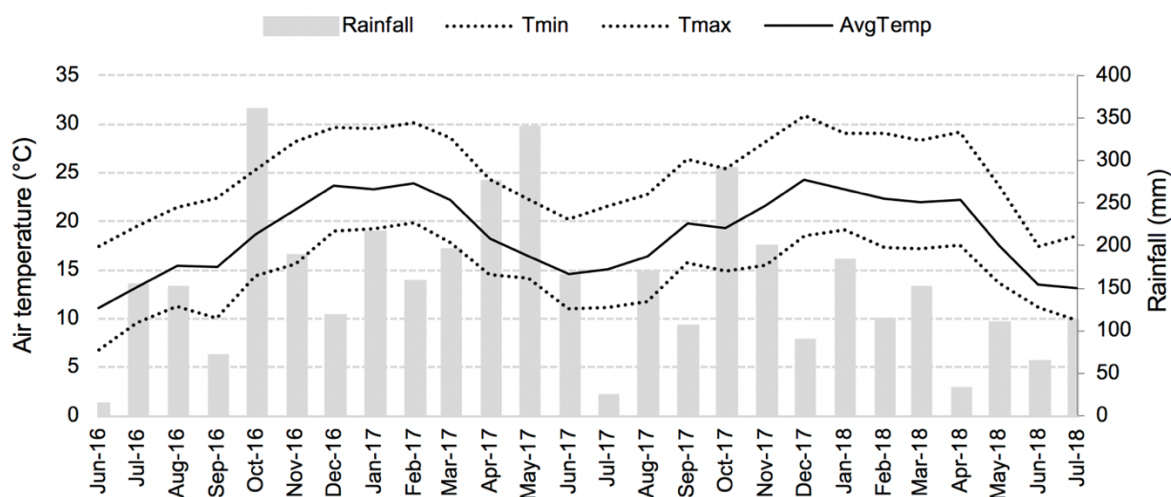
## Tables and Figures

**Table 1.** Plant population density (plants m<sup>-2</sup>) and mass of individual plants (g DM plant<sup>-1</sup>) of Italian ryegrass (*Lolium multiflorum* Lam.) over time during pasture establishment (prior to the beginning of stocking period) following different grazing intensities [10 cm sward height, or intense grazing (G10); 20 cm sward height, or moderate grazing (G20); 30 cm sward height, or moderate-light grazing (G30); 40 cm sward height, or light grazing (G40); and ungrazed cover crops (UG)] in the previous winter stocking period of an integrated beef-soybean system in São Miguel das Missões, RS, Brazil.

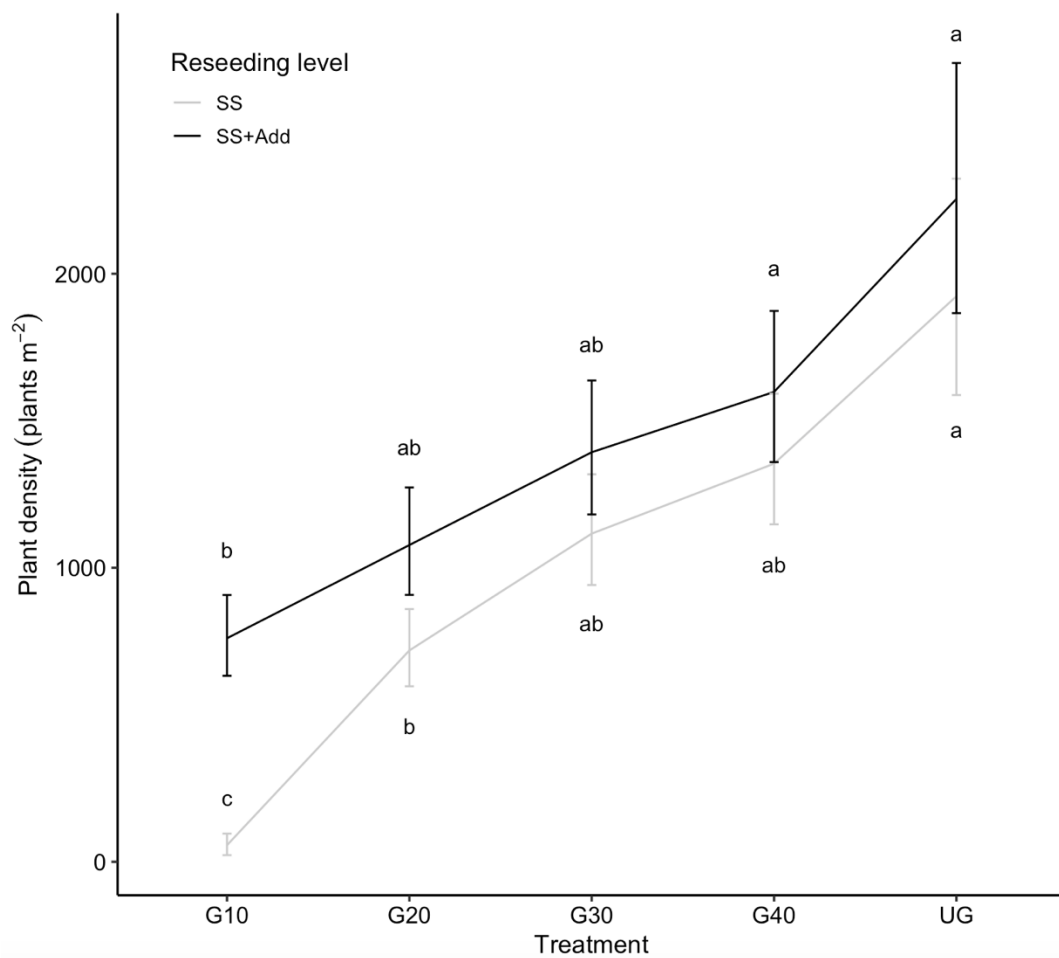
| Plant population density (plants m <sup>-2</sup> ) |           |         |          |          |         |         |
|--|-----------|---------|----------|----------|---------|---------|
| Sampling period                                    | Treatment |         |          |          |         | Average |
|  | G10       | G20     | G30      | G40      | UG      |         |
| 1  | 218 f     | 783 de  | 1208 bcd | 1295 bcd | 3104 a  | 1322    |
| 2  | 294 f     | 927 cd  | 1367 bcd | 1596 abc | 2006 ab | 1238    |
| 3  | 393 ef    | 948 bcd | 1177 bcd | 1540 abc | 1433 bc | 1098    |
| Average  | 302       | 886     | 1251     | 1477     | 2181    | 1219    |
| SEM <sup>†</sup>                                   | 0.12      | 0.12    | 0.12     | 0.12     | 0.15    |         |
| Mass per plant (g DM plant <sup>-1</sup> )         |           |         |          |          |         |         |
| Sampling period                                    | Treatment |         |          |          |         | Average |
|  | G10       | G20     | G30      | G40      | UG      |         |
| 1  | 0.37      | 0.21    | 0.13     | 0.12     | 0.05    | 0.18 b  |
| 2  | 0.13      | 0.17    | 0.11     | 0.13     | 0.10    | 0.13 b  |
| 3  | 0.45      | 0.28    | 0.22     | 0.22     | 0.24    | 0.28 a  |
| Average  | 0.32 a    | 0.22 ab | 0.15 ab  | 0.16 ab  | 0.13 b  | 0.20    |
| SEM  | 0.25      | 0.25    | 0.25     | 0.25     | 0.30    |         |

Different letters represent significant differences among treatments according to the Tukey test ( $\alpha = 0.05$ ).

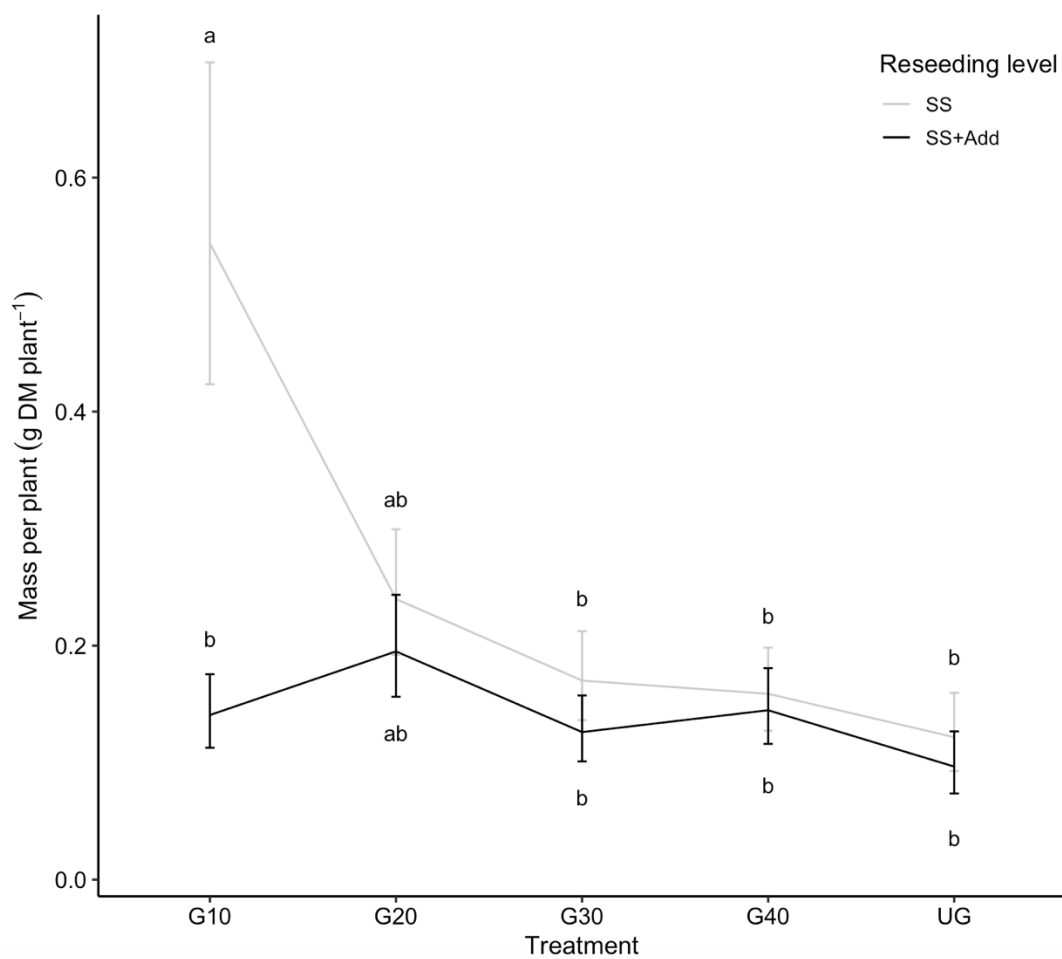
<sup>†</sup>Standard error of the mean from pairwise comparisons of log transformed data.



