

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE AGRONOMIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA DO SOLO

**ESTABILIZAÇÃO E SATURAÇÃO DE CARBONO EM SOLO SUBTROPICAL
EM PLANTIO DIRETO POR LONGO PRAZO**

**Lucas Antonio Telles Rodrigues
(Tese de Doutorado)**

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Engenheiro-Agrônomo (UNIPAMPA)

Tese apresentada como um dos
requisitos à obtenção do Grau de
Doutor em Ciência do Solo

Porto Alegre (RS) Brasil
Junho de 2021

CIP - Catalogação na Publicação

Telles Rodrigues, Lucas Antonio
ESTABILIZAÇÃO E SATURAÇÃO DE CARBONO EM SOLO
SUBTROPICAL SOB PLANTIO DIRETO POR LONGO PRAZO / Lucas
Antonio Telles Rodrigues. -- 2021.
86 f.

Orientador: Cimélio Bayer.

Tese (Doutorado) -- Universidade Federal do Rio
Grande do Sul, Faculdade de Agronomia, Programa de
Pós-Graduação em Ciência do Solo, Porto Alegre, BR-RS,
2021.

1. Saturação de C. 2. Estabilização de C. 3.
Plantio direto. 4. Qualidade dos resíduos. 5. Isótopo
13C. I. Bayer, Cimélio, orient. II. Título.

LUCAS ANTONIO TELLES RODRIGUES
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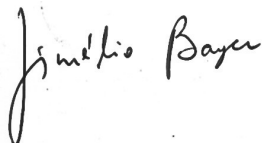
TESE

Submetida como parte dos requisitos
para obtenção do Grau de

DOCTOR EM CIÊNCIA DO SOLO

Programa de Pós-Graduação em Ciência do Solo
Faculdade de Agronomia
Universidade Federal do Rio Grande do Sul
Porto Alegre(RS), Brasil

Aprovado em: 18/06/2021
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“Essentially, all life depends upon the soil... There can be no life without soil and no soil without life; they have evolved together.”

- Dr. Charles E Kellogg

*Dedico a minha mãe Cleci
Bernadete Rodrigues, e ao meu pai
Noil Telles Rodrigues*

Agradecimentos

À minha família, pelo apoio e incentivo que me dedicaram durante todo o período de desenvolvimento e execução deste trabalho e por entenderem minha ausência muitas vezes.

A minha namorada Renata pelo amor, pelo companheirismo, e apoio nas horas boas e nas ruins, quando se fez presente ao meu lado quando mais precisei.

Ao meu orientador Cimélio Bayer, pela confiança e oportunidade a mim concedida e por todo o apoio em todas as fases da execução das pesquisas e por compartilhar seu magnífico conhecimento acerca da Ciência do Solo.

Ao prof. Sandro Giacomini por todo o apoio e suporte prestado para a realização das análises de ^{13}C e pela contribuição na consolidação das pesquisas.

A prof. Déborah Dick pelo apoio prestado para a realização das análises de fracionamento físico e pelo compartilhamento do seu conhecimento.

Ao Tio Zé, por todo o apoio prestado durante a condução das atividades de pesquisa. És muito prestativo e gente boa.

Ao Marcelo Schmidt, quem me apoiou quando cheguei a Porto Alegre quando eu não conhecia muita coisa, pela amizade, e pelo companheirismo durante os dois anos em que dividimos moradia.

Aos colegas e amigos Cícero, Anaí, Jessica Pereira, Caroline, Jessica Cavalcante, Murilo Veloso, Vitor Ambrosini e Guilherme, pelos vários momentos de compartilhamento de conhecimento e amizade.

Aos bolsistas IC do grupo de pesquisa, em especial ao Geam e ao Alexssander pela ajuda prestada na condução das atividades de pesquisa.

Aos professores do PPGCS da UFRGS, pelas contribuições que proporcionaram o meu crescimento acadêmico.

Ao Departamento de Solos, em nome da Faculdade de Agronomia e da Universidade Federal do Rio Grande do Sul, pela estrutura e recursos que possibilitaram o desenvolvimento deste trabalho e da realização do curso.

À Capes, e ao CNPq e Fapergs, pelo apoio financeiro.

ESTABILIZAÇÃO E SATURAÇÃO DE CARBONO EM SOLO SUBTROPICAL SOB PLANTIO DIRETO POR LONGO PRAZO¹

Autor: Lucas Antonio Telles Rodrigues
Orientador: Prof. Dr. Cimélio Bayer

RESUMO

Solos subtropicais sob plantio direto associado a sistemas de culturas diversificados podem atuar como um grande sumidouro de C. No entanto, pouco se sabe sobre a saturação de C nessas condições, especialmente em Argissolos resilientes. Dois estudos foram conduzidos em experimento de longa duração (36 anos) sobre um Argissolo vermelho no sul do Brasil, os quais tiveram como objetivo (i) avaliar o efeito de sistemas de culturas a base de leguminosas de cobertura de solo em plantio direto no déficit de saturação de C na matéria orgânica associada aos minerais (<20 μ m, DSC_{moM}) e na capacidade remanescente de estabilização de C (estudo 1); (ii) investigar o efeito do DSC_{moM} na estabilização do C recém adicionado, proveniente de resíduos vegetais de diferentes qualidades e enriquecidos com ¹³C, em diferentes frações da matéria orgânica do solo (MOS), em um experimento de incubação no campo por 15 meses (estudo 2). Diferentes frações da MOS foram isoladas por fracionamento físico, e a avaliação de C e do isótopo ¹³C derivado dos resíduos foi realizada nessas frações da MOS no primeiro e no segundo estudo, respectivamente. Os principais resultados indicam que os sistemas de cultura à base de leguminosas de cobertura de solo (lablab, guandu e feijão-caupi) com alta adição de C resultaram em alto acúmulo de C na moM e em níveis de C nesta fração próximos a saturação, principalmente na camada de 0-2,5 cm. Além disso, foi observado um decréscimo no DSC_{moM} em todas as camadas de solo até 20 cm. A capacidade remanescente de estabilização de C na moM diminuiu 90-97% quando comparada ao solo descoberto na camada de 0-2,5 cm. A maior estabilização do ¹³C na moM foi observado no C derivado da ervilhaca do que da aveia na camada superficial do solo (0-2,5 cm), mas apenas quando o solo apresentava alta capacidade de estabilização de C (níveis alto e muito alto de DSC_{moM}). Quando a estabilização do C novo na moM foi limitada pela saturação, a incorporação do ¹³C foi intensificada nas frações intra e inter-agregados. Os resultados destacam que os fatores sistemas de cultivos diversificados, o DSC_{moM} e a qualidade dos resíduos impulsionam a estabilização do C nas camadas superficiais do solo sob plantio direto. Estudos futuros de incubação investigando o efeito da DSC_{moM} na migração de C no perfil de solo sob plantio direto a longo prazo são necessários para entender melhor a dinâmica de sequestro de C neste ambiente.

Palavras-chave: saturação de C, estabilização de C, plantio direto, qualidade dos resíduos, isótopo ¹³C

¹Tese de Doutorado em Ciência do Solo-Programa de Pós-Graduação em Ciência do Solo. Faculdade de Agronomia. Universidade Federal do Rio Grande do Sul. Porto Alegre (87 p.), Junho de 2021.

CARBON STABILIZATION AND SATURATION IN A NO-TILL SUBTROPICAL SOIL IN LONG-TERM²

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ABSTRACT

Subtropical no-till soils combined with diversified cropping systems can act as a large C sink. However, very little is known about the C saturation in these conditions, especially in resilient Acrisols. Two studies were performed based on a long-term (36 years) no-till experiment on a Acrisol in Southern Brazil. These studies aimed to evaluate the (i) effect of long-term no-till legume-based cropping systems on C saturation deficits in mineral-associated organic matter (<20 μ m, CSD_{mOM}) and the remaining capacity of C stabilization (study 1); (ii) investigate the effect of CSD_{mOM} on C stabilization from different quality ¹³C-labeled litters in different fractions of soil organic matter (SOM) in a 15 months *in situ* experiment (study 2). Different fractions of SOM were isolated by physical fractionation, and the assessment of C and litter-derived ¹³C in these SOM fractions were performed in the first and second study, respectively. The main findings indicate that legume-based cropping systems with high C input led to high C stabilization in mineral-associated organic matter (mOM) and resulted in C levels in this fraction close to saturation, mainly in the 0-2.5 cm soil layer. Also, a decrease of CSD_{mOM} was observed in all soil layers up to 20 cm. The capacity to further stabilize carbon in mOM decreased by 90-97% when compared to bare soil in the 0-2.5 cm layer. Higher C stabilization in the mOM was observed for vetch-than oat-derived ¹³C in the 0-2.5 cm, but only when soil presents a high capacity of C stabilization in the mOM fraction (*high* and *very high* levels of CSD_{mOM}). When the litter-derived C stabilization in mOM was limited by its previous C level close to saturation, ¹³C incorporation was intensified in the intra- and inter-aggregate SOM fractions. The results highlight that the factors diversified cropping system, CSD_{mOM} and residue quality drive the C stabilization in surface soil layers of no-till subtropical soils. Future *in situ* studies investigating the effect of C saturation on migration of C in profile of long-term no-till subtropical soils are required to better understand the dynamic of C sequestration in this environment

Keywords: C saturation, C stabilization, no-tillage, residue quality, ¹³C isotope

²Doctoral thesis in Soil Science. Graduate Program in Soil Science, Faculty of Agronomy, Universidade Federal do Rio Grande do Sul. Porto Alegre (87 p.), June 2021.

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CAPÍTULO I – Introdução Geral

À nível global, o carbono orgânico (C) armazenado na matéria orgânica do solo (MOS) é duas vezes maior do que o C contido na atmosfera, e três vezes o C presente na vegetação (LAL, 2016). No contexto das mudanças climáticas em nível global, estimativas recentes indicam que a América do Sul contribui com aproximadamente 31% (0,34 Pg C) (1 Pg= 1 bilhão de toneladas) da emissão anual de gases de efeito estufa - GEE (1.1 Pg C) através das mudanças no uso da terra (GLOOR et al., 2012). Em contrapartida, o potencial de acúmulo adicional de C nos solos desta região foi estimado em aproximadamente 8,24 Pg de C, sendo passível de ser alcançado entre os anos de 2016 a 2050 por meio da adoção de estratégias do plano da Agricultura de Baixa Emissão de C (Plano ABC) (SÁ et al., 2017). Esse potencial de acúmulo de C representa, substancialmente, uma importante contribuição para mitigação das mudanças climáticas.

A identificação de estratégias sustentáveis e eficientes em promover o sequestro de C em compartimentos com elevado tempo de residência no solo (*passive pools*) têm sido objeto de esforços da comunidade científica nas últimas décadas. Nesse sentido, estudos recentes têm mostrado que o manejo de solos agrícolas no sistema de plantio direto (SPD) associado ao uso de plantas de cobertura com alto aporte de resíduos promovem incrementos elevados nos estoques de C no solo, e inclusive favorecem o sequestro de C em compartimentos estáveis do solo, resultando numa mitigação das emissões de GEE quanto comparados a sistemas convencionais ou tradicionais de manejo do solo.

Embora o solo seja um dos principais destino do CO₂ atmosférico capturado via processo de fotossíntese vegetal, fortes evidências sugerem a existência de uma capacidade finita de acúmulo de C no solo, especialmente o C retido em complexos organominerais na fração fina do solo (< 20µm), cuja estabilização baseia-se em processos físico-químicos. A intensidade do processo proteção de C e/ou saturação de C pode variar, especialmente, com tipo de minerais (argilominerais 2:1, 1:1, ou óxidos de Fe e Al, principalmente) e sua proporção no solo. Na condição de saturação de C na fração mineral do solo, pode haver decréscimo na eficiência de sequestro do C no solo. Em contrapartida, evidências indicam que nessa condição o solo continua acumulando C em compartimentos menos protegidos.

A maior parte dos estudos conduzidos para investigar os conceitos de saturação de C no solo foram realizados em regiões de clima temperado, onde predominam minerais 2:1. Por outro lado, em ambiente (sub)tropical, onde predominam minerais 1:1 com presença de óxidos de Fe e Al na fração silte+argila, estudos investigando a saturação do C e seus efeitos são escassos e a maior parte deles foram conduzidos em Latossolos argilosos cujo resultados evidenciaram alta capacidade remanescente de estabilização de C. Além disso, apesar de ser altamente representativo dos solos agrícolas brasileiros, os Argissolos cauliniticos com textura média ou arenosa na camada superficial foram poucos investigados, e evidências sugerem que a saturação de C na fração argila pode ocorrer em períodos relativamente curtos de tempo (17 anos) nas camadas superficiais de solos quando em plantio direto associado a sistemas de cultura com plantas de cobertura com alto aporte de resíduos (DIECKOW et al., 2005).

Em geral, a manutenção dos níveis de matéria orgânica (MO) em solos subtropicais depende de uma adição anual de aproximadamente 10 Mg ha⁻¹ de matéria seca para compensar a taxa de perdas de C pela mineralização da MO pelos microrganismos decompositores (BAYER et al., 2006a). Contudo, além da quantidade, a qualidade do resíduo vegetal pode influenciar positivamente a proporção do C adicionado que é incorporado e estabilizado na MOS. Estudos recentes conduzidos em Argissolo subtropical cultivado com leguminosas de cobertura têm reportado elevado sequestro de C no solo, cujos

efeitos foram atribuídos a alta qualidade dos resíduos dessas espécies leguminosas. Esse efeito tem sido atribuído a alta eficiência do metabolismo microbiano em processar compostos de fácil degradação comparado a compostos mais recalcitrantes. Entretanto, apesar do efeito da qualidade dos resíduos na estabilização de C ser cientificamente bem fundamentado, resultados não conclusivos e bastante variáveis foram reportados na literatura recente, cujo às causas ainda necessitam ser elucidadas. Uma das possíveis explicações baseia-se na hipótese de que o grau de saturação de C no solo, também referido como déficit de saturação de C (DSC) (diferença entre o conteúdo de C no ponto de saturação e o nível de C atual) atua como um mecanismo primeiro no controle de transferência e estabilização do C proveniente de resíduos vegetais de alta e baixa qualidade.

Diante do exposto, evidenciou-se a necessidade de um estudo sistemático sobre tema saturação de C em solos de ambientes (sub)tropicais visando resposta a alguns questionamentos: (i) em que condições e, em que camada do solo pode haver saturação do C em Argissolos em PD sob alto aporte de biomassa vegetal? (ii) existe relação entre saturação de C e os mecanismos de estabilização da MOS? (iii) quais compartimentos da MOS podem saturar nesses ambientes? (iv) o efeito da qualidade dos resíduos vegetais na estabilização do C no solo depende do nível de saturação de C no solo? Compreender melhor esses aspectos pode ajudar a identificar práticas de manejo mais eficientes em aumentar o C em um determinado solo. Além disso, o conhecimento dos níveis de saturação de C ou dos deficit de saturação de C no solo podem ser usados para mapear solos agrícolas quanto ao seu potencial de sequestro de C.

Os objetivos gerais da presente tese foram: (i) Avaliar o impacto de sistemas de culturas com inclusão de leguminosas de cobertura em sistema de plantio direto a longo prazo (36 anos) na saturação do C associado ao minerais da fração fina solo (silt fino+argila, < 20 μ m), no déficit de saturação de C e na capacidade remanescente de estabilização de C; (ii) avaliar o efeito de diferentes deficits de saturação de C e de resíduos de diferentes qualidades no acúmulo do C em diferentes compartimentos da MOS.

CAPÍTULO II – Revisão Bibliográfica

1. Estabilização e saturação de C em solos subtropicais

Os solos formados em ambientes subtropicais são mediana/altamente intemperizados, e diferem quanto as suas características químicas e mineralógicas dos solos formados sob clima temperado, onde o grau de intemperismo é menos intenso. Os Argissolos são solos altamente representativos de regiões subtropicais, ocupando uma área de aproximadamente 1 bilhão de hectares no mundo (FAO, 2006) e aproximadamente 23 milhões hectares no Brasil, o que corresponde a 26% da área superficial do país (EMBRAPA, 2006). A mineralogia predominante nos Argissolos é composta por caulinita e óxidos de Fe e Al (PEDRON; LOURENZI; CERETTA, 2018). A caulinita é um mineral 1:1 não expansivo, e possui baixa área superficial específica (ASE), $\sim 15 \text{ m}^2 \text{ g}^{-1}$ de argila. Por outro lado, dentre outras características morfoestruturais, a baixa cristalinidade e alta porosidade (poros $< 10 \text{ nm}$) dos óxidos de Fe e Al os conferem maior ASE do que na caulinita, sendo reportado valores de 200 e $800 \text{ m}^2 \text{ g}^{-1}$ de óxido para goetita e ferridrita, respectivamente (KLEBER et al., 2015). Portanto, mesmo em menor proporção do que a caulinita, essas características dos óxidos de Fe e Al conferem aos solos tropicais e subtropicais maior ASE ($\sim 40 \text{ m}^2 \text{ g}^{-1}$ solo) do que é comumente reportado para a caulinita.

Os compostos orgânicos interagem com os grupos funcionais da fração mineral do solo por meio de uma série de mecanismos, incluindo adsorção física ou interação através das forças de Van der Waals, interações eletrostáticas (troca catiônica e aniônica), pontes de cátions pelo qual o metal polivalente forma

uma ponte entre a molécula orgânica e a superfície inorgânica dos minerais, pontes de hidrogênio, entre outras (ESSINGTON, 1997). O tipo de mecanismo predominante na interação MO-argilomineral é controlado, principalmente, pelo tipo de mineral presente, sua abundância e pelas características de carga dos grupos funcionais de superfície (KLEBER et al., 2015; SARKAR et al., 2018). Na caulinita interação das moléculas orgânicas com o grupos funcionais, e.g. com o silanól ocorre principalmente por complexo de esfera externa, sendo essa interação de baixa estabilidade (KLEBER et al., 2015). No entanto, nos óxidos de Fe e Al ocorrerem ligações covalentes entre os grupos funcionais dos óxidos e os grupos funcionais da MOS, e.g. o carboxílico com o ferrol dos óxido de ferro, as quais apresentam elevadas constantes de estabilidade (KLEBER et al., 2015). Portanto, essas interações são mais significativas do que aquelas que se verifica com a fração mineral de solos temperados, onde as reações predominantes envolvem interação eletrostática e.g. pontes de cátions (KÖGEL-KNABNER et al., 2008). Nesse contexto, essas propriedades podem influenciar a capacidade de proteção exercida pelo solo no C e são limitadas por suas características, o que é consistente com o fenômeno de saturação de C (HASSINK, 1997; SIX et al., 2002a; STEWART et al., 2008a).

1.1 Mecanismos de estabilização da matéria orgânica do solo

A MOS é protegida da decomposição microbiana em diferentes níveis pela atuação de três principais mecanismos de proteção no solo, sendo: (i) Recalcitrância bioquímica das moléculas orgânicas; (ii) Proteção física pelos agregados do solo; e (iii) Proteção química pela associação do C com os argilominerais (i.e. silte, argila, óxidos de Fe e Al) (SOLLINS; HOMANN; CALDWELL, 1996).

A recalcitrância bioquímica têm sido considerada de pouca importância na proteção da MOS por longo tempo no solo (MARSCHNER et al., 2008; MIKUTTA et al., 2006), pois os microrganismos são capazes de decompor substâncias complexas, como a lignina, desde que satisfeitas as condições necessárias para atividade microbiana como a disponibilidade de C solúvel (KLOTZBÜCHER et al., 2011).

A proteção física do C nos agregados dos solos, em especial nos microagregados (DENEFF et al., 2007) ocupa uma posição intermediária em

termos de importância na resistência da MOS a decomposição microbiana ao longo do tempo, sendo atribuída a (i) compartimentação do substrato e da biomassa microbiana (inacessibilidade espacial) e (ii) redução da difusão de oxigênio que conduz a uma atividade reduzida de microrganismos (SIX et al., 2002a). Em síntese, esse mecanismo atua protegendo o C da decomposição microbiana devido a restrição de acesso e a presença de condições não favoráveis ao crescimento microbiano nos componentes estruturais do solo.

A estabilização química do C pelas associações com os argilominerais têm sido considerada o principal mecanismo que atua na proteção da MOS por longo tempo (KLEBER, 2010; KLOTZBÜCHER et al., 2011) e que controla a máxima capacidade que o solo tem em sequestrar C (HASSINK, 1997; SIX et al., 2002a; STEWART et al., 2008a). Como descrito acima, o tipo de ligação e sua estabilidade varia como o tipo de argilomineral presente no solo. Portanto, a magnitude de atuação desse mecanismo depende consideravelmente do tipo de solo, o que inclui a textura e mineralogia.

1.2 Sequestro de C em solos tropicais e subtropicais sob plantio direto

O sequestro de C no solo pode ser influenciado por muitos fatores, incluindo o clima regional, as propriedades físicas e químicas do solo e as práticas de manejo. No que se refere as práticas agrícolas de manejo, cabe destaque ao tipo de preparo (i.e preparo convencional, plantio direto), que têm efeitos significativos nos estoques (BAYER et al., 2006a; VELOSO et al., 2018) e sequestro de C no solo (VELOSO; CECAGNO; BAYER, 2019). O elevado grau de perturbação no solo sob preparo convencional (PC) afeta negativamente o C, aumentando o *turnover* dos macroagregados e diminuindo a proteção da matéria orgânica particulada (MOP) da decomposição microbiana. Por outro lado, em sistema de plantio direto (SPD) o ambiente menos oxidativo devido ao baixo grau de perturbação do solo aumenta a labilidade da MOS, evidenciado pela relação entre compartimentos lábeis e não lábeis (DIECKOW et al., 2005). Nesse sistema há, também, aumento na formação de agregados estáveis, o que propicia efeitos positivos no acúmulo de C pelo efeito da proteção física (CONCEIÇÃO; DIECKOW; BAYER, 2013; DENEFF, SIX, JOHAN; MERCKX, 2004) e da proteção química por favorecer o acúmulo de C por interação

organomineral (CONCEIÇÃO; DIECKOW; BAYER, 2013; VELOSO; CECAGNO; BAYER, 2019). Outros fatores como o cultivo de leguminosas de cobertura em SPD e manejo da fertilidade do solo influenciam positivamente o aumento do C no solo (BAYER et al., 2009; FERREIRA et al., 2018; VELOSO et al., 2018).

A formação de agregados nos solos tropicais inclui a atuação e interação de agentes biológicos e processos físico-químicos, conforme mostrado na revisão de SIX et al., (2002a). Os agregados formados biologicamente provêm da interação de um conjunto de fatores, incluindo a (i) atuação de fungos e bactérias, (ii) o crescimento, produção de mucilagens e exsudação de compostos orgânicos pelas raízes e (iii) a atuação da fauna do solo. A formação de agregados por processos físico-químicos é uma particularidade química-mineralógica dos solos tropicais e subtropicais. A presença de óxidos de Fe e Al induz a formação de agregados pela interação química com a caulinita, devido as diferenças nos pontos de carga zero desses minerais que fazem com que predominem cargas negativas nos grupos funcionais da caulinita e positiva nos óxidos em condições de pH dos solos agrícolas. Esse processo favorece a formação de macroagregados devido a atração eletrostática das partículas desses dois argilominerais durante os ciclos de umedecimento e secagem do solo. Entretanto, os agregados formados por processos físico-químicos são menos funcionais e menos estáveis comparado aos agregados formados biologicamente (SIX et al., 2002b)

Em condições tropicais e subtropicais alguns estudos tem demonstrado efeito positivo do plantio direto e alto aporte de resíduos na proteção física da matéria orgânica, devido a oclusão de C nos agregados, em especial nos microagregados (CONCEIÇÃO; DIECKOW; BAYER, 2013; DENEFF, SIX, JOHAN; MERCKX, 2004; DENEFF et al., 2007). A fragmentação da MOP grossa em MOP mais fina pelos microrganismos no interior dos macroagregados e a formação de microagregados envolvendo a MOP fina dentro dos macroagregados foi proposto por SIX et al., (2002a). Os microagregados protegem MOP fina da rápida decomposição microbiana, permitindo a formação de associações organominerais (CONCEIÇÃO et al., 2013). DENEFF, SIX, JOHAN; MERCKX, (2004) comparando o SPD ao PC em um Latossolo de Passo Fundo, RS, observarem que a MOP fina contida nos microagregados dentro dos

macroagregados correspondem a 50% do C total na camada de 0-5 cm, e que aproximadamente 85% do C total corresponde ao C associado aos minerais nos microagregados na camada de 0-20 cm. Em adição, CONCEIÇÃO et al., (2013) avaliando um Argissolo sob plantio direto com inclusão de leguminosas de cobertura verificaram que a maior parte do C estabilizou por associações organominerais e que a oclusão do C nos microagregados é de igual importância. As leguminosas de cobertura têm papel importante nesse efeito. VELOSO; CECAGNO; BAYER, (2019) em um estudo posterior no mesmo experimento, verificaram que as leguminosas de cobertura favorecem a formação de associações organominerais nos microagregados. Portanto, juntos esses resultados sugerem que em solos tropicais e subtropicais sob SPD a proteção física e a proteção química do C são os mecanismos predominantes na estabilização da MOS, com destaque para o acúmulo de C nos microagregados.

1.3 Saturação e estimativa da capacidade protetiva de C no solo

O termo saturação de C no solo refere-se ao conteúdo limite de C o qual o solo é capaz de acumular, a partir dessa condição havendo aumento da adição de C, não há resposta em aumento nos estoques de C no solo, ou em um compartimento específico que compõe parte do C do solo como um todo (SIX et al., 2002a; STEWART et al., 2007). Em condições naturais em que as entradas e saídas de C são equivalentes e permanecem constantes no tempo, o C do solo encontra-se no estado estável (*steady state*) (STEWART et al., 2007). Por outro lado, quando o solo é convertido da condição natural para lavoura, às práticas de manejo, especialmente o sistema de preparo e os sistemas de cultivos, determinam alterações nas entradas e saídas de C no solo e conseqüentemente os estoques de C respondem negativa ou positivamente (WEST; SIX, 2007). Após longo período (> 20 anos) sob manejo constante, o solo alcança um nível de C correspondente a um novo *steady state* (Figura 1), condição na qual as entradas e saídas de C são equivalentes (WEST; SIX, 2007), porém a magnitude das alterações no conteúdo de C do solo dependem especialmente do efeito das práticas de manejo nas entradas e saídas de C no solo ao longo do tempo (STEWART et al., 2007).

Práticas de manejo que reduzem as perdas de C, e aumentam a adição determinam um conteúdo de C no solo na condição *steady state* acima do nível anterior (Figura 1), e múltiplos níveis de C na condição *steady state* podem ser alcançados (STEWART et al., 2007; WEST; SIX, 2007). Entretanto, na condição em que o C do solo não aumenta proporcionalmente ao aumento da adição de C imposta pelas mudanças nas práticas de manejo na condição *steady state*, esse fenômeno é indicativo do efeito da saturação no acúmulo de C (STEWART et al., 2007; WEST; SIX, 2007). Esse entendimento é válido para o conteúdo de C nos diferentes compartimentos da MOS (SIX et al., 2002a).

