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Dissertação

**Estoques e frações de Carbono e Nitrogênio do solo sob diferentes sistemas
agropecuários na metade Sul do Rio Grande do Sul**

Robson Bosa dos Reis

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Resumo

REIS, Robson Bosa dos. **Estoques e frações de Carbono e Nitrogênio do solo sob diferentes sistemas agropecuários na metade Sul do Rio Grande do Sul.** Orientador: Filipe Selau Carlos. 2026. 88f. Dissertação (Mestrado em Manejo e Conservação do Solo e da Água) - Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas, 2026.

Sistemas de integração lavoura-pecuária (ILP) promovem a diversificação dos agroecossistemas e contribuem para a sustentabilidade agrícola; entretanto, seus efeitos sobre a dinâmica do carbono (C) e do nitrogênio (N) do solo ainda são pouco documentados em ambientes subtropicais. Esta dissertação teve como objetivo avaliar os impactos de diferentes sistemas de ILP sobre as frações e os estoques de C e N do solo em dois sistemas agrícolas representativos do sul do Brasil: áreas de várzea sob cultivo de arroz irrigado e áreas de coxilha sob sistemas baseados na cultura da soja. O primeiro estudo avaliou um experimento de ILP conduzido por oito anos em um Planossolo, comparando o monocultivo de arroz com sistemas integrados diversificados, envolvendo soja, milho, sudão, pastagens perenes e forrageiras de inverno sob pastejo. O segundo estudo investigou o impacto de sistemas ILP baseados em soja, implantados em áreas originalmente ocupadas por campos nativos, comparando o monocultivo de soja com sistemas integrados que incluíram a sucessão de soja com gramíneas de inverno sob pastejo, em um Argissolo. Amostras de solo foram coletadas em diferentes camadas para a determinação dos teores e estoques totais de C e N, bem como suas frações lábeis ($<53\ \mu\text{m}$) e não lábeis ($>53\ \mu\text{m}$). No estudo com soja, também foram avaliados a densidade e a porosidade do solo, além do Índice de Gestão de Carbono. De modo geral, os sistemas de ILP, especialmente aqueles com maior diversidade de culturas e pastagens, promoveram aumentos significativos nos teores e estoques de C e N do solo em todas as camadas avaliadas, em comparação aos sistemas em monocultivo. Os maiores efeitos foram observados nas camadas superficiais, com incrementos tanto na fração lábil quanto não lábil. Em média, os sistemas de ILP aumentaram os estoques de C total em até 22% nos sistemas com arroz irrigado e em até 56% nos sistemas com soja, na camada de 0-40 cm, em relação aos monocultivos. Os resultados evidenciam que a integração lavoura-pecuária constitui uma estratégia eficiente para aumentar o sequestro de C e N no solo e promover a sustentabilidade de sistemas produtivos de arroz e soja em regiões subtropicais do sul do Brasil.

Palavras-Chaves: sequestro de carbono no solo; integração lavoura-pecuária; matéria orgânica do solo; manejo conservacionista; sustentabilidade agrícola.

Abstract

REIS, Robson Bosa dos. **Stocks and fractions of soil carbon and nitrogen under different agricultural systems in the southern region of Rio Grande do Sul.** Advisor: Filipe Selau Carlos. 2026. 88f. Dissertation (Master's Degree in Soil and Water Management and Conservation) - Eliseu Maciel Faculty of Agronomy, Federal University of Pelotas, Pelotas, Brazil, 2026.

Integrated crop-livestock systems (ICLS) promote agroecosystem diversification and contribute to agricultural sustainability; however, their effects on soil carbon (C) and nitrogen (N) dynamics are still poorly documented in subtropical environments. This dissertation aimed to evaluate the impacts of different ICLS on soil C and N fractions and stocks in two representative agricultural systems of southern Brazil: lowland areas under irrigated rice cultivation and upland areas under soybean-based systems. The first study evaluated an eight-year ICLS experiment conducted on a Albaqualf, comparing rice monocrop with diversified integrated systems involving soybean, maize, sudangrass, perennial pastures, and grazed winter forages. The second study investigated the impact of soybean-based ICLS established in areas originally occupied by native grasslands, comparing soybean monocrop with integrated systems that included soybean in succession with winter grasses under grazing, in an Typic Hapludult. Soil samples were collected at different depths to determine the total C and N contents and stocks, as well as their labile (<53 μm) and non-labile (>53 μm) fractions. In the soybean-based study, bulk density and soil porosity were also evaluated, along with the Carbon Management Index. Overall, ICLS, particularly those with greater crop and pasture diversity, promoted significant increases in soil C and N contents and stocks across all evaluated soil layers compared with monocrop systems. The greatest effects were observed in surface layers, with increases in both labile and non-labile fractions. On average, ICLS increased total soil C stocks by up to 22% in irrigated rice systems and by up to 56% in soybean-based systems at the 0-40 cm soil depth relative to monocrop. These results demonstrate that crop-livestock integration is an effective strategy to enhance soil C and N sequestration and to promote the sustainability of rice and soybean production systems in subtropical regions of southern Brazil.

Keywords: soil carbon sequestration; crop-livestock integration; soil organic matter; conservation management; agricultural sustainability.

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Lista de abreviaturas e siglas

C	Carbono
N	Nitrogênio
GEE	Gases de Efeito Estufa
COS	Carbono Orgânico do Solo
NTS	Nitrogênio Total do Solo
RS	Rio Grande do Sul
MOS	Matéria Orgânica do Solo
MOP	Matéria Orgânica Particulada
MAM	Matéria Orgânica Associada aos Minerais
COT	Carbono Orgânico Total
CO ₂	Dióxido de Carbono
ILP	Integração Lavoura-Pecuária
Mt	Milhões de toneladas

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1. INTRODUÇÃO

Os solos constituem o maior reservatório terrestre de carbono (C) e desempenham papel central no ciclo biogeoquímico do nitrogênio (N), sendo componentes-chave no balanço global de gases de efeito estufa (GEE) e na mitigação das mudanças climáticas (Georgiou *et al.*, 2022; Nazir *et al.*, 2024). Em ecossistemas naturais, como campos nativos, os estoques de carbono orgânico do solo (COS) e de nitrogênio total do solo (NTS) tendem a permanecer relativamente estáveis, em função do equilíbrio entre os aportes de resíduos orgânicos e os processos de decomposição microbiana. Entretanto, a conversão dessas áreas para uso agrícola pode romper esse equilíbrio, resultando em perdas expressivas de C e N, com impactos diretos na qualidade do solo e no aquecimento global (Houghton e Castanho, 2023; Huang *et al.*, 2024).

No contexto dessas transformações no uso da terra, na metade sul do Rio Grande do Sul (RS), região predominantemente inserida no Bioma Pampa, extensas áreas de campos naturais vêm sendo historicamente convertidas em sistemas agrícolas. Destaca-se, em décadas passadas, a expansão do arroz irrigado (*Oryza sativa* L.), especialmente em áreas de terras baixas, e, mais recentemente, o avanço do cultivo de soja (*Glycine max* L.) em terras altas, intensificando a ocupação agrícola regional.

Nesse cenário de expansão agrícola, grande parte desses sistemas produtivos ainda é conduzida sob monocultivo, frequentemente associado ao preparo convencional do solo. O revolvimento mecânico intensifica a mineralização da matéria orgânica do solo (MOS), acelera a oxidação do C e altera atributos estruturais, comprometendo os ciclos biogeoquímicos e a qualidade do solo (Carlos *et al.*, 2020). Além disso, a ocorrência de períodos de pousio durante o inverno, após a colheita das culturas de grãos, reduz os aportes de resíduos vegetais e limita a reposição da MOS (Martins *et al.*, 2017). Em conjunto, essas práticas favorecem a simplificação dos sistemas produtivos e estão associadas à degradação do solo e à perda de atributos essenciais à sustentabilidade agrícola (Alves *et al.*, 2025; Ambus *et al.*, 2023).

Em contraste com esses sistemas agrícolas, áreas nativas pampeanas apresentam elevados estoques de C e N, especialmente nos primeiros 40 cm do perfil do solo, desempenhando relevante função ambiental e climática (Azevedo *et al.*,

2023). Esse cenário reforça a necessidade de estratégias de manejo que conciliem produção agrícola e conservação dos recursos naturais.

Como alternativa aos modelos convencionais, sistemas de integração lavoura-pecuária (ILP) têm sido amplamente reconhecidos como estratégias eficazes de intensificação sustentável da agricultura (Martins *et al.*, 2017; Carlos *et al.*, 2020). Esses sistemas promovem maior eficiência no uso dos recursos, aumentam a resiliência produtiva e contribuem para a mitigação das mudanças climáticas por meio do incremento dos estoques de C e N no solo. A inclusão de forrageiras pastejadas em rotação com culturas anuais amplia o aporte de biomassa, estimula a atividade biológica e favorece a dinâmica da MOS, refletindo positivamente nos teores e estoques de C e N (Carvalho *et al.*, 2021; Alves *et al.*, 2025).

Entretanto, para compreender de forma mais abrangente os impactos desses sistemas, a análise dos estoques totais de C e N, embora essencial, não é suficiente. A separação da MOS em frações lábeis, como a matéria orgânica particulada (MOP), e em frações mais estáveis, como a matéria orgânica associada aos minerais (MAM), permite detectar de forma mais sensível as respostas do solo às práticas de manejo e às mudanças no uso da terra (Ferreira *et al.*, 2024; Yu *et al.*, 2022). Essas frações estão intimamente relacionadas aos atributos físicos do solo, como densidade, porosidade total e distribuição de macro e microporos, os quais regulam processos como infiltração de água, aeração e proteção física do C e do N contra a decomposição microbiana (Ma *et al.*, 2025).

Apesar dos avanços observados em outras regiões brasileiras, especialmente no Cerrado, a adoção de sistemas integrados ainda é incipiente na metade sul do RS (Martins *et al.*, 2017; Soares *et al.*, 2024). Consequentemente, ainda são escassas as informações sobre os efeitos desses sistemas nos estoques e frações de C e N, bem como nos atributos físicos do solo em ambientes subtropicais do sul do Brasil.

Diante desse contexto, torna-se fundamental avaliar como diferentes sistemas de uso e manejo do solo influenciam os estoques de C e N, a distribuição das frações desses elementos ao longo do perfil e a qualidade física dos solos da região, contribuindo para o desenvolvimento de estratégias agrícolas mais sustentáveis, resilientes às mudanças climáticas e alinhadas à conservação dos recursos naturais.

1.1 Objetivos

1.1.1. Geral

Avaliar os efeitos da adoção de sistemas de integração lavoura-pecuária sobre os estoques e frações de carbono e nitrogênio do solo, em áreas agrícolas da Metade Sul do Rio Grande do Sul tradicionalmente manejadas sob monocultivo.

1.2.2. Específicos

- Quantificar e comparar os estoques totais de carbono e nitrogênio ao longo do perfil do solo em sistemas de integração lavoura-pecuária sob plantio direto e em monocultivo de grãos.
- Caracterizar o fracionamento físico da matéria orgânica do solo, determinando a contribuição relativa da MOP e da MAM na distribuição vertical do carbono e do nitrogênio em função do sistema de manejo.
- Avaliar a qualidade de manejo do solo por meio do Índice de Manejo do Carbono, comparando sistemas de sucessão soja-pastagens hibernais sob plantio direto e monocultivo.
- Analisar a associação entre estoques e frações de carbono e nitrogênio e os atributos físicos do solo, incluindo densidade e distribuição de poros, sob diferentes sistemas de manejo.

1.2 Hipóteses

- A adoção de sistemas de integração lavoura-pecuária sob plantio direto aumenta os estoques totais de carbono e nitrogênio do solo, em comparação ao monocultivo de grãos.
- Sistemas ILP promovem maior acúmulo de carbono e nitrogênio na fração MOP em camadas superficiais do solo e maior estabilização desses elementos na fração MAM em camadas subsuperficiais, quando comparados ao monocultivo.

- Sucessões anuais envolvendo soja e pastagens hibernais sob plantio direto aumentam a estabilidade do carbono no solo, refletida em maiores valores do Índice de Manejo do Carbono, em comparação ao monocultivo.
- Melhorias nos atributos físicos do solo sob sucessão anual de soja e pastagens hibernais sob plantio direto estão associadas à manutenção de cobertura vegetal contínua e ao maior aporte de carbono e nitrogênio ao solo, em comparação ao monocultivo.

2. REVISÃO DE LITERATURA

2.1 Áreas da metade sul do RS: contexto com a produção de arroz irrigado e soja

Historicamente, a metade Sul do RS apresenta condições edafoclimáticas e socioeconômicas singulares no contexto da agricultura brasileira, sendo marcada, por longo período, pela predominância da pecuária extensiva baseada em campos naturais do bioma Pampa (Rocha, 2000; Hettwer, 2025). Nas últimas décadas, entretanto, observa-se uma intensificação dos sistemas de uso do solo, com destaque para a expansão da agricultura de grãos, especialmente o arroz irrigado e a soja (Ribas *et al.*, 2021). Nesse cenário, a região assume papel estratégico tanto na produção nacional de arroz quanto como fronteira agrícola emergente para a cultura da soja (Goulart *et al.*, 2020).

Fora do continente asiático, o Brasil se destaca como o maior produtor mundial de arroz, com produção média anual de aproximadamente 12,6 Mt, das quais cerca de 70% são oriundas do RS (IRGA, 2025). No estado, predomina o cultivo de arroz irrigado sob sistema de inundação contínua, conduzido em áreas de várzea que ocupam aproximadamente 1 milhão de hectares (IRGA, 2025). Essas áreas caracterizam-se pela predominância de médias e grandes propriedades rurais, geralmente entre 150 e 750 ha por produtor, com elevado nível de mecanização, diferindo substancialmente dos sistemas orizícolas tradicionais observados em países asiáticos.

As áreas de várzea do RS, tradicionalmente denominadas solos arrozeiros, apresentam atributos físicos e hidrológicos específicos, como elevada densidade do solo, baixa taxa de infiltração de água, reduzida proporção de macroporos em relação aos microporos e topografia predominantemente plana (SOSBAI, 2014). Essas características limitam a drenagem natural, favorecendo a permanência de condições de alagamento por períodos prolongados, o que cria um ambiente propício ao cultivo do arroz irrigado e explica o predomínio histórico dessa cultura na região (Rocha, 2000; SOSBAI, 2014).

Até meados dos anos 2000, o sistema produtivo predominante nas áreas de várzea do sul do Brasil era caracterizado pelo monocultivo de arroz irrigado, com uma

única safra anual (Ribas *et al.*, 2021). No entanto, o aumento da pressão de plantas daninhas, a seleção de biótipos resistentes a herbicidas e a elevação dos custos de produção passaram a comprometer a sustentabilidade econômica e agrônômica desse sistema (Quevedo *et al.*, 2022). Diante desse contexto, a introdução da soja em rotação com o arroz emergiu como uma alternativa viável, visando a diversificação dos sistemas produtivos, a redução de problemas fitossanitários e o aumento da rentabilidade das propriedades rurais (Quevedo *et al.*, 2022).

Atualmente, estima-se que aproximadamente 38,6% da área cultivada com arroz irrigado no Rio Grande do Sul adote a rotação bienal soja-arroz, na qual as culturas são conduzidas de forma sequencial na mesma área, com uma cultura por ano agrícola (IRGA, 2025). De modo geral, o cultivo da soja apresenta maior simplicidade operacional em comparação ao arroz irrigado, sobretudo por não demandar manejo contínuo de lâmina de água. Entretanto, o estabelecimento da soja em ambientes de terras baixas impõe desafios agrônômicos significativos, relacionados ao excesso de umidade, à drenagem deficiente, à baixa estruturação do solo e à limitada capacidade de retenção de água (Rocha, 2000). Além disso, a compactação decorrente de preparos mecânicos frequentes, a elevada acidez e a disponibilidade restrita de cultivares adaptadas a solos hidromórficos constituem entraves adicionais à consolidação desse sistema (Ribas *et al.*, 2021; Balest *et al.*, 2022). Esses fatores ajudam a explicar por que grande parte das áreas de várzea ainda não aderiu plenamente à rotação soja-arroz, apesar de seus benefícios agrônômicos potenciais.

Paralelamente, a cultura da soja tem apresentado expressiva expansão em áreas de terras altas da metade Sul do RS, historicamente ocupadas sistemas de pecuária extensiva sobre campos nativos (Hettwer, 2025). Essa expansão representa uma mudança estrutural no uso e na ocupação do solo da região, impulsionada por fatores econômicos, avanços tecnológicos e melhorias na infraestrutura logística (Hettwer, 2025). Embora essas áreas apresentem menor risco de encharcamento, seus solos são, em geral, rasos, com baixa fertilidade natural e elevada suscetibilidade à degradação física e à erosão, o que exige práticas de manejo assertivas para assegurar a sustentabilidade dos sistemas agrícolas.

No entanto, a substituição parcial da pecuária extensiva por sistemas agrícolas baseados no monocultivo de grãos, especialmente a soja, tem provocado alterações expressivas nesses agroecossistemas (Hettwer, 2025). Entre os principais impactos,

destacam-se mudanças nos teores de C e N do solo, na estrutura física, na dinâmica hídrica e na biodiversidade edáfica (Galarza *et al.*, 2023; Machado *et al.*, 2024). Essas alterações reforçam a necessidade de adoção de sistemas de produção mais diversificados e resilientes, capazes de conciliar produtividade agrícola com a conservação dos recursos naturais.

Nesse contexto, sistemas ILP, têm sido adotados como alternativas promissoras para a metade Sul do RS, tanto em ambientes de terras baixas (Martins *et al.*, 2017) quanto de terras altas (Viana; Silveira; Antunes, 2022). A integração entre culturas anuais e pastagens favorece a diversificação do uso do solo, o aumento do aporte de resíduos orgânicos, a melhoria da estrutura física e maior eficiência na ciclagem de nutrientes, contribuindo para a manutenção e, potencialmente, o incremento dos teores de C e N do solo (Martins *et al.*, 2017; Rodrigues *et al.*, 2021; Alves *et al.*, 2025).

Dessa forma, compreender o papel da produção de arroz irrigado e soja, bem como as transformações recentes nos sistemas de uso do solo da zona Sul do estado, é fundamental para subsidiar estratégias de manejo que promovam a sustentabilidade ambiental, a resiliência dos agroecossistemas e a manutenção da produtividade agrícola no longo prazo.