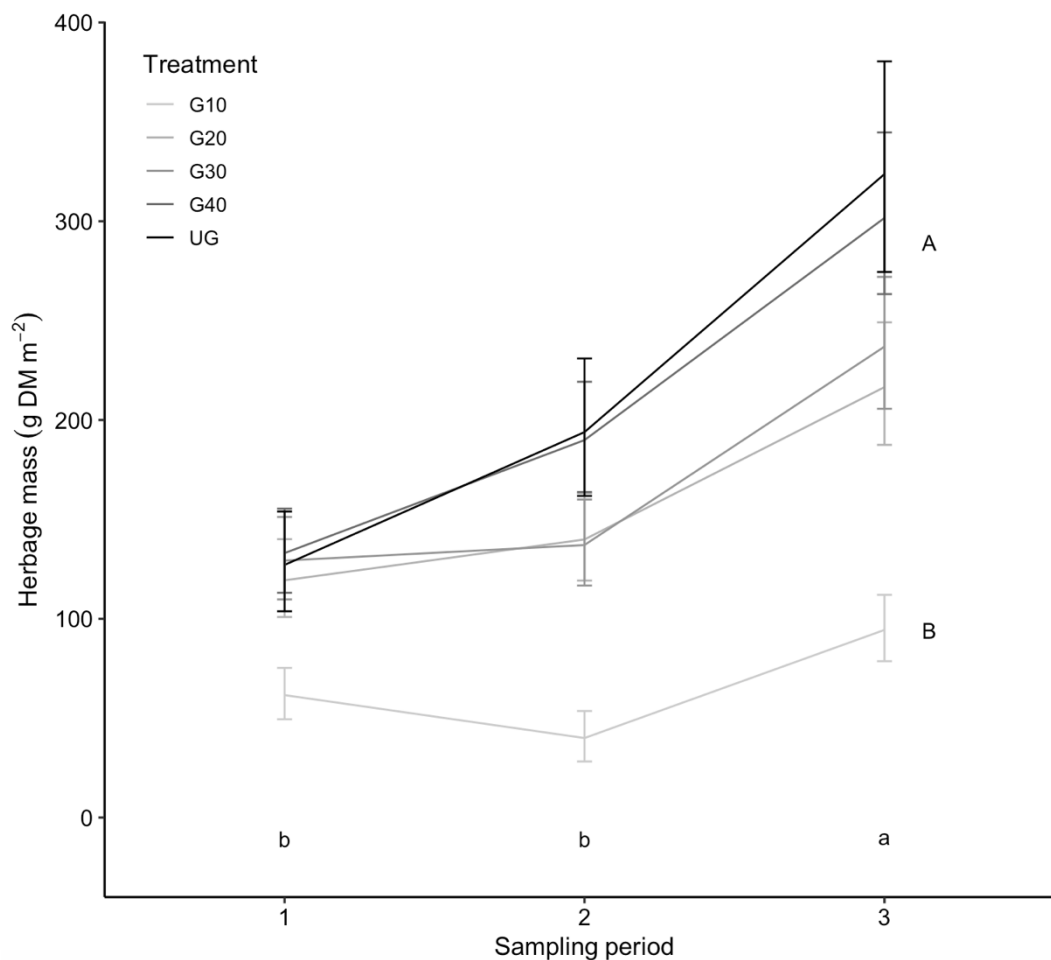
**Figure 1.** Monthly average environmental conditions at the station nearest to the experimental site (National Institute of Meteorology, Cruz Alta, 78 km from the study site) for the period of June 2016 to July 2018. Black continuous line shows the monthly average air temperature ( $^{\circ}\text{C}$ ). Grey bars show the monthly average rainfall (mm). Dashed, black lines show minimum (bottom) and maximum (top) air temperatures.



**Figure 2.** Plant population density (plants m<sup>-2</sup>) of Italian ryegrass (*Lolium multiflorum* Lam.) established by self-seeding only (SS) or self-seeding with addition of seeds (SS+Add) following different grazing intensities [10 cm sward height, or intense grazing (G10); 20 cm sward height, or moderate grazing (G20); 30 cm sward height, or moderate-light grazing (G30); 40 cm sward height, or light grazing (G40); and ungrazed cover crops (UG)] in the winter stocking period of an integrated beef-soybean system in São Miguel das Missões, RS, Brazil.

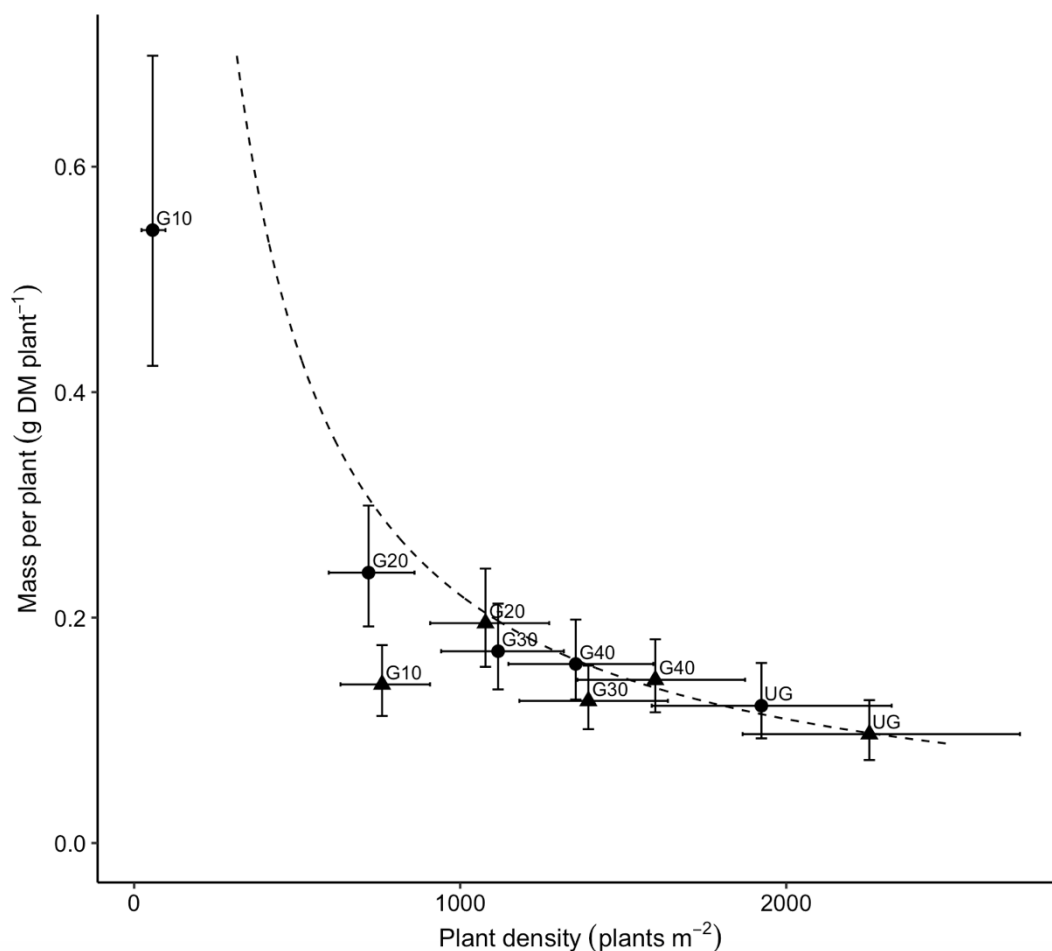


**Figure 3.** Mass of individual plants (g DM plant<sup>-1</sup>) of Italian ryegrass (*Lolium multiflorum* Lam.) established by self-seeding only (SS) or self-seeding with addition of seeds (SS+Add) following different grazing intensities [10 cm sward height, or intense grazing (G10); 20 cm sward height, or moderate grazing (G20); 30 cm sward height, or moderate-light grazing (G30); 40 cm sward height, or light grazing (G40); and ungrazed cover crops (UG)] in the winter stocking period of an integrated beef-soybean system in São Miguel das Missões, RS, Brazil.



**Figure 4.** Herbage mass (g DM m<sup>-2</sup>) of Italian ryegrass (*Lolium multiflorum* Lam.) over time during pasture establishment (prior to the beginning of stocking period) following different grazing intensities [10 cm sward height, or intense grazing (G10); 20 cm sward height, or moderate grazing (G20); 30 cm sward height, or moderate-light grazing (G30); 40 cm sward height, or light grazing (G40); and ungrazed cover crops (UG)] in the previous winter stocking period of an integrated beef-soybean system in São Miguel das Missões, RS, Brazil. Data are averages of the reseeding levels. Periods that do not share any common lower-case letter and grazing treatments that do not share any common upper-case letter are significantly different from each other. The upper case “A” is associated with each grazing treatment except G10.





**Figure 5.** Relationship between individual plant mass (g DM plant<sup>-1</sup>) and plant population density (plants m<sup>-2</sup>) of Italian ryegrass (*Lolium multiflorum* Lam.) across treatments averaged over periods and years. Circles represent treatments without addition of seed (self-seeding only) and triangles represent treatments with addition of ryegrass seed. Vertical and horizontal lines are standard errors of the means. The dashed line is an "iso-yield" curve where the total herbage mass is constant at 220 g m<sup>-2</sup>. Labels next to each point are grazing treatments [10 cm sward height, or intense grazing (G10); 20 cm sward height, or moderate grazing (G20); 30 cm sward height, or moderate-light grazing (G30); 40 cm sward height, or light grazing (G40); and ungrazed cover crops (UG)].

#### **4. CAPÍTULO IV**

#### 4.1 CONSIDERAÇÕES FINAIS

No Capítulo II, foi apresentado aquele que possivelmente seja o primeiro estudo oriundo de um banco de dados de um experimento de longa duração sobre a estabilidade produtiva de indicadores de produção vegetal (produtividade de soja e produção total de pasto), animal (ganho de peso vivo por área) e de sistema (produção de proteínas digestíveis pelo ser humano e lucratividade) quando animais em pastejo são integrados a sistemas puramente agrícolas (compondo um sistema integrado de produção agropecuária, SIPA) sob diferentes intensidades de pastejo (ou ausência do mesmo) e condições ambientais ao longo do tempo.

Verificou-se que intensidades de pastejo moderadas favorecem a estabilidade dos atributos de sistema e da produtividade de grãos de soja frente às oscilações verificadas no período estudado (2001-2016). Corroborando estudos anteriores, o pastejo não prejudica a produção de soja subsequente em SIPA, mas reduz a probabilidade de fracasso em anos desfavoráveis. Alturas de pasto mais elevadas (pastejo leve, a 40 cm de altura, ou ausência de pastejo) favorecem a estabilidade da produção de pasto e a cobertura do solo, mas comprometem significativamente a lucratividade do sistema, principalmente pela limitação da produtividade animal. Em contrapartida, apesar de ser também estável em termos de produção de pasto comparativamente aos pastejos moderados, o pastejo intenso (pastos manejados a 10 cm de altura) mostrou-se mais vulnerável às oscilações climáticas no que diz respeito à produção animal, além de constituir um risco à sustentabilidade do sistema pela baixa cobertura vegetal fruto do superpastejo.

Este estudo salientou a importância de protocolos experimentais e bases de dados de longa duração para entender respostas sistêmicas ao longo do tempo, o que é fundamental para responder questões relacionadas, por exemplo, ao funcionamento

de sistemas de produção em um cenário de mudanças climáticas. Metodologias de análise como a utilizada aqui podem ser extrapoladas para outros sistemas de produção e/ou protocolos experimentais de longa duração, a fim de investigar o comportamento destes sistemas frente a diferentes condições ambientais quando submetidos a diferentes manejos, buscando encontrar soluções sustentáveis para a produção de alimentos em um cenário de crescente demanda de alimentos e incertezas climáticas.