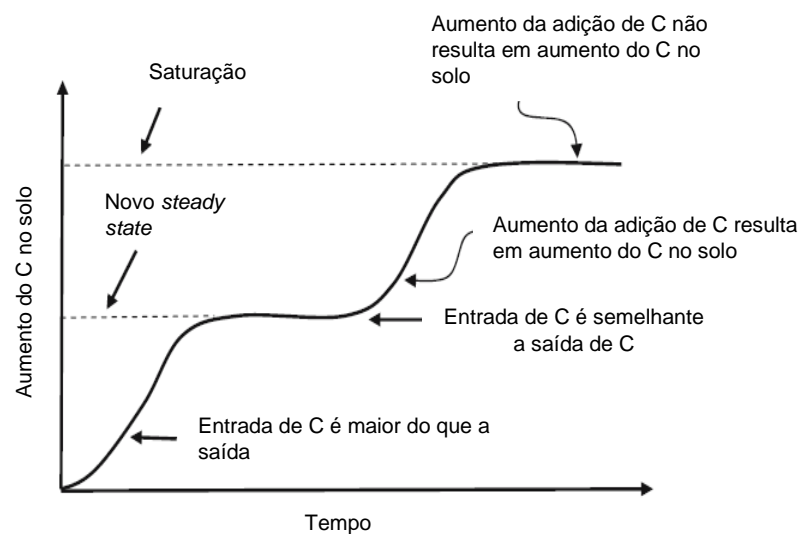


Figura 1. Dinâmica do C no solo após mudanças no manejo destinados a sequestrar C. Um novo *steady state* no solo C é alcançado após uma mudança no uso da terra. Se o solo estiver saturado de C, não ocorrerá acúmulo no solo após uma mudança no uso da terra destinada a sequestrar C. Se o solo não estiver saturado de C, novos *steady states* poderão ser alcançados antes da saturação de C após mudanças sucessivas no manejo. Adaptado de WEST; SIX, (2007)

Apesar de ser extremamente importante no contexto agronômico e ambiental, o tema saturação de C no solo vem sendo modestamente estudado pela comunidade científica a partir do estudo de HASSINK (1997) “*The capacity of soils to preserve organic C and N by their association with clay and silt particles*”. Nesse estudo, o autor avaliou a relação entre a textura do solo e a quantidade máxima de C e N que pode ser sorvida na fração silte e argila a partir de um conjunto de solos não cultivados e sob pastagem natural. Além disso, foi

verificado relação linear entre a proporção da fração mineral fina do solo ($< 20 \mu\text{m}$, %) e a concentração de C nessa fração e sugeriu o uso da inclinação da equação linear ($4.09 + 0.37 \times (< 20 \mu\text{m}, \%)$) como a máxima capacidade de sequestro de C (37 g C kg^{-1} de fração $< 20 \mu\text{m}$) e a proporção da fração fina no solo inteiro ($< 20 \mu\text{m}$, %) para estimar a capacidade protetiva dos solos em nível global (ANGERS et al., 2011; GEUSS; WIESMEIER; HU, 2014; MEYER et al., 2017).

Entretanto, cabe ressaltar que Hassink não observou diferenças significativas na capacidade de sequestro de C entre os solos com mineralogia predominantemente compostas por minerais 2:1 e 1:1 sob climas temperado e tropicais, o que contradiz alguns estudos porque os minerais 2:1 e 1:1 diferem largamente na sua área superficial específica, quantidade e tipos de grupos funcionais capazes de sorver compostos orgânicos (KAISER; GUGGENBERGER, 2003; KLEBER et al., 2015; MIKUTTA et al., 2007). Além disso, não há informações sobre o grau de saturação dos solos utilizados no estudo de Hassink. Portanto existem incertezas sobre a eficácia da equação de Hassink em estimar a capacidade protetiva de C dos solos em nível global (SIX et al., 2002b).

A estimativa da capacidade protetiva de C dos solos foi revisada, utilizando um amplo conjunto de dados, os quais incluíram solos sob diferentes usos e mineralogia contrastante, por SIX et al., (2002b). Nesse estudo os autores verificaram diferenças nos parâmetros das regressões para o tamanho das partículas (< 53 e $< 20 \mu\text{m}$), mineralogia 2:1 e 1:1 e uso (agricultura, floresta e pastagem). Além disso, o conceito de saturação de C da fração mineral ($< 53 \mu\text{m}$ ou $< 20 \mu\text{m}$) foi estendido para o solo inteiro, incluindo compartimentos da MOS protegidos por diferentes mecanismos e com diferentes tempos médios de residência.

Entretanto, posteriormente FENG; PLANTE; SIX, (2013) mostrou que os valores da inclinação das equações lineares de HASSINK, (1997) e SIX et al., (2002b) (0.37 e 0.21-41, respectivamente) podem subestimar a estimativa da capacidade protetiva dos solos por serem relativamente baixos comparados a algumas observações reportadas na literatura. Nesse contexto, os autores testaram dois métodos adicionais para melhorar a estimativa da capacidade

protetiva dos solos, sendo o método de carregamento de C “*C loading*” e análise de linha limítrofe “*boudary line analysis*”.

Conforme FENG; PLANTE; SIX, (2013), o método *C loading* considera 1 mg C m⁻² de área superficial específica como máxima quantidade de C que pode ser estabilizada nas superfícies minerais. Essa quantidade foi baseada em estudos de sedimentos marinhos considerando uma monocamada de sorção nos minerais. Além disso, esse método é baseado em observações de que a área superficial específica dos minerais é positivamente correlacionada com o C no solo e na fração mineral (WISEMAN; PUTTMANN, 2005). Por sua vez, o método *boudary line analysis* é a técnica usada para estimar o limite inferior e superior em resposta a uma variável independente. Conforme descrito por FENG; PLANTE; SIX, (2013), os pontos no limite superior do ajuste da reta entre o C presente na fração < 20 µm e a proporção dessa fração no solo inteiro (%) representam os solos que atingiram o máximo de C estabilizado na fração mineral e, portanto, os parâmetros da equação linear gerada a partir desse conjunto de dados pode ser mais apropriada para estimar a capacidade protetiva dos solos do que aquelas reportadas nos estudos de Hassink e Six.

1.4 Evidências de saturação de C no solo

A ocorrência da saturação de C no solo e seus reflexos na dinâmica e estabilização do C no solo têm sido testadas nas últimas décadas. Contudo, a maior parte das observações provêm de solos sob clima temperado, cuja mineralogia é predominantemente composta por argilominerais 2:1 (CHUNG et al., 2010; DI et al., 2017; GULDE et al., 2008; KONG et al., 2005; STEWART et al., 2008b). Embora o aumento linear do C do solo e/ou em diferentes compartimentos da MOS seja frequentemente observado (BAYER et al., 2006a; KONG et al., 2005; VIEIRA et al., 2009), o aumento assintótico da concentração de C em diferentes classes de agregados, e especialmente com o C associado aos minerais têm sido reportada devido ao efeito do fenômeno de saturação de C (GULDE et al., 2008; STEWART et al., 2008a). Contudo, as evidências de saturação de C no solo inteiro são escassas e foram observadas especialmente em solos de clima temperado (CAMPBELL et al., 1991; CHUNG; GROVE; SIX, 2008). Esses resultados são indicativos da capacidade finita de acúmulo de C no solo (HASSINK, 1997; STEWART et al., 2007). Quanto mais

próximo da saturação, menor será eficiência de acúmulo adicional do C no solo na condição *steady state* (STEWART et al., 2007).

Em geral, as frações menos protegidas da MOS, i.g. a MOP, respondem linearmente ao aumento na adição de C, o que sugere a ausência de saturação nesses compartimentos (SANTOS et al., 2011; STEWART et al., 2008a). Além disso, na condição de saturação do C associado aos minerais, um aumento exponencial crescente da MOP pode ocorrer (DIECKOW et al., 2005). GULDE et al., (2008) isolaram diferentes compartimentos da MOS em um solo no Canadá sob adição de diferentes quantidades de dejetos por longo tempo (> 30 anos) (0, 60, 120, e 180 Mg ha⁻¹ ano⁻¹) e verificaram que não houve aumento na concentração de C nas frações de macroagregados pequenos (250-2000 µm), microagregados (53-250 µm) e na fração silte+argila (<53 µm) no tratamento com adição de 180 Mg ha⁻¹ ano⁻¹ de dejetos, indicando efeito da saturação de C em diferentes compartimentos da MOS. Nesse estudo, a MOP recuperada nos macroagregados grandes (>2000 µm) aumentou linearmente em resposta a maior adição de dejetos. Portanto, esses resultados sugerem que a saturação controla a dinâmica de acúmulo de C em compartimentos lábeis e estáveis da MOS (GULDE et al., 2008). Após a saturação da fração silte + argila, o acúmulo de C continua no compartimento MOP (CARTER et al., 2003). Além disso, a saturação controla o *turnover* de compartimentos lábeis e estáveis da MOS (MEYER et al., 2017).

Em solos tropicais e subtropicais, o efeito da saturação no acúmulo de C no solo foi pouco estudado até o momento (BRIEDIS et al., 2016, 2018; CORBEELS et al., 2016; DIECKOW et al., 2005; REIS et al., 2014). A relação linear entre a adição de C e a concentração de C total no solo têm sido observada, indicando não haver efeito da saturação de C (BRIEDIS et al., 2016; DIECKOW et al., 2005). BRIEDIS et al., (2016) avaliaram o efeito do DSC e da escassez de nutrientes no acúmulo de C através de adições crescentes de C (0, 6, 12 e 24 Mg C ha⁻¹) em diferentes camadas (0–20, 20–40 e 40–100 cm) de três Latossolos em um estudo de laboratório. Os autores verificaram relação linear entre as adições de C e o C orgânico no solo, indicando que esses solos têm alta capacidade de sequestro de C na camada de 0-20. Além disso, houve maior eficiência de acúmulo de C nas camadas superficiais, efeito que foi

atribuído a maior eficiência microbiana na superfície devido a sua melhor fertilidade do que no subsolo. As limitações de acúmulo de C devido a saturação de C são mais evidentes em camadas mais superficiais e em compartimentos da MOS com *turnover* lento, e mais protegidos da decomposição microbiana (DIECKOW et al., 2005). Embora os resultados do estudo de BRIEDIS et al., (2016) indiquem que o solo não atingiu o nível de saturação, há ausência de informações sobre qual compartimento da MOS (lábeis ou estáveis) foi influenciado pela adição de C.

Em um Argissolo subtropical no sul do Brasil cultivado sob plantio direto com diferentes sistemas de culturas e com variado aporte de resíduos, DIEKOW et al., (2005b) fracionaram a MOS em MOP, fração associada ao silte e fração associada a argila. Os autores verificaram que a fração argila atingiu o nível de saturação de C predito de 48,8 g C kg⁻¹, ou 4,0 Mg C ha⁻¹ na camada superficial do solo de 0-2.5 cm. Mas por outro lado, o C associado a fração silte e a MOP tiveram melhor ajuste linear e exponencial crescente, respectivamente, indicando alta capacidade de acúmulo de C em compartimentos mais lábeis, mas capacidade limitada de acumular C em compartimento estável na camada superficial do solo 0-2.5 cm. BRIEDIS et al., (2018) avaliaram o efeito de diferentes sistemas de manejo, como preparo convencional, sistema de plantio direto e pastagem nativa em três Latossolos, no município de Ponta Grossa (MT), Londrina (PR) e Lucas do Rio Verde (MT), respectivamente. Os autores verificaram melhor ajuste assintótico entre o C total do solo e o C na fração associada aos minerais, indicando efeito da saturação de C no sequestro de C no solo, entretanto, o C mensurado nessa fração estava distante do nível correspondente a saturação teórica predita, indicando que esses solos possuem alta capacidade adicional de estabilização de C. Além disso, esses autores verificaram que a adoção do sistema de plantio direto aumentou o sequestro de C em frações lábeis da MOS, e a proteção física nesse sistema favoreceu a interação do C com a superfície dos minerais, diminuindo o DSC.

1.5 Qualidade dos resíduos vegetais e estabilização de C no solo

A composição química e estrutural dos resíduos vegetais varia entre as espécies (ADAIR et al., 2008). Os resíduos de espécies que possuem elevado

teor polifenóis, celulose e alta relação Lig:N e relação C:N, decompõe-se lentamente e são considerados de baixa qualidade (i.e. resíduos de gramíneas, tecidos lenhosos) (CASTELLANO; MUELLER, 2015; ZHANG et al., 2008). Por outro lado, resíduos vegetais que possuam baixa relação Lig:N, altos teores de N e baixa relação C:N são considerados de alta qualidade e decompõe rapidamente (i.e. resíduos de leguminosas) (KLEBER, 2010). Além disso, a parte aérea das plantas (talos e folhas) são consideradas menos recalcitrantes do que as raízes (BERTRAND et al., 2006).

Anteriormente acreditava-se que apenas o C presente na fração dos resíduos considerada bioquimicamente resistente à decomposição microbiana acumulava na MOS, enquanto o C presente nas frações mais lábeis era totalmente mineralizado a CO₂. Entretanto, essa visão tradicional não considerava a incorporação de C no solo pela percolação de compostos solúveis e posterior estabilização pela interação com os minerais do solo (MIKUTTA et al., 2006), bem como a estabilização da MOP via proteção física no interior dos agregados de solo (COTRUFO et al., 2009, 2015)

A MOS é formada através da decomposição e transformação parcial de resíduos de plantas pelos microrganismos do solo (COTRUFO et al., 2015; KALLENBACH; GRANDY; FREY, 2016). Portanto, os fatores (i.e. temperatura, umidade e composição bioquímica dos resíduos, disponibilidade de nutrientes), que afetam a eficiência com que os microrganismos metabolizam os compostos orgânicos presentes em resíduos vegetais podem influenciar a formação da MOS (ADAIR et al., 2008; HATTON et al., 2015). Tradicionalmente, a dinâmica de decomposição de resíduos vegetais tem sido avaliada separadamente dos estudos de estabilização da MOS e, enquanto muito se sabe sobre o impacto do clima, composição dos resíduos vegetais e comunidade de microrganismos na taxa de decomposição da liteira (ADAIR et al., 2008; COTRUFO; DEL GALDO; PIERMATTEO, 2009), os fatores que controlam a proporção de C e N dos resíduos que é incorporado em compartimentos estáveis da MOS permanecem ainda pouco entendidos (COTRUFO et al., 2013; PRESCOTT, 2010).

Para instigar os cientistas de solos direcionar suas pesquisas a avaliar o efeito da qualidade dos resíduos no acúmulo de C no solo, COTRUFO et al., (2013) propôs o conceito *Microbial Efficiency-Matrix stabilization framework* que

é baseado em dois princípios: 1) as associações do C com os minerais é o principal mecanismo de estabilização da MOS por longo prazo (KÖGEL-KNABNER et al., 2008; SIX et al., 2002b; VON LÜTZOW et al., 2008) e 2) a maior parte da MOS estabilizada na matriz mineral do solo é de origem microbiana (KALLENBACH; GRANDY; FREY, 2016). De acordo com esse *framework* a composição bioquímica dos resíduos vegetais, especialmente a maior proporção de compostos lábeis (i.e. carboidratos, lipídios, compostos solúveis) em relação aos recalcitrantes (i.e. lignina, celulose, tanino) pode afetar a eficiência do uso do C (EUC) pelos microrganismos (relação entre a quantidade de C incorporado na biomassa microbiana (BM) e o C metabolizado e mineralizado a CO₂) (MOORHEAD et al., 2013; SINSABAUGH et al., 2013) e, portanto, afeta a taxa que o C é transformado, incorporado e estabilizado na matriz mineral do solo (HATTON et al., 2015).

Por longo tempo acreditou-se que a formação da MOS fosse originada da preservação de polímeros bioquimicamente recalcitrantes de plantas no solo, como a lignina (WAKSMAN, 1936; FLAIG et al., 1975). Nos estudos de decomposição em *litter bags*, considerava-se que a fração dos resíduos que era metabolizada após um tempo, era mineralizada a CO₂, enquanto a fração que permanecia (fração bioquimicamente recalcitrante) formaria a MOS (COTRUFO; DEL GALDO; PIERMATTEO, 2009). Entretanto, estudos recentes têm quebrado esse paradigma através do uso de técnicas isotópicas (¹³C) e espectroscópicas. O estudo de PRESTON; NAULT; TROFYMOW, (2009) demonstra que lignina não é preservada durante a decomposição dos resíduos vegetais. A decomposição microbiana da lignina é dependente de uma fonte de C disponível extra (KLOTZBÜCHER et al., 2011). Portanto, as evidências sugerem que a recalcitrância bioquímica não conduz a estabilização da MOS por longo tempo (KIEM; KÖGEL-KNABNER, 2003; MARSCHNER et al., 2008), sendo que o tempo médio de residência de compostos derivados da lignina no solo fica na faixa de 20 a 38 anos (RASSE et al., 2006).

A maior proporção de compostos lábeis em relação a recalcitrantes, e/ou a baixa relação Lig:N e razão C:N dos resíduos vegetais, favorece o maior EUC pelos microrganismos decompositores (SINSABAUGH et al., 2013), aumentando a BM (SHAHBAZ et al., 2017). Apesar da BM ser rapidamente

mineralizada no solo devido à favorável produção de energia e a baixa C:N (BLAGODATSKY; HEINEMEYER; RICHTER, 2000) a BM e os bioprodutos gerados podem ser seletivamente preservados e estabilizado via interação organomineral no solo (COTRUFO et al., 2013; HECKMAN et al., 2013). RUBINO et al., (2010) verificaram que dois terços da massa dos resíduos de folhas de *populus nigra* marcadas com ^{13}C foram incorporados no solo, provavelmente na forma de C orgânico dissolvido e materiais fragmentados, enquanto apenas um terço do C foi mineralizado a CO_2 . COTRUFO et al., (2015) avaliaram a decomposição *in situ* de resíduos de *Andropogon Girardi* (Poaceae) marcados com ^{13}C e a incorporação do C na MOS durante 3 anos, no Kansas, USA, e verificaram que nos estágios iniciais de decomposição a fração lábil do resíduo C acumulou no solo por interação organomineral.

O efeito da qualidade dos resíduos também pode influenciar a seleção de populações de microrganismos que apresentam EUC distintas, e isso pode ter impacto na eficiência que o C é incorporado na MOS (ROLLER; SCHMIDT, 2015). Ao nível de espécies de microrganismos individuais a variabilidade na EUC são manifestadas com base na história de vida (BEARDMORE et al., 2011; ROLLER; SCHMIDT, 2015). Os microrganismos copiotróficos selecionados em ambientes ricos em substrato são caracterizadas por rápida taxa de produção microbiana (TPM) (duplicação de células ou crescimento de hifas de fungos por unidade de tempo), porém com baixa EUC em relação ao microrganismos oligotróficos, que possuem uma taxa de produção lenta, mas com alta EUC (i.e. alguns fungos e actinomicetos) (BEARDMORE et al., 2011; LIPSON et al., 2009). Por exemplo, KALLENBACH; GRANDY; FREY, (2016) demonstram através da análise de biomarcadores em um estudo de incubação que na presença de compostos recalcitrantes ocorre alto desenvolvimentos de fungos, e esses são positivamente correlacionados com a EUC e com o acúmulo de C no solo.

A EUC e a TPM também pode variar com a variação do ambiente. Por exemplo, a EUC e a TPM aumentam conforme ocorre elevação da concentração do substrato (ROLLER; SCHMIDT, 2015) e com o aumento da qualidade do substrato (i.e. energia livre, baixa C:N, Lig:N) (SINSABAUGH et al., 2013). O estudo de KALLENBACH et al., (2015) demonstra que o uso de plantas de coberturas como leguminosas com baixa C:N proporciona substrato rico em

nutrientes e energia comparado a resíduos de milho e trigo, favorecendo o aumento da EUC e TPM. Além disso, nesse estudo os autores demonstram que a fisiologia da população microbiana é diretamente influenciada pelas práticas de manejo (com ou sem revolvimento do solo) e que a eficiência e a taxa com que o C adicionado é utilizado pelos microrganismos para formar a BM pode ser um importante mecanismo de acúmulo de C no solo. Esse entendimento pode explicar porque são observados maiores acúmulos de C em solos em condições tropicais e temperadas, sob plantio direto e com rotação de culturas incluindo leguminosas de cobertura (BAYER et al., 2009, 2016; DIEKOW et al., 2005; FERNÁNDEZ et al., 2017; VELOSO et al., 2018, 2020; VELOSO; CECAGNO; BAYER, 2019).

Embora exista bom embasamento científico que pode justificar o maior efeito de resíduos vegetais de maior qualidade na estabilização de C no solo, a revisão de CASTELLANO e MUELLER, (2015) mostra que os efeitos da qualidade dos resíduos no C do solo não são consistentes, além disso são altamente variáveis. Ainda, os autores propuseram um modelo conceitual para linkar a qualidade dos resíduos vegetais com o conceito de saturação de C no solo, o qual considera a separação dos compartimentos de C associado aos minerais, que é predominantemente C de origem microbiana, da matéria orgânica particulada que é predominantemente composta por resíduos de plantas e é protegida fisicamente. Além disso, esses autores sugerem que o efeito da qualidade dos vegetais é dependente do DSC e que solos que receberam adição de resíduos de alta qualidade irão saturar com menores taxas de entrada de C do que solos que receberam resíduos de baixa qualidade. Portanto, a qualidade dos resíduos vegetais deve afetar a quantidade de C estabilizado na fração mineral apenas em solos que apresentam expressivo DSC, e não em solos saturados ou próximo da saturação.

CAPÍTULO III – Legume-based cropping systems induce high C sequestration in no-till soil, but its potential decreases in long term due to saturation of fine silt plus clay size fraction²

1. Introduction

Soil organic matter (SOM) is the largest C reservoir in the terrestrial environment (SCHLESINGER, 1990), and efforts have been directed to identify strategies of increasing the capture of atmospheric carbon dioxide (CO₂) into the SOM pool (SMITH, 2016), especially into the more stable pools (LAL, 2004). Shoot and root litter are the primary source of soil organic C (SOC), so that sequestration of atmospheric CO₂-C into SOM means that the amount of plant-C fixed by photosynthesis and added to soil must be higher than the amount of C lost by microbial mineralization (BAYER et al., 2006b).

While C input into soil may be improved by cropping systems with high net primary productivity enabled by winter and summer cover crops combined with high input cash crops like maize, the SOM mineralization may be decreased by no-till (NT) management and its minimal soil disturbance (Bayer et al., 2000; Ferreira et al., 2018; Veloso et al., 2018). Legume-based cropping systems favor C stabilization in organo-mineral associations (VELOSO; CECAGNO; BAYER, 2019) because the quality of legume residues enhances the C use efficiency by soil microbes (COTRUFO et al., 2013, 2015) and thus the SOM formation by the microbial pathways (KALLENBACH; GRANDY; FREY, 2016; KLEBER; SOLLINS; SUTTON, 2007). The combination of high input cropping systems and no-till under the conservation agriculture principles is reported to promote soil C

sequestration at rates varying from 0.19 to 0.51 Mg ha⁻¹ year⁻¹ in tropical and subtropical soils in Brazil (BAYER et al., 2006b; VELOSO et al., 2018), similar or even higher than 0.24 Mg ha⁻¹ year⁻¹ observed in temperate soils (Lal et al., 1999).

The potential of C sequestration in soils under conservation agriculture is influenced by the capacity of soil C stabilization, which is mainly determined by the chemical association and physical protection of SOM in fine silt- and clay-size particles (< 20µm) and microaggregates (Six et al., 2002a). A high capacity of soil C stabilization has been observed in clayey highly weathered soil from humid tropical and subtropical regions (BRIEDIS et al., 2016; REIS et al., 2014), and it is mainly related to the high density of surface functional groups in Fe and Al oxides and sesquioxides that establish a strong linkage with SOM (MIKUTTA et al., 2006). However, the soil C stabilization capacity is finite and depends not only from physical-chemical soil properties, but it is proportional to the C saturation deficit (CSD) (Six et al., 2002a; Stewart et al., 2007) that represents the difference between current C content and the maximum C stabilization capacity (Hassink, 1997; Six et al. 2002a). In addition to stable SOM fractions, the CSD can also influence the dynamics of C incorporation in unprotected SOM fractions, even though the mechanisms involved are not clearly understood (GULDE et al., 2008; STEWART et al., 2008a).

The capacity of soil C stabilization has been estimated from the slope of the linear regression obtained from the relationships between the proportion of fine fraction (< 20µm) in the bulk soil as predictor variable and the current C stabilized in that fraction as response variable for a range of soil texture (HASSINK, 1997), mineralogy (2:1 vs 1:1) and land use in different climate zones (Six et al., 2002a). However, some soils used for predictions of soil C stabilization capacities cannot be reached their maximum stabilization capacity in the mineral-associated SOM and this may result in an underestimation of the SOC stabilization potential (FENG; PLANTE; SIX, 2013). More accuracy for estimation of CSD and the SOC stabilization potential can be reached by using the boundary lines analysis, which consists on the use of soils from upper limit of data set, which theoretically represent soils that reached the stabilization potential (Feng et al., (2013).

In recent studies, clayey Oxisols have been assessed in relation to their improved capacity of C stabilization (Briedis et al., 2018, 2016; Santos et al., 2011). However, there are few studies conducted on very resilient sandy or loam kaolinitic Ultisols, which represent more than 30 percent of the subtropical agricultural area in southern Brazil. Due to lower clay content and kaolinite predominance, the C saturation of the clay fraction ($< 2 \mu\text{m}$) in surface soil layers under no-till system is attained after few decades (Dieckow et al., 2005) and, thus, can impact negatively on C accumulation rates due to lower availability of functional groups in mineral surfaces for SOC stabilization against the microbial decomposition (KLEBER et al., 2015; SARKAR et al., 2018).

We assessed the hypothesis of C saturation in the fine silt plus clay-size fraction of surface soil layers of a kaolinitic Acrisol (220 g kg^{-1} clay) subjected to no-till cropping systems for long-term. We also evaluated the relationship of CSD with C accumulation in non-protected SOM fractions. This study, based on a 36-years field experiment, aimed to investigate how no-till cropping systems impact (i) C storage in soil, (ii) C stabilization in the fine silt plus clay-size ($< 20 \mu\text{m}$) fraction and its relationship with the decrease of C saturation deficit (CSD) in this fraction, and (iii) on C accumulation in labile fractions of soil organic matter (SOM) in 0-2.5, 2.5-5, 5-10 and 10-20 cm soil layers.