2.2 Sistemas de Integração lavoura-pecuária na metade sul do RS

A integração entre atividades agrícolas e pecuárias constitui uma prática historicamente associada ao desenvolvimento dos sistemas de produção de alimentos (Tui *et al.*, 2020). Desde períodos pré-históricos, a associação entre o cultivo de plantas e a criação de animais baseou-se, principalmente, na capacidade desses sistemas em promover a ciclagem de nutrientes e o aproveitamento eficiente dos recursos naturais disponíveis (Duarte *et al.*, 2018). Animais domesticados passaram a consumir vegetação não utilizada diretamente na alimentação humana e, por meio de seus excrementos, contribuíram para a reposição de nutrientes no solo, favorecendo o crescimento das culturas agrícolas. Essas interações configuram as formas mais primitivas dos atuais sistemas integrados de produção agropecuária (Anghinoni *et al.*, 2013).

Com o avanço da Revolução Verde, a agricultura passou por um intenso processo de modernização, caracterizado pela mecanização, pelo uso intensivo de fertilizantes minerais, corretivos e defensivos agrícolas, bem como pela especialização dos sistemas produtivos (Pingali, 2012). Embora essas inovações tenham contribuído significativamente para o aumento da produção e da segurança alimentar em escala global, também promoveram a dissociação entre agricultura e pecuária, favorecendo sistemas de monocultivo altamente dependentes de insumos externos (Liu *et al.*, 2020; Matos, 2010).

Na metade sul do RS, a pecuária extensiva baseada em campos nativos constitui uma atividade histórica e culturalmente consolidada (Caballero *et al.*, 2023). Por décadas, esses campos sustentaram sistemas pecuários caracterizados pelo uso de raças bovinas adaptadas às condições edafoclimáticas locais, desempenhando papel fundamental na conservação da biodiversidade, na ciclagem de nutrientes e na manutenção dos estoques de carbono no solo (Overbeck *et al.*, 2007). Entretanto, nas últimas décadas, a expansão da agricultura, especialmente do arroz irrigado em áreas de terras baixas e da soja em terras altas, tem promovido a conversão de extensas áreas de campos naturais em sistemas agrícolas intensivos.

Essa substituição dos campos nativos por monocultivos anuais, frequentemente conduzidos sob preparo convencional do solo e com períodos de pousio, tem resultado em profundas alterações nos agroecossistemas do Pampa (Overbeck *et al.*, 2007; Nicolas *et al.* 2022; Machado *et al.* 2024). Entre os principais impactos destacam-se a redução da diversidade biológica, o aumento da suscetibilidade à erosão, a degradação da estrutura do solo e a diminuição dos estoques de matéria orgânica, carbono e nitrogênio do solo (Nicolas *et al.* 2022; Machado *et al.* 2024). Esses efeitos comprometem não apenas a sustentabilidade produtiva, mas também os serviços ecossistêmicos associados aos solos dessa região.

Nesse contexto, os sistemas ILP emergem como uma estratégia promissora para a intensificação sustentável da produção agropecuária da região (Martins *et al.*, 2017). Esses sistemas baseiam-se na alternância temporal ou espacial entre culturas agrícolas e pastagens, promovendo maior diversidade biológica, aumento dos aportes de resíduos vegetais e intensificação da ciclagem de nutrientes (Carlos *et al.*, 2020; Carvalho *et al.*, 2021). A presença de pastagens, especialmente quando associada ao pastejo, estimula a atividade biológica do solo, favorece a redistribuição de nutrientes

via excretas animais e contribui para a melhoria da qualidade física, química e biológica do (Carlos *et al.*, 2020; Carvalho *et al.*, 2021).

Em áreas de terras baixas, tradicionalmente ocupadas pelo cultivo de arroz irrigado, a adoção de sistemas de ILP, com a inserção de pastagens no período de entressafra, tem sido apontada como alternativa para reduzir a degradação do solo, aumentar a cobertura vegetal e melhorar a ciclagem de nutrientes (Martins *et al.*, 2017; Carlos *et al.*, 2020; Carvalho *et al.*, 2021). Já em terras altas, onde predomina o cultivo de soja, a integração com pastagens de inverno tem demonstrado potencial para aumentar a eficiência do uso de fertilizantes, reduzir a dependência de insumos externos e incrementar os estoques de carbono do solo (Soares *et al.*, 2024; Reis *et al.*, 2025).

De modo geral, os sistemas de ILP promovem a formação de ciclos produtivos mais fechados, nos quais a pastagem fornece cobertura ao solo e resíduos para o sistema plantio direto, a atividade pecuária intensifica a ciclagem de nutrientes por meio das excreções animais e o uso de fertilizantes na produção de grãos beneficia o desenvolvimento das forrageiras subsequentes (Silva *et al.*, 2014; Franzluebbers *et al.*, 2014). Assim, a integração entre lavoura e pecuária representa uma alternativa estratégica visando conciliar produtividade agrícola, conservação do solo e manutenção dos estoques de carbono e nitrogênio nos agroecossistemas.

2.3 Estoques e frações de Carbono e Nitrogênio do solo e a contextualização com as mudanças climáticas

O aumento da intensificação agrícola, aliado às mudanças climáticas globais, tem provocado alterações significativas nos ecossistemas terrestres, com impactos diretos sobre a qualidade e a funcionalidade do solo (Houghton e Castanho, 2023; Wang *et al.*, 2025). Nesse contexto, o solo assume papel estratégico tanto como base da produção agrícola quanto como um dos principais reservatórios terrestres de C e N, elementos essenciais à fertilidade do solo e ao equilíbrio climático global (Georgiou *et al.*, 2022). A dinâmica dos estoques desses elementos é fortemente influenciada pelos sistemas de manejo e uso da terra, refletindo-se diretamente na sustentabilidade dos sistemas produtivos (Lal, 2021; Nicolas *et al.*, 2022; Machado *et al.*, 2024).

Historicamente, a conversão de áreas naturais em sistemas agrícolas tem resultado em perdas expressivas de C e N do solo, principalmente em função da

remoção da cobertura vegetal e da intensificação do revolvimento do solo (Nicolas *et al.*, 2022; Machado *et al.*, 2024). Estima-se que práticas agrícolas convencionais tenham sido responsáveis por reduções substanciais nos estoques de COS ao longo do último século, contribuindo para o aumento das concentrações atmosféricas de CO₂ (Söderström *et al.*, 2014). Em contrapartida, estratégias de manejo conservacionistas vêm sendo amplamente estudadas e adotadas como alternativas para reverter esse cenário, promovendo o acúmulo de C e N no solo e fortalecendo o papel da agricultura na mitigação das mudanças climáticas (Soares *et al.*, 2024; Reis *et al.*, 2025).

A MOS constitui o principal compartimento de armazenamento de C e N e exerce influência direta sobre a fertilidade e a qualidade do solo. O COS atua como agente estruturante, favorecendo a agregação e a estabilidade estrutural, enquanto o N orgânico representa a principal fonte desse nutriente para as plantas, sendo disponibilizado gradualmente por meio dos processos de mineralização (Yudina & Kuzyakov, 2023). Assim, os estoques de C e N estão intimamente associados à eficiência do uso de nutrientes, à produtividade das culturas e à resiliência dos agroecossistemas frente a estresses ambientais (Carlos *et al.*, 2020; Carvalho *et al.*, 2021; Ribas *et al.*, 2021).

Além de seus efeitos sobre a fertilidade, a MOS exerce papel central nos atributos físicos e biológicos do solo. O aumento do teor de C orgânico favorece a formação e a estabilização de agregados, aumenta a macroporosidade, a infiltração e a retenção de água, bem como a resistência do solo à compactação e à erosão (Yudina & Kuzyakov, 2023). Do ponto de vista biológico, a MOS constitui a principal fonte de energia para os microrganismos do solo, regulando a biomassa microbiana, a atividade enzimática e os processos de ciclagem de nutrientes, fundamentais para a manutenção da funcionalidade do solo (Carlos *et al.*, 2020).

Para compreender de forma mais detalhada a dinâmica do C e do N no solo, a avaliação das frações físicas da MOS tem se mostrado uma abordagem sensível às mudanças de manejo (Bayer *et al.*, 2002). O fracionamento em MOP e MAM permite distinguir compartimentos com diferentes graus de labilidade, proteção e tempo de residência no solo (Cambardella & Elliott, 1992). A MOP apresenta rápida ciclagem e elevada sensibilidade às práticas de manejo, estando diretamente relacionada ao aporte recente de resíduos vegetais, enquanto a MAM apresenta maior estabilidade,

desempenhando papel central no sequestro de C e N em longo prazo (Cotrufo *et al.*, 2019).

Sistemas baseados no preparo convencional promovem a ruptura de agregados e a exposição da MOS à oxidação, resultando em perdas expressivas de C e N, especialmente nas frações mais lábeis (Houghton e Castanho, 2023). Em contraste, sistemas conservacionistas, como o plantio direto, favorecem o acúmulo de resíduos vegetais na superfície do solo e a proteção física da MOS, promovendo o aumento gradual dos estoques de C e N (Martins *et al.*, 2017; Cotrufo *et al.*, 2019).

Além do manejo, características intrínsecas do solo, como textura, mineralogia e teor de óxidos de ferro e alumínio, exercem papel determinante na capacidade de estabilização da MOS. Solos com maior teor de argila e maior reatividade superficial tendem a apresentar maior eficiência na proteção do C e do N associados à fração mineral (Bayer *et al.*, 2006), reforçando a importância da avaliação integrada entre tipo de solo e sistema de manejo.

Nesse contexto, sistemas ILP, têm se destacado como estratégias eficazes para aumentar os estoques e melhorar a qualidade da MOS (Martins *et al.*, 2017; Alves *et al.*, 2025). A inclusão de pastagens promove maior aporte de biomassa, diversificação de resíduos e intensificação do crescimento radicular, estimulando a atividade microbiana e favorecendo a estabilização do C e do N no solo (Carvalho *et al.*, 2021).

Do ponto de vista das mudanças climáticas, o aumento dos estoques de C e N no solo representa uma estratégia relevante de mitigação, ao promover o sequestro de carbono atmosférico e reduzir a dependência de fertilizantes nitrogenados, além de contribuir para a redução das emissões de óxidos de nitrogênio (N₂O) (IPCC, 2021; Ferreira *et al.*, 2024). Assim, o manejo adequado da MOS assume papel central na construção de sistemas agrícolas de baixo carbono.

Embora a quantificação dos estoques totais de C e N seja fundamental, alterações significativas nesses estoques podem demandar longos períodos para serem detectadas. Nesse sentido, a análise das frações físicas da MOS surge como ferramenta complementar essencial, permitindo identificar mudanças precoces decorrentes do manejo e fornecer subsídios para a avaliação da qualidade do solo e do potencial de sequestro de carbono (Bayer *et al.*, 2002; Cotrufo *et al.*, 2019).

3. CAPÍTULO I - INTEGRATED CROP-LIVESTOCK SYSTEMS WITH CROP DIVERSIFICATION INCREASE SOIL C LEVELS AND STOCKS IN SUBTROPICAL PADDY FIELDS IN BRAZIL

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Abstract: Integrated crop-livestock systems (ICLS) promote diversification in agricultural ecosystems; however, few studies address the impact of ICLS on soil carbon (C) and nitrogen (N) contents, especially in paddy fields in subtropical climates. In this context, the objective of this study was to evaluate the effects of different ICLS on soil C and N fractions and stocks. The long-term (eight-year) ICLS field trial under no-tillage, in a Alvaquarf, is located in southern Brazil. The treatments are different production systems: S1 - rice monocrop; S2 - manual rice in succession with winter ryegrass; S3 - annual rotation between soybean and rice with winter ryegrass; S4 - rotation between rice, soybean, corn, and Sudangrass in summer, with winter ryegrass and white clover; S5 - Three consecutive years of perennial pasture in summer, followed by one year of rice with ryegrass, white clover, and lotus in winter. With the exception of S1, all systems included pasture during the winter season, with grazing animals present. Soil samples were collected from different layers, and stocks and fractions of total C and N, labile SOM (<53µm), and non-labile SOM (>53µm) were evaluated. The results indicated that ICLS systems under high diversity (S4 and S5) favored soil C and N contents and total stocks in all layers evaluated, while S1 presented the lowest values. Labile and non-labile C and N fractions were also positively affected, mainly at the surface (0-0.1m) under ICLS use. ICLS contribute to increasing soil C and N and the sustainability of rice production systems in subtropical lowlands. Thus, integrated rice cultivation with crop diversity increases, on average, the stocks of labile C, non-labile C and total C at a depth of 0-0.4 m by 30%, 20% and 22%, respectively, compared to rice under monocrop.

Keywords: Soil organic matter; soil carbon; soil management; agricultural sustainability.

3.1. Introduction

Integrated crop-livestock systems (ICLS) have been adopted in several countries as strategies for sustainable agricultural intensification (Garrett *et al.*, 2017; Soares *et al.*, 2024). These systems promote high efficiency in the use of natural resources, increase production resilience, and contribute to the mitigation of climate change in agriculture (Carvalho *et al.*, 2021; Franzluebbers and Hendrickson, 2024; Reis *et al.*, 2025). In Brazil, the expansion of ICLS has intensified in the last decade (Reis *et al.*, 2025); however, in subtropical regions focused on rice (*Oryza sativa* L.) production, ICLS adoption is still incipient (Martins *et al.*, 2017).

In lowlands, flood-irrigated rice monocrop has historically predominated (Sousa *et al.*, 2021), generally conducted under conventional practices, which basically involve soil disturbance before rice sowing (SOSBAI, 2018). This type of intensive management affects soil biochemical cycles (Linh *et al.*, 2015; Linh *et al.*, 2016), accelerates carbon (C) losses (Martins *et al.*, 2017) and favors degradation processes, resulting in reduced soil quality (Carlos *et al.*, 2020; Ambus *et al.*, 2023).

Soils represent the largest terrestrial C reservoir (Georgiou *et al.*, 2022), and soil organic carbon (SOC) sequestration is a fundamental strategy for maintaining greenhouse gas (GHG) balance (Nazir *et al.*, 2024). ICLS promote soil-plant-animal-environment interactions (Carvalho *et al.*, 2021) and represent a promising alternative to increase ecosystem resilience and act as C sinks (Piva *et al.*, 2020; Soares *et al.*, 2024). Studies have reported increased SOC in areas under ICLS (Piva *et al.*, 2020; Liebig *et al.*, 2020) and indicate that these stocks can be important indicators of the sustainability of agricultural systems (Rodrigues *et al.*, 2021; Alves *et al.*, 2025).

However, the consequences of adopting these systems on SOC fractions, as well as the persistence and functionality of stored C, remain poorly understood, especially in grain production areas in subtropical and lowland regions. Understanding C storage in different soil organic matter (SOM) fractions and how these fractions respond to soil management and cultivation strategies is essential for assessing soil quality (Murindangabo *et al.*, 2023; Leuthold *et al.*, 2023). The physical separation of SOM into particulate organic matter (POM) and mineral-associated organic matter (MAOM) helps understand SOM maintenance and dynamics, providing more assertive management recommendations for soil C storage (YU *et al.*, 2022).

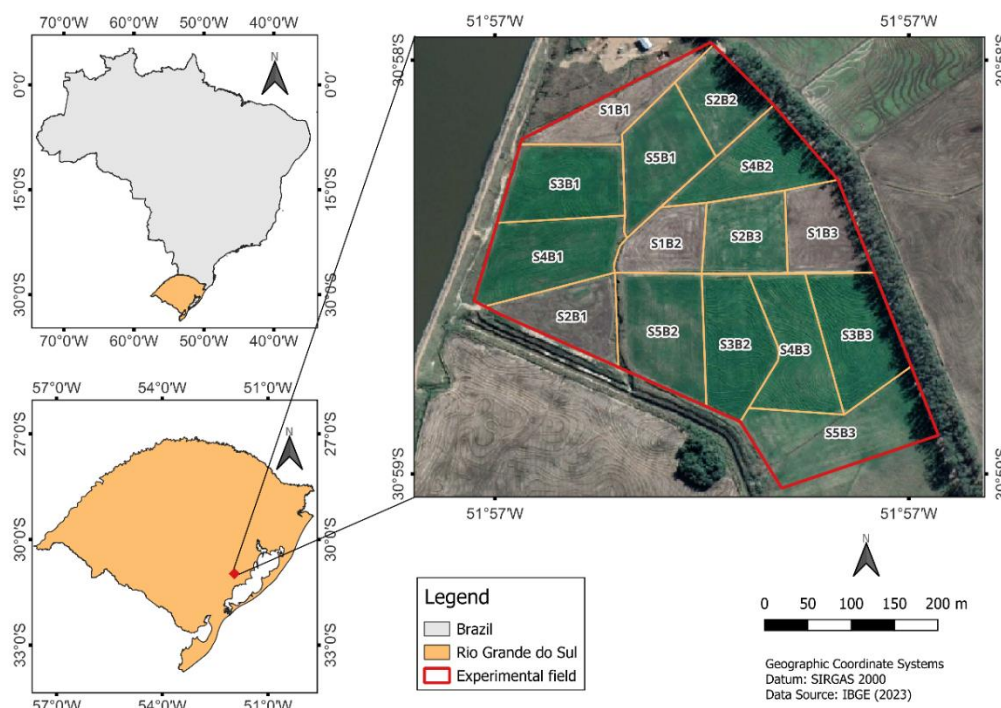
In the face of the global climate crisis, the adoption of efficient and integrated agricultural systems capable of optimizing the use of natural resources and contributing to mitigating the global climate crisis is essential (Roser, 2024; Foley *et al.*, 2011). In this context, this study aimed to evaluate the influence of the use of different ICLS under no-tillage over the long term (8 years) on soil C and N fractions under irrigated rice production in lowland subtropical regions. We believe that the long-term use of ICLS in lowland areas contributes to increasing C and N contents and stocks in the different fractions of the SOM.

3.2. Materials and methods

3.2.1. Site description

The research was conducted in a field trial (8 years) at Fazenda Corticeiras, in southern Brazil (30°57'13"S, 51°57'20"W) (Fig. 1). The site has an average elevation of 28 m above sea level and the soil is characterized as Albaqualf (USDA, 2014), with a sandy clay loam texture and limited drainage capacity (Martins *et al.*, 2017). According to the Köppen climate classification, the climate is Cfa, with hot and humid summers, with an average annual precipitation and temperature of 1500 mm and 18.3°C, respectively (Wrege *et al.*, 2011).

Figure 1 - Location of the long-term field trial evaluating the adoption of different integrated crop-livestock systems in an Albaqualf, in Cristal, Rio Grande do Sul, Brazil.