No Capítulo III, discutiu-se que apesar de sua reconhecida importância como planta forrageira no sul do Brasil, pouco se sabia sobre a efetividade da ressemeadura natural do azevém anual (*Lolium multiflorum* Lam.) quando submetido a intensidades de pastejo contrastantes. Neste manuscrito, verificamos que o pastejo intenso (pastos manejados a 10 cm de altura durante o inverno) não é uma alternativa sustentável quando o objetivo é o estabelecimento da pastagem, no ano seguinte, por ressemeadura natural.

Foram verificadas plantas mais pesadas, mas menor densidade populacional durante o estabelecimento, no ano seguinte, como resultado deste manejo. A realização de sementeira suplementar neste tratamento aumentou a densidade populacional a valores comparáveis à intensidade de pastejo moderada, mas reduziu a massa individual de plantas, resultando em menor massa de forragem total até o final do período de estabelecimento do pasto e revelando esta ser uma alternativa insustentável ao comprometer um fundamento importante para a autossuficiência destes sistemas. Em contrapartida, a combinação de densidade e massa individual de plantas seguindo intensidades de pastejo moderadas a leves (pastos manejados a 20, 30 ou 40 cm de altura) é suficiente para manter a massa de forragem comparável ao controle sem pastejo somente por ressemeadura natural.

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## APÊNDICES

**Apêndice 1** – Material suplementar do manuscrito “*Livestock integration improves long-term stability of yields and profitability of soybean systems*” (Capítulo II).

Supplementary information for:

**Livestock integration improves long-term stability of yields and profitability of soybean systems**

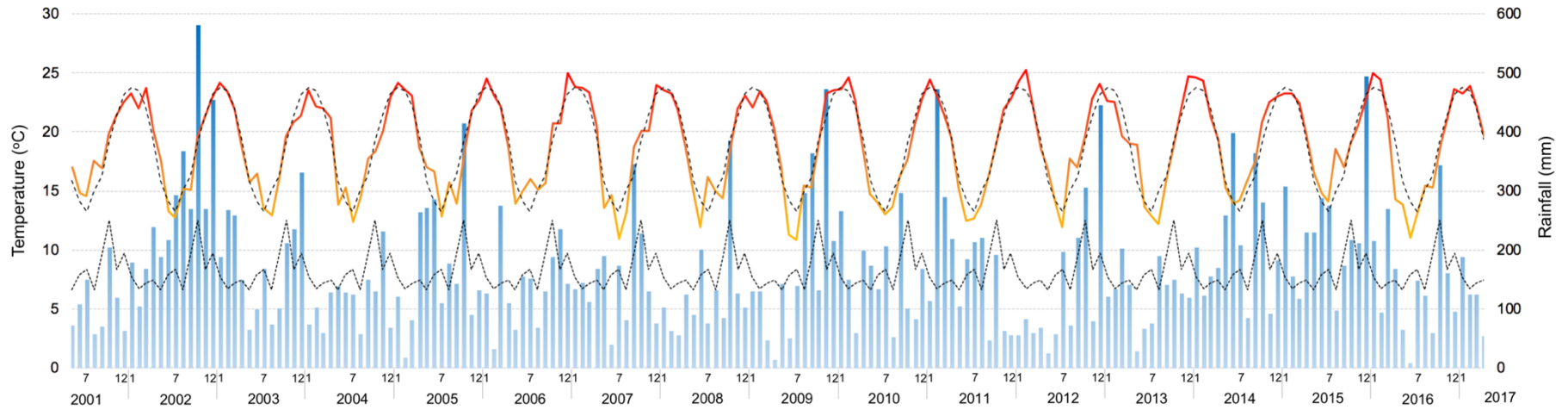
Pedro A. de A. Nunes<sup>1,\*</sup>, Emilio A. Laca<sup>2</sup>, Paulo C. de F. Carvalho<sup>1</sup>, Meng Li<sup>2</sup>, William de Souza Filho<sup>1</sup>, Taise R. Kunrath<sup>1</sup>, Amanda P. Martins<sup>3</sup> and Amélie C. M. Gaudin<sup>2</sup>

<sup>1</sup>Department of Forage Plants and Agrometeorology, Federal University of Rio Grande do Sul, Porto Alegre, RS 91540-000, Brazil

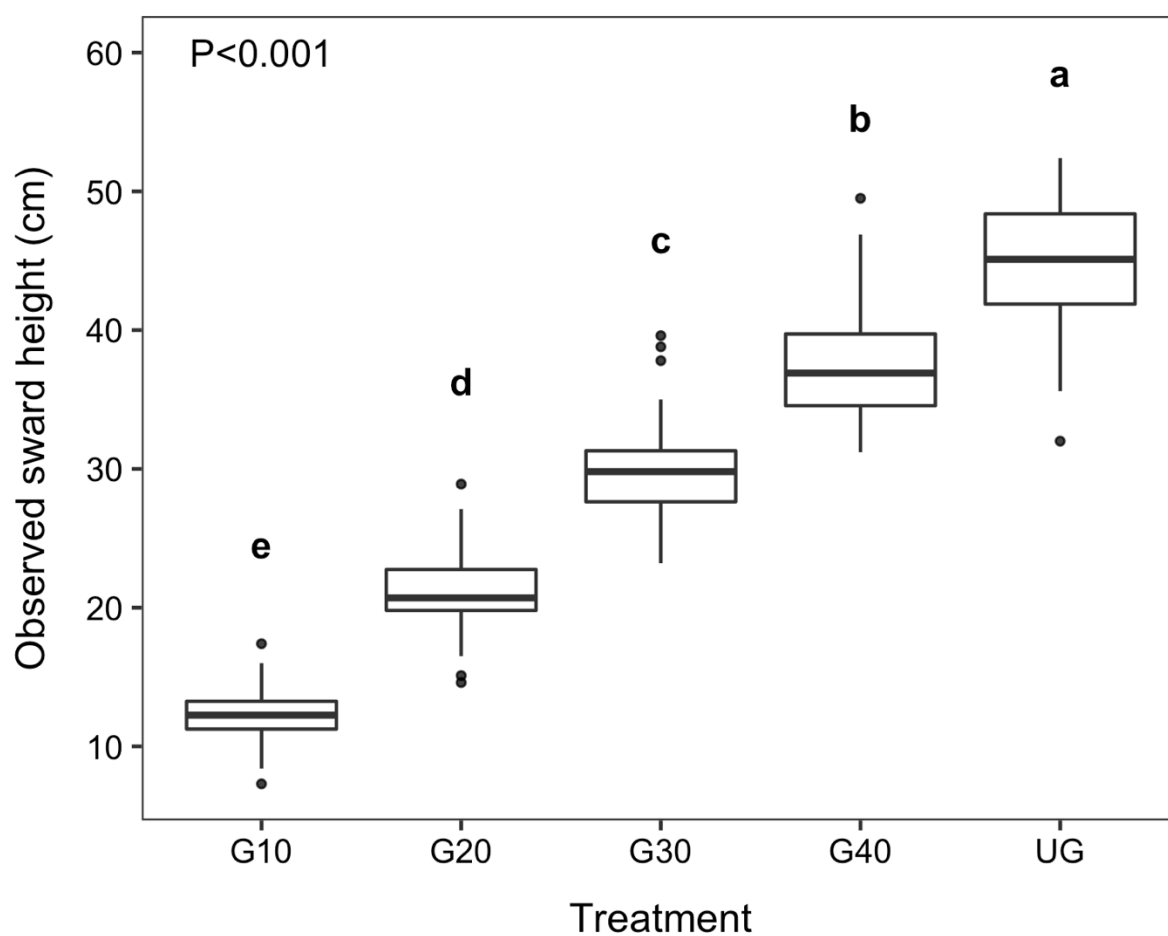
<sup>2</sup>Department of Plant Sciences, University of California - Davis, Davis, CA 95616, USA

<sup>3</sup>Department of Soil Science, Federal University of Rio Grande do Sul, Porto Alegre, RS 91540-000, Brazil

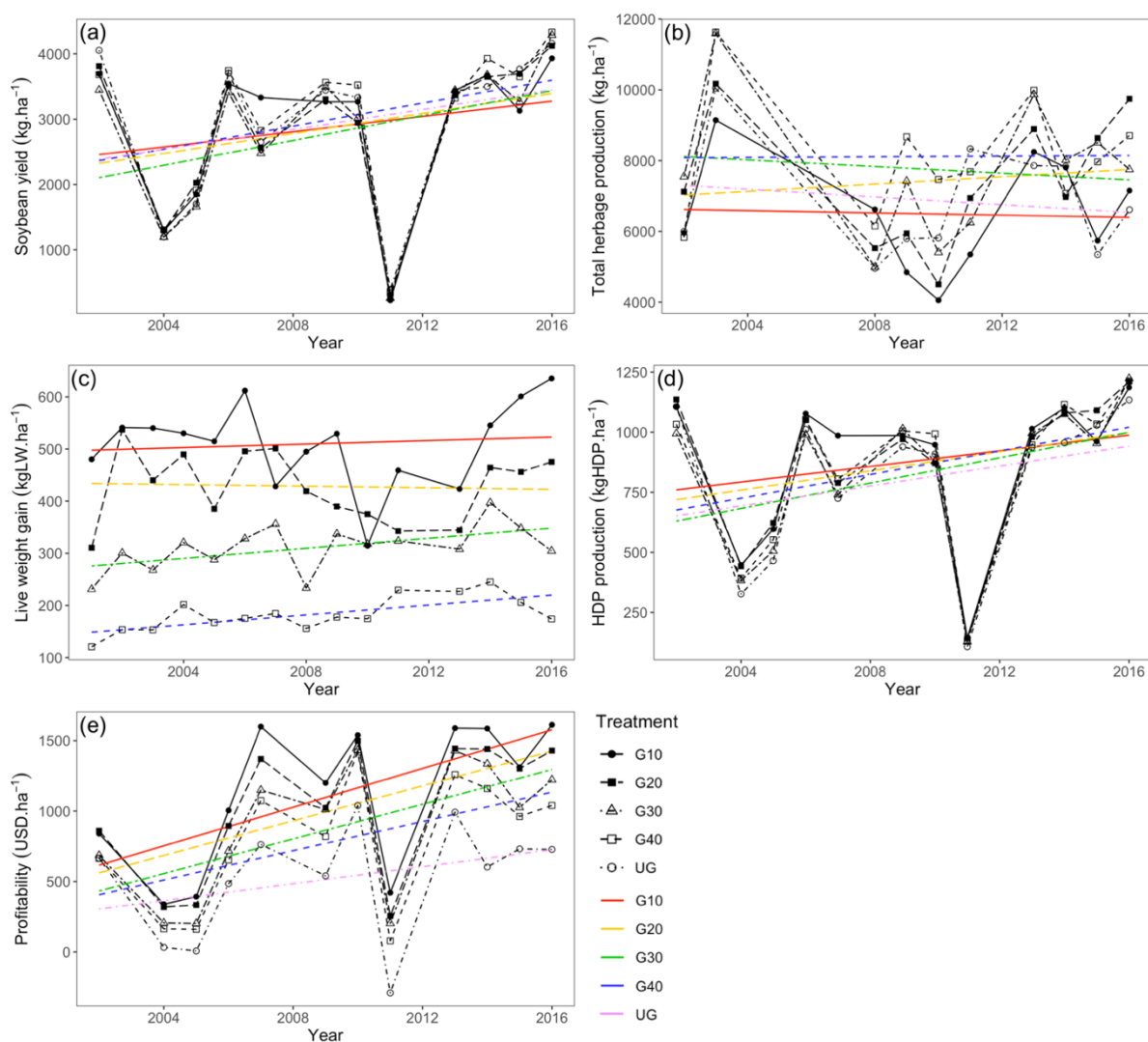
\*Corresponding author: [pedro\\_nuness@hotmail.com](mailto:pedro_nuness@hotmail.com)



Supplementary Figure S1. Monthly averaged environmental conditions at the experimental site (São Miguel das Missões, Rio Grande do Sul State, Brazil) for the period of May 2001 to April 2017. Orange continuous line shows air temperature (°C) and blue bars show rainfall (mm). Dashed, black lines show the historical monthly averages for air temperature (top) and rainfall (bottom) for the experimental period.