2. Material e methods

2.1 Field experiment

The study was conducted in a long-term field experiment located at the Agronomic Station of the Federal University of Rio Grande do Sul, Eldorado do Sul (RS), southern Brazil ($30^{\circ}51'S$ and $51^{\circ}38'W$). The climate is subtropical humid, *Cfa* type according to the Köppen classification, with a mean annual temperature of 19.4°C and annual rainfall of 1440 mm. The soil was classified as Typic Paleudult, according to the Soil Taxonomy, or as a sandy loam Acrisol, according to the WRB classification. The clay content was 220 g kg^{-1} , mainly comprised by kaolinite (720 g kg^{-1}) and iron oxides ($109 \text{ g kg}^{-1} \text{ Fe}_2\text{O}_3$) (BAYER et al., 2001).

The native vegetation of grassland was removed in 1967 and then the soil was used for cropland and subjected to conventional tillage. Plowing and disking operations were performed twice a year, for winter and summer crops,

leading to removal of mulch residue and structural degradation of soil. In 1983 the experiment was established to evaluate the capacity of no-till cropping systems to promote soil amelioration (BAYER et al., 2000).

The experiment comprised ten no-till cropping systems constituted of winter and or summer cover crops and of maize as summer cash crop. For this study five treatments were selected: i) Bare soil, a control treatment without plant growing and thus without canopy or mulch cover and carbon input, being spontaneous plants controlled mechanically or with glyphosate herbicide; ii) O/M, black oat (*Avena strigosa* Schreb.) as winter cover crop and maize (*Zea mays* L.) in summer; iii) O+V/M+C, black oat plus vetch (*Vigna sativa* L.) as winter cover crops and maize plus cowpea (*Vigna unguiculata* (L.) Walp.) in summer, with cowpea serving as cover crop during and after maize cropping, until the next frost; iv) M+LL, maize plus lablab (*Lalab purpureus* (L.) Sweet ssp. *Purpureus*), with lab-lab serving as cover crop during and after maize cropping, until next frost; and v) M+P, maize plus pigeon pea (*Cajanus cajan* (L) Mill sp.], with pigeon pea serving as cover crop during and after maize cropping, until next frost.

Cropping systems were arranged with two levels of urea-N to maize (0 and 180 kg N ha⁻¹) in a split-plot randomized block design, with three replicates (BURLE; MIELNICZUK; FOCCHI, 1997). Main plots of 5×16 m contained the cropping systems and subplots of 5×8 m contained the N levels. For this study, we used the 180 kg N ha⁻¹ level.

Black oat and vetch were sown in April–May every year using a tractor-mounted seed drill. Seed rate of black oat was 80 kg ha⁻¹, when grown alone (O/M), or 30 kg ha⁻¹ when intercropped with vetch (O+V/M+C), and in this case vetch was seeded at 50 kg seed ha⁻¹. Maize was sown in Oct-Nov every year at a density of 60.000-70.000 plants ha⁻¹. The summer cover crops cowpea, lab-lab and pigeon pea (in O+V/M+C, M+LL and M+P) were sown 20–30 days after maize emergency, at a rate of 3-4 seeds per hole between the maize lines, spaced 0.4 m between holes.

2.2 Annual C inputs by cropping systems

The aboveground biomass of cover crops were calculated from original data of previous studies conducted in the same experiment (Burle et al., 1997; Vieira et al., 2009; Zanatta et al., 2007). The aboveground biomass of

maize was estimated from data of grain yields obtained in 1983-2019 and by using the equation $\text{maize biomass} = 0.84Y_{\text{grain}} + 2.9$, developed by Lovato et al. (2004) in the same experimental site. The root biomass of either cover crops or maize was considered as being 30% of the respective aboveground biomass (ZANATTA et al., 2007). The carbon inputs were then calculated by considering the overall biomass of aboveground plus roots and by assuming a carbon concentration of 400 g C kg^{-1} biomass.

2.3 Soil sampling and fractionation of soil organic matter

Soil samples were collected from the 0-2.5, 2.5-5, 5-10 and 10-20 cm depths in May 2019, when the experiment was 36 years old. Twelve sub-samples, collected randomly in each plot using iron augers of 5 cm in diameter, comprised a composite soil sample. After air drying, the soil samples were sieved in a 2 mm mesh sieve and subjected to physical fractionation. The method of fractionation was similar to that of Sohi et al., (2001) and aimed to obtain the following physical fractions: i) light particulate organic matter (IPOM), ii) sand particulate organic matter (sPOM), iii) coarse silt organic matter, 20-53 μm (cSiltOM) and iv) mineral associated organic matter in fine silt plus clay, <20 μm (mOM) (Figure 2). Briefly, ten grams of air-dried soil were added to a falcon-type centrifuge bottle (50 ml). After adding 40 mL of sodium polytungstate solution (SPT, $\rho = 2.0 \text{ kg dm}^{-3}$), the bottle was closed and manually inverted five times. Complete soil dispersion was achieved by applying 240 J cm^{-3} of ultrasonic energy, according to previous calibration for 95% of clay dispersion (INDA JUNIOR et al., 2007). After soil dispersion, the bottles were centrifuged for 90 min at $2.000 \times g$ and the IPOM fraction was recovered by filtration of the SPT supernatant using fiberglass filters. Those filters were previously dried and weighed. The IPOM was washed with 150 mL of distilled water and 50 mL of 0.01 M CaCl_2 solution to remove excess of SPT, then it was oven dried at $50 \text{ }^\circ\text{C}$ for 24 h. The remaining pellet containing the heavy fraction in the bottle was resuspended manually in 40 mL of distilled water and subjected to 80 J cm^{-3} ultrasonic dispersion. Then, this suspension was sieved through a 53- μm mesh sieve fitted on top of a 1 L measuring cylinder, and the recovered sPOM was transferred to an aluminum pan and oven dried at $50 \text{ }^\circ\text{C}$ for 48 h

(CAMBARDELLA; ELLIOTT, 1992). The summation of IPOM and sPOM represented a total POM fraction.

The suspension that passed the 53- μm mesh sieve in the previous step was subjected to sedimentation-resuspension cycles in 15-cm high cylinders to obtain the mOM fraction, in accordance with the Stocks' Law. Cycles were repeated daily until the supernatant was clear. The remaining cSiltOM fraction in the bottom of the sedimentation cylinder was transferred to an aluminum pan. Soil suspensions containing these fractions were separately flocculated using 10 mL of 1 M CaCl_2 solution and, after decantation for few hours, the water was partially removed, and the fractions were oven dried at 50 °C for 96 h.

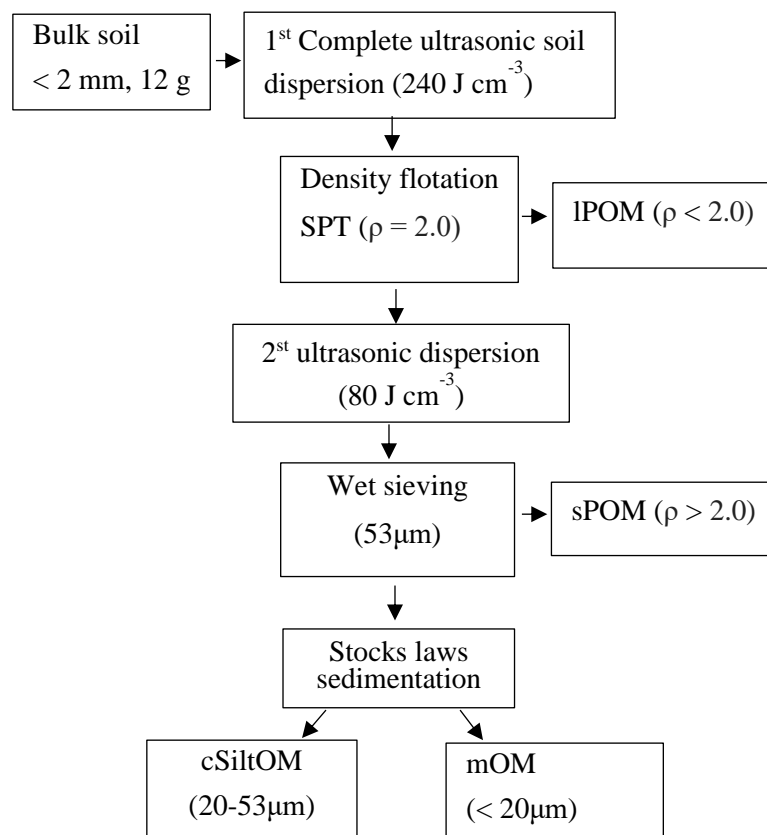


Figure 2. Schematic representation of the physical fractionation of soil to isolate the light particulate organic matter (IPOM); sand particulate organic matter, >53 μm , $\rho > 2.0$ (sPOM); coarse silt organic matter, 20-53 μm (cSiltOM); and mineral associated organic matter, < 20 μm (mOM).

The four obtained fractions were finely ground in agate mortar, and then analyzed for C content determination in a dry combustion analyzer (Fisher Scientific Flash 2000). The recovery rate of the initial soil mass through the

fractionation process was on average 97%, and that of total C content was on average 95%.

2.4 Evaluation of C saturation in labile and stable fractions of soil organic matter

Despite the C input by cropping systems is available, this data was not used for interpretation of C saturation in individual SOM fractions because different plants species (legumes vs poaceae) and its parts (shoot vs root) have different decomposition rates and its derived C is not uniformly incorporated in distinct fractions of SOM (Stewart et al., 2008). Therefore, the evidence of C saturation of individual fractions of SOM was analyzed by the linear or asymptotic relationship with the C content in the bulk soil (g C kg^{-1} soil) because of balance between C input and decomposition process to normalize the decomposition rates (Stewart et al., 2008) across NT cropping systems.

The equations used to evaluate the C saturation behavior of individual fractions of SOM were adjusted by using the total soil C as independent variable and the C of individual SOM fractions as response variable in each soil layer, as follow:

Linear model:

$$C_f = \alpha + \beta C_{soil} \quad (\text{Eq. 1})$$

Where the C_f and C_{soil} are the C quantified in the fraction (g kg^{-1}) and the bulk-soil C, respectively; α e β are the intercept and straight slope, respectively. The linear relationship indicates a same proportion of C increment in SOM fraction and bulk soil and, thus, that the C accumulation in SOM fraction is not being limited by saturation degree.

Exponential rising to maximum value:

$$C_f = C_{f_{max}} (1 - e^{-kC_{soil}}) \quad (\text{Eq. 2})$$

When the $C_{f_{max}}$ is the maximum C accumulation capacity in the fraction (g kg^{-1}) and k is the first order constant. The asymptotic relationship indicates that C accumulation in SOM did not increase in the same proportion of the SOC increase, and thus, the C accumulation in SOM fraction is being limited by C saturation, until a maximum C content is attained (STEWART et al., 2008a).

Exponential grown model:

$$C_f = I e^{(gC_{soil})} \quad (\text{Eq. 3})$$

Where, I is the initial C content in the SOM fraction and g is the grown rate. The grown model was observed previously by Dieckow et al. (2005) and suggest that C saturation of some stable SOM fractions may impact positively on C accumulation in labile SOM fractions. In this study, IPOM and sPOM were summed to obtain the total POM, which was tested for exponential grown model.

2.5 Assessment of C saturation deficit and stabilization potential in the mineral-associated SOM

The C saturation deficit in mineral-associated (<20 μm , fine silt+clay size fraction) SOM (CSD_{mOM}) was estimated by difference between Cf_{max} and current C measured in the SOM fraction. The Cf_{max} was estimated using the proportion of fine fraction <20 μm (Table 1) and the slope (0.43) of boundary lines analysis (BL) method reported by Feng et al. (2013) for 1:1 soils aiming to have a CSD_{mOM} comparable to published data (Eq. 3). The authors compared the BL to methods of linear regression methods reported by Hassink (1997) and Six et al., (2002a) and conclude that BL result in a best estimation of CSD for 1:1 soils.

Thus, the CSD_{mOM} was estimated by using the equations 3 and 4, as follow:

$$Cf_{\text{max}} (\text{g kg}^{-1} \text{ soil}) = 0.43 * f \quad (\text{Eq. 4})$$

Where 0.43 is the slope of BL regression; f is the proportion (%) of fine fraction (<20 μm) in the bulk-soil.

$$\text{CSD}_{\text{mOM}} (\text{g kg}^{-1} \text{ soil}) = Cf_{\text{max}} - Cf_{\text{current}}. \quad (\text{Eq. 5})$$

Where the Cf_{current} is the current C measured in the fine soil fraction (<20 μm).

The CSD_{mOM} was also expressed in %, using equation 6

$$\text{CSD}_{\text{mOM}} (\%) = 100 - \left[\left(\frac{Cf_{\text{current}}}{Cf_{\text{max}}} \right) \times 100 \right] \quad (\text{Eq. 6})$$

Where, CSD_{mOM} near to 0% indicate that the soil has low CSD_{mOM} or is theoretically saturated when $\text{CSD}_{\text{mOM}} < \text{or} = 0\%$. In contrast, the CSD_{mOM} near to 100% indicate that the soil is far from saturation, and has high unsatisfied C stabilization capacity (high CSD_{mOM})

Based on the CSD_{mOM} and soil bulk density, we estimated the remaining soil potential of SOC stabilization in different cropping systems and soil layers, according to equation 6:

$$\text{Remaining SOC stabilization potential (Mg C ha}^{-1}\text{)} = \frac{\text{CSD}_{\text{mOM}} \times \text{BD} \times \text{ST} \times 10000}{1000} \quad (\text{Eq. 7})$$

Where, CSD_{mOM} was expressed in g C kg^{-1} soil, BD is bulk soil density (kg cm^{-3}), and ST is the soil thickness (m).

2.6 Statical analysis

Data normality and variance homogeneity were checked by Shapiro-Wilk and Levene tests, respectively. The data were adjusted to models using the significance and magnitude of adjusted coefficient of determination (R^2) as the first criteria. When the adjusted R^2 was similar between models, the model with lower root square mean error (RSME) was selected. The data of CSD_{mOM} and the remaining soil C sequestration potential were subjected to analysis of variance and when significance differences were found, the difference between means were tested by Tukey ($\alpha < \text{or} = 0.05$). Lastly, Pearson and Spearman correlation analysis were applied to test the relationships between pairs of variables. Presumptions of analysis of variance and Tukey test were performed using the package SAS® v.9.4 (Statistical Analysis System Institute Cary, North Carolina). Fitting models and relationship between variables were performed using the software Sigma Plot v.14.0.

3. Results and Discussion

3.1 C input by cropping systems

The total carbon input by cropping systems, except bare soil, varied from 7.0 to 9.8 $\text{Mg C ha}^{-1} \text{ yr}^{-1}$ (Figure 3). The contribution by cover crops ranged widely from 1.97 $\text{Mg C ha}^{-1} \text{ yr}^{-1}$, in O/M, to 5.20 $\text{Mg C ha}^{-1} \text{ yr}^{-1}$, in M+P, and respectively represented 28% to 52% of that total input. As maize contributed a rather constant input of about 5 $\text{Mg C ha}^{-1} \text{ yr}^{-1}$ across systems, the main cause of the variation in total input was related to the variation of inputs by cover crops. Bare soil had the lowest input of 0.5 $\text{Mg C ha}^{-1} \text{ yr}^{-1}$, attributed to sporadic growing of spontaneous plants.

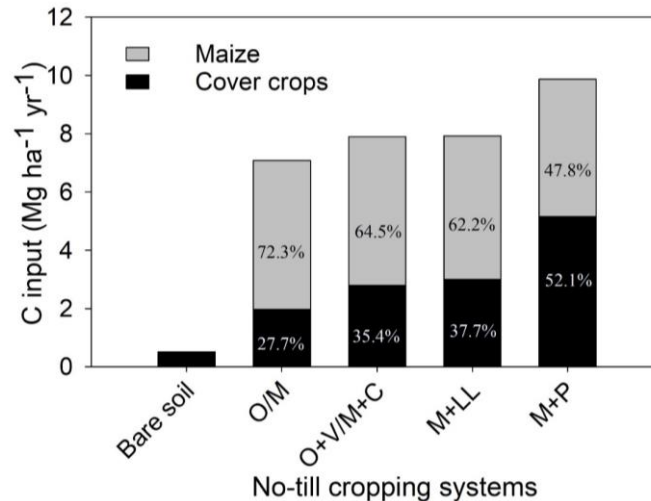


Figure 3. Annual C input in bare soil and of cover crops and maize in the no-till cropping systems: black oat/maize (O/M), black oat + vetch /maize + cowpea (O+V/M+C), maize + lablab (LL+M) and pigeon pea+maize (P+M).

3.2 Carbon content in whole soil and physical fraction fractions

The cropping systems changed considerably the C content in whole soil and in the physical fractions mOM, cSiltOM and tPOM; mainly to 10 cm depth (Table 1). Those changes were related to the C input by cropping systems, but especially by the cover crops, as C content in the top 2.5 cm soil showed a closer relationship with C input by cover crops ($R^2=0.96$, $p=0.002$) than with total C input ($R^2=0.53$, $p=0.09$). This result is derived likely from the higher variation of C input by cover crops than by maize (Figure 3) and may be also related to the higher quality of cover crops residues with potential higher impact on soil C pool (Veloso et al., 2018, 2019).

The C content in physical fractions showed a similar pattern between cropping systems as that observed for total soil C (Table 1), with higher C contents in the high-C input cropping systems of M+LL and M+P. The C content in tPOM decreased substantially with soil depth, and the effect of cropping systems were restricted to 10 cm depth, mainly in the 0-2.5 and 2.5-5 cm layers. In general, the same pattern was observed for cSiltOM (53-20 μ m) and mOM (fine silt+clay fraction), but these fractions presented C contents much higher than those of tPOM, except for upper 2.5 cm soil, where tPOM stored up to 60% of total soil C, as in the M+P cropping system (Table 1).

Table 1. Carbon content in bulk-soil and in physical fractions of a subtropical Acrisol under long-term no-till cropping systems

Cropping Systems	SOC pools				Relative proportion to SOC			ρMass <20μm	BD (Mg m ⁻³)
	Total soil C	†tPOM	cSiltOM	mOM	mOM	tPOM	cSilt		
	---g C kg soil ⁻¹ ---	---g C kg soil ⁻¹ ---	-g C kg fraction ⁻¹ -		----- %-----				
0-2.5 cm									
M+P	38.6±2.3	21.5±3.1	24.1±1.2	42.3±3.6	33.6	60.8	5.5	34.3	1.1
M+LL	23.5±2.0	7.7±0.8	21.6±3.6	40.7±2.6	58.6	33.5	7.8	34.1	1.1
O+V/M+C	21.1±1.5	6.7±1.3	8.6±2.2	35.3±0.8	61.4	34.9	3.7	33.2	1.1
O/M	17.9±2.6	5.4±1.4	6.9±0.1	30.7±3.8	63.6	32.5	3.9	33.2	1.4
Bare soil	11.7±1.4	2.9±0.6	4.3±1.7	25.0±2.4	72.5	24.4	3.0	34.3	1.5
2.5-5 cm									
M+P	21.8±4.3	5.8±1.5	13.4±5.5	36.0±0.7	65.2	29.0	5.7	35.7	1.4
M+LL	16.0±0.5	2.5±0.4	9.1±0.4	32.5±1.5	77.1	17.6	5.3	34.8	1.4
O+V/M+C	17.2±1.2	3.3±0.9	3.8±0.4	29.9±0.2	74.8	23.0	2.3	36.0	1.3
O/M	12.3±1.6	2.3±0.2	2.9±0.6	23.6±0.5	77.3	20.3	2.4	36.8	1.5
Bare soil	7.9±1.1	1.6±1.1	1.7±0.7	18.9±1.8	79.6	18.7	1.6	36.9	1.3
5-10 cm									
M+P	13.9±3.2	2.3±0.6	4.0±1.9	26.3±4.6	79.2	18.2	1.4	38.0	1.5
M+LL	11.0±0.9	1.6±0.3	3.3±0.5	23.7±0.4	82.5	14.8	2.1	36.7	1.5
O+V/M+C	10.9±0.9	1.7±0.4	1.6±0.2	21.6±1.3	81.7	16.9	1.4	38.3	1.4
O/M	7.8±1.2	1.2±0.2	2.1±0.2	17.2±0.3	82.9	15.0	2.6	39.4	1.6
Bare soil	7.0±0.8	1.1±0.1	1.1±0.2	14.6±1.0	82.0	16.6	2.6	37.7	1.5
10-20 cm									
M+P	8.0±1.5	1.2±0.2	1.4±0.6	16.6±1.8	82.8	15.8	1.5	39.8	1.6
M+LL	8.5±0.3	1.0±0.1	1.4±0.2	17.1±0.3	85.0	13.6	1.5	39.1	1.5
O+V/M+C	7.4±0.2	0.9±0.1	1.4±0.4	14.9±0.2	84.8	13.4	1.7	39.4	1.6
O/M	6.9±0.8	1.1±0.2	1.1±0.5	14.2±0.2	82.5	16.1	1.4	40.5	1.6
Bare soil	6.1±1.1	1.0±0.2	1.0±0.3	13.0±0.4	81.7	17.0	1.3	40.1	1.6

*Total particulate organic matter (tPOM) was obtained after soil dispersion, and it consist of sum of light POM with density <2 g cm³ (free and inter-aggregate) (IPOM) and the coarse POM > 2 g cm³ recovered in sandy soil particles (sPOM); C in coarse silt organic matter 20-53 μm (cSiltOM); C in fine silt plus clay fraction <20μm (mOM). ρ Mass proportion of <20μm particles in the bulk soil. Soil bulk density (BD) was determined using volumetric steem rings.

3.3 Evidence of C saturation in mineral-associated organic matter

With respect to the relationship between C content in physical fraction and C content in whole soil, the best fit for the mOM fraction (<20μm) was the asymptotic non-linear model (saturation model) (R² =0.84 - 0.93), compared to

the linear model ($R^2=0.73 - 0.92$); and this was observed in the 0-2.5, 2.5-5 and 5-10 cm layers (Table 2). However, the highest carbon saturation level in mOM was found in the 0-2.5 cm, where the C content of $42.3\pm 2.3 \text{ g C kg}^{-1}$ fraction (Table 1) was the closest to the maximum C stabilization capacity of 47.4 g C kg^{-1} fraction predicted by the saturation model (data not shown). This stabilization capacity was similar to the $48.8 \text{ g C kg clay}^{-1}$ ($< 2\mu\text{m}$) estimated for the 0-2.5 cm layer when the same experiment was 17 years old (Dieckow et al. 2005).

When combining the data from the 0-2.5, 2.5-5 and 5-10 cm layer into a single layer of 0-10 cm, the saturation model also showed the best fit ($R^2 = 0.93$, $p < 0.0001$), with saturation being approached by high input cropping systems like P+M (Figure 4a). Although the C saturation tendency was more evident in the first 2.5 cm soil, the fit of the saturation model in the 2-5 and 5-10 cm layers suggests that those layers also have a limit to stabilize new C (STEWART et al., 2007).

Table 2. Statistics fits for the linear, non-linear (saturation) and exponential grown (exp. grown) models when explored the relationships between soil organic matter fractions and total soil C

Layer (cm)	Linear mOM (< 20 μ m)		Saturation		Linear cSitOM (20-53 μ m)		Saturation		Linear tPOM		Exp. grown		Saturation	
	<i>p</i>	adjR ²	<i>p</i>	adjR ²	<i>P</i>	adjR ²	<i>p</i>	adjR ²	<i>p</i>	adjR ²	<i>p</i>	adjR ²	<i>p</i>	adjR ²
0-2.5	**	0.72	***	0.83	**	0.69	***	0.64	**	0.92	***	0.95	***	0.75
2.5-5	**	0.87	***	0.90	**	0.68	***	0.51	**	0.78	***	0.86	***	0.72
5-10	**	0.92	***	0.93	**	0.66	***	0.57	**	0.86	***	0.87	***	0.85
10-20	**	0.82	***	0.77	ns	0.12	ns	0.11	ns	0.10	ns	0.11	ns	0.07

The higher adjusted R square (adjR²) and lower error means square were used as a criterion to define the best fit model. Special characters ** and *** indicate the significance level of the models $p < 0.001$ and $p < 0.0001$, respectively. ns: non-significant mean when $p > 0.05$. The F test was applied to the linear model and the t test to the non-linear model to test the null hypothesis that the coefficients of determination are not significantly different from zero.

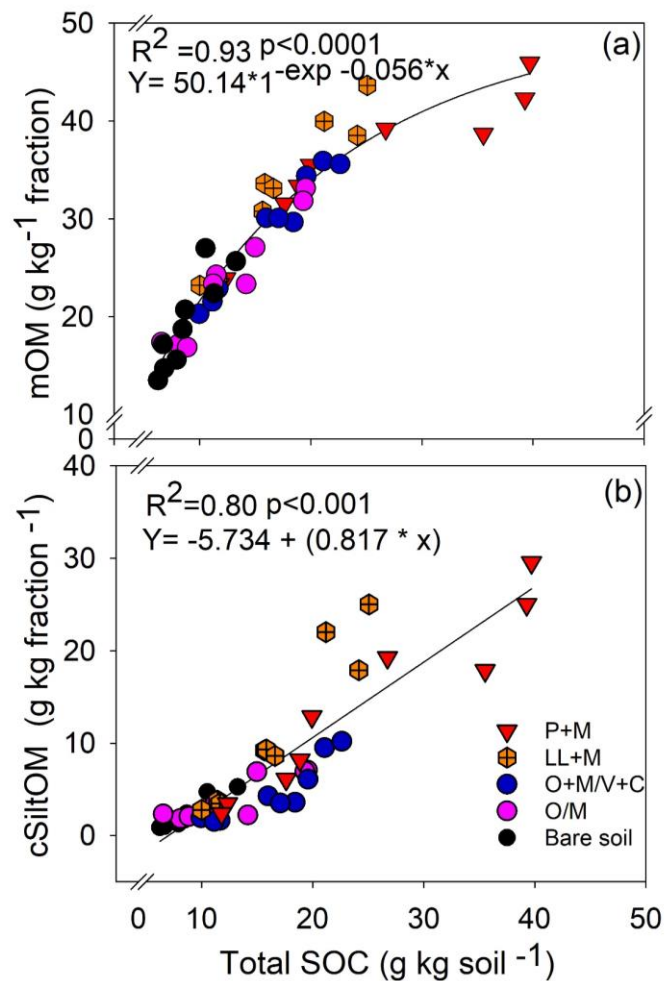


Figure 4. Nonlinear regression represented by the exponential rise to maximum (saturation model) between the total soil C and C content in the mineral associated organic matter (mOM) stabilized in the fine fraction (a); and linear fit between total soil C and C associated in the coarse silt organic matter fraction (cSiltOM) (b) of a subtropical Acrisol subjected to long term no-till cropping systems. The analysis included 45 observations of each soil fraction obtained from the 0-2.5, 2.5-5, and 5-10 cm layers of the evaluated cropping systems: [i] Bare-soil (Bare), without crops cultivation, [ii] black oat/maize (O/M), [iii] black oat+vetch /maize+cowpea (O+V/M+C), [iv] maize + lablab (L+M) and [v] pigeon pea +maize (P+M).