3.2.2. Historical characterization of the experimental area

Between 1960 and 2009, the experimental trial site was used for annual irrigated rice cultivation during the summer, with a fallow period during the winter. After the 2009 harvest, agricultural activities were interrupted, and the area remained fallow between 2010 and 2013. Agricultural use resumed in the 2013 agricultural year, with the implementation of the study. Prior to the implementation of the field trial, soil samples were collected at the site to determine chemical parameters, which are detailed in Table 1. Aiming to increase the soil pH (potential of hydrogen) to 6.0, 4.5 Mg ha⁻¹ of limestone was applied and incorporated into the soil, with a PRNT (Relative Total Neutralizing Power) of 70%, following the recommendations of the CQFS-RS/SC (2016).

Table 1 - Chemical characteristics of the soil before the start of the experiment, agricultural year 2013, at Corticeiras farm, Cristal, Rio Grande do Sul, Brazil.

Layer	SOM	pH	P	K	Ca	Al	Mg	S	CEC pH 7,0	V
(m)	%	(1:1 soil:water)	mg dm ⁻³		cmol _c dm ⁻³					(%)
0.2	1.8	5.5	10	76	3.5	0.1	2.3	13.5	10.6	56

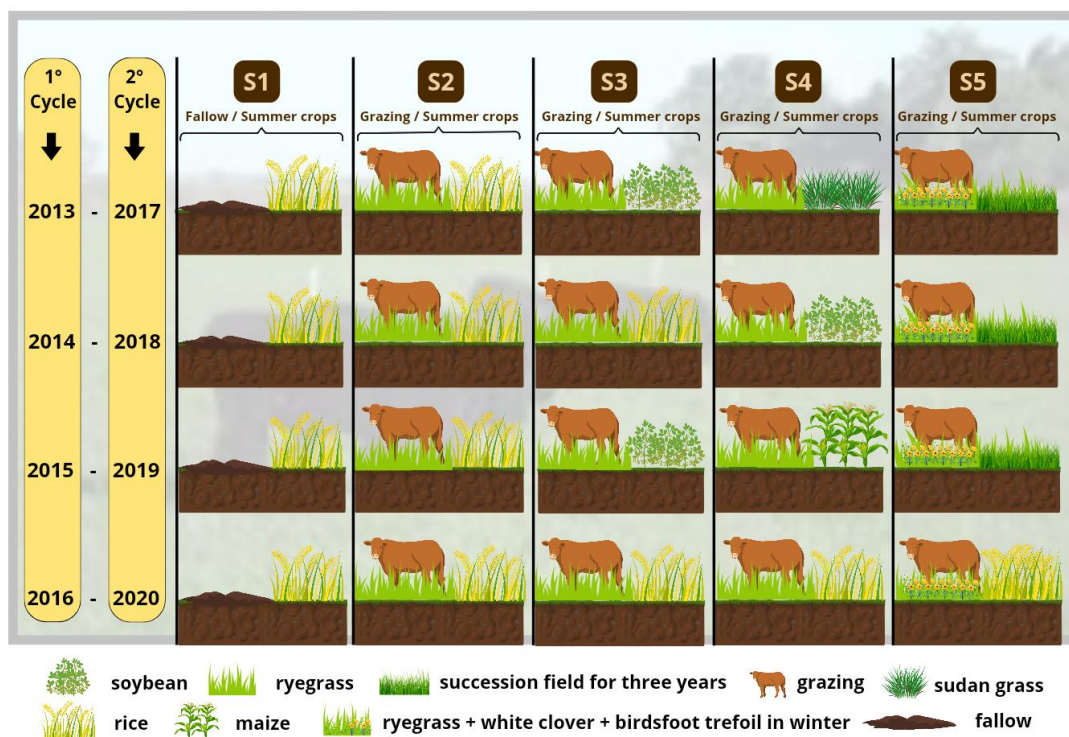
Soil organic matter (SOM), phosphorus (P), potassium (K), calcium (Ca), aluminum (Al), magnesium (Mg), sulfur(S), cation exchange capacity at pH 7,0 (CEC) and base saturation(V).

3.2.3 Experimental design and treatments

The trial was conducted over two cycles, each lasting four years: 2013-2016 and 2017-2020, in a randomized complete block design with three replicates, totaling 15 experimental units with an average area of 1.1 ha each. The study evaluated four ICLS with different arrangements, in relation to rice monocrop, which varied in crop diversity, soil tillage, and temporal frequency of irrigated rice cultivation. The pastures used during the winter period varied in each cycle according to the ICLS. The system combinations included: (S1) - Irrigated rice monocrop and winter fallow (rice crop residues and spontaneous vegetation). Control management, with harrowing (0-0.2 m) and soil preparation operations immediately after harvest, as a means of early preparation for the next harvest. In this system, fallow is adopted during the off-season, therefore, there is no animal grazing; (S2): Annual rice cultivation in summer and ryegrass (*Lolium multiflorum* L.) under winter grazing; (S3): Annual rotation between rice and soybean (*Glycine max* L.) in summer and ryegrass cultivation under winter grazing; (S4): Encompassing a cycle, rotation between sudangrass (*Sorghum sudanense*), soybean, corn (*Zea mays* L.) and rice in summer and pasture of ryegrass and white clover (*Trifolium repens*) in 2013 or ryegrass and Persian clover (*Trifolium resupinatum* L.) from 2014 to 2019 in the winter period, both under winter grazing. In this system, sudangrass is used as forage in summer grazing; and (S5): comprising a cycle, in the summer, successional field during the first three years and a fourth year with irrigated rice cultivation, while in the winter ryegrass + white clover + birdsfoot trefoil (*Lotus corniculatus* L.) were cultivated. In S5, with the exception of the rice cultivation cycle (in the fourth year of each cycle), the remaining periods remained

under grazing (winter and summer). With the exception of S1, the ICLS systems were conducted under no-tillage. Full details of the cropping systems are shown in Figure 2.

Figure 2 - Graphical description of the treatments involving different irrigated rice cultivation systems in lowland areas in southern Brazil.



3.2.4 Management of production systems

Fertilization consisted of $150 \text{ kg ha}^{-1} \text{ N}$ and, on average, 99-106, 150-170, 160-165, 163-177, and 156-159 $\text{kg ha}^{-1} \text{ K}_2\text{O-P}_2\text{O}_5$ for systems S1, S2, S3, S4, and S5, respectively, following the recommendations of CQFS RS/SC (2016). Nitrogen fertilizer was applied only to grasses, whereas legumes met their N requirements through biological fixation. Detailed cultivation parameters are provided in Supplementary Table S1.

3.2.5 Grazing

Grazing was managed using a continuous variable stocking method, following Mott and Lucas (1952). Each experimental unit contained three fixed animals, supplemented with regulator animals introduced or removed as needed to maintain

sward height. Newly weaned juvenile steers (~10 months old; average live weight ~200 kg) were used. Grazing commenced when sward height reached ~0.15 m, corresponding to ~1,500 kg dry matter ha⁻¹, and was maintained throughout the grazing season from early June to mid-October. Sward height was monitored every 15 days using a sward stick (Barthram, 1985), measured from the soil surface to the first fully expanded leaf blade.

3.2.6 Soil sampling

The soil was sampled in October 2020, eight years after the experiment was installed. In each experimental unit, four replicates (soil samples) were collected for SOM fractionation using a cutting shovel and screw auger. Each sample was established by collecting 10 subsamples in the 0-0.4 m layer, stratified into 0-0.025, 0.025-0.05, 0.05-0.075, 0.075-0.1, 0.1-0.15, 0.15-0.2, and 0.2-0.4 m. After collection, the soil samples were identified and taken to the laboratory of the soil department of the Federal University of Pelotas, where they were air-dried and sieved through sieves (mesh = 2 mm diameter). To quantify soil density (Supplementary Table 3), 8 samples were collected (rings measuring 0.067 m in diameter and 0.081 m in height), in layers 0-0.1, 0.1-0.2 and 0.2-0.3 m, according to the methodology described by Blake and Hartge (1986).

3.2.7 Analysis

3.2.7.1 Aboveground biomass

To estimate aboveground biomass (AGB), annual samples were collected from the aerial parts of grain/pasture (summer crop) and forage (winter crop) crops when they reached full maturity and pre-flowering stages, respectively. Eight random samples were collected from each experimental unit using an iron structure measuring 0.25 x 0.25 m², corresponding to the aerial part of the plants cut close to the ground. These samples were subsequently dried at 60°C and their weight converted to Mg ha⁻¹. The annual averages over the eight years of the study are shown in Figure 3.

3.2.7.2 Physical fractionation of soil organic matter

The analysis consisted of adding 10 g of soil and 30 mL of sodium hexametaphosphate solution (5 g L⁻¹) to 100 mL snap-cap flasks, followed by horizontal shaking for 16 h. The resulting suspensions were sieved through a 53 µm mesh using distilled water. The material retained on the sieve consisted of particulate organic matter (POM) (labile fraction), and the material that passed through the sieve was defined as mineral-associated organic matter (MAOM) (non-labile fraction). After sieving, the samples were oven-dried at 60 °C and quantified by mass. The mineral-associated C (MAOM-C) content was calculated as the difference between total organic carbon (TOC) and POM-C content (equation 1). For the final quantification of TOC, approximately 10 g of soil from the initial sample was used, which was processed to a mesh size ≤ 0.053 mm in an agate mortar, as well as the POM-C sample, and subsequently the contents were quantified by the dry combustion method (Elementar Vario EL Analyzer) (Cambardella & Elliott, 1992). The quantifications of total nitrogen (TN), mineral-associated nitrogen (MOAM-N) and particulate organic nitrogen (POM-N) are related and similar to the C analyses.

$$MAOM(g\ kg^{-1}) = TOC - POM \quad \text{Equation 1}$$

C and N stocks were calculated based on the mass equivalent method (Ellert & Bettany, 1995) in each analyzed layer (equation 2):

$$C\ stocks\ (Mg\ ha^{-1}) = \frac{C(g\ kg^{-1}) \times Bd(Mg\ m^{-3}) \times \left(\frac{Bd_{ref}}{Bd} * e\right)}{10} \quad \text{Equation 2}$$

Where:

C = C or N concentration (g kg⁻¹);

Bd = Soil bulk density (Mg m⁻³);

Bd ref = Reference soil bulk density (Mg m⁻³); in our study, we used S1 as a reference;

E = Thickness of the evaluated soil layer (m).

The bulk densities of the soil for each system are described in supplementary table S2.

3.2.7.3 Statistical analysis

The normal distribution of the data was assessed using the Shapiro-Wilk test. Complying with the assumptions of normality, the studied variables were subjected to analysis of variance (ANOVA), and when significant ($p < 0.05$), the data were subjected to the Duncan test (0.05). Statistical analyses were performed using the R® statistical program and the agricolae package (R Core Team, 2017).

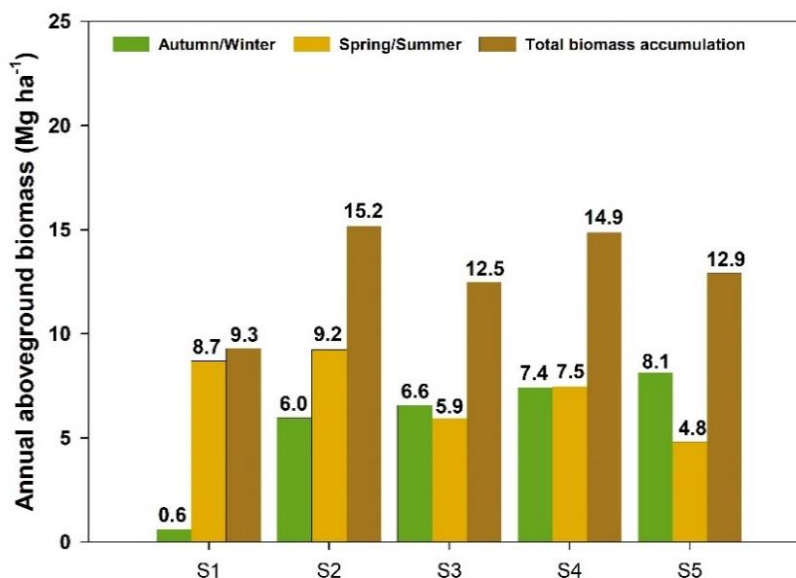
3.3 Results

3.3.1. Aboveground biomass

Annual mean AGB, evaluated in spring-summer and autumn-winter, varied among systems depending on the crops used (Figure 3). System S1, consisting of rice monocrop, did not include forage or pasture in the winter, resulting in an annual mean of 9.3 Mg ha⁻¹ AGB. This represents 5.9, 3.2, 5.6, and 3.6 Mg ha⁻¹ lower AGB than S2, S3, S4, and S5, respectively. Among systems with winter cover crops, AGB production increased with greater species diversity (Figure 3).

Figure 3 - Aboveground biomass under different crop-livestock integration systems involving rice cultivation in southern Brazil.

S1: rice monocrop; S2: rice-ryegrass integration with beef cattle; S3: soybean/rice-ryegrass rotation with beef cattle; S4: annual rotation of rice/soybean/corn/Sudangrass-ryegrass + white clover or Persian clover with beef cattle; S5: three years of perennial pasture followed by one year of irrigated rice-ryegrass + white clover + birdsfoot trefoil with beef cattle.



3.3.2. Soil organic matter fractionation

3.3.2.1. Total organic carbon and total nitrogen

After eight years of ICLS implementation, the results evidenced a general trend of declining TOC and TN contents along the soil profile (Table 2). In the 0-0.25 m layer, S5 exhibited the highest TOC content (37.0 g kg^{-1}), with statistically significant mean increases of 24.2% compared with the other systems. In the subsequent 0.025-0.05 m layer, S5 again presented the highest TOC value (27.1 g kg^{-1}), showing a statistically significant difference exclusively relative to S1 (47.35%). TOC contents in the intermediate layer (0.05-0.20 m) did not differ significantly among production systems, ranging from 10.9 to 21.3 g kg^{-1} . Conversely, in the deepest layer (0.20-0.40 m), S4 substantially enhanced TOC content, being statistically distinct from S1 and S3, with mean increases of 93.4% and 125.6%, respectively (Table 2).

Regarding TN contents, the affected soil layers followed the same patterns observed for TOC, with minimal variation in the statistical outcomes. In the 0-0.025 m layer, the highest TN content was observed under S5 (3.5 g kg^{-1}), with statistically significant mean increases of 58.2% compared with the other treatments. In the 0.025-0.05 m layer, S5 enhanced TN contents relative to S1 and S2, with increases of 56.2% and 39.0%, respectively. In the 0.20-0.40 m layer, a pattern similar to that observed for TOC was found, where S4 markedly increased TN contents relative to S1 and S3, with gains exceeding 100% (Table 2).

Table 2 - Total Organic Carbon (TOC), C and N in Particulate Organic Matter (POM-C and POM-N), C and N in Mineral-Associated Organic Matter (MAOM-C and MAOM-N), Total Nitrogen (TN), and Carbon/Nitrogen (C/N) ratio under different Integrated Crop-Livestock Production Systems in lowland areas. Corticeiras Farm - Cristal, RS, Brazil.

ICLS	TOC	POM-C	MAOM-C	TN	POM-N	MAOM-N	C/N
	g Kg ⁻¹						
0-0.025 m							
S1	18.7c	7.3c	11.4a	1.7c	0.4b	1.2b	11.2
S2	24.7bc	13.9b	10.8a	2.3bc	1.1a	1.2b	10.8
S3	24.8bc	14.1b	10.6a	2.4b	1.0a	1.4b	10.3
S4	28.3b	16.9ab	11.3a	2.7b	1.3a	1.4ab	10.6
S5	37.0a	20.4a	16.6a	3.5a	1.3a	2.2a	10.5
0.025-0.05 m							
S1	18.4b	4.9b	13.5a	1.6b	0.3b	1.4a	11.2
S2	20.1ab	7.0ab	13.2a	1.8b	0.5ab	1.3a	11.2
S3	20.8ab	6.7b	14.2a	2.0ab	0.5ab	1.5a	10.5
S4	21.6ab	7.6ab	14.0a	2.0ab	0.6ab	1.5a	10.6
S5	27.1a	11.9a	15.2a	2.5a	0.9a	1.7a	10.6
0.05-0.1 m							
S1	17.6a	3.4a	14.2a	1.6a	0.2a	1.4a	11.3
S2	18.1a	5.2a	12.9a	1.7a	0.4a	1.3a	10.8
S3	16.3a	2.5a	13.8a	1.6a	0.2a	1.3a	10.4
S4	16.6a	3.8a	12.8a	1.6a	0.3a	1.3a	10.6
S5	21.3a	6.8a	14.6a	2.0a	0.5a	1.5a	10.7
0.1-0.15 m							
S1	16.6a	3.0a	13.5a	1.5a	0.2a	1.3a	11.2
S2	16.4a	3.3a	13.0a	1.5a	0.3a	1.3a	10.6
S3	13.5a	1.1a	12.4a	1.3a	0.1a	1.2a	10.3
S4	13.8a	1.4a	12.3a	1.3a	0.1a	1.1a	11.0
S5	16.3a	1.6a	14.7a	1.5a	0.1a	1.4a	10.7
0.15-0.2 m							
S1	15.6a	1.8a	13.8a	1.5a	0.2a	1.3a	10.8
S2	14.6a	2.4a	12.2a	1.4a	0.2a	1.2a	10.5
S3	10.9a	0.7a	10.2a	1.2a	0.1a	0.9a	10.7
S4	11.2a	1.0a	10.1a	1.1a	0.1a	1.0a	10.7
S5	16.0a	3.5a	12.5a	1.5a	0.2a	1.2a	10.9
0.2-0.4 m							
S1	9.1b	1.2a	7.9ab	0.8b	0.1a	0.7a	11.0
S2	10.9ab	0.8a	10.1ab	0.9ab	0.2a	0.8a	11.5
S3	7.8b	0.4a	7.4b	0.7b	0.1a	0.6a	11.4
S4	17.6a	1.2a	16.3a	1.6a	0.2a	1.4a	11.2
S5	10.7ab	0.7a	10ab	1.0ab	0.1a	0.9a	10.6

S1: rice monocrop; S2: rice-ryegrass integration with beef cattle; S3: soybean/rice-ryegrass rotation with beef cattle; S4: annual rotation of rice/soybean/corn/Sudangrass-ryegrass + white clover or Persian clover with beef cattle; S5: three years of perennial pasture followed by one year of irrigated rice-ryegrass + white clover + birdsfoot trefoil with beef cattle.