Supplementary Fig. S2. Mean sward heights (cm) for each treatment over 16 experimental years (2001-2016). G10: intense grazing (10 cm sward height); G20: moderate grazing (20 cm sward height); G30: moderate-light grazing (30 cm sward height); G40: light grazing (40 cm sward height); UG: ungrazed cover crop. Different letters indicate significant differences between means according to the Tukey test ( $\alpha = 0.05$ ).



Supplementary Fig. S3. Long-term yield trajectories of (a) soybean yield (kg grain ha<sup>-1</sup>), (b) total herbage production (kg dry matter ha<sup>-1</sup>), (c) animal live weight (LW) gain (kg LW ha<sup>-1</sup>), (d) human-digestible protein (HDP) production (kg HDP ha<sup>-1</sup>) and (e) profitability (USD ha<sup>-1</sup>) from 2001 to 2016. Colored lines are the trends over the 16 years for the different treatments: G10, intense grazing (10 cm sward height); G20, moderate grazing (20 cm sward height); G30, moderate-light grazing (30 cm sward height); G40, light grazing (40 cm sward height); UG: ungrazed cover crop.



Supplementary Table S1. Analysis of variance (ANOVA) of the model for mean soybean yield, total herbage production, animal live weight gain, human-digestible protein production and profitability.

| Variable                            | Source         | Sum of Squares | Mean Squares | Degrees of Freedom | F-value | p-value |
|-------------------------------------|----------------|----------------|--------------|--------------------|---------|---------|
| Soybean yield                       | Year           | 30933.800      | 2812.160     | 11                 | 313.895 | < 0.001 |
|                                     | Treatment      | 43.500         | 10.880       | 4                  | 1.215   | 0.375   |
|                                     | Year*Treatment | 329.200        | 7.480        | 44                 | 0.835   | 0.747   |
| Total herbage production            | Year           | 2.888e+08      | 3.209e+07    | 9                  | 21.870  | < 0.001 |
|                                     | Treatment      | 4.927e+07      | 1.232e+07    | 4                  | 8.396   | < 0.001 |
|                                     | Year*Treatment | 9.4223+07      | 2.617e+06    | 36                 | 1.784   | 0.015   |
| Live weight gain                    | Year           | 2.647e+05      | 18907.000    | 14                 | 8.007   | < 0.001 |
|                                     | Treatment      | 2.3953+06      | 7.983e+05    | 3                  | 338.099 | < 0.001 |
|                                     | Year*Treatment | 3.395e+05      | 8083.000     | 42                 | 3.423   | < 0.001 |
| Human-digestible protein production | Year           | 65.802         | 5.982        | 11                 | 448.304 | < 0.001 |
|                                     | Treatment      | 0.181          | 0.045        | 4                  | 3.386   | 0.065   |
|                                     | Year*Treatment | 0.514          | 0.012        | 44                 | 0.875   | 0.686   |
| Profitability                       | Year           | 9244.800       | 840.440      | 11                 | 263.352 | < 0.001 |
|                                     | Treatment      | 1695.700       | 423.920      | 4                  | 132.837 | < 0.001 |
|                                     | Year*Treatment | 532.400        | 12.100       | 44                 | 3.792   | < 0.001 |

Supplementary Table S2. Analysis of variance (ANOVA) of the model for yield trends of soybean yield, total herbage production, animal live weight gain, human-digestible protein production and profitability.

| Variable                            | Source         | Sum of Squares | Mean Squares | Degrees of Freedom | F-value | p-value |
|-------------------------------------|----------------|----------------|--------------|--------------------|---------|---------|
| Soybean yield                       | Year           | 1.383e+22      | 1.383e+22    | 1                  | 24.042  | < 0.001 |
|                                     | Treatment      | 1.250e+21      | 3.125e+20    | 4                  | 0.543   | 0.704   |
|                                     | Year*Treatment | 1.250e+21      | 3.125e+20    | 4                  | 0.543   | 0.704   |
| Total herbage production            | Year           | 4.281e+05      | 4.281e+05    | 1                  | 0.106   | 0.745   |
|                                     | Treatment      | 4.111e+06      | 1.028e+06    | 4                  | 0.254   | 0.907   |
|                                     | Year*Treatment | 4.125e+06      | 1.031e+06    | 4                  | 0.255   | 0.906   |
| Live weight gain                    | Year           | 26904.000      | 26903.900    | 1                  | 5.466   | 0.021   |
|                                     | Treatment      | 22017.000      | 7339.100     | 3                  | 1.491   | 0.219   |
|                                     | Year*Treatment | 21169.000      | 7056.300     | 3                  | 1.434   | 0.235   |
| Human-digestible protein production | Year           | 7.539e+18      | 7.539e+18    | 1                  | 24.090  | < 0.001 |
|                                     | Treatment      | 6.568e+17      | 1.642e+17    | 4                  | 0.525   | 0.718   |
|                                     | Year*Treatment | 6.574e+17      | 1.643e+17    | 4                  | 0.525   | 0.717   |
| Profitability                       | Year           | 3.199e+13      | 3.199e+13    | 1                  | 67.929  | < 0.001 |
|                                     | Treatment      | 4.558e+12      | 1.140e+12    | 4                  | 2.420   | 0.046   |
|                                     | Year*Treatment | 4.601e+12      | 1.150e+12    | 4                  | 2.443   | 0.044   |

**Apêndice 2** – Código suplementar (.R) do manuscrito “*Livestock integration improves long-term stability of yields and profitability of soybean systems*” (Capítulo II).

```
##### Title: Script yield distribution (probability of extreme yield events)
##### Author: Emilio A. Laca (UC Davis) and Pedro A. de A. Nunes (UFRGS)
##### Adapted from: Timothy Bowles (UC Berkeley) and Amelie Gaudin (UC Davis)
##### Last updated: October 01, 2019
```

**# Description of method**

This is a distribution-free, simulation-based method to test the hypothesis that there are no treatment or system effects on the probability of extreme yields. The method is based on generating a null distribution of the statistic of interest for each treatment while the null hypothesis is known to be true, because treatments are ignored in the creation of the distribution by resampling. Then, the observed values of the statistic are compared to the null distribution. If the observed values are more extreme than the 10 or 90 % (or other levels) of the distribution, they are considered "significantly" different and the null is rejected. Although the code also calculates the null distribution of proportion of observations below the median, the explanation is focused on the distribution of proportions below the 10th and above the 90th percentiles.

The statistic of interest is itself a probability or proportion of yield values that fall below a critical level considered "extreme." It is best to think of it as a proportion to avoid confusion with the probability of the statistic.

1. Yields were detrended and converted to deviations.
2. Threshold deviations (data values) were defined as the observed 10, 50 and 90% quantiles of the pooled set of deviations (all data pooled) ignoring treatments. (threshB1, threshB2, threshB3)
3. Kernel densities of deviations were estimated for each treatment and the proportions of values more extreme than the thresholds were calculated for each treatment. These are the OBSERVED values of the statistics of interest. (results)
4. Generate null distribution of statistics of interest ignoring treatments:
  - a. Generate R (5000) random sets of deviations data by resampling with replacement from the data ignoring treatments and within blocks. (ryields)
  - b. Estimate a kernel density for each random data set. (dens.rnd)

c. Calculate the statistics of interest (proportions of density beyond thresholds) for each density.

5. For each treatment, compute the proportion of the null distribution that is more extreme than the observed values of the statistic of interest.

```
# R packages
```

```
library(tidyverse)
```

```
library(sandwich)
```

```
library(reshape2)
```

```
library(car)
```

```
##### Read detrended yield data #####
```

```
# These data were provided by the code that Pedro used.
```

```
# Yields are corrected for treat*year but not for block
```

```
d.soy <- read.csv("yield.unconditional.csv") %>%
```

```
select(year = Year, treat = Treat, block = Block, yield = detrend2.Yield) %>%
```

```
filter(!is.na(yield))
```

```
### detrended Block Corrected Yield #####
```

```
# The original data are read and I calculate the detrended, block-corrected data.
```

```
soy.eal <- read.csv("PlanilhaTupaSoy.csv")
```

```
soy.eal <- soy.eal %>%
```

```
  mutate(block = factor(Block),
```

```
    treat = Treat,
```

```
    year = Year,
```

```
    yield = Yield) %>%
```

```
filter(year != 2001 & !is.na(yield)) %>%
```

```
select(year, block, treat, yield)
```

```
str(soy.eal)
```

```
sm1 <- lm(yield ~ treat*year + block, soy.eal)
```

```
sm2 <- lm(yield ~ treat*year, soy.eal)
```

```
anova(sm1)
```

```
anova(sm2)
```

```
summary(sm1)
```

```

soy.eal <- soy.eal %>%
  mutate(dYield1 = residuals(sm1) + mean(yield), # Block-corrected
         dYield2 = residuals(sm2) + mean(yield)) # Block uncorrected

pairs(soy.eal[, 4:6], main = "Determine if data in d.soy were block corrected")
write.csv(soy.eal, "soy.eal.csv")

# Here I simulated random detrended data without any treatment or block effects.
# These data can be used to check the method when original data have no effects.
set.seed(33)
soy.rnd <- expand.grid(year = c(2002, 2004, 2005, 2006, 2007, 2009,
                             2010, 2011, 2012, 2013, 2014, 2015, 2016),
                    block = c(1, 2, 3),
                    treat = c("10", "20", "30", "40", "sp")) %>%
  mutate(yield = rnorm(length(year), mean = 3142, sd = 1082))

#####
# Modify this section by commenting/uncommenting to pick the data set desired.
# Data for this analysis

ddd <- d.soy      # OPTION 1: use treat*year detrended data

# ddd <- soy.eal %>%      # OPTION 2: use treat*year + block detrended data
# as.tbl() %>%
# select(year, block, treat, yield = dYield1) %>%
# filter(!is.na(yield))
#
# ddd <- soy.rnd      # OPTION 3: use random simulated data

# Leave this as it is to get the right number of rows later.
ddd <- as.data.frame(arrange(ddd, block, treat, year))

ddd.wide <- spread(ddd, treat, yield) %>%
  select(year, block, T10 = '10', T20 = '20', T30 = '30', T40 = '40', sp)

# R original densities
summary(ddd$yield)