The mOM fraction has a turnover time ranging from decades to millennium (CARTER, 2002; VON LÜTZOW et al., 2008). Thus, the saturation of this pool of SOM suggests that the contribution of this soil under no-till to C sequestration is limited on soil surface layers, and mainly dependent on the

storage of stable C in deeper soil layers in long term as evidenced by Veloso et al. (2018).

Similar results were found by other studies in tropical and subtropical soils under long-term no-till, where the saturation model also showed the best fit to the variation of mOM (<20 or <53 μ m) in clayed soils (FERREIRA et al., 2018; REIS et al., 2014; SANTOS et al., 2011). However, in these studies the C content in the mOM fraction was well below the C saturation level, even in the topmost soil layers (up to 10 cm) (BRIEDIS et al., 2018; REIS et al., 2014). This difference may be explained by the greater protective capacity in their soils due to the greater proportion of fine fraction (HASSINK, 1997). Furthermore, the effect of soil mineralogy, cropping intensity and climate variations should be considered (OGLE; BREIDT; PAUSTIAN, 2005).

In this study, the soil mineralogy was predominantly composed by kaolinite (720 g kg soil⁻¹), followed by iron oxides (109 g kg soil⁻¹) (BAYER et al., 2001). The characteristics as non-expansivity, negligible isomorphous substitution and low specific surface area lead to low C stabilization capacity by interaction with kaolinite particles (KAISER; GUGGENBERGER, 2003; SARKAR et al., 2018). However, the coating of this clay by the Fe and Al oxides results in changes in its electrochemical properties, which may increase the C sorption (ARIAS; BARRAL; DIAZ-FIERROS, 1995). Further, the high surface area and chemical affinity for C in clayey soils with high content of Fe and Al oxides result in a high stabilization capacity of tropical soils, mainly Oxisols (DICK et al., 2005; THAYMUANG et al., 2013).

In the 10-20 cm layer, the C content in mOM fraction best fit linear model than saturation model ($R^2 = 0.82$ vs 0.77) (Table 2), indicating that C saturation in this deeper layer is less evident than in topmost layers, possibly because C input by aboveground and roots is higher in the first top centimeters of soils under no-tillage, limiting C accumulation and consequently the possibility of saturation in this soil depth.

3.4 C accumulation in coarse silt did not reveal saturation

The C content in cSiltOM fraction best fitted the linear model instead of the saturation model, and this was observed in the 0-2.5, 2.5-5 and 5-10 cm soil layers (Table 2, Figure 4b), while in the 10-20 cm the C content in cSiltOM

fitted no model ($p > 0.05$) (Table 2). As observed in the other physical fractions, the highest C contents in cSiltOM fraction were observed in the high input cropping systems of P+M and LL+M, while the lowest occurred in bare soil (Table 1). These results suggest that there is no saturation limit for C accumulation in cSiltOM fraction, so that it continues to accumulate C after saturation of mOM fraction, as clearer in the 0-2.5 cm.

A previous study in the same experiment indicated a lower content of kaolinite and Fe oxides this coarse silt fraction relative to clay fraction (BAYER et al., 2001), what suggests that the organo-mineral interaction may be not the main mechanism of C stabilization in coarse silt. Moreover, Dieckow et al., (2005) also reported a linear relationship between the C content in the silt-size fraction and total soil C content and observed with microscope that it was composed mainly by POM material (plant debris). We suggest that the cSiltOM is a combination of materials derived from plant and microorganisms. However, the quantitative importance of this cSiltOM fraction to the full storage of C in each soil layer is rather little once it represents on average only 4.8% of the total C content in the 0-2.5 cm layer and 1.4% in the 10-20 cm layer.

3.5 Carbon saturation deficit and implications on remaining C stabilization potential

As no-till cropping systems had strong impact on SOC and SOM fractions, the CSD_{mOM} was also affected ($p < 0.001$) up to 20 cm soil depth (Figure 5). Since most of the crop residues were added on soil surface (aboveground) and in the first centimeter's soil (roots), the more expressive decrease in the CSD_{mOM} across cropping systems was observed in the 0-2.5 cm, where the CSD_{mOM} of 42% in bare soil decreased to 1.6% in the P+M system (Figure 5). In general, CSD_{mOM} was negatively related to the annual input by cropping systems and decreased according to order: bare soil > O/M > O+V/M+C > LL+M > P+M in all soil layers (Figure 5). In 10-20 cm layer, the CSD_{mOM} varied from 70% in bare soil to 61% in higher C input cropping systems (average of LL+M and P+M).

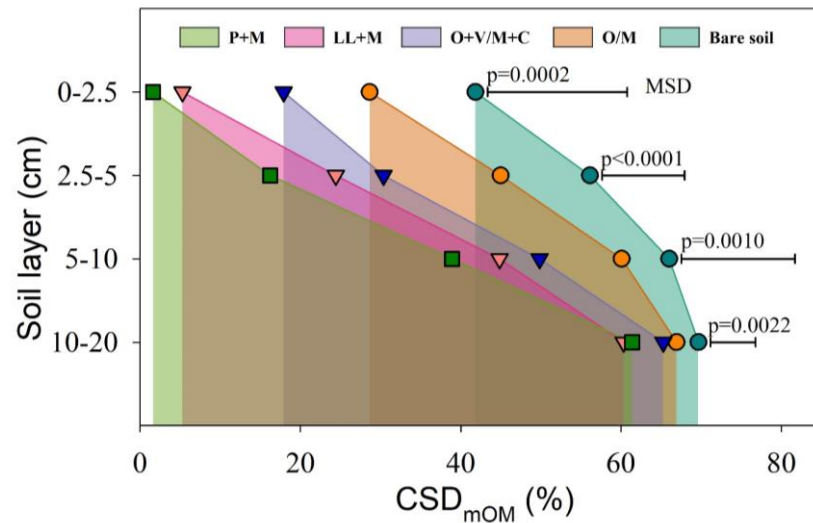


Figure 5. Carbon saturation deficit in the mineral associated organic matter (CSD_{mOM}) stabilized in the fine silt + clay fraction ($<20\mu m$) at different soil depths after 36 years of subtropical no-till cropping systems: [i] Bare-soil (Bare), without crops cultivation, [ii] black oat/maize (O/M), [iii] black oat+vetch /maize+cowpea (O+V/M+C), [iv] maize + lablab (L+M) and [v] pigeon pea +maize (P+M). The horizontal bars mean the minimum significant difference (MSD) by Tukey test at 5% of probability ($p < 0.05$) at each soil depth.

As expected, the decrease of the CSD_{mOM} diminished the potential capacity of the fraction to further stabilize new carbon calculated as the difference between Cf_{max} and $Cf_{current}$, mainly in upper layers and high C input cropping systems (Figure 6). In the 0-2.5 cm layer, the remaining capacity of C stabilization in the P+M and LL+M were 0.06 and 0.22 Mg C ha⁻¹, respectively, which means a reduction of 97 and 90% in comparison to the remaining capacity of C stabilization of 2.33 Mg C ha⁻¹ in bare soil (Figure 6). In the O/M the remaining capacity of C stabilization was equivalent to 65% (1.53 Mg C ha⁻¹) than observed in the bare soil for 0-2.5 cm layer.

A significant and negative correlation was observed between the CSD_{mOM} in the 0-2.5 cm and the total annual C input by cropping systems ($R = -0.88$, $p = 0.0457$, according to Pearson analysis), showing that increasing C inputs in the long-term resulted in a decrease in CSD_{mOM} (Figure 5) and therefore a decrease in the potential capacity of the mOM additionally stabilizes carbon (Figure 6). Moreover, we found an even more significant correlation between

CSD_{mOM} and the annual C input by cover crops (-0.93, $p=0.0233$), reinforcing the hypothesis that the C accumulation and, thus, the decrease of CSD_{mOM} may be more related to C input by cover crops than by maize.

The remaining C stabilization capacity in the mOM of the whole 0-20 cm layer was also affected by long-term cropping systems. That remaining stabilization capacity varied from 22.5 Mg C ha⁻¹ in P+M, to 30.8 Mg C ha⁻¹ in the O/M and to 32.8 Mg C ha⁻¹ in the bare soil, and most of this capacity (58-75%) was found in the deeper 10-20 cm layer (Figure 6). These results are consistent with studies developed in field experiments in tropical and subtropical soils, in which was observed that C accumulation in deeper soil layers (30-100 cm) is equivalent in long-term to those observed in 0-30 cm layer of soil (BODDEY et al., 2010; VELOSO et al., 2018). In addition to higher CSD in deeper soil layers, a higher efficiency of C-derived from roots and high interactive capacity of minerals as iron oxide in tropical and subtropical soils can explain partially these results (SANTOS et al., 2011).

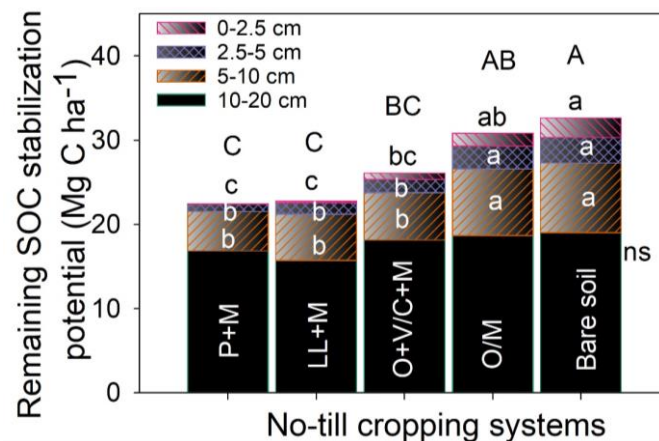


Figure 6. Remaining soil C sequestration potential in the fine silt plus clay particles (<20 μm) at different depths of subtropical Acrisol subjected to long-term no-till cropping systems: [i] Bare soil (Bare), without crops cultivation, [ii] black oat/maize (O/M), [iii] black oat+vetch /maize+cawpea (O+V/M+C), [iv] maize + lablab (L+M) and [v] pigeon pea +maize (P+M). The lower-case letter compares the effect of cropping systems at each soil depth and capital letter compare the effect of cropping systems in whole soil depth (0-20) by Tukey test at 5% of probability ($p<0.05$).

The effects of residue quality at increasing the C stabilization by organic-mineral interaction were reported by (COTRUFO et al., 2013; VELOSO; CECAGNO; BAYER, 2019), but those effects are potentially dependent of the C stabilization capacity (CASTELLANO; MUELLER, 2015). In the long term, the decrease of CSD can have a negative impact on additional C accumulations, mainly in surface soil layers up to 10 cm. On the other hand, the saturation of the functional groups on clay surfaces in the upper soil layer (0-2.5 cm) may have favored the translocation and stabilization of C into unsaturated mineral surfaces in deeper layers (NICOLOSO et al., 2018). This finding is supported by a linear relationship between CSD_{mOM} in the 0-2.5 cm and C content in the mOM fraction of 10-20 cm layer ($R^2=0.77$, $p<0.001$) (Figure 7), suggesting that the C increase in soil at depth can be related to C saturation in the soil surface. The downward movement of dissolved organic matter may be a form of carrying C from top to deeper layers (KAISER; KALBITZ, 2012) and may also serve as a pathway of mineral associated organic matter formation (SOKOL; SANDERMAN; BRADFORD, 2019), contributing to C accumulation in deeper soil layers of subtropical soil under long-term no-till (VELOSO et al., 2018). In addition, the root C deposition was reported to be related to accumulation and posterior saturation of C onto silt + clay particles in subtropical no-till soils (SANTOS et al., 2011).

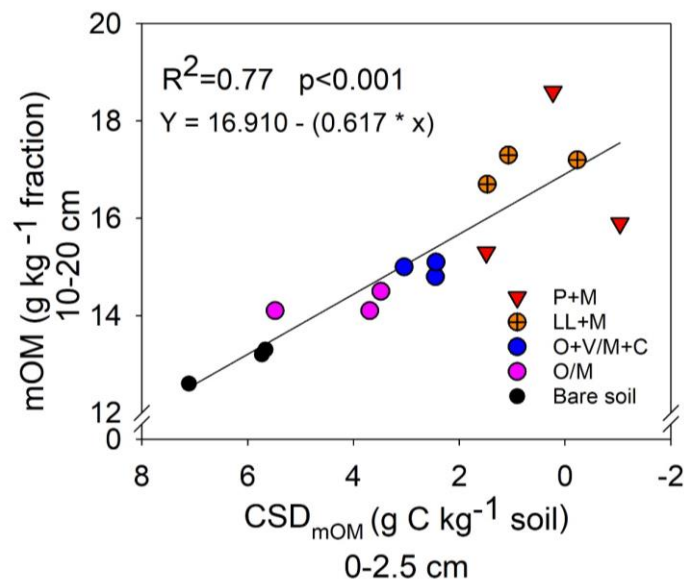


Figure 7. Linear relationship between mineral associated organic matter (mOM) stabilized in the fine fraction < 20 μ m at 10-20 cm soil layer of with C saturation deficit in mineral associated organic matter (CSD_{mOM}) at 0-2.5 cm layer of subtropical Acrisol under long term no-till cropping systems.

3.6 Carbon accumulates as POM after saturation of mineral-associates fraction

The C content of the tPOM fraction of 0-2.5 cm, 2.5-5 cm and 5-10 cm soil layers best fit the exponential growth model ($R^2=0.86 - 0.95$, $p < 0.0001$) (Table 2), indicating that the increase observed in total soil C after the saturation of mOM occurred mainly as POM ($R^2=0.95$ $p < 0.001$) (Figure 8). Further, the CSD_{mOM} decreased exponentially ($R^2=0.92$, $p=0.001$) as total soil C increased, suggesting some possible relationship between increase of tPOM and saturation of C associated to fine silt + clay fraction (STEWART et al., 2008a).

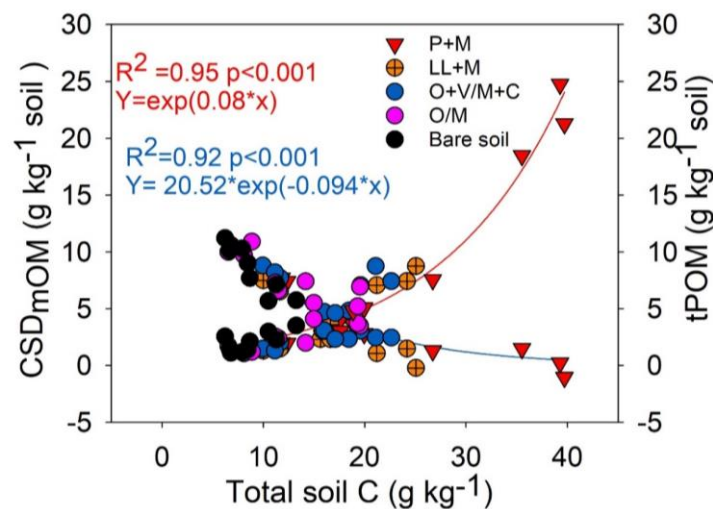


Figure 8. Relationship between of total particulate organic matter (tPOM) and total soil C in the 0-10 cm soil layer given by the nonlinear exponential growth (red line) and relationship between C saturation deficit in mineral associated organic matter (CSD_{mOM}) and bulk-soil C given by the nonlinear exponential decay (blue line). The analysis includes 45 observations from the 0-2.5 cm, 2.5-5 cm and 5-10 cm soil layer of long-term subtropical no-till crop systems analyzed together: [i] Bare-soil (Bare), without crops cultivation, [ii] black oat/maize (O/M), [iii] black oat + vetch /maize + cowpea (O+V/M+C), [iv] lablab + maize (LL+M) and [v] pigeon pea +maize (P+M).

We performed a Spearman correlation analysis, which establish non-parametric relationships between pair of variables (SCHOBBER; SCHWARTE; SCHWARTE, 2018), to investigate the relationships between tPOM and CSD_{mOM} in the 0-2.5 cm, 2.5-5 cm, and 5-10 cm soil layers. This analysis indicated a strong negative relationship between these two variables (-0.90 , $p < 0.0001$) reinforcing

our hypothesis that tPOM accumulation was positively related to the saturation of mineral-associated SOM fraction, even the mechanisms involved are not well understood.

An exponential or linear increase of POM while C associated to fine soil particles tends towards saturation has been previously reported in different agroecosystems (Carter, 2002; Dieckow et al., 2005; Gulde et al., 2008; Santos et al., 2011; Stewart et al., 2008), including agricultural tropical and subtropical soils (Dieckow et al., 2005; Santos et al., 2011). Most plausible explanation for this effect can be related to hierarchical saturation, from smaller to largest soil structures (GULDE et al., 2008). The smaller size fractions are likely to reach the saturation first than larger size fractions due to hierarchy of aggregate formation (TISDALL; OADES, 1982). As the C accumulated in smaller size particles (as clay particles) has a slow turnover time than large size soil structures, the saturation of this fractions can occur first (CHUNG; GROVE; SIX, 2008). In temperate agroecosystem the mOM is likely to reach the saturation at low rates of C input than in large size aggregates (GULDE et al., 2008). In our study, an indication of saturation in mOM fraction only at high C input (P+M cropping system) can be due to faster decomposition of SOM in (sub)tropical soils compared to temperate regions (Six, et al., 2002b), suggesting that it is necessary much higher steady state C input levels to saturate the C physically protected in large size soil structures in this environment and variable charge soils.

However, the tPOM reported in this study resulted from the sum of the IPOM free and physically protected in soil aggregates < 2 mm and sPOM. Thus, a more detailed fractionation is required to investigate the C saturation of physically protected C in soil aggregates sizes (GULDE et al., 2008; STEWART et al., 2008a). We hypothesized a possible saturation of physically-protected SOM in microaggregates in the cropping systems that shown saturation of fine soil particles, while an exponential increase of tPOM must be due to increase of POM in lesser protected fractions, as macro aggregate structures (CHUNG; GROVE; SIX, 2008). One of most beneficial effects of no-till is related to physical protection of C due to increasing of soil aggregates stability and decreasing of aggregates turnover (CONCEIÇÃO; DIECKOW; BAYER, 2013; DENEFF, SIX, JOHAN; MERCKX, 2004; DENEFF et al., 2007). Although not evident in this

study, the saturation of POM considers a limit of soil C physical protection capacity, especially for microaggregates (Gulde et al., 2008; J Six et al., 2002a).

4. Conclusions

In long-term (36 yrs.), the high annual C input via legume-based cropping system under no-till improved the C storage in soil but led the C content in the fine silt plus clay-size fraction towards a saturation level, mainly at surface top layer (0-2.5 cm). Despite the decrease of the remaining capacity of C stabilization in mineral-association soil organic matter in soil layers up to 20 cm in long-term, the C accumulation in labile and non-protected soil organic matter fractions was preserved in this no-till soil after three decades. Moreover, the capacity of C stabilization in no-till soil was practically preserved in deeper soil layers (10-20 cm), suggesting the focus of soil management practices on abundant root systems aiming to prolong the C sequestration in no-till soils.

CAPÍTULO IV – Carbon saturation deficit and litter quality drive the stabilization of litter-derived ¹³C in mineral-associated organic matter of surface layers of a long-term no-till soil³

1. Introduction

Promote the atmospheric carbon dioxide (CO₂) sequestration in soils under conservation agriculture, especially in mineral-associated organic matter (mOM) passive pool, is an important mitigation pathway to slow down the global warming (BOSSIO et al., 2020; LAL, 2004, 2008; MACÍAS; ARBESTAIN, 2010; ZOMER et al., 2017). Mineral-associated organic matter is a slow turnover C pool that can persist in the soils from decades to millenniums, and the mechanisms involved in its stabilization in soil are mainly related to chemical bond with functional groups of minerals from clay-size fraction (KLEBER et al., 2015, 2021) and/or substrate compartmentation referred as C occlusion especially in microaggregates (MIKUTTA et al., 2006; VON LÜTZOW et al., 2008).

Soils may act as atmospheric CO₂ sinks via adoption of sustainable management practices (FAO, 2019) that reduces C losses through adoption of no-till system and that increases the annual C and N inputs by intensive and diversified cropping systems (FERREIRA et al., 2018; SÁ et al., 2017; VELOSO et al., 2018). However, despite the potential of agricultural soils to sequester C under conservation agriculture, some studies have shown a nonlinear soil organic C (SOC) accumulation in response to long-term C input. It suggests that soil's capacity to stabilize C is not infinite (CAMPBELL et al., 1991; NICOLOSO et al., 2018), promoting an intense debate about how long soils can act as CO₂ sink

(POWLSON; WHITMORE; GOULDING, 2011). Although, the Intergovernmental Panel on Climate Change (IPCC) assumes as a default value that soil C stock achieves a new steady-state after 20 years under improved agricultural management (HIRAISHI et al., 2013), this time can be longer according to regional soil and climate conditions and cropping system management (WEST; SIX, 2007).

The asymptotic increase of C stocks in soils, or absence of C increase in response to increase of steady state C inputs, it has been defined as the soil C saturation phenomenon (Six et al., 2002; Stewart et al., 2007; West and Six, 2007). In temperate agroecosystems, studies have shown that the limited soil's capacity for C stabilization on mOM pool is stronger than for physically-protected and unprotected or whole soil pools (CHUNG; GROVE; SIX, 2008; GULDE et al., 2008; KOOL et al., 2007; STEWART et al., 2007). Accordingly, the C saturation is to occur first in smaller than larger soil structures, indicating a hierarchy of saturation in soils (GULDE et al., 2008; KOOL et al., 2007). In addition, a recent literature review shows that mOM and particulate organic matter (POM) fractions vary in terms of formation, persistence and function in the soil, and consequently affect the soil's capacity to accumulate C over time (LAVALLEE; SOONG; COTRUFO, 2020)

In tropical and subtropical regions, highly weathered soils, with predominance of Fe and Al oxides and sesquioxides in the clay fraction, have pronounced capacity of C stabilization by organo-mineral association (BRIEDIS et al., 2016; FERREIRA et al., 2018; SANTOS et al., 2011). In contrast, despite of very representative soil classes in tropical and subtropical environment, resilient kaolinitic-clay loam Acrisols keep still little investigated with respect to saturation. Some evidences suggest that clay soil particles are C saturated after 17 years of no-tillage adoption in these soils (DIECKOW et al., 2005) and the saturation is extended to fine silt plus clay fraction (<20 μ m) in long-term and related to high input of high quality litters by legume cover crops as verified in our previous study in the same experiment. In no-tilled soils, the high input of crop residues can influence the C saturation deficit (C_{sd}) (*i.e.*, the difference between maximum soil C saturation level and the current soil C saturation level) of soil layers until 20 cm, but the more intense effects are concentrated in the first centimeters of soil. Therefore, considering all shoot biomass-C input occur on

surface in no-till soils, the decrease of C_{sd} of these soil surface layers can impact the stabilization of added C in soil. Moreover, C illuviation can contribute to C accumulation in deeper soil layers with higher C_{sd} (NICOLOSO et al., 2018).

Recent studies have highlighted the fundamental role of soil microorganisms and biochemical quality of substrate in the process of slow-cycling mOM formation (COTRUFO et al., 2013, 2015; KALLENBACH; GRANDY; FREY, 2016). High litter quality, which present higher nitrogen (N) content (i.e., low C:N ratio), high soluble fraction, and low lignin:N ratio is expected to be used more efficiently by soil microbes (higher proportion of C converted in microbial biomass than respired to CO_2) and induces large C stabilization in mOM per unit of added C (COTRUFO et al., 2013). However, faster, and more efficient accumulation of high-quality litter-derived C in the mOM can be highly dependent of the available capacity of mineral surfaces to interact and stabilize C as in soils with high C saturation C (Castellano et al., 2015).

Based on the complex soil-litter interactions that affects soil C stabilization in long-term no-tillage soils, we established two hypotheses:(i) stabilization of ^{13}C litter-derived in mineral-associated SOM fraction (silt plus clay-size) has a positive relationship with C_{sd} ; and (ii) the ^{13}C stabilization is more pronounced for vetch (legume)- than black oat (grass)-litters, mainly when C_{sd} is larger. To test our hypotheses, we conducted a field experiment to evaluate the impact of variable C_{sd} in surface layers of a no-till subtropical soil on the stabilization of ^{13}C labelled shoot biomass of grass and of legume 15-months after their addition on soil surface.

2. Material and methods

2.1 Site and field experiment description

The study was conducted in a long-term field experiment (36 years) at the Agronomic Station of the Federal University of Rio Grande do Sul, Eldorado do Sul (RS), southern Brazil (30° 51' S and 51°38' W). The climate is subtropical humid, Cfa type according to the Köppen classification, with a mean temperature of 19.4 °C and annual rainfall of 1440 mm. The soil is a Typic Paleudult according to the Soil Taxonomy, or a sandy loam Acrisol, according to the WRB classification, with 540 g kg⁻¹ sand, 240 g kg⁻¹ silt and 220 g kg⁻¹ clay. The clay

fraction is composed mainly by kaolinite (720 g kg⁻¹) and iron oxides (109 g kg⁻¹ Fe₂O₃) (BAYER et al., 2001).

The local land was converted from natural grassland to cropland in 1969, and then cultivated for 15 years under conventional tillage with plowing and disking operations for each of the two crops per year. The experiment was established in 1983 to evaluate the capacity of no-till cropping systems comprising different combinations of winter and summer cover crops with maize to improve the quality of a previously degraded soil (BAYER et al., 2000).

The field experiment is composed by ten no-till cropping systems (BURLE; MIELNICZUK; FOCCHI, 1997) in main plots (5x16 m), and two level of urea-N (0 and 180 kg de N ha⁻¹) for maize crop in the sub-plots (5 x 8 m), according to a split-plot randomized block design, with three field replicates. For this present study, we selected five no-till cropping systems with a wide range of annual C inputs: [i] Bare soil (BS), without crops cultivation except spontaneous plants controlled with herbicide glyphosate; [ii] black oat [*Avena strigosa* Schreb.]/maize [*Zea mays* L.] (O/M); [iii] black oat+vetch [*Vigna sativa* L.]/maize+cowpea [*Vigna unguiculate* (L.) Walp.] (O+V/M+C); [iv] maize+lalab [*Lalab purpureus* (L.) Sweet ssp. *Purpureus*] (L+M); and [v] pigeon pea [*Cajanus cajan* (L.) Mill sp.]+maize (P+M). Those treatments were selected because they represent a range of C saturation deficit (C_{sd}) in the fine silt plus clay fraction (<20 µm) of SOM, as identified our previous study conducted in the same experiment and created by the range of annual C inputs. Only sub-plots with N fertilization were included in the study. Some soil characteristics in each of the selected treatments when this study was started are given in Table 3.