3.3.2.2. C and N in the labile fraction of SOM

The highest POM-C contents were observed in the surface layer, regardless of the management system adopted (Table 2). In the surface layer (0-0.025 m), S5 exhibited the highest content (20.4 g kg⁻¹), with statistically significant mean increases compared with S1, S2, and S3, whose contents ranged from 7.3 to 14.1 g kg⁻¹. In the 0.025-0.05 m layer, a similar pattern was observed; however, S5 (11.9 g kg⁻¹) significantly increased POM-C contents only relative to S1 and S3, which exhibited 4.9 and 6.7 g kg⁻¹, respectively. Consistent with TOC, no statistically significant differences were detected among treatments in the 0.05-0.20 m layer, with POM-C contents ranging from 0.7 to 6.8 g kg⁻¹. Likewise, the 0.20-0.40 m layer was not affected by the management systems, with POM-C contents ranging from 0.4 to 1.2 g kg⁻¹ (Table 2).

Similar patterns were observed for POM-N indices, with variations restricted to the surface layer. In the surface layer (0-0.025 m), the adoption of both ICLS systems (1.0-1.3 g kg⁻¹) resulted in significant increases compared with S1 (0.4 g kg⁻¹). In contrast, in the 0.025-0.05 m layer, only S5 (0.9 g kg⁻¹) differed significantly from S1 (0.3 g kg⁻¹), showing statistically significant gains (Table 2).

3.3.2.3 C and N in the non-labile fraction of SOM

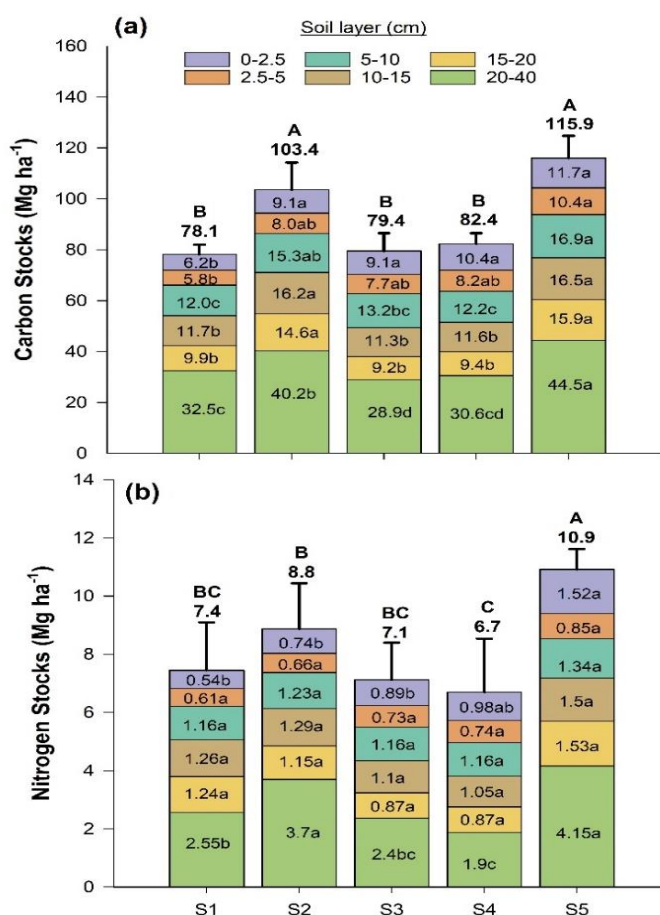
The contents of mineral-associated organic carbon (MAOM-C) were affected by the adoption of different ICLS systems only in the deepest layer (0.20-0.40 m) (Table 2). At this depth, the use of S4 (16.3 g kg⁻¹) significantly increased MAOM-C contents compared with S3 (7.4 g kg⁻¹), but showed no pronounced effect relative to the other systems. In the layers between 0-0.20 m, only minor variations were observed, with MAOM-C contents ranging from 10.1 to 16.6 g kg⁻¹. Similarly, MAOM-N contents varied only in the surface layer (0-0.025 m), where S5 (7.4 g kg⁻¹) significantly increased MAOM-N relative to S1, S2, and S3 (1.2-1.4 g kg⁻¹) (Table 2).

3.3.3. Total C and N stock

C and N stocks varied according to the ICLS systems (Figures 4a and 4b). Regarding total C and N stocks, on average, the adoption of S2 (103.4 Mg ha⁻¹) and S5 (115.9 Mg ha⁻¹) significantly increased total C stocks compared with S1, S3, and S4 (78.1, 79.4, and 82.4 Mg ha⁻¹, respectively), representing a mean accumulation of 37.1% (29.7 Mg ha⁻¹) (Figure 4a). When C stocks were evaluated in stratified layers, S5 exhibited the highest accumulations across all layers, with statistically significant differences relative to the other systems (S1-S4) (Figure 4a). In the surface layer (0-0.025 m), all treatments associated with integrated systems significantly increased C stocks compared with the monocrop system (S1), with S5 showing the highest accumulation (11.7 Mg ha⁻¹), corresponding to an increment of 5.5 Mg ha⁻¹ relative to the conventional system. Similar results were observed in the 0.025-0.05 m layer, where S5 reached 10.4 Mg ha⁻¹, significantly exceeding S1 (8.0 Mg ha⁻¹), but not differing statistically from S2 (8.0 Mg ha⁻¹), S3 (7.7 Mg ha⁻¹), or S4 (8.2 Mg ha⁻¹). In the subsequent layers, the superiority of S5 remained evident (Figure 4a). In the 0.05-0.10, 0.10-0.15, and 0.15-0.20 m layers, S5 accumulated 16.9, 16.5, and 15.9 Mg ha⁻¹, respectively, showing significant gains compared with S1, S3, and S4 (Figure 4a). In the 0.05-0.20 m segment, S2 also exhibited notable C accumulation, particularly relative to S3 and S4. Both S2 and S5 contributed continuously to C accumulation throughout the soil profile, enhancing total stocks (Figure 4a). At depth (0.20-0.40 m), the highest C accumulations were again observed under S2 (40.2 Mg ha⁻¹) and S5 (44.5 Mg ha⁻¹), with an average increase of 38.1% (11.7 Mg ha⁻¹) relative to S1 (32.5 Mg ha⁻¹), S3 (28.9 Mg ha⁻¹), and S4 (30.6 Mg ha⁻¹) (Figure 4a). Regarding total N stocks, the highest accumulations were observed under S5 (10.9 Mg ha⁻¹), with gains relative to the other treatments. Notably, high values were also recorded under S2 (8.8 Mg ha⁻¹), representing substantial accumulations, particularly when compared with S4 (6.7 Mg ha⁻¹) (Figure 4b). When stratified N stocks were evaluated, a pattern similar to that of C was observed, with S5 standing out across all layers (Figure 4b). In the surface layer (0-0.025 m), S5 reached 1.52 Mg ha⁻¹, significantly exceeding S1 (0.54 Mg ha⁻¹), S2 (0.74 Mg ha⁻¹), and S3 (0.89 Mg ha⁻¹), corresponding to a mean increase of 110.2% (0.80 Mg ha⁻¹) relative to the treatments. In the 0.025-0.20 m layers, the highest mean N stocks were again observed under S5 (1.3 Mg ha⁻¹), followed by S2 (1.08 Mg ha⁻¹) > S1 (1.06 Mg ha⁻¹) > S3 (0.97 Mg ha⁻¹) > S4 (0.90 Mg ha⁻¹), although differences were not statistically significant. In contrast, in the deepest layer (0.20-0.40

m), S2 and S5 significantly increased N stocks, with an average gain of 71.9% (1.64 Mg ha⁻¹) compared with S1, S3, and S4 (Figure 4b).

Figura 4 - Total carbon (a) and nitrogen (b) stocks stratified in a 0-0.4 m soil profile on an Albaqualf, 8 years after adopting different rice cultivation systems in southern Brazil. Different lowercase letters indicate significant differences between treatments within the same soil layer, while uppercase letters indicate significant variation in the total stock (0-0.4 m) according to Duncan's test ($p < 0.05$). S1: rice monocrop; S2: rice-ryegrass integration with beef cattle; S3: soybean/rice-ryegrass rotation with beef cattle; S4: annual rotation of rice/soybean/corn/Sudangrass-ryegrass + white clover or Persian clover with beef cattle; S5: three years of perennial pasture followed by one year of irrigated rice-ryegrass + white clover + birdsfoot trefoil with beef cattle.



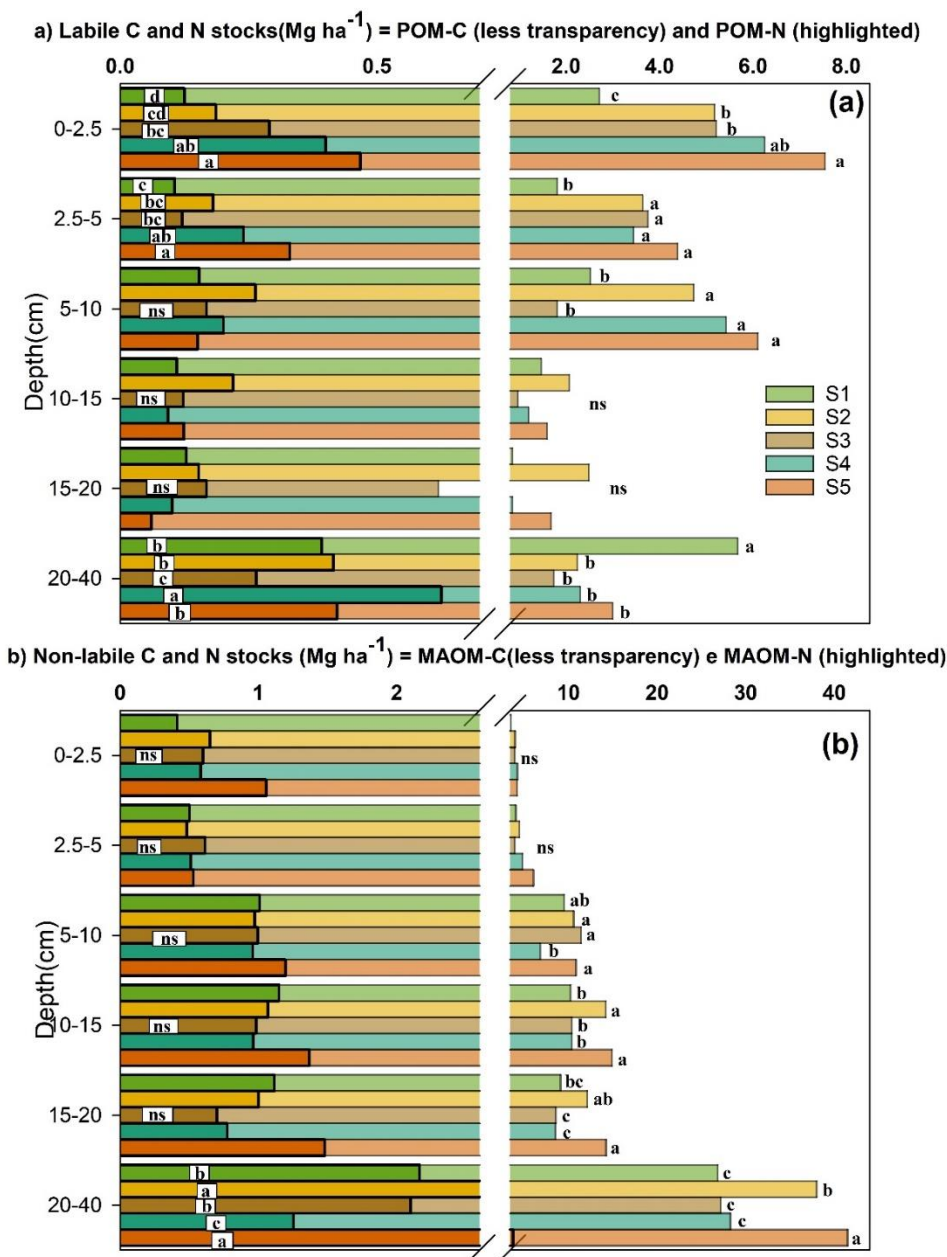
3.3.4 Fractionated C and N stocks

POM-C/POM-N and MAOM-C/MAOM-N stocks varied across the analyzed layers and according to the adopted ICLS systems (Figures 5a and 5b). In the surface layer (0-0.05 m), labile C and N stocks were higher under S5 and S4, with significant increases compared with S1 and S2, corresponding to a mean accumulation of 62.2%

(2.1 Mg ha⁻¹) for labile C and 140% (0.210 Mg ha⁻¹) for labile N (Figure 5a). In the subsequent layer (0.05-0.10 m), significant differences were observed only for labile C, where S5, S4, and S2 (6.1, 5.4, and 4.7 Mg ha⁻¹, respectively) increased, on average, by 151.2% (3.25 Mg ha⁻¹) relative to S1 (2.5 Mg ha⁻¹) and S3 (1.8 Mg ha⁻¹) (Figure 5a). In the 0.10-0.20 m layer, no significant differences were detected among treatments, despite high mean values under S2, with 0.187 and 2.3 Mg ha⁻¹ for POM-N and POM-C, respectively (Figure 5a). In the deepest layer, the highest POM-N stocks were observed under S4 (0.62 Mg ha⁻¹), whereas the highest POM-C values were recorded under the monocrop system (S1, 5.7 Mg ha⁻¹), creating a contrasting pattern (Figure 5a). Non-labile C and N stocks varied mainly in the deeper layers (Figure 5b). In general, within the 0-0.20 m layers, the highest MAOM-N stocks were observed under S5; however, no statistically significant differences were detected among treatments (Figure 5b). In contrast, in the 0.20-0.40 m layer, the highest MAOM-N stocks were recorded under S5 (3.73 Mg ha⁻¹) and S2 (3.3 Mg ha⁻¹), with a statistically significant mean increase of 90.7% (1.7 Mg ha⁻¹) relative to S1, S3, and S4 (Figure 5b). Regarding MAOM-C stocks, no differences were observed among treatments in the surface layer (0-0.05 m). Conversely, in the subsequent layers (0.05-0.40 m), the highest MAOM-C stocks were recorded under S5 and S2, with these systems differing particularly from S3 (Figure 5b).

Figura 5 - Stocks of POM-C/POM-N (a) and MAOM-C/MAOM-N (b) stratified in a 0-0.4 m soil profile on a Albaqualf, 8 years after adopting different rice production systems in southern Brazil.

POM-C: Particulate organic carbon; MAOM-C: Mineral-associated organic carbon; POM-N: Particulate organic nitrogen; MAOM-N: Mineral-associated organic nitrogen. Duncan ($p < 0.05$) = averages followed by the same letters do not differ from each other within the same layer. S1: rice monocrop; S2: rice-ryegrass integration with beef cattle; S3: soybean/rice-ryegrass rotation with beef cattle; S4: annual rotation of rice/soybean/corn/Sudangrass-ryegrass + white clover or Persian clover with beef cattle; S5: three years of perennial pasture followed by one year of irrigated rice-ryegrass + white clover + birdsfoot trefoil with beef cattle.



3.4 Discussion

The Pampa Biome encompasses the rice-growing fields of southern Brazil, where native vegetation has been altered due to agricultural expansion, particularly soybean and rice cultivation (Azevedo *et al.*, 2023). In areas with stable native cover, the Pampa Biome is able to store, on average, 49 t ha⁻¹ of TOC in the first 0.30 m of the soil profile, representing the second-highest rate among Brazilian biomes (MAP BIOMAS, 2023). Developing production systems that enable more sustainable agriculture represents a major challenge for the agribusiness sector, especially given the resistance of producers rooted in traditional farming methods. Designing crop combinations that increase C input into the soil is an essential step in the transition toward more balanced agricultural production systems. Identifying and implementing agricultural practices that provide economic gains while maintaining and/or improving soil attributes - particularly TOC - at levels comparable to those found in natural systems is one of the primary goals of the agribusiness sector (Snapp *et al.*, 2005; Power, 2010). The adoption of no-tillage, winter pasture cultivation, crop diversification, and livestock grazing over the long term (8 years) has resulted in improvements in soil C contents in integrated crop-livestock systems (ICLS) (Table 2). In food production environments aiming for environmental and economic sustainability, understanding the combined performance of rice-pasture systems with respect to the dynamics and functions of soil C and N fractions and stocks is particularly relevant, as these are key indicators of soil quality (Martins *et al.*, 2017; Leuthold *et al.*, 2023; Murindangabo *et al.*, 2023).

3.4.1. Aboveground biomass

The introduction of larger amounts of plant residues into the soil is often used as an effective and isolated strategy to increase SOC content (Amelung *et al.*, 2020; Fujisaki *et al.*, 2018), without considering the source or quality of the residues. The introduction of winter (fall-winter) pastures was observed to increase AGB, leading to high accumulation of plant residues on the soil surface (Figure 3). On average, the four ICLS systems produced 13.9 Mg ha⁻¹ yr⁻¹ of SPS, with variations depending on the characteristics of the pastures/forages used. In addition to increasing C sequestration, the use of cover crops provides other benefits, including improved nutrient cycling and availability, improved soil structure, and increased microbial density through photosynthesis or decomposition of crop residues after senescence (Griffiths *et al.*,

2022; Hussain *et al.*, 2021; Koudahe *et al.*, 2022). Systems S2 and S5 exhibited the highest total biomass accumulation, as well as the highest total C and N stocks in the evaluated profile (115.9-10.9 and 103.4-8.8 Mg ha⁻¹, respectively) (Figure 3). These high levels of aboveground biomass help reduce soil water evaporation, increasing the photosynthetic activity of plants, which capture atmospheric CO₂ and fix it in their tissues or exude it directly into the soil profile through their root systems in a process known as rhizodeposition (Kusumi *et al.*, 2012; Hussain *et al.*, 2021). Therefore, the higher the AGB, the higher the expected accumulations of C and N. However, changes in SOM fractions are not limited to biomass accumulation alone. In ICLS systems, animal grazing influences system dynamics, as pastures and/or forages stimulate root growth through tillering in grazed plant tissues (Anghinoni *et al.*, 2013). Another significant factor is the nutrient cycling facilitated by animals in the environment (Rodrigues *et al.*, 2021; Ferreira *et al.*, 2022). Ruminant excreta are released directly into the field, creating a dynamic three-way interaction between soil, plants, and animals. When high levels of AGB are present in systems with a wide range of grazing species and animals, numerous compounds accumulate (lignin, cellulose, flavonoids, feces, urine, amino acids, lipids, proteins), each with distinct biochemical characteristics, promoting microbial diversity in the soil. These microorganisms, in turn, transform crop residues, further increasing SOM content (Denardin *et al.*, 2020; Sedlář *et al.*, 2023).