```

```

n = 25001
width <- 0.25
from = 0
to = from + (n - 1) * width

densO <- by(ddd, ddd$treat,
  function(x) {
    density(x$yield,
      bw = "SJ",
      kernel = "gaussian",
      n = n,
      from = from,
      to = to)})

plot(densO[[1]], main = "")
for (i in 2:5) lines(densO[[i]], lwd = 1.5 * i)

lines(density(ddd$yield,
  bw = "SJ",
  kernel = "gaussian",
  n = n,
  from = from,
  to = to),
  col = "red",
  lwd = 2)

# Get 0.10, 0.50 and 0.90 quantiles from ALL yield data rounded to integer
# Note that treatments are ignored
# Quantiles of pooled data, ignoring treatments
threshB1 <- round(quantile(ddd$yield, 0.1))
threshB2 <- round(quantile(ddd$yield, 0.5))
threshB3 <- round(quantile(ddd$yield, 0.9))

abline(v = c(threshB1, threshB2, threshB3),
  col = "blue",
  lwd = 2)

```

```
# Calculate the probabilities of the (pooled) empirical quantiles for each treatment
# using the "observed" kernel densities.
```

```
results <- data.frame(
  p.10 = round(
    sapply(
      densO,
      function(d) {
        sum(d$y[which.min(d$x):which(d$x == threshB1)]*width)
      }) * 100, 1),
  p.med = round(
    sapply(
      densO,
      function(d) {
        sum(d$y[which.min(d$x):which(d$x == threshB2)]*width)
      })*100, 1),
  p.90 = round(
    sapply(
      densO,
      function(d) {
        sum(d$y[which(d$x == threshB3):which.max(d$x)]*width)
      })*100, 1))
```

```
nt <- nlevels(ddd$treat)
```

```
##### Yield randomization #####
```

```
# For our data we need to sample a random treatment for each year and block.
# We also need to accommodate missing treatments or plots in certain years.
# Each year and block combination has a set of yields for the treatments
# observed that year in that block. We take bootstrap samples from the set
# ignoring treatments to generate a null distribution for each statistic of interest.
```

```
nr <- 5000 # define the number of bootstrap samples to use
```

```
nestd <- as_tibble(ddd) %>% nest(treat, yield) # nested tibble to facilitate
# bootstrapping within year and block
```

```

# Create empty matrix to receive random data realizations in columns
ryields <- matrix(NA, nrow = nrow(ddd.wide), ncol = nr)

# Fill matrix with nr independent realizations.
# Sample WITHIN blocks with replacement.
# sp has missing values in some block-year combinations.
# In those cases, the sample of size nt is obtained from fewer than nt values.
for (j in seq(from = 1, to = nr, by = nt)) {
  ryields[, j:(j + (nt - 1))] <-
    map(nestd$data, ~ sample(.x$yield, size = nt, replace = TRUE)) %>%
    do.call(rbind, .)
}

# ryields is a matrix where each column is a randomized yield data set where
# yields were sampled with replacement from each block-year combination.

##### Estimate kernel density for randomized yields #####
# This generates a density function for each set of random yields where treatments
# are ignored.

dens.rnd <- apply(ryields, 2,
  function(x) density(x, bw = "SJ",
    kernel = "gaussian",
    n = n,
    from = from,
    to = to))

# Check a few densities by plotting
plot(dens.rnd[[1]])
for (i in 2:16) lines(dens.rnd[[i]], col = "green")

##### Randomized proportions #####

# For each random density, this generates a null pdf of the percentage of yields
# expected to be below (above) each threshold. There is one percentage for each
# threshold (column) and random density (row).

```



```

results.rnd <- data.frame(
  p.10 = round(sapply(dens.rnd,
    function(d) sum(d$y[which.min(d$x):which(d$x == threshB1)]*width))*100, 1),
  p.med = round(sapply(dens.rnd,
    function(d) sum(d$y[which.min(d$x):which(d$x == threshB2)]*width))*100, 1),
  p.90 = round(sapply(dens.rnd,
    function(d) sum(d$y[which(d$x == threshB3):which.max(d$x)]*width))*100, 1)
)

```

```

# Finally, the OBSERVED percentage of yields below (above) the threshold are
# placed in the null distributions to determine the null probabilities of
# observing more extreme values.

```

```

# These are the null distributions of the statistics of interest with the
# observed values as vertical lines. Narrowest is for the first treatment
# and width increases with the default treatment order.

```

```

# Null distribution of the percentage of yields falling below threshold1
plot(density(results.rnd$p.10))
abline(v = results$p.10, lwd = 1:5*2)

```

```

# Compare to results of other method

```

```

# lines(density(100 * rnd_r[1, ]), col = "red")

```

```

# Null distribution of the percentage of yields falling below threshold2
plot(density(results.rnd$p.med))
abline(v = results$p.med, lwd = 1:5*2)
# lines(density(100 * rnd_r[2, ]), col = "red")

```

```

# Null distribution of the percentage of yields falling above threshold3
plot(density(results.rnd$p.90))
abline(v = results$p.90, lwd = 1:5*2)
# lines(density(100 * rnd_r[3, ]), col = "red")

```

```

p_val.rnd <- data.frame(p.10 = apply(results, 1, function(x) sum(results.rnd[,1] <
x[1])/ncol(ryields)),
                      p.50 = apply(results, 1, function(x) sum(results.rnd[,2] < x[2])/ncol(ryields)),
                      p.90 = apply(results, 1, function(x) sum(results.rnd[,3] > x[3])/ncol(ryields)))

# p.10 = null probability that the percentage of yields below threshold1 is smaller than
# observed.
# p.50 = null probability that the percentage of yields below threshold2 is smaller than
# observed.
# p.90 = null probability that the percentage of yields above threshold3 is greater than
# observed.

print(p_val.rnd)
print(results)

# Graphs with different colors for the random distribution and treatments
plot(dens.rnd[[1]], col="darkgray")
lapply(dens.rnd, lines, col="darkgray")
lines(densO[[1]], col="darkorange2", lwd=2)
lines(densO[[2]], col="goldenrod1", lwd=2)
lines(densO[[3]], col="forestgreen", lwd=2)
lines(densO[[4]], col="royalblue", lwd=2)
lines(densO[[5]], col="plum1", lwd=2)
abline(v=c(thresh1, thresh2, thresh3))

```

**Apêndice 3** – Material suplementar do manuscrito “*Intense winter grazing impairs Italian ryegrass cover crop reestablishment by self-seeding in a no-till soybean-beef cattle system*” (Capítulo III).

Supporting information for:

**Intense winter grazing impairs Italian ryegrass cover crop reestablishment by self-seeding in a no-till soybean-beef cattle system**

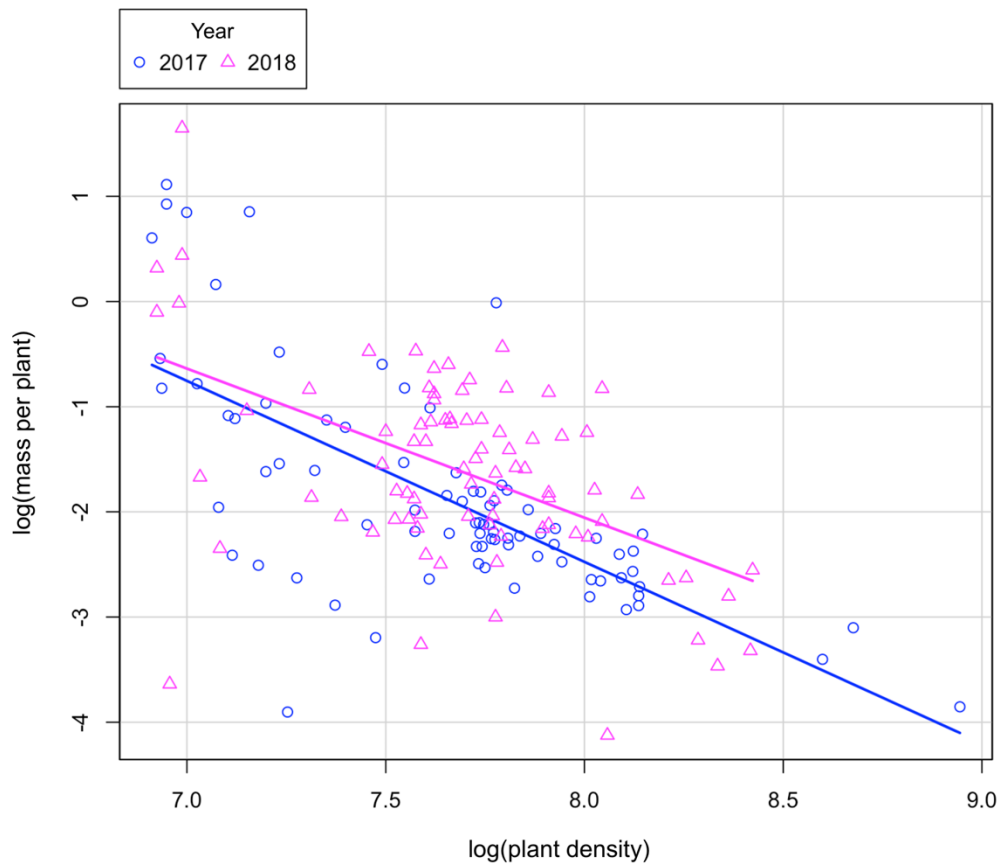
Pedro Arthur de Albuquerque Nunes<sup>1,\*</sup>, Emilio Andrés Laca<sup>2</sup>, Taise Robinson Kunrath<sup>1</sup>, William de Souza Filho<sup>1</sup>, Amanda Posselt Martins<sup>3</sup>, Paulo César de Faccio Carvalho<sup>1</sup>

<sup>1</sup>Department of Forage Plants and Agrometeorology, Federal University of Rio Grande do Sul, Porto Alegre, RS 91540-000, Brazil

<sup>2</sup>Department of Plant Sciences, University of California - Davis, Davis, CA 95616, USA

<sup>3</sup>Department of Soil Science, Federal University of Rio Grande do Sul, Porto Alegre, RS 91540-000, Brazil

\*Corresponding author: pedro\_nuness@hotmail.com (P. A. A. Nunes)



**Supplementary Figure 1.** Scatterplot of log-log relationship between individual plant mass (g DM plant<sup>-1</sup>) and plant population density (plants m<sup>-2</sup>) during the establishment phase of Italian ryegrass (*Lolium multiflorum* Lam.) in the experimental years of 2017 and 2018, regardless of grazing treatments or reseeding levels. Plant density followed the self-thinning rule when data were log transformed and plotted as a log-log chart, with a slope close to  $-3/2$ .