The maximum C concentration to be reached in the fine silt plus clay fraction (<20 µm) of this soil, namely the C saturation level for this fraction, was estimated as 47 mg C g⁻¹ fraction. However, instead of the local C_{f_{max}}, we estimate C_{f_{max}} using the proportion of fine fraction <20µm and the slope (0.43) of boundary lines analysis (BL) method reported by Feng et al. (2013) for 1:1 soils aiming to have a CSD_{mOM} comparable to published data, as was described in our previous study. Considering the current C concentrations in this fraction across the five selected cropping systems, the estimated C saturation deficits in this fraction varied from 1.6 to 42% in the 0-2.5 cm layer and increased to 61 to 70% in the 10-20 cm layer (Table 3). Among the selected cropping systems, the C

saturation deficit in the silt plus clay fraction decreased as BS > O/M > O+V/M+C > L+M > P+M; and hereafter those treatments are respectively referred according to their C_{sd} level: *very high*, *high*, *moderate*, *low* and *very low*, respectively. Specific values of C_{sd} for each treatment or level are provided in Table 3.

Table 3. Initial soil and plant litter characterization before the field experiment in a subtropical Acrisol under no-tillage

Cropping	$\delta^{13}C$ (‰)	Total C -----g kg ⁻¹ -----	Total	C:N	Bsd Mg m ⁻³	C_{sd} %	C_{sd} level
0-2.5 cm							
BS	-20.61	11.8	1.39	8.5	1.51	41.8	<i>Very High</i>
O/M	-18.72	17.9	1.61	11.2	1.45	28.6	<i>High</i>
O+V/M+C	-19.98	21.1	1.85	11.4	1.13	20.9	<i>Moderate</i>
LL+M	-20.98	23.5	2.15	10.9	1.12	5.3	<i>Low</i>
G+M	-22.33	38.2	3.11	12.3	1.07	1.6	<i>Very Low</i>
2.5-5 cm							
BS	-19.63	7.93	1.06	7.4	1.35	56.1	<i>Very High</i>
O/M	-17.99	12.3	1.24	9.9	1.53	45.0	<i>High</i>
O+V/M+C	-20.34	17.2	1.55	11.1	1.32	30.0	<i>Moderate</i>
LL+M	-17.66	16.0	1.60	10.0	1.44	24.4	<i>Low</i>
G+M	-21.04	21.8	1.75	12.5	1.36	16.2	<i>Very Low</i>
Plant litter							
Grass (black)	1046.24	401.56	28.5	14.08	-	-	
Legume (vetch)	924.37	377.0	47.50	7.90	-	-	
Biochemical							
	NDF	ADF	Hem	Lig	Lig:N	-	
g kg ⁻¹						
Grass (black)	542.83	267.65	275.3	29.0	0.98	-	
Legume (vetch)	366.9	225.1	141.8	51.33	0.92	-	

Biochemical parameters of plants litters as neutral detergent fibers (NDF), acid detergent fibers (ADF), hemicellulose (Hem), lignin (Lig), lig nitrogen ratio (Lig:N) were determined according to Valsøy methods. Bulk soil density (Bsd) was determined using volumetric cores in the soil adjacent and out to the PVC tubes. C_{sd} : C saturation deficit in de fine soil fraction <20µm

2.2 ¹³C-residue labelling and analysis

Black oat (grass) and vetch (legume) plants were ¹³C-labelled by pulse application of ¹³CO₂ pulses using a 33% ¹³C atoms enriched sodium bicarbonate solution in an air sealed, expansible and sun light transparent glass chamber (40 cm high x 105 cm wide and 105 cm long) located inside a greenhouse. The labeling method we used was adapted from those of Bromand et al. (2001) and Tahir et al. (2018). Briefly, the two species were separately sown in sixty-six 8-L

plastic pots containing a mixture of soil (50%) and water washed sand (50%). The ^{13}C pulse labelling started thirty days after emergency, when photosynthesis rate was detected sufficient to decrease the CO_2 level inside the chambers from ~430 to ~327 ppm, as measured with infrared gas analyzer (IRGA - EXTECH, SD800). The ^{13}C pulses were performed between 9h and 11h pm, once a week, until the flowering stage. In each labeling event, after the chambers were closed and the CO_2 level decreased to 327 ppm by photosynthesis, a 99% ^{13}C atoms enrichment solution was applied directly to one plastic recipient containing 50 ml 2M HCl acid to form an atmospheric enrichment of 33% $^{13}\text{CO}_2$ inside the chamber. This procedure was repeated and the apparent CO_2 atmosphere concentration inside the chamber was maintained between 250 ppm and 350 ppm (BROMAND et al., 2001).

By the end of labeling, plants were cut close to the soil surface, partitioned into leaves and stems, air dried and cut into 3 cm length pieces. The plants parts were mixed forming the shoot biomass of black oat (12.86% panicles, 55.83% leaf and 31.31% stems) and vetch (44.8% leaf and 55.22% stems). Litter water content was measured on an oven-dried (65 °C) litter subsamples for dry-weight correction and a subsample was finely ground and subjected to total C and N, and $\delta^{13}\text{C}$ analysis in a mass spectrometer (IRMS) (Delta V Advantage, Thermo Fisher Scientific Inc. Germany). The biochemical composition of the studied plants was performed by method of Van Soest (Van Soest et al., 1991), as reported in Table 3.

2.3 Experiment with ^{13}C labeled residue addition and decomposition in the field conditions

The ^{13}C labeled residue trial was conducted for 15 months in the field experiment, where the five C_{sd} levels, given by the five selected cropping systems, were combined with the two types of labeled residue. A 5x2-factorial scheme was used (C_{sd} levels x residue type), in a complete randomized block design and three replicates.

For each plot of a given C_{sd} level, three open PVC cylinders (\varnothing 10 cm x 25 cm high) were inserted 20 cm into the soil to serve as incubation spots. One cylinder was used for grass residue, another for legume residue and the third cylinder did not receive any litter and was used as reference of the $\delta^{13}\text{C}$ natural

abundance of soil. The original surface residue from previous crops in each PVC spot was carefully removed. Then, the ^{13}C labeled residue of grass and legume were placed onto the soil surface of their respective cylinder, as to simulate the litter cover on no-till soil. The residues were applied at an equivalent rate of 4.5 Mg C ha^{-1} . After residue application, the top of each PVC cylinder was covered with a 3-mm mesh screen, to prevent the entry and exit of residue; and was perforated with small openings ($\varnothing 5 \text{ mm}$) at the soil surface level, to prevent internal surface water accumulation. During irrigation events, cylinders were covered with plastic films to prevent variation of soil water content that irrigation might cause.

2.4 Soil sampling and physical fractionation of soil organic matter

After the 15-month experiment, the remaining litter residue was collected from the soil surface, but since it represented only 1.4% (0.5 to 3%) of the applied residue, it was not quantified for ^{13}C and was assumed as fully decomposed.

The PVC cylinders were carefully removed by manual excavation around them. Then they were longitudinally cut using an electric saw and each soil core was divided into the 0-2.5, 2.5-5, 5-10 and 10-20 cm layers. The soil samples were gently broken into aggregates $< 9.75 \text{ mm}$ sieve, air dried and stored in plastic pots at room temperature.

Aggregate samples were physically fractionated into i) free-light fraction (FLF), ii) intra-aggregate light fraction (IALF), iii) sand particulate organic matter, $> 53\mu\text{m}$ (sPOM), and iv) mineral-associated organic matter, $< 53\mu\text{m}$ (mOM) (Figure 9), according to the procedure proposed by Sohi et al., (2001). Briefly, 12 grams of air-dried soil ($<9.75\text{mm}$) were added to a glass centrifuge bottle (100 ml). After 50 ml of sodium polytungstate solution (SPT) ($\rho = 2.0$) was added (CONCEIÇÃO et al., 2008), the bottles were closed with a rubber stopper and carefully inverted five times. Then, bottles were centrifuged for 90 min at $2.000 \times g$ and the FLF ($\rho < 2.0$) was recovered by filtration of the PTS suspension in a Millipore system (Aseptic Sterifil, 47 mm; Millipore Burlington, Massachusetts, EUA) using previously dried fiberglass filters (47 mm diameter, 2 microns, Whatman type GF/A).

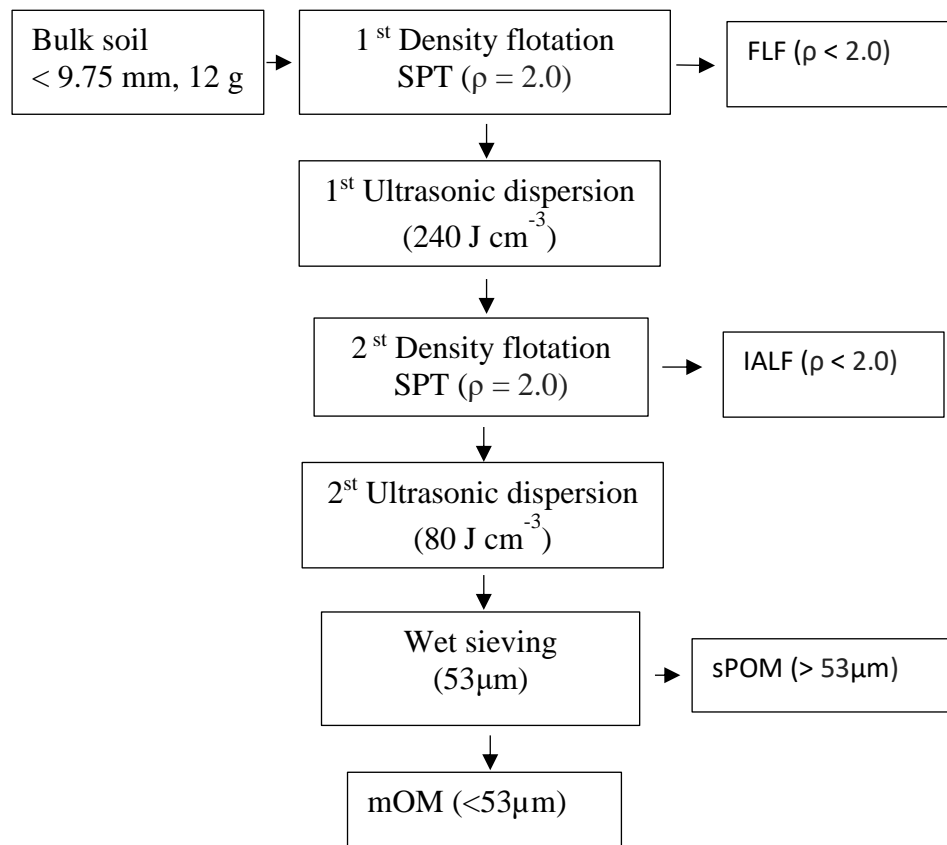


Figure 9. Soil physical fractionation scheme for the isolation of organic matter fractions: free light fraction (FLF), intra-aggregate light fraction (IALF), sand particulate organic matter (sPOM) and mineral-associated organic matter (mOM).

The FLF was washed with 200 ml of distilled water and 50 ml of 0.01 M CaCl₂ solution to remove the excess of PTS, then it was dried at 50 °C for 24 h. Afterwards, the soil was dispersed by applying 240 J cm⁻³ of ultrasonic energy, which was previously determined sufficient to produce 95% of clay dispersion (INDA JUNIOR et al., 2007). After dispersion, the IALF was recovered as described above for FLF. The remaining heavy fraction in the bottle was manually resuspended, washed three times and 50 mL of distilled water was added and the tube was submitted to 15 min centrifugation at 2500 x g. The clean supernatant was discarded, and the heavy fraction was resuspended again and subjected to 80 J cm⁻³ ultrasonic dispersion and passed through a 53μm mesh sieve. The sPOM was retained on the sieve and then transferred to an aluminum pan and oven-dried at 50 °C for 48 h (CAMBARDELLA; ELLIOTT, 1992). The

mOM fraction that passed the sieve was transferred to another aluminum pan and oven-dried at 50 ° C for 96 h. The recovery of the initial soil mass averaged 97% and that of total C averaged 95%.

2.5 Total C and ¹³C analysis, and ¹³C litter-derived C calculations

The whole soil and the physical fractions were oven-dried at 60 °C, finely ground in agate mortar, and total organic C and N were analyzed by dry combustion and δ¹³C isotope signature using an isotope ratio mass spectrometer (IRMS) (Delta V Advantage, Thermo Fisher Scientific, Bremen, Germany) interfaced with an elemental analyser (Flash EA 2000, IRMS Thermo Fisher Scientific Inc. Germany). The C concentration in each SOM fraction (g C kg⁻¹ fraction) was multiplied by its mass proportion in whole soil and divided by initial soil mass to obtain the C concentration of each fraction on the soil basis (g C kg⁻¹ soil).

The proportion of litter-derived C (*fC*) in the whole soil and SOM fractions was calculated by a mixed model, as follow.

$$fC = \frac{(^{13}C_{sr} - ^{13}C_s)}{(^{13}C_r - ^{13}C_s)} \quad (\text{Eq. 1})$$

where ¹³C_{sr} is the δ¹³C of the whole soil or SOM fractions in litter amended soil, ¹³C_s is the δ¹³C natural abundance of unamended control soil, and ¹³C_r is the δ¹³C of labelled litter of the grass or legume. The litter-derived C concentration (LC) in the whole soil and SOM fractions was calculated by multiplying the *fC* by the total C concentration in the sample (g kg⁻¹ soil).

The litter-derived C stock (LC stock) in the whole soil and in the SOM fractions was calculated using Eq. 2:

$$\text{LC stock (kg C ha}^{-1}\text{)} = \frac{\text{LC} \times \text{BD} \times \text{dp} \times 10000}{1000} \quad (\text{Eq. 2})$$

where, BD is de bulk soil density (kg m⁻³, Table 3) and dp is the soil depth (m).

Litter-derived C conversion to whole soil and to mOM fraction was calculated using Eq. 3:

$$\text{Litter conversion (\%)} = \frac{\text{LC stock}}{C \text{ input}} \times 100 \quad (\text{Eq. 3})$$

where *the C input* is the litter C input (kg C ha⁻¹).

The litter-derived C distribution (LCD) in each SOM fractions was calculated using Eq. 4:

$$\text{LCD (\%)} = \frac{\text{LCfraction} \times 100}{\Sigma \text{LCfractions}} \quad (\text{Eq. 4})$$

where, LC_{fraction} is the litter-derived C stock in the fraction, i.e., FLF, and $\Sigma \text{LC}_{\text{fractions}}$ is the summatory of litter-derived C stock of all SOM fractions.

2.6 Statical analysis

Data was tested for and fulfilled the assumption of normality and homogeneity of variance, according to Shapiro-Wilk's and Levene's tests, respectively. The PROC MIXED procedure on SAS was used to compare the effects of C_{sd} and litter type on the response variables. This procedure considers the main factors (C_{sd} and litter type) and their interactions as fixed factors and the blocks and the experimental errors as random factors. The litter-derived C data was subjected to analysis of variance and when significant ($p < 0.05$), the difference between means were evaluated by Tukey's test ($p < 0.05$). Linear regression analysis was also performed to explore the relationship between litter-derived C and C_{sd} level. All analyses were performed using the SAS statistical package® v.9.4 (Statistical Analysis System Institute, Cary, North Carolina), except for regression analysis that was performed using SigmaPlot (SigmaPlot for Windows v.12.0, Systat Software, Inc., San Jose, CA).

3. Results

3.1 Soil C content and stocks

After the 15-month under field conditions, the litter-derived C that entered the soil accumulated mainly in the top 0-2.5 cm layer and to some extent in the 2.5-5 cm but was almost not detected in the 5-10 and 10-20 cm (Figure 10). Across the five cropping systems representing the C_{sd} gradient, the content of litter-derived C in the responsive top layer ranged from 1.33 to 2.74 $g\ kg^{-1}$ under grass litter, and from 1.39 to 2.59 $g\ C\ kg\ soil^{-1}$ under legume litter (Figure 10).

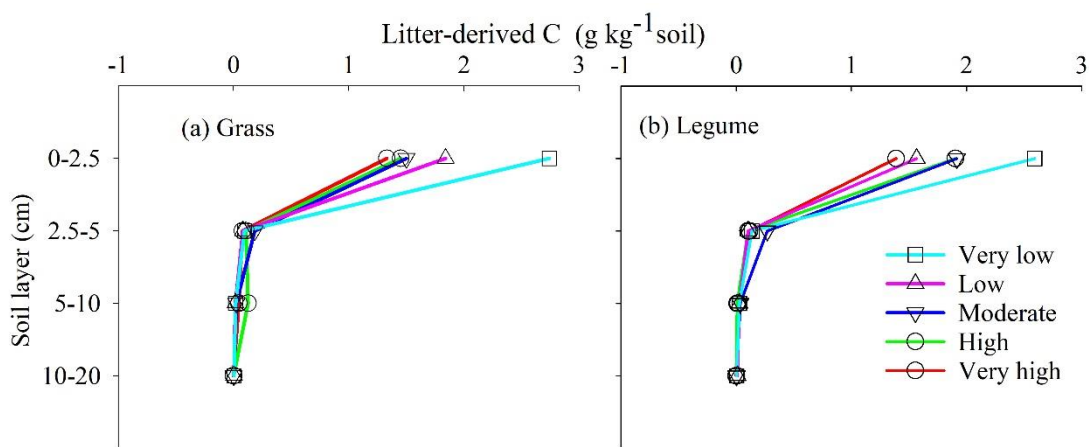


Figure 10. Content of litter-derived C in soil after 15-months *in situ* field experiment with ^{13}C -labeled of grass (black oat) and legume (vetch) litters in a subtropical Acrisol under no-till with a gradient of C saturation deficit.

The stock of litter-derived C in the top 0-2.5 cm was influenced by the C_{sd} ($p=0.0364$), but not by the litter type ($p=0.3975$); and this trend was also observed when considering the whole 0-20 cm layer (Table 4). Most of the litter-derived C stocks in the whole 0-20 cm layer was stored in the top 0-2.5 cm (85-92%), while only a small portion of it (8-15%) accumulated in the deeper layer of 2.5-20 cm (Figure 11). Although the effect of C_{sd} ($p=0.0425$) on litter-derived C stock at 0-20 cm soil layer was significant (Table 4), there was not a clear tendency of stabilization of the litter-derived C across the C_{sd} gradient (Figure 11), and no relationship was found between C_{sd} and litter-derived C stocks in the 0-2.5 cm (Figure 12).

Table 4. Results of analysis of variance on the effect of carbon saturation deficit (C_{sd}), litter type and interaction of these factors on residue ^{13}C -derived C stock in the bulk-soil, physical fractions of soil organic matter and carbon conversion after 15 months experiment in the field.

Soil layer (cm)	nC_{sd}	Litter type	$C_{sd} \times$ Litter type
-----p value-----			
C stocks			
Whole soil			
0-2.5	0.0364*	0.3975 ^{ns}	0.3979 ^{ns}
2.5-20	0.3252 ^{ns}	0.4890 ^{ns}	0.0228*
0-20	0.0425*	0.3222 ^{ns}	0.5032 ^{ns}
FLF			
0-2.5	0.0112*	0.9179 ^{ns}	0.1428 ^{ns}
2.5-5	0.0351*	0.0539 ^{ns}	0.0574 ^{ns}
0-5	0.0111*	0.9555 ^{ns}	0.1484 ^{ns}
IALF			
0-2.5	0.9252 ^{ns}	0.1352 ^{ns}	0.2117 ^{ns}
2.5-5	0.0160*	0.0182*	0.9261 ^{ns}
0-5	0.9396 ^{ns}	0.1209	0.2428
sPOM			
0-2.5	0.0410*	0.1271 ^{ns}	0.1060 ^{ns}
2.5-5	0.5500 ^{ns}	0.3390 ^{ns}	0.2589 ^{ns}
0-5	0.0462*	0.1539 ^{ns}	0.1312 ^{ns}
mOM			
0-2.5	<.0001***	<.0001***	0.0022**
2.5-5	0.3035 ^{ns}	0.0084**	0.0391*
0-5	<.0001***	<.0001***	0.0049**
Litter C conversion			
Whole soil 0-20 cm	0.0421*	0.3234	0.5025
mOM 0-5 cm	<.0001***	<.0001***	0.0055*
Litter-derived C distribution			
FLF	0.0088**	0.2079 ^{ns}	0.2594 ^{ns}
IALF	0.1460 ^{ns}	0.6554 ^{ns}	0.0930 ^{ns}
sPOM	0.0729 ^{ns}	0.8349 ^{ns}	0.8768 ^{ns}
mOM	0.0063**	0.0632 ^{ns}	0.1758 ^{ns}

n carbon saturation deficit (C_{sd}). Free light fraction-FLF; intra-aggregate light fraction-IALF; carbon recovered in the sand (>53 μ m) referred as particulate organic matter (sPOM), mineral-associated organic matter (mOM) in the silt plus clay particles (<53 μ m). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ means significant effects at 5%, 1% and 0.1% of significance level, respectively.

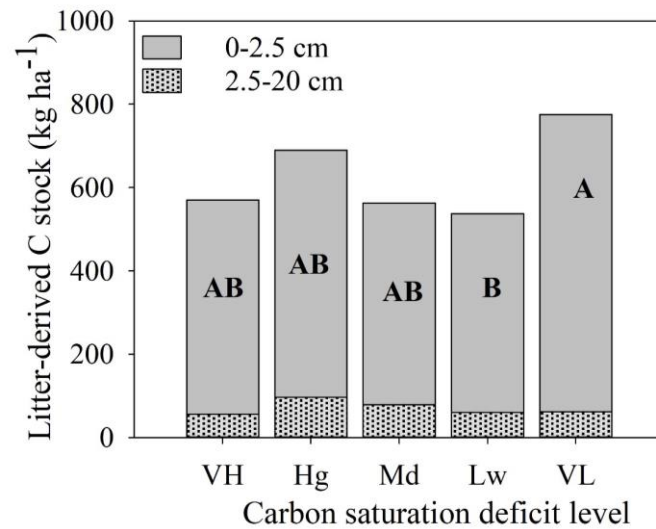


Figure 11. Litter-derived C stock after 15-months ^{13}C labeled litter decomposition in the field experiment in a subtropical Acrisol under no-till with a gradient of C saturation deficit. Means followed by same letters did not differ when evaluated the effect of carbon saturation deficit on litter-derived C in whole soil C stock in the 0-2.5 cm. There was no significant effect of the individual factors on the litter-derived C stocks in the 2.5-20 cm nor in 0-20 cm soil layer. Differences between means were tested according to Tukey's test ($p \leq 0.05$)

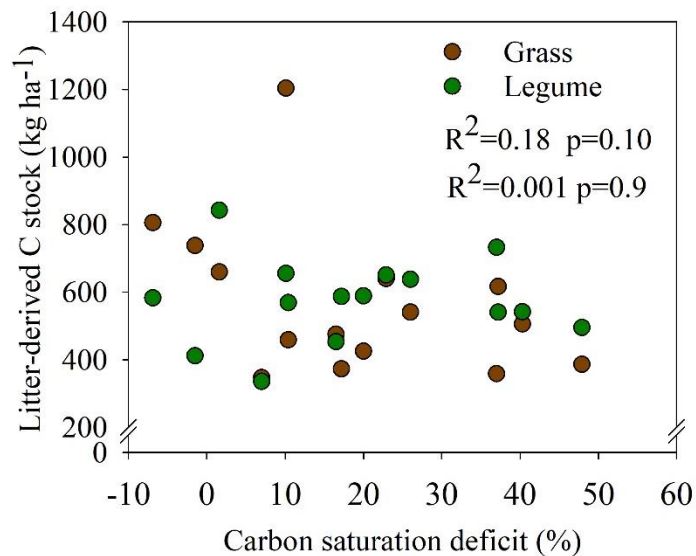


Figure 12. Relationship between C saturation deficit and litter-derived C stock in the whole soil. The analysis includes 30 observations from 0-2.5 cm layer after 15-months in situ field experiment with ^{13}C -labeled grass (black oat) and legume (vetch) litters in subtropical Acrisol under no-till.

3.2 Effects of C_{sd} and litter type on soil organic matter fractions

3.2.1 Non-protected (FLF) and physically protected C (IALF)

The amount of litter-derived C stored as FLF was influenced by the C_{sd} , as observed in the 0-2.5 cm ($p=0.0112$), 2.5-5 cm ($P=0.0351$) and, thus, in the whole 0-5 cm depth ($p=0.0111$) (Table 4). In those layers, more litter-derived C was stored in the FLF as lower was the C_{sd} (Figures 13a and 13b); and this result was confirmed by the negative linear relationship between the C_{sd} and litter-C stored as FLF in the 0-2.5 cm ($R^2=0.28$, $p=0.041$) and 2.5-5 cm soil layers ($R^2=0.59$, $p<0.001$) (data not shown). On average, the FLF accounted for 50% of total litter-derived C stored in the first 5 cm soil (adapted from results in Figure 13).

The litter type, however, did not affect the C stored as FLF, neither in the 0-2.5 cm ($p=0.9179$) nor in the 2.5-5 cm ($p=0.0539$) (Table 4). Accordingly, there was no interaction between C_{sd} and litter type ($p>0.05$) in the litter-derived C storage in FLF (Table 4).

The -derived C stored as physically protected fraction (IALF) was not affected by C_{sd} or litter type in the uppermost soil layer (0-2.5 cm) (Table 4). Across the C_{sd} gradient, litter-derived C which accumulated in the IALF of the 0-2.5 cm was on average 100 kg ha⁻¹ (Figure 13c) and accounted for 19% of the total litter-derived C stored in this soil layer. In the 2.5-5 cm, though, both C_{sd} ($p=0.0160$) and litter type ($p=0.0182$) affected the litter-derived C incorporation in the IALF (Table 4), with greater incorporation in the *very low* compared to *very high* and *high* C_{sd} levels (Figure 13d). Regarding the effect of litter type, legume derived C was 43% greater than grass-derived C in this fraction and layer (Figure 13e).

The litter-derived C stock recovered in the sand particulate organic matter (sPOM) varied from 5.9 to 18.5 kg C ha⁻¹ in the 0-5 cm (Figure 14) and was weakly influenced by C_{sd} in both 0-2.5 cm ($p=0.0410$) and 0-5 cm layers ($p=0.0462$) (Table 4). Litter type did not affect the C stocks in sPOM fraction, regardless of soil layer (Table 4). In terms of C_{sd} gradient, it was observed greater sPOM value in *high* than in *very low* level (Figure 14). Nevertheless, there was no linear relationship between sPOM and C_{sd} in the 0-2.5 cm ($R^2=0.22$ $p=0.076$) nor in the 0-5 cm ($R^2=0.22$ $p=0.075$) (data no show).