3.4.2 C and N contents in SOM fractions

The present study indicates that increasing species diversity in ICLS enhances C and N contents in the surface layers (0-0.05 m) (Table 2). Analyzing the soil profile, the observed tendency of decreasing C and N contents with depth is consistent with findings reported by other authors in ICLS studies (Piva *et al.*, 2020; Liebig *et al.*, 2020). When evaluating the contributions of ICLS to soil C and N inputs, S5 provided the highest C and N contents, 37 and 3.5 g kg⁻¹, respectively (Table 2). The presence of plants such as irrigated rice + ryegrass + white clover + birdsfoot trefoil, combined with animal grazing, favored high C and N inputs, even though this ICLS did not produce the highest AGB. (Figure 3), highlighting that in addition to quantity, the quality of the plant cover is important for promoting higher soil C and N inputs. In general, the intercropping of forage legumes with winter grasses in cropping systems has

demonstrated a high potential to promote C sequestration due to functional complementarity among these plant groups. Studies show that combinations of grasses and legumes directly influence C dynamics, although most investigations have focused on semi-arid areas (Li *et al.*, 2023; Wang *et al.*, 2023). The observed variation in TOC and TN contents results from the balance between C input processes (plant residues, animal excreta, root exudates, and external sources), microbial decomposition, and stabilization of C in the soil (Jastrow, Amonette, & Bailey, 2006). These gain and loss processes determine C and N contents in the labile (POM) and non-labile (MAOM) SOM fractions, which contribute to a more accurate description of soil C accumulation, persistence, and the response to N availability (Cotrufo *et al.*, 2019). Regarding the fractions, it was observed that in the first layer (0-0.025 m), all ICLS systems except S1 exhibited the highest C contents in POM-C, which represents the primary pathway of C entry into the soil (Rocci *et al.*, 2021), whereas N showed the opposite trend, with higher contents in the MAOM-N fraction at the surface. Legumes generally have a lower C/N ratio than grasses (Spady *et al.*, 2018), which facilitates decomposition and N release to the soil, increasing N contents in MAOM-N, in contrast to the labile POM-C fraction (Córdova *et al.*, 2018). Grasses, with their dense fibrous root systems, contribute high non-labile C inputs and favor the formation of stable soil aggregates, while legumes, through biological nitrogen fixation, increase N availability in the system, stimulating grass biomass growth and, consequently, the input of organic residues into the soil. This synergy not only enhances C entry via roots and litter but also improves the efficiency of converting plant biomass into stable soil C, resulting in high C accumulation over time. In general, C and N fractions vary over time and are influenced by plant biomass, residue accumulation rate, microbial activity, and soil physical properties, such as aggregation and structure (Singh & Benbi, 2018). In the surface layer (0-0.1 m), the highest TOC and TN contents are associated with elevated labile fractions (POM-C and POM-N) (Table 5). Higher concentrations of labile SOM fractions are directly linked to litter and decomposing root systems derived from crop residues used as soil cover (Zeng *et al.*, 2024). The surface soil layer also exhibits the highest microbial activity within the profile (Graaff *et al.*, 2010). This combination of factors ultimately increases labile C and N fractions in the uppermost centimeters of the soil profile. Some authors attribute the success of ICLS in accumulating SOM to C incorporated by the roots of forage grasses used as cover and/or pasture (Martins *et al.*, 2017; Piva *et al.*, 2020). For others, the high accumulation of TOC and TN in ICLS

areas can indeed be explained by the substantial contribution of organic residues from forage grasses, although the process is enhanced mainly by the absence of soil disturbance, which allows for better profile structuring and increased dynamics during the decomposition of crop residues (Carlos *et al.*, 2020). The majority of soil C and N is provided by biomass produced within the soil profile, as succession fields produce high root mass compared to areas under annual crops, increasing microbial activity and promoting POM formation (DuPont *et al.*, 2014; Mazzilli *et al.*, 2015). This phenomenon occurs due to the synergistic effect of soil cover and minimal or absent agricultural practices, which increase the input of organic residues and their persistence in the environment, enhancing soil C levels (Martins *et al.*, 2017; Carlos *et al.*, 2020; Liebig *et al.*, 2020; Piva *et al.*, 2020). However, at high depths, surprisingly, conventional management did not show significant losses in labile or non-labile C and N fractions (Table 2). In this system, areas remain fallow during the winter, and residues present consist of rice crop leftovers. Soil preparation with a harrow incorporates residues into the 0-0.2 m layer, creating a transition zone where lower material is brought to the surface and residues are incorporated at depth. This practice may be directly related to C and N fraction contents in the intermediate layer (0.1-0.2 m), due to the high C/N ratio of rice residues, which slows mineralization and contributes to the accumulation of labile SOM fractions. Additionally, the monocrop history of rice in the area, spanning over four decades, may explain the accumulation of recalcitrant SOM fractions.

3.4.2. Stocks de C e N

After eight years of ICLS implementation on an Albaqualf, an increase in C and N stocks was observed compared to the rice monocrop (S1) in the 0-0.4 m layer (Figures 4a and 4b). These results can be attributed to the system management, which was carried out without soil disturbance (no-tillage), combining grain crops in succession with pastures. When implemented together, these conservation practices provide high benefits to soil quality compared to when applied individually (Anghinoni *et al.*, 2013; Carvalho *et al.*, 2010). The increase in C and N stocks is directly linked to the previously mentioned labile and recalcitrant fractions, mediated by the balance between C and N accumulation and losses (Chenu *et al.*, 2019). With the exception of S1, all other systems included cover crops during the winter period, increasing plant

biomass both above and belowground. DuPont *et al.* (2014) and Mazzilli *et al.* (2015) report that grasses can develop extensive root systems, which serve as the primary source of decomposition, providing C and N to the soil, particularly in labile fractions, as illustrated in Figure 5a. Surprisingly, the implementation of ICLS with an annual rice-soybean rotation did not contribute to increased C and N stocks at depth compared to the monocrop system, resulting in similar total stocks between the two systems (Figures 4a and 4b). These results may be related to the characteristics of soybean straw and the production environment, particularly the soil. Soybean straw has a lower C/N ratio and low lignin content, making it easily mineralizable, which releases large amounts of CO₂ into the atmosphere and contributes little to soil C and N stocks (Hassan, 2013). In contrast, rice contains higher levels of compounds that slow its decomposition and has a higher C/N ratio, retaining more C and N in the soil. Another factor that helps explain the values observed in Figures 4a and 4b is the hydromorphic environment. Under continuous flooding of irrigated rice, anaerobic conditions reduce residue decomposition, promoting high C and N inputs to the soil profile (Neue, 1993). When analyzing the entire soil profile, the highest total C and N stocks were observed under systems S5 and S2 (Figures 4a and 4b), and their interpretation can vary according to the characteristics of each system. The values observed for S5 are most likely associated with the wide range of crops and conservation practices implemented, which enhanced species diversity at the site, resulting in increased soil profile stocks. The results indicate that N introduced into the high-diversity system by legumes, likely through biological nitrogen fixation, promoted N distribution throughout the soil profile and outweighed potential losses via leaching. This feature tends to be unique in layers where legumes are intercropped with grasses. In contrast, in S2, the amount of above- and belowground plant biomass produced by the annual rice-ryegrass succession, combined with grazing and the dynamic effects that animals exert on the system, creates an environment with high residue input, which is central to understanding the observed values (Bayer *et al.*, 2011). Although other studies have shown that pasture rotations can provide significant ecosystem services, including climate regulation, nutrient cycling, and food production (Carvalho *et al.*, 2021; Franzluebbers *et al.*, 2014), there is also a growing demand for intensification and diversification of agricultural systems to mitigate economic challenges associated with high production costs and reduced profit margins for rice producers in South America. The results of this study, along with other long-term research, are important for reducing producer

resistance to the adoption of ICLS in rice monocrop areas, contributing to increased crop profitability due to improvements in soil quality while simultaneously providing more balanced growing conditions in subtropical lowland areas. The findings highlight the role of integrated systems in the dynamics of labile C (POM-C), particularly in the surface layers. In the 0-0.025 m layer, system S5 exhibited the highest particulate organic carbon (POM-C) content, reaching 20.4 g kg^{-1} , indicating that more complex and diverse systems favor the accumulation of more sensitive forms of C. Labile C fractions (POM-C), due to their rapid turnover, represent an important energy reserve for microorganisms, actively participating in nutrient cycling and contributing to the maintenance of soil fertility and quality (Hussain *et al.*, 2021; Koudahe *et al.*, 2022). These fractions are particularly sensitive to changes in land use and management, quickly reflecting alterations induced by different agricultural practices or production systems. The higher presence of POM-C fractions in surface layers may be associated with the deposition of higher-quality plant residues, as occurs in systems integrating legume species. These plants contribute rapidly decomposable organic material and increase the input of soluble compounds into the soil, which may explain the elevated labile C contents observed in more complex systems, such as S5. As pointed out by Dhakal and Islam (2018), root exudates, particularly those produced by legumes, can reach deeper soil layers and contribute to carbon input in these regions. However, in this study, such effects were minimal at the highest depths analyzed. These results reinforce that the labile fractions of POM-C and POM-N in the soil are highly sensitive to management practices adopted in integrated systems. In particular, system S5, by promoting high organic matter input and likely stimulating higher biological activity, demonstrated the high potential for accumulation of these fractions, especially in the surface layers. Thus, as POM-C decomposes, it associates with MAOM, which enhances the protection and stability of C because it becomes less available for microbial decomposition (Cotrufo *et al.*, 2013). This pattern is evident in our results, with increases of up to 50% in non-labile C stocks (MAOM-C) compared to labile C stocks (POM-C) (Figure 5).

3.5. Conclusions

The adoption of ICLS under no-tillage, with cover crops under grazing during the winter period, is capable of increasing total C and N contents, stocks, and both

labile and non-labile fractions in the profile of an Albaqualf, compared to rice monocrop areas. Integrated rice cropping with diverse species increases, on average, labile C, non-labile C, and total C stocks at 0-0.4 m depth by 30%, 20%, and 22%, respectively, compared to monocrop rice.

Thus, the use of ICLS that incorporate forages, animal dynamics, and rice cultivation represents a conservation management strategy capable of enhancing soil organic matter in production fields in southern Brazil.

4. CAPÍTULO II - SOYBEAN-LIVESTOCK INTEGRATION AS A STRATEGY TO INCREASE SOIL CARBON AND NITROGEN STOCKS IN THE BRAZILIAN PAMPA

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Abstract:

Temperate South American grasslands play a relevant role in soil carbon (C) and nitrogen (N) sequestration. Their conversion to croplands, especially soybean monocrops, reduces soil C and N stocks and can intensify climate instability. Management strategies such as integrated crop-livestock systems (ICLS) can mitigate these losses by increasing soil C and N sequestration and improving soil physical quality. In the Brazilian Pampa biome, however, studies on ICLS remain scarce. The objective of this study was to evaluate soil C and N fractions and stocks under different soybean-based ICLS involving grazed grasses in succession, in native grasslands converted to croplands. Four systems were assessed in Rio Grande do Sul, southern Brazil: native vegetation (NV); soybean monocrop with winter fallow (SM); soybean followed by grazed annual ryegrass (ICLS1); and soybean followed by grazed black oat (ICLS2). Total soil C and N stocks, their labile and non-labile fractions, bulk density, and soil porosity were quantified across multiple soil depths. Results showed that ICLS increased soil C and N contents and stocks at all depths, whereas SM presented the lowest values. Labile and non-labile C and N fractions, the Carbon Management Index (CMI), bulk density, and soil porosity were positively influenced by ICLS. On average, soybean cultivated in ICLS with winter grasses increased labile C, non-labile C, and total C stocks at 0-0.40 m by 71%, 48%, and 56%, respectively, compared to the monocrop.

Keywords: soil carbon sequestration; integrated crop-livestock systems; conservation-oriented management; agricultural sustainability.

4.1 Introduction

Land-use changes directly affect soil organic carbon (SOC) and soil total nitrogen (STN) reservoirs, intensifying the global climate crisis (Houghton and Castanho, 2023; Wang *et al.*, 2025). Soil is the largest terrestrial C reservoir and a key component of the biogeochemical N cycle, both central to the global balance of greenhouse gases (GHG) (Georgiou *et al.*, 2022). In natural ecosystems, SOC and STN tend to remain relatively stable due to the balance between organic inputs and microbial decomposition; conversion to agriculture generally leads to substantial losses, particularly of C (Huang *et al.*, 2024). Soils under grasslands store 25-34% of global SOC and represent important reservoirs of organic N (Bai and Cotrufo, 2022).

In South America, the conversion of grasslands and native vegetation to croplands, particularly for soybean (*Glycine max* L.) production, has reached unprecedented levels over the past decade (Bortolo *et al.*, 2025; Popin *et al.*, 2025). Among the most affected ecosystems, the Pampa biome has undergone substantial transformation, estimated at 2.85 million hectares (Azevedo *et al.*, 2023). Temperate South American grasslands span northeastern Argentina, Uruguay, Paraguay, and southern Brazil, where they are restricted to the state of Rio Grande do Sul, covering approximately ~60% of its territory (Verdum *et al.*, 2019). In preserved areas, the Pampa stores on average 49 t ha⁻¹ of SOC in the upper 0.30 m of soil (MapBiomass, 2023).

Agricultural practices that increase soil C and N stocks are essential for mitigating GHG emissions and enhancing system resilience and stability (Sooryamol *et al.*, 2023). Integrated crop-livestock systems (ICLS), which combine grain crops in succession with grazed forages, have been adopted worldwide as an efficient and conservation-oriented intensification strategy (Garrett *et al.*, 2017; Soares *et al.*, 2024). In Brazil, ICLS have shown promising results compared to continuous monocrop systems, particularly in the Cerrado biome (Sant-Anna *et al.*, 2016; Soares *et al.*, 2024). By promoting soil-plant-animal interactions, ICLS increase biomass inputs, stimulate biological activity, and improve soil structure, thereby enhancing soil organic matter (SOM) dynamics (Carvalho *et al.*, 2021).

Soil physical attributes also play a decisive role in controlling C and N stabilization (Ma *et al.*, 2025). Bulk density, total porosity, and the distribution of macro-

and micropores regulate aeration, water infiltration, and GHG diffusion, all of which influence SOM decomposition and the sequestration of C and N (Ferreira *et al.*, 2024; Ma *et al.*, 2025). Well-structured soils, characterized by greater macroporosity and lower bulk density, facilitate residue incorporation and the formation of stable aggregates, protecting C and N from microbial decomposition and contributing to the buildup of the stable SOC and STN fractions (Ferreira *et al.*, 2024; Ma *et al.*, 2025).

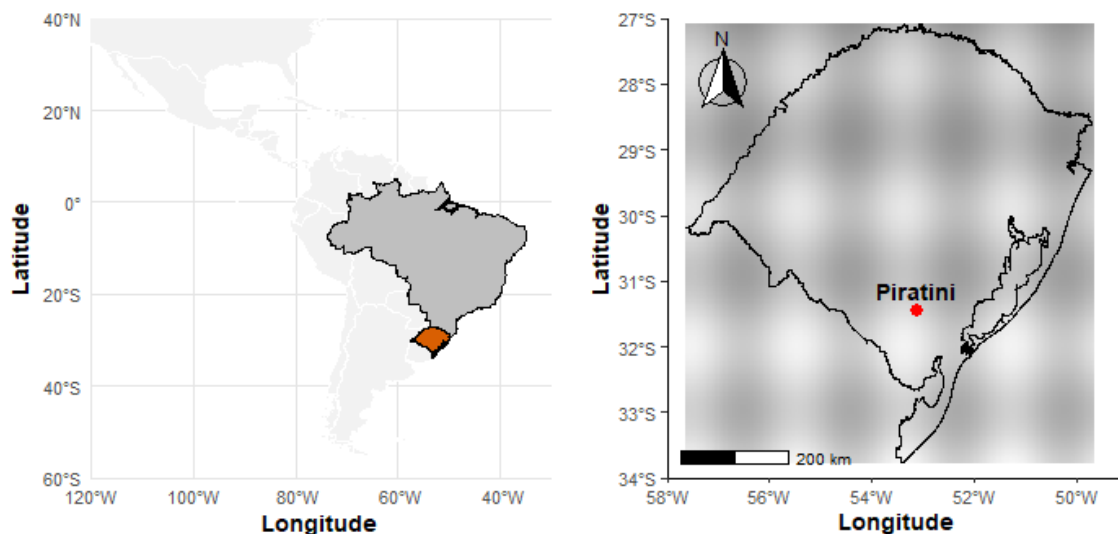
In Brazil, the adoption of ICLS has expanded over the last decade (Soares *et al.*, 2024; Reis *et al.*, 2025). However, in Pampean regions focused on soybean production, implementation of the practice remains incipient (Pereira *et al.*, 2025). Consequently, information on how different ICLS arrangements simultaneously affect soil C and N stocks and soil physical properties is still scarce. Therefore, this study evaluated native grasslands converted into three systems: soybean monocrop with winter fallow, soybean followed by grazed annual ryegrass, and soybean followed by grazed black oat, both under ICLS. Total soil C and N stocks, size-fractionated SOM (labile and stable fractions), bulk density, and soil porosity were quantified. We hypothesize that ICLS increase soil C and N stocks and improve soil structure due to greater plant residue inputs and the presence of grazing animals during the winter period.

4.2 Materials and methods

4.2.1 Site description

The study was conducted at Agropecuária Thomazi (~350 ha), located in the municipality of Piratini, Rio Grande do Sul, southern Brazil (31°35'45.6" S; 53°04'58.1" W; 235 m above sea level) (Figure 6), as part of a medium-term trial (5 years). The local climate is classified as Cfa (hot and humid summer) according to Köppen, with a mean annual temperature of 18.3 °C and annual precipitation of 1460.8 mm (Wrege *et al.*, 2011). The soil in the area is classified as a Typic Hapludult (Soil Survey Staff, 2014).

Figure 6 - Location of the field trial on livestock integration into soybean production systems in a Typic Hapludult, in the municipality of Piratini, southern Brazil.