## **Apêndice 4 – Normas para elaboração e submissão de trabalhos científicos ao periódico *Scientific Reports*.**

### **Format of articles**

*Scientific Reports* publishes original research in one format, Article. In most cases we do not impose strict limits on word count or page number. We do, however, strongly encourage authors to write concisely and to adhere to the guidelines below.

Articles should ideally be no more than 11 typeset pages in length. As a guide, the main text (not including Abstract, Methods, References and figure legends) should be no more than 4,500 words. The maximum Article title length is 20 words. The Abstract — which must be no more than 200 words long and contain no references — should serve both as a general introduction to the topic and as a brief, non-technical summary of the main results and their implications.

For the main body of the text, there are no explicit requirements for section organization. According to the authors' preference, the text may be organized as best suits the research. As a guideline and in the majority of cases, however, we recommend that you structure your manuscript as follows:

Introduction

Results (with subheadings)

Discussion (without subheadings)

Methods

A specific order for the main body of the text is not compulsory and, in some cases, it may be appropriate to combine sections. Figure legends are limited to 350 words. As a guideline references should be limited to 60 (this is not strictly enforced). Footnotes should not be used.

We suggest that Articles contain no more than 8 display items ([figures](#) and/or [tables](#)). In addition, a limited number of uncaptioned molecular structure graphics and numbered mathematical equations may be included if necessary. To enable typesetting of papers, the number of display items should be commensurate with the word length — we suggest that for Articles with less than 2,000 words, no more than 4 figures/tables should be included. Please note that schemes are not used and should be presented as figures.

Authors must provide a competing interests statement within the manuscript file.

Submissions should include a cover letter, a manuscript text file, individual figure files and optional supplementary information files. For first submissions (i.e. not revised manuscripts), authors may incorporate the manuscript text and figures into a single file up to

3 MB in size; the figures may be inserted in the text at the appropriate positions, or grouped at the end. Supplementary information should be combined and supplied as a single separate file, preferably in PDF format.

The following file types can be uploaded for Article text:

txt, doc, docx, tex, (pdf [first submissions only])\*

\*We are unable to accept PDF files for article text for revised manuscripts.

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Abbreviations, particularly those that are not standard, should also be kept to a minimum. Where unavoidable, abbreviations should be defined in the text or legends at their first occurrence, and abbreviations should be used thereafter. The background, rationale and main conclusions of the study should be clearly explained. Titles and abstracts in particular should be written in language that will be readily intelligible to any scientist. We strongly recommend that authors ask a colleague with different expertise to review the manuscript before submission, in order to identify concepts and terminology that may present difficulties to non-specialist readers.

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In most cases we do not impose strict limits on word counts and page numbers, but we encourage authors to write concisely and suggest authors adhere to the guidelines below. For a definitive list of which limits are mandatory please visit the [submission checklist page](#).

Articles should be no more than 11 typeset pages in length. As a guide, the main text (not including Abstract, Methods, References and figure legends) should be no more than 4,500 words. The maximum title length is 20 words. The Abstract (without heading) - which must be no more than 200 words long and contain no references - should serve both as a general introduction to the topic and as a brief, non-technical summary of the main results and their implications.

The manuscript text file should include the following parts, in order: a title page with author affiliations and contact information (the corresponding author should be identified with an asterisk). The main text of an Article can be organised in different ways and according to the authors' preferences, it may be appropriate to combine sections.

As a guideline, we recommend that sections include an Introduction of referenced text that expands on the background of the work. Some overlap with the Abstract is acceptable. This may then be followed by sections headed Results (with subheadings), Discussion (without subheadings) and Methods.

The main body of text must be followed by References, Acknowledgements (optional), Author Contributions (names must be given as initials), Additional Information (including a Competing Interests Statement), Figure Legends (these are limited to 350 words per figure) and Tables (maximum size of one page). Footnotes are not used.

**For first submissions** (i.e. not revised manuscripts), authors may choose to incorporate the manuscript text and figures into a single file up to 3 MB in size in either a Microsoft Word, LaTeX, or PDF format - the figures may be inserted within the text at the appropriate positions, or grouped at the end.

**For revised manuscripts** authors should provide all textual content in a single file, prepared using either Microsoft Word or LaTeX. We do not accept PDF files for article text for revised manuscripts. Figures should be provided as individual files.

Supplementary Information should be combined and supplied as a separate file, preferably in PDF format. The first page of the Supplementary Information file should include the title of the manuscript and the author list.

Authors who do not incorporate the manuscript text and figures into a single file should adhere to the following: all textual content should be provided in a single file, prepared using either Microsoft Word or LaTeX; figures should be provided as individual files. The manuscript file should be formatted as single-column text without justification. Pages should be numbered using an Arabic numeral in the footer of each page. Standard fonts are recommended and the 'symbols' font should be used for representing Greek characters.

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or human samples must include a statement of ethical approval in the Methods section (see [our detailed requirements](#) for further information on preparing these statements).

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References should be numerical within square brackets and numbered sequentially, first throughout the text, then in tables, followed by figures; that is, references that only appear in tables or figures should be last in the reference list. Only one publication is given for each number. Only papers or datasets that have been published or accepted by a named publication, recognized preprint server or data repository should be in the numbered list; preprints of accepted papers in the reference list should be submitted with the manuscript. Published conference abstracts and numbered patents may be included in the reference list. Grant details and acknowledgements are not permitted as numbered references. Footnotes are not used.

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Published papers:

Printed journals

Schott, D. H., Collins, R. N. & Bretscher, A. Secretory vesicle transport velocity in living cells depends on the myosin V lever arm length. *J. Cell Biol.* **156**, 35-39 (2002).

Online only

Bellin, D. L. *et al.* Electrochemical camera chip for simultaneous imaging of multiple metabolites in biofilms. *Nat. Commun.* **7**, 10535; [10.1038/ncomms10535](https://doi.org/10.1038/ncomms10535) (2016).

For papers with more than five authors include only the first author's name followed by 'et al.'.

#### Books:

Smith, J. Syntax of referencing in *How to reference books* (ed. Smith, S.) 180-181 (Macmillan, 2013).

#### Online material:

Babichev, S. A., Ries, J. & Lvovsky, A. I. Quantum scissors: teleportation of single-mode optical states by means of a nonlocal single photon. Preprint at <https://arxiv.org/abs/quant-ph/0208066> (2002).

Manaster, J. Sloth squeak. *Scientific American Blog Network*

<http://blogs.scientificamerican.com/psi-vid/2014/04/09/sloth-squeak> (2014).

Hao, Z., AghaKouchak, A., Nakhjiri, N. & Farahmand, A. Global integrated drought monitoring and prediction system (GIDMaPS) data sets. *Figshare* <https://doi.org/10.6084/m9.figshare.853801> (2014).

### Acknowledgements

Acknowledgements should be brief, and should not include thanks to anonymous referees and editors, or effusive comments. Grant or contribution numbers may be acknowledged. Assistance from medical writers, proof-readers and editors should also be acknowledged here.

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The author(s) declare no competing interests.

#### Competing interests

Dr X's work has been funded by A. He has received compensation as a member of the

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Refer to each piece of supplementary material at the appropriate point(s) in the main article. Be sure to include the word "Supplementary" each time one is mentioned. Please do not refer to individual panels of supplementary figures.

Use the following examples as a guide (note: abbreviate "Figure" as "Fig." when in the middle of a sentence): "Table 1 provides a selected subset of the most active compounds. The entire list of 96 compounds can be found as Supplementary Table S1 online." "The

biosynthetic pathway of L-ascorbic acid in animals involves intermediates of the D-glucuronic acid pathway (see Supplementary Fig. S2 online). Figure 2 shows..."

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File sizes should be as small as possible, with a maximum size of 50 MB, so that they can be downloaded quickly.

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Figure legends begin with a brief title sentence for the whole figure and continue with a short description of what is shown in each panel in sequence and the symbols used; methodological details should be minimised as much as possible. Each legend must total no more than 350 words. Text for figure legends should be provided in numerical order after the references.

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Figures divided into parts should be labelled with a lower-case bold a, b, and so on, in the same type size as used elsewhere in the figure. Lettering in figures should be in lower-case type, with only the first letter of each label capitalized. Units should have a single space between the number and the unit, and follow SI nomenclature (for example, ms rather than msec) or the nomenclature common to a particular field. Thousands should be separated by commas (1,000). Unusual units or abbreviations should be spelled out in full or defined in the legend. Scale bars should be used rather than magnification factors, with the length of the bar defined on the bar itself rather than in the legend. In legends, please use visual cues rather than verbal explanations such as "open red triangles".

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Stereo diagrams should be presented for divergent 'wall-eyed' viewing, with the two panels separated by 5.5 cm. In the final accepted version of the manuscript, the stereo images should be submitted at their final page size.

## **Statistical guidelines**

Every article that contains statistical testing should state the name of the statistical test, the *n* value for each statistical analysis, the comparisons of interest, a justification for the use of that test (including, for example, a discussion of the normality of the data when the test is

appropriate only for normal data), the alpha level for all tests, whether the tests were one-tailed or two-tailed, and the actual P value for each test (not merely "significant" or " $P < 0.05$ "). It should be clear what statistical test was used to generate every P value. Use of the word "significant" should always be accompanied by a P value; otherwise, use "substantial," "considerable," etc.

Data sets should be summarized with descriptive statistics, which should include the n value for each data set, a clearly labelled measure of centre (such as the mean or the median), and a clearly labelled measure of variability (such as standard deviation or range). Ranges are more appropriate than standard deviations or standard errors for small data sets. Graphs should include clearly labelled error bars. Authors must state whether a number that follows the  $\pm$  sign is a standard error (s.e.m.) or a standard deviation (s.d.).

Authors must justify the use of a particular test and explain whether their data conform to the assumptions of the tests. Three errors are particularly common:

**Multiple comparisons:** When making multiple statistical comparisons on a single data set, authors should explain how they adjusted the alpha level to avoid an inflated Type I error rate, or they should select statistical tests appropriate for multiple groups (such as ANOVA rather than a series of t-tests).

**Normal distribution:** Many statistical tests require that the data be approximately normally distributed; when using these tests, authors should explain how they tested their data for normality. If the data do not meet the assumptions of the test, then a non-parametric alternative should be used instead.

**Small sample size:** When the sample size is small (less than about 10), authors should use tests appropriate to small samples or justify their use of large-sample tests.

### **Chemical and biological nomenclature and abbreviations**

Molecular structures are identified by bold, Arabic numerals assigned in order of presentation in the text. Once identified in the main text or a figure, compounds may be referred to by their name, by a defined abbreviation, or by the bold Arabic numeral (as long as the compound is referred to consistently as one of these three).

When possible, authors should refer to chemical compounds and biomolecules using systematic nomenclature, preferably using [IUPAC](#). Standard chemical and biological abbreviations should be used. Unconventional or specialist abbreviations should be defined at their first occurrence in the text.

### **Gene nomenclature**

Authors should use approved nomenclature for gene symbols, and use symbols rather than italicized full names (for example Ttn, not titin). Please consult the appropriate nomenclature databases for correct gene names and symbols. A useful resource is LocusLink.