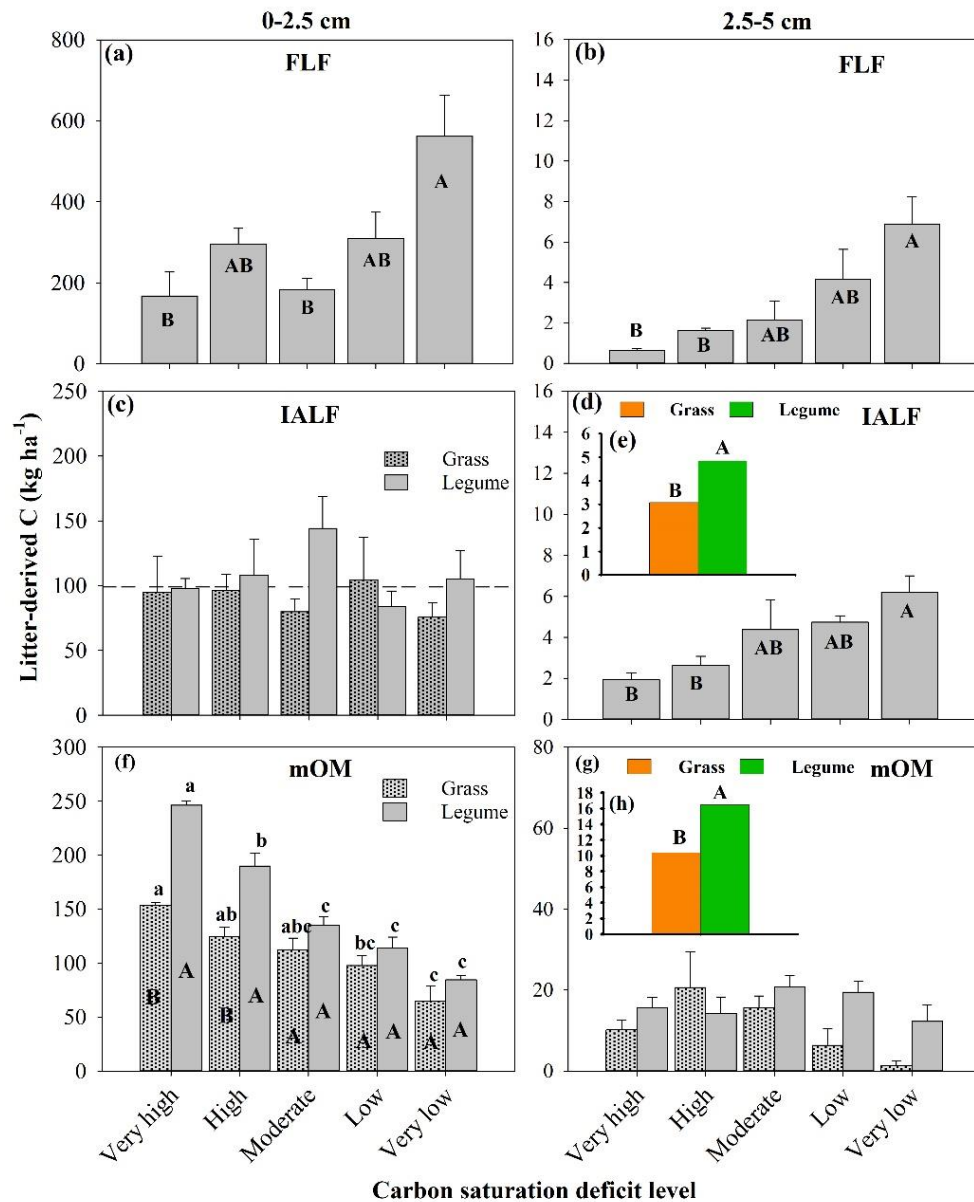


Figure 13. Effect of carbon saturation deficit at 0-2.5 cm (a), and 2.5-5 cm soil layer (b) and litter type (e, h) at 2.5-5 cm, and interaction factors (deficit x litter type) effect (f) on litter-derived C stock accumulated in the soil organic matter fractions: Free (FLF) and intra-aggregate (IALF) light fractions and mineral-associated organic matter (mOM) after 15-months *in situ* ^{13}C -labeled litter decomposition. Means represented by bars labeled with different letters are significantly different ($p < 0.05$) according to Tukey's test in the figures a, b, d, e, f and h. Lower case letters compare the levels of C saturation deficit for each litter type and capital letters compare the litter types for each C saturation deficit by Tukey's test ($p < 0.05$) in figure f.

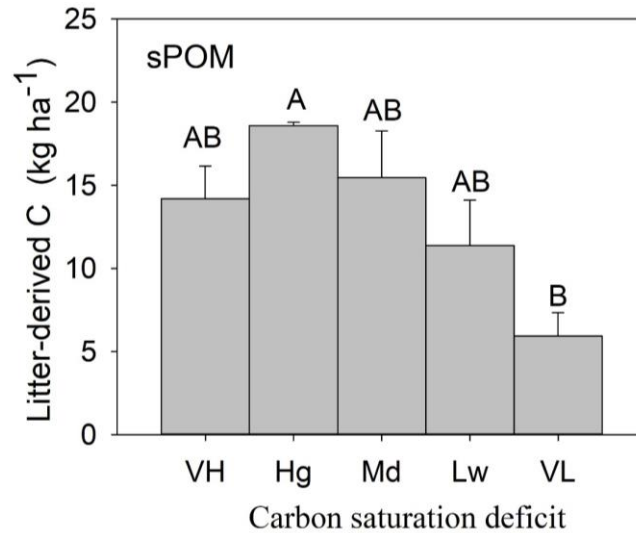


Figure 14. Effect of C saturation deficit on litter-derived C stock recovered in sand-size fraction in the 0-5 cm soil after 15-months ¹³C-labeled grass (black oat) and legume (vetch) litters decomposition *in situ* in a subtropical Acrisol under no-till. Means represented by bars labeled with different letters differ statistically by Tukey test ($p \leq 0.05$).

3.2.2 Mineral-associated organic matter (mOM)

Litter-derived C stored in the mOM fraction was influenced by both C_{sd} and litter type, either in the 0-2.5 cm ($p=0.0022$) or cumulatively in the 0-5 cm depth ($p=0.0049$) (Table 4). In top layers with *very high* or *high* C_{sd} , the incorporation and stabilization of legume-derived C in the mOM was 44-53% higher than that derived from grass (Figure 13f). With *moderate*, *low* or *very low* C_{sd} levels, the stabilization of litter-derived C did not differ between grass- and legume-litter. Overall, the stock of litter-derived C in the mOM fraction of the 0-5 cm layer increased linearly with C_{sd} , but at a rate that was twice as high under legume litter than under grass litter (3.7 vs 1.76 g C kg⁻¹ C added) (Figure 15).

Despite the significant effect of the interaction between C_{sd} and litter type on the litter-derived C accumulated in mOM ($p=0.0391$, Table 4), the effect of the C_{sd} on litter-C stabilization was not clear for the 2.5-5 cm layer (Figure 13g).

However, the litter-C stored in the mOM fraction of this 2.5-5 cm layer was 63% higher when it came from legume than from grass (Figure 13h).

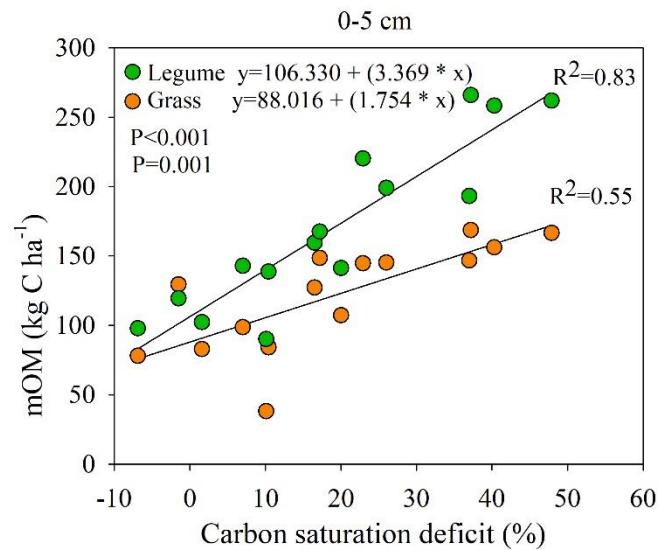


Figure 15. Relationship between C saturation deficit and litter-derived C stock in the silt plus clay-size particles in the 0-5 cm layer of a subtropical Acrisol under no-till after 15-months ¹³C-labeled grass (black oat) and legume (vetch) litters decomposition *in situ*.

3.3 Efficiency of litter-C conversion

There was no clear effect of C_{sd} on the conversion of litter-C into soil-C for the 0-20 cm layer (Figure 16a), which was also indicated by the non-significant relationship between C_{sd} and litter-C conversion to whole soil-¹³C stocks at 0-20 cm (Figure 12). Neither litter type affected this litter-C conversion into soil C ($p=0.7875$) (Table 4). On average across the five C_{sd} levels and the two litter types, 14% of added litter-C was converted into soil C pool (Figure 16a).

However, focusing only on the mOM fraction of the combined 0-5 cm layer, the conversion of litter-C into soil C was higher for legume litter than for grass litter, mainly in the highest levels of C_{sd} ($p=0.0055$) (Table 4, Figure 16b). The conversion rate of litter-C into mOM-C ranged from 1.42 to 5.81% (Figure 16b). In the two highest C_{sd} levels, the amount of litter-C accumulated in the mOM fraction was 22-40% higher under legume litter than grass-litter (Figure 16b).

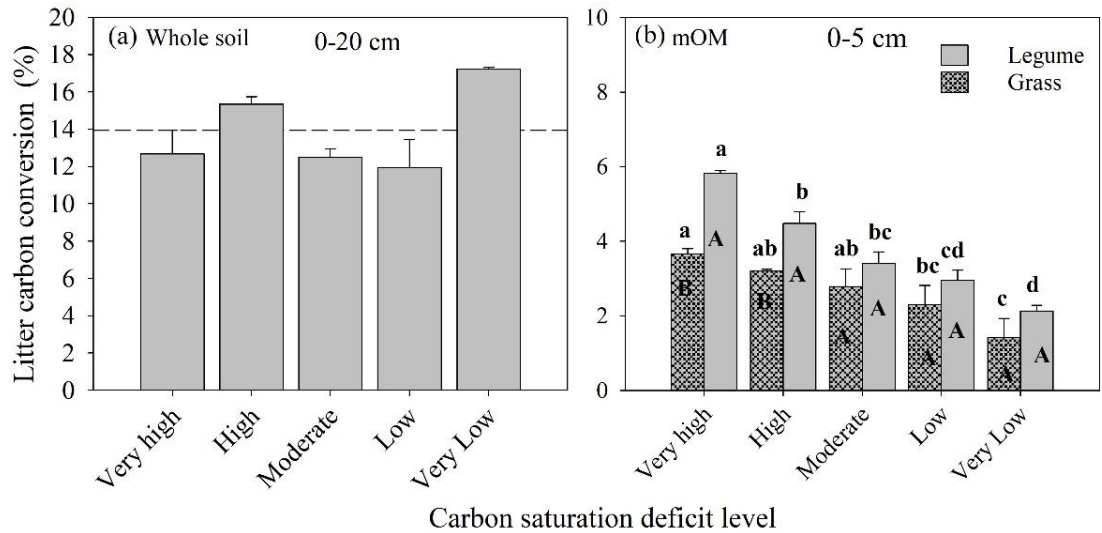


Figure 16. Litter-C conversion to whole soil (0-20 cm) (a) and to mineral-associated C fraction (b) at 0-5 cm soil layer after 15-months ^{13}C -labeled litter decomposition in a subtropical Acrisol under no-till with a gradient of carbon saturation deficit. Means represented by bars labeled with different letters differ statistically by Tukey test ($p \leq 0.05$). Lowercase letters compares the levels of carbon saturation deficit within the levels of litter quality and capital letters compares the levels of litter type within levels of carbon saturation.

In contrast, for the lower C_{sd} levels, the conversion of litter-C into mOM-C was not significantly different between legume- and grass-litter (Figure 16b). For each litter type, the magnitude of differences in litter-C conversion among C_{sd} was higher for legume than for grass. There was higher conversion of litter-C to mOM-C in the *very high* than all other C_{sd} levels for legume litter, while for grass litter the C conversion in the *very high* C_{sd} was greater only than *low* and *very low* deficit levels (Figure 16b).

3.4 Litter-derived C distribution into soil organic matter fractions

The distribution of litter-C in the FLF and mOM was affected by the C_{sd} ($p < 0.01$), regardless of the litter type (Table 4). The proportion of litter-C stored as FLF ranged from 33 to 74% of total litter-C converted to SOM across C_{sd} levels, reducing the proportion according increase the C_{ds} level (Figure 17). The proportion of ^{13}C present in this fraction of SOM increased linearly with decrease of C_{sd} in the 0-5 cm soil layer ($R^2 = 0.54$, $p = 0.02$) (data not shown). When the proportion of FLF was high in the lowest C_{sd} level, the mOM fraction accounted for a small portion of total litter-C in the soil. The proportion of mOM ranged from 11 to 48% of total litter-derived C stock (Figure 17). Moreover, ^{13}C contents present in this fraction of SOM decreased linearly with decrease of C_{sd} ($R^2 = 0.64$ $p < 0.001$) in the soil surface (0-5 cm) (data no shown).

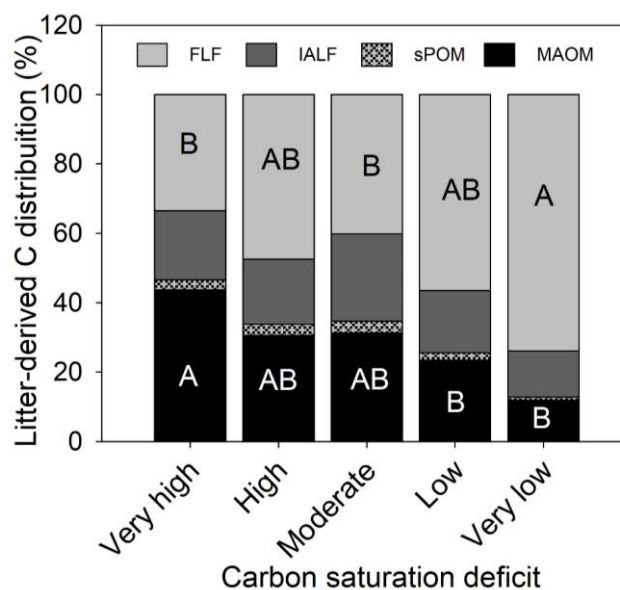


Figure 17. Effect of C saturation deficit on the distribution of soil organic matter (SOM) fractions [free- and intra-aggregate light fractions (FLF and IALF), (>53 μ m) particulate organic matter (sPOM) and mineral-associated SOM < 53 μ m, (mOM)] relative to total C recovered after SOM fractionation. Means represented by bars labeled with different letters differ statistically by Tukey test

The proportion of litter-derived soil C recovered as IALF and as sPOM ranged from 13 to 25% and from 1 to 3%, respectively; but these fractions were influenced neither by C_{sd} nor by litter type (Table 4, Figure 17).

4. Discussion

4.1 Litter-derived C in soil C stock

For a field assessment of C_{sd} concepts, a broad range of steady state C input in different long-term cropping systems under no-till in a kaolinitic subtropical soil was evaluated in a long-term experiment in southern Brazil. To the best of our knowledge, this is a pioneer study in which the influence of C_{sd} on ^{13}C litter-derived SOC incorporation and distribution within SOM fractions was investigated directly at the field using ^{13}C -labeled litters, mainly in a (sub)tropical environment.

Results indicated that the litter-derived C accumulated mainly in the top 2.5 cm of this no-till soil but was not related to C_{sd} nor influenced by litter type (0-20 cm) (Figure 11). Carbon accumulation in the surface soil layers has been typically reported for subtropical no-till soil, which is related to the litter-C addition on soil surface, without being incorporation into the soil (e.g., Conceição et al., 2013; Rodrigues et al., 2021), as well it converges with global pattern of soil C accumulation as a function of soil depth (BALESDENT et al., 2018).

Although C movement from surface to subsurface layers can be intensified with saturation in the soil surface in temperate environment (NICOLOSO et al., 2018), in our study, the long-term (36-years) crops C input on soil surface in the field experiment decreased C_{sd} in thin surface layers, but no influence of C_{sd} was observed in the ^{13}C movement to deeper soil layers (> 5 cm). These results suggest that C saturation in the surface soil layer may not increase the C movement to deeper soil layer (Figure 10). It is possible that C accumulation in deeper soil layer in long-term no-till soil can be derived from plants roots (TAHIR et al., 2016) and from the influence of biological activity, that in this study was restricted. However, future *in situ* studies with ^{13}C labeled shoot and root at field are required (KONG; SIX, 2010; TAHIR et al., 2016). Moreover, repeated ^{13}C -shoot addition and longer experiment time is also required to confirm this hypothesis.

The lack of relationship between litter-derived C stocks and C_{sd} (Figure 12) indicated that the previous level of C in silt plus clay-size soil particles did not affect the litter-derived C storage in the whole soil. This result corroborates with those reported in the study of Hassink (1997), in which the authors did not found

relationship between a broad range of fine soil particles proportion (range of C_{sd}) (<20 μm) and C storage in the whole soil. In our study, lower ^{13}C stabilization in mOM in lower C_{sd} levels was compensated by higher ^{13}C incorporation in labile or non-protected SOM fractions, which resulted on increase of whole soil C stocks, as discussed in the next item. Our findings revealed that C_{sd} drives the preferential mechanism of C stabilization in soil. Lavalley et al. (2020) reported that the soil's capacity to store mOM is finite, whereas soil is not subjected to saturation by continuous inputs of POM. Nevertheless, the accumulation of POM fractions depend on physical protection mechanisms (occlusion in macroaggregates) (VON LÜTZOW et al., 2007), which is fostered in no-tillage farming due to absence of soil disturbance and breakdown of large aggregates (SIX; ELLIOTT; PAUSTIAN, 2000).

4.2 Litter-derived C allocation in unprotected SOM fractions in response to C_{sd}

The results showed that litter-derived C stored as FLF, usually referred as unprotected particulate organic matter, increased linearly with decrease of C_{sd} , so that the FLF stored most the litter-derived C in the lowest C_{sd} (Figures 13a and 13b). These results indicate that in soils in which the mOM fraction is reaching saturation, the C from short-term inputs of aboveground material is to accumulate primarily in the unprotected fraction of SOM. Similar results were found in response to long-term increased manure application on well-structured rich C soil (GULDE et al., 2008) and in soil under elevated CO_2 and C input (KOOL et al., 2007) from temperate ecosystems.

The FLF is a transitory C pool between fragmented fresh litters of plant origin and stabilized C in soil (GOLCHIN et al., 1994). Its increase in response to decrease of C_{sd} in the 2.5-5 cm soil layer (Figure 13b) suggest that litter was fragmented and physically transferred to deeper layer into the soil (COTRUFO et al., 2015). However, this effect was very little, because most litter-derived C accumulated in the uppermost soil layer (0-2.5 cm) and was not impacted by litter type (Figure 13a). In the soil, the particulate organic matter can be protected by its inherent recalcitrance (VON LÜTZOW et al., 2008), fragmented and protected by spatial inaccessibility during aggregates formation (Golchin et al., 1994; Six et al., 2000), or even be completely mineralized to CO_2 and lost to the atmosphere

in the short term. The results suggest that most of the litter-derived C stored in soils of lowest C_{sd} levels may be more susceptible to microbial degradation, once the saturation of the mOM may limit additional C transferences and stabilization into this fraction.

Considering the existence of an hierarchy of saturation from small to large soil structure units (KOOL et al., 2007), the lack of C_{sd} effect on litter-derived C stored as IALF in the upper soil layer (0-2.5 cm) (Figure 13c) indicated physical protection mechanism of SOM is the main pathway through which soil C has been stabilized. However, considering that the IALF was obtained after complete soil dispersion in our study, a more detailed assessment of the effect of C_{sd} on physically-protected fractions is required, especially regarding C incorporation in macro and microaggregates (GULDE et al., 2008; KOOL et al., 2007; SIX et al., 2002a). Nevertheless, the increase of IALF in the 2.5-5 cm in response to decrease of C_{sd} level suggests that the recently formed aggregates and occlusion of litter-derived C was greater in low than high C_{sd} in this soil layer (Figure 13d). In addition, legume litter can contribute more to physically protected fraction in depth than grass litter (Figure 13e). Cotrufo et al. (2015) reported that in the latter stages of litter decomposition, the fragmented particulate organic matter was transferred and accumulated at depth. In this sense, our results illustrated that this process was more intense for legume- than grass-litter (Figure 13e). An investigation of ^{13}C and ^{15}N -labelled maize litter incorporation in water-stable macroaggregates of SOC-rich topsoil and SOC-poor subsoil revealed that water-stable macroaggregate formation per unit of litter-C added was greater in the SOC-poor subsoil than in the SOC-rich topsoil (POIRIER et al., 2020).

Physical protection of SOM has been typically identified as one of the strongest effects of long-term no-till cropping systems in subtropical environment due to its effect in soil aggregation (CONCEIÇÃO; DIECKOW; BAYER, 2013; VELOSO et al., 2020). The saturation of physically-protected fraction was proposed due to limit of soil aggregation (Six et al., 2002a). Chung et al. (2008) provided an evidence of whole soil C saturation in a long-term grain production system with wide range of soil C inputs due to a combination of straw retention, tillage, and N fertilization treatments in Canada. In their study, large and small macroaggregates did not sequester additional SOC in response to higher soil C inputs (Chung et al., 2008). In contrast, in tropical and subtropical soils there are

no evidence of saturation of this SOM fraction. It is plausible that due to higher rates of SOM decomposition in the (sub)tropical than temperate environment (Six et al., 2002b), a high steady state C input is also required to saturate the physically-protected C fractions, especially in those soils with well-developed structure.

4.3 Incorporation of recent added C inputs in mineral-associated organic matter (mOM) fraction depends on litter type and C saturation deficit

Litter type clearly affected the C stabilization in the mOM fraction in this subtropical no-till soil (Figure 13f), with greater C contribution from legume than from grass litter. As most of the mineral-accumulated SOM is of microbial origin (KALLENBACH; GRANDY; FREY, 2016; MAMBELLI et al., 2011), greater C stabilization from high- than low-quality litters may occur due to the high proportion of C incorporated into the microbial cells per unit of C uptake, what is typically referred as C use efficiency (COTRUFO et al., 2013). Despite legume litter had higher lignin content than grass litter, both had similar lignin-to-nitrogen ratio (Lig:N) (Table 3). Further, legume litter had greater N content and lower C:N ratio, and higher soluble fraction and thus it has higher quality than grass litter (Table 3). The high-quality litter is expected to be easily decomposed by soil microorganism, due to its great proportion of low-molecular weight substances. Therefore, less energy is needed by soil microbes to decompose these compounds, resulting in greater microbial biomass and microbial-derived compounds that can be stabilized in soil matrix per unit of C added (Castellano et al., 2015; Cotrufo et al., 2013).

High efficiency of SOM formation through the early stages of labile litter decomposition was reported in a ^{13}C -labeled litter *in situ* field experiment (RUBINO et al., 2010). Dissolved organic matter-microbial pathway to mineral-associated C fraction has been reported as important mechanisms of stable SOM formation (COTRUFO et al., 2015; KAISER; KALBITZ, 2012). In addition, recalcitrant compounds as lignin have not been selectively preserved during litter decomposition (MARSCHNER et al., 2008), so that its degradation can be faster in the presence of easily decomposable C sources (KLOTZBÜCHER et al., 2011). Yet, corroborating our hypothesis, the effect of high quality-litter on C

stabilization in mOM depends on the C_{sd} , and this is firstly noted in the soil surface (Figure 13f). In other words, in soil with mOM saturated or close to saturation, C from higher quality-litter accumulates in the mOM fraction at amounts not greater than C from low quality-litters in subtropical environments. This effect is related to limitation imposed by saturation in the availability of functional groups in the silt plus clay-size particles for stabilization of added C, regardless of litter type (Figure 13f). Our results corroborate those reported by Castellano et al., (2015), in which the effect of litter quality on C stabilization in the mineral-associated SOM occurs mainly for soil with available capacity for C stabilization, i.e. high C_{sd} .

A greater conversion of litter-C into mOM in soil was verified for legume- than grass-litter only in the high C_{sd} levels (Figure 16b), suggesting that soils receiving high-quality litter over long long term will present lower C_{sd} (Castellano et al., 2015). This result corroborates our previous findings in the same experiment, in which we showed lower C_{sd} in the long-term legume-based cropping systems. Furthermore, recent studies at the same experimental site showed that legume cover crops and no-tillage combined favor the C stabilization by organo-mineral interactions (VELOSO; CECAGNO; BAYER, 2019) and, in which, the processes are most mediated predominantly by fungi than bacteria (VELOSO et al., 2020). Fungal population decomposes lignin derivatives more efficiently than bacteria (KALLENBACH; GRANDY; FREY, 2016). Thus, our field study results provide evidence that litter quality and C_{sd} control the C stabilization in the mineral-associated C fraction (Figure 13f and Figure 15). These findings can serve as base to establish best management practices to improve the C sequestration in (sub)tropical soils, and then, mitigate climate changes.

4.4 The C_{sd} controls the allocation of recent added C within SOM fractions

Our results indicate that the C_{sd} affected the allocation of litter-derived C within labile and stable SOM fractions (Figure 17). The limitation imposed by the level of stable C_{sd} on incorporation and stabilization of litter-derived C in silt plus clay-size particles and the increase as FLF suggests that previous level of C_{sd} controlled the mechanisms of protection of recently added C (Figure 17). In soil with *very high* C_{sd} , the litter-derived C was likely protected mainly by physical-chemical protection in silt plus clay-size particles. In contrast, with decreasing C_{sd}

the litter-derived C is to be protected preferentially by other mechanisms, including physical protection in soil aggregates (GULDE et al., 2008; STEWART et al., 2008a) and inherent biochemical recalcitrance of particulate organic matter, although the latter do not lead to long-term SOM stabilization (MARSCHNER et al., 2008).

Occluded-light fraction is composed mainly by old fragmented particulate organic matter, from both roots and aboveground litter (GOLCHIN et al., 1994) physically-protected within soil aggregates, especially in microaggregates (Six et al., 2002). The absence of C_{sd} effect in the proportion of litter derived ILAF in the uppermost soil layer (0-2.5 cm) can be related to insufficient time for fully litter fragmentation, decomposition, and incorporation into IALF fraction (Figure 17). In addition, only aboveground plant litters were incubated in the soil surface and, thus, the additional impact of roots on SOM fractions was not assessed in this study. Fine-plant roots and their exudates are possibly contributing to aggregates formation, especially to large aggregates (> 2000 μ m) (SMUCKER, 2003; TISDALL; OADES, 1982). As a source of C, roots and aboveground litters also provide a food source to the microflora and fauna which contribute to structure formation and stabilization (ANGERS; CARON, 1998). Thus, the combine effects of shoot and root biomass on physically-protected C fractions should be addressed in future studies using *in situ* ^{13}C labeled plant (KONG; SIX, 2010; TAHIR et al., 2016).