4.2.2 Historical characterization of the area and management of the production systems

Between the mid-1960s and 2017, the property was predominantly used for extensive cattle grazing on native grasslands of the Pampa biome, characterized by a diverse plant composition, with grasses and legumes predominating and accompanied by shrubs and herbaceous species (Overbeck *et al.*, 2007). The conversion of native grassland to soybean cultivation occurred in the 2018 growing season. For this study, four land-use types within the property were selected: native vegetation of the Pampa biome without anthropogenic interference (NV); soybean monocrop under conventional tillage prior to sowing, followed by fallow during the off-season (SM); and two ICLS under no-till commonly used in the region, consisting of annual soybean cultivation in succession with grazed ryegrass (*Lolium multiflorum* L.) (ICLS1) and soybean in succession with grazed common oat (*Avena sativa* L.) (ICLS2). Each experimental unit comprises approximately 4 ha. The general chemical characterization of the area prior to the implementation of the experiment is presented in Table 3. Subsequently, the native vegetation was removed, and the soil was mechanically prepared with plowing and disking to a depth of 0.40 m for the establishment of the SM, ICLS1, and ICLS2 management systems. Soil pH was corrected to 6.0, and 6.0 Mg ha⁻¹ of lime with a 71% relative neutralizing value (RNV) was applied and incorporated into the soil, following the recommendations of CQFS-

RS/SC (2016). A detailed description of the production arrangements, fertilization rates and timing, and cropping parameters is provided in Table 4. Fertilization schedules and rates varied according to the treatments and were applied according to CQFS-RS/SC (2016), except for NV. Annually, phosphorus and potassium fertilization for soybean and winter forages was applied in rows, whereas nitrogen fertilization for winter crops was broadcast. Continuous grazing with variable stocking rate was carried out using three weaned, castrated steers per ha (~10 months old; ~200 kg), supplemented by regulator animals to maintain pasture height. Grazing began when the pasture reached approximately 0.15 m in height, equivalent to ~1,500 kg of dry matter ha⁻¹, and this height was maintained throughout the grazing period until mid-October or November. Canopy height was evaluated biweekly using a graduated sward stick, measuring from the soil surface to the first fully expanded leaf blade (Barthram, 1985).

Table 3 - Chemical characteristics of the soil before the start of the experiment, agricultural year 2018, at Thomazi farm, Piratini, Rio Grande do Sul, Brazil.

Layer	pH	P	K	Ca	Al	Mg	S	CEC pH 7,0	V
(m)		mg dm ⁻³				cmol _c dm ⁻³			(%)
0.02	5.6	10	70	7.0	0.1	2.8	13.5	10.6	56

Potencial hidrogenionico (pH) - (1:1 soil:water), phosphorus (P), potassium (K), calcium (Ca), aluminum (Al), magnesium (Mg), sulfur(S), cation exchange capacity at pH 7,0 (CEC) and base saturation(V).

Table 4 - Description of cropping parameters in Integrated Crop-Livestock Systems (ICLS) aimed at soybean production at Thomazzi Farm - Piratini, RS, Brazil.

Crop information	Crops		
	Soybean	<i>Lolium Multiflorum</i> L.	<i>Avena Sativa</i> L.
Sowing Density (¹ Kg ha ⁻¹ / ² Plants m ²)	30 ²	30 ¹	30 ¹
Row Spacing (m)	0.45	a	a
Cultivar	BMX FIBRA	BRS Ponteio®	URS Corona®
Annual Fertilization Rate (N-P ₂ O ₅ -K ₂ O) (Kg ha ⁻¹)	00-130-110	100-90-80	100-90-80
Sowing Period	Oct/Nov	May/jun	May/jun
Harvest Period	Apr/may	-	-

a: Sowing done by broadcasting

4.2.3 Soil Sampling

Soil sampling was conducted in June 2023, five years after the establishment of the experiment. For each experimental unit, eight subsamples were collected from the 0-0.40 m soil layer using a spade and a screw auger. Soil depth was stratified into 0-0.025, 0.025-0.05, 0.05-0.075, 0.075-0.1, 0.1-0.15, 0.15-0.2, 0.2-0.3, and 0.3-0.4 m intervals, and the subsamples were then homogenized in a bucket to form a composite sample per plot. Samples were labeled and transported to the Soil Laboratory at UFPel, where they were air-dried and sieved through a 2 mm mesh. For the determination of soil bulk density, macro- and microporosity, and total porosity, six metal rings (0.049 m in diameter and 0.0498 m in height) were collected at the midpoint of the 0-0.1 m, 0.1-0.2 m, and 0.2-0.4 m layers, following the method described by Blake & Hartge (1986).

4.2.4. Analyses

4.2.4.1 Size fractionation of SOM

SOM fractions were obtained by dispersing 10 g of soil in 30 mL of a sodium hexametaphosphate solution (5 g L⁻¹) inside 100 mL snap-cap flasks. The samples

were shaken horizontally for 16 h to ensure complete disaggregation of soil aggregates. After dispersion, the suspensions were wet-sieved through a 53 µm mesh under a gentle stream of distilled water. The material that remained on the sieve was classified as particulate organic matter (POM), representing the labile SOM pool, whereas the fraction that passed through the mesh was designated as mineral-associated organic matter (MAOM), corresponding to the non-labile pool. POM samples were oven-dried at 60 °C and weighed to determine their mass. Carbon associated with the mineral fraction (MAOM-C) was calculated as the difference between total organic carbon (TOC) and the carbon measured in the POM fraction (POM-C), following Equation 1. For TOC and total nitrogen (TN) determinations, approximately 10 g of soil from each sample were finely ground in an agate mortar to a particle size ≤ 0.053 mm; the same preparation was used for POM samples. TOC, TN, POM-C, and POM-N concentrations were quantified via dry combustion using an Elementar Vario EL Analyzer (Cambardella & Elliott, 1992). Soil C and N stocks were computed using the equivalent mass approach, accounting for differences in bulk density among sampling depths (Ellert & Bettany, 1995; Equation 2). For interpretation and presentation of results, the stratified soil profile (0-0.40 m) was consolidated into three depth intervals: surface (0-0.025, 0.025-0.05, 0.05-0.075, 0.075-0.10 m), intermediate (0.10-0.15, 0.15-0.20 m), and deep (0.20-0.30, 0.30-0.40 m).

$$MAOM(g\ kg^{-1}) = TOC - POM \quad \text{Equation 1}$$

$$C\ stocks\ (Mg\ ha^{-1}) = \frac{c(g\ kg^{-1}) \times Bd(Mg\ m^{-3}) \times \left(\frac{Bd_{ref}}{Bd} * E\right)}{10} \quad \text{Equation 2}$$

Where:

C = C or N concentration ($g\ kg^{-1}$);

Bd = Soil bulk density ($Mg\ m^{-3}$);

Bd_{ref} = Soil Bulk Density - Reference ($Mg\ m^{-3}$) - in our study, we used NV as a reference;

E = Sampled layer thickness (m).

4.2.4.2 Physical attributes

For the determination of macroporosity, undisturbed samples (collected in aluminum rings) were saturated with water by capillarity for 24 h and subsequently placed on a tension table, where they reached equilibrium at a tension of 6 kPa. Equilibrated samples were then oven-dried at 105 °C until constant weight for the determination of microporosity and soil bulk density. Total porosity was calculated as the sum of macroporosity and microporosity (EMBRAPA, 1997).

4.2.4.3 Carbon Management Index (CMI)

The Carbon Management Index (CMI) was calculated following Blair *et al.* (1995), with modifications proposed by Diekow *et al.* (2005). The calculation used TOC, POM-C, and MAOM-C contents from each system, with NV serving as the reference treatment (Equation 3). The other indices were calculated as follows: Carbon Stock Index (CSI) (Equation 4), Carbon Lability Index (CLI) (Equation 5), and Soil Carbon Lability (CL) (Equation 6):

$$CMI = CSI \times CLI \times 100 \quad \text{Equation 3}$$

where:

$$CSI = \frac{\text{treatment total C stock}}{\text{reference total C stock}} \quad \text{Equation 4}$$

$$CLI = \frac{\text{treatment CL}}{\text{reference CL}} \quad \text{Equation 5}$$

$$CL \text{ (Lability of C)} = \frac{\text{labile C stock}}{\text{Non - labile C stock}} \quad \text{Equation 6}$$

4.2.4.4 Statistical analyses

Data normality was assessed using the Shapiro-Wilk test. When the normality assumptions were met, the studied variables were subjected to analysis of variance (ANOVA). When significant ($p < 0.05$), means were compared using the Tukey HSD test (0.05) for C and N analyses, whereas soil physical attributes were compared using the least significant difference (LSD) test. Pearson correlation and principal component

analysis (PCA) were also performed. All statistical analyses were conducted using R® statistical software (R Core Team, 2017).

4.3. Results

4.3.1. Soil organic matter fractionation

4.3.1.1. Total Organic Carbon and Total Nitrogen in Soil

A significant effect ($p < 0.05$) was observed, with variation among treatments and soil layers (Table 5). Data obtained across the soil profile indicated higher TOC and TN contents in the surface layers (Table 5). In the 0-0.025 m layer, the highest TOC content was found in ICLS1, with 44.0 g kg^{-1} , followed by 38.4, 25.0, and 21.3 g kg^{-1} in NV, ICLS2, and SM, respectively (Table 5). The lowest TOC values observed in the SM system represent a reduction of over 50% compared to ICLS1 (Table 5). Similarly, in the 0.025-0.05, 0.05-0.075, and 0.075-0.1 m layers, ICLS1 also exhibited the highest TOC contents, 39.9, 33.7, and 16.8 g kg^{-1} , respectively, corresponding to an average increase of 65% compared with the other systems. In the 0.1-0.15 m layer, ICLS2 showed the highest TOC value, 17.6 g kg^{-1} , representing a 49.1% increase relative to the other treatments. At greater depths (0.15-0.4 m), ICLS1 again presented the most significant TOC values, averaging 12.3 g kg^{-1} (Table 5). Regarding TN contents, the majority of the evaluated layers followed similar patterns to those observed for TOC. In the surface layer (0-0.10 m), the highest TN content was observed under ICLS1, with an average value of 3.1 g kg^{-1} , representing a significant average increase of over 35.4% compared with the other treatments. At greater depths, low TN contents under SM were notable (Table 5).

Table 5 -Total Organic Carbon (TOC), Particulate Organic Carbon (POM-C), Mineral-Associated Organic Carbon (MAOM-C), Total Nitrogen (TN), Particulate Nitrogen (POM-N), and Mineral-Associated Nitrogen (MAOM-N) under different Integrated Crop-Livestock Systems in a Typic Hapludult. Thomazi Farm - Piratini, RS, Brazil.

ICLS	TOC	POM-C	MAOM-C	NT	POM-N	MAOM-N
.....g Kg ⁻¹						
0-0.025 m						
NV	38.4b	16.2c	22.2a	3.61b	1.4b	2.21a
SM	21.3d	15.1c	6.2c	1.6d	1.0c	0.60c
ICLS1	44.0a	27.0a	17.1b	4.53a	2.74a	1.79b
ICLS2	25.0c	20.5b	4.5c	2.68c	1.4b	1.38b
0.025-0.05 m						
NV	24.7c	14.4bc	10.3b	2.6b	1.3b	1.3a
SM	17.1d	11.3c	5.8c	1.1d	0.5d	0.5b
ICLS1	39.9a	28.7a	11.3b	3.60a	2.6a	1.0a
ICLS2	28.7b	14.8b	13.9a	2.2c	1.0c	1.3a
0.05-0.075 m						
NV	15.1c	6.5c	8.5c	1.2c	0.5b	0.6b
SM	16.3c	2.8d	13.5a	1.3bc	0.2c	1.1a
ICLS1	33.7a	23.3a	10.4bc	2.7a	1.7a	1.0ab
ICLS2	27.3b	14.6b	12.7ab	1.4b	0.3bc	1.14a
0.075-0.1 m						
NV	15.2a	7.5a	7.7a	1.2ab	0.5ab	0.7a
SM	5.7b	2.4b	3.4b	0.4c	0.1c	0.2b
ICLS1	16.8a	7.2a	9.6a	1.6a	0.6a	0.9a
ICLS2	14.1a	4.0b	10.2a	1.2b	0.3bc	0.9a
0.1-0.15 m						
NV	11.7b	2.9b	8.9a	1.3a	0.5a	0.8a
SM	12.7b	3.2b	9.5a	1.0a	0.2b	0.8a
ICLS1	11.0b	3.4b	7.6a	0.9a	0.2b	0.7a
ICLS2	17.6a	7.8a	9.9a	1.2a	0.3ab	0.8a
0.15-0.2 m						
NV	11.8a	2.0a	9.8a	0.8a	0.1a	0.6a
SM	9.3a	2.9a	6.4b	0.7a	0.2a	0.5a
ICLS1	11.7a	1.9a	9.8a	0.9a	0.1a	0.8a
ICLS2	8.9a	1.5a	7.5ab	1.0a	0.2a	0.8a
0.2-0.3 m						
NV	11.1ab	1.5a	9.6ab	0.9a	0.1a	0.8a
SM	8.3b	1.8a	6.5c	0.5a	0.1a	0.4a
ICLS1	12.6a	1.0a	11.5a	0.9a	0.1a	0.8a
ICLS2	9.1ab	0.8a	8.3bc	0.7a	0.1a	0.6a
0.3-0.4 m						
NV	14.1a	0.9a	13.1a	1.3a	0.1a	1.2a
SM	5.5b	1.2a	4.4c	0.5c	0.1a	0.4c
ICLS1	12.6a	1.8a	10.8a	0.9ab	0.1a	0.8ab
ICLS2	8.5b	1.0a	7.6b	0.6bc	0.1a	0.5bc

NV: native vegetation; SM: soybean monocrop with hibernal fallow; ICLS1: annual soybean cultivation in rotation with grazed ryegrass; ICLS2: annual soybean cultivation in rotation with grazed oat.

4.3.1.2. Labile fractions of C and N

POM-C and POM-N contents were significantly higher in the surface layers of the ICLS1 system (Table 5). In the 0-0.025, 0.025-0.05, 0.05-0.075, and 0.075-0.1 m layers, ICLS1 increased POM-C and POM-N contents by an average of 48.3% and 98%, respectively. In the 0.1-0.15 m layer, ICLS2 showed a notable increase in POM-C compared with the other treatments, while NV exhibited higher POM-N. At greater depths, no significant changes were observed for both labile fractions. Nevertheless, low POM-C contents under ICLS2 and low POM-N under ICLS1 were notable in the deeper layers (Table 5).

4.3.1.3. Non-labile fractions of C and N

MAOM-C and MAOM-N contents did not follow a consistent pattern, varying according to the production systems within each evaluated layer (Table 5). The highest non-labile C and N values were observed in the surface layer (0-0.025 m) under NV, with 22.2 and 2.21 g kg⁻¹, respectively. In the 0.025-0.05 and 0.075-0.1 m layers, high MAOM-C and MAOM-N values were observed under ICLS2, whereas in the 0.05-0.075 m layer, the gains occurred under SM. In the deeper layers (0.15-0.4 m), high MAOM-C contents were observed under ICLS1 and NV, representing an average increase of 61.8% compared with SM and ICLS2; similarly, NV and ICLS1 exhibited substantial increases in MAOM-N, particularly at greater depth (0.3-0.4 m) (Table 5).

4.3.2. C and N stocks

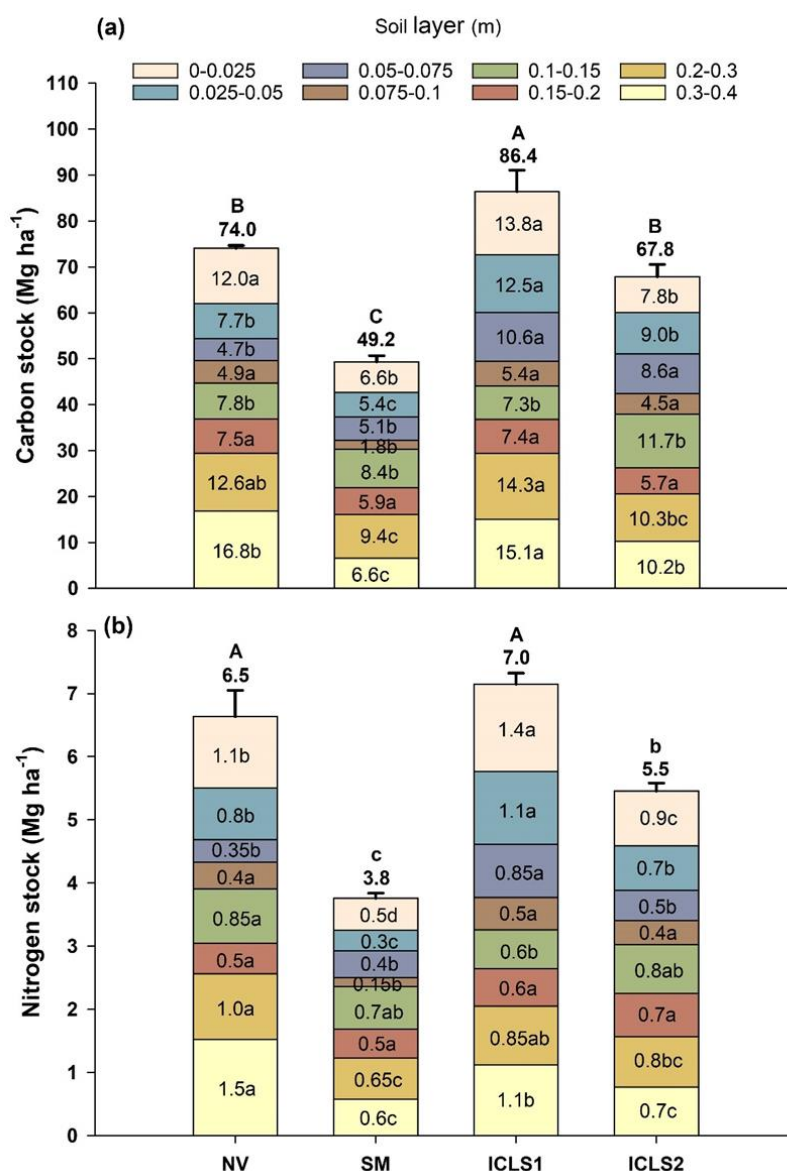
4.3.2.1. Stock de C-Total e de N-Total

Differences in total C and N stocks among the production systems were significant ($p < 0.05$) in most soil layers, with varying response patterns according to soil depth. The ICLS1 system exhibited the highest total stocks, reaching 86.4 Mg ha⁻¹ of C and 7.0 Mg ha⁻¹ of N, followed by NV (74.0 and 6.5 Mg ha⁻¹), ICLS2 (67.8 and 5.5 Mg ha⁻¹), and SM, which showed the lowest values (49.2 and 3.8 Mg ha⁻¹) (Figures 7a and 7b). In the surface layer, treatments differed from each other, with the highest

C and N stocks under ICLS1 (42.3 and 3.9 Mg ha⁻¹), followed by ICLS2 (29.9 and 2.4 Mg ha⁻¹) > NV (29.3 and 2.7 Mg ha⁻¹) > SM (19.0 and 1.4 Mg ha⁻¹). In contrast, in the intermediate layer, ICLS2 exhibited the highest stocks, with average increases of 27.8% for C and 33.3% for N compared with the other treatments. In the deep layer, ICLS systems increased C stocks by an average of 56% and N stocks by 49.2% relative to SM (16.0 and 1.2 Mg ha⁻¹).

Figura 7 - Total soil carbon (a) and nitrogen (b) stocks stratified in the 0-0.4 m layer in a Typic Hapludult, five years after the adoption of different soybean-focused integrated crop-livestock systems (ICLS).