Approved human gene symbols are provided by HUGO Gene Nomenclature Committee (HGNC), e-mail: [hgnc@genenames.org](mailto:hgnc@genenames.org); see also [www.genenames.org](http://www.genenames.org). Approved mouse symbols are provided by The Jackson Laboratory, e-mail: [nomen@informatics.jax.org](mailto:nomen@informatics.jax.org); see also [www.informatics.jax.org/mgihome/nomen](http://www.informatics.jax.org/mgihome/nomen).

For proposed gene names that are not already approved, please submit the gene symbols to the appropriate nomenclature committees as soon as possible, as these must be deposited and approved before publication of an article.

Avoid listing multiple names of genes (or proteins) separated by a slash, as in 'Oct4/Pou5f1', as this is ambiguous (it could mean a ratio, a complex, alternative names or different subunits). Use one name throughout and include the other at first mention: 'Oct4 (also known as Pou5f1)'.

## **Characterization of chemical and biomolecular materials**

*Scientific Reports* is committed to publishing technically sound research. Manuscripts submitted to the journal will be held to rigorous standards with respect to experimental methods and characterization of new compounds. Authors must provide adequate data to support their assignment of identity and purity for each new compound described in the manuscript. Authors should provide a statement confirming the source, identity and purity of known compounds that are central to the scientific study, even if they are purchased or resynthesized using published methods.

### **1. Chemical identity**

Chemical identity for organic and organometallic compounds should be established through spectroscopic analysis. Standard peak listings (see formatting guidelines below) for <sup>1</sup>H NMR and proton-decoupled <sup>13</sup>C NMR should be provided for all new compounds. Other NMR data should be reported (<sup>31</sup>P NMR, <sup>19</sup>F NMR, etc.) when appropriate. For new materials, authors should also provide mass spectral data to support molecular weight identity. High-resolution mass spectral (HRMS) data are preferred. UV or IR spectral data may be reported for the identification of characteristic functional groups, when appropriate. Melting-point ranges should be provided for crystalline materials. Specific rotations may be reported for chiral compounds. Authors should provide references, rather than detailed procedures, for known compounds, unless their protocols represent a departure from or improvement on published methods.

### **2. Combinational compound libraries**

Authors describing the preparation of combinatorial libraries should include standard characterization data for a diverse panel of library components.

### **3. Biomolecular identity**

For new biopolymeric materials (oligosaccharides, peptides, nucleic acids, etc.), direct structural analysis by NMR spectroscopic methods may not be possible. In these cases,



authors must provide evidence of identity based on sequence (when appropriate) and mass spectral characterization.

#### 4. Biological constructs

Authors should provide sequencing or functional data that validates the identity of their biological constructs (plasmids, fusion proteins, site-directed mutants, etc.) either in the manuscript text or the Methods section, as appropriate.

#### 5. Sample purity

Evidence of sample purity is requested for each new compound. Methods for purity analysis depend on the compound class. For most organic and organometallic compounds, purity may be demonstrated by high-field  $^1\text{H}$  NMR or  $^{13}\text{C}$  NMR data, although elemental analysis ( $\pm 0.4\%$ ) is encouraged for small molecules. Quantitative analytical methods including chromatographic (GC, HPLC, etc.) or electrophoretic analyses may be used to demonstrate purity for small molecules and polymeric materials.

#### 6. Spectral data

Detailed spectral data for new compounds should be provided in list form (see below) in the Methods section. Figures containing spectra generally will not be published as a manuscript figure unless the data are directly relevant to the central conclusions of the paper. Authors are encouraged to include high-quality images of spectral data for key compounds in the Supplementary Information. Specific NMR assignments should be listed after integration values only if they were unambiguously determined by multidimensional NMR or decoupling experiments. Authors should provide information about how assignments were made in a general Methods section.

Example format for compound characterization data. mp: 100-102 °C (lit.<sup>ref</sup> 99-101 °C); TLC ( $\text{CHCl}_3$ :MeOH, 98:2 v/v):  $R_f = 0.23$ ;  $[\alpha]_D = -21.5$  (0.1 M in n-hexane);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.30 (s, 1H), 7.55-7.41 (m, 6H), 5.61 (d,  $J = 5.5$  Hz, 1H), 5.40 (d,  $J = 5.5$  Hz, 1H), 4.93 (m, 1H), 4.20 (q,  $J = 8.5$  Hz, 2H), 2.11 (s, 3H), 1.25 (t,  $J = 8.5$  Hz, 3H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  165.4, 165.0, 140.5, 138.7, 131.5, 129.2, 118.6, 84.2, 75.8, 66.7, 37.9, 20.1; IR (Nujol):  $1765\text{ cm}^{-1}$ ; UV/Vis:  $\lambda_{\text{max}}$  267 nm; HRMS (m/z):  $[\text{M}]^+$  calcd. for  $\text{C}_{20}\text{H}_{15}\text{Cl}_2\text{NO}_5$ , 420.0406; found, 420.0412; analysis (calcd., found for  $\text{C}_{20}\text{H}_{15}\text{Cl}_2\text{NO}_5$ ): C (57.16, 57.22), H (3.60, 3.61), Cl (16.87, 16.88), N (3.33, 3.33), O (19.04, 19.09).

#### 7. Crystallographic data for small molecules

Manuscripts reporting new three-dimensional structures of small molecules from crystallographic analysis should include a .cif file and a structural figure with probability ellipsoids for publication as Supplementary Information. These must have been checked using the IUCR's [CheckCIF](#) routine, and a PDF copy of the output must be included with the submission, together with a justification for any alerts reported. Crystallographic data for small molecules should be submitted to the [Cambridge Structural Database](#) and the

deposition number referenced appropriately in the manuscript. Full access must be provided on publication.

#### **8. Macromolecular structural data**

Manuscripts reporting new structures should contain a table summarizing structural and refinement statistics. Templates are available for such tables describing [NMR](#) and [X-ray crystallography](#) data. To facilitate assessment of the quality of the structural data, a stereo image of a portion of the electron density map (for crystallography papers) or of the superimposed lowest energy structures ( $\geq 10$ ; for NMR papers) should be provided with the submitted manuscript. If the reported structure represents a novel overall fold, a stereo image of the entire structure (as a backbone trace) should also be provided.

## **Apêndice 5 – Normas para elaboração e submissão de trabalhos científicos ao periódico *Grass and Forage Science*.**

### **1. SUBMISSION**

Authors should kindly note that submission implies that the content has not been published or submitted for publication elsewhere except as a brief abstract in the proceedings of a scientific meeting or symposium.

**Once the submission materials have been prepared in accordance with the Author Guidelines, manuscripts should be submitted online at <http://mc.manuscriptcentral.com/gfs>.**

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### **2. AIMS AND SCOPE**

*Grass and Forage Science* publishes the results of research and development in all aspects of grass and forage production, management and utilization, reviews of the state of knowledge on relevant topics and book reviews. Authors are also invited to submit papers on non-agricultural aspects of grassland management such as bioenergy, equine, recreational and amenity use and the environmental implications of all grassland systems. The Journal considers papers from all climatic zones. Originality is required in papers submitted for publication but this does not preclude the publication of material of a developmental nature.

As a guide to authors the Editors would make the following suggestions:

- Experiments that are sensitive to environmental interactions, such as quantitative measurements, must be repeated over time and/or space.

- Experiments that primarily describe the results of in vitro or controlled environment research should fully describe the conditions of the experiment and also the practical application of the research must be clear.
- Papers that describe the routine evaluation of new cultivars, inoculant strains, seed coatings or similar will not be considered unless a high level of novelty and broad impact can be demonstrated.

### **3. MANUSCRIPT CATEGORIES AND REQUIREMENTS**

The main document must be uploaded as an editable Word document (.doc, .docx) with continuous line numbering at 1.5 line spacing. All tables, figures, supporting information and bibliographic entries must have a reference in the text. Tables should be included in the main document after the reference list, each on an individual page alongside their legend. A list of figure captions should be included at the end of the main document. Figures should not be included in the main document and should instead be uploaded as individual files.

Note: Authors submitting papers to *Grass and Forage Science* are strongly urged to read *An international terminology for grazing lands and grazing animals* by the Forage and Grazing Terminology Committee. The article should be used as a guide to the correct use of terminology in grazing studies, and can be accessed for free [here](#).

#### **i. Original Articles**

A full length research paper that describes novel research that is within the scope of Grass and Forage Science. There is no word limit for Original Articles which should be using the following general structure:

*Manuscript structure:* Abstract (250 words maximum); Keywords; Introduction; Materials and Methods; Results; Discussion; Conclusion (if applicable)\*; Acknowledgements (if applicable); References.

#### **ii. Short Communications**

A short communication is a short paper that describes timely results from an experiment testing a novel hypothesis. The short communication is subject to the same review standards as an Original Article with the acknowledgment that the results may be more limited in scope or serve to prompt further research. A short communication may also be suitable for the publication of negative results. The format of the short communication is the same as an original article without additional subheadings, while Results & Discussion should be combined. As a guide a short communication should contain a total of no more than 5 tables and figures.

### **iii. Methods and Techniques Notes**

A methods and techniques note is a paper that describes a new method or technique or a significant improvement in a recognised method. The paper should state the importance of the methodology to grassland or forage science and the situations in which it can be applied.

Authors must provide sufficient details of methods and results (including controls, accuracy, precision) to allow the method to be assessed and repeated. Where possible the results of the new method or technology should be compared to existing methods.

Authors should make clear during submission whether the manuscript is to be considered for publication as a full paper, short communication, or methods and techniques note.

### **iv. Review Article**

Full length review papers are welcomed.

*Manuscript structure:* Abstract (250 words maximum); keywords; Introduction; Content-appropriate headings; References

### **v. Book Reviews**

Book reviews (1000 words maximum) may be commissioned by the Editor.

*Manuscript structure:* No specified structure.

## **4. PREPARING THE SUBMISSION**

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### **Cover Letters**

Cover letters are not mandatory; however, they may be supplied at the author's discretion.

### **Parts of the Manuscript**

The manuscript should be submitted in separate files: main text file including title page and tables; figures; supplementary files.

### **Main Text File**

The text file should be presented in the following order:

- i. Title
- ii. A short running title of less than 40 characters
- iii. The full names of the authors
- iv. The author's institutional affiliations where the work was carried out, with a footnote for the author's present address if different from where the work was carried out
- v. The corresponding author and their contact email address.
- vi. Acknowledgments
- vii. Abstract and keywords
- viii. Main text
- ix. References
- x. Tables (each table complete with title and footnotes)
- xi. Figure legends
- xii. Appendices (if relevant). Figures and supporting information should be supplied as separate files.

**Title.** The title should be short and informative, containing major keywords related to the content. The title should not contain abbreviations (see [Wiley's best practice SEO tips](#)).

**Authorship.** For details on eligibility for author listing, please refer to the journal's Authorship policy outlined in the [Editorial Policies and Ethical Considerations](#) section.

**Acknowledgements.** Contributions from individuals who do not meet the criteria for authorship should be listed, with permission from the contributor, in an Acknowledgements section. Financial and material support should also be mentioned. Thanks to anonymous reviewers are not appropriate.

**Conflict of Interest Statement.** Authors will be asked to provide a conflict of interest statement during the submission process. See 'Conflict of Interest' section in [Editorial Policies and Ethical Considerations](#) for details on what to include in this section. Authors should ensure they liaise with all co-authors to confirm agreement with the final statement.