5. Conclusions

The field study using ^{13}C -litter provides evidence that soil C saturation deficit is a factor that controls the litter-C stabilization in the mineral-associated SOM fraction and influences the litter-C incorporation in the labile SOM fractions in a subtropical no-till Acrisol. Soil C saturation deficit was the main controller of the conversion of litter-C into mineral-associated-C. In soil with high C saturation deficit, litter-C from legume (vetch) is converted into soil mineral-associated-C at a greater rate than litter-C from grass (black oat). This implies that adopting legume-based cropping systems with high input of legume residue in C-poor soils (high C saturation deficit) can be a good strategy for atmospheric CO_2 sequestration in the soil.

In contrast, soils with very low C saturation deficit does not convert greater amount of C incorporation to mineral-associated C fraction when receive high-quality litter. In C-rich soils, additional C incorporation occurs mainly in the inter- and intra-aggregates SOM fractions. Despite these fractions of SOM are more accessible to microbial decomposition, they have key eco-functions (see Hoffland et al., 2020) such as nutrient cycling, microbial activity and soil aggregation, which benefits plant growth. Finally, we highlight the relevance of adopting diversified cropping systems, including cover crops in the off-season period to protect the soil surface, promote constant C input and enhances soil health in this subtropical ecosystem.

CAPÍTULO V – Conclusões Gerais

Os principais resultados da presente pesquisa demonstram que sistemas de culturas à base de leguminosas de cobertura de solo (lablab, guandu e feijão-caupi) com alta adição de C proporcionaram alta estabilização de C na matéria orgânica associada aos minerais (silte e argila) e resultaram em níveis de C nesta fração próximos a saturação, principalmente na camada superficial do solo (0-2,5 cm). Além disso, houve diminuição do déficit de saturação de C em todas as camadas de solo avaliadas até 20 cm de profundidade após longo tempo de cultivo combinado a sistemas de cultura com elevado aporte de biomassa vegetal em plantio direto.

A capacidade remanescente de estabilização de C na fração associada aos minerais na camada superficial do solo (0-2,5 cm) diminuiu 90-97% quando comparada ao solo descoberto. Entretanto, apesar dessa limitação de sequestro de C na fração de *turnover* lento, os resultados sugerem que outras frações mais lábeis da matéria orgânica, como a matéria orgânica particulada, tendem a incrementar após a saturação da fração associada aos minerais, explicando a continuidade no aumento do C observado no solo inteiro.

Os resultados encontrados no segundo estudo, onde foram utilizados resíduos enriquecidos com isótopo ^{13}C incubados em tratamentos com diferentes déficits de saturação de C a nível de campo, corroboram com as indicações encontradas no primeiro estudo. O déficit de saturação de C na camada superficial do solo em sistema de plantio direto por longo tempo controla a transferência e estabilização do C novo recém adicionado para as diferentes frações da MOS, especialmente a fração de *turnover* lento. Quando a estabilização do C novo na fração associada aos minerais foi limitada pela

saturação de C, o aumento do C novo (^{13}C) foi intensificado nas frações intra e inter-agregados. Além disso, foi constatado maior estabilização do C derivado de ervilhaca do que de aveia na fração associada aos minerais na camada superficial do solo (0-2,5 cm), mas apenas quando o solo apresentava alta capacidade de estabilização de C, ou seja, níveis alto e muito alto de déficit de saturação de C

Em síntese, os resultados encontrados evidenciam que a saturação de C em solo subtropical sob plantio direto ocorre principalmente na camada superficial do solo (0-2.5 cm), enquanto nas camadas subsuperficiais (10-20 cm) o solo apresenta alto potencial de sequestro de C após quase quatro décadas de cultivo em plantio direto. Além disso, o déficit de saturação de C e a qualidade dos resíduos influenciam significativamente a estabilização do C nas camadas superficiais dos solos subtropicais sob plantio direto a longo prazo.

Estudos futuros de incubação investigando o efeito da déficit de saturação de C na migração de C no perfil de solo, bem como no sequestro de C em frações funcionais da matéria orgânica, considerando especialmente a preservação da estrutura e agregação do solo, são necessários nesses ambientes subtropical sob plantio direto de longo prazo para entender melhor a dinâmica do acúmulo de C e identificar práticas de manejo mais eficientes em promover o sequestro de C, especialmente visando o aumento do C em profundidade nestes ambientes.

REFERÊNCIAS

ADAIR, E. C. *et al.* Simple three-pool model accurately describes patterns of long-term litter decomposition in diverse climates. **Global Change Biology**, Oxford, v. 14, n. 11, p. 2636–2660, 2008.

ANGERS, D. A. *et al.* Estimating and mapping the carbon saturation deficit of French agricultural topsoils. **Soil Use and Management**, Wallingford, v. 27, n. 4, p. 448–452, 2011.

ANGERS, D. A.; CARON, J. Plant-induced changes in soil structure: processes and feedbacks. **Biogeochemistry**, Dordrecht, v. 42, n. 1, p. 55–72, 1998.

ARIAS, M.; BARRAL, M. T.; DIAZ-FIERROS, F. Effects of iron and aluminium oxides on the colloidal and surface properties of kaolin. **Clays and Clay Minerals**, London, v. 43, n. 4, p. 406–416, 1995.

BALESDENT, J. *et al.* Atmosphere–soil carbon transfer as a function of soil depth. **Nature**, London, v. 559, n. 7715, p. 599–602, 2018.

BAYER, C. *et al.* Organic matter storage in a sandy clay loam Acrisol affected by tillage and cropping systems in southern Brazil. **Soil and Tillage Research**, Amsterdam, v. 54, n. 1/2, p. 101–109, 2000.

BAYER, C. *et al.* Changes in soil organic matter fractions under subtropical no-till cropping systems. **Soil Science Society of America Journal**, Madison, v. 65, n. 5, p. 1473–1478, 2001.

BAYER, C. *et al.* A method for estimating coefficients of soil organic matter dynamics based on long-term experiments. **Soil and Tillage Research**, Amsterdam, v. 91, n. 1/2, p. 217–226, 2006a.

BAYER, C. *et al.* Carbon sequestration in two Brazilian Cerrado soils under no-till. **Soil and Tillage Research**, Amsterdam, v. 86, n. 2, p. 237–245, 2006b.

BAYER, C. *et al.* Cover crop effects increasing carbon storage in a subtropical no-till sandy Acrisol. **Communications in Soil Science and Plant Analysis**, Basingstoke, v. 40, n. 9/10, p. 1499–1511, 2009.

BAYER, C. *et al.* Mitigating greenhouse gas emissions from a subtropical Ultisol by using long-term no-tillage in combination with legume cover crops. **Soil and Tillage Research**, Amsterdam, v. 161, p. 86–94, 2016.

BEARDMORE, R. E. *et al.* Metabolic trade-offs and the maintenance of the fittest and the flattest. **Nature**, London, v. 472, n. 7343, p. 342–346, 2011.

BERTRAND, I. *et al.* Can the biochemical features and histology of wheat residues explain their decomposition in soil? **Plant and Soil**, The Hague, v. 281, n. 1/2, p. 291–307, 2006.

BLAGODATSKY, S. A.; HEINEMEYER, O.; RICHTER, J. Estimating the active and total soil microbial biomass by kinetic respiration analysis. **Biology and Fertility of Soils**, Berlin, v. 32, n. 1, p. 73–81, 2000.

BODDEY, R. M. *et al.* Carbon accumulation at depth in Ferralsols under zero-till subtropical agriculture. **Global Change Biology**, Oxford, v. 16, n. 2, p. 784–795, 2010.

BOSSIO, D. A. *et al.* The role of soil carbon in natural climate solutions. **Nature Sustainability**, London, v. 3, n. 5, p. 391–398, 2020.

BRIEDIS, C. *et al.* Can highly weathered soils under conservation agriculture be C saturated? **Catena**, Amsterdam, v. 147, p. 638–649, 2016.

BRIEDIS, C. *et al.* How does no-till deliver carbon stabilization and saturation in highly weathered soils? **Catena**, Amsterdam, v. 163, p. 13–23, 2018.

BROMAND, S. *et al.* A pulse-labelling method to generate ¹³C- enriched plant materials. **Plant and Soil**, The Hague, v. 235, n. 2, p. 253–257, 2001.

BURLE, M. L.; MIELNICZUK, J.; FOCCHI, S. Effect of cropping systems on soil chemical characteristics, with emphasis on soil acidification. **Plant and Soil**, The Hague, v. 190, n. 2, p. 309–316, 1997.

CAMBARDELLA, C. A.; ELLIOTT, E. T. Particulate soil organic-matter changes across a grassland cultivation sequence. **Soil Science Society of America Journal**, Madison, v. 56, n. 3, p. 777–783, 1992.

CAMPBELL, C. A. *et al.* Effect of crop rotations and cultural practices on soil organic matter, microbial biomass and respiration in a thin Black Chernozem. **Canadian Journal of Soil Science**, Ottawa, v. 71, n. 3, p. 363–376, 1991.

CARTER, M. R. Soil quality for sustainable land management: organic matter and aggregation interactions that maintain soil functions. **Agronomy Journal**, Madison, v. 94, n. 1, p. 38–47, 2002.

CARTER, M. R. *et al.* Characterizing organic matter retention for surface soils in eastern Canada using density and particle size fractions. **Canadian Journal of Soil Science**, Ottawa, v. 83, n. 1, p. 11–23, 2003.

CASTELLANO, M. J. *et al.* Integrating plant litter quality, soil organic matter stabilization, and the carbon saturation concept. **Global Change Biology**, Oxford, v. 21, n. 9, p. 3200–3209, 2015.

CHUNG, H. *et al.* Evidence for carbon saturation in a highly structured and organic-matter-rich soil. **Soil Science Society of America Journal**, Madison, v. 74, n. 1, p. 130, 2010.

CHUNG, H.; GROVE, J. H.; SIX, J. Indications for soil carbon saturation in a temperate agroecosystem. **Soil Science Society of America Journal**, Madison, v. 72, n. 4, p. 1132–1139, 2008.

CONCEIÇÃO, P. C. *et al.* Fracionamento densimétrico com politungstato de sódio no estudo da proteção física da matéria orgânica em solos. **Revista Brasileira de Ciência do Solo**, Viçosa, MG, v. 32, n. 2, p. 541–549, 2008.

CONCEIÇÃO, P. C.; DIECKOW, J.; BAYER, C. Combined role of no-tillage and cropping systems in soil carbon stocks and stabilization. **Soil & Tillage Research**, Amsterdam, v. 129, p. 40–47, 2013.

CORBEELS, M. *et al.* Evidence of limited carbon sequestration in soils under no-tillage systems in the Cerrado of Brazil. **Scientific Reports**, London, v. 6, [art.] 21450, [p. 1–8], 2016.

COTRUFO, M. F. *et al.* The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? **Global Change Biology**, Oxford, v. 19, n. 4, p. 988–995, 2013.

COTRUFO, M. F. *et al.* Formation of soil organic matter via biochemical and physical pathways of litter mass loss. **Nature Geoscience**, London, v. 8, n. 10, p. 776–779, 2015.

COTRUFO, M. F.; DEL GALDO, L.; PIERMATTEO, D. Litter decomposition: concepts, methods and future perspectives. *In*: KUTSCH, W. L.; BAHN, M.; HEINEMEYER, A. (ed.). **Soil carbon dynamics: an integrated methodology**. Cambridge: Cambridge University Press, 2009. p. 76–90.

DENEF, K. *et al.* Carbon sequestration in microaggregates of no-tillage soils with different clay mineralogy. **Soil Science Society of America Journal**, Madison, v. 68, p. 1935–1944, 2004.

DENEF, K. *et al.* Microaggregate-associated carbon as a diagnostic fraction for management-induced changes in soil organic carbon in two Oxisols. **Soil Biology & Biochemistry**, Oxford, v. 39, n. 5, p. 1165–1172, 2007.

DI, J. *et al.* Soil organic carbon saturation deficit under primary agricultural managements across major croplands in China. **Ecosystem Health and Sustainability**, Baltimore, v. 3, n. 7, [art.] 1364047, 2017.

DICK, D. P. *et al.* Characteristics of soil organic matter of different Brazilian

Ferralsols under native vegetation as a function of soil depth. **Geoderma**, Amsterdam, v. 124, p. 319–333, 2005.

DIECKOW, J. *et al.* Carbon and nitrogen stocks in physical fractions of a subtropical Acrisol as influenced by long-term no-till cropping systems and N fertilisation. **Plant and Soil**, The Hague, v. 268, n. 1, p. 319–328, 2005.

DIECKOW, J. *et al.* Soil C and N stocks as affected by cropping systems and nitrogen fertilisation in a southern Brazil Acrisol managed under no-tillage for 17 years. **Soil and Tillage Research**, Amsterdam, v. 81, n. 1, p. 87–95, 2005.

ESSINGTON, M. E. **Environmental soil chemistry: an integrative approach**. Boca Raton: Taylor & Francis, 2005.

FAO. **World reference base for soil resources 2006: a framework for international classification, correlation and communication**. Rome: FAO, 2006.

FAO. **Recarbonization of global soils: a tool to support the implementation of the Koronivia Joint Work on Agriculture**. Rome: FAO, 2019. Disponível em: <http://www.fao.org/documents/card/en/c/ca6522en/>. Acesso em: 2 jun. 2021.

FERREIRA, A. O. *et al.* Driving factors of soil carbon accumulation in Oxisols in long-term no-till systems of South Brazil. **Science of the Total Environment**, New York, v. 622/623, p. 735–742, 2018

FENG, W.; PLANTE, A. F.; SIX, J. Improving estimates of maximal organic carbon stabilization by fine soil particles. **Biogeochemistry**, Dordrecht, v. 112, p. 81–93, 2013.

FERNÁNDEZ, R. *et al.* Soil quality and productivity under zero tillage and grazing on Mollisols in Argentina – A long-term study. **Geoderma Regional**, Amsterdam, v. 11, p. 44–52, 2017.

GLOOR, M. *et al.* The carbon balance of South America: a review of the status, decadal trends and main determinants. **Biogeosciences**, Katlenburg-Lindau, v. 9, n. 12, p. 5407–5430, 2012.

GOLCHIN, A. *et al.* Study of free and occluded particulate organic matter in soils by solid state ¹³C cp/mas nmr spectroscopy and scanning electron microscopy. **Australian Journal of Soil Research**, Melbourne, v. 32, n. 2, p. 285–309, 1994.

GULDE, S. *et al.* Soil carbon saturation controls labile and stable carbon pool dynamics. **Soil Science Society of America Journal**, Madison, v. 72, n. 3, p. 605-612, 2008.

HASSINK, J. The capacity of soils to preserve organic C and N by their association with silt and clay particles. **Plant and Soil**, The Hague, v. 191, n. 1, p. 77–87, 1997.

HATTON, P. J. *et al.* Litter type control on soil C and N stabilization dynamics in

a temperate forest. **Global Change Biology**, Oxford, v. 21, n. 3, p. 1358–1367, 2015.

HECKMAN, K. *et al.* Sorptive fractionation of organic matter and formation of organo-hydroxy-aluminum complexes during litter biodegradation in the presence of gibbsite. **Geochimica et Cosmochimica Acta**, Amsterdam, v. 121, p. 667–683, 2013.

HIRAISHI, T. *et al.* (ed.). **2013 Revised supplementary methods and good practice guidance arising from the Kyoto Protocol**. Geneva: IPCC, 2014.

HOFFLAND, E. *et al.* Eco-functionality of organic matter in soils. **Plant and Soil**, The Hague, v. 455, n. 1/2, p. 1–22, 2020.

INDA JUNIOR, A. V. *et al.* Variáveis relacionadas à estabilidade de complexos organo-minerais em solos tropicais e subtropicais brasileiros. **Ciência Rural**, Santa Maria, v. 37, n. 5, p. 1301–1307, 2007.

KAISER, K.; GUGGENBERGER, G. Mineral surfaces and soil organic matter. **European Journal of Soil Science**, Oxford, v. 54, n. 2, p. 219–236, 2003.

KAISER, K.; KALBITZ, K. Cycling downwards - dissolved organic matter in soils. **Soil Biology and Biochemistry**, Oxford, v. 52, p. 29–32, 2012.

KALLENBACH, C. M. *et al.* Microbial physiology and necromass regulate agricultural soil carbon accumulation. **Soil Biology and Biochemistry**, Oxford, v. 91, p. 279–290, 2015.

KALLENBACH, C. M.; GRANDY, A.; FREY, S. D. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. **Nature Communications**, London, v. 7, [art.] 13630, [p. 1–10], 2016.

KIEM, R.; KÖGEL-KNABNER, I. Contribution of lignin and polysaccharides to the refractory carbon pool in C-depleted arable soils. **Soil Biology and Biochemistry**, Oxford, v. 35, n. 1, p. 101–118, 2003.

KLEBER, M. What is recalcitrant soil organic matter? **Environmental Chemistry**, Boca Raton, v. 7, n. 4, p. 320–332, 2010.

KLEBER, M. *et al.* Mineral-organic associations: formation, properties, and relevance in soil environments. **Advances in Agronomy**, San Diego, v. 130, p. 1-140. 2015.

KLEBER, M. *et al.* Dynamic interactions at the mineral–organic matter interface. **Nature Reviews Earth & Environment**, London, v. 2, n. 6. p. 402-421, 2021.

KLEBER, M.; SOLLINS, P.; SUTTON, R. A conceptual model of organo-mineral interactions in soils: self-assembly of organic molecular fragments into zonal structures on mineral surfaces. **Biogeochemistry**, Dordrecht, v. 85, n. 1, p. 9–24, 2007.

KLOTZBÜCHER, T. *et al.* A new conceptual model for the fate of lignin in decomposing plant litter. **Ecology**, Brooklyn, v. 92, n. 5, p. 1052–1062, 2011.

KÖGEL-KNABNER, I. *et al.* Organo-mineral associations in temperate soils: integrating biology, mineralogy, and organic matter chemistry. **Journal of Plant Nutrition and Soil Science**, Weinheim, v. 171, n. 1, p. 61–82, 2008.

KONG, A. Y. Y. *et al.* The relationship between carbon input, aggregation, and soil organic carbon stabilization in sustainable cropping systems. **Soil Science Society of America Journal**, Madison, v. 69, n. 4, p. 1078-1085, 2005.

KONG, A. Y. Y.; SIX, J. Tracing root vs. residue carbon into soils from conventional and alternative cropping systems. **Soil Science Society of America Journal**, Madison, v. 74, n. 4, p. 1201-1210, 2010.

KOOL, D. M. *et al.* Hierarchical saturation of soil carbon pools near a natural CO₂ spring. **Global Change Biology**, Oxford, v. 13, n. 6, p. 1282–1293, 2007.

LAL, R. Soil carbon sequestration impacts on global climate change and food security. **Science**, New York, v. 304, n. 5677, p. 1623–1627, 2004. Disponível em: <http://www.jstor.org/stable/3837021>. Acesso em: 2 fev. 2021.

LAL, R. Carbon sequestration. **Philosophical Transactions of the Royal Society B: Biological Sciences**, London, v. 363, n. 1492, p. 815-830, 2008.

LAL, R. Soil health and carbon management. **Food and Energy Security**, Malden, v. 5, n. 4, p. 212–222, 2016.

LAVALLEE, J. M.; SOONG, J. L.; COTRUFO, M. F. Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. **Global Change Biology**, Oxford, v. 26, n. 1, p. 261–273, 2020.

LIPSON, D. A. *et al.* The trade-off between growth rate and yield in microbial communities and the consequences for under-snow soil respiration in a high elevation coniferous forest. **Biogeochemistry**, Dordrecht, v. 95, n. 1, p. 23–35, 2009.

LOVATO, T. *et al.* Carbon and nitrogen addition related to stocks of these elements in soil and corn yield under management systems. **Revista Brasileira de Ciência do Solo**, Viçosa, MG, v. 28, p. 175–187, 2004.

MACÍAS, F.; ARBESTAIN, M. C. Soil carbon sequestration in a changing global environment. **Mitigation Adaptation Strategies for Global Change**, Dordrecht, v. 15, n. 6, p. 511–529, 2010.

MAMBELLI, S. *et al.* Relative contribution of foliar and fine root pine litter to the molecular composition of soil organic matter after in situ degradation. **Organic Geochemistry**, Oxford, v. 42, n. 9, p. 1099–1108, 2011.

MARSCHNER, B. *et al.* How relevant is recalcitrance for the stabilization of

organic matter in soils? **Journal of Plant Nutrition and Soil Science**, Weinheim, v. 171, n. 1, p. 91–110, 2008.

MEYER, N. *et al.* Carbon saturation drives spatial patterns of soil organic matter losses under long-term bare fallow. **Geoderma**, Amsterdam, v. 306, p. 89–98, 2017.

MIKUTTA, R. *et al.* Stabilization of soil organic matter: association with minerals or chemical recalcitrance? **Biogeochemistry**, Dordrecht, v. 77, p. 25–56, 2006.

MIKUTTA, R. *et al.* Biodegradation of forest floor organic matter bound to minerals via different binding mechanisms. **Geochimica et Cosmochimica Acta**, Amsterdam, v. 71, p. 2569–2590, 2007.

MOORHEAD, D. L. *et al.* Calculating co-metabolic costs of lignin decay and their impacts on carbon use efficiency. **Soil Biology and Biochemistry**, Oxford, v. 66, p. 17–19, 2013.

MORDACQ, L.; MOUSSEAU, M.; DELEENS, E. A ¹³C method of estimation of carbon allocation to roots in a young chestnut coppice. **Plant, Cell and Environment**, Oxford, v. 9, n. 9, p. 735–739, 1986.

NICOLOSO, R. S. *et al.* Carbon saturation and translocation in a no-till soil under organic amendments. **Agriculture, Ecosystems and Environment**, Amsterdam, v. 264, p. 73–84, 2018.

OGLE, S. M.; BREIDT, F. J.; PAUSTIAN, K. Agricultural management impacts on soil organic carbon storage under moist and dry climatic conditions of temperate and tropical regions. **Biogeochemistry**, Dordrecht, v. 72, n. 1, p. 87–121, 2005.

PEDRON, F. D. A. *et al.* Clay mineralogy of subtropical soils under long-term organic fertilization in no-tillage systems. **Revista Brasileira de Ciência do Solo**, Viçosa, MG, v. 42, [art.] e0170092, [p. 1–14], 2018.

POIRIER, V. *et al.* Organo-mineral interactions are more important for organic matter retention in subsoil than topsoil. **Soil Systems**, Basel, v. 4, n. 1, [art.] 4, [p. 1-17], 2020.

POWLSON, D. S.; WHITMORE, A. P.; GOULDING, K. W. T. Soil carbon sequestration to mitigate climate change: a critical re-examination to identify the true and the false. **European Journal of Soil Science**, Oxford, v. 62, n. 1, p. 42–55, 2011.

PRESCOTT, C. E. Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? **Biogeochemistry**, Dordrecht, v. 101, p. 133–149, 2010.

PRESTON, C. M.; NAULT, J. R.; TROFYMOW, J. A. Chemical changes during 6 years of decomposition of 11 litters in some canadian forest sites. Part 2. ¹³C abundance, solid-state ¹³C NMR spectroscopy and the meaning of “Lignin”.

Ecosystems, New York, v. 12, n. 7, p. 1078–1102, 2009.

RASSE, D. P. *et al.* Lignin turnover in an agricultural field: from plant residues to soil-protected fractions. **European Journal of Soil Science**, Oxford, v. 57, n. 4, p. 530–538, 2006.

REIS, C. E. S. *et al.* Carbon sequestration in clay and silt fractions of Brazilian soils under conventional and no-tillage systems. **Scientia Agricola**, Piracicaba, v. 71, n. 4, p. 292–301, 2014.

RODRIGUES, L. A. T. *et al.* Short- and long-term effects of animal manures and mineral fertilizer on carbon stocks in subtropical soil under no-tillage. **Geoderma**, Amsterdam, v. 386, [art.] 114913, 2021.

ROLLER, B. R. K.; SCHMIDT, T. M. The physiology and ecological implications of efficient growth. **ISME Journal**, London, v. 9, n. 7, p. 1481–1487, 2015.

RUBINO, M. *et al.* Carbon input belowground is the major C flux contributing to leaf litter mass loss: evidences from a ¹³C labelled-leaf litter experiment. **Soil Biology and Biochemistry**, Oxford, v. 42, n. 7, p. 1009–1016, 2010.

SÁ, J. C. M. *et al.* Low-carbon agriculture in South America to mitigate global climate change and advance food security. **Environment International**, New York, v. 98, p. 102–112, 2017.

SANTOS, N. Z. *et al.* Forages, cover crops and related shoot and root additions in no-till rotations to C sequestration in a subtropical Ferralsol. **Soil and Tillage Research**, Amsterdam, v. 111, n. 2, p. 208–218, 2011.

SARKAR, B. *et al.* Clay minerals — organic matter interactions in relation to carbon stabilization in soils. *In*: GARCIA, C.; NANNIPIERI, P.; HERNANDEZ, T. (ed.). **The future of soil carbon: its conservation and formation**. Cambridge: Academic Press, 2018. cap. 3, p. 71-86.

SCHLESINGER, W. H. Evidence from chronosequence studies for a low carbon-storage potential of soils. **Nature**, London, v. 348, n. 6298, p. 232–234, 1990.

SCHÖBER, P.; BOER, C.; SCHWARTE, L. A. Correlation coefficients: appropriate use and interpretation. **Anesthesia and Analgesia**, Ames, v. 126, n. 5, p. 1763–1768, 2018.

SHAHBAZ, M. *et al.* Microbial decomposition of soil organic matter is mediated by quality and quantity of crop residues: mechanisms and thresholds. **Biology and Fertility of Soils**, Berlin, v. 53, n. 3, p. 287–301, 2017.

SINSABAUGH, R. L. *et al.* Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. **Ecology Letters**, Oxford, v. 16, n. 7, p. 930–939, 2013.

SIX, J. *et al.* Stabilization mechanisms of soil organic matter: Implications for C-

saturation of soils. **Plant and Soil**, The Hague, v. 241, p. 155–176, 2002a.

SIX, J. *et al.* Soil organic matter, biota and aggregation in temperate and tropical soils - Effects of no-tillage. **Agronomie-Sciences des Productions Vegetales et de l'Environnement**, Paris, v. 22, n. 7/8, p. 755–776, 2002b.

SIX, J.; ELLIOTT, E. T.; PAUSTIAN, K. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. **Soil Biology & Biochemistry**, Oxford, v. 32, p. 2099–2103, 2000.

SMITH, P. Soil carbon sequestration and biochar as negative emission technologies. **Global Change Biology**, Oxford, v. 22, n. 3, p. 1315–1324, 2016.

SMUCKER, A. J. M. Root carbon contributions to soil aggregate formation and function. *In*: ABE, J. (ed.). **Roots: the dynamic interface between plants and the earth**. Dordrecht: Kluwer Academic, 2003. cap. 3, p. 421–426.

SOHI, S. P. *et al.* A procedure for isolating soil organic matter fractions suitable for modeling. **Soil Science Society of America Journal**, Madison, v. 65, n. 4, p. 1121–1228, 2001.

SOKOL, N. W.; SANDERMAN, J.; BRADFORD, M. A. Pathways of mineral-associated soil organic matter formation: integrating the role of plant carbon source, chemistry, and point of entry. **Global Change Biology**, Oxford, v. 25, n. 1, p. 12–24, 2019.