Different lowercase letters indicate significant differences between treatments within the same soil layer, while uppercase letters indicate significant differences for total stock (0-0.4 m) according to Tukey's test ($p < 0.05$). NV: native vegetation; SM: soybean monocrop with fallow during the winter season; ICLS1: annual soybean cultivation in succession with grazed ryegrass; ICLS2: annual soybean cultivation in succession with grazed common oat.



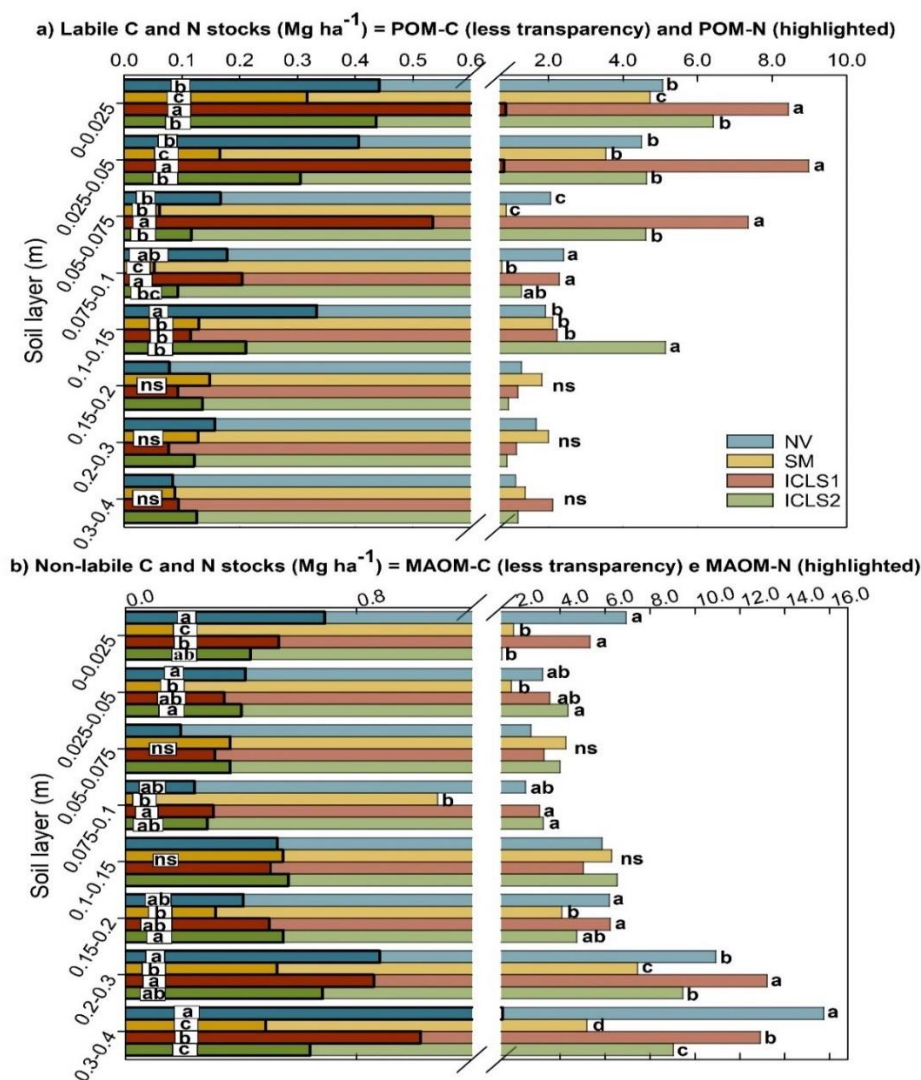
4.3.2.2. Stock of C and N Labile fraction

Labile C and N stocks differed only in the surface and intermediate layers (Figure 8a). In the 0.0-0.1 m layer, the highest labile C and N stocks were observed

under ICLS1, with 27.1 and 2.4 Mg ha⁻¹, respectively. However, in the intermediate layer, ICLS2 showed the highest labile C stock (6.7 Mg ha⁻¹), while NV exhibited the highest labile N stock (0.4 Mg ha⁻¹) (Figure 8a). At greater depths, contrasting trends were observed under SM, with high C stocks and low N stocks, although these differences were not significant (Figure 8a).

Figura 8 - Labile (a) and non-labile (b) C and N stocks stratified in the 0-0.4 m layer in a Typic Hapludult, five years after the adoption of different soybean-focused integrated crop-livestock systems (ICLS).

NV: native vegetation; SM: soybean monocrop with winter fallow; ICLS1: annual soybean cultivation in succession with grazed ryegrass; ICLS2: annual soybean cultivation in succession with grazed common oat. Means followed by different letters within each soil layer are significantly different according to Tukey's test at $p \leq 0.05$. ns: no significant difference.



4.3.2.3. Stock de C e de N non-labile fraction

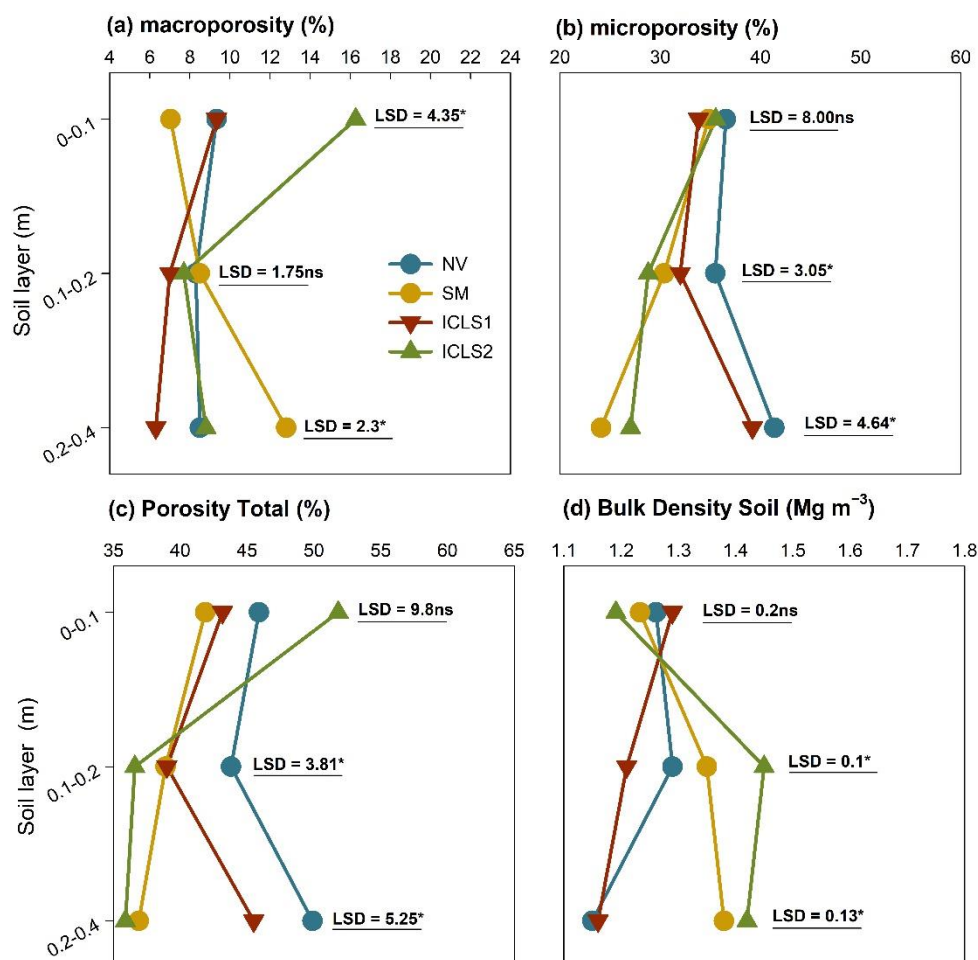
Non-labile C and N stocks varied across treatments in both assessments, showing similar trends across the evaluated depths (Figure 8b). In the surface layer, both ICLS treatments increased non-labile C and N stocks by an average of 59.3% and 87.5%, respectively, compared with SM (Figure 8b). In contrast, no significant changes were observed in the intermediate layer. At greater depths, lower non-labile C and N stocks were again observed under SM, while ICLS systems increased these stocks by an average of 76.3% and 59.4%, respectively (Figure 8b).

4.3.3. Soil Physical Attributes

A significant interaction ($p > 0.05$) was observed between production systems and soil layers, varying according to each physical property analyzed (Figures 9a, 9b, 9c, and 9d). In the surface layer, macroporosity was highest under ICLS2, followed by ICLS1, NV, and SM, with values of 16.3%, 9.3%, 9.2%, and 7.0%, respectively. In the intermediate layer, the lowest values were observed under ICLS1 (7.0%), representing an average difference of 17% compared with the other systems. At greater depth (0.2-0.4 m), SM exhibited the highest macroporosity (12.8%), an average increase of 62.9% relative to the other treatments (Figure 9a). In both layers, the highest microporosity values were found under NV; however, in the surface layer (0-0.1 m), no significant differences were observed among treatments. In the intermediate and deep layers, with values of 35.5% and 41.4%, NV increased microporosity by an average of 16.8% and 37.5%, respectively, compared with the other production systems (Figure 9b). Total porosity also varied among treatments. In the 0-0.10 m layer, ICLS2 exhibited the highest total porosity (51.8%), on average 18.7% greater than the other systems. Similarly, in the intermediate and deep layers, NV showed higher total porosity values of 43.8% and 49.9%, representing average increases of 14.7% and 26.6%, respectively, compared with the other systems (Figure 9c). Regarding soil bulk density, values in the 0-0.1 m layer were similar across treatments, approximately 1.25 Mg m^{-3} . In the 0.10-0.20 m layers, ICLS2 exhibited the highest bulk densities, 1.45 and 1.42 Mg m^{-3} , representing significant increases compared with the other systems (Figure 9d).

Figura 9 - Microporosity (a), macroporosity (b), total porosity (c), and soil bulk density (d) stratified in the 0-0.3 m layer in a Typic Hapludult, five years after the adoption of different soybean-focused integrated crop-livestock systems (ICLS).

LSD: least significant difference. NV: native vegetation; SM: soybean monocrop with winter fallow; ICLS1: annual soybean cultivation in succession with grazed ryegrass; ICLS2: annual soybean cultivation in succession with grazed common oat. $p < 0.05$; ns: no significant difference.



4.3.4. Carbon Indicators

The different production systems influenced ($p < 0.05$) CL, CLI, CSI, and CMI, with variation depending on the soil depth, taking the native vegetation soil profile as the reference for calculation (CLI/CSI: 1.00; CMI: 100%) (Table 6). CL values decreased with increasing soil depth; in the surface layer, the highest values were observed under ICLS, whereas at greater depths, higher CL values were generally observed under SM (Table 6). Similarly, CLI values were higher under ICLS in the surface layers and lower at greater depths, indicating higher C activity at depth due to

a lower ratio between labile and non-labile C fractions (Table 6). Across the evaluated layers, CSI values were generally higher under ICLS, highlighting the efficiency of these systems in increasing total C stocks in both layers assessed (Table 6). CMI varied according to soil depth, with the highest CMI values observed under ICLS, particularly in the surface layer. At greater depths, elevated CMI values were noted under SM, primarily associated with the higher carbon lability indices found in this system (Table 6).

Table 6 - Soil carbon lability (CL), carbon lability index (CLI), carbon stock index (CSI), and carbon management index (CMI) in soil layers of a Typic Hapludult under soybean-livestock integration systems with no-tillage and different grazing intensities, using native forest as reference.

Treatment	CL	CLI	CSI	CMI (%)
0-0.025m				
NV	0.7d	1.0c	1.0a	100.0b
SM	2.4b	3.3b	0.5b	174.8ab
ICLS1	1.6c	2.2bc	1.2a	266.2a
ICLS2	4.6a	6.2a	0.5b	322.7a
0.025-0.05m				
NV	1.4c	1.0a	1.0b	100.0b
SM	2.0b	1.4a	0.7c	104.4b
ICLS1	2.6a	2.0a	1.7a	329.2a
ICLS2	1.1c	0.8a	1.1b	91.7b
0.05-0.075m				
NV	0.8b	1.0b	1.0c	100.0bc
SM	0.2c	0.3b	1.1c	29.7c
ICLS1	2.2a	2.9a	2.2a	657.5a
ICLS2	1.2b	1.5ab	1.6b	250.6b
0.075-0.1m				
NV	1.0a	1.0a	1.0ab	100.0a
SM	0.7ab	0.7a	0.4c	26.3a
ICLS1	0.8ab	0.8a	1.1a	86.0a
ICLS2	0.4b	0.4a	0.8b	33.1a
0.1-0.15m				
NV	0.3a	1.0a	1.0b	100.0b
SM	0.3a	1.1a	1.1b	118.5b
ICLS1	0.5a	1.5a	1.0b	153.3b
ICLS2	0.8a	2.6a	1.6a	437.3a
0.15-0.2m				
NV	0.2a	1.0a	1.0a	100.0a
SM	0.5a	2.2a	0.8a	185.5a
ICLS1	0.2a	1.1a	1.0a	108.1a
ICLS2	0.2a	1.1a	0.9a	94.4a
0.2-0.3m				
NV	0.2a	1.0	1.0ab	100.0a
SM	0.3a	1.9	0.9b	171.2a
ICLS1	0.1a	0.6	1.2a	73.0a
ICLS2	0.1a	0.6	1.0ab	66.4a
0.3-0.4m				
NV	0.1a	1.0b	1.0a	100.0a
SM	0.3a	4.3a	0.5c	188.7a
ICLS1	0.2a	2.5b	0.9ab	221.6a
ICLS2	0.1a	2.0b	0.7b	142.4a

NV: native vegetation; SM: soybean monocrop with hiberna fallow; ICLS1: annual soybean cultivation in rotation with grazed ryegrass; ICLS2: annual soybean cultivation in rotation with grazed oat.

4.3.5. Pearson Correlation and Principal Component Analysis (PCA)

Figure 10 presents the Pearson correlation coefficients between the stocks of C and N fractions in the 0-0.40 m layer and the soil bulk density and porosity variables assessed across the soil profile. According to the results, a very strong positive correlation was observed between total C stock and MAOM-C ($r = 0.91$), POM-N ($r = 0.93$), and total N ($r = 0.97$), as well as a strong correlation with POM-C ($r = 0.81$) and MAOM-N ($r = 0.85$). When correlated with physical attributes, moderate positive correlations were found with M_i ($r = 0.51$) and T_p ($r = 0.50$). In addition, total N exhibited very strong positive correlations with MAOM-C ($r = 0.96$), POM-N ($r = 0.89$), and MAOM-N ($r = 0.93$); strong correlation with POM-C ($r = 0.68$); and moderate correlations with M_i ($r = 0.61$) and T_p ($r = 0.52$). Very strong positive interactions were also found among the stable SOM fractions, particularly between MAOM-C and MAOM-N ($r = 0.95$), and between T_p and M_i ($r = 0.94$). Regarding the stable SOM pool, MAOM-C showed strong positive correlations with POM-N ($r = 0.77$), M_i ($r = 0.76$), and T_p ($r = 0.69$), along with a moderate negative correlation with B_d ($r = -0.53$), indicating that increases in B_d lead to structural degradation of the soil profile and consequently reduce the stocks of C and N fractions. Similarly, MAOM-N displayed moderate positive correlations with M_i ($r = 0.66$) and T_p ($r = 0.52$). Moreover, moderate negative correlations were observed between B_d and POM-N ($r = -0.53$), and between M_a and POM-N ($r = -0.61$). Figure 11 shows the PCA of soil C fraction stocks and physical attributes in the 0-0.4 m layer, five years after ICLS adoption. PC1 and PC2 explained 80.4% of the observed variance. PCA revealed variation in the clustering of different ICLS systems. Overlap was observed between ICLS2 and NV management groups, with natural pasture strongly associated with T_p and M_i vectors. In contrast, ICLS1 and SM systems showed no overlap. Vectors representing total C and N and their labile fractions were strongly aligned with the ICLS1 cluster, clearly indicating the system with the highest C and N stocks in the evaluated profile. Similarly, vectors associated with non-labile C and N fractions tended to point toward ICLS systems but were closer to NV, reflecting the higher input of residues in these systems, which explains their stronger interactions with the more recalcitrant fractions.

Figura 10 - Pearson correlation between the average stocks of soil carbon fractions and soil physical attributes in the 0-0.4 m layer, five years after the adoption of soybean-based integrated crop-livestock systems (ICLS).

POM.C: labile carbon stock; MAOM.C: non-labile carbon stock; C.TOTAL: total carbon stock; POM.N: labile nitrogen stock; MAOM.N: non-labile nitrogen stock; N.TOTAL: total nitrogen stock; Ma: macroporosity; Mi: microporosity; Tp: total porosity; Bd: soil bulk density.