### **Abstract**

Please provide an abstract of no more than 250 words containing the major keywords.

### **Keywords**

Please provide up to six keywords.

### **Main Text**

For information on structure of manuscripts, please view [Section 3. MANUSCRIPT CATEGORIES AND REQUIREMENTS](#).

If you are submitting an Original Article, please take note of the following additional advice pertaining to structure:

**Introduction:** The Introduction of the paper should explain briefly the reasons for conducting the investigation and its nature: a full review of the literature is not necessary.

**Materials and Methods:** The Materials and methods section of the paper should describe the experimental details so that the study could be repeated.

**Results:** Experimental results should be presented in either tabular or diagrammatic form but not in both forms.

**Discussion:** The Discussion of the results should conclude with a clear statement of their importance and application.

## **References**

References should be prepared according to the Publication Manual of the American Psychological Association (6th edition). This means in text citations should follow the author-date method whereby the author's last name and the year of publication for the source should appear in the text, for example, (Jones, 1998), (Jones and Smith, 2000), (Jones et al, 2002).

Please note: For peer-review, authors should ensure they use the basic (name, year) method described above. However should a paper be accepted, the typesetters will ensure citations and references follow full APA style and so you may see some changes in citation format during proof stage. This is because according to APA style, citation format changes depending on the number of authors listed on the cited paper. More information on citation format can be found on the APA website.

The complete reference list should appear alphabetically by name at the end of the paper. A sample of the most common entries in reference lists appears below. Please note that a DOI should be provided for all references where available. For more information about APA referencing style, please refer to the [APA FAQ](#). Please note that for journal articles, issue numbers are not included unless each issue in the volume begins with page one.

### *Journal article*

Beers, S. R. , & De Bellis, M. D. (2002). Neuropsychological function in children with maltreatment-related posttraumatic stress disorder. *The American Journal of Psychiatry*, 159, 483–486. doi:10.1176/appi.ajp.159.3.483

### *Book*

Bradley-Johnson, S. (1994). *Psychoeducational assessment of students who are visually impaired or blind: Infancy through high school* (2nd ed.). Austin, TX: Pro-ed.

### *Internet Document*

Norton, R. (2006, November 4). How to train a cat to operate a light switch [Video file]. Retrieved from <http://www.youtube.com/watch?v=Vja83KLQXZs>

## **Tables**

Tables should be self-contained and complement, not duplicate, information contained in the

text. They should be supplied as editable files, not pasted as images. Legends should be concise but comprehensive – the table, legend, and footnotes must be understandable without reference to the text. All abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶, should be used (in that order) and \*, \*\*, \*\*\* should be reserved for P-values. Statistical measures such as SD or SEM should be identified in the headings. Tables should be numbered consecutively with Arabic numerals and all must be referred to in the main text.

### **Figure Legends**

Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement. Figures should be numbered consecutively with Arabic numerals and all must be referred to in the main text.

### **Figures**

Although authors are encouraged to send the highest-quality figures possible, for peer-review purposes, a wide variety of formats, sizes, and resolutions are accepted. [Click here](#) for the basic figure requirements for figures submitted with manuscripts for initial peer review, as well as the more detailed post-acceptance figure requirements.

**Figures submitted in colour** may be reproduced in colour online free of charge. Please note, however, that it is preferable that line figures (e.g. graphs and charts) are supplied in black and white so that they are legible if printed by a reader in black and white. If an author would prefer to have figures printed in colour in hard copies of the journal, a fee will be charged by the Publisher.

### **Guidelines for Cover Submissions**

If you would like to send suggestions for artwork related to your manuscript to be considered to appear on the cover of the journal, please follow these general

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### **Additional Files**

#### **Appendices**

Appendices will be published after the references. For submission they should be supplied as separate files but referred to in the text.

#### **Supporting Information**

Supporting information is information that is not essential to the article, but provides greater depth and background. It is hosted online and appears without editing or typesetting. It may include tables, figures, videos, datasets, etc. [Click here](#) for Wiley's FAQs on supporting information.



Note: if data, scripts, or other artefacts used to generate the analyses presented in the paper are available via a publicly available data repository, authors should include a reference to the location of the material within their paper.

### **General Style Points**

The following points provide general advice on formatting and style.

**Abbreviations:** In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Initially, use the word in full, followed by the abbreviation in parentheses. Thereafter use the abbreviation only. With regards to composition of fertilisers, the abbreviations N, P, P<sub>2</sub>O<sub>5</sub>, K and K<sub>2</sub>O may be used without definition at the first occurrence, but P and K should not be used to indicate phosphorus and potassium quantities or contents calculated in P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O respectively.

**Units of measurement:** Measurements should be given in SI or SI-derived units. Visit the Bureau International des Poids et Mesures (BIPM) website at [www.bipm.fr](http://www.bipm.fr) for more information about SI units. Proportions, rather than percentages, should be used except where there is a scientific convention to use percentages, e.g. cover and germination rate. The 24-hour clock should be used for time.

**Numbers:** numbers under 10 are spelt out, except for: measurements with a unit (8mmol/l); age (6 weeks old), or lists with other numbers (11 dogs, 9 cats, 4 gerbils).

**Trade Names:** Chemical substances should be referred to by the generic name only. Trade names should not be used. Drugs should be referred to by their generic names. If proprietary drugs have been used in the study, refer to these by their generic name, mentioning the proprietary name and the name and location of the manufacturer in parentheses.

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The journal supports the [Resource Identification Initiative](#), which aims to promote research resource identification, discovery, and reuse. This initiative, led by the [Neuroscience Information Framework](#) and the [Oregon Health & Science University Library](#), provides unique identifiers for antibodies, model organisms, cell lines, and tools including software and databases. These IDs, called Research Resource Identifiers (RRIDs), are machine-readable and can be used to search for all papers where a particular resource was used and to increase access to critical data to help researchers identify suitable reagents and tools.

Authors are asked to use RRIDs to cite the resources used in their research where applicable in the text, similar to a regular citation or Genbank Accession number. For antibodies, authors should include in the citation the vendor, catalogue number, and RRID both in the text upon first mention in the Methods section. For software tools and databases, please provide the name of

the resource followed by the resource website, if available, and the RRID. For model organisms, the RRID alone is sufficient.

Additionally, authors must include the RIIDs in the list of keywords associated with the manuscript.

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US authors should cite compliance with the US National Research Council's [Guide for the Care and Use of Laboratory Animals](#), the US Public Health Service's [Policy on Humane Care and Use of Laboratory Animals](#), and [Guide for the Care and Use of Laboratory Animals](#).

UK authors should conform to UK legislation under the [Animals \(Scientific Procedures\) Act 1986 Amendment Regulations \(SI 2012/3039\)](#).

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Upon its first use in the title, abstract, and text, the common name of a species should be followed by the scientific name (genus, species, and authority with correct use of parentheses; date of species description is not required) in parentheses. For well-known species, however, scientific names may be omitted from article titles. If no common name exists in English, only the scientific name should be used.

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Been involved in drafting the manuscript or revising it critically for important intellectual content;

Given final approval of the version to be published. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content; and

Agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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## VITA

Pedro Arthur de Albuquerque Nunes, filho de Maria Beatriz Soares Albuquerque e Ricardo Nunes, nasceu no dia 23 de julho de 1990, em Cachoeira do Sul, Rio Grande do Sul, Brasil. cursou Ensino Fundamental e Médio no Colégio Sinodal Barão do Rio Branco, em Cachoeira do Sul, onde concluiu seus estudos no ano de 2007. Após cursar um semestre letivo no curso de Ciências Biológicas da Universidade Federal do Rio Grande do Sul (UFRGS), seguindo o passos de seu tio Biólogo e Entomologista, Gilberto, optou por trilhar o caminho das ciências agrárias, pelas quais também sempre teve apreço devido ao forte vínculo à Fazenda São Nicolau, propriedade da Família Albuquerque, localizada no distrito de Cordilheira, em Cachoeira do Sul. Ingressou, em 2009, na 83ª turma do curso de Agronomia da Universidade Federal de Santa Maria (UFSM), onde imediatamente iniciou suas atividades como voluntário de iniciação científica no Laboratório de Bovinocultura de Corte, sob supervisão dos professores Dr. Dari Celestino Alves Filho e Dr. Ivan Brondani. Teve passagem pelo Setor de Ovinocultura, sob supervisão do professor Dr. Sérgio Machado e, posteriormente, ingressou no Laboratório de Biotecnologia Vegetal, sob orientação do professor Dr. Fernando Teixeira Nicoloso e supervisão da Dra. Júlia Gomes Farias (ainda mestranda naquela ocasião). Neste local, trabalhou por dois anos como bolsista CNPq de iniciação científica, com a temática da fisiologia vegetal e nutrição de plantas em solos contaminados por metais pesados, principalmente as culturas do arroz irrigado e da batata. No sétimo semestre do curso de Agronomia, passou a trabalhar no Laboratório de Pastos e Suplementos, sob orientação das professoras Dra. Luciana Potter e Dra. Marta Gomes da Rocha, onde permaneceu como bolsista de monitoria até o final da graduação. Formou-se Engenheiro Agrônomo em janeiro de 2014. Em abril de 2014, deu início ao curso de Mestrado em Zootecnia na UFRGS, sob orientação do Dr. Paulo César de Faccio Carvalho, líder do Grupo de Pesquisa em Ecologia do Pastejo (GPEP). Em 2015, ainda durante o Mestrado, participou de Mestrado Sanduíche pelo período de 3 meses na *Facultad de Ciencias Veterinarias da Universidad Nacional del Centro de la Provincia de Buenos Aires* (UNCPBA), em Tandil, Argentina, sob a supervisão do Dr. Horacio Leandro Gonda. Tornou-se Mestre em Zootecnia em março de 2016. Em abril de 2016, iniciou seus estudos de Doutorado em Zootecnia na UFRGS, ainda sob orientação do Dr. Paulo César de Faccio Carvalho. Esteve por 6 meses como aluno visitante no *Department of Plant Sciences da University of California Davis*, através do Programa de Doutorado Sanduíche no Exterior (PDSE-CAPES), sob coorientação da Dra. Amélie C. M. Gaudin, mas trabalhando também muito próximo ao Dr. Emilio Andrés Laca. Durante todo o período de pós-graduação na UFRGS, interessou-se pela temática do pastejo em diferentes ecossistemas pastoris, integrados ou não com a agricultura, estudando principalmente o efeito de diferentes intensidades de pastejo em sistemas integrados de produção agropecuária. Em 2017, durante a 54ª Reunião Anual da Sociedade Brasileira de Zootecnia, em Foz do Iguaçu, recebeu o “Prêmio Novos Talentos”, conferido ao conferencista pela melhor apresentação de trabalho na forma oral naquele evento. Até o momento da publicação deste documento, tem em seu currículo 12 artigos científicos e 4 capítulos de livros publicados, 1 capítulo de livro no prelo, 5 artigos científicos em tramitação e dezenas de resumos publicados em anais de congressos. Foi submetido à banca de defesa da Tese de Doutorado no dia 27 de março de 2020.