SOLLINS, P.; HOMANN, P.; CALDWELL, B. A. Stabilization and destabilization of soil organic matter: mechanisms and controls. **Geoderma**, Amsterdam, v. 74, n. 1/2, p. 65–105, 1996.

STEWART, C. C. E. *et al.* Soil carbon saturation: linking concept and measurable carbon pools. **Soil Science Society of America Journal**, Madison, v. 72, n. 2, p. 379, 2008a.

STEWART, C. E. *et al.* Soil carbon saturation: concept, evidence and evaluation. **Biogeochemistry**, Dordrecht, v. 86, n. 1, p. 19–31, 2007.

STEWART, C. E. *et al.* Soil carbon saturation: evaluation and corroboration by long-term incubations. **Soil Biology and Biochemistry**, Oxford, v. 40, n. 7, p. 1741–1750, 2008b.

TAHIR, M. M. *et al.* In situ roots decompose faster than shoots left on the soil surface under subtropical no-till conditions. **Biology and Fertility of Soils**, Berlin, v. 52, n. 6, p. 853–865, 2016.

TAHIR, M. M. *et al.* Field¹³C pulse labeling of pea, wheat, and vetch plants for subsequent root and shoot decomposition studies. **Revista Brasileira de Ciência do Solo**, Viçosa, MG, v. 42, p. 1–14, 2018.

THAYMUANG, W. *et al.* Organic matter stabilization by iron oxides in upland tropical red soils under tropical monsoonal and tropical savanna climates. **Thai**

Journal of Agricultural Science, Bangkok, v. 46, n. 3, p. 119–130, 2013.

TISDALL, J. M.; OADES, J. M. Organic matter and water-stable aggregates in soils. **Journal of Soil Science**, Oxford, v. 33, n. 2, p. 141–163, 1982.

VELOSO, M. G. *et al.* High carbon storage in a previously degraded subtropical soil under no-tillage with legume cover crops. **Agriculture, Ecosystems and Environment**, Amsterdam, v. 268, p. 15–23, 2018.

VELOSO, M. G. *et al.* Carbon accumulation and aggregation are mediated by fungi in a subtropical soil under conservation agriculture. **Geoderma**, Amsterdam, v. 363, [art.] 114159, 2020.

VELOSO, M. G.; CECAGNO, D.; BAYER, C. Research Legume cover crops under no-tillage favor organomineral association in microaggregates and soil C accumulation. **Soil & Tillage Research**, Amsterdam, v. 190, p. 139–146, 2019.

VIEIRA, F. C. B. *et al.* Building up organic matter in a subtropical paleudult under legume cover-crop-based rotations. **Soil Science Society of America Journal**, Madison, v. 73, n. 5, p. 1699–1706, 2009.

VON LÜTZOW, M. *et al.* Stabilization mechanisms of organic matter in four temperate soils: development and application of a conceptual model. **Journal of Plant Nutrition and Soil Science**, Weinheim, v. 171, n. 1, p. 111–124, 2008.

WAKSMAN, S. A. **Humus**: origin, chemical composition, and importance in nature. Baltimore: The Williams & Wilkins Company, 1936.

WEST, T. O.; SIX, J. Considering the influence of sequestration duration and carbon saturation on estimates of soil carbon capacity. **Climatic Change**, Dordrecht, v. 80, n. 1/2, p. 25–41, 2007.

WIESMEIER, M. *et al.* Carbon sequestration potential of soils in southeast Germany derived from stable soil organic carbon saturation. **Global Change Biology**, Oxford, v. 20, p. 653–665, 2014.

WISEMAN, C. L. S.; PUTTMANN, W. Soil organic carbon and its sorptive preservation in central Germany. **European Journal of Soil Science**, Oxford, v. 56, p. 65–76, 2005.

ZANATTA, J. A. *et al.* Soil organic carbon accumulation and carbon costs related to tillage, cropping systems and nitrogen fertilization in a subtropical Acrisol. **Soil and Tillage Research**, Amsterdam, v. 94, n. 2, p. 510–519, 2007.

ZHANG, D. *et al.* Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. **Journal of Plant Ecology**, Oxford, v. 1, n. 2, p. 85–93, 2008.

ZOMER, R. J. *et al.* Global sequestration potential of increased organic carbon in cropland soils. **Scientific Reports**, London, v. 7, [art.] 15554, [p. 1–8], 2017

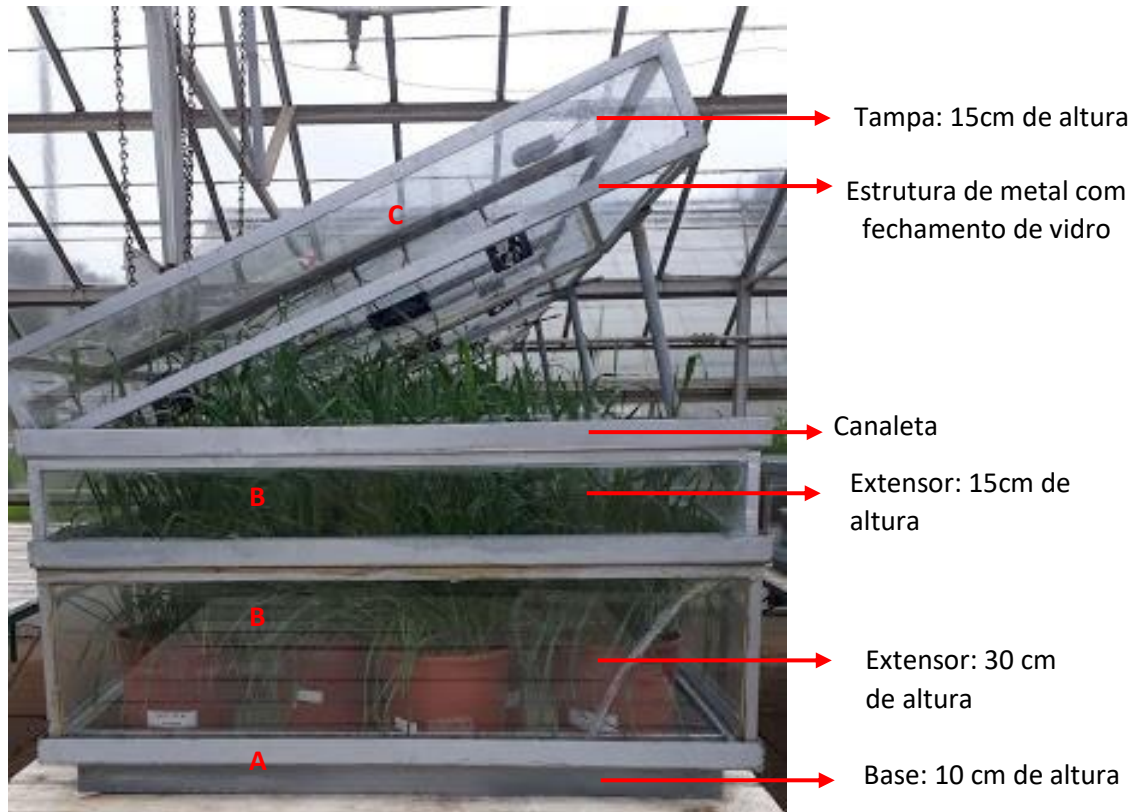
APÊNDICES

Enriquecimento de aveia e ervilhaca com isótopo estável ^{13}C

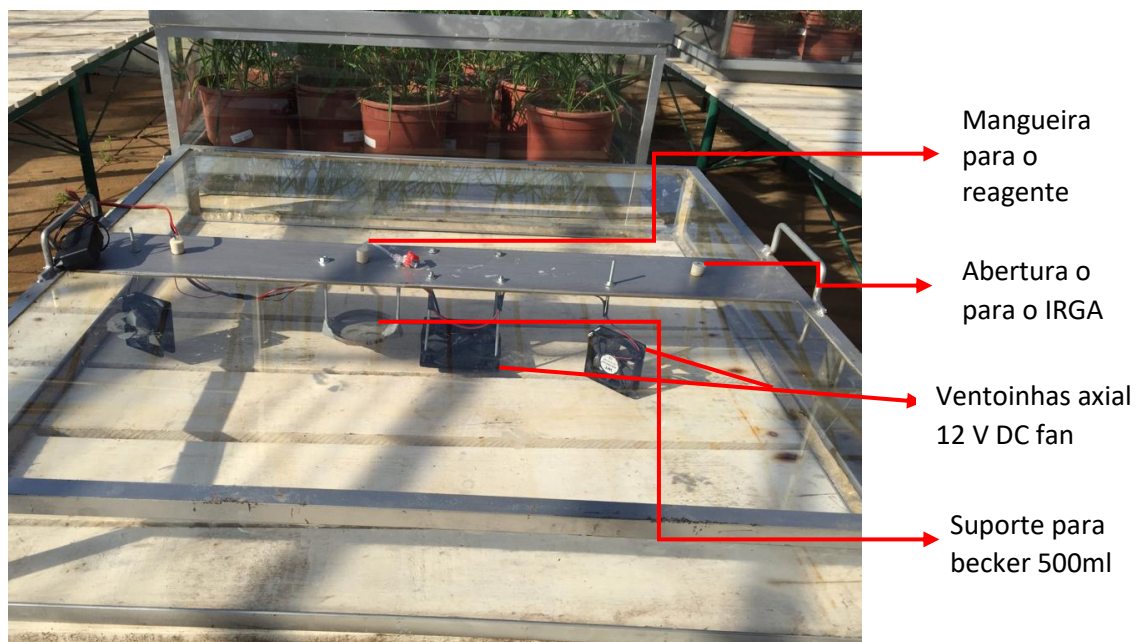
Câmaras de enriquecimento

As câmaras de enriquecimento foram confeccionadas em vidro colados numa estrutura em metal galvanizado, sendo compostas por diferentes partes, incluindo: Base de metal de 5 mm de espessura, com dimensões de 105 cm x 105 cm x 10 cm (Apêndice 1A); Extensores com dimensões 105 cm x 105 cm x 15 ou 30 cm (Apêndice 1B) e tampas com 105 cm de largura por 105 cm de comprimento e 15 cm de altura (Apêndice 1C). Na superfície de cada uma das partes, exceto na tampa, confeccionou-se uma canaleta com a finalidade de selar a atmosfera interna da câmara com uso de água.

Na tampa foram fixadas três ventoinhas axial 12 V DC fan, uma delas, com dimensões 10 cm x 10 cm, foi posicionada no centro, e as outras duas, com dimensões 8 cm x 8 cm, foram posicionadas nas extremidades, em direções opostas para realizar a circulação e homogeneização da atmosfera interna da câmara. Além disso, na tampa foram confeccionados dois orifícios por onde passaram o sensor de CO_2 do IRGA e uma mangueira direcionada ao becker de 500 ml fixado internamente para a adição da solução contendo ^{13}C (Apêndice 2).



Apêndice 1. Câmara de enriquecimento composta por base de metal (A), extensor de 15 ou 30 cm (B) e tampa de 15 cm de altura (C).



Apêndice 2. Detalhes dos componentes da tampa da câmara de enriquecimento

Condições de cultivo

O cultivo das espécies foi realizado em casa de vegetação no Departamento de Solos da Faculdade de Agronomia na Universidade Federal do Rio Grande do Sul, em Porto Alegre, RS, na Região Sul do Brasil. As espécies cultivadas foram a aveia preta (*Avena strigosa*) e a ervilhaca (*Vicia sativa*), escolhidas com objetivo de se obter uma variação na qualidade de resíduos (Gramínea x leguminosa) e por serem espécies comumente utilizadas como cobertura de solo no Brasil. As sementeiras da ervilhaca e da aveia preta foram realizadas em 29 de maio e 07 de junho de 2018, respectivamente, na densidade de seis sementes por vaso. O substrato utilizado foi uma mistura de solo < 2 mm (Argissolo Vermelho) + areia lavada em água corrente, na proporção 1:1, cujos parâmetros químicos e de fertilidade são apresentados a seguir no Apêndice 3.

Apêndice 3- Caracterização química da mistura de solo com areia utilizada no cultivo de aveia e ervilhaca vasos na casa de vegetação

P	K	S	Zn	Cu	B	Mn
mg/dm ³						
8,9	74	20	6,7	0,6	0,3	18
Argila	M.O	V	m	pH	SMP	
%						
16	2,4	77	0,0	5,8	6,8	
Al	Ca	Mg	CTC	H+Al		
cmol _c /dm ³						
0,0	3,5	2,1	7.6	1,7		

A adubação das plantas foi realizada segundo a recomendação da Comissão de Química e Fertilidade do Solo para o RS e SC (SBCS-CQFS, 2016) conforme a análise química da mistura. O P e o K foram aplicados em quantidades equivalentes a 5 vezes a recomendação da adubação de campo (95 kg de P₂O₅ e 95 kg de K₂O ha⁻¹) na formulação 0-20-20. A aplicação da quantidade equivalente a 5 vezes a recomendação de campo foi definido arbitrariamente para evitar a deficiência de nutrientes considerando à baixa relação solo: raiz explorado pelo sistema radicular no vaso comparado ao cultivo das plantas a campo. O nitrogênio foi aplicado em cobertura, equivalente a 95 kg ha⁻¹ na forma de ureia, parcelado em 6 aplicações, sendo: 15.8 kg ha⁻¹ na sementeira, 15.8 kg ha⁻¹ no estágio fenológico V4, e o restante das doses a cada 15 dias. Além disso, realizou-se a aplicação de solução com micronutrientes no

estádio V3 e posteriormente a cada 20 dias. O controle de doenças foliares foi realizado com aplicação fungicida epoxiconazol + piraclostrobina (opera).

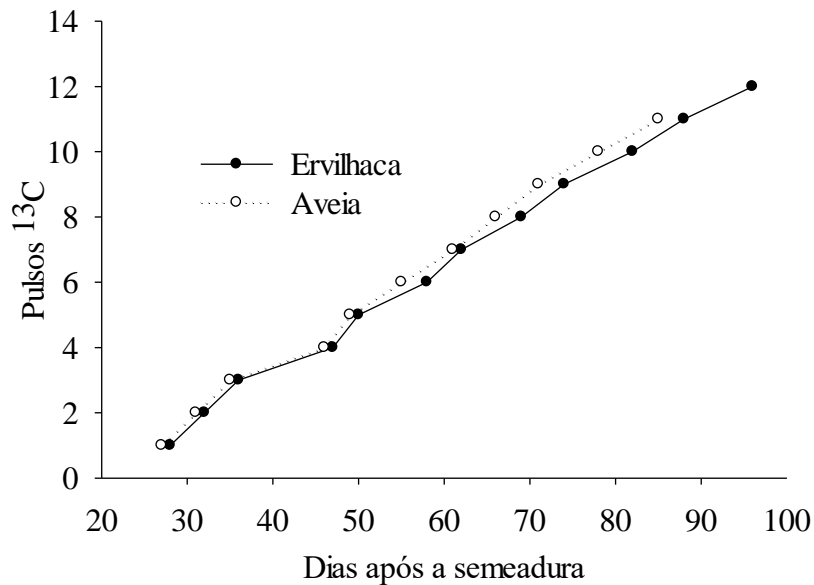
Para o enriquecimento das espécies, foram utilizadas 4 câmaras para aveia e duas câmaras para ervilhaca, contendo 10 vasos em cada (Apêndice 4). Além disso, três vasos de aveia preta e dois vasos de ervilhaca foram cultivados fora das câmaras como controle, os quais não foram expostos ao $^{13}\text{CO}_2$. Os vasos foram rotacionados semanalmente a fim de diminuir o efeito do sombreamento na eficiência fotossintética e de obter uma boa homogeneidade de enriquecimento entre as plantas.



Apêndice 4. Imagem das câmaras de enriquecimento contendo vasos de ervilhaca e de aveia preta na casa de vegetação da Faculdade de Agronomia – UFRGS.

Aplicação de pulsos de ^{13}C

O método de enriquecimento utilizado foi adaptado de BROMAND et al., (2001) e TAHIR et al., (2018). O enriquecimento das plantas de aveia e da ervilhaca iniciaram nos dias 10 e 11 de julho de 2018, respectivamente. Foram realizadas aplicações de pulsos de $^{13}\text{CO}_2$ uma vez por semana até o início do florescimento (Apêndice 5), normalmente realizados entre 9:00h e 11:00h da manhã (Apêndice 6).

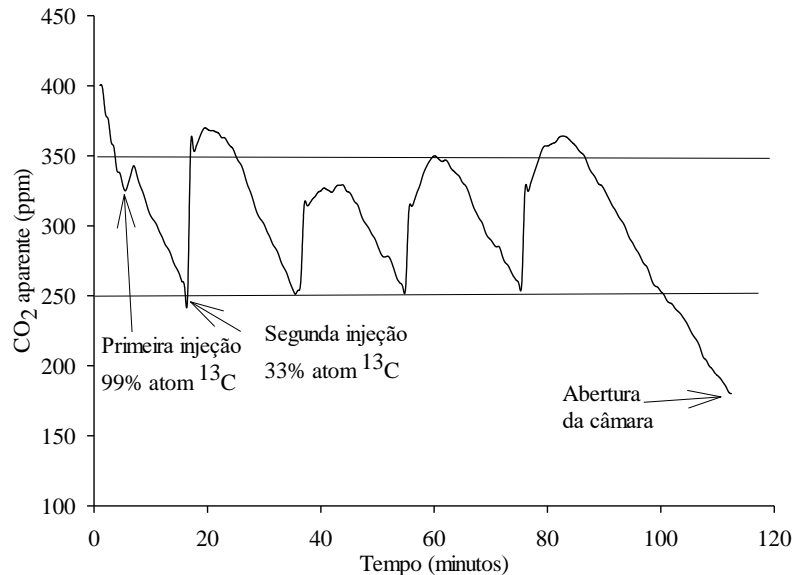


Apêndice 5. Número de pulsos de $^{13}\text{CO}_2$ realizados ao longo do ciclo das culturas da aveia e ervilhaca

O enriquecimento das plantas por aplicação de pulsos de $^{13}\text{CO}_2$ consistiu na formação de uma atmosfera enriquecida com ~ 33% atom ^{13}C no interior das câmaras. O $^{13}\text{CO}_2$ foi gerado a partir do uso de duas soluções NaHCO_3 1M, sendo a primeira com ~ 99% atom ^{13}C e a segunda com ~ 33% atom ^{13}C . As soluções foram preparadas através do uso de bicarbonato de sódio altamente enriquecido com ^{13}C (99% atom ^{13}C , CLM-441-5, USA) e não enriquecido ^{12}C . A concentração de $^{12}\text{CO}_2$ no interior das câmaras foi monitorada usando um analisador de gás infravermelho (IRGA - EXTECH, SD800). Devido a diferença no espectro de absorvância entre o $^{13}\text{CO}_2$ em relação ao $^{12}\text{CO}_2$, o IRGA detecta uma pequena porção da concentração $^{13}\text{CO}_2$. Portanto, foi estimado que o IRGA detecta aproximadamente 72% do CO_2 no interior da câmara gerado após a primeira injeção de $^{13}\text{CO}_2$ (BROMAND et al., 2001) e, portanto, subestima a concentração real de CO_2 (MORDACQ; MOUSSEAU; DELEENS, 1986).

Para formar uma atmosfera enriquecida com ~ 33% atom $^{13}\text{CO}_2$, as câmaras com volume inicial de $0,4 \text{ m}^3$ foram fechadas e após a concentração de CO_2 diminuir de ~ 430 ppm até ~ 327 ppm pela fotossíntese (Figura 5), foram

injetados 3 ml (3 mmol de $\text{NaH}^{13}\text{CO}_3$) da solução 99% atom ^{13}C em cada um dos beakers contendo 50 ml de solução de ácido clorídrico ($2 \text{ mol L}^{-1} \text{ HCl}$).



Apêndice 6. Dinâmica da concentração de CO_2 no interior de uma câmara de enriquecimento. Dados registrados pelo IRGA durante a realização de um pulso de $^{13}\text{CO}_2$ na cultura da aveia.

Após a concentração de CO_2 diminuir a 250 ppm (Apêndice 6), 4 ml (1,3 mmol de $\text{NaH}^{13}\text{CO}_3$) da solução com 33% atom ^{13}C foram injetados nos beakers, e esse processo foi repetido durante ~1 hora e 30 min. Após a última injeção da solução de 33% as câmaras foram mantidas fechadas até a concentração de CO_2 diminuir até ~170 ppm quando foram abertas (Apêndice 6). No final do dia do enriquecimento ao pôr do sol, as câmaras eram fechadas novamente para que o $^{13}\text{CO}_2$ respirado durante a noite fosse fotossintetizado na manhã seguinte. Após a concentração de CO_2 diminuir a 170 ppm, as câmaras eram abertas novamente. Esse procedimento visou aumentar a eficiência de incorporação de ^{13}C nas diferentes partes das plantas.

Para manter a temperatura amena ($\sim 20^\circ\text{C}$) no interior das câmaras a fim de maximizar a fotossíntese durante o processo de enriquecimento, foram utilizadas garrafas pets de 2L contendo gelo. Durante o ciclo de crescimento e desenvolvimento das espécies, à medida que havia aumento na estatura das plantas (principalmente para aveia) foi necessário aumentar o volume das

câmaras para 0,5, 0,65 e 0,8 m³ por meio do uso dos extensores e o volume de solução com ¹³C utilizado foi aumentado proporcionalmente.

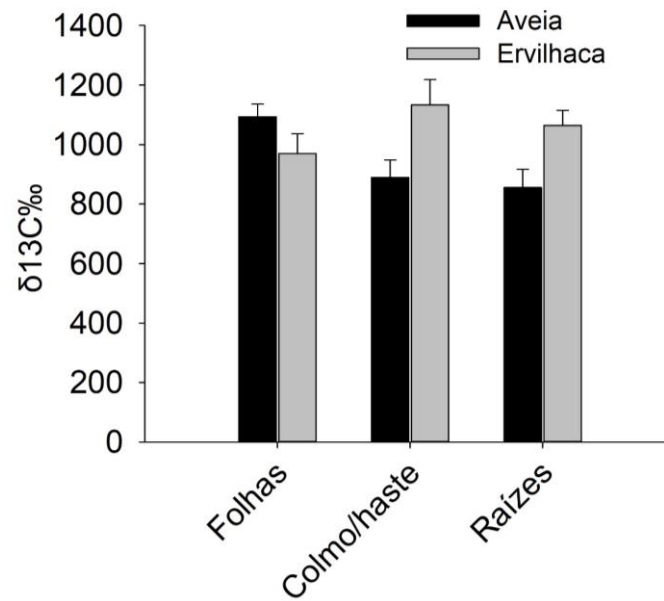
Avaliação do enriquecimento dos resíduos

Após o florescimento as plantas foram cortadas rente à superfície do solo, secas em casa de vegetação a temperatura ambiente, separadas em colmos/hastes, folhas e raízes e quantificadas quanto a sua massa. Uma sub amostra foi seca a 60 °C em estufa para correção da umidade e determinação da matéria seca. Outra sub amostra de cada parte (folhas, colmos e raízes) de 5 plantas escolhidas aleatoriamente em cada câmara foi seca em estufa a 45°C até peso constante, moídas em moedor de café e posteriormente em moinho de bolas. Em seguida, os resíduos de cada parte das plantas foram submetidos a análise isotópica do ¹³C em analisador elementar (Flash 2000 IRMS, Thermo Fisher Scientific, Bremen, Germany) acoplado a um espectrômetro de massa de razão isotópica (DELTA V Advantage, Thermo Fisher Scientific, Bremen, Germany) no Laboratório de Análises de Isótopos Estáveis da Universidade Federal de Santa Maria.

Nível de enriquecimento obtido

Os resíduos de aveia apresentaram valores médios de $\delta^{13}\text{C}$ de 1093,7‰ nas folhas, 889,2‰ nos colmos e 855,4‰ nas raízes (Apêndice 7). Os resíduos de ervilhaca apresentaram valores similares de $\delta^{13}\text{C}$ nas folhas (970,6‰), hastes (1133,0‰) e raízes (1064,7‰). Em experimento similares, como no estudo de THAIR et al., (2018) foram obtidos valores de $\delta^{13}\text{C}$ em plantas de trigo em torno de 493,4‰ nas folhas, 357,4‰ nos colmos e 479,9‰ nas raízes e para plantas de ervilhaca em torno de 254,8‰ nas folhas, 315,1‰ nas hastes e 340,6‰ nas raízes. Verificando assim que a concentração de ¹³C nos resíduos de aveia preta e ervilhaca do nosso estudo foram considerados altos (>850‰) quando comparados a outros estudos em condições de casa de vegetação (BROMAND et al., 2001) e de campo (TAHIR et al., 2018). Portanto, o método de enriquecimento dos resíduos das duas espécies foi eficiente e

permite a sua utilização nos estudos da dinâmica da MOS a campo em ambiente controlado em laboratório.



Apêndice 7. Enriquecimento $\delta^{13}\text{C}$ nas diferentes partes (folha, colmo/ haste e raiz) das plantas de aveia preta (*Avena strigosa*) e de ervilhaca (*Vicia sativa*).

RESUMO BIOGRÁFICO

Lucas Antonio Telles Rodrigues, filho de Noil Telles Rodrigues e Cleci Bernadete Rodrigues, agricultores, nasceu em 13 de dezembro de 1989, em Jóia, Rio Grande do Sul. Estudou nos colégios Orestes Ghisleni, Dr. Augusto Nascimento e Silva, Júlio Biasi e, Macedo Beltrão do Nascimento, onde completou seus estudos de primeiro e segundo graus. Em 2008 ingressou no serviço militar obrigatório no 9º BLOG-Santiago-RS, como soldado enfermeiro, onde permaneceu por 1 ano, e obteve certificado de honra ao mérito concedido pelo Exército Brasileiro pela conduta exemplar de respeito a hierarquia e disciplina durante o serviço militar. Em 2009 ingressou no curso de Agronomia da URI, campus Santiago e em 2010 transferiu-se para o curso de agronomia da UNIPAMPA, onde graduou-se como Engenheiro Agrônomo em dezembro de 2014. Em 2015 iniciou seus estudos de Mestrado em Ciência do Solo no Programa de Pós-Graduação em Ciência do Solo da UFSM sob orientação do Prof. Dr. Carlos Alberto Ceretta, onde desenvolveu sua pesquisa de dissertação sobre o efeito do uso de dejetos de animais e fertilizante mineral a longo prazo nos estoques de C no solo e em frações físicas da matéria orgânica, cujo trabalho compôs um dos requisitos para obtenção do título de Mestre em Ciência do Solo naquela instituição em março de 2017. Em maio de 2017 iniciou o curso de Doutorado em Ciência do solo na Universidade Federal do Rio Grande do Sul sob orientação do Prof. Dr. Cimélio Bayer, cuja pesquisa foi direcionada a avaliação da dinâmica de estabilização e saturação de C no solo.