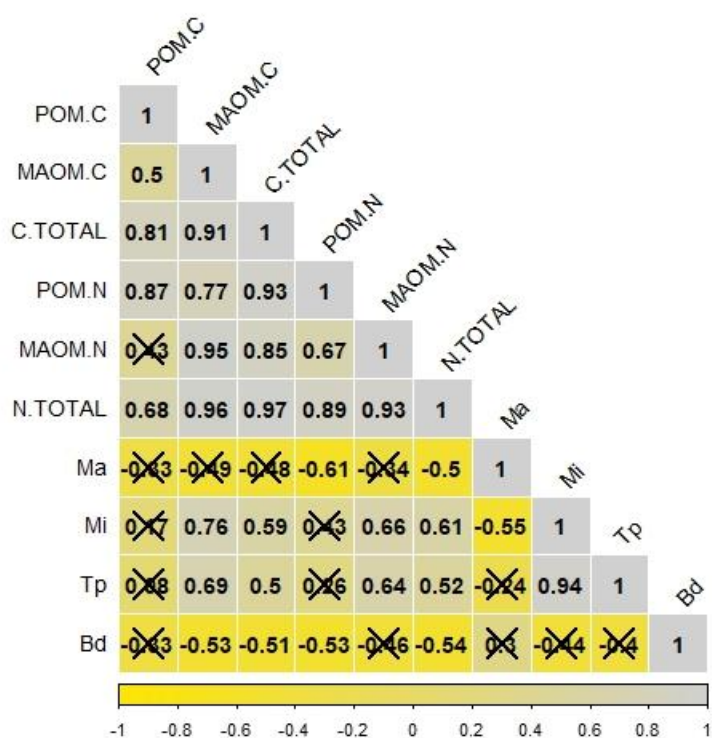
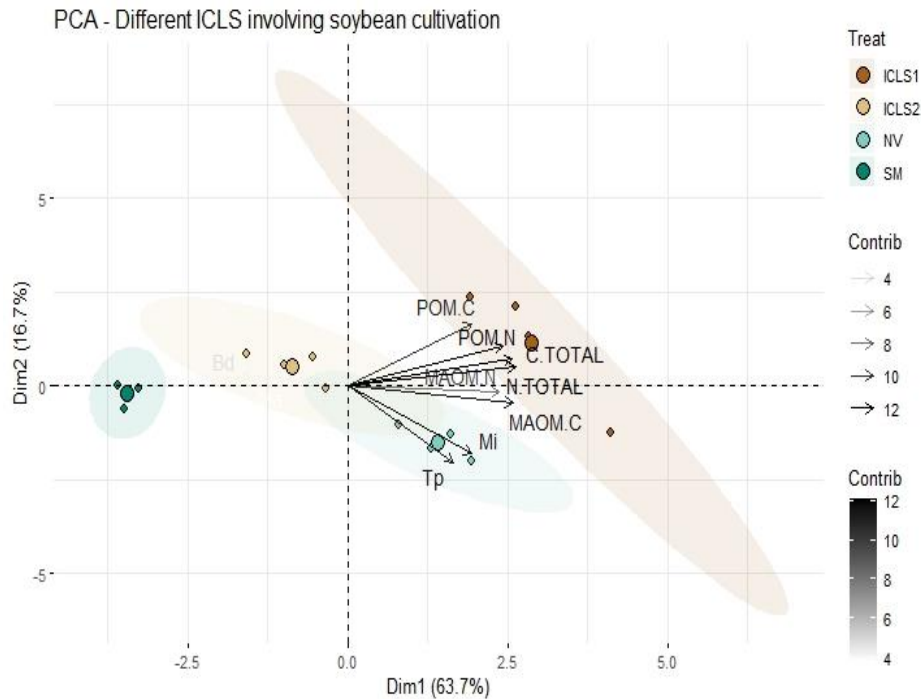


Figura 11 - Principal component analysis (PCA) of the average stocks of soil carbon fractions and soil physical attributes in the 0-0.4 m layer, five years after the adoption of soybean-based integrated crop-livestock systems (ICLS).

POM.C: labile carbon stock; MAOM.C: non-labile carbon stock; C.TOTAL: total carbon stock; POM.N: labile nitrogen stock; MAOM.N: non-labile nitrogen stock; N.TOTAL: total nitrogen stock; Ma: macroporosity; Mi: microporosity; Tp: total porosity; Bd: soil bulk density. NV: native vegetation; SM: soybean monocrop with hibernal fallow; ICLS1: annual soybean cultivation in rotation with grazed ryegrass; ICLS2: annual soybean cultivation in rotation with grazed oat.



4.4. Discussion

The conversion of temperate Pampa grasslands into agricultural systems significantly affects soil C and N dynamics. This trend highlights the vulnerability of the Pampa, the biome with the smallest territorial representation in Brazil (Lanfranco *et al.*, 2022), in the face of recent agricultural expansion, primarily driven by soybean and rice monocrops (Alves, 2024; Caballero *et al.*, 2023). It was observed that ICLS systems exhibited higher C and N stocks and contents compared to SM, indicating that the use of grasses under grazing in rotation contributes to mitigating SOM losses associated with native area conversion. The maintenance or increase of C and N stocks under ICLS is related to the continuous input of plant residues and the greater stability of non-labile soil fractions. These results suggest that the adoption of ICLS can reduce the negative impact of agricultural expansion in the Pampa, functioning as

an effective strategy for C sequestration and improvement of soil physical quality. These findings corroborate previous studies highlighting the role of integrated systems in conserving C and N in subtropical and temperate environments (Carvalho *et al.*, 2016; Koga *et al.*, 2020), reinforcing the importance of sustainable agricultural practices for mitigating greenhouse gas emissions (Lal, 2004; Bonannella *et al.*, 2023).

4.4.1 C and N contents in SOM physical fractions

In the present study, we observed that the conversion of NV into different soybean production systems influenced total, labile, and non-labile C and N contents, particularly in the surface soil layer (Table 5). The decreasing trend of C and N contents with soil depth aligns with previous reports from ICLS studies (Piva *et al.*, 2020; Liebig *et al.*, 2020). Broadly, TOC and TN compartments result from processes of C and N input, loss, and stabilization in the soil, which are determined by their recalcitrant and bioavailable fractions (Jastrow *et al.*, 2007; Cotrufo *et al.*, 2019). These fractions exhibit distinct dynamics in the environment and are influenced by multiple factors, collectively contributing to a more comprehensive understanding of soil C (Cotrufo *et al.*, 2019). In this context, our findings are consistent with previous studies in native areas with high clay content that were converted into croplands (Cotrufo *et al.*, 2019; YU *et al.*, 2022). Notably, in our study, the high TOC and TN contents in the topsoil (0-0.1 m) under ICLS were directly associated with elevated levels of labile C and N fractions at the same depth, whereas contents decreased significantly in deeper layers (Table 5). These labile fractions are generally sensitive to land-use and management changes (Hondroudakos *et al.*, 2024). Additional factors contributing to these results include the presence of grazing animals and the physical characteristics of the evaluated soil profile, which, beyond being deep (~150 cm), exhibited good structure, high porosity, and a clayey texture, as previously noted. These conditions directly and indirectly affect soil C and N contents by promoting biomass accumulation, SOM incorporation, stabilization, and protection, while reducing mineralization rates, thus creating C and N accumulation gradients. Moreover, grazing animals can modify soil dynamics by enhancing forage regrowth and supplying organic inputs through excreta (Alves *et al.*, 2025; Li *et al.*, 2025). Conversely, the lowest C and N contents were observed under monocrop (Table 5). In these systems, the soil is continually disturbed by tillage, which

may have contributed to C and N losses through increased mineralization (Huang *et al.*, 2024). Similar reductions in TOC under monocrop have been reported by Nicolas *et al.* (2022) and Machado *et al.* (2024). Significant variations were also observed among different ICLS compositions (Table 5), likely attributable to the higher residue input from ryegrass compared to common oat. Ryegrass generally provides greater above- and below-ground biomass and has chemical properties that confer higher resistance to decomposition, creating conditions more favorable for C and N accumulation in the soil (Choi *et al.*, 2023). The high root biomass contribution is a key factor for increasing soil C, as root-derived C represents the most effective source for SOM formation, explaining the pronounced differences observed between the evaluated systems.

4.4.2. Effects of land use on soil C and N stocks

The conversion of NV into soybean production fields significantly affected soil C and N stocks in the 0-0.4 m layer (Figures 7a and 7b). The cultivation of legumes combined with cattle grazing altered the budgets and distribution of SOM forms, creating differentiated gradients across the evaluated soil layers. In more dynamic production systems, changes in C and N stocks relative to grain monocrops are generally associated with the input of winter cover crops, as observed in our study in ICLS1 and ICLS2; however, little attention is usually given to the source and quality of the plant residues added to the system (Amelung *et al.*, 2020; Fujisaki *et al.*, 2018). Selecting forages with high root development potential may be key to increasing soil C and N inputs. Studies show that the highest soil C and N accumulations are directly linked to root system decomposition (Alves *et al.*, 2025; Yang *et al.*, 2023). In addition to annual renewal of plant biomass through crop rotation, cattle grazing stimulates tillering and vegetation renewal, increasing aboveground and root residue deposition (Alves *et al.*, 2025). Another factor influencing the C cycle, and consequently C storage, is the soil's physical properties, particularly density and porosity (Chirol *et al.*, 2025). Pedological horizons with higher clay content (clayey profiles) also favor SOC stabilization by providing greater protection to its fractions (Bayer *et al.*, 2006). In our study, the soil was classified as Argissol, characterized by high clay content, which plays a fundamental role in SOM stabilization. Fine clay particles provide both physical and chemical protection of C by forming organo-mineral complexes and

microaggregates, limiting microbial access and SOM decomposition (Six *et al.*, 2002). Moreover, the presence of iron and aluminum oxides typical of these soils enhances adsorption of organic compounds, reinforcing chemical protection of C and contributing to the maintenance of higher C stocks in the evaluated systems (Lehmann and Kleber, 2015). This may be particularly relevant for SM, which, although providing lower C stocks compared to more dynamic systems, exhibited values similar to those reported by the MapBiomass Project, which indicates an average of 49 Mg C ha⁻¹ (0-30 cm) in areas of native vegetation converted to different land uses in the South American Pampa biome. Despite lower stocks in SM compared to ICLS1, ICLS2, and NV, the results suggest that soil and management factors can partially mitigate C losses associated with the conversion of native areas to other land uses. Similarly, N stocks followed patterns analogous to C, reflecting the high correlation between C and N (Li *et al.*, 2023). Microbial mineralization uses C as an energy source and N as an essential element for biomass synthesis, explaining their positive correlation. Thus, the C:N ratio of residues influences decomposition rate and the accumulation of labile C fractions in the soil (Chen *et al.*, 2018). Our findings complement previous studies conducted in the Pampa biome (Machado *et al.*, 2024; Nicolas *et al.*, 2022). High rates of C depletion under SM, resulting in reduced soil C storage capacity, have also been observed in Argentine Pampean grasslands, where significant C losses occurred in native areas converted to grain production, especially under intensive soybean cultivation (Nicolas *et al.*, 2022). Similarly, Machado *et al.* (2024) investigated C stocks in southern Brazilian grasslands, comparing native areas with croplands. The study encompassed different soil classes (Kandiudox, Hapludox, Hapludult, Argiudoll), and no significant differences were observed between C stocks under ICLS and native vegetation. The management practices applied in those studies were similar to our investigation, with annual soybean cultivation in summer and cover crops such as ryegrass and oats in winter, grazed throughout most of spring. Higher labile C and N stocks in the surface layers can be associated with the deposition of higher-quality plant residues, as occurs in ICLS integrating leguminous and winter grass species (Figures 8a and 8b). As previously noted, POM-C and POM-N respond sensitively to land use and management changes, quickly reflecting the effects of different agricultural practices or production systems (Hondroudakakis *et al.*, 2024). Generally, legumes contribute rapidly decomposable organic material due to their lower C:N ratio, promoting microbial activity and C and N release in the soil. This process tends to

increase the labile C fraction, as accelerated decomposition generates less stabilized organic compounds more susceptible to mineralization (Wu *et al.*, 2022). Conversely, grasses under grazing provide higher inputs of soluble compounds and fine roots, also supporting the more dynamic SOM fraction. Together, these factors explain the elevated labile C contents observed in more complex systems, such as ICLS1 and ICLS2. In addition to residues, vegetation releases root exudates capable of increasing C input, even at greater depths (Panchal *et al.*, 2022). These results reinforce that labile SOM fractions are highly sensitive to management practices in integrated systems. Accordingly, POM-C, due to its rapid turnover, constitutes an important energy source for soil microorganisms, playing a key role in nutrient cycling and maintaining soil fertility and quality (Koudahe *et al.*, 2022). As POM-C decomposes, it associates with MAOM, enhancing C protection and stability, as C becomes less available for microbial decomposition (Cotrufo *et al.*, 2013). This was evident in our results, with MAOM-C stocks increasing relative to POM-C, particularly at greater depths (Figures 8a and 8b).

4.4.3 Physical attributes

The results indicated variations in soil porosity and bulk density depending on the production systems and soil layers evaluated (Figures 9a, 9b, 9c, and 9d). In the surface layer (0-0.10 m), a noticeable reduction in macropore volume was observed under monocrop, a trend that reversed at greater depths, where conventional management led to increased macroporosity. These changes can be attributed to the use of implements such as harrows and plows in soil preparation under SM, which disturb the 0-0.20 m layer, altering the structural arrangement and directly impacting macropore distribution and volume (Silva *et al.*, 2022). Micropore formation is closely linked to the arrangement of primary soil particles, such as clay and SOM, as well as soil structure and microbial activity. During SOM decomposition, microorganisms secrete substances that promote soil aggregation and the formation of micropores within aggregates. More stable systems, such as NV, with years of biomass accumulation and well-structured soil, facilitate the formation of a broad range of pore sizes, contributing to soil physical quality (Yudina & Kuzyakov, 2023). While macropores support the entry and incorporation of plant residues into the soil,

micropores provide protection to the existing C fractions in the profile. Assessing microporosity is particularly important in compaction studies, as it determines the amount of water the soil can retain, which in turn affects root growth and crop productivity (Lima *et al.*, 2006). The importance of total porosity quantification is related to studies on water and air storage and movement in the soil, and consequently to root system development (Liu *et al.*, 2020). Bulk density, therefore, provides critical information about soil conservation status, particularly its influence on properties such as water infiltration and retention, root development, gas exchange, and susceptibility to erosive processes, being widely used to evaluate soil compaction and/or densification (Coelho *et al.*, 2020a, 2020b)

4.4.4 Carbon Indicators

The results indicate that the conversion of NV into ICLS significantly altered soil C dynamics, as evidenced by the CL, CLI, CSI, and CMI indicators (Table 6). NV, used as a reference, exhibited low CL and CLI values, but CSI remained stable along the profile, reflecting a more recalcitrant C fraction resistant to decomposition. This pattern confirms the higher resilience of soils under native vegetation to land-use changes (Guo and Gifford, 2002). In the surface layer (0-0.025 m), ICLS1 and ICLS2 exhibited the highest CL and CLI values, indicating a greater proportion of labile C. Among the integrated systems, ICLS2 stood out, suggesting that this species promotes the incorporation of more easily mineralizable C. The increase in CL reflects higher nutrient availability and microbial activity, resulting in improvements in aggregation, cation exchange capacity, and soil structure. These effects are consistent with the physical gains discussed previously (Figures 9a-9d). In contrast, CSI was higher in ICLS1, indicating greater total C accumulation in the surface layer compared to SM. This trend supports studies showing that integrating annual crops with pastures increases the input of organic residues and root biomass, promoting higher C stocks and lability in the soil (Alves *et al.*, 2025). In the subsurface layers (0.025-0.1 m), CMI remained high in the ICLS systems, whereas SM values were significantly lower, particularly at 0.05-0.075 m (29.7%). This demonstrates that ICLS helps maintain and enhance C sequestration potential in deeper layers, while monocrop reduces both the quality and quantity of available C. In the intermediate and deep layers (>0.1 m), ICLS2 showed

the highest CMI, notably at 0.1-0.15 m (437.3%), indicating greater C incorporation at depth. This result is likely due to higher root biomass and organic residue deposition from oats throughout the profile. Such C redistribution was not observed in SM, reinforcing the positive effect of integrating forage crops on soil C conservation (Lal, 2021). CMI proved to be a sensitive indicator for distinguishing the effects of management systems on C dynamics. Higher values in ICLS1 and ICLS2 reflect greater sequestration potential and better soil C quality, whereas SM exhibited significant reductions, suggesting loss of labile C and lower system sustainability. Beyond the absolute values, differences in CMI between land uses highlight the impact of management on C balance. These findings corroborate studies demonstrating that diversified systems with permanent vegetation cover promote higher SOM stocks and stability (Guo and Gifford, 2002; Zanatta *et al.*, 2019). Additionally, forage quality strongly influenced the surface layer, with ICLS2 promoting greater lability and CMI than ICLS1. This demonstrates that the choice of forage species directly affects C dynamics and highlights the importance of management strategies that maximize C sequestration and the sustainability of integrated systems (Lal, 2020).

4.5. Conclusion

Integrated systems involving soybean cultivation in succession with winter cover crops under grazing increase the labile, non-labile, and total fractions of C and N in temperate South American grasslands converted into croplands. The use of ICLS increases, on average, labile C, non-labile C, and total C stocks at a depth of 0-0.4 m by 71%, 48%, and 56%, respectively, compared to soybean monocrop.

Bulk density, soil porosity, and the Carbon Management Index were influenced by ICLS; however, the successive variations across soil layers indicate the need for long-term studies.

These results demonstrate that sustainable intensification through ICLS represents a promising strategy to reconcile agricultural production with the maintenance of soil carbon and nitrogen stocks in subtropical areas of the Brazilian Pampa biome.

5. CONSIDERAÇÕES FINAIS

Sistemas de integração lavoura-pecuária constituem uma estratégia eficiente para melhorar a qualidade do solo e aumentar os estoques de carbono e nitrogênio em ambientes subtropicais do sul do Brasil. Em comparação aos monocultivos, os sistemas integrados proporcionaram incrementos expressivos nos estoques de carbono total na camada de 0-40 cm, alcançando aumentos de 22% em áreas com arroz irrigado e 56% em áreas com soja

Os aumentos observados nas frações MOP e MAM da matéria orgânica indicam que os sistemas integrados favorecem tanto o aporte quanto a estabilização do carbono ao longo do perfil do solo, contribuindo para a manutenção da resiliência dos agroecossistemas.

De modo geral, os resultados confirmam que a integração lavoura-pecuária é uma estratégia eficaz para aumentar o estoque e a estabilidade do carbono no solo, contribuindo para a sustentabilidade produtiva e para a mitigação das mudanças climáticas em sistemas agrícolas subtropicais.

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Anexos

Anexo A - Tabela Suplementar referente ao Capítulo I

Table S1. Description of cultivation parameters for integrated crop-livestock production systems in lowland areas in southern Brazil. Corticeiras Farm - Cristal/RS.

Crop	Seeding density (¹ Kg ha ⁻¹ / ² Plant m ²)	Row spacing (m)	Variety (anos)	Sowing	Harvest
Rice	100 ¹	0.17	IRGA 424® (2013/14 - 2014/15) IRGA 430® (2015/16) IRGA 424 RI® (2016-2020)	October	April
Soybean	30 ²	0.45	TEC IRGA 6070 RR® (2013/14- 2017/2018) BS IRGA 1642 IPRO® (2018/19- 2020)	November	April
Ryegrass	30 ¹	a	BRS Ponteio®	May	-
White clover	4 ¹	a	BRS URS Entrevero®	June	-
Persian clover	8 ¹	a	BRS Resteveau®	July	-
Bird's-foot trefoil	8 ¹	a	São Gabriel®	September	-

Anexo B - Tabela Suplementar referente ao Capítulo I

Table S2. Soil bulk density at depths of 0-0.1, 0.1-0.2, and 0.2-0.3 m under different Integrated Crop-Livestock Production Systems in lowland areas. Corticeiras Farm - Cristal/RS, Brazil.

Soil depth (m)	Integrated Crop-Livestock Systems				
	S1	S2	S3	S4	S5
Bulk Density (Mg m ⁻³)					
0-0.1	1.5	1.6	1.5	1.4	1.3
0.1-0.2	1.7	1.6	1.6	1.6	1.5
0.2-0.4	1.7	1.7	1.7	1.6	1